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POULTRY JOURNAL

The Book of Abstracts



XVI European Poultry  
Conference

VALENCIA

24<sup>th</sup> 28<sup>th</sup> June 2024

education  
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**XVI European Poultry Conference of the WPSA  
Valencia - Spain  
June 24 – 28, 2024**

## **Book of abstracts**

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**Hosted by the:**

**AECA - WPSA: Asociación Española de Ciencia Avícola  
Spanish Branch of the World's Poultry Science Association**



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Email: [secretaria@wpsa-aeca.es](mailto:secretaria@wpsa-aeca.es)



## Welcome Letter to the XVI European Poultry Conference of the WPSA, Valencia 2024



Dear Colleague and Friend,

We are honoured to welcome you to the XVI European Poultry Conference, taking place in Valencia from 24th to 28th June 2024. This event, organized for this edition by the Asociación Española de Ciencia Avícola (the Spanish branch of the WPSA), under the auspices of the European Federation of the World's Poultry Science Association (WPSA).

The European Poultry Conference stands as one of the leading gatherings in the poultry sector, bringing together delegates from across Europe and beyond to participate in a comprehensive and dynamic program. Our event builds upon the successes of previous conferences held every four years in major European cities, maintaining a tradition of excellence and innovation in poultry science and industry.

We are privileged to host this year's edition in the vibrant and historically rich city of Valencia, known for its stunning architecture, beautiful beaches, and lively arts scene. Valencia provides an inspiring backdrop for scientific exchange and professional networking.

We have curated an engaging scientific program featuring renowned speakers from around the world, who will share their latest research findings and insights on various topics relevant to poultry science. The conference will also offer ample opportunities for industry professionals, researchers, university faculty, and students to connect, collaborate, and share knowledge. We particularly welcome the involvement of graduate and PhD students, who represent the future of our field and bring fresh perspectives to our shared challenges and opportunities.

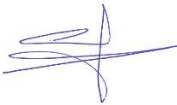
Our heartfelt gratitude goes out to the companies sponsoring this event, whose generous support has been instrumental in making this conference possible. An integral part of our conference is to give visibility to these sponsors, who have prepared an engaging commercial exhibition. We also

extend our thanks to the Valencian and Spanish institutions for their invaluable assistance and collaboration. We appreciate the dedicated efforts of the scientific and organizing committees in curating a high-quality conference.

In addition to the academic sessions, we have arranged a series of social events designed to showcase the best of Valencia's multicultural charm and Spanish hospitality. These events will offer participants the opportunity to experience the local culture and forge lasting relationships with colleagues from across the globe.

We eagerly anticipate a highly successful conference and urge all attendees to actively engage and contribute to discussions, with a particular emphasis on the involvement of numerous members of WPSA and enthusiasts of poultry science.

With warm regards,



Prof. Dr. Carlos Garcés-Narro

President of the XVI European Poultry Conference.

## **Welcome message of the Scientific Committee Chairperson**



Dear Colleagues and Friends,

It is my pleasure, as President of the Scientific Committee, to welcome you to the European Poultry Conference 2024. This event promises to be an exceptional opportunity to explore the latest scientific advancements in poultry science, share cutting-edge research, and foster synergies and knowledge transfer to the industry.

The scientific committee, composed of renowned specialists, has carefully crafted a program of great scientific interest and quality. Experts will impart their insights in key areas such as physiology, breeding and genetics, incubation and fertility, management and smart farming, among others. In addition, innovative strategies will be presented to improve the health, nutrition and welfare of poultry and, of course, to provide high quality meat and eggs, which will contribute to greater efficiency and sustainability of the poultry sector.

During this edition, 13 main speakers distributed in 6 plenary sessions will share their knowledge on the most important topics of poultry science. In addition, the conference will host parallel sessions, allowing for more focused discussions on specific subjects. These sessions will include some keynotes and the presentation of more than 200 short oral communications, highlighting the most important new findings. Furthermore, we are excited to showcase over 300 posters, which will be displayed throughout the duration of the conference. There will be four specific poster sessions where you can discuss and engage the authors directly. These more than 500 papers have been carried out by researchers affiliated with universities, research centres, and companies from all over Europe and beyond, sharing new perspectives, emerging challenges and open new avenues for research and collaboration.



We are committed to fostering an interactive and engaging environment. Each session, whether plenary or parallel, will facilitate direct interaction with the speakers. Attendees will have the opportunity to ask questions in real-time or via our dedicated app.

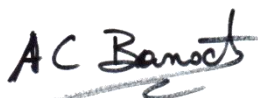
I would like to highlight the Youth Program, which has enabled more than 40 doctoral and postdoctoral students from around the world to participate in the conference, expanding their network of knowledge and contacts, and improving their preparation and skills for integration into the industrial and research sectors. This program is undoubtedly a benefit to society as a whole.

My deepest thanks to all the people who have supported this event: speakers, moderators, members of the abstract review and evaluation committee, organizers of the Youth Program, and the chairpersons who contribute to the smooth running of the program. Special recognition is due to the support of the members of the various WPSA committees (International Board, European Federation) and especially to AECA, the Spanish branch of WPSA, and to the organizing committee of this conference.

We believe that this conference will not only broaden your scientific knowledge, but also offer numerous opportunities for professional growth and collaboration. We look forward to your active participation in the stimulating discussions that will arise from this convergence of specialists in the field of poultry science.

Welcome to Valencia, and welcome to the European Poultry Conference 2024.

Prof. Dr. Ana C. Barroeta

A handwritten signature in black ink that reads "AC Barroeta". The signature is written in a cursive style with a horizontal line underneath the name.

Chairperson of the Scientific Committee of the XVI European Poultry Conference

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## INDEXES

## Content

### ***PLENNARY SESSIONS and KEYNOTE SPEAKERS***

<b>Slot</b>	<b>Abstract</b>	<b>Title (Presenter)</b>	<b>Page</b>
K01-01	131331	How can Precision Livestock Farming help improve Sustainable Poultry Production? (Tomas Norton)	3
K01-02	131027	A holistic and pragmatic view of sustainable development pathways in poultry production (Rick Kleyn)	4
K02-01	131123	Chicken-derived airway organoids: a model to study viral dynamics for infectious bronchitis, Newcastle disease, and avian influenza viruses (Ferran Tarrés-Freixas)	5
K03-01	131343	Optimizing poultry production through feed technology: It's all about structure (Birger Svihus)	6
K04-01	131354	Nourished mind: Egg Nutrients and Cognitive Function (Ana Maria Lopez Sobaler)	7
K05-01	131348	All Chickens Fast and Slow- assessing the “embryonic developmental tempo” phenotype of poultry breeds (Megan Davey)	8
K06-01	131329	Breeding for new requirements and future production systems. Laying hens (Teun van de Braak)	9
K06-02	131332	Breeding for new requirements and future production systems. Broilers Breeding for welfare in meat poultry (Anne-Marie Neeteson)	10
K06-03	131330	Sexual dimorphism during egg incubation and development of ovosexing methods (Sophie Réhault-Godbert)	11
K08-01	131339	Antibiotic resistance, the big one health deal (Bruno González Zorn)	12
K09-01	131345	Farm animal welfare assessment and certification (Antonio Dalmau)	13
K10-01	130837	Heritability estimates for movement of laying hens within a quasi-commercial aviary barn (Michael Toscano)	14
K11-01	131327	The microbiome-gut-brain axis in poultry production: what we know and don't know and why it matters (Mark Lyte)	15
K11-02	131336	Fostering embryonic development in chickens: maternal and in ovo nutritional strategies (Eugeni Roura)	16
K13-01	131344	Transfer of undesirable substances from feed into meat and eggs - from experimental data to predictive models (Robert Pieper)	17
K14-01	131346	Innovation in sensory assessment of poultry meat and meat products (Sonia Ventanas)	18
K15-01	131352	Growing broilers during tough economic conditions (Andrew Catlett)	19
K15-08	131350	Cost factors in broiler meat production. An alternative analysis (Jose Ignacio Barragan Cos)	26
K16-01	131328	Vaccination against avian influenza: adapting our strategy to changing challenges (Jean-Luc Guérin)	21



K16-02	131335	Unlocking Immune System Potential in Chickens through Gene Editing: A Promising Frontier (Benjamin Schusser)	22
K17-01	131353	The role of plant-based antinutritional factors in poultry diets. New progress on their effects on chicken growth and health (Gonzalo González Mateos)	23
K19-01	131351	Dealing with Global Supply Chain Realities, Layer Genetics (Sijne van der Beek)	24
K20-01	131347	Greenhouse gas emissions (Salvador Calvet Sanz)	25
K21-01	131337	Global Poultry Outlook - Consequences of political tensions in the poultry sector (Nan-Dirk Mulder)	26
K21-02	131338	Trends for the future of the Poultry sector: Education, Employment, and Research (Vincent Guyonnet)	27
K22-01	130981	Phage Therapy Against Superbugs: Promising Strategies and Insights (Sandra Sevilla-Navarro)	28
K23-01	131357	Early feeding nutrition of minerals and vitamins: implications and applications (Zehava Uni)	29
K24-01	131039	Beak trimming or breeding for better beaks? A genetic approach to reducing feather pecking damage (Sarah Struthers)	30
K25-01	131287	Towards a Humane and Sustainable Solution: A Genetic Model to Eliminate Male Chicks Culling in the Egg Industry (Yuval Cinnamon)	31
K26-01	131333	Strengths and weaknesses of poultry meat quality attributes for meeting the increasing global protein demand (Stefaan De Smet)	32
K26-02	131334	Bone and eggshell quality in hens in extended laying cycles (Alejandro Rodríguez Navarro)	33
K28-01	131340	Enterococcus cecorum, an emerging pathogen of meat type chickens (Arne Jung)	34

### **SHORT ORAL COMMUNICATIONS**

<b>Slot</b>	<b>Abstract</b>	<b>Title (Presenter)</b>	<b>Page</b>
O02-04	131116	An integrated approach for the management of avian influenza information (Giacomo Nalesso)	37
O02-05	130984	A chicken blood transcriptomic signature related to adaptive immune responses is associated with resistance to highly pathogenic avian influenza virus infection (Maria Jose Valdez-May)	38
O02-06	131216	Pathobiology and variable genetic determinants for virulence, transmission and tissues tropism of H7N7 avian influenza virus in chickens, turkeys and different duck breeds (El-Sayed Abdelwhab)	39
O02-07	131305	Efficacy of an Inactivated Monovalent AIV-H5 Vaccine against early challenge with HPAI-H5N8 clade 2.3.4.4b (Ricardo Neto)	40
O02-08	130751	A comparative study of epigenetic and pathogenesis mechanisms between the virulent and avirulent strains of Newcastle disease viruses using bioinformatics analysis (Aleksandar Dodovski)	41
O02-09	131162	First report of molecular detection of aMPV circulation in domestic and wild birds in Colombia (David Escobar)	42
O02-10	131185	Avian metapneumovirus circulation in broiler farms in Italy (Luis Agosto Pantoja Millas)	43

O02-11	131241	Infectious bronchitis virus surveillance in layers and broiler breeders in Spain from 2020 to 2023 (Angela Blanco)	44
O03-04	130977	Effects of polihherbal based product supplementation on growth performance, and gut health in broiler chickens challenged with <i>Eimeria</i> spp (Cinta Sol)	45
O03-05	130867	Dietary phytogetic inclusion level affects performance and expression of heat shock and inflammatory genes in the whole blood of cyclic heat-stressed broilers (Eugenia Iliopoulou)	46
O03-06	131080	Growth performance and antioxidant response of broiler chicken fed oxidized lipids with or without phytogetic feed additives (Fernando Sevillano)	47
O03-07	130939	Effect of dietary supplementation and in ovo feeding of menthol on the immunocompetence traits, mineral composition and fatty acid profile of breast and thigh muscle of commercial broilers (Amitav Bhattacharyya)	48
O03-08	131015	Quantitative and qualitative evaluation of plant intake in laying hens: n-alkanes as predictive fecal markers for dietary composition assessment (Laid Dardabou)	49
O03-09	131042	Effects of dietary supplementation of essential oils, lysozyme and vitamins blend on layer hen performance, viral vaccinal response and egg quality characteristics (Ilias Giannenas)	50
O03-10	131254	Application of cannabis-originated products in turkey nutrition (Pawel Konieczka)	51
O03-11	130795	In ovo delivery of carvacrol triggered the expression of antimicrobial peptides in the yolk sac of broiler embryos (Mila Meijer)	52
O04-04	131300	A sustainable and efficient strategy to enrich eggs with n-3 LC-PUFA (Jesús Villora)	53
O04-05	130807	Effect of activated vitamin D3 on eggshell quality and performance in brown laying hens to 100 weeks of age (Christine Clark)	54
O04-06	130894	Effects of hen age and egg storage conditions on the quality and protein composition of the vitelline membrane (Océane Hervé)	55
O04-07	130916	Nutritional value and sensory characteristics of eggs from laying hens fed hempseed (Mattia Pravato)	56
O04-08	131279	Determination of elemental composition of chicken eggs using X-ray fluorescence spectroscopy (Maryam Mehri)	57
O04-09	131044	Incidence of internal spots in commercial eggs in Spain (Maria Del Mar Campo)	58
O04-10	130880	Cut-off rate of floor eggs in the cage-free system: exploring possible contributing factors (Claudia Ciarelli)	59
O04-11	131099	Impact of eggshell translucency and trace mineral supplementation on eggshell quality parameters (Duarte Neves)	60
O05-04	130825	Non-invasive early assessment of chicken egg fertilization through VOC profiling using HSSE-GC-MS (Matthias Corion)	61
O05-05	131053	Non-invasive embryonic blood vessel detection during automated in-ovo sexing (Carla Van Der Pol)	62
O05-06	131100	Epigenetic programming of avian hypothalamus by in-ovo green monochromatic illumination and its effect on growth, appetite and metabolism in broilers (Joanna Bartman)	63
O05-07	130895	Effect of thermal manipulation of slow-growing broiler chickens on chick quality and physiology (Roos Molenaar)	64
O05-08	131102	Impact of feed restriction on testicular parameters in broiler breeder roosters during the production period (Silvia Elena Palao)	65
O05-09	131189	Incubation of chicken eggs should take 24 days instead of 21 days (Jan Wijnen)	66
O05-10	131138	Rediscovering Italian poultry treasures: reviving the Millefiori Piemontese breed (Eleonora Erika Cappone)	67
O05-11	131029	Carvacrol fed to broiler breeder hens was transferred to fertile eggs enhancing embryonic gut development (Shahram Niknafs)	68

O07-01	131117	The influence of nutrition on body composition and allometric growth patterns in laying hen pullets (Lieske Van Eck)	69
O07-02	130908	Effect of dietary balanced protein levels during rearing on early egg production and egg quality of white and brown egg-type strains (Jo Ann Chew)	70
O07-03	131057	Adequate dietary amino acid supply allows to moderately reduce dietary crude protein at the onset of lay without affecting laying performance of Isabrown layers (Maeva Durand)	71
O07-04	131103	Impact of soybean origin and harvest year on chemical composition, amino acid profile, and protein quality indicators of soybean meals collected from 2007 to 2023 (Nereida Luna Corrales)	72
O07-05	131045	The impact of digestible lysine on egg weight control and eggshell quality in old Lohmann Brown hens during 82 to 92 weeks (Reza Akbari Moghaddam Kakhki)	73
O07-06	131070	Cytokine expression during a mixed Eimeria infection in broilers given low protein diets supplemented with methionine or threonine (James Taylor)	74
O07-07	131156	Is the expression of markers for breast muscle development in broiler chickens affected by breed, sex, age, and dietary energy level? (Regiane Santos)	75
O07-08	130755	Comparative effects of low-protein soybean meal or high-protein soybean meal with wheat bran or prebiotics in broilers challenged with Eimeria using immune response and bacterial antimicrobial resistance gene abundance (Oluyinka Olukosi)	76
O07-09	131120	Caecal microbiota composition in chickens of different genotypes fed low-input diets (Giulia Zardinoni)	77
O07-10	131033	Effects of in ovo feeding of sericin on hatching performances and hepatic gene expression of broilers (Vaishali Gupta)	78
O07-11	131194	Dietary amino acid density affects growth rate and body composition of fast- and slow-growing broiler chickens (Hsuan Chen)	79
O07-12	130882	Nutritional needs of slow-growing broilers (Saskia Leleu)	80
O08-04	130941	Association between genotypic and phenotypic characteristics of E. coli strains isolated from broiler breeder flocks (Lica Lozica)	81
O08-05	130985	Full characterization of 23 salmonella wild type bacteriophages suitable for the eradication of the most relevant salmonella zoonotic serovars (Jan Torres-Boncompte)	82
O08-06	130722	In ovo carvacrol delivery downregulated splenic inflammatory responses after a post-hatch lipopolysaccharide challenge in broiler chicks (Mila Meijer)	83
O08-07	130785	Understanding the mode of action of Bacillus subtilis 29784 poultry probiotic through its secreted metabolites: A resilient approach (Nuria Vieco-Saiz)	84
O08-08	130817	Microbiome Modulation by a precision biotic lead to increased production of short-chain fatty acids in the intestine of broiler chickens (Cristiano Bortoluzzi)	85
O08-09	131013	Probiotics or Prophybiotics? Validation of effects of in-ovo administration on gut health and production of broiler chickens (Ramesha Nirmali Wishna Kadawarage)	86
O08-10	131182	An assessment of the antibacterial properties of black soldier fly larvae (Hermetia illucens) meal against several pathogenic bacteria found in poultry (Dumeshi Ellawidana)	87
O08-11	130914	Butyric acid and medium-chain fatty acids improve vaccine efficacy in broiler chickens by modulating the adaptive immune system (David Hermans)	88
O09-04	130784	Use of artificial intelligence tools to simplify broiler welfare assessment protocols (Xavier Averós)	89
O09-05	131202	Welfare evaluation in the main Italian rearing system for poultry meat production by ebene app (Alice Cartoni Mancinelli)	90
O09-06	130901	Broiler welfare assessment: footpad and hock health analysis in Austrian	91

		abattoirs, 2020-2022 (Janja Sirovnik)	
O09-07	131082	Interobserver reliability of a scoring system to evaluate bruises in turkey carcasses (Luisa Raederscheidt)	92
O09-08	131239	LEGMONI, a ready-to-use tool for evaluating laying hen welfare at the slaughterhouse (Anneleen Watteyn)	93
O09-09	131152	Determination of ideal perch space allowance for pullets in Canada (Carolyn Adler)	94
O09-10	131244	Direct and carry-over effects of feeding pullets during sensitive periods in early rearing (Annemarie Mens)	95
O09-11	131066	Status quo of light conditions in turkey barns to identify light-associated risk factors of behavioral disorders in turkey flocks (Kathrin Toppel)	96
O09-12	131215	Will turkey hens utilize a perch when given the opportunity? (Kailyn Buchynski)	97
O10-04	130810	Genetics of feeding behavior in medium-growing broiler chickens (Sandrine Mignon-Grasteau)	98
O10-05	131228	Evaluation of selection indices for adoption in dual-purpose and specialized breeding goals in indigenous chicken breeding programmes (Sophie Miyumo)	99
O10-06	131168	Morphological characteristics of breeder males at 60 weeks of age and their relationship with fertility and testicular development (Cristina Guerra)	100
O10-07	131267	Genetic evaluation of clutch traits in laying hens (Roberto Espinola)	101
O10-08	131131	Bone quality evaluation by keel bone palpation and live tibial X-ray density measurement in a White Leghorn pure line population (Björn Andersson)	102
O10-09	131118	Genome-wide association study on egg quality, laying performance and body weight in a backcrossing scheme between Araucana chicken and white layers (Chi Mei Sun)	103
O10-10	131174	Studying glycerol toxicity on frozen chicken sperm and strategies to mitigate it (Hsiu-Lien Herbie Lin)	104
O10-11	131130	The antibody response to experimental <i>Ascaridia galli</i> infection in three local chicken breeds (Emel Zergani)	105
O10-12	131095	New insight into Wooden breast myopathy by physics behind type III collagen structure (Eleonora Iaccheri)	106
O12-01	131129	Adenoviral gizzard erosion complicated with <i>Clostridium Perfringens</i> ventriculitis in a laying hen flock (Alex Cobos Arnalot)	107
O12-02	131055	Evaluating the impact of intermittent multi-mycotoxin exposure on layer breeder performance, egg quality, and hatchability: insights from an intervention study (Marisabel Caballero)	108
O12-03	131084	Impact of moulting on the immune system of commercial layers (Silke Rautenschlein)	109
O12-04	130858	Protection against experimental infection by <i>Salmonella Gallinarum</i> biovar <i>Gallinarum</i> using different live vaccination programs (Rafael Penha)	110
O12-05	130997	Combination of live and inactivated <i>Salmonella</i> vaccines to control Fowl Typhoid in laying hens (Jesus Rubio)	111
O12-06	131319	Avian intestinal spirochaetosis in poultry in Ireland – preliminary results (Sebastian Alessandro Mignacca)	112
O12-07	131190	Compatibility of mixing of several respiratory vaccines in spf chickens (Luis Augusto Pantoja Millas)	113
O12-08	130917	Molecular characterization of <i>Mycoplasma gallisepticum</i> and <i>Mycoplasma synoviae</i> in clinical samples from Spanish poultry flocks (Laura Digon)	114
O12-09	131255	Comparison of fowl adenovirus ELISA and virus neutralization titers in commercial broiler breeder flocks with distinct vaccination schemes against avian adenovirus (Roser Dolz)	115
O12-10	130871	IBDV detection in Northwestern Germany: Epidemiology and correlation to broiler performance (Violette Naccache)	116
O12-11	131031	Pathology of colibacillosis and its molecular detection from recent outbreaks in Chicks (Md. Golam Haider)	117

O12-12	131273	Morphological and immunological effect of in ovo administration in chicken of two different compound with probiotic and postbiotic feature (Lucia Biagini)	118
O13-04	130996	Lipids: Consistently inconsistent – Variability in commercial fats and oils (David Gonzalez Sanchez)	119
O13-05	130905	Evaluation of the simple and interactive effect of corn and oil quality in 21-day-old broiler chickens (Sunday Adedokun)	120
O13-06	131249	Inclusion of a stabilized emulsifier in low-energy diet for broilers: effects on nutrients retention, excreta trait and growth performances (Luca Marchetti)	121
O13-07	130727	Intelligent emulsifier addition to broiler feed formulations to improve FCR (Daphne Michels)	122
O13-08	130940	The effect of a combination of organic acids and medium chain fatty acid mono esters administered via water on broiler performance, mortality rate, and foot pad score (Pedro Medel)	123
O13-09	130838	Influence of free fatty acid content and degree of fat saturation in laying hen diets on bone quality (María Palomar)	124
O13-10	131151	Apparent fatty acid digestibility in laying hens fed medium-chain fatty acid-rich diets (Araceli Orozco Mas)	125
O13-11	131299	Dietary inclusion of Echium plantagineum oil and metabolism modulation of n-3 LC-PUFA in Canarian chicken (Jesús Villora)	126
O13-12	131077	Effect of a high-energy diet on liver stress and lipid metabolisms, meat and carcass traits in a slow-growing chicken breed (Nadia Stoppani)	127
O14-04	130848	Computed tomography as a non-invasive tool to characterize and advance on chicken breast myopathies (Cristina Zomeño)	128
O14-05	131079	Use of shear-wave elastography to predict in vivo the presence and severity of the wooden breast defect in chickens (Cécile Berri)	129
O14-06	130803	Chicken breast myopathies affect colour acceptance and willingness to purchase during shelf life (Míriam Muñoz-Lapeira)	130
O14-07	131135	Gene expression patterns associated with processing yield of marinated breast meat in broilers affected by white striping and wooden breast defects (Martina Bordini)	131
O14-08	130814	Dietary specification on meat quality of fast and slow growing broilers (Hannah Scott-Cook)	132
O14-09	130965	Meat quality: electrical water bath stunning vs. CO2 gas stunning in broiler welfare assessment (Jolien Hacker)	133
O14-10	130816	Effects of provisioning black soldier fly whole larvae or larvae meal on carcass and meat quality traits in fast-growing broilers (Elisabeth Baeza)	134
O14-11	130763	Palm kernel fatty acid distillates: revalorizing this by-product as feeding fat. Effects on lipid composition, oxidation and sensory acceptance of chicken meat. (Laura Parro)	135
O14-12	130743	Whole dehydrated and live black soldier fly larvae in autochthonous chicken breeds, effects on slaughtering performance and meat quality (Edoardo Fiorilla)	136
O15-04	130970	Economic effects of EFSA's welfare recommendations (Simon Fourdin)	137
O15-05	130820	HPAI vaccination in France: Concept, Experiences during the first stages and economic effects of the choice for vaccination (Simon Fourdin)	138
O15-06	130878	Economic perspectives of the Dutch broiler sector (Luuk Vissers)	139
O15-07	131161	Additional costs of turkey hens with untrimmed beaks in enriched housing conditions (Petra Thobe)	140

O15-11	131111	BroilerNet- Practice and Science Broiler production innovation network (Natàlia Majó)	144
O15-12	131004	Animal health law: Qualification and current skills regarding biosecurity in commercial poultry farms (Luisa Raederscheidt)	145
O17-04	130764	Diet composition influences probiotic and postbiotic effects on broiler growth, physiology and microbiota (Samuel Jansseune)	143
O17-05	131133	Transcriptomics reveals different modes of action of three probiotic strains compared with an antibiotic growth promoter in broiler chickens (Muzahir Hussain)	144
O17-06	130961	Dietary muramidase improves apparent ileal energy digestibility, gut functionality and increases broiler growth performance independent of diet nutrient density (Aurelia A. Séon Simon)	145
O17-07	131122	The effect of NSPase and feeding regimen on performance of broilers fed diets based on high and low viscosity barley (Muhammad Ahmad)	146
O17-08	130989	Differences in origin and dose of dietary endoxylanases result in distinct arabinoxylan degradation patterns in the gi tract of broilers fed wheat-based diets (An Bautil)	147
O17-09	131219	Assessment of in vitro fermentation efficacy of yeast-derived $\beta$ -glucans with different molecular mass and solubility using broilers' caecal content (Yunfeng Gu)	148
O17-10	131180	Impacts of early nutritional strategies on digestive homeostasis of chicks (Vincent Jonchère)	149
O17-11	131025	Effect of the dietary supplementation with sodium butyrate on gut morphology and caecal microbiota of broiler chickens (Almudena Huerta)	150
O17-12	130845	In-ovo injection of sodium butyrate improves growth performance in underweight broilers by modulating microbiota and gut barrier gene expression (Muhammad Zeeshan Akram)	151
O18-01	130757	A walk through the broiler breeder life (Käthe Kittelsen)	152
O18-02	131181	Contact with an adult hen after hatching promotes maturation of the chick caecal microbiota and has a greater effect than hatching systems (Laurence Guilloteau)	153
O18-03	131062	Microbiome diversity along the GIT of indigenous chicken (Armel Tangomo Ngnintedem)	154
O18-04	131158	The microbial evaluation of the Boschveld chickens fed Sorghum-based diets (Monnye Mabelebele)	155
O18-05	131317	The effect of non-starch polysaccharide degrading enzymes (NSPase) on gut leakage and intestinal histology in young broilers fed high-fiber, viscous, diets (Ester Vinyeta)	156
O18-06	131009	Effects of electrolyzed-reduced water and functional amino acid supplementation in drinking water to alleviate heat stress in broilers (Renée De Baets)	157
O18-07	130960	Exploring the avian breath: a comprehensive meta-analysis of factors shaping chicken respiratory microbiota (Alejandro Moreno)	158
O18-08	131048	Potential biosecurity breaches in laying hen farms: characterization of the poultry-wildlife interface through a camera trap study (Giulia Graziosi)	159
O18-09	131128	The biofilm removing capacity of drinking water disinfectants in an in vitro model (Daisy Roijackers)	160
O18-10	130932	A comprehensive review on backyard poultry: exploring non intensive production systems (Fernando Carrasquer Puyal)	161
O18-11	131083	Light as an enrichment tool: effects on animal health and behavior in commercial turkey production (Silke Rautenschlein)	162
O18-12	131238	Effects of Aflatoxin B1 and Ochratoxin on the Fitness and Neonatal Growth of White Pekin Ducks: An In Ovo Injection Study with and without	163

		Ameliorants, Investigating Mycotoxin-Threshold and Toxin-Toxin Interaction (Navniet Mishrra)	
O19-04	130862	Spatial patterns in the use of aviaries in relation to body condition and production – A study on individual laying hens (Sabine Gebhardt-Henrich)	164
O19-05	130893	Influence of light exposure and early feed access on the stress response during the developmental and early posthatch period in laying hen chicks (Catharina M. H. Broekmeulen)	165
O19-06	131018	Cost-benefit analysis of the upright versus conventional method for catching and loading end-of-lay hens (Femke Delanglez)	166
O19-07	130912	Long-term effects of ramps provided during rearing and laying phases in laying hens (Alex Johny)	167
O19-08	131145	Exercise using wing-assisted incline running during rearing affects initial descent velocity in adult laying hens (Grace Hong)	168
O19-09	131209	Access to outdoor space for laying hens: evaluating motivation and behavior of the animals (Marco Birolo)	169
O19-10	130946	Using a validated x-ray method on individual living hens, bone density does not decline over their lifetime (Ian Dunn)	170
O19-11	130903	Occurrence of keel bone lesions measured at the slaughterhouse in laying hens: analysis of risk factors for on farm animal welfare (Angela Trocino)	171
O19-12	130804	The effect of hypocalcaemia on keel bone radiodensity and damage (Maisarah Maidin)	172
O20-04	130928	Analyzing attitudes and satisfaction level of digital on-farm technologies for climate change adaptation and mitigation in poultry farming (Pia Münster)	173
O20-05	130958	Comparing Soybean Meal Reduction on Performance of Fast and Slow-Growing Chickens (Clara Alfonso-Carrillo)	174
O20-06	131298	Performance and environmental implications of modulating the metabolizable energy content in broiler chicken diets (Marco Zampiga)	175
O20-07	131065	Ammonia emissions can be reduced through a quality-controlled bedding material in broiler housing systems (Benedikt Thesing)	176
O20-08	130980	Impact of slower growing broilers and reduced stocking density on ammonia emission and animal welfare (Neil Van Den Broeck)	177
O20-09	130978	Feasibility of purified rainwater as drinking water for broilers (Neil Van Den Broeck)	178
O20-10	130874	Reusing cleaning water from broiler farms: on-site biological treatment and membrane filtration (Bleyen Peter)	179
O20-11	131023	Comparative life-cycle assessment for the sustainable production of fermentation-based L-methionine (L-Met) (Behnam Saremi)	180
O20-12	131303	Exploring BroilerNet stakeholders' attitudes towards the sustainability of broiler industry in Greece (Evangelia Sossidou)	181
O22-04	130783	Antimicrobial resistance in eggs: the role of layer production system. Preliminary results (Laura Montoro-Dasi)	182
O22-05	130802	Salmonella Infantis rules the roost: a continuous cycle of contamination between broiler farms and slaughterhouses (Lisa De Witte)	183
O22-06	131234	A double encapsulation of bioactives to reduce salmonella livingstone shedding in artificially infected broiler chickens (Stephanie Ladirat)	184
O22-07	131167	Aerotolerance and haemolytic activity of invasive and non-invasive campylobacter coli strains from broiler chickens (María Pilar González Navarro)	185
O22-08	131207	Colonization by different Campylobacter jejuni strains produces distinct effects on broiler chickens performance (Borja Vilà)	186
O22-09	131199	Host immune response of broiler chickens to campylobacter jejuni strains with different pathogenic potential (Alicia Manzanares)	187

O22-10	130935	Effect of a bacterial probiotic on <i>Campylobacter</i> spp. cecal carriage (Elen Rondel)	188
O22-11	131263	Assessing the impact of a commercial water disinfectant, blend of hydrogen peroxide, acetic, and peracetic acid on the performance and <i>Campylobacter jejuni</i> counts in experimentally challenged broiler chicks (Tilemachos Mantzios)	189
O23-04	130937	Effect of dietary supplementation and in ovo feeding of alpha ketoglutaric acid on the immunocompetence traits, mineral composition and fatty acid profile of breast and thigh muscle of commercial broilers (Amitav Bhattacharyya)	190
O23-05	131284	Assessing the impact of feeding different, highly available sources of essential trace minerals delivered from a premix blend on broiler performance (Gavin Boerboom)	191
O23-06	130782	A combination of 25-Hydroxycalciferol, carotenoid, organic copper and glycans improved broiler breeder performance, egg quality and hatchability traits (Konstantinos Mountzouris)	192
O23-07	130790	Broiler breeder hens significantly benefit by replacing inorganic trace minerals with glycinate (Susanne Rothstein)	193
O23-08	131178	Reducing breast myopathies with supplementation of <i>Solanum glaucophyllum</i> (Katia Pedrosa)	194
O23-09	131170	Enhancing feed digestibility: Exploring the potential of calcium humophosphate as a novel source of phosphorus on broiler nutrition (Amélie Juanchich)	195
O23-10	130840	Changes in the acid-base balance of broiler chickens after a substitution of peptide-bound with free amino acids in the diet (Wolfgang Siegert)	196
O23-11	130950	Laying hens require a minimum supplementation of riboflavin to maintain body weight, egg weight, and optimize egg riboflavin content (Matheus Reis)	197
O24-04	130971	Breaking the beak conundrum: navigating laying hen welfare in beak-trimmed and non-trimmed flocks (Niels Demaître)	198
O24-05	131143	Associations between the gut-brain axis and feather pecking in laying hens (Alexandra Harlander)	199
O24-06	130812	Toe injuries are linked to altered movement behavior in laying hens in a semi-commercial aviary system (Michael Toscano)	200
O24-07	130811	Lower stocking densities support broiler welfare – A proof of concept of the behavioural space model (Inga Tiemann)	201
O24-08	130805	A slow-growing broiler strain has more beneficial effects on broiler welfare than providing environmental enrichment (Ingrid De Jong)	202
O24-09	131134	Multifactorial evaluation of poultry welfare in different genotypes and farming systems (Cecilia Mugnai)	203
O24-10	130949	The effect of on-farm hatching on spatial traits in a quasi-commercial barn (Matthew Petelle)	204
O24-11	130891	Effects of providing uniform or gradient light intensity on behaviour and welfare of fast- and slower-growing broiler chickens (Ingrid De Jong)	205
O24-12	130920	Stunning effectiveness: electrical water bath stunning vs. CO <sub>2</sub> gas stunning in broiler welfare assessment (Yukari Togami)	206
O25-04	130721	Exogenous supplementation of additional level of arginine (L_Arg) in corn-soy based diets is required for broiler breeder hens during late production phase (Gautham Kolluri)	207
O25-05	130889	Expression of genes involved in calcium and phosphorus homeostasis in hen's kidney: effects of sexual maturity and PO stage (Baraa Ezzo)	208
O25-06	130938	Neural regulation of the stress response in chicken (Loreta Medina)	209
O25-07	131040	Long-term effects of prenatal temperature stimulation on mRNA expression of <i>Bdnf</i> , <i>Npy</i> , and <i>Pomc</i> in the chicken hypothalamus (Tzschentke Barbara)	210



O25-08	131191	Comparative analysis of transcriptional profiles of chicken jejunum and - derived organoids collected early post-hatch (Vincent Jonchère)	211
O27-01	131073	Comparative analysis of caeca microbiota composition in broiler chickens fed diets with alternative protein sources (Alessandra De Cesare)	212
O27-02	131288	Impact of protein source and digestibility marker on nutrient digestibility in broilers (Ibtissam Kaikat)	213
O27-03	131141	Are heat treated mixed crops of faba beans and wheat suitable for broiler feeds? (Noémie Van Noten)	214
O27-04	131056	Maintaining an adequate amino acid profile allows to successfully reduce dietary crude protein in broiler chickens in both wheat or corn-based diets (William Lambert)	215
O27-05	131275	The inclusion of full-fat Black Soldier fly larvae in broiler's feed in different forms (meal and whole): effects on growth performance and breast fatty acids' composition (Alba Cerisuelo)	216
O27-06	130744	Assessing the environmental impact of poultry feed in meat type autochthonous chicken farming (Edoardo Fiorilla)	217
O27-07	130774	Bran particle size impacts the precaecal fibre particle size distribution, caecal influx and caecal fibre fermentation differently with age (Paulien Vanderghinste)	218
O27-08	130801	Effect on degradation and passage of oat hulls through digestive tract of broilers at different time points (Shlesha Ghimire)	219
O27-09	131155	Assessing the flow of digesta and digestive dynamics in broiler chickens: A comparative study with growing pigs (Lucas Bassi)	220
O27-10	131179	Influence of coarse mash and crumbled diets on productive performance and nutrient digestibility of laying hens from 74 to 85 weeks of age (Jihed Zemzmi)	221
O27-11	131097	Use of almond shells as insoluble fibre source for 5 weeks old pullets feeding: effect of particle size and inclusion levels (Eduardo Ruiz Herrero)	222
O28-04	131093	Establishment of an immunological cell model for investigating amyloid arthropathy in chickens (Nora-Fabienne Paul)	223
O28-05	130947	Gut microbiota and host interactions in chickens: a dynamic interplay with performance implications (Ester Arevalo Sureda)	224
O28-06	130775	Antimicrobial resistance profiles of Enterococcus spp. associated to poultry diseases and their susceptibility to probiotic Bacillus (Kirsty Gibbs)	225
O28-07	130986	Study of the combined therapy of antibiotics and bacteriophages for the control of enterococcus spp. in poultry farming (Josep Garcia-Llorens)	226
O28-08	130910	Air sample based detection of Eimeria spp. in broiler houses (Julia Skov)	227
O28-09	130898	Turkey productivity improvement and poult gut health support during Eimeria spp. challenge using a poultry specific synbiotic (Luis Valenzuela)	228
O28-10	131184	Coniferous rosin-based soap formulations inhibit the sporulation and infectivity of Eimeria tenella oocysts in vitro (Hannele Kettunen)	229
O28-11	131256	Investigation of the dietary supplementation of lauric and butyric acid glycerides on the performance, intestinal gross lesions and cecal microbiota in a subclinical necrotic enteritis challenge model in broiler chicks (Vasileios Tsiouris)	230
O28-12	131323	Induction of protective immunity against necrotic enteritis by delivering live Clostridium perfringens by the intrapulmonary route at hatch (Susantha Gomis)	231
O29-01	130885	Navigation ability of white and brown hens in an aviary system during laying phase (Francesco Bordignon)	232
O29-02	130924	Tag 'n' track: simplifying the validation of animal behaviour tracking technologies with an automated annotation method (Serge Alindekon)	233
O29-03	131020	Development of a temperature based non-invasive sensor system for controlling heart rate during temperature training of broiler embryos in the hatching phase (Hans-Ullrich Balzer)	234

O29-04	131290	Enhancing broiler welfare through precision classification of stress calls in varied environmental conditions (Shelly Druyan)	235
O29-05	130724	Autonomous bioacoustics system for monitoring broiler welfare in hatcheries using artificial intelligence (Gerardo José Ginovart-Panisello)	236
O29-06	131054	Does distribution of light intensity impact broiler production or welfare? (Tory Shynkaruk)	237
O29-07	131196	Stocking density and access to open-air influences feed efficiency in a strain of slow-growing naked neck chicken (Chaumont Salomé)	238
O29-08	131204	Relationships between range use, performances and health and welfare related traits in four strains of organic broilers (Elisabeth Le Bihan-Duval)	239
O29-09	131165	Performance of dual-purpose chickens in organic farming (Philipp Hofmann)	240
O29-10	131060	Egg and meat performance of three dual-purpose hen genotypes reared under free-range conditions (Alexandr Torres)	241
O29-11	131281	Starling movements: connectivity at the human-domestic animal -wildlife interface (Alberto Sanchez Cano)	242
O29-12	130786	Bonelli's eagles as a biomarker: multi-resistant strains and biosecurity in a One Health framework (Barbara Martin-Maldonado)	243

### **DIGITAL POSTERS SESSIONS**

Slot	Abstract	Title (Presenter)	Page
<i>Poultry nutrition</i>			
P1-24	130716	Effects of Quillaja saponaria and Yucca schidigera saponins and polyphenols on intestinal health, welfare, and performance of broilers under commercial conditions in Belgium (Vasil Stanev)	270
P1-25	130718	Effect of Dietary Supplementation of Arjuna (Terminalia arjuna) Bark and Sahjan (Moringa oleifera) Leaf Powder on the Growth Performance and Egg Quality of Uttara chicken (Ankit Sharma)	271
P1-26	130758	Production performance and egg quality parameters of laying hen supplemented with black turmeric under heat stress (Vinay Singh)	272
P1-27	130865	Dietary phytogetic inclusion level improves performance and egg quality by regulating cytoprotective and inflammatory responses in the ovary and duodenum of laying hens (Ioannis Brouklogiannis)	273
P1-28	130943	Standardized Natural Citrus Extract stimulates the production of endogenous butyric acid in the gut (Juan Manuel García Coronel)	274
P1-29	131052	Enhancing Reproductive Performance of Broiler Breeders and Progeny Quality and Performance Through Phytomolecules (Twan Van Gerwe)	275
P1-30	131063	Dietary manipulations with herbal feed additives to produce value added egg from laying chicken (MSK Sarker)	276
P1-31	131071	Proving the use of nutritional matrix values for Phytogetic Feed Additives through modeling (Manu De Laet)	277
P1-32	131101	Effects of feeding oxidized lipids and an extract from an olive oil by-product, rich in Hydroxytyrosol, on cecal fermentation in broilers at 21 days of age (Joel Alexis Maldonado)	278
P1-33	131108	Effects of a Chinese herbal extract on gut microbiota, production performance, immune organ indices, meat quality, and serum biochemical indices of white carneau squabs (Zhixuan Chen)	279
P1-34	131119	Effects of supplementation of salinomycin and herbal feed additive on growth performance and oocyst shedding in broiler chickens challenged with Eimeria spp (Gabriel Moyano)	280

P1-35	131173	Artichoke extract ( <i>Cynara scolymus</i> L.) improves production performance of laying hens after long-term stress of feeding mycotoxin-contaminated diets (Konkawat Rassmidatta)	281
P1-36	131225	Effects of an encapsulated phytogetic and phycogenic solution when used in a diet formulated with or without matrix value on performance, egg quality and nutrient digestibility of laying hens (Stephanie Ladirat)	282
P1-37	131231	Effects of natural extracts on the oxidative stability of poultry feeds (Marta Blanch)	283
P1-38	131295	Influence of purslane extract and probiotic on welfare related parameters and droppings characteristics of broilers at high stocking density (Ahmad Tatar)	284
P2-08	130720	The impact of different types of serine proteases on growth performance and jejunal morphology in broilers (Reza Akbari Moghaddam Kakhki)	324
P2-09	130737	Microbiota profile and antibiotic resistance genes in the caecal content of broiler chickens are influenced by dietary protein content and the plant protein source (Oluyinka Olukosi)	325
P2-10	130742	Ileal amino acid digestibility of extracted sunflower meal, determined with layer type pullets (Nikoletta Such)	326
P2-11	130780	Maternal supplementation of guanidino acetic acid: Influence on egg composition and chick development (Corey-Ann Firman)	327
P2-12	130902	Neuropeptide hormone responses and jejunal absorption rate in broiler chickens receiving reduced protein diets: A comparison of branched-chain amino acids with lysine as proteinogenic amino acids (Oluyinka Olukosi)	328
P2-13	130926	Microbiome implications of total replacement of soybean meal in fast growing male broilers (Kirsty Gibbs)	329
P2-14	130929	Energy utilization and jejunal mRNA expression of nutrient transporters in broiler chickens fed for different feeding lengths diets containing resistant starches from raw potato or high amylose corn (Oluyinka Olukosi)	330
P2-15	130933	Novel sfericase protease improves amino acid digestibility of soybean meal and rapeseed meal in broiler chickens (Aurelia Seon)	331
P2-16	130944	Effect of rearing substrate on amino acid digestibility of back soldier fly larvae fed to broiler chicks (Emily Burton)	332
P2-17	130951	Response of broilers to dietary metabolizable energy levels from 0 to 4 days old (Matheus Reis)	333
P2-18	130972	Nutritional Potential of African Edible insects as food and feed (Sekobane Daniel Kolobe)	334
P2-19	131008	Alternative protein sources in broiler diets: effects on bird performance, welfare status and development of the gastro-intestinal tract (Tom Vingerhoets)	335
P2-20	131017	A new fermentation-based L-methionine (L-Met eco) has a better efficacy compared to petro-chemical based DL-methionine (DL-Met) (Behnam Saremi)	336
P2-21	131058	Dietary amino acid profile boosted in arginine and threonine improves growth performance of turkeys fed reduced crude protein diets (William Lambert)	337
P2-22	131081	Effect of solid-state fermentation on nutritional properties of oilseed cakes and in vitro digestibility in poultry (María Cambra-López)	338
P2-23	131085	Methionine requirements of slower growing broilers in the starter (d 0-10), grower 1 (d 10-24), grower 2 (d 24-38) and finisher (d 38-50) phases (Lotte Stokvis)	339
P2-24	131127	The use of an olive by-product as a source of insoluble fibre for pullet feeding (Eduardo Ruiz Herrero)	340
P2-25	131136	Study on <i>Ganoderma lucidum</i> plus phytase to solid-state ferment agricultural by-products producing functional feed (Tzu Tai Lee)	341
P2-26	131142	Development and application of fermented soybean hull by <i>Bacillus velezensis</i> as functional feedstuff (Tzu Tai Lee)	342
P2-27	131154	Effect of incorporating mango ( <i>Mangifera indica</i> ) feed and boiled mango seed	343

		kernel as partial substitutes for maize on the zootechnical performance of broilers (Hippolyte Mekuiko Watson)	
P2-28	131200	Effect of including dehydrated <i>Hermetia illucens</i> larvae on the productive performance of Isazul laying hens (27- to 38-week-old) (Ana Montalbán Pérez)	344
P2-29	131205	Grape and Apple Pomace Antioxidant Capacity: Potential Inclusion in Poultry Diets (Gabriela Maria Cornescu)	345
P2-30	131221	Effect of different levels of amino acids on broiler performance (Karine Bebin)	346
P2-31	131224	Efficacy evaluation of iron amino acid complexes (Fe-Lys-Glu) in laying hens: effects on laying performance, egg iron content and blood biochemical parameters (Cibele Torres)	347
P2-32	131259	Amino acid-complexed minerals supplementation improves laying hen performance, egg and bone Quality of 98-week-old hens (Cibele Torres)	348
P2-33	131269	Effect of partial or total replacement of soybean oil by <i>Hermetia illucens</i> larvae fat in layers diets on performance and apparent ileal digestibility of nutrients (Aleksandra Dražbo)	349
P2-34	131302	In-ovo feeding by glucose and its effect in improving hatchability of Fayoumi breeder (Amira Mahmoud)	350
P2-35	131306	The impact of fiber supplementation on energy metabolizability and the production of short-chain organic acids in broilers, depending on their age (Martina Lichovnikova)	351
P2-36	131324	Standardised ileal amino acid digestibility of maize grains dried at two different temperatures and supplemented or not with protease (Sebastian Kaczmarek)	352
P2-37	130890	Evaluation of dietary supplementation of garlic powder ( <i>Allium sativum</i> ) on the growth performance, carcass traits and meat quality of Japanese quail ( <i>Coturnix coturnix japonica</i> ) (Hassan Jalal)	353
P2-38	131148	Effects of dietary mango seed kernel and peel extracts supplementation as an alternative to antibiotics on growth performance, carcass characteristics and gut health of broilers (Zafar Hayat)	354
P2-39	131296	Protection efficiency of different inactivated AIV-H5 vaccines against challenge with recent H5N1 clade 2.3.4.4 circulating virus (Wael Elfeil)	355
P2-40	130723	An overview of transmissible viral Proventriculitis in broiler chickens in Poland (Marcin Smialek)	356
P2-41	131192	Host mi RNA profiling of Vero cells during Avian orthoreovirus infection (Deepak Kumar)	357
P3-23	130836	Broiler chickens fed a low digestible diet improved intestinal health and growth performance when supplemented with a multi-component solution alone or combined with a protected blend of organic acids and essential oils (Heitor Vieira Rios)	410
P3-24	130870	Butyric and valeric glycerides alleviate sub-clinical necrotic enteritis effect on performance in broiler (Sm Mostafizur Rahaman Sumon)	411
P3-25	130881	Medium chain fatty acids reduce oxidative stress in intestinal Caco-2 cells (Laura Parro)	412
P3-26	130956	Effect of dietary enrichment with n-3 fatty acids by using lipid-based nanoparticles on growth performance, carcass composition, meat quality and fatty acid profile of breast meat in slow-growing Korat chickens (Wittawat Molee)	413
P3-27	130957	Optimizing the utilization of n-3 fatty acids for enhanced functional meat production in slow-growing Korat chickens involves evaluating the characteristics of glucose transporter-targeted lipid nanoparticles (Piyaradtana Homiyok)	414
P3-28	130964	Effect of double buffered sodium butyrate associated with low energy-protein diets on small intestinal morphology and microbiota in broilers in a context of	415

		coccidiosis challenge (Xavier Roulleau)	
P3-29	130994	Supplementation of a combination of lysolecithins, a synthetic emulsifier and monoglycerides on broiler performance and profitability during heat stress (David Gonzalez Sanchez)	416
P3-30	130995	Supplementation of a combination of lysolecithins, a synthetic emulsifier and monoglycerides to diets of broilers containing phytase in super dosing, on performance and profitability (David Gonzalez Sanchez)	417
P3-31	131003	In feeding line application of organic acids improved feed hygiene and growth performance in broiler chickens (Sandra Van Kuijk)	418
P3-32	131005	The effect of a selected blend of organic acids on controlling Salmonella Infantis in broiler chickens (Sandra Van Kuijk)	419
P3-33	131038	Effects of medium-chain fatty acids rich diets on intestinal morphology, microbiology and volatile fatty acids in young broiler chickens (Mateo Espinosa De Los Monteros Peñafiel)	420
P3-34	131236	Protected sodium butyrate improves productive performance and carcass characteristics in broiler chickens (Beatriz Saldaña Mancebo)	421
P4-01	130706	The positive impact of two Bacillus-based products on broiler performance and lesion scoring during necrotic enteritis-induced challenge (Florence Barbe)	456
P4-02	130707	Effect of yeast cell wall supplementation on broiler performance: analysis of 3 trials (Florence Barbe)	457
P4-03	130708	Reality of feed intake in layers related to zootechnical performances and improvement of feed intake quality with an innovative hydrolyzed yeast (Florence Barbe)	458
P4-04	130766	A global cross-study analysis in broilers of a multi-enzyme combination and Bacillus probiotic (Sasha Van Der Klein)	459
P4-05	130776	Relationships between microbiota and zootechnical parameters in laying hens after supplementation with probiotic <i>Pediococcus acidilactici</i> CNCM I-4622 (Florence Barbe)	460
P4-06	130777	Efficacy of manganese sources for broilers: impact on performance and manganese accumulation (Ian Cockshott)	461
P4-07	130787	Probiotics in broiler chicks' diet fed with different sources of protein (Ciurescu Georgeta)	462
P4-08	130788	Effects of microencapsulated <i>Lactobacillus</i> spp. and cowpea seeds in broiler chickens' diets on production parameters, health status, and intestinal microbial population (Aurelia Nicoleta Lefter)	463
P4-09	130789	Effect of <i>Lactobacillus plantarum</i> on performance and gut microflora populations of broiler fed with alternative protein source (Mihaela Dumitru)	464
P4-10	130806	Elemental selenium limits the utilization of supplemented selenium in broilers (Amine Hachemi)	465
P4-11	130815	Reducing feed costs with phytase and NSP enzymes for rearing male layer chickens to potentially avoid day old culling (Saad Gilani)	466
P4-12	130821	The effect of synbiotic and probiotic preparations on the concentration of ochratoxin in meat, blood, liver and kidney of turkeys (Krzysztof Lipinski)	467
P4-13	130822	Effects on growth performance of enzyme feed addition for coccidiosis vaccinated Ross 308 broilers (Nadine Cariou)	468
P4-14	130826	Broiler chickens undergoing an intestinal challenge and supplemented with a bacterial xylanase demonstrated better nutrient digestibility, intestinal health and growth performance (Heitor Vieira Rios)	469
P4-15	130828	Effect of am/pm diet on laying performance and egg quality of laying hens (Nasima Akter)	470
P4-16	130847	Does the latest NASEM vitamin A requirement estimate support optimal growth in broiler chickens? (Yauheni Shastak)	471
P4-17	130852	Effect of the age and duration of the trial in the evaluation of the adequate mineral content and phytase dose in broilers (Mehran Javadi)	472

P4-18	130853	Evaluation of postbiotics on production performance in broiler chicken as an alternative to Antibiotic Growth Promoters (Monika M)	473
P4-19	130854	A novel phytase allows for higher level of inorganic phosphate substitution with no detrimental effect in hens during the late laying period (Cristiano Bortoluzzi)	474
P4-20	130884	The effect of muramidase in broiler feed on broiler performance in semi-field conditions (Necmettin Ceylan)	475
P4-21	130887	Muramidase supplementation enhances laying hen performance: a comprehensive analysis of three trials (Irene Eising)	476
P4-22	130897	The production benefits of full matrix application for a consensus bacterial 6-phytase variant alone or in combination with an enzyme mix of xylanase, amylase, and protease: review of two studies (Rachael Hardy)	477
P4-23	130904	The use of fibre-degrading enzymes in broiler feed containing wheat distiller's dried grains with solubles (Eunjoo Kim)	478
P4-24	130942	Source of limestone affects growth performance and bone mineralization of broilers (Cristiano Bortoluzzi)	479
P4-25	130954	Increasing the dose of a novel consensus bacterial 6-phytase variant improved ileal digestibility of Na and Mg in broilers (Yueming Dersjant-Li)	480
P4-26	130959	A novel phytase improves the responses of laying hens fed an inorganic phosphorus-free diet from 25 to 45 wk of age (Anna Maria Pérez-Vendrell)	481
P4-27	130993	Impact of added phytase and dietary InsP6 on the structure and the core microbiota of the digestive tract of broilers (Ismael Rubio-Cervantes)	482
P4-28	131030	Dietary synbiotics improve feed utilization, eggshell quality, and fecal ammonia emission of laying hens under tropical conditions (Yuwares Ruangpanit)	483
P4-29	131069	Effect of supplementing multicomponent mycotoxin detoxifying agent on performance, carcass variables, immune responses, and liver function variables in broiler chicken fed known concentrations of aflatoxin b1, deoxynivalenol and T-2 toxin (Jog Raj)	484
P4-30	131075	Bacillus licheniformis DSMZ 28710 to support high-producing laying hens during lay (Wouter Van Der Veken)	485
P4-31	131112	A meta-analysis evaluating the effect of <i>Pediococcus acidilactici</i> CNCM I-4622 on performances of laying hens (Florence Barbe)	486
P4-32	131160	The effect of yeast $\beta$ -1,3/1,6 glucan administration on the growth performance of broilers in a model of subacute necrotic enteritis (Tri Duong)	487
P4-33	131176	The supplementation with glycinate of Zinc enhances the immune response and the antioxidant capacity in broilers (Javier López-Paredes)	488
P4-34	131203	NMR-based metabolic characterisation of broiler serum (Katie Wilshaw)	489
P4-35	131212	Solid state fermentation of wheat bran by <i>Phellinus linteus</i> applied in broiler diet (Tzu-Tai Lee)	490
P4-36	131220	Optimizing the nutritive value of broiler wheat-based diets through enzyme containing endo(xylo)-glucanase and beta-mannanase (Sara Llamas-Moya)	491
P4-37	131235	Evaluation of supplemented zinc-complexed minerals on performance of broilers fed diets formulated to maximize phytate hydrolysis in the intestinal tract (Cibele Torres)	492
P4-38	131289	Estimations of Ca digesta flows in broilers fed different dietary Ca and P levels: likely implications on digestibility studies (Ibtissam Kaikat)	493
 <b><i>Poultry health</i></b>			
P1-39	130725	Live Salmonella vaccine compatibility with different microbial based solutions (Florence Barbe)	285
P1-40	130745	Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for H5 avian influenza A (Stephanie Lesceu)	286
P1-41	130793	Evaluation of inactivated vaccine formulated with new polymeric adjuvant	287

		against Salmonellosis in layers (Andra Corder)	
P1-42	130808	compatibility of innovax-nd-ibd + nobilis h9-nd p vaccine against 3 challenge strains (ibd, nd, ai) in spf birds at t=3 or t=4 (ibd at 3 weeks, nd and ai at 4 weeks) (Rüdiger Raue)	288
P1-43	130855	Evaluation of compatibility and production performance in commercial layers immunized with vectorized vaccines rHVT-H7 and rHVT-F and immune-complex vaccine against Gumboro applied simultaneously in the hatchery (Ricardo Franco)	289
P1-44	130860	Evaluation of vaccination strategies against highly pathogenic avian influenza H5 in Spanish poultry production: application of the EVACS tool (Marta Perello)	290
P1-45	130919	Study to assess the correlation between Infectious Bronchitis live vaccine volume applied by spray to day-old chick boxes and the percentage of positivity and Ct values (Alberto Giner)	291
P1-46	130923	Bacillus coagulans DSM 32016 controls Salmonella Typhimurium-Caused performance decline in broiler through improved gut histomorphology and beneficial microbiota modulation (Rubén Crespo Sancho)	292
P1-47	131006	Development of a new probiotic feed additive based on the microbial consortium of Limosilactobacillus fermentum and Ligilactobacillus salivarius for the prevention of salmonellosis in broiler chickens (Ilya Nikonov)	293
P1-48	131210	Effect of in feed galactomannans in broiler chickens challenged with different Campylobacter jejuni strains (Borja Vilà)	294
P1-49	131245	comparative analysis of resistance to salmonella enterica serovar enteritidis in guinea fowl and chicken (Girraj Goyal)	295
P1-50	131266	Assessment of the activity of Lactobacillus spp commercial against Salmonella Minnesota with multiple drug resistance profile (Bruno Milagres)	296
P1-51	131304	Protective Efficacy of Inactivated Trivalent-H5 Vaccine Against Challenge with Both HPAIV-H5N1 and H5N8 Viruses in Turkey Poults Under Both Laboratory and Field Conditions (Wael Elfeil)	297
P2-42	130888	Exploring iron scavenging as an under-explored mode for pathogen elimination by Bacillus-based probiotics (Krithika Venkatasubramanian)	358
P2-43	130936	Characterization of Escherichia coli MG1655 resistant variant after extended exposure to essential oils mixture (Claire Carlu)	359
P2-44	131014	Antimicrobial resistance among of Gallibacterium anatis biovar haemolytica isolated from chickens in Poland (Olimpia Kursa)	360
P2-45	131088	Positive effect of Euglena gracilis derived paramylon on immunoglobulin Y content of egg yolk (Valentine Van Hamme)	361
P2-46	131091	Immuno-boosting effects of lysozyme, essential oils, vitamins mixture of additives in response to live E. coli vaccine (Jasna Bošnjak-Neumüller)	362
P2-47	131208	Antimicrobial resistance of Escherichia coli isolates from broilers (Isabel Fabra)	363
P2-48	131253	In vitro comparison and evaluation of antimicrobial activity of ammonium and sodium formate combined with short-chain fatty acid salts in different microorganisms (Abdelhacib Kihal)	364
P2-49	131271	Mucolytic activity of essential oils, lysozyme, and vitamins' premix of additives intended for pigs and poultry (Jasna Bošnjak-Neumüller)	365
P2-50	131282	Probiotic in the control of mortality and lethality in experimentally challenged broiler chickens with avian pathogenic escherichia coli (Bruno Milagres)	366
P2-51	131283	Evaluation of the persistence of commercial lactobacillus spp in experimentally contaminated chicken litter with APEC (Bruno Milagres)	367
P2-52	131307	Quercetin can mitigate lipopolysaccharide induced apoptosis and oxidative stress in bursal and thymic lymphocytes (Vladimir Zmrhal)	368
P3-35	130726	Adaptation of Avian Orthoreovirus strain DVB 04 in Vero cells and its titration (R. Prarthana)	422

P3-36	130740	Genetic variability of infectious bursal disease virus (IBDV) in the Iberian peninsula (José Luis Arnal)	423
P3-37	130749	First report of Marek's disease virus in commercial turkeys in Slovenia (Zoran Žlabravec)	424
P3-38	130769	Efficacy of Probiotic via Drinking Water on Growth Performance of Broiler Chickens during Fowl Adenovirus Infection in UAE (Merghani Adam)	425
P3-39	130819	Epidemiology of infectious bursal disease virus in portugal: replacement of local strains by Northwestern European reassortants as dominant field type (Matteo Legnardi)	426
P3-40	130824	Disease diagnosis through analysis of chicken mycoplasma antigens in 9th Industrialization and preservation-maintenance line of Korean Native Chickens(KNC) (Are-Sun You)	427
P3-41	130834	Immune status and reproductive performance in Broiler breeders (Joaquim Tarres)	428
P3-42	130983	Hyperpigmentation of laying hens at slaughterhouse associated with lesions in the reproductive tract. (Alex Cobos Arnalot)	429
P3-43	131024	Efficacy of different IBD vaccines against the current IBDV circulating strains in the Benelux area (Nikolai Ongena)	430
P3-44	131034	Efficacy of different infectious bursal disease vaccines administered in the hatchery against a very virulent bursal disease challenge in broiler chickens (Luis Augusto Pantoja Millas)	431
P3-45	131046	Evaluation of organ lesions in broiler chickens exposed to mycotoxins (Damien Preveraud)	432
P3-46	131092	Molecular longitudinal surveys reveal circulation of chicken anemia virus genogroups IIIa and IIIb in Greece broiler breeder (Giulia Quaglia)	433
P3-47	131096	Extensive adenovirus molecular survey demonstrates predominant circulation of FAdV-D in Italian broiler flocks (Gabriele Lizzi)	434
P3-48	131104	Cranial osteomyelitis and meningoencephalitis associated with Pasteurella canis in broiler chickens (Leandro Cadiz Nuñez)	435
P3-49	131171	Foot pad dermatitis survey on three hungarian broiler farms (Márk Tóth)	436
P3-50	131229	Evaluation of haemagglutination Inhibition Assay (using FAdV- sensitized tanned chicken RBCs) to determine Humoral response to the challenge of novel Fowl Adenoviruses isolated from Pakistan during 2019- 2022 (Iqra Zaheer)	437
P3-51	131232	Phylogenetic analysis of partial sigma $\sigma$ A of avian orthoreovirus (Surabhi Kashyap)	438
P3-52	131237	Comparative analysis of a partial fragment of $\sigma$ and $\lambda$ gene of avian reovirus (Laxmi Rathore)	439
P3-53	131277	Circulating genotypes of Mycoplasma spp. and their molecular profiles of antimicrobial resistance as a contribution to the diagnosis of avian mycoplasmosis in Colombia (Sara Forero)	440
P4-39	130760	The ast evaluation of the resistance eimeria spp. isolated from the southern part of thailand against commercial anticoccidial drugs (Arithat Limsatanun)	494
P4-40	130771	Butyric glycerides act directly and indirectly to protect chicken intestine from pathogens colonization (Amine Mellouk)	495
P4-41	130832	Combining the benefits of postbiotics and phytochemicals to promote early gut microbiota maturation (Anne Goderis)	496
P4-42	130864	Assessment of coccidiosis in broilers in france based on autopsy data from 2018 to 2022 (Sarah Betz)	497
P4-43	130872	Comparison of the efficacy of different ionophores on the control of E. acervulina (Ben Dehaeck)	498
P4-44	130873	The Impact of E. acervulina on FCR (Ben Dehaeck)	499
P4-45	130879	Saponin-rich plants mixture supports coccidiosis vaccination program in broiler chickens: a case study of floor pen challenge model. (Mohammed El	500



## Amine Benarbia)

P4-46	130900	Effect of uncoated-buffered sodium butyrate supplementation in diet on small intestinal morphology under high stocking density of broilers (Julián Melo)	501
P4-47	130925	Supplementation of a synbiotic and a precision biotic improves the growth performance of broiler chickens submitted to coccidiosis vaccination (Bortoluzzi Cristiano)	502
P4-48	130934	Evaluation of the effects of a bacterial probiotic in experimentally induced necrotic enteritis (Elen Rondel)	503
P4-49	130945	Standardized workflow for microbiome analysis – methodology and application in commercial broiler farms (Sergi Raurich)	504
P4-50	130948	Effects Of Dietary Polyphenol Extracts And Zinc Bacitracin On The Caecal Microbiome Of Young Broilers (Saheed Salami)	505
P4-51	130967	Effect of a triple-strain bacillus-based probiotic on in vitro inhibition of <i>Enterococcus cecorum</i> isolated from clinical broilers (Sarah Betz)	506
P4-52	130975	Exploring the antimicrobial potential of glycerides of medium chain fatty acids in a simulated poultry gastrointestinal environment (Nuria Vieco-Saiz)	507
P4-53	131016	Intestinal microbiota responses to the dietary supplementation of bacillus sp. pb6 and fiber level in broiler chickens (Karen Vermeulen)	508
P4-54	131028	Effects of phytobiotics based on tannins, saponins, and organic acids on performance and intestinal health of broiler chickens infected with different <i>Eimeria</i> species (Marko Pajic)	509
P4-55	131049	Safety and immunogenicity of the associated administration of an in ovo coccidiosis vaccines, an infectious bursitis disease immunocomplex vaccine and a rHVT-ND vaccine in commercial broilers (Luis Augusto Pantoja Millas)	510
P4-56	131051	Evaluation of the productive performance of commercial broilers vaccinated against coccidiosis with an in ovo vaccine in comparison with a coccidiosis coarse spray vaccine (Luis Augusto Pantoja Millas)	511
P4-57	131064	Floor pen study to compare the effects of different in-feed treatments against a mixed <i>eimeria</i> spp. inoculum challenge (André Pereira)	512
P4-58	131072	<i>Bacillus licheniformis</i> DSMZ 28710 to mitigate gut health challenges in high-producing broilers (Wouter Van Der Veken)	513
P4-59	131157	Identification of different <i>Eimeria</i> species presence and enumeration of coccidia oocysts in layer poultry flocks in Greece (Evgenios Chalvatzis)	514
P4-60	131261	Broiler performance and health feeding different sources of minerals (Luigi Moreira)	515

***Poultry welfare***

P1-10	130731	Pecking behaviour of turkey hens in proximity to the feeding pan – any influence of beak condition? (Nina Volkmann)	256
P1-11	130866	Effect of stocking density on performance and footpad dermatitis in broilers (Lidija Peric)	257
P1-12	130927	Effects of a twin shell building and proactive ventilation on litter humidity and health of broiler chicken (Stephanie Schäfers)	258
P1-13	130969	Hanging poultry scales as a model for environmental enrichments in commercial broiler houses (Brenda Hernandez Tapia)	259
P1-14	131007	Welfare of turkey hens with untrimmed beaks in enriched housing conditions (Birgit Spindler)	260
P1-15	131012	Hatching on-farm under practical conditions - first experiences on the behavior in on-farm hatched and hatchery-hatched broiler chicks (Sabine Vossler)	261
P1-16	131022	Effect of bedding material on leg quality in broilers (Sava Spiridonovic)	262
P1-17	131036	Climate change: heat stress trends in German regions of intensive turkey production (Nina Volkmann)	263

P1-18	131068	Effect anti-stressor supplementation on body weight changes, SOD (Superoxide Dismutases), and Glucocorticoids (GC) gene expression in transport stressed broiler chicken (Jaydip Rokade)	264
P1-19	131086	On-farm hatching in slow growing broilers: an experience out of the practice (Anna Schwarz)	265
P1-20	131098	Stripped to the Bone: Comparing Broiler Bone Strength and Bone Density Across German Fattening Methods (Jolien Hacker)	266
P1-21	131153	Effect of different litter types on occurrence and severity of pododermatitis in broiler production (Slobodan Knežević)	267
P1-22	131211	Effects of a triple-strain Bacillus-based probiotic in ameliorating stress-related impacts on broiler gut microbiota for improved well-being and productivity (Antoine Meuter)	268
P1-23	131246	Expression of regulatory neuropeptides in broiler breeder females fed diets with decreased energy and protein content (Gillian Cherrie)	269
P3-01	130750	Growth performance of male layer-type chickens reared at different stocking density (Evgeni Petkov)	388
P3-02	130809	What emergency killing methods should be used for poultry weighing less than or more than 5kg? (Marie Bourin)	389
P3-03	130876	Weight lifting hens? The impact of sensor weight on the movements of aviary housed hens (Maja Makagon)	390
P3-04	130886	Development of an automatic device for the administration of mother hen uropygial secretion analogue in hatching eggs and its effect on vocalization at hatch (Marta Yerpes)	391
P3-05	130992	Differential effect of two different doses of organic selenium on production performance in White Leghorn layers (Anand Laxmi N)	392
P3-06	131050	Assessment of welfare and meat quality parameters of backyard native birds reared under deep litter versus cage rearing system (Asawaree Hable)	393
P3-07	131074	Stakeholders' attitudes toward painfulness of keel bone damage in laying hens: an empirical study from Serbia (Vesna Rodic)	394
P3-08	131132	Efficacy of alliaceous extracts in modulating cytokine response under heat stress in poultry: insights from in vitro macrophage studies (María Arántzazu Aguinaga Casañas)	395
P3-09	131144	Early characterization of behavioural reactivity in two Italian chicken breeds: a preliminary study (Stefano Paolo Marelli)	396
P3-10	131149	Measuring the chronic metabolic demands of keel bone fractures in brown-feathered end-of-lay hens (Jacob Brost)	397
P3-11	131166	Occurrence of feather damage, skin injuries, and keel bone deviations in dual-purpose hens in organic farming (Philipp Hofmann)	398
P3-12	131172	The effect of crossbreeding of local chicken breeds with commercial parental lines on animal welfare (Sonja Hillemacher)	399
P3-13	131177	Judgement bias test in dual-purpose chickens exposed to different environments (Josefine Stuff)	400
P3-14	131187	Field study on the behaviour, feed consumption and performance of laying hens in an aviary system (Julie Laclau)	401
P3-15	131197	Investigation of the use of different environmental enrichment elements in the case of different hens genotypes (preliminary results) (Lilla Petò)	402
P3-16	131198	Effect of including whole <i>Hermetia illucens</i> larvae in the diet on the stress levels in laying hens (Ana Montalbán Pérez)	403
P3-17	131226	Investigation of the use of different environmental enrichment elements for various waterfowl genotypes (a preliminary study) (Sándor Szász)	404
P3-18	131247	Welfare of spent hens in Flemish slaughterhouses: current situation (Anneleen Watteyn)	405
P3-19	130915	Gene expression of two egg proteins, VMO1 and AvBD11, in the laying hen (Magali Chessé)	406

***Meat quality***

P3-54	130759	Chemical and fatty acid profile of chicken meat from different housing systems (Mirjana djukic Stojcic)	441
P3-54	131258	Detection of antibodies against the chicken infectious anaemia Virus (CAV) in backyard birds in rural areas in Pichincha province, ecuador (Marco Cisneros)	441
P3-55	130772	Multi-omics analyses of chicken breast with spaghetti meat myopathy (Byungwhi Kong)	442
P3-56	130813	The impact of sustainable protein sources on meat quality of Redbro broiler chickens (Hannah Scott-Cook)	443
P3-57	130921	Effects of dietary inclusion of different lentil varieties on broilers' meat quality (Gabriela Maria Cornescu)	444
P3-58	131043	Nuclear Magnetic Resonance and Dielectric Spectroscopy as valuable tools to detect Wooden Breast abnormality in broiler chickens (Massimiliano Petracci)	445
P3-59	131089	Myopathies in broiler chicken breasts in a slaughterhouse from Argentina: prevalence, severity, and attributes contributing to the occurrence (Florenxia Graciela Campostrini)	446
P3-60	131175	Nutritional value and quality of poultry meat affected by white striping, wooden breast and spaghetti meat myopathies (Marija Boškovic Cabrol)	447
P3-61	131201	Comparison of meat quality traits among the main chicken categories marketed in Italy: 1. Technological properties (Mara Antonia Gagliano)	448
P3-62	131242	Comparison of meat quality traits among the main chicken categories marketed in Italy: 2. lipid and protein oxidative stability (Emilia Luigia Antenucci)	449
P3-63	131243	Breast quality of poultry fed with diet supplemented with live black soldier fly larvae (Niko Gioacchino Zeni)	450
P3-64	131264	Effect of the application of pulsed electric fields in combination with salts on the drip water loss, texture and appearance of wooden breast meat in broilers (Tamara Anahí López)	451
P3-65	131293	Supplementation of olive oil-derived phytocomplexes in broiler chicken diets: effects on breast meat quality and oxidation changes of thigh meat during refrigerated storage (Emilia Luigia Antenucci)	452
P3-66	131294	Effect of the dietary inclusion of <i>Hermetia illucens</i> oil on fatty acid composition and technological properties of chicken meat (Mara Antonia Gagliano)	453
P3-67	131301	Effect of palm kernel fatty acid distillates and Black Soldier fly larvae oil on meat quality of Broiler Chickens (Laura Parro)	454

***Egg quality***

P1-62	130800	The fatty acid composition of egg yolk from hens fed diets differing in maize hybrid, rapeseed oil level and addition of emulsifier (Nives Marušic Radocic)	308
P1-63	130851	Effect of a new 3-phytase on egg production and quality and bone mineralisation traits in laying hens (Mehran Javadi)	309
P1-64	130913	Eggshell quality in extended laying cycle (Nicolas Guyot)	310
P1-65	131035	Influence of natural pigments on the shelf life of eggs from Isa Brown layers (Cristina-Camelia Matache)	311
P1-66	131121	Evaluating the impact of temperature and storage duration on egg quality under tropical climatic conditions (Sonale Nagesh)	312
P1-67	131183	The effect of experimental <i>Ascaridia galli</i> infection on egg quality in three local chicken breeds (Leopold Schwarz)	313
P1-68	131188	From colorimetry to carotenoids deposition in egg yolk: impact of carotenoids combination on egg yolk pigmentation (Aurelia A. Séon Simon)	314

P1-69	131248	Protected sodium butyrate improves productive performance and egg quality in old laying hens (Beatriz Saldaña)	315
P1-70	131313	Influence of age on eggshell colour persistency in greenshell and blueshell layers (Filip Dyttrt)	316

### *Food Safety*

P1-52	130741	Efficacy of the application of an attenuated Salmonella Typhimurium vaccine in a broiler farm to reduce excretion and colonization of field strains after and experimental infection (Lourdes Sisquella)	298
P1-53	130781	Stability of a bivalent live Salmonella vaccine in drinking water over a 24-hour period, with and without the addition of a water stabilizer (Doris Mueller-Doblies)	299
P1-54	130827	Removal of Aflatoxin B1 Using Alfalfa Leaves as an Adsorbent Material: A Comparison between Two In Vitro Experimental Models (María De Jesús Nava Ramírez)	300
P1-55	130835	Influence of inactivated Salmonella vaccine in breeders on the vertical transference of MDA in day-old chicks (Santiago De Castro)	301
P1-56	130850	Prevalence and characterization of ESBL producing Klebsiella pneumoniae from Sonali chicken meat in Narsingdi district, Bangladesh (Muhammad Ali)	302
P1-57	130962	Phage Therapy Modifies the Host's Intestinal Ecosystem by Modulating the Microbiome: A Meta-Analysis Approach (Laura Lorenzo-Rebenaque)	303
P1-58	130974	Prevalence and antibiotic resistance of Enterococcus faecium /faecalis isolated from broiler chicken meat in Poland (Olimpia Kursa)	304
P1-59	131109	Multidrug resistant escherichia coli and salmonella isolated from water, litter and meat in commercial poultry farms bio-security concern and emerging public health threat in pakistan (Aayesha Riaz)	305
P1-60	131114	Characterization of the Clostridium perfringens and Clostridioides difficile hazards in broiler slaughterhouses (Adeline Huneau-Salaün)	306
P1-61	131257	Identification and antibiotic susceptibility profiling of bacteria isolated from backyard poultry (Aayesha Riaz)	307

### *Breeding and genetics*

P2-57	130843	Research of breeding and improvement immune system in poultry (Ekaterina Barkunova)	373
P2-58	130990	Effects of stocking density and sex ratio on the reproductive parameters of breeding geese in indoor keeping system (Barbara Vegi)	374
P2-59	130998	GWAS for FCR and breast meat yield traits in Pekin ducks (Julien FABLET)	375
P2-60	131001	Decoding selective sweeps associated with tropical adaptability in Guinea fowl through whole-genome sequencing (Azhaguraja Manokaran)	376
P2-61	131002	Molecular characterization of Chicken B cell marker (ChB6) in native chicken of Poonch region from international borders of India and Pakistan (Mandeep Singh Azad)	377
P2-62	131041	Novel MHC-B haplotypes in Chantecler chickens (Jean-Marc Larivière)	378
P2-63	131047	Inclusion of eggshell thickness in a genetic improvement program in laying hens (Tomasz Szwaczkowski)	379
P2-64	131113	Application of impedance spectroscopy for the determination of body condition and pelvic fat content in breeder broiler hen (Cristina Guerra)	380
P2-65	131147	Semen quality parameters of Italian chicken breeds: characterization at the molecular level by proteomics (Silvia Cerolini)	381

P2-66	131164	Single Nucleotide Polymorphism related to maternal ability trait of Thai and Laos native chickens (Amonrat Molee)	382
P2-67	131206	Comparison of the nesting behaviour of white and brown pure line laying hens (Lina Hattendorf)	383
P2-68	131213	Key miRNA of chicken seminal plasma extracellular vesicles related with sperm motility regulation (Jilan Chen)	384
P2-69	131222	Genetics of daily feed efficiency in broiler lines divergent for digestive efficiency (Sandrine Mignon-Grasteau)	385
P2-70	131274	A crossbreeding as a proposal in a conservation program of Spanish 'Murciana' chicken breed (Laura Martínez Martínez)	386
P2-71	131311	Detection of male gametes from successful primordial germ cell implantation in a sterile interspecies hybrid (Eszter Várkonyi)	387

### ***Incubation and Fertility***

P2-53	130792	Effects of short period of incubation during storage of eggs from different breeder ages on chick characteristics and nutrient transporters (Servet Yalcin)	369
P2-54	131061	Effects of breeder age on plasma glucose and lactate of newly hatched chicks (José Henrique Stringhini)	370
P2-55	131090	Use of dimethylacetamide for cryopreserving rooster sperm from an endangered breed: effects on sperm quality and sperm fertilizing ability (Eva Mocé)	371
P2-56	130823	Preliminary investigations on Mulard duck, as sterile recipient in gonadal tissue transplantation (Kitti Buda)	372

### ***Poultry physiology***

P3-20	131010	Herbal adaptogen feed-additive modulates the hypothalamic expression of feeding-related neuropeptides in heat-stressed broilers (Sami Dridi)	407
P3-21	131278	Bone quality and incidence of urolithiasis in male broiler breeders fed a female or male ration (Nicola Tyler)	408
P3-22	131067	Role of novel natural nutritional emulsifier in optimizing fat utilization and energy saving in poultry diets (Shivi Maini)	409

### ***Smart Farming, Housing and Management***

P2-01	130856	Design of a moist feeding system for poultry (Salma Rian)	317
P2-02	130982	The effect of different indoor layers' housing systems on the changing of live weight of laying hens and its uniformity (Lilla Pető)	318
P2-03	131115	Productive and reproductive performance, dressing yield and chemical composition of different varieties of guinea fowl (Nushrat Nourin Lisa)	319
P2-04	131223	Promoting an innovative heating system for broiler chickens in rural communities in Cameroon: a preliminary study (Achille Schiavone)	320
P2-05	131227	Validation of an automated scale for continuous monitoring of the body weight in broiler chicken farming (Maria Roda Tena)	321
P2-06	131251	Thermographic analysis in 11-week-old turkeys (Ezequiel Sánchez)	322
P2-07	131285	Poultry thermoregulation in the heat: seasonal acclimatization and partitioning of evaporative water losses (Thobela Nkukwana)	323

***Sustainability***

P1-01	130719	Study of economic parameters in Uttara chicken: A promising native chicken of Uttarakhand state of India (Anil Kumar)	247
P1-02	130799	Sea urchin waste as a sustainable source of calcium in laying hens production (Francesca Leone)	248
P1-03	130849	Effects on the carbon footprint of chicken meat by an infection with Eimeria spp (Julia Gickel)	249
P1-04	130979	Feasibility of purified rainwater as drinking water for laying hens (Neil Van Den Broeck)	250
P1-05	131150	Improve broiler production sustainability reducing N and P excretion through diet optimization with multi-enzymatic complex with high doses of phytase (Davide Militello)	251
P1-06	131195	Comparing the improvement in feed sustainability through multi-carbohydrase complex with high doses of phytase using two different GFLI databases (Davide Militello)	252
P1-07	131240	Effect of the addition of protected sodium butyrate in broiler diet on nitrogen and phosphorus excretion (Beatriz Saldaña Mancebo)	253
P1-08	131262	Sustainable utilisation of eggs lost during further processing and packaging (Simone Pauling)	254
P1-09	131291	Effect of genotype on human-edible protein conversion efficiency: valorisation of local chicken breeds through cross-breeding strategy (Federica Volpe)	255

***Economics and Marketing***

P4-75	130966	Empowering consumers to choose safer chicken in informal markets in Ouagadougou, Burkina Faso (Gemma Tacken)	530
P4-76	131137	Economic efficiency of males of dual-purpose genotypes in organic poultry production in Germany (Petra Thobe)	531

***Education and Information***

P4-73	130952	Development of a virtual poultry slaughterhouse simulator to train veterinary students in Europe (Alessandra De Cesare)	528
P4-74	131276	Agripreneurship intentions in the poultry sector of youth in a tertiary institution (Harry Swatson)	529

***Other***

P4-61	130906	In vitro efficacy assessment of sodium dichloroisocyanurate (SDIC) with potassium monopersulphate (PMP) disinfectant against poultry viruses, yeast, and fungi (Jia Hui Tee)	516
P4-62	130907	In vitro bactericidal activities of sodium dichloroisocyanurate (SDIC)/potassium monopersulfate (PMP) disinfectant against common poultry bacteria (Wei Shen Tong)	517
P4-63	130918	Does essential oils inhibition of Escherichia coli is dependant of cassia oil? (Claire Carlu)	518
P4-64	130976	Characterization of the chemical contents of selected insects as food and feed (Emmanuel Malematja)	519
P4-65	130988	Elimination of mycoplasma species from the semen of Hungarian native goose (Barbara Vegi)	520

P4-66	131019	Genetic and acoustic characters of Indonesian long crowing chicken for contest purposes (Maria Ulfah)	521
P4-67	131059	Comparison of four non-linear growth models in free-ranging lohmann dual cockerels (Alexandr Torres)	522
P4-68	131218	Comparative study on production performance of brown and white variety of Japanese quail ( <i>Coturnix coturnix japonica</i> ) (Shahbaz Haroon Khan)	523
P4-69	131230	Effects of different housing systems on the performance of laying quails (Philipp Hofmann)	524
P4-70	131280	Characterizing the interface: Seasonal changes in bird communities on poultry farms and house sparrow - wild bird contacts revealed by camera trapping (Alberto Sanchez Cano)	525
P4-71	131286	Comparative analysis of growth performance and economics in various germplasm of chicken for backyard farming (Rajshree Dhoulpuri)	526
P4-72	131076	Poultry in vitro digestion model allows a relevant evaluation of raw materials nutritional value (Stéphanie Klein)	527



**XVI European Poultry Conference**  
VALENCIA, SPAIN 24<sup>th</sup>-28<sup>th</sup> June 2024



### Authors' index

#### A

J. C. Abad, 65, 100, 380  
 A. E. Abd El-Hakim, 350  
 M. Abd El-maged, 350  
 A. Abd El-Wahab, 249  
 N. Abdeleel, 332  
 E. Abdelwhab, 39  
 A. Abellán, 209  
 V. Abramov, 293  
 C. Achard, 460  
 N. G. Acosta, 53, 126  
 M. Adam, 425  
 S. Adedokun, 120  
 C. Adler, 94, 260  
 R. K. Agarwal, 357, 439  
 M. A. Aguinaga Casañas, 395  
 L. Aguirre, 23, 72  
 M. Ahmad, 146  
 K. Ahmed, 231  
 A. Ajao, 325, 328  
 V. Akaram, 247  
 R. Akbari Moghaddam  
   Kakhki, 73, 324  
 M. Z. Akram, 151  
 N. Akter, 470  
 A. Alberdi Estibaritz, 144  
 J. A. Alencar, 251, 252  
 P. Alfonso, 41  
 C. Alfonso Carrillo, 33, 73  
   174  
 M. Alhussien, 22  
 M. Ali, 302  
 Z. Ali, 32  
 A. Alid, 40  
 S. Alindekon, 233  
 A. Alkhtib, 332  
 V. Allamand, 129  
 N. Aloy, 185, 187, 298  
 R. M. Alsina-Pagès, 236  
 D. Álvarez, 42  
 S. Álvarez, 53, 126, 241, 522  
 B. Ampe, 93, 166, 405  
 E. Anagnostopoulos, 46, 192,  
   273  
 Z. Ancsin, 436  
 V. Anderle, 316  
 K. B. Andersen, 227  
 B. Andersson, 30, 102  
 R. Andersson, 92, 96, 145, 176  
 P. Anfossi, 512  
 R. Angel, 492

S. Ania, 114, 423  
 G. Annonay, 153  
 E. L. Antenucci, 448, 449, 452,  
   453  
 E. Antenucci, 125  
 N. Antilles, 44, 363  
 G. Antonissen, 157, 166, 432  
 Z. Antoszkiewicz, 467  
 T. Applegate, 74  
 M. Ardakani, 407  
 E. Arevalo Sureda, 224  
 J. Argilaguët, 38  
 A. Ariyachayut, 414  
 E. Armero Ibáñez, 386  
 J. L. Arnal, 114, 423  
 J. M. Arroyo, 278  
 T. Ashok Kumar, 312  
 A. H. Ataei, 385  
 M. Auerbach, 109  
 R. Aureli, 145, 314  
 S. Avendano, 269  
 X. Averós, 89  
 N. Avital Cohen, 63  
 L. Ayala, 222, 344, 340, 403  
 L. Ayalew, 231  
 T. Ayats, 185, 186, 187 294  
 M. S. Azad, 377  
 M. Azam, 354

#### B

B. Babarczy, 372  
 E. Babini, 106  
 C. Baes, 14  
 E. Baéza, 134, 413, 414  
 C. Bagnard, 153  
 R. M. Baig, 307  
 M. Baladelli, 362  
 G. Balbiani, 111  
 L. Baldinger, 531  
 M. Ball, 74  
 H. Balzer, 234  
 A. Baños, 395  
 A. Baptista, 296, 366, 367  
 M. Baratelli, 431  
 T. Barbara, 210  
 A. Barbe, 468  
 F. Barbe, 285, 456, 457, 458,  
   460, 486  
 F. Barbut, 306  
 E. Barkunova, 373  
 J. Barna, 374, 520  
 J.I. Barragan Cos, 26  
 A. C. Barroeta, 124, 221, 454  
 J. Bartman, 63  
 L. Bassi, 220  
 M. J. Batista Dos Santos, 348  
 A. Bautil, 147, 148, 218  
 A. Bazida, 40  
 K. Bebin, 346  
 S. Bechis, 320  
 M. Bedford, 147, 218  
 S. Bellezza Oddon, 450  
 A. Bello, 156, 329, 477 480  
 P. Belloir, 134  
 D. Belloumi, 216  
 I. Belo, 338  
 M. Beltran, 42, 440  
 J. Ben Arous, 287  
 M. E. A. Benarbia, 274, 500  
 C. Benavides, 33, 124  
 A. Benito, 114, 423  
 J. Bennewitz, 99  
 E. Ben-Tal Cohen, 31  
 A. Benteo, 367  
 D. Berdejo, 359  
 S. Bergmann, 524  
 J. Berk, 162  
 J. Bernard, 129  
 M. Bernardeau, 225  
 N. Bernardet, 55, 406  
 M. Bernardino De Lima, 315  
 C. Berri, 32, 129, 134, 239,  
   413, 414  
 K. Bertran, 5, 38  
 M. Betancor, 126  
 S. Betz, 268, 497, 506  
 C. K. Beura, 163  
 H. Bharati, 272  
 J. Bhati, 376  
 A. Bhattacharyya, 48, 78, 190  
 L. Biagini, 118  
 C. Biard, 195  
 M. Biarnés, 107, 115, 363  
 I. Biasato, 217, 450  
 K. Bierinckx, 119  
 M. Birolo, 77, 150, 169, 447  
 V. Biselo, 251, 252  
 A. Biswas, 473  
 F. Blanc, 143  
 E. Blanch, 371  
 M. Blanch, 283  
 A. Blanco, 44, 115, 363  
 C. Blaschka, 105, 313

- R. Blatchford, 390  
P. Blavy, 98, 385  
E. Blesbois, 104  
P. Bleyen, 178, 250  
C. Bôa-Viagem Rabello, 348  
D. Bochynska, 112  
L. G. Bódog, 318  
G. Boerboom, 191, 515  
S. Bonaspetti, 270  
B. B. Bondar, 48, 190  
V. Bongiorno, 67, 136 217  
C. Bonnefous, 153, 239  
V. Bontempo, 121  
W. Boonsathitanan, 113, 431  
C. Borbély, 402  
G. Borchardt, 268  
T. Bordeau, 129, 134, 149  
F. Bordignon, 56, 77, 150, 171, 232, 447  
M. Bordini, 106, 131, 445  
C. Bortoluzzi, 85, 474, 475, 479, 502  
M. Boškovic Cabrol, 56, 150, 447  
J. Bošnjak-Neumüller, 50, 362, 365, 484  
N. Botteldoorn, 183  
C. Boudry, 469  
A. Bourdillon, 527  
M. Bourin, 129, 389  
M. Bovo, 255  
B. Bowker, 442  
J. Boyd, 489  
K. Brijs, 148  
C. M. H. Broekmeulen, 165  
L. Broer, 176  
J. Brost, 397  
I. Brouklogiannis, 46, 192, 273  
R. M. Bruckmaier, 165  
B. Brun, 128  
M. Brunon, 188, 503  
P. Brusi, 221  
K. Buchynski, 94, 97  
K. Buda, 372  
S. Budnik, 352  
D. Bueno Alguacil, 73  
K. Bühler, 194  
H. Bui, 500  
R. Bunnom, 382  
T. Burnside, 10  
E. Burton, 122, 332, 489  
W. Büscher, 201, 399, 400  
M. Busquet Solé, 43, 113, 431, 511
- F. Bussière, 149  
J. Buyse, 147  
K. Buyse, 214
- C**  
M. Caballero, 108, 275  
I. Cabarkapa, 365  
L. Cadiz Nuñez, 435  
D. Cadogan, 327, 470  
E. Cailleau-Audouin, 149, 153  
L. Calandrea, 239  
F. Calenge, 143  
A. Calik, 471  
M. Call, 321  
A. Callejo, 47  
S. Calvet Sanz, 25  
M. A. Calvo, 364  
M. C. Camacho, 525  
L. Cámara, 23, 72  
A. Camarina-Silva, 482  
M. Cambra-López, 309, 338, 472  
M. Campelo Silva, 426  
M. D. M. Campo, 58  
F. G. Campostrini, 446 451  
L. Canaple, 495  
C. Cano, 216  
S. Canogullari Dogan, 353  
J. Cao, 347  
L. C. Capern, 506  
E. E. Cappone, 67, 136, 217  
A. Caralt, 135  
E. Carceller, 43, 113  
T. Cardona Cabrera, 242, 525  
D. Cardoso, 465  
L. Cardoso, 479  
N. Cariou, 468  
C. Carlu, 359, 518  
N. Carmona-Vicente, 5  
L. Carpintero, 309, 472  
F. Carrasquer Puyal, 161  
M. D. Carro, 278  
M. Carroll, 60  
L. Carroy, 527  
A. Cartoni Mancinelli, 90, 169, 448, 449  
I. Carvalhido, 69  
C. Casarotto, 37  
J. Caspers, 254  
L. Castellanos, 289  
C. Castellini, 169, 239  
M. Castex, 285, 456, 457, 458, 460
- L. Castillejos, 221, 321, 420  
N. Castro, 241  
P. Catalá-Gregori, 82, 226  
E. Catelli, 159, 433, 434  
A. Catlett, 19  
D. Cavallini, 353  
D. Caverio, 101, 102, 103  
G. Ceada, 5  
F. Ceccaroni, 175  
M. Cecchinato, 43, 426  
J. Celej, 51  
R. Cepero, 58  
M. Cerdà-Cuellar, 185, 186  
A. Cerisuelo, 216, 309, 472  
S. Cerolini, 381, 396, 450  
N. Ceylan, 475, 485  
T. Chabrilat, 518  
P. Chacana, 111  
G. Chacón, 423  
M. Chagunda, 99  
H. Chai, 41  
A. Chakrabarti, 272  
D. Chakraborty, 377  
E. Chalvatzis, 514  
T. Chalvon-Demersay, 195  
B. Chanchayanon, 494  
S. C. Chang, 490  
S. Chang, 341  
N. Charoenvisal, 113, 431  
M. Charriere, 501  
P. Chartrin, 134, 149  
S. Chaumont, 98  
D. Chen, 279  
H. Chen, 69, 79, 279  
J. Chen, 384  
Y. Chen, 342  
Z. Chen, 279  
S. Cheng, 41  
P. Cheong, 516, 517  
G. Cherrie, 269  
M. Chessé, 55, 310, 406  
E. Chevaux, 285, 456, 457 458, 486  
J. A. Chew, 70  
L. Chew, 505  
D. Chiattelli, 90, 169  
C. Chibanda, 531  
M. Choct, 478  
J. Choi, 442  
Y. Choi, 78  
H. Choo, 427  
A. Choudhary, 393  
B. Choudhury, 272  
L. Chouhan, 523, 526

- E. Christodoulou, 514  
C. Ciarelli, 59, 171, 232  
O. Ciervo, 298  
Y. Cinnamon, 31  
A. Cismileanu, 345, 444  
M. Cisneros, 441  
F. Cisneros-Gonzalez, 192  
S. Cisse, 274  
G. Ciurescu, 463, 464  
E. Claeys, 430  
C. Clark, 54  
À. Cobos Arnalot, 107, 429  
I. Cockshott, 461  
C. Coello, 191  
I. Cohen, 31  
J. Collet, 98, 239  
A. Collin, 64, 153, 239  
S. Combes, 153  
Ö. Consortium, 313  
J. Consuegra, 84, 495, 507  
A. Corder, 287  
M. Corion, 61  
L. Cornaille, 149, 211  
G. M. Cornescu, 311, 345, 444  
J. Cortes, 136  
J. Costa, 296, 366, 367  
H. Cotta, 391  
A. Cottin-Tillon, 469  
E. Coudert, 195  
C. Coudron, 136  
N. Couroussé, 153  
C. M. Courtin, 147, 148, 218  
N. Covreur, 527  
A. Cowieson, 145, 220, 331  
R. Crespo Sancho, 292  
J. L. Criado, 43, 113  
D. Croonen, 148  
I. Cruvinel Di Castro, 370  
P. Cruz-Dousdebes, 391  
D. Cudnik, 325, 76  
B. Curtis, 347  
A. Czepiel, 418
- D**  
A. Dal Bosco, 90  
F. Dalla Favera, 159  
M. Dalla Rosa, 106  
A. Dalmau, 13  
F. S. Dalolio, 253  
K. Dalsgaard, 227  
K. Damme, 240, 398  
T. H. Dao, 470  
T. Dapcevic Hadnadjev, 365
- L. Dardabou, 49  
M. Dardi, 301, 431  
A. Darji, 185, 187  
C. Darrington, 378  
B. Das, 272  
G. Das, 105  
G. Dauphin, 290  
M. Davey, 8  
R. Davin, 75, 339  
K. De Baere, 80, 177  
R. De Baets, 157  
D. De Bellis, 118  
H. H. De Carvalho Mello, 370  
S. De Castro, 301  
A. De Cesare, 212, 528  
A. De Grande, 214  
M. De Gussem, 432  
I. De Jong, 95, 144, 201, 202, 205  
B. De Ketelaere, 61  
M. De Laet, 277  
J. De Oliveira, 496  
T. De Rauglaudre, 149  
K. De Reu, 183  
C. De Schepper, 148  
S. De Smet, 32  
M. De Soler Piart, 431  
M. De Souza Vieira, 410, 469  
E. De Vet, 530  
L. De Vriendt, 179  
L. De Witte, 183  
R. D'Eath, 269  
P. Debnath, 272  
J. Degroote, 157  
B. Dehaeck, 498, 499  
F. Delanglez, 166  
E. Delezie, 80, 157, 166, 177  
N. Demaitre, 93, 166, 198, 250, 405  
V. Demey, 285, 456, 457, 458, 486  
S. Deori, 272  
M. Derks, 317  
Y. Dersjant-Li, 156, 329, 466, 477, 480  
C. Deschamps, 465  
E. Desfilis, 209  
J. Deutsch, 233  
C. Deuttner Neumann Barroso, 362  
I. Devine, 54  
N. Dewe Alfin, 510  
P. Dhaese, 270  
R. Dhoulpuri, 523, 526
- M. Di Benedetto, 508  
G. Di Martino, 37, 171  
A. Dias, 338  
J. Dickson, 291  
C. Dierks, 105  
L. Digon, 114, 423  
Z. Ding, 41  
M. Dione, 530  
L. Dixon, 269  
A. Djikeng, 154  
F. Djitie Kouatcho, 343  
M. Djordjevic, 267  
M. Djukic Stojcic, 257 262, 267, 394, 441  
B. Djurdjevic, 509  
L. Dóbbé, 402, 404  
A. Dodovski, 41  
S. Dokou, 189  
R. Dolz, 44, 107, 115  
J. Domelevo Entfellner, 154  
A. Donadeu, 309, 472  
C. Donaldson, 277  
M. Doret-Aubertot, 527  
G. Dosa, 159  
D. Douangvilay, 382  
A. Dovc, 424  
E. Drackova, 316, 351  
D. Dragotoiu, 311  
A. Drazbo, 51, 349  
S. Dridi, 407  
Á. Drobnýák, 372, 374, 387, 520  
S. Druyan, 31, 235  
I. Duarte, 426  
S. Dube, 408  
K. Dublec, 49, 326  
A. Dubois, 277  
M. J. Duclos, 211, 208  
P. Duklong, 43  
M. Dumitru, 463, 464  
A. Dunislawska, 151  
I. Dunn, 30, 102, 170, 172, 269  
T. Duong, 487, 491  
J. Dupuy, 309, 472  
M. Durand, 71  
F. Dytrt, 316
- E**  
V. Economou, 189  
I. Eising, 85, 145, 475, 476, 502  
W. El Feilb, 40  
M. Z. El-Abideenc, 40

- W. Elfeil, 297, 355  
D. Ellawidana, 87  
D. N. Enea, 343  
H. Enting, 69, 79  
M. Erdélyi, 436  
M. Erhard, 133, 206  
D. Escobar, 42  
S. Escobar Sánchez, 386  
C. Escoda, 44, 115  
F. Esperon, 243  
R. Espinola, 101  
M. Espinosa De Los Monteros Peñafiel, 420  
C. Esteve, 371  
A. Esteve-Codina, 38  
I. Estevez, 89  
M. Estévez, 18  
J. Estevinho, 293  
U. S. Ety, 276  
C. Evans, 459  
N. Everaert, 61, 148, 151, 224  
B. Ezzo, 208
- F**  
J. Fablet, 375  
I. Fabra, 363  
P. Fabrega, 421  
F. Fagnoul, 129, 238  
N. S. Fagundes, 251, 252  
V. Fain Binda, 501  
J. Faivre, 527  
B. G. Falcini Girrach, 370  
C. Falker-Gieske, 223  
J. Faltin-Schnitzer, 162  
M. Farahat Selem Zahran, 421  
A. Farajallah, 521  
T. P. Farkas, 318, 402, 404  
G. Farre, 221  
S. Faruque, 276, 319  
V. B. Fascina, 474  
C. Favier, 527  
K. Fayolle, 518  
N. Fejszák, 387  
C. Fernández, 216  
V. Ferrante, 248  
L. Ferrari, 248  
P. Ferrari, 144  
J. Ferrer, 58  
P. Ferrer, 309, 472  
R. Ferrer, 412  
J. I. Ferrero, 253  
C. Ferriz-Nuñez, 303  
A. Fickler, 478
- J. D. D. Figueroa Cárdenas, 300  
E. Fiorilla, 67, 127, 136, 203, 217, 344, 403  
A. Fireman, 348  
C. Firman, 327  
O. Firmesse, 306  
P. J. Fito, 106  
L. Fitz, 240, 398  
E. Folegatti, 476, 512  
E. Fonsatti, 150  
F. Font-i-Furnols, 128  
M. Font-i-Furnols, 130  
R. Forder, 327  
S. Forero, 440  
D. Fornasiero, 37  
R. Fortina, 320  
P. Fortomaris, 189, 230  
D. Foukas, 181  
S. Fourdin, 137, 138  
Y. Fournis, 496  
R. Franco, 289  
R. Franco-Rosselló, 174, 419  
M. Fresno, 241, 522  
M. Frikha, 100, 380  
M. Frisch, 292  
J. Fulton, 378  
L. Fumero, 241, 522  
I. Fusaro, 353
- G**  
M. A. Gagliano, 90, 448, 449, 453  
G. Gagliardi, 90  
F. Gai, 67, 136  
M. Gaimard, 286  
A. Galanis, 230  
G. Gallina, 512  
L. Galosi, 118  
S. Gangwar, 271  
C. Gao, 41  
G. Garavini, 159  
C. Garcés-Narro, 124, 125  
G. García, 429  
C. García, 44  
P. A. García, 446  
J. M. García Coronel, 274  
A.I. García Ruiz, 33, 419  
J. A. García-Bautista, 124, 125  
D. Garcia-Gonzalo, 359  
J. Garcia-Llorens, 82, 226  
A. I. Garcia-Ruiz, 197, 333, 73, 174, 324
- A. García-Vara, 412  
M. Gariglio, 67, 136, 217  
R. Gatti, 106  
H. Gautam, 231  
X. Gautier, 389  
J. Gautron, 208, 310, 406  
S. Gebhardt-Henrich, 14 164, 165, 201  
M. Gebaska, 144  
J. Geibel, 103  
O. Genin, 31  
N. Gentile, 161  
C. Georgeta, 462  
S. Gerard, 314  
C. Gérard, 134  
K. Germain, 153, 239  
M. Gesek, 356  
A. Ghane, 477  
K. Gharib-Naseri, 411  
A. Gheorghe, 463  
S. Ghimire, 219  
M. R. Ghorbani, 284  
M. Giammarco, 353  
E. Giampieri, 528  
I. Giannenas, 50, 189, 230  
K. Gibbs, 156, 225, 329, 459  
S. Gibson, 285  
J. Gickel, 249  
L. Giehl, 240, 398  
M. Gierus, 49  
V. Gigaud, 314  
S. Gilani, 466, 477  
Q. Gilbert, 290  
A. Gilberto Bertechini, 253  
N. Gilles, 225  
A. Gimenez Mascarenhas, 370  
R. Gimenez-Rico, 477  
A. Giner, 291  
G. J. Ginovart-Panisello, 236  
C. Girard, 359, 518  
M. Gitau Gicheha, 154  
E. Gócza, 387  
A. Goddard, 299  
A. Goderis, 496  
G. L. Godoy, 251, 252  
W. L. Goh, 516, 517  
K. Goitom Tewelde, 326  
L. Gomez, 270  
A. Gómez, 42, 440  
E. A. Gómez, 216, 317  
I. S. Gómez, 82  
Y. Gómez, 165, 200  
L. Gomez-Osorio, 50  
S. Gomis, 231

- J. González, 187  
G. González Mateos, 23, 72  
M. P. González Navarro, 185  
G. Gonzalez Ortiz, 147, 218  
D. Gonzalez Sanchez, 119, 416, 417  
B. González Zorn, 12,  
A. Gonzalez-Mohíno, 18  
D. Goo, 280  
T. Goossens, 84, 230, 495, 507  
A. Gordillo, 471  
Ž. Gottstein, 81  
G. Goyal, 295, 523, 526  
B. Grabkowsky, 173  
D. Grace, 530  
I. Grasseau, 104  
F. Grassi Salvini, 381  
J. Grau Pla, 301, 511  
T. Grauwet, 122  
G. Graziosi, 159, 434  
A. Green, 242  
E. Greene, 407  
B. Grenier, 504  
E. Griela, 273  
P. Groot Koerkamp, 317  
P. J. Groves, 59  
S. Grubac, 509  
A. Grund, 109  
Y. Gu, 148  
F. Guardiola, 135, 412, 454  
D. Gudyñaite, 112  
J.L. Guérin, 21  
C. Guerini, 401  
C. Guerra, 100, 380  
V. Guesdon, 239  
E. Guettier, 238  
P. Guevellou, 401  
C. Guidou, 134  
E. Guillamón, 395  
L. Guilloteau, 149, 153, 239  
T. Gumpenberger, 145  
S. Gunnarsson, 144, 181  
H. Gunnink, 202, 205  
R. Günther, 162  
O. Gunturkun, 475  
P. K. Gupta, 357, 439  
V. Gupta, 78  
V. Guyonnet, 27  
N. Guyot, 55, 310, 406  
M. H Kogut, 144
- H**  
M. Habeanu, 463  
A. Hable, 393  
A. Hachemi, 465  
J. Hacker, 133, 206, 266  
M. Hadnadjev, 365  
M.G. Haider, 117  
I. Halachmi, 235  
S. Haldar, 477  
L. Hall, 478  
J. J. Hamming, 123  
X. Han, 384  
J. Hankel, 249  
R. Hardy, 477  
B. Hargis, 97  
J. A. Harijaona, 471  
A. Harlander, 168, 199  
A. Haron, 31, 235  
R. Harrison, 299  
L. Hattendorf, 383  
C. Hautefeuille, 290  
Z. Hayat, 354  
M. Heincinger, 374  
M. Hejdysz, 352  
W. Hendriks, 143  
M. Hennies, 105  
K. Heo, 427  
D. Hermans, 88  
F. Hernandez, 136, 217, 222, 340  
E. Hernández, 322  
D. Hernández Patlán, 300  
F. Hernández Ruipérez, 344, 403  
B. Hernandez Tapia, 259  
A. Herr, 94  
M. Hertog, 61  
O. Hervé, 55, 406  
E. Herwig, 94  
C. Hess, 81  
R. M. Hickey, 86  
S. Hillemacher, 399  
B. Hillen, 477  
D. Hinrichs, 399  
U. Höfle, 242, 525  
P. Hofmann, 240, 398, 524  
M. Hohenfeld, 524  
P. Homyok, 413, 414  
A. Hondelatte, 153  
G. Hong, 168  
G. Horst, 361  
F. Horta, 45  
S. Hossain, 276  
J. Houdijk, 329  
A. Huerta, 150, 447  
H. Hufen, 109
- A. Huneau-Salaün, 306  
E. Hunter, 489  
M. Hussain, 144  
Y. Hwang, 78
- I**  
C. I Ju, 483  
E. Iaccheri, 106, 445  
C. Iaconis, 504  
N. Iaffaldano, 381  
M. Ibañez, 283  
M. Á. Ibañez, 49, 72  
A. Ibrahim, 196, 307  
M. Ibrahim, 40  
P. Idziaszek, 379  
B. Iglesias, 501  
M. Ignatova, 388  
E. Ilatsia, 99  
G. Ilboudo, 530  
S. Ilic, 504  
E. Iliopoulou, 46  
C. Im, 427  
V. Indio, 212  
M. Ingberman, 362  
V. Inhuber, 327  
F. Iquierdo, 468  
I. Iriondo Sanz, 236  
M. A. Islam, 319  
K. Itani, 146, 219  
M. Iuspa, 502  
A. Ivanona, 293  
O. Ivanona, 293
- J**  
J. Jacob, 120  
L. Jacobsen, 145  
V. Jacquier, 88  
H. Jalal, 353  
A. S. Jalali, 167  
S. Jansseune, 143  
M. Jasinski, 491  
M. Javadi, 309, 472  
N. Jaworski, 324  
R. Jaydip, 312  
S. Jaypong, 281  
R. J. Jaywant, 207  
L. Jeay, 208  
G. R. Jena, 163  
L. Jessus, 149  
S. Jhetam, 97  
L. Jiang, 384  
S. Jiang, 41

- J. Jimenez, 55, 149, 310  
P. Joaquim, 111  
A. Jofré, 130  
J. Jofré, 128  
A. Johnny, 167  
V. Jonchère, 149, 211  
J. Jordá, 182, 243  
R. Jové, 363  
D. Józefiak, 349  
A. Juanchich, 195  
A. Jung, 34  
P. Jurankova, 368  
L. Justino, 366, 367
- K**  
S. R. R. K., 392  
S. K. A., 376  
S. Kaczmarek, 219, 220, 352  
P. Kaewsatuan, 382  
I. Kaikat, 213, 493  
J. Kaliniewicz, 467  
L. Kalvelage, 116  
A. H. M. Kamal, 302  
M. A. Kamal, 292  
A. Kamkaew, 413, 414  
V. Kaneko, 296, 366, 367  
K. Kanjanavaikoon, 494  
S. Kashyap, 438  
Y. Katagiri Dalmoro, 469  
M. G. Katchoua Tchualeu, 343  
F. Kaufmann, 92, 145  
C. Keambou Tiambo, 154  
E. Kebreab, 175  
L. Keeling, 201  
G. Kefalas, 46, 273  
K. Keirsschieter, 336  
S. Keller, 193  
I. Kempen, 93, 166, 177, 178, 179, 198, 250, 405  
N. Kemper, 92, 256, 258, 260, 261, 263, 265  
S. Kerros, 359, 518  
H. Kettunen, 229  
S. H. Khan, 523, 526  
S. Khare, 526  
S. Khariief, 287  
A. A. Khaskheli, 52, 83  
J. Khatun, 276  
A. R. Kherde, 48, 190  
G. Khillare, 207  
P. Khoutsavang, 382  
A. Kihal, 364  
W. Kilany, 40, 297, 355
- E. Kim, 478  
H. Kim, 427, 180  
J. Kim, 180  
W. Kim, 45, 74  
W. K. Kim, 280  
M. Kinsner, 51  
D. Kioussi, 230  
S. Kirwan, 361  
K. Kiskinis, 189, 230  
B. Kiss, 326  
É. Kissné Váradi, 374, 520  
K. Kittelsen, 152  
S. Klein, 527  
M. Kleinke, 254  
R. Kleyn, 4  
K. Kljak, 308  
F. Kloska, 116  
D. Klosterhalfen, 116  
S. Knežević, 267, 509  
T. Knight-Jones, 530  
R. Kobayashi, 366  
K. Kochanowski, 5  
G. Kolluri, 207  
S. Kolobe, 519  
S. D. Kolobe, 334  
A. Koncicki, 356  
B. Kong, 442  
P. Konieczka, 51, 349  
S. Koprivec, 424  
S. Kostka, 360  
K. Koszela, 379  
J. Kouamo, 343  
K. Koutoulis, 426, 433  
D. Koutsianos, 514  
M. Kovács-Weber, 374, 436  
J. Kowalczyk, 356  
E. Kowalski, 32  
K. Kozłowski, 51, 349  
M. Kramer, 260  
S. Kreuzer-Redmer, 88  
M. Kritsa, 181  
P. Kuhne, 430  
S. Kujawa, 379  
S. Kulcsár, 436  
U. Kulsoom, 305  
A. Kumar, 295, 312, 411, 247, 271  
D. Kumar, 357, 422, 438, 439, 247, 163  
G. R. Kumar, 295  
M. Kumar, 48  
S. Kumar, 247  
V. Kumar, 190  
O. Kursa, 304, 360
- T. Kushnir, 31  
V. Kuttappan, 496  
R. Kwakkel, 69, 95, 317, 335  
P. Kwiatkowski, 491  
I. Kyriazakis, 74
- L**  
V. Labas, 55  
J. Laclau, 401  
S. Lacroix-Lamande, 149, 211  
S. Ladirat, 184, 282  
L. Laghi, 445  
V. Lagos, 75  
L. Lahaye, 469  
W. Lambert, 71, 215, 337  
E. Lamed, 31  
A. Lammers, 143  
J. Lammertyn, 61  
B. Landwehr, 193  
J. Langa, 144  
J. Langbein, 233  
J. Larivière, 378  
J. Laurain, 274  
J. L. Lavín, 158  
J. Lawrence, 76, 325  
B. Lázár, 387  
S. Lázaro, 114, 423  
R. Lázaro García, 23  
E. Le Bail, 468  
E. Le Bihan-Duval, 129, 238, 239  
S. Le Bouquin, 306  
J. Le Douce, 188, 503  
C. Le Marechal, 306  
S. Lecuelle, 71, 337  
J. Lee, 45, 280  
T. Y. Lee, 483  
T. T. Lee, 341, 342  
T. Lee, 490  
A. Lefort, 389  
G. Lefort, 149, 211  
A. N. Lefter, 463  
N. Lefter, 464  
M. Legnardi, 426  
I. Lehoczky, 372  
E. Leishman, 168, 397  
S. Leleu, 80  
O. Lemâle, 84, 230, 495, 507  
M. Lemos De Moraes, 410, 469  
J. Lemoufouet, 343  
F. Leone, 248  
S. Lesceu, 286

- T. Lev Ron, 235  
L. Li, 411  
Y. Li, 384  
M. Lichovnikova, 316, 351, 368  
A. Limsatanun, 494  
H. Lin, 378  
H. H. Lin, 104  
M. J. Lin, 490  
M. Lin, 341  
Y. Lin, 279  
L. Linares, 492  
M. Lindemann, 120  
R. Lindenwald, 109, 162  
S. Lioliopoulou, 189, 230  
K. Lipinski, 467  
K. Liptói, 372, 374, 387  
M. Lisowski, 379  
G. Liu, 280  
H. Liu, 331  
S. Liu, 480  
X. Liu, 41  
G. Lizzi, 433, 434  
S. Llamas-Moya, 487, 491  
B. Lloret, 182  
K. Long, 237  
C. Longoni, 288, 512  
J. C. Lopes Brasileiro, 370  
C. Lopez, 259  
R. López, 5  
T. A. López, 446, 451  
C. López Coello, 300  
A. Lopez Karolys, 418  
A. M. Lopez Sobaler, 7  
C. Lopez-Calderon, 242  
M. D. C. López-Luján, 338  
J. López-Paredes, 488  
L. Lorenzo-Rebenaque, 182, 243, 303  
R. Losa, 194  
M. Lourenço, 80  
H. Louton, 133, 206, 233, 266  
L. Lozica, 81  
K. Lucas, 175  
A. Lucchi, 212  
B. Lumpkins, 456, 487  
N. Luna Corrales, 72  
Y. Luo, 474  
C. Lupini, 159, 433, 434  
V. Lux, 210  
M. Lyte, 15
- M**
- A. K. M, 357  
M. M, 264, 473  
R. K. M., 392  
S. M., 392  
E. M. Machuka, 154  
K. Maak, 254  
M. Mabelebele, 155, 334, 519  
E. Macchi, 203  
W. MacEline, 54  
G. Machova, 351  
J. Macías-Vidal, 309, 472  
A. M. Mackenzie, 132, 443  
K. Macklin, 60  
M. Madeddu, 381, 396  
D. Madjdian, 530  
J. Madrid, 136, 217, 222, 340, 344, 403  
B. Maertens, 229  
J. Maertens, 224, 358  
L. Maesela, 529  
T. Mafuna, 155  
S. Magamage, 87  
A. Mahmoud, 350, 350  
M. Maidin, 30, 172  
J. Maina Kagira, 154  
S. Maini, 409  
N. Majó, 5, 38, 107, 144, 158, 429  
M. Makagon, 390  
A. Makahlehe, 40  
B. Mankanjuola, 14  
J. Malchow, 144, 201  
J. A. Maldonado, 47, 278, 283  
E. Malematja, 519, 334  
J. J. Mallo, 364, 488  
Y. J. Manaig, 461  
G. Manca, 37  
G. Manfreda, 212  
A. Mannelli, 159  
A. Manokaran, 376  
S. C. Mansbridge, 132, 443  
W. Mansilla, 197, 324, 333  
T. Mantzios, 50, 189, 230  
A. Manzanares, 186, 187, 294  
G. Marappan, 207  
L. Marchal, 329, 480  
L. Marchetti, 121  
A. Marco, 63  
A. Marco-Fuertes, 182, 243, 303  
F. Marco-Jiménez, 303  
A. Marcos Fuertes, 161  
C. Marecaille, 415  
S. P. Marelli, 381, 396, 450
- M. Maresca, 495  
J. Marguerie, 389,  
D. Maric, 257, 262  
M. Marien, 498, 499  
C. Marin, 161, 182, 243, 303  
S. Mariotti, 169  
L. Martelli, 37  
Í. Martín, 380  
S. Martinez, 222, 340  
J. C. Martínez, 49  
M. Martínez, 216  
L. Martínez Martínez, 386  
S. Martinez-Miró, 136, 217, 344, 403  
A. Martínez-Talaván, 371  
G. Martini, 159  
B. Martin-Maldonado, 243  
R. Martin-Venegas, 412  
N. Marušić Radocic, 308  
S. Marzorati, 248  
B. Masy'ud, 521  
C. C. Matache, 311, 345, 444  
A. Mathiaud, 71  
G. Mathis, 456  
C. Matias, 479  
O. Matika, 30  
F. Matté, 296, 366, 367  
S. Mattioli, 239  
B. Matton, 336  
M. Maudoux, 496  
M. Mauric Maljkovic, 81  
A. Maxey, 332  
M. Mazur-Kuśnir, 467  
M. Mazzucato, 37  
C. Mbajirgu, 155  
H. McCormack, 30, 102, 170, 172  
M. J. McGrew, 387  
A. McKechnie, 323  
S. McPhee, 94  
B. Méda, 134  
P. Medel, 123  
H. Medic, 308  
L. Medina, 209  
M. Mehri, 57  
M. Meijer, 16, 52, 68, 83  
H. Mekuiko Watsop, 343  
S. Melis, 202, 205  
A. Mellouk, 495  
J. Melo, 501  
N. Mème, 129, 134  
L. Menchetti, 90  
A. Méndez Albores, 300  
O. Mendoza, 315

- F. Menegon, 171  
D. Menoyo, 47, 278, 283  
A. Mens, 95  
Y. Mercier, 74, 339  
N. Merino, 359  
P. Mermillod, 104  
G. Mescolini, 433  
A. H. Metwalli, 209  
A. Meuter, 268, 506  
Á. Mezòlaki, 326  
D. Mezoszentgyörgy, 402, 404  
L. Miccio, 43  
V. Michel, 201, 495  
D. Michels, 122  
S. A. Mignacca, 112  
S. Mignon-Grasteau, 55 98,  
238, 239, 310, 385  
J. Miguel, 322  
D. Mihaela, 462  
B. Milagres, 296, 366, 367  
D. Militello, 251, 252  
M. Mimon, 31  
A. Minetto, 461  
C. Mingala, 41  
B. Miniscalco, 203  
S. Mioletti, 203  
S. Mishra, 163  
V. K. Mishra, 272  
N. Mishra, 163  
P. Mitsch, 91  
S. Miyumo, 99, 383  
E. Mocé, 371  
M. L. Mocé, 371  
B. Mocholi Herrero, 511  
B. Mohamed Bawish, 421  
J. Mohan, 207  
M. Mohl, 228  
A. Molee, 382, 413, 414  
W. Molee, 382, 413, 414  
R. Molenaar, 64  
F. Molist, 75  
J. Molist Badiola, 510, 511  
M. Molnár, 387  
Z. Molnár, 374  
B. C. Mondal, 271  
A. Montalban, 222, 340  
A. Montalbàn, 217  
A. Montalbán Pérez, 344, 403  
C. Montalcini, 204  
R. Montanhini Neto, 277  
A. Monteiro, 461  
V. Montoro, 525  
L. Montoro Dasi, 161, 182,  
243, 303  
R. Moore, 390  
P. Moquet, 215, 337  
H. Morales, 111  
R. Morató Molet, 510  
T. Moreau, 55, 406  
L. Moreira, 191, 515  
A. Moreno, 5, 158  
J. A. Moreno, 209,  
L. Morgans, 144, 181  
J. Moriarty, 112  
D. Mörlein, 313  
C. Morris, 60  
V. Moset, 338  
A. F. Moss, 470  
A. Mott, 313  
A. C. Mott, 105  
K. Mountzouris, 46, 192, 273  
G. Moyano, 280  
J. Mu, 41  
W. Mueller, 379  
D. Mueller-Doblies, 299  
C. Mugnai, 127, 203  
W. Muir, 54  
W. I. Muir, 59  
N.D. Mulder, 26  
J. Muñoz, 100, 380  
M. Muñoz-Lapeira, 128, 130  
P. Münster, 173  
R. Murphy, 293  
E. R. Mussari, 528  
N. Mut-Salud, 395  
K. Mutucumarana, 87
- N**  
A. L. N, 392  
E. N'guetta, 508  
G. H. Nacamura Da Silva, 315  
V. Naccache, 116  
S. Nagesh, 312, 473  
N. Nagy, 387  
P. K. Naik, 163  
G. Nakazato, 366  
G. Nalesso, 37, 171  
A. Narcy, 98, 149, 208, 385  
D. Narinc, 373  
K. Nasr Eldin Fahmy, 421  
M. D. J. Nava Ramírez, 300  
M. Navarro, 16  
A.M. Neeteson, 10  
N. Nemukondeni, 155,  
R. Neto, 40, 361  
D. Neves, 60  
T. Nevitt, 505,
- M. Newcomb, 79  
L. Nicoleta, 462  
J. Niczyporuk, 304  
A. Niederberger, 200  
P. Niewind, 140, 256, 260  
H. Nijs, 177  
S. Niknafs, 52, 68, 83  
I. Nikonov, 293  
T. Nkukwana, 323  
S. Nodland, 487  
M. Nofrarias, 186, 294, 5, 38,  
185, 187  
S. Nonnis, 381  
M. Noor, 302  
T. Norton, 3  
N. Nourin Lisa, 319  
S. Novoa, 100, 380  
P. Nowak, 379  
A. Nozeran, 486  
J. Numata, 17  
S. Nurisso, 127  
Y. Nys, 55, 310
- O**  
C. Obsil, 91  
A. Oladeinde, 76, 325  
C. Oliveri, 212  
O. Olukosi, 74, 76, 325, 328,  
330  
I. Oluseyifunmi, 330  
S. Omerovic, 112  
J. S. Ong, 172  
N. Ongena, 430  
A. Orbán, 318, 402  
A. Orea, 289  
L. Orellana, 60  
J. Orengo Femenía, 386  
A. Orozco, 124, 125  
L. Ozella, 203
- P**  
V. Padmini, 108  
C. Paes, 149  
E. Pagan, 359  
R. Pagan, 359  
M. Pagès Bosch, 510  
E. Pagot, 188, 503  
M. Pajic, 50, 267, 362, 509  
L. Pál, 326  
K. Palanisamy, 108, 275  
S. E. Palao, 65



- N. Pálincás-Bodzsár, 520  
M. Palomar, 124, 125  
S. Palussiere, 208  
T. Panaite, 345  
T. D. Panaite, 311, 444  
M. Pandur, 372  
A. Panin, 293  
T. Panisello Monjo, 236  
I. Panitsidis, 50  
L. A. Pantoja Millas, 43, 113, 430, 431, 510, 511  
T. Pap, 374, 436  
G. Papadopoulos, 50, 189, 230  
K. Papageorgiou, 189  
T. Paparounis, 433  
T. Papazyan, 293  
V. Paraskeuas, 46, 192, 273  
H. Parnas, 63  
L. Parro, 135, 412, 454  
M. Parsons, 237  
J. J. Pascual, 309, 338, 472  
G. Pasquali, 478  
J. J. Pastor, 283  
P. Patel, 264  
I. Patounas, 181  
A. Patsias, 50, 181  
N. Paul, 223  
P. Paulet, 110  
S. Pauling, 57, 254  
H. Pawar, 409  
S. Pedrazzoli, 433, 434  
K. Pedrosa, 194  
R. Pelle, 154  
W. Pelletier, 471  
W. Pellikaan, 335  
R. Penha, 110  
A. Pennings, 62, 66  
A. Pereira, 512  
E. Pereira Da Silva, 315  
M. Perello, 290  
J. A. Pérez, 53, 126  
J. F. Pérez, 213, 493  
M. Pérez, 5, 38, 5  
A. Pérez De Rozas, 187, 298  
A. M. Pérez-Vendrell, 481  
L. Peric, 257, 262, 394, 441  
S. Peris, 352  
A. Perlas, 5  
A. Perlas-Puentes, 38  
V. Perricone, 121  
A. Pescatore, 120  
M. Petelle, 164, 204  
B. Peter, 179  
J. Petit, 288  
E. Petkov, 388  
L. Petò, 318, 402, 404  
S. Petow, 266  
M. Petracci, 32, 90, 106, 131, 194, 255, 445, 447, 448, 449, 452, 453  
N. Petrick, 210, 234  
E. Petridou, 189, 230  
D. Petzoldt, 112  
M. Peyre, 290  
T. Pézier, 211  
A. Philatha, 483  
V. Pimphachanhvongsod, 382  
T. Phonxaiya, 382  
C. Piantoni, 121  
R. Pieper, 17  
M. Pikkemaat, 304  
M. Pinard Van Der Laan, 143  
C. Pineda Vadillo, 527  
S. Pinto, 501  
L. Piquer, 216  
O. Piquer, 124  
V. Pirgozliev, 132, 443, 491  
R. Piró, 391  
R. Piscitelli, 255  
M. Pizarro, 65, 100  
P. Plumstead, 492  
H. Pluschke, 531  
P. Pola, 171  
F. Poletto, 426  
K. Poltowicz, 86, 379  
K. Pongmanee, 281, 483  
T. Popova, 388  
S. Popowich, 231  
E. Posadas, 322  
S. Potot, 192  
M. Poujol, 195  
D. Powers, 168, 397  
O. Prakash, 271  
R. Prarthana, 422  
W. Prasad, 312  
C. Praud, 129  
M. Pravato, 56, 447  
P. Preesong, 410  
R. Preisinger, 102, 103, 109  
Z. Prentza, 433  
D. Preveraud, 432  
B. Prió, 283  
K. Promkhun, 382  
P. Promvijit, 494  
A. Pross, 209  
T. Prucha, 432  
B. Prunic, 509  
L. Puillet, 385  
A. Pullin, 390  
B. Puppe, 233  
M. Puyalto, 364, 488
- Q**  
G. Quaglia, 433, 434  
J. Quinteros A., 54
- R**  
R. R, 357, 439  
S. Radwan, 88  
L. Raederscheidt, 92, 145  
M. Rafecas, 135, 454  
L. Ragni, 445  
A. A. Rahman, 117  
J. Raj, 50, 362, 365, 484  
M. Raju, 484  
G. Ramirez, 42, 440  
J. Ramirez, 24  
Y. Ramiro, 525  
L. Ramon, 44  
J. Ramos, 380  
S. V. R. Rao, 484  
M. A. Rashid, 319  
F. Raspa, 127  
K. Rassamidatta, 483  
G. Rasschaert, 183  
K. Rassmidatta, 281, 282  
Y. Rather, 523  
L. Rathmann, 524  
L. Rathore, 357, 422, 438, 439  
E. Rauch, 133, 206, 266  
R. Raue, 288  
S. Raurich, 504  
S. Rautenschlein, 109, 116, 162  
L. Ravon, 153, 239  
C. Raybaud, 71, 215, 337  
F. X. Rebordosa Trigueros, 510  
R. Rebucci, 121  
E. T. Reddy, 484  
S. Réhault-Godbert, 11, 55, 406  
M. Reichelt, 64  
C. Reimer, 103  
A. L. Reimers, 96  
M. Reis, 197, 333  
Y. Ren, 41  
V. Reñé, 321  
M. Reverchon, 239  
S. Rey Antón, 386

- M. Rezvani, 419  
S. Rian, 317  
D. A. Riaz, 305, 307  
T. Riaz, 354  
M. Ribeiro Alves Lourenço, 214  
A. B. Riber, 201  
P. Riboulet, 129  
A. Rieder, 136  
A. Riggi, 188, 503  
A. Ritchie Martinez, 5  
S. Riva, 236  
R. C. Rivero, 446, 451  
S. Rizzo, 37  
P. Robert, 385  
R. Roca, 363, 107  
S. Rochana, 521  
M. Roda Tena, 321  
R. Rodbotten, 136  
M. Rodehutsord, 196, 482  
B. Rodenburg, 64, 233  
V. Rodic, 394  
A. Rodiles, 460  
M. Rodrigues Barros, 348  
R. Rodrigues Gomes, 370  
C. Rodríguez, 53, 126  
E. Rodríguez Gallego, 144  
A. Rodríguez Navarro, 33, 124  
I. A. Rodríguez Osuna, 451  
C.Z.Y. Rodriguez-Rubio, 236  
P. Roieski, 367  
D. Roijackers, 160  
F. Rojo, 289  
S. Rojo, 216  
J. Rokade, 264, 473  
J. Romero, 441  
E. Rondel, 188, 503  
P. Rose, 491  
T. Rosenberg, 63  
C. Rosique, 217,  
G. Rossi, 118  
S. Rothstein, 193  
X. Roulleau, 415  
E. Roura, 16, 52, 68, 83  
I. Rozenboim, 63  
E. Rožmanec, 424  
Y. Ruangpanit, 281, 282, 410, 483  
A. Ruano, 182  
J. Rubio, 111, 298  
I. Rubio-Cervantes, 482  
J. Ruckebusch, 228  
E. Ruiz Herrero, 222, 340, 344, 403  
D. RuwandEEPika, 87  
Z. Ružic, 267  
A. Rybicka, 123  
K. Rybski, 51
- S**  
J. K. S., 392  
P. Sacakli, 471  
A. Sacy, 285, 456, 457, 458, 460, 486  
W. Saeton, 281  
M. Safwat, 355  
L. Sahoo, 272  
S. Saka, 100, 380  
B. Sake, 263  
S. Sakib, 276  
P. Sakkas, 346  
C. Sala, 212  
R. Sala, 124, 135, 420  
S. Salami, 505  
B. Saldaña, 253, 315, 421  
K. Saleem, 354  
K. Saleemi, 437  
M. Salewski, 17  
J. M. Salgado, 338  
C. Salomé, 238  
C. J. Sanchez, 222, 340  
J. Sanchez, 253, 315, 421  
E. Sánchez, 322  
A. Sanchez Cano, 242, 525  
C. J. Sánchez Parra, 344, 403  
V. Sandilands, 30  
D. Sandvang, 506  
J. Sansano, 243  
E. Santolini, 255  
L. Santos, 367  
R. Santos, 75, 156  
S. Santos, 61  
R. Sanz García, 469  
J. Sarabia, 65, 110, 289  
S. Saraswathy, 324  
B. Saremi, 180, 336  
M. S. K. Sarker, 319  
M. Sarker, 276  
J. Sasipreeyajan, 113, 431  
A. Saurina, 58  
S. Savvidou, 50  
A. Sawicka-Durkalec, 360  
V. K. Saxena, 295  
S. Schäfers, 258  
A. Schattner, 314  
C. C. Schebor, 451  
W. Schelstraete, 498, 499  
A. Schiavone, 67, 127, 136, 217, 320, 344, 403  
A. Schlagheck, 193, 292  
S. Schleibinger, 22  
H. Schliessnig, 91  
M. W. Schmid, 49  
M. Schmutz, 30, 102, 383  
J. Schoenebeck, 30  
D. Scholey, 122, 332  
C. Schouler, 153  
L. Schrader, 201  
R. Schreiter, 240, 398, 524  
J. Schulz, 263  
B. Schusser, 22  
A. Schwarz, 261, 265  
C. Schwarz, 49  
L. Schwarz, 105, 313  
K. Schwean-Lardner, 94, 97, 237  
F. Scolamacchia, 37  
H. Scott-Cook, 132, 443  
E. Sebastià Pujol, 510  
N. Sebola, 334, 519  
A. Sedeek, 355  
A. Sedeik, 297  
A. Seemacharoensri, 410  
V. Segers, 166  
P. Segobola, 145, 502, 512  
A. Seguino, 528  
M. Segura-Wang, 504  
P. Selle, 480  
R. Selvaraj, 74, 76, 325,  
A. Seon, 331  
A. A. Séon Simon, 145, 314  
A. Serraino, 528  
L. Sesti, 110  
S. Sevilla-Navarro, 28, 82, 226  
F. Sevillano, 47, 278, 283  
Y. Sha, 41  
M. Shakeri, 442  
R. Shanmugasundaram, 76, 325  
A. R. Sharifi, 105  
A. Sharma, 247, 271  
R. K. Sharma, 271, 247  
F. Sharmin, 276, 319  
A. Sharp, 112  
Y. Shastak, 471  
M. Shazaib Ramay, 471  
S. Shigut, 241  
A. Shinde, 523, 526  
P. K. Shukla, 48, 190  
T. Shynkaruk, 94, 237  
H. Sid, 22

- A. Sieczkowska, 360  
W. Siegert, 196  
J. M. Siegford, 167  
H. Simianer, 103  
A. Simongiovanni, 71, 337  
A. K. Singh, 376  
B. Singh, 271  
K. Singh, 488  
M. Singh, 272  
P. Singh, 523  
V. Singh, 272  
D. Singhare, 409  
P. Sinpru, 382  
J. Sirovnik, 91  
F. Sirri, 175, 194, 212, 255, 452, 453  
L. Sisquella, 298  
M. Siwek, 86  
K. Skiba, 256, 260  
E. Skotarczak, 379  
J. Skov, 227  
S. Sky, 264  
P. Slama, 368  
B. Slavec, 424  
N. Sleenckx, 93, 166, 177, 198, 405  
K. Slizewska, 467  
N. Smeets, 224, 358  
M. Smialek, 356  
P. Smid, 418  
A. Smith, 219, 474, 479, 481  
J. Snijders, 62, 66  
H. Snoek, 530  
G. Soares, 296  
R. Soares, 289  
L. Socas, 111  
D. Soglia, 67, 127  
F. Soglia, 90, 106, 131, 445, 448, 449, 452, 453  
B. Sohnrey, 105  
C. Sol, 45  
X. Soldevila, 221, 321  
M. Solé Berga, 43, 301, 430, 511  
J. Soler, 321  
M. D. Soler, 124, 125, 420  
B. Solís Cruz, 300  
F. Somers, 416, 417  
S. Sonal, 295  
N. Sonale, 264  
J. M. Soriano, 82, 226  
N. Sosa, 446, 451  
E. Sossidou, 181  
D. Sousa, 338  
M. Souza, 366  
L. Souza Olegario, 18  
R. Spaepen, 122  
S. Sparesato, 171  
B. Spindler, 92, 256, 260, 261, 265  
S. Spiridonovic, 257, 262, 441, 509  
A. Sriprad, 494  
P. Srivastav, 438  
V. Stanev, 270  
L. Star, 70, 466  
N. Stark, 227  
M. Stasinopoulou, 514  
C. Stefanello, 251, 252, 469, 479  
F. Steinhoff, 146  
W. Stertenbrink, 116  
P. Stevanato, 77, 150  
T. Stoeger, 41  
L. Stokvis, 339, 466  
N. Stoppani, 67, 127  
A. Stratmann, 167  
J. H. Stringhini, 370  
S. Struthers, 30, 102  
J. Stuff, 400  
I. Stylianaki, 50  
I. Subhasinghe, 231  
N. Such, 326  
M. Sugni, 248  
K. Sukkasem, 281  
N. Sultana, 276  
S. M. R. Sumon, 411  
C. M. Sun, 103  
Y. Sun, 384  
S. Suradker, 409  
C. Sürrie, 109  
D. S. Suryawanshi, 488  
Z. Süto', 318, 402, 404  
B. Svihus, 6, 146, 219, 220  
B. K. Swain, 163  
H. Swam, 288  
A. Swat, 379  
H. Swatson, 529  
B. Syed, 502  
R. Szabó, 374  
Z. Szabó, 372, 520  
S. Szász, 318, 402, 404  
D. Szkopek, 51  
Z. Szöke, 374  
N. Sztán, 387, 520  
T. Szwaczkowski, 379
- T**  
E. Tabanera, 100  
F. Tabassum, 319  
G. Tacken, 530  
G. B. Tactacan, 410  
F. Tahamtani, 152  
R. Takumbo Takam, 320  
G. Talegón, 23, 72  
E. Talsma, 530  
A. Tangomo Ngnintedem, 154  
Z. Tariq, 354  
J. Tarradas Font, 144  
K. Tarrant, 259  
J. Tarres, 428  
F. Tarrés-Freixas, 5  
B. Tas, 505  
P. Tassinari, 255  
C. Tassinato, 37  
A. Tatar, 284  
J. Taylor, 74  
G. Tedeschi, 381  
J. H. Tee, 516  
R. Tellez, 191  
G. Téllez Isaías, 300  
I. P. Teodósio Da Nóbrega, 474  
J. Tetens, 102, 105, 223, 313  
B. Thesing, 176  
P. Thobe, 140, 531  
J. M. Thoby, 145  
M. Thoeleke, 116  
I. Thøfner, 152  
K. Thumanu, 382  
I. Tiemann, 105, 201, 260, 313, 399, 400  
S. Tikoo, 231  
A. K. Tiwari, 376  
B. Tobalske, 168, 397  
D. Todorovic, 509  
Y. Togami, 133, 206, 266  
M. Toghyani, 54, 480  
C. Tognoli, 381, 396, 450  
A. L. Toledo, 253  
S. Tomar, 376  
D. Tomas, 55  
C. Tomás, 371  
G. Tomczyk, 360  
W. S. Tong, 517  
K. Toppel, 96, 176, 372, 520  
D. Torreggiani, 255  
A. Torres, 53, 126, 241, 522  
C. Torres, 60, 347, 348, 492  
Í. Torres, 363  
J. Torres-Boncompte, 82, 226

- A. B. Toscan, 251, 252  
M. J. Toscano, 14, 165, 167, 164, 200, 204  
M. Tóth, 436  
A. Travel, 468  
A. Tres, 135, 412, 420, 454  
C. Trespeuch, 134  
G. Trevisani, 159  
M. Tristan, 44  
A. Trocino, 56, 77, 150, 171, 232, 447  
E. Tseren-Ochir, 41  
B. Tshingilane, 323  
V. Tsiouris, 50, 189, 230  
A. Tsitsos, 189  
C. M. Tucciarone, 43, 426  
C. M. Tung, 461  
G. Turgay Izzetoglu, 369  
F. Tuytens, 93, 166, 405  
J. Tyagi, 473  
J. S. Tyagii, 207  
N. Tyler, 408  
B. Tzschentke, 234
- U**  
M. Ubieto, 114, 423  
M. Ulfah, 521  
S. Ullah, 354  
M. Umar Faruk, 192, 314, 331, 476  
Z. Uni, 29  
A. E. Untea, 463  
V. Upmanyu, 295  
R. Urbani, 171
- V**  
M. J. Valdez-May, 38  
L. Valenzuela, 228  
R. Valle, 5, 38  
A. Vallecillos Quijada, 386  
M. Van Asseldonk, 530  
J. Van Baal, 143  
T. van de Braak, 9  
I. Van Den Anker, 64  
J. Van Den Borne, 335,  
H. Van Den Brand, 52, 64 83  
N. Van Den Broeck, 80, 177, 178, 179, 198, 250  
M. Van Den Nieuwenhof, 149  
L. Van Den Tweel, 62  
S. van der Beek, 24  
J. Van Der Eijk, 202, 205  
M. Van Der Eycken, 183  
B. Van Der Hee, 5  
S. Van Der Klein, 459  
C. Van Der Pol, 62, 66  
W. Van Der Veken, 485 513  
E. Van Donselaar, 418  
L. Van Eck, 69, 79  
T. Van Gerwe, 108, 275  
V. Van Hamme, 361  
T. Van Hattum, 202  
T. Van Hecke, 32  
F. Van Immerseel, 183  
S. Van Kuijk, 418, 419  
H. Van Meirhaeghe, 166  
N. Van Noten, 93, 214 405  
J. Van Raemdonck, 183  
N. Van Staaveren, 168 199, 397  
L. Vandenberghe, 505  
P. Vanderghinste, 218  
S. Vanhooydonck, 179  
E. Várkonyi, 387  
G. Vasconcellos, 251, 252  
G. Vasdal, 152  
M. Vasiljevic, 50, 362, 365, 484  
S. Vaswani, 48, 190  
A. Vázquez Durán, 300, 322  
S. Vega, 243  
B. Vegi, 374, 520, 372, 387  
S. Veluri, 76  
K. Venkatasubramanian, 358  
S. Ventanas, 18  
K. Venter, 477, 492  
M. Verdú Piquer, 221, 321  
M. Vereecken, 498, 499  
J. Vergara-Alert, 5  
M. Verhaagh, 140  
S. Verkempinck, 122  
S. Vermaut, 123  
K. Vermeulen, 122, 508  
N. Versillé, 287  
J. Vervloesem, 88  
G. Vestergaard, 268  
S. Vichi, 135, 454  
S. Vidakovic Knežević, 267  
E. Vidal, 429  
E. Vidal Hernandez, 419  
N. Vieco-Saiz, 84, 495 507, 410, 469  
H.K. Vikkula, 22  
B. Vilà, 186, 187, 294  
A. Villa, 290  
E. Villalbí, 44, 107  
G. Villalobos, 45  
J. Villora, 53, 126  
T. Vingerhoets, 335  
E. Vinyeta, 156  
C. Visscher, 249  
L. Vissers, 139  
A. Vitorino Carvalho, 104  
P. A. Vlaicu, 463  
W. Vogt-Kaute, 399  
N. Volkmann, 256, 263  
F. Volpe, 255  
T. von Heyl, 22  
K. Von Zuben Augusto, 191  
S. Vossler, 261, 265  
R. K. G. Vpps, 376  
L. Vrhovec, 308  
J. Vuorenmaa, 229
- W**  
C. Walk, 479  
M. Wang, 41  
Q. Wang, 329  
S. Wang, 331, 41  
L. Warin, 90  
C. Wasike, 99  
M. Watson, 85  
A. Watteyn, 93, 166, 405  
B. Waweru, 154  
A. Wealleans, 508, 122  
T. Wei, 490  
J. Weidemann, 256  
S. Weigend, 103, 105, 399  
R. Weiss, 63  
C. Wekere, 343  
J. Wells, 5  
K. Weyermair, 91  
T. Widowski, 168, 397  
A. Wiedemann, 211  
J. Wijnen, 62, 66  
H. Williams, 489  
N. Willson, 327  
K. Wilshaw, 489  
P. Wilson, 172, 269, 30, 102, 170  
R. N. Wishna Kadawarage, 86  
A. Wojciechowski, 418  
L. Wolf, 524  
S. Wolfrum, 482  
W. Won, 180  
M. Woodward, 430, 431  
S. Wu, 411

**X**

G. Xiccato, 56, 77, 150, 171,  
232, 447

**Y**

A. Yadav, 393  
S. Yalcin, 369  
W. Yan, 41  
T. Yata, 413, 414  
M. Yerpes, 391  
R. Yin, 41  
Y. Yitzhaky, 235  
A. You, 427  
A. Yousaf, 305  
H. Youssef, 297  
D. Yu, 347  
A. Yuste, 364  
F. Yusuf, 505

**Z**

A. Zaghi, 212  
I. Zaheer, 437  
M. Zain El-Abideen, 355  
S. Zakariab, 40  
V. Zambotto, 67, 127, 136  
M. Zampiga, 175, 212, 255,  
452, 453  
L. Zaniboni, 381, 396, 450  
M. Zappaterra, 131  
M. Zaragozí, 391  
G. Zardinoni, 77, 150  
M. Zeeshan Akram, 353  
L. Zeibich, 292  
J. Zemzmi, 221  
N. G. Zeni, 450  
C. Zenner, 22

E. Zergani, 105, 313  
Q. Zhang, 474, 479, 481  
X. Zhao, 378  
Y. Zhao, 384  
Q. Zhou, 347  
J. Zhu, 347  
H. Zhuang, 442  
D. Žikic, 257, 262  
M. A. Zinnah, 117  
M. Živkov-Baloš, 267  
Z. Žlabravec, 424  
V. Zmrhal, 368  
G. Zock, 76, 325  
C. Zomeño, 128, 130  
Y. Zong, 384  
O. Zorman Rojs, 424  
M. Zuidhof, 70



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## PLENARY SESSIONS

Ordered chronologically by Slot (Time of presentation)



## **How can Precision Livestock Farming help improve Sustainable Poultry Production?**

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Farmed poultry is an animal category that represents 70% of all birds on the planet. The demand for poultry-derived proteins will continue growing with income countries, while protein consumption, in general, will become more strongly linked with the sustainability of the livestock sector. Recent citizen-driven initiatives such as End the Cage Age or the Better Chicken Commitment indicate how Western society values the lives of farm animals. Furthermore, increasing the welfare standards will have a positive influence on the welfare standards in lower-income countries. Consequently, there is a growing need for better technologies to support farmers in managing environmental emissions, animal feeding, health, and welfare. Precision Farming (PF) is a field where the aim is to exploit Information and Communications Technologies (ICTs) to facilitate high-resolution planning and management of farming operations. PF has seen great success in the crop production sectors, where it can be used to maximize the spatial-temporal productivity of the crop within certain environmental constraints. However, the application of precision farming to livestock production, i.e. precision livestock farming (PLF), has been more challenging to implement with the same level of success. As a result, while there are various PLF technologies under research for poultry health, welfare, and productivity monitoring and management, the translation to functioning robust farm technologies that capture and act on information is still taking time. Part of the problem is the difficulty in demonstrating the value for the farmer while creating better living standards for the animal. Answers are still needed as to how PLF technology can help the sector become more sustainable while at the same time giving farmers a livelihood worth pursuing. This talk will look to the opportunities provided by PLF for the poultry sector, the recent state of the art as well as some pitfalls to consider when translating research into functioning solutions.

**Keywords:** Precision livestock farming, poultry production, health and welfare



## **A holistic and pragmatic view of sustainable development pathways in poultry production**

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Sustainability should be the primary objective of agricultural production. Sustainable poultry development is achieved via three pathways: the environment, in terms of demand for scarce resources and environmental degradation; the ethical and welfare aspects of both the animals being produced and the humans consuming animal products; and the economic robustness of the entire supply chain. Often, advances in one aspect of sustainability negatively impact the others. All aspects of the supply chain need to be considered. This includes the production of feed ingredients and feed and manufacture, poultry production and processing and the marketing and distribution of products. True sustainability will only be achieved in systems not impacted by human activity. Continuous improvement in all aspects of sustainability will be essential as we shift to more sustainable poultry production. All role players, producers and consumers, must know that sustainability is multifactorial. Rather than focusing on a single aspect, a holistic, pragmatic approach is required to drive sustainable development pathways in poultry production.

Keywords: environment; ethics; economics; holistic; pragmatic

## **Chicken-derived airway organoids: a model to study viral dynamics for infectious bronchitis, Newcastle disease, and avian influenza viruses**

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Organoids are becoming an increasingly popular model to study the pathophysiology of many diseases. They are complex 3D cultures that recapitulate the architecture of the tissue of origin, and hence they can be used to study viral dynamics at the cellular level. Despite being extensively used in the biomedical field, fewer efforts have been invested in the development of animal organoids, and even less for avian species. Here, we report the development of organoids from the lung of adult chickens. We demonstrate that our models are composed of different cell lineages present in the tissue of origin (i.e., basal cells, ciliated cells, and goblet cells) and that they maintain their functionality by means of mucin-secretion and ciliated movement. These organoids can sustain long-term passaging and can be cultured for up to a month using complex techniques such as air-liquid interface (ALI) systems that display an apical-basal polarisation. Furthermore, we tested their susceptibility against three respiratory viruses of importance in the poultry industry: i) a highly pathogenic avian influenza virus (HPAIV) H5N8 strain; ii) a Newcastle disease virus (NDV) strain (Herts 33/56); and iii) an infectious bronchitis virus (IBV) strain (Massachusetts 41). Upon infecting ALI cultures on the apical side with an MOI=0.01, we observed histopathological changes at 48 hours post-infection (hpi) such as deciliation and intercellular oedema in H5N8- and IBV-infected organoids and scattered apoptotic cells in NDV-infected organoids. Trans-epithelial electrical resistance measurements demonstrated no disruption of the epithelial barrier at 48 hpi. Finally, all three viruses were detected by qPCR on the apical side of the membrane, with lower CTs at 48 hpi than 24 hpi, while only NDV and H5N8 were also detected on the basal membrane. These results match with NDV and H5N8 HPAIV in vivo capability to rapidly cause systemic dissemination and viraemia, whereas IBV pre-eminently affects the respiratory tract. Overall, these results highlight the importance of avian organoids to study viral tropism, cytopathogenicity, and host-pathogen interactions. Moreover, they can be further developed to screen for antiviral treatments or to test vaccine efficacy.

**Keywords:** lung; organoids; ALI culture; viral dynamics; IBV; NDV; H5N8; HPAIV; influenza.

## **Optimizing poultry production through feed technology: It's all about structure**

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Probably, no animal is fed a diet so perfectly matched to its nutrient requirement as poultry. Progress in optimization of the physical structure of poultry diets, however, has not improved similarly. Reduction in particle size through grinding was adapted early, and were soon followed by the realization that the resulting mash should be pelleted. Unfortunately, these developments were to a large extent pragmatic practical adaptations which were not supported by research to the same extent as nutrition. The improvement in feed intake, at least for broilers, when diets were pelleted, is due to the peculiar feed intake pattern of poultry, where particles are grasped one by one in a series of rapid pecks. Thus, as particle size increases, the feed intake per time unit increases. In broilers, this is important, since the willingness to increase the percentage time feeding is limited, despite using less than 10 % of the time feeding when given pellets. Although macrostructure standards for size of pellets and the maximum amount of fines have been established, they are not solidly supported by targeted research and have been challenged recently. While it has been recommended to limit the amount of particles > 3 mm to 15 % for broilers in the starter phase, recent experiments have shown that birds willingly eat particles > 4 mm even during the first week of age. Similarly, while it has been recommended to not exceed a diameter of 4 mm in grower diets, feeding a 5 mm diameter pellets did not negatively affect performance when fed from 10 days of age. Even increasing the length from 6 to 10 mm did not deter the birds from maximizing the feed intake. In fact, particle selection data even indicated that the birds preferred these large particles at later stages of growth. The tolerance to fines also seems to be higher than currently assumed. While recommendations are that the amount of particles < 1 mm should be less than 10 %, it has recently been shown that the amount of fines can increase to 20-30 % without negatively affecting feed intake. As these results with broilers indicate, birds appear to be much more robust to changes in macrostructure than currently assumed. Thus, more research is needed to optimize macrostructure, including the pellet size and the amount of fines in the diet for minimized processing costs and optimized broiler growth.

Keywords: Processing; particle size; weight gain, pelleting, fines

## **Nourished mind: Egg Nutrients and Cognitive Function**

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The study of the relationship between nutrition and cognitive function is an area that has aroused great interest in recent years. In this regard, eggs are a very nutritionally complete food, providing high-quality proteins, fats mainly unsaturated, and highly bioavailable vitamins and minerals. These nutrients play a fundamental role in the maintenance and functioning of the nervous system and cognitive function. For example, egg proteins are especially rich in tryptophan. This amino acid is necessary for the synthesis of serotonin, which is a neurotransmitter related to appetite, emotions and mood; and melatonin, which regulates sleep/wake cycles. Eggs are also one of the best choline sources, which is need for the synthesis of the neurotransmitter acetylcholine, involved in memory and learning, and synthesis of phosphatidylcholine, which is the main phospholipid of cell membranes. In addition, choline participates in methionine metabolism and in 1-C metabolism, especially when the diet is insufficient in folates. Eggs also provide the diet with a significant amount of vitamins such as B12 or D, which are involved in brain function. And they are a good source of other bioactive compounds such as lutein and zeaxanthin, which have antioxidant and anti-inflammatory activity, and whose intake has been linked to improved cognitive function. Finally, some proteins with immunomodulatory and neuroprotective activities have recently been identified in eggs, participating in neuron's activity and survival, inhibiting neurodegeneration processes. Prospective cohort studies suggest that moderate egg intake (between 2 and 6 eggs/week) has a neutral effect or an inverse association with the risk of cognitive impairment or mortality from neurodegenerative diseases, including Alzheimer's Disease. However, more longer-term and well--designed studies are needed to examine the effect of egg consumption and changes in cognitive function. In conclusion, eggs contain nutrients of great interest to maintain cognitive function and prevent neurological deterioration at all stages of life. Its moderate consumption, in the context of a balanced diet, is associated with benefits for health and cognitive function.

Keywords: Eggs; Nutrition; Cognitive function

## **All Chickens Fast and Slow- assessing the “embryonic developmental tempo” phenotype of poultry breeds**

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Embryonic development is a process of orchestrated progressive steps, which lead to the generation of patterned structures. While the sequence of developmental events is highly conserved, the speed at which they occur or “tempo” is a unique feature of a species and is highly variable. Embryonic tempo is a relatively unexplored field presenting an opportunity to investigate and develop a fundamental area of biology with far reaching implications for many fields, including selective breeding. But what does "embryonic time" look like and how can it be measured? In the study of developmental tempo, we search for the development of identical physical phenotypes over different time functions between individual embryos. The avian embryo, developing outside the mother, in a stereotypical way that varies little between individuals, is an excellent model. With advances in sequencing, genome engineering, advances in high resolution live cell microscopy and machine learning, it is now possible to explore the question of tempo. My lab is currently developing this field, first by characterising the differences in embryonic tempo between avian species (quail, chicken) as well as using live embryo microscopy of transgenic chicken embryos over days to establish a high resolution developmental time-line. Alterations in the rate of embryonic development and patterning have been observed in breeds of chickens selected for divergent size (LWS/HWS) and in my laboratory we have noted differences in the rate of patterning between Bantam and Broiler chickens. Using our system to define and study the development of ‘Fast and ‘Slow’ chickens breeds may provide a unique insight into the genetics of embryonic tempo as well as associated selectable phenotypes of developmental tempo, such a time to hatch.

Keywords: tempo, embryo, development

**Breeding for new requirements and future production systems. Laying hens****T. van de Braak<sup>1</sup>**<sup>1</sup>Hendrix Genetics, Boxmeer, Netherlands*Presenting author: Teun.van.de.Braak@hendrix-genetics.com*

In the dynamic landscape of poultry breeding, the integration of high-tech innovations in animal husbandry poses challenges and opportunities. Focused on enhancing animal genetics and poultry breeding, breeding companies navigate complexities arising from farm environments, varying scales, and unpredictable animal behavior. Collaboration and open innovation extend beyond traditional approaches, enabling the adoption of unconventional techniques. Machine vision emerges as a transformative tool in laying hen breeding, automating egg grading and ensuring consistent, objective evaluations. This innovation minimizes human error and subjectivity, facilitating more accurate data on external egg phenotypes. Additionally, Magnetic Resonance Imaging (MRI) is introduced, providing non-destructive insights under the eggshell. Despite initial high costs, affordable AI-driven MRI tools are developed for hatcheries, enabling fertility checks and gender identification without egg breakage. Pioneering research projects like IMAGEN and HenTrack demonstrate the integration of sensing, AI, and breeding to address challenges in transitioning to sustainable livestock production. IMAGEN focuses on understanding animal behavior in welfare-friendly systems, while HenTrack employs innovative tracking for comprehensive observations in commercial facilities. In this era of evolving societal demands and environmental considerations, the adoption of new traits and technologies underscores the essential role of genetic variation in maintaining viable poultry populations. The balance in breeding and selection practices remains pivotal for meeting the needs of a rapidly changing society.

**Keywords:** Poultry; genetic selection; phenotypes; laying hens; SMART farming

## **Breeding for new requirements and future production systems. Broilers Breeding for Welfare in Meat Poultry**

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Animal breeding operates at the start of the food supply chain. The resulting crossbreeds disseminate permanent and cumulative genetic change across the production base. We will address breeding for welfare and sustainability as part of balanced breeding, meaning that welfare, production, environmental impact, reproductive fitness and environmental adaptability are improved simultaneously and continuously every generation. Over the last 4-5 decades meat poultry breeding evolved from focusing on production and reproduction, to covering several groups of biological traits: welfare, robustness, health, environmental impact and quality have increasingly larger emphasis in the breeding goal. This was enabled by continuous availability of phenotypes for all traits and development of novel quantitative and genomic analytical tools. Increased computing power allowing management of large amounts of data has leveraged the scope for managing trait antagonisms and increases in prediction accuracy across traits in broad breeding goals. We will provide examples and obtained achievements. Ongoing investments in research and innovation will drive further improvements. The outlook into the future is exciting with more options to better understand the biology of the bird and how it interacts with its environment individually or in a group. Poultry is an affordable, healthy animal protein, with little/no religious or cultural limitations. It is our role to enable a safe and secure supply of healthy birds to help feed a growing global population with a sustainable supply of breeding stock from large diverse gene pools to the wider industry. We support producers with latest management and technical advice to allow optimum expression of genetic potential in production conditions. As part of setting and managing breeding goals we incorporate feedback from a wide range of stakeholders (the market itself, customers and society in general). A concept Aviagen has laid out in its Top 5 Commitments. Poultry is taking up a larger share of current and future animal protein. While it is difficult to predict the exact future or relative representation of each crossbreed type globally or regionally, there will be room for different genotypes including conventional and slow growing. The focus from the breeder will be to offer genetic potential suited to all market segments while fulfilling sustainability requirements from economical, biological, welfare and environmental considerations.

**Keywords:** Balanced breeding, selection, health welfare and sustainability, meat poultry, broiler

## **Sexual dimorphism during egg incubation and development of ovosexing methods**

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One of the main problems facing the poultry industry is the lack of economic value of male chicks from the laying hen production chain, due to their low meat yield and poor quality. Indeed, as the selection of breeding hens is based on productivity and egg quality criteria, the adults from laying strains are animals with poorly developed carcasses and low muscle yields. As a result, while the females are bred to produce table eggs, the males cannot be sold for their meat, which does not comply with European meat sales standards. Prior to the publication of government decrees in certain European countries, male chicks were killed one day after hatching. In France, this issue concerned nearly 50 million male chicks. This awareness which has been widely publicized in recent years and questioned the ethics of laying hens production, has led to the publication of a French decree on February 5, 2022, banning the killing of male chicks from *Gallus gallus* breeds selected for the production of table eggs. Three alternatives have been proposed: 1) raising the brothers of laying hens and identifying new economic markets, 2) evaluating the performances of new genetic crossbreeds between broiler and layer lines, where the females would be raised for their eggs, and the males for their meat (dual-purpose breeds), 3) developing ovosexing tools to detect then eliminate male eggs rather than male chicks. The latter approach has been the most popular, as it possesses many advantages in terms of implementation at the hatchery level and acceptability to consumers, citizens, and professionals. This review will first introduce the socio-economic context associated with the production of egg-laying hens. It will detail a few physiological landmarks related to avian embryology and sexual dimorphism during embryonic development, which will help to better understand the technical bases and constrains for the development of ovosexing methods. The presentation will also give an overview of the recommendations for euthanasia of sorted male eggs that depend on the developmental stage of the embryo, and the management of hatchery by-products. Now that several ovosexing methods are on the market, the next challenge is to improve existing methods or develop new ones so that they are effective at very early stages of development, on all types of egg-laying strains, while remaining affordable for professionals and consumers.

Keywords: ovosexing, male chicks



## **Antibiotic resistance, the big one health deal**

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Antibiotic resistance (AR) represents one of the most pressing public health challenges of the 21st century, transcending the boundaries between humans, animals, and the environment, and being currently the paradigm of One Health. The escalation of resistant pathogens has been precipitated by decades of antibiotic misuse and overuse, coupled with a significant decline in the development of novel antibiotics. Here I will talk about the current knowledge, impacts, strategies, and future directions necessary for the containment and management of antibiotic resistance within the One Health framework. AR poses probably the most important threat to modern medicine, rendering common infections increasingly difficult and sometimes impossible to treat, thereby increasing morbidity, mortality, and healthcare costs. The One Health approach is critical in addressing the multifaceted nature of antibiotic resistance. The environmental dimension of AR cannot be underestimated, as water bodies, soils, and wildlife serve as reservoirs and conduits of resistant bacteria and resistance genes, influenced by agricultural run-offs, inadequate waste management, and pollution. The transmission of AR between environments, animals, and humans highlights the need for stringent regulatory frameworks, improved waste management practices, and global surveillance systems. Policies promoting the judicious use of antibiotics in the poultry sector, also in LMICs, in agriculture, alongside the development of alternative approaches such as vaccines, probiotics, and improved management practices, are crucial. These strategies could reduce the reliance on antibiotics, thereby diminishing the selection pressure for resistance development. Human health impacts are equally severe, as AR compromises the efficacy of antibiotics, making standard treatments less effective and increasing the risk of disease spread, severe illness, and death. The healthcare sector must enhance infection prevention and control measures, stewardship programs to optimize antibiotic use, and invest in research and development of new antibiotics and alternative therapies. Global collaboration and coordination are imperative to tackle AR effectively. This includes strengthening international partnerships to share data, standardize surveillance methods, improve regulatory practices, and foster global and regional policies and frameworks that mitigate risks associated with antibiotic use and resistance. Furthermore, public education and engagement are essential to raise awareness about the responsible use of antibiotics among prescribers, veterinarians, and the public. Behavioral changes, influenced by greater awareness and understanding, can significantly contribute to the reduction of inappropriate antibiotic use. In conclusion, antibiotic resistance is a complex, multifactorial problem that demands a concerted One Health approach to effectively manage and mitigate its impacts. The commitment of all sectors, including social sciences such as anthropology, economics or sociology is crucial for the long-term containment of this growing threat to public health.

Keywords: antimicrobial resistance; One Health; transmission; genomics

## Farm animal welfare assessment and certification

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Traditionally, monitoring systems and legislation largely relied on examination of inputs, ‘what’ or ‘how much’ of different resources are given to animals. These parameters are easy to define, to measure and have a high repeatability. However, these measures are a poor guarantee for good animal welfare, as animals may experience the same situation or handling procedure differently depending on previous experiences. Since welfare is a condition of the individual animal, an assessment should place its emphasis on animal-based measures (also called “outcome” measures). In fact, outcome measures are likely to be the most direct reflection of their actual welfare state. However, the selection of these outputs should be based on three aspects: validity, repeatability, and feasibility, and the development of the protocol for a certification scheme in other two: balance between the different dimensions of animal welfare and a robust training program of assessors. In determining welfare, there are several domains that are generally regarded as areas of concern and of importance for good animal welfare. A good exercise on how to address these different domains was the Welfare Quality architecture, that tried to develop a new way of assessing farm animal welfare that was both scientifically rigorous and reflected broader public concerns. The welfare Quality project developed protocols for different species, including one applied to broiler chicken and one for laying hens that were published in 2009. The protocol for laying hens was reviewed and published as a new version for the Welfare Quality Network in 2019 and contain a number of animal-based measures or outcomes. In recent years and based in the same approach that Welfare Quality, the Animal Welfare Program of IRTA has developed a protocol for meat and laying quails. These new protocols for quail were created within the scope of a certification scheme that was created in 2014 in Spain for the implementation of the Welfare Quality protocols in the real market. The goal was to demonstrate that it was possible to use these animal-based protocols created by researchers for certification purposes and in fact, that it was fairer for the animal than using just resource-based or management-based protocols as has been done historically. In fact, this pilot project that sought to implement the protocols of Welfare Quality became the seal called Welfair® in 2019 (from adding the words welfare + fair).

Keywords: Animal welfare; Handling procedures; Transport; Outcome Measures.

## **Heritability estimates for movement of laying hens within a quasi-commercial aviary barn**

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Cage-free and cage housing for laying hens are very different environments, although the vast majority of phenotyping for breeding takes place using relatively small groups or even individual hens in cages. To provide laying hen genetics that are robust within cage-free housing systems, novel methods that allow for phenotyping within cage-free housing are needed. We have been developing -sensor-based technology to track location of laying hens within a quasi-commercial aviary that can then be related to various health, production, and welfare traits for breeding selection. The objectives of this study were to estimate genetic parameters for duration of time spent at different locations (top, middle, and lower tiers, litter, wintergarden) within an aviary using a multivariate repeatability model and to compare correlations between time spent in these locations. For this study, a total of 4800 white laying hens arrived at our site as hatchlings from crosses of pure lines. Each chick wore a wing tag that indicated the sire and was placed into eight pens with 600 animals each in an on-site rearing barn, stratified for sire with 100 sires in total. Just before transfer to the production barn, 1,124 pullets from 25 of the 100 sires were fitted with a passive Radio Frequency Identification transponder (125 kHz) and a pen-specific color leg band. The allocation of pullets to five pens of 225 birds in the laying barn was stratified for sire and pen in the rearing barn. Within the laying barn, hens had full access to all barn areas. Hens were in the aviary for a total of 290 days and daily records of duration in each area were collected for each hen culminating in a total of 937,740 records. Heritability estimates ranged from 0.05 (0.01) to 0.28 (0.03) where the lowest heritability was time spent at the lower tier while the highest was time spent in the litter. A moderately high negative genetic correlation of -0.59 (0.08) was observed between time spent in the top tier and litter areas, while a positive correlation of 0.37 (0.14) was found between time spent in the lower tier and the winter garden. The findings of this study show that the duration of time spent in different zones within an aviary has a genetic basis and can provide phenotypes for animal selection. Our group is continuing to link relevant behavioral metrics to health and welfare measures that could be used within modern breeding programs for cage-free laying hens.

**Keywords:** phenotype; sensor technology; tracking; breeding; heritability; aviary

## **The microbiome-gut-brain axis in poultry production: what we know and don't know and why it matters**

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The gut is a highly dynamic environment where elements of the neurophysiological, microbiological, immunological and nutritional systems interact. Due to the extensive neurophysiological innervation of the gut, these interactions are directly monitored by the brain often resulting in changes in behaviour. All of these elements, therefore, combine to create the microbiome-gut-brain axis. The critical importance of the microbiome-gut-brain axis in poultry production is only now being fully realised due to the removal of antibiotics from feed. In endeavouring to understand the mechanisms governing such complex interactions, a mechanistic approach has been utilised that employs a common evolutionary-based language that unites all the systems. That common uniting element is a shared, evolutionary-based neurochemistry. Neurochemicals, which are ubiquitous throughout nature, are found not only in feed but also are produced by gut bacteria as well as by immune cells and enteric gut neurons. As part of our ongoing examination of the microbiota-gut-brain axis in poultry, we have utilised a diet-inducing inflammation model that produces a chronic inflammatory response that closely replicates what occurs in the poultry industry resulting in decreased growth performance. Following feeding of a high non-starch polysaccharides (NSP) diet composed of 30% rice bran for 28-days, the temporal relationships between neurochemical composition of the gut, immune status, and growth performance were measured. The NSP diet induced a dramatic increase in the production of norepinephrine (1000's-fold increase) at the same time inducing immune dysregulation leading to a low grade, chronic inflammatory intestinal response. Further, the critical role that neurochemicals play in the development of gut inflammation was demonstrated through the use of a dopamine-producing probiotic that completely abrogated the ability of the NSP diet to induce an increase in norepinephrine that is a hallmark of NSP-diet induced inflammation. In this keynote, I will discuss how neurochemical-based interactions between food, gut bacteria, the gut's immune and enteric nervous systems and the brain influence poultry health disease progression and behaviour. Critically, an understanding of this neurochemical bridge between all the systems provides for the development of new approaches to poultry production that will improve health and behaviour while at the same time lessening disease progression.

**Keywords:** Microbiome-gut-brain axis; nutrition; inflammation; stress; neurophysiology; infectious disease; behaviour

## **Fostering embryonic development in chickens: maternal and in ovo nutritional strategies**

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Up to 5% of hatchlings do not survive the first days post-hatch. The peri-hatching period is critical for the development of the gastrointestinal tract (GIT) and the immune system. From a nutritional perspective this period is determined by the transition in nutrient sources from the endogenous liquid yolk to exogenous solid feed. This review is about novel strategies investigated to overcome the constraints of the post-hatching period including transgenerational nutrition and conditioning, in ovo functional feeding, and the early modulation of the microbiome. The nutritional status of broiler breeders affects the nutrient composition of the egg and ultimately embryonic development. Maternal programming mechanisms play a fundamental role in the early post-hatching hours by conditioning feeding choices and appetite. These transgenerational mechanisms evolved as evolutionary strategies to optimise the survival of the progeny in front of anticipated environmental challenges. For example, the adaptation to shortages of dietary protein and amino acids (AA) are of interest to commercial broiler production where a mismatch of 40% or more in protein content between maternal and offspring diets is common practice. This mismatch may result in altered metabolic phenotypes that interfere in broiler growth. The involvement of epigenetic processes warrants investigation. Another aspect of maternal conditioning relates to chemosensory pathways around external volatile compounds. A plant volatilome is the complete profile of volatile compounds released by a plant. When consumed by the hen, part of the volatilome is transferred to the fertile egg helping define the innate food preferences in the progeny. Plant derived volatile compounds may include components of essential oils (EO). The application of EO using in ovo feeding techniques has been developed in recent years uncovering novel aspects that improve chicken development such as the GIT and the immune system. To finalise, a new concept around fostering a healthy microbiome early in the life of the chicken will be explained. Like eukaryotic cells, bacteria require energy and AA to survive. Some commensal bacteria show dependence on exogenous AA sources (essential AA), a requirement not always observed in non-commensal bacteria. These properties indicate that AA have the potential to be used as a novel class of prebiotics (“aminobiotics”) to modulate the chicken microbiota.

**Keywords:** transgeneration nutrition; maternal conditioning; in ovo; embryonic development; broiler chickens; aminobiotic

**Transfer of undesirable substances from feed into meat and eggs - from experimental data to predictive models****R. Pieper<sup>1</sup>, M. Salewski<sup>1</sup>, J. Numata<sup>1</sup>**<sup>1</sup>Department Safety in the Food Chain, German Federal Institute for Risk Assessment (BfR), Max-Dohrn-Str. 8-10, Berlin, Germany*Presenting author: robert.pieper@bfr.bund.de*

Natural or anthropogenic substances with potential adverse effects on animal or human health are found throughout the environment, including in animal feed. Regulations have long been in place in the European Union to ensure that animal and human health is not negatively affected by exposure to these undesirable substances. Undesirable substances include (semi)metals, persistent organic pollutants (POPs), process contaminants and natural toxins. Some of these substances have the ability to accumulate along the soil/water - plant - (livestock) animal - food pathway. This transfer of undesirable substances from the oral exposure of animals to food of animal origin poses a number of challenges for actors along the food chain, risk assessors and risk managers. Predicting the transfer behavior of undesirable substances into food of animal is often a daunting task because it depends on the complex interplay of the absorption, accumulation into selected tissue components (specific proteins, fat, etc.), metabolism and excretion. Experimental data from feeding trials with laying hens or fattening chicken exposed to polychlorinated biphenyls (PCBs) or per- and polyfluoroalkyl substances (PFAS) in a controlled manner can be used to generate predictive toxicokinetic models. We have derived such predictive models and translated them into an easy-to-use computer tool called ConTrans (“Contaminant Transfer Predictor”). ConTrans can be used to predict the consequences of real incidents in which contaminated feed is (accidentally) used in poultry at a given concentration and over a given period of time; in such cases, ConTrans can predict the concentration of the contaminant in edible tissues or eggs. Although this does not replace official controls, it can be an important decision support tool for farmers and risk management authorities. It can, for example, help to assess whether the maximum level of a substance in these foods is likely to be exceeded. The program is freely available at <https://contrans.bfr.bund.de>. The necessary access data can be requested at <https://limesurvey-001.bfr.berlin/427925>.

**Keywords:** Undesirable substances, Contaminants, Contaminant Transfer Predictor

## **Innovation in sensory assessment of poultry meat and meat products**

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Obtaining sensory profiles of foods using traditional descriptive sensory techniques such as Quantitative Descriptive Analysis (QDA) implies a prior training of the participants which involves time, effort and cost. Training a panel comprises different stages, starting with the familiarization of the participants with the sensory characteristics of the product to be evaluated, then to reach a consensus of the descriptors to be included in the evaluation sheet and finally to learn how to use the quantification scales. Moreover, this technique can be considered as “static” since assessors integrate their response to provide a time-average single intensity value. However, sensory experience is a dynamic process which cannot be recorded using these classical descriptive techniques. In the last decades, innovative sensory techniques have been emerged to overcome the main handicaps of the classical descriptive techniques. These advanced means of assessment reduce the time exerted to train panellists and enable the inclusion of consumers’ opinion at all stages of the foods’ production and marketing. In this context, several quick-fast descriptive techniques as CATA (check all that apply), JAR (just about right) scales, Napping or Flash Profile have been successfully introduced to a variety of muscles foods, including poultry. These rapid descriptive techniques enable featuring consumers (non-trained participants) in the sensory characterization process and to quickly develop the evaluations in one or two sessions. Moreover, Time-intensity can be considered as the first dynamic sensory technique developed for assessing the evolution of intensity perception of an attribute over time during food consumption. Later, new dynamic techniques have been implemented as the multi-attribute technique Temporal Dominance of Sensation (TDS) and its most recent variant Multiple intake TDS. Dynamic techniques allow obtaining a more realistic sensory characterization of food products. Some studies focused on the comparison of the dynamic sensory profile of both poultry and chicken plant-based analogues will be addressed. The study of emotions evoked during food consumption has gained importance in the last decade mainly due to their role as a key drivers and predictors to decode food choices. Several methodologies have been developed to register the emotions elicited including both implicit and explicit methods. Among these methods, the ESense25 procedure, has been successfully used to define the emotional profile of poultry products. Moreover, these new sensory approaches have been introduced to explore consumers’ perceptions and behaviour towards poultry and meat products and to evaluate the impact of information and context.

Keywords: Meat; Poultry Products; CATA-check; sensory approaches

## **Growing broilers during tough economic conditions**

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Growing Broilers During Tough Economic Conditions Andrew Catlett Cobb Europe, Severalls Business Park, 770 The Crescent, Highwoods, Colchester CO4 9YQ It is well known and widely accepted that feed costs contribute between 60-70% of the total production costs, therefore it is important to be able to optimize production costs by changing feed specifications without adversely affecting performance significantly while still producing quality products. When practically assessing formulations the three primary components that drive the costs of the different feed phases are Energy, Available Phosphorus and Digestible Amino Acids. A study was conducted to evaluate the effect of reduced specifications on physical performance. Study was compared to the Cobb 2022 Nutrient Specifications (control, T1). The first experiment comprised of a control and four treatments, Treatment two considered Available phosphorus 0.54%, 0.35%, 0.30% and 0.25% and Calcium 0.96%, 0.76%, 0.54% and 0.5% in the starter, grower 1, grower 2 and finisher phases respectively. Treatments three to five had the same reduced available Phosphorus and Calcium and 3% less energy (kcal/kg). Treatments three and four had reduced digestible amino acids by 3% and 6% respectively. Although the experiment showed no significant effect on body weight, there was a significant effect on feed conversion ratio from 14 days through to 34 days of age where Treatment three and four were significantly different from the control. There was no effect on reducing the available phosphorus and calcium on feed conversion ratio. From this experiment it can be concluded there is scope to reduce specifications in diets being fed to Cobb 500 birds especially during times when feed prices are high.

**Keywords:** Nutrient Specifications; calcium; available phosphorus; energy



## **Cost factors in broiler meat production. An alternative analysis**

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The real impact of zootechnical parameters on the production cost of chicken meat is a multifaceted issue that requires careful analysis. While it's clear that technical parameters play a crucial role in determining the efficiency of production, they alone do not define the economic viability of a poultry enterprise. Profitability, the ultimate measure of success, hinges not just on technical efficiency but also on the delicate balance between production costs and the market value of the end product. This balance is influenced by a myriad of factors, some of which lie beyond the immediate control of field technicians. In the complex equation of poultry production, certain costs stand out for their direct impact on profitability. These include the cost of feed, which represents a significant portion of total expenses, the cost of chicks, and various operational costs associated with running a farm. Among the technical parameters, live weight and feed conversion ratio are particularly noteworthy for their influence on production costs. These factors are not static; they are influenced by market demands and the specific requirements of slaughterhouses, which in turn dictate the optimal live weight for chickens. To navigate this complex landscape, it is imperative for poultry producers to not only focus on maximizing technical efficiency but also to develop a nuanced understanding of the market forces at play. Optimizing feed conversion ratios, for example, can lead to substantial cost savings and improved profitability. However, this requires a holistic approach that considers both the technical aspects of poultry production and the broader economic context in which it operates. In conclusion, the real impact of zootechnical parameters on the production cost of chicken meat is a topic that encompasses both technical and economic dimensions. By understanding and optimizing these parameters, poultry producers can enhance their profitability and ensure the sustainability of their operations in a competitive market.

**Keywords:** Economy; Meat cost; Technical performances

## **Vaccination against avian influenza: adapting our strategy to changing challenges**

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High pathogenicity avian influenza viruses (HPAIVs) have caused major epizootics in the last years with devastating consequences for poultry and wildlife worldwide. Domestic and wild ducks can be highly susceptible to HPAIVs, and infection leads to efficient viral replication and massive shedding (i.e., high titres for an extended time), contributing to widespread viral dissemination. Importantly, ducks are known to shed high amounts of virus in the earliest phase of infection, although the dynamics and impact of environmental contamination in the epidemiology of HPAIV outbreaks is still poorly understood. Based on epidemiological modelling at the regional scale, it has been clear that the control of HPAI in the specific sector of duck industry was critically important in France to reach a global control in the other poultry sectors. So far, the main doctrine of HPAI control has been based on early detection of outbreaks and their control through rapid culling, with a sparing use of vaccination. Mass vaccination for HPAI has therefore been only used in some low- and middle-income countries, where biosecurity and early detection could not be applied, with poor or mixed benefits. Recent advances make now possible to implement vaccination with a strict control: the marketing of next-generation vaccines (RNA-based, sub-units, recombinant herpesviruses, ...) opens new avenues to keep under a strict control the potential circulation of wild avian influenza viruses in vaccinated flocks. Molecular testing is now widely available to be implemented at a mass-scale. Altogether, these improvements in vaccines, diagnostic tools and our knowledge make possible a safe and efficient implementation of vaccination in the framework of a global control strategy. Based on these considerations, a vaccination plan focused on ducks has been prepared and implemented in France since October 2023, after a risk assessment and complementary vaccine trials in field conditions. A comprehensive analysis of the outcomes of this vaccination plan after its first year will be very important to assess the relevance of this strategy, at least in the specific context of the French poultry production. On a long-term perspective, we should remember that no single strategy will be sufficient to tackle the threat of HPAI clade 2.3.4.4.b, but rather a combination of the different leverages (biosecurity, regulation of farms density, surveillance) along with vaccination when relevant.

**Keywords:** Avian influenza; vaccination; ducks; surveillance

## Unlocking Immune System Potential in Chickens through Gene Editing: A Promising Frontier

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Genetically modified animals have been instrumental in enhancing our understanding of immunity, infectious diseases, neurology, behavior, and developmental biology. Knowledge of the immune system is crucial for discovering innovative health interventions for humans and animals alike, with the CRISPR/Cas9 system revolutionizing this domain by facilitating accurate gene alterations. Chickens are a crucial global protein source and serve as valuable models for studying vertebrate developmental biology. However, compared to mammals, creating genetically modified chickens has historically been challenging due to the complex nature of chicken zygotes. The ability to modify primordial germ cells (PGCs) and reintroduce them into the embryonic vasculature has facilitated the production of transgenic chickens. Interferons (IFNs) play a critical role in the innate immune response against viral infections. This response encompasses a complex mixture of type I IFN (IFN- $\alpha$ ) and type III IFN (IFN- $\lambda$ ). The objective of this project is to generate IFN- $\alpha$  and IFN- $\lambda$  receptor knockout (KO) chickens to dissect the role of avian IFNs in viral infections. PGCs were co-transfected with a CRISPR/Cas9 vector expressing a single guide RNA and Cas9-2A-eGFP targeting the coding region within exon1 of IFNAR1 chain of the IFN- $\alpha$  receptor and the epithelium-specific chain (IFNLR1) of the IFN- $\lambda$  receptor. Western blot and qPCR studies confirmed the deletion of IFNAR1 and IFNLR1 receptors and reduced expression of Mx in response to recombinant IFN treatment under in vitro and in ovo conditions. Both IFNAR<sup>-/-</sup> and IFNLR<sup>-/-</sup> chickens displayed normal development and body weight compared to wild-type (WT) chickens. Homozygous roosters and hens were bred within each line to assess fertility in terms of producing fertile sperm and eggs. Eggs were collected, hatched, and the experiment showed normal production of fertilized eggs. The chicks resulting from breeding homozygous roosters and hens exhibited normal health and growth. The IFN KO chickens provide a novel tool to explore the avian IFN system and understand the antiviral properties of IFNs, essential for developing therapeutics targeting a broad spectrum of viruses with veterinary and zoonotic significance.

Keywords: genetically modified chickens, CRISPR/Cas9, immune system, primordial germ cells

## **The role of plant-based antinutritional factors in poultry diets. New progress on their effects on chicken growth and health**

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Antinutritional factors (ANF) of plant origin are very abundant in nature, with negative effects on feed intake and on physiological and metabolic processes that affect the availability of nutrients for poultry. Traditionally, chicken growth and efficiency relied on the use of in feed antibiotics but after the ban, in 2016, the absence of the growth promoters in the EU-28, resulted in a series of problems for the poultry industry. In fact, in relation with a greater incidence of wet litter, breast blisters, and pododermatitis at the farms, and in an increase in the use of antibiotics as curative. The situation was improved with flock management, including changes in feeding programs, feed form, and dietary nutrient specifications. Additionally, the industry became interested in the use of plant-based feed supplements to retain or recover bird health and productivity. At that time, our knowledge on the mode of action and how the new additives interfere with feed utilization and animal health, was very limited. New studies on the effects of the inclusion of secondary plant metabolites on voluntary feed intake and their potential to act as an ANF or as an enhancer of different physiological and metabolic processes, were conducted at Universities and Research Institutions all over the world. It became apparent that the effects of plant components, such as tannins, flavonoids, saponins,  $\alpha$ -galactosides, haemagglutinins, and trypsin inhibitors, present in numerous feeds, could be positive or negative, both in humans and animals, depending on the dose. In fact, at high doses, most of the ANF present in plants, resulted in a loss of growth and poor health status of the birds. However, the same ANF, at low levels of inclusion, prevented or reduced oxidative and inflammatory processes in the birds. Even more, further studies in humans, showed a reduction of common metabolic diseases, such as diabetes mellitus, coronary heart disease, and cancer, with the inclusion of some of these additives in the diet. Because of the high biological activity when consumed in adequate amounts, the term plant “antinutritional factor”, commonly used in animal feeding for most of the components or secondary metabolites present in ingredients and diets, become to be named, “bioactive compounds” or “pharmacologically active agents”. These new metabolites of plant origin are currently used to improve not only poultry production but also to ameliorate the health status of animals and humans

**Keywords:** antinutritional factor; bioactive compound; poultry production

## **Dealing with Global Supply Chain Realities, Layer Genetics**

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The Egg Layer breeding pyramid is a key to the success and competitiveness of the egg sector. The breeding pyramid allows for: specialization, economies of scale and high biosecurity which enables a sustainable egg production system with low carbon footprint and high animal welfare status. It further allows for the formation and growth of specialized genetic companies, distribution companies and egg producers. Driven by specialization and economies of scale, the Egg Layer Breeding Pyramid is now fully globalized. Global genetic companies concentrate their breeding activities in a few specialized pure-line facilities. From there they supply grandparent customers and strategically located grandparent hubs. From grandparent distribution hubs genetic companies supply parent stock to their customers. Those customers usually operate within one country and are the suppliers of egg layer flocks to egg producers. The Egg layer Breeding Pyramid results in a global supply chain that crosses many borders, and bridges long distances. This creates challenges in terms of health status, animal welfare, logistics and product market fit. To overcome these challenge in logistics, animal welfare and health status challenges, genetic companies have been investing in: spreading pure-line facilities across countries and continents; setting up more and smaller grandparent distribution hubs; more collaborations with more airlines resulting in more and better facilities at airports. To overcome the product-market fit challenge, genetic companies have been investing in expanding the number of products they produce, setting up product test stations in several countries in several environments ranging from tropical open sided cage housing to confined large scale aviaries in temperate zones, collecting more field data from customers. These developments have changed the Egg Layer Breeding Pyramid: it is now complemented by an Egg Industry Information Funnel, that channels information from global markets to the Breeding Pyramid resulting in a more flexible and adaptive Pyramid that is able to deal with the todays Global Supply Chain realities and to contribute to a sustainable egg production system.

**Keywords:** Egg Layer Breeding Pyramid; Supply Chain; Biosecurity; Global Trade; Sustainable Egg Production

## **Greenhouse gas emissions**

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Greenhouse gas emissions have received growing interest in the last years. The global climate crisis affects all sectors, and consequently mitigation and adaptation measures must be implemented. This paper reviews the relevance of poultry production in global atmospheric emissions, in the European Union context. Poultry is a relatively minor source of direct greenhouse gas emissions. This is a consequence of the animal digestive system and the usual manure management, which do not provide the conditions for methane formation. However, relevant amounts of nitrous oxide are generated when managing manure. Indirect emissions must be considered as a more relevant concern for poultry production. These indirect emissions come mainly from obtaining and processing the ingredients required for feed production. Apart from greenhouse gases, ammonia is a very relevant atmospheric pollutant produced in all livestock houses, including poultry. Specific commitments to reduce ammonia involve adapting mitigation strategies on the farm, which include nutrition strategies, housing management, manure processing and land application to crops.

**Keywords:** methane; nitrous oxide; ammonia; direct emissions; indirect emissions

## **Global Poultry Outlook - Consequences of political tensions in the poultry sector**

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Poultry and eggs will keep their global positions as fastest fastest-growing animal protein in the next decade. Global demand is expected to grow by around 2% YOY compared to 1.2% YOY for animal protein. Most of the growth will be in emerging markets (>90%) with Asia (60%) in the lead. Growth will be driven by population growth, higher incomes, and changing preferences and the specific poultry benefits in terms of pricing, health and convenience, and more limited cultural restrictions for consumption of poultry compared to beef and pork. The global context will also be changing with a changing social context with more emphasis on social concerns in terms of environment, human and animal health, and social awareness. A key challenge for global poultry and egg producers will be how to supply future market growth. The fact that most of the growth is in Asia, a region with limited resources will require a higher emphasis on strategic supply chain management. In general, this context of growing demand and more limited resources will lead to a higher focus on improving yields and efficiency with a higher emphasis on sustainability and other social concerns. Rising global political tensions will impact future investment directions. Tensions as seen in Eastern Europe and the Middle East are having a big impact on feed and feed ingredient markets, logistics (Red Sea Channel), meat trade, and other input markets. This will lead to changing perspectives on food security, resource security, and the future role of trade. It is also expected to impact global investment decisions, with likely a higher emphasis on local investments in poultry and egg production instead of global trade. This has a big impact on future investments by local and global investors who have to reconsider their investment strategy.

**Keywords:** Global Poultry Outlook Political Tensions

## **Trends for the future of the Poultry sector: Education, Employment, and Research**

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While contributing to feed 9 billion people by 2050, the Poultry sector faces some challenges related to changes in the global population, both in terms of consumer needs and expectations as well as recruiting and training future generations to work in the sector. The rise of Artificial Intelligence (AI) with Machine Learning and Large Language Models will change the way people access and use new knowledge. New approaches towards learning, including the continuing education of professionals after graduation (e.g. WVEPAH), must respond to the expectations of younger generations as well as cope with the ever increasing amount of new research published. Teaching techniques, including Virtual Reality tools and AI-generated individual Online learning programs, will be incorporated into the curriculum while maintaining the opportunities for real field experiences. Dedicated Poultry AI Chatbots will allow instant access to accurate and relevant information, requiring the Poultry sector to adjust its decision-making processes. In many countries, the sector is already struggling to recruit new talents. While the digitalization of the Poultry sector will attract professionals with new skill sets and offer more stimulating positions to young graduates, the automation of several tedious or painful processes is also required and will limit job opportunities. The use of robots, video cameras, sensors and internet of things devices will change the role of farm workers but will not replace them in the near future. However, AI-derived predictions will augment their ability to make decisions. Research should focus on addressing the needs of consumers and their key food buying factors: price, taste, convenience, healthiness, and sustainability. Taste and convenience will allow the sector to capture more “share of stomach” and increase meal opportunities for poultry and eggs. While the healthiness of products from the sector is well established, consumer perceptions about plant-based alternatives create new challenges. The demand for more sustainable food choices offers a wide range of research options, from more sustainable feed ingredients and poultry diets to identifying revenue streams from waste materials (e.g. eggshells). With consumers and, at times governments, making decisions based on emotions and not science, research should also focus on new communication tools and messaging to help the sector face an audience more and more removed from the rural environment.

Keywords: Education; Employment; Research: Trends; Poultry; WVEPAH; Artificial Intelligence



## Phage Therapy Against Superbugs: Promising Strategies and Insights

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Antibiotic resistance stands as one of the foremost threats to public health, causing over 1.2 million deaths annually. It is estimated that this figure could rise to 10 million annual deaths by 2050 if a solution is not found. Overuse of antimicrobials, both in human medicine and livestock, contributes significantly to this crisis, fostering the selection and dissemination of resistant bacteria throughout the food chain. To address this multifaceted challenge, a One Health approach is imperative, emphasizing collaborative efforts across human, animal, and environmental health sectors. Alongside this approach, the development of innovative biocontrol protocols is essential to prevent or eliminate multidrug-resistant (MDR) bacteria. In response to this urgent need, bacteriophages, viruses that specifically target bacteria, emerge as highly promising tools for preventing, treating, and eradicating resistant bacterial infections. Unlike broad-spectrum antibiotics, bacteriophages offer targeted action, minimizing collateral damage to the host microbiota. Their efficacy extends to biofilm removal, facilitated by phage-derived enzymes that disrupt biofilms effectively. Furthermore, bacteriophages play a crucial role in ecosystem equilibrium, coevolving with bacteria and contributing to the natural regulation of bacterial populations. In certain regions, such as the United States, regulatory bodies like the Food and Drug Administration (FDA) have already approved the use of phage cocktails to control pathogens like *Listeria* and *Salmonella* in poultry and ready-to-eat products. However, regulatory approval for phage-based interventions has been slower in other regions, like the European Union (EU). Nevertheless, recent developments, such as the European Medicines Agency's 2023 guideline on bacteriophages in veterinary medicine, signal progress towards authorizing phage-based strategies against MDR bacteria in Europe. In conclusion, the threat of antibiotic resistance underscores the urgent need for innovative solutions. Bacteriophages offer a targeted and environmentally sustainable approach to combating MDR bacteria, with potential applications across human and veterinary medicine as well as food safety. While challenges persist, collaborative efforts and evolving regulatory frameworks hold promise for harnessing the full potential of bacteriophages in the fight against antibiotic resistance.

Keywords: Phage Therapy; Superbugs; Poultry; Antimicrobial Resistance; One Health

## **Early feeding nutrition of minerals and vitamins: implications and applications**

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The fertile egg contains many important metabolites such as proteins, fats, vitamins and minerals however, in the pre-post hatch period, levels and availability of several minerals and vitamins are limited. Accordingly, especially in our fast growing broiler breeds, early feeding strategies are essential practice in poultry production for supporting the development of critical tissues (intestine, breast muscle, leg bones) in the newly hatched chicks. This practice is setting the stage for optimal performance throughout their lifespan. To address the minerals/vitamins nutritional limitations and for supporting the nutritional needs of chicks before-and-after hatch it is important to provide a formulated solution, for in ovo feeding, or a formulated diet for immediate post hatch chicks,. Key nutrients supplemented in in-ovo and early-feed include vitamins A, E, C, and B-complex vitamins, along with essential minerals like zinc, iron, copper, and manganese for skeletal development, immune function, and growth. Overall, in ovo feeding holds great promise as a versatile and innovative approach with numerous potential applications for improving poultry production efficiency, sustainability, and also animal welfare. Applied research with industry is likely to uncover new opportunities and further refine the use of in ovo technology in the poultry industry. Combining in-ovo-vaccination technology with in-ovo-feeding technology can be applied in hatcheries worldwide using the automated egg injection machines. Application by industry will exposed new opportunities to the poultry industry.

Keywords: Early feeding, Minerals, Vitamins, Poultry

## **Beak trimming or breeding for better beaks? A genetic approach to reducing feather pecking damage**

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The beak serves many important functions, including feeding and preening. Beyond these functions, commercial poultry species, particularly laying hens, can develop maladaptive beak-related behaviours such as injurious pecking (IP). IP has serious welfare consequences as it can result in wounds, cannibalism, and mortality. IP is difficult to prevent and control due to its multifactorial nature, and there has been considerable effort, both genetic and environmental, to address its underlying causes. Despite the significant advancements in improving bird management to reduce IP, the behaviour still prevails. Currently, the most effective management method is beak trimming; however, due to its own perceived welfare and ethical concerns, there is significant pressure to eliminate the practice and rely on alternative approaches, one of which is selective breeding. Selective breeding against IP behaviour is possible; however, behavioural phenotypes can be difficult to collect and implement into commercial breeding practices. Selecting for proxy traits such as plumage cover and liveability shows promise but their disadvantage often is only the victims of IP can be detected and not the perpetrators. Beak shape is another proxy trait that could be selected for but has received less research focus. Considerable variation in natural beak shape and size has been observed between White Leghorn pure lines, with distinct maxillary beak phenotypes ranging from long and narrow with pointed beak tips to short and wide with more curved beak tips. Aspects of beak shape are moderately heritable ( $h^2 = 0.11$  to  $0.34$ ), suggesting that genetic progress is possible. However, negative genetic correlations between beak length, curvature, and egg production ( $h^2 = -0.56$  to  $-0.83$ ) hint that selection for certain phenotypes (e.g., short and curved) could impact egg production. Going forward, the challenge is confirming the link between form and function in order to know which phenotypes to measure and which birds to use as selection candidates. Like IP, the beak's ability to inflict damage is likely multifactorial. Preliminary studies demonstrate that birds with naturally blunter beaks cause less damage; however, how and why this occurs remains unclear.

**Keywords:** injurious pecking; laying hen; welfare; geometric morphometrics

## **Towards a Humane and Sustainable Solution: A Genetic Model to Eliminate Male Chicks Culling in the Egg Industry**

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Unwanted by the meat and eggs industries, approximately 7 billion day-old male chicks are annually sorted and culled worldwide in the table eggs industry. In terms of sheer numbers, this issue constitutes a significant animal welfare concern in livestock, posing economic and sustainability challenges for farmers. Previous attempts to address this problem have been largely unsuccessful, primarily focusing on early-stage sorting of male embryos. However, given that there are no overt and practical differences between genders until late stages of development and that sex determination in chickens relies on sex chromosome segregation, a genetic solution has been devised to prevent the development of male embryos. Enter the GOLDA hens, which lay eggs that hatch only female layers. Importantly, both the layer hens and their table eggs are non-GMO and indistinguishable from conventional production. The system boasts 100% accuracy, allowing for the removal of undeveloped male eggs from the incubator as early as 48-72 hours, thereby potentially doubling the incubation space capacity. In chickens, sex determination relies on two sex chromosomes, namely W and Z. Males possess ZZ chromosomes, while females possess WZ chromosomes. Consequently, the common factor among all males is the maternal Z chromosome, whereas all females carry the maternal W chromosome. The GOLDA hens solution is rooted in a genetic modification that targets the maternal Z chromosome. Specifically, an inducible trait is inserted, which selectively halts the development of males during the early embryogenesis stages. Importantly, the genetic modification is limited to the maternal Z chromosome, leaving the maternal W chromosome unaltered in females. The inducible component of the system operates through light induction using an optogenic molecular mechanism, allowing for activation, through the eggshell prior to incubation and seamless integration into the hatchery workflow.

**Keywords:** Animal welfare; Table eggs industry; Culling day-old male chicks; Genetic solution; Sex chromosome segregation

## **Strengths and weaknesses of poultry meat quality attributes for meeting the increasing global protein demand**

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Poultry meat has become the most consumed meat in the world. Compared to other meats, it is more affordable for consumers due to lower production costs resulting from high feed conversion and reproductive efficiency. It is easy to prepare for fresh consumption and can be further processed. As with other meats, sensory attributes are among the most important quality attributes for consumers. Sensory and technological traits are complex and determined by many factors, including genetics, feeding and management. As a result, sensory and technological meat quality is variable and several meat quality defects have emerged, particularly in intensive production systems. A major strength of poultry meat is its universal acceptance and healthy image. As with other meats, poultry meat is a good source of high-quality protein and essential micronutrients, which contributes to ensuring adequate nutrition and avoiding nutrient deficiencies for the growing world population. Unlike red and processed meats, consumption of this white meat is not associated with the risk of developing chronic diseases. On the contrary, some reports suggest a protective effect. Further mechanistic studies on the contribution of poultry meat consumption to human health are needed, e.g. on the role of bioactive compounds, which remain largely unexplored. However, the need for animal-source foods in general, and meat in particular, is now being debated in high-income countries. The quality of poultry meat is no longer limited to intrinsic quality attributes but also includes extrinsic quality attributes such as environmental impact and animal welfare. As the most prominent example of intensive livestock production, poultry production is particularly vulnerable to concerns about animal welfare. In response to these changing societal demands, production systems are now diversifying away from the largely dominant intensive indoor systems. The supply of meat from diverse production systems provides both opportunities and challenges to meet future consumer demands. Therefore, tools to comprehensively assess all quality attributes of poultry meat should be developed. In this ‘One-Quality’ concept, relationships between production practices and intrinsic and extrinsic poultry quality traits, as well as synergies and trade-offs between traits, are currently being investigated in the EU INTAQT project.

**Keywords:** chicken meat; nutrition; sensory quality; human health; technological quality; production systems

## Bone and eggshell quality in hens in extended laying cycles

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Egg laying is highly demanding and female birds have developed specific physiological adaptations for it. Laying hens need a large and continuous supply of calcium (about 2 g of calcium daily for eggshell formation, equivalent to 10 % of total body calcium). During lay, a stimulated production of vitamin D leads to an increase in calcium absorption by intestinal and uterine tissues. Also, hens develop a new type of bone within the marrow cavities of their long bones (medullary bone) that serves as a calcium reservoir for eggshell calcification during the night when hens are not eating and the intestinal calcium supply is exhausted. The formation and resorption of medullary bone is synchronized with the egg daily cycle. Thus, bone and eggshell mineralization are interrelated and it is clear that egg laying, and high calcium requirements, makes laying hens more susceptible to develop skeletal problems (osteoporosis, keel damage) than non-laying birds (male chickens). However, recent studies challenge the general concept that selection for high egg production is responsible for skeletal weakness in laying hens. In fact, recent studies reveal that there are no evident relationships between bone quality and egg production or eggshell quality. Therefore, it seems that bone and egg quality are mostly independent and can be improved separately. Cracked and damaged eggs increase with hen age and the downgraded percentage can rise from 6 % up to 20-30 % of eggs at 70 weeks of age; being one of the main reasons to limit the production cycle to one year. Nowadays, the industry is aiming to extend the egg-laying period from about 70 weeks until 100 weeks, to make egg production more sustainable. Modern laying hens have been selected for extended laying cycles (100 weeks) but achieving the genetic potential is challenging. Egg laying causes a general deterioration of the hen health during normal egg production period and even more in extended cycles. During the intensive egg laying period, medullary bone is formed at the expense of cortical bone resulting in a progressive loss of structural bone and to a general deterioration of skeletal integrity with hen age (osteoporosis). Poor bone quality increases the incidence of skeletal deformation and bone fractures and is one of the most relevant welfare problems facing the egg industry today. On the other hand, the decrease of eggshell quality during the production cycle, is a major concern for food safety, as eggs with a damaged eggshell, or without cuticle, are more easily contaminated with bacteria (i.e., Salmonella); trans-shell penetration is considered to be the prevalent route for bacterial contamination. Thus it is of great importance to maintain eggshell quality, and skeletal integrity, as well as the overall hen health during the extended production cycles. In any case, all studies show that bone quality, and eggshell characteristics, are highly variable and are influenced by a wide array of factors including genetics, nutrition, age and housing. In this work, we review how bone structure and mineralization, and eggshell quality, changes during extended production cycles (100 weeks) and how bone mineralization at the start of laying can determine eggshell quality at the end of the laying period. We revise how adequate nutrition and management can help maintain bone quality and ensure hen laying persistency, egg quality and hen health. We also review new developments in the study of bone, and eggshell, structure, mineralization and quality assessment.

Keywords: laying hens; bone quality; osteoporosis; eggshell quality; aging

**Enterococcus cecorum, an emerging pathogen of meat type chickens****A. Jung<sup>1</sup>**<sup>1</sup>Clinic for Poultry, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

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Enterococcus cecorum (EC) is a Gram positive ovoid coccus and belongs to the lactic acid bacteria. Over the years, EC has become one of the most important bacterial pathogens of broiler type chickens worldwide leading to increased mortality, treatment cost and higher condemnation rates at the processing plants. The pathogenesis consists of following steps: oral uptake, intestinal colonization, translocation from the intestine, septic phase, skeletal infection. The infection takes place in the first week of life and one inoculation of  $10^6$  CFU is enough to induce disease experimentally. All birds are colonized in the intestine for several weeks, but translocation starts already a few days after infection. Intestinal damage is not required for translocation. During the septic phase, some animals may develop pericarditis and hepatitis. The free thoracic vertebra and the femoral heads of broiler type chickens are especially exposed to torsional and sheer stress which leads to the development of osteochondrotic clefts in the cartilage. These lesions are colonized by septic EC and osteomyelitis develops which leads to lameness and hind-limb paresis of the affected chickens. To answer the question how susceptible slow growing broilers are to EC infection, we conducted an animal trial where we infected Ross 308 and slow growing bird Hubbard JA 757, non-infected controls were included. The slow growing birds showed less pathological changes and EC positive organs. Therefore we concluded that slow growing broilers can develop EC associated disease but are less susceptible than fast growing broilers. It can be speculated that the reason may be less stress on the skeletal system for the slow growing birds. We conducted a further study where we examined if antibodies can protect against EC infection. We produced a hyperimmune serum in vaccinated SPF chickens and used the serum for subcutaneous application at day 1 and day 2 in Ross 308 chickens. Directly after the second application, animals were infected with EC. The administration of the hyperimmune serum led to a significant reduction of pathological bone lesions, bacteriological positive chickens and serum antibody development after infection. Further work concerning antigenic proteins and vaccine development is needed. Although some knowledge concerning EC associated disease was generated in the last years, more research is still needed to unravel epidemiology and answer open questions of the pathogenesis.

Keywords: Enterococcus cecorum; broilers; chickens, osteomyelitis, pathogenesis



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**SHORT ORAL**

**COMMUNICATIONS**

Ordered chronologically by Slot (Time of presentation)





**An integrated approach for the management of avian influenza information****G. Nalesso<sup>1</sup>, C. Tassinato<sup>1</sup>, G. Di Martino<sup>1</sup>, S. Rizzo<sup>1</sup>, M. Mazzucato<sup>1</sup>, C. Casarotto<sup>1</sup>, L. Martelli<sup>1</sup>, D. Fornasiero<sup>1</sup>, F. Scolamacchia<sup>1</sup>, G. Manca<sup>1</sup>**<sup>1</sup>Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, ItalyPresenting author: [gnalesso@izsvenezie.it](mailto:gnalesso@izsvenezie.it)

Avian influenza (AI) is a critical poultry disease with a worrying epidemic and zoonotic potential. Since 1997, the densely populated poultry area of Northeastern Italy has been affected by large and recurrent epidemics of AI, resulting in severe direct and indirect economic losses. The Epidemiology department of the “Istituto Zooprofilattico Sperimentale delle Venezie” (IZSVe) developed various tools to manage AI data. The tools included (i) a data-warehouse that integrates poultry farm data with LIMS and geospatial information, (ii) an outbreaks data manager dedicated to manage AI outbreaks data (e.g. date of suspect, date of cleansing and disinfection, number of death animals, etc.), (iii) a document management system organized to support and store the various document produced during the disease, and (iv) a WebGIS called IZSVeGIS Avian Influenza (IZSVeGIS-AI), aimed at real-time monitoring the epidemiological situation in Italy and at supporting the definition of control measures. IZSVeGIS-AI allows the measurement of linear distances between farms and their selection within a defined distance (e.g.; in fixed distance buffers). The tool provides information on animals and premises (e.g. current number, age and type of housed animals, farm size, production system) and permits searching information by spatial location (address or geographical coordinates), by registry ID and by owner. Moreover, adding multiple layers directly on the map to visualize risk zones, the presence of wetlands, synchronous re-stocking areas, and restriction zones is possible. It also allows mapping creation and downloading according to specific layers and filters. Furthermore, the stage of each outbreak is identified (suspected, confirmed, and extinct). It enables the visualization of the restriction zones established by the health authority after confirming the outbreak. IZSVeGIS-AI is made available to all stakeholders, particularly the supply chains and regional and local veterinary services, to enhance collaboration among the actors involved, which is essential during AI epidemics.

Keywords: Avian Influenza, GIS (Geographic information system), Epidemiology

## **A chicken blood transcriptomic signature related to adaptive immune responses is associated with resistance to highly pathogenic avian influenza virus infection**

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Avian influenza (AI) is a viral disease that has significant economic consequences for the global poultry industry. While most chickens infected with highly pathogenic AI viruses (HPAIV) develop a lethal disease in a few days, a significant percentage of birds can survive the infection, illustrating a complex process that involves intricate interactions between the virus and the host's immune response. Here we aimed to investigate blood transcriptomic signatures associated with H7N1 HPAIV infection outcome early after challenge. Chickens were inoculated with 10<sup>5</sup> ELD<sub>50</sub> of H7N1 HPAIV through the intra-choanal route. Blood samples were collected and analyzed from non-infected controls (n=8) before challenge, and at 8 (n=17), 24 (n=17), and 48 (n=28) hours post-inoculation (hpi) from inoculated birds. Susceptible chickens were classified based on mortality, clinical signs, viral shedding measured by PCR, macroscopic and histopathological lesions consistent with HPAIV infection, and the presence of AIV nucleoprotein antigen determined by immunohistochemistry. Birds that did not meet any of these criteria were categorized as resistant. Differentially expressed genes (DEGs) were identified by RNA-sequencing in blood samples of resistant and susceptible chickens compared to non-infected controls. At 48 hpi, we identified 3528 and 5631 DEGs in resistant and susceptible chickens, respectively. While gene-ontology enrichment analysis revealed the presence of genes related to innate immunity in both groups, a specific transcriptomic signature with features of adaptive immune responses was identified in the resistant group. Prominent terms included B cell receptor responses, T cell differentiation, Pattern recognition receptors (PRRs), innate immune receptors, apoptosis, and endocytosis. From these terms, we selected 38 genes to be analyzed using Fluidigm Multiplex Quantitative PCR at 8 and 24 hpi. This analysis showed the dynamic expression profile of these key genes during the early stages of infection. Overall, these findings suggest that the early differential expression of certain genes and pathways associated with the adaptive immune response may play a critical role in determining resistance or susceptibility to HPAIV infection in chickens.

Keywords: Avina influenza; HPAIV; Transcriptomic; Resistant genes

## **Pathobiology and variable genetic determinants for virulence, transmission and tissues tropism of H7N7 avian influenza virus in chickens, turkeys and different duck breeds**

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Highly pathogenic (HP) avian influenza viruses (AIV) cause severe mortality in chickens (*Gallus gallus domesticus*) and turkeys (*Meleagris gallopavo*), although turkeys are more sensitive than chickens. Little is known about the virulence determinants in both bird species, beyond the polybasic cleavage site (CS) in the hemagglutinin (HA). In 2015, HPAIV H7N7 and a low pathogenic (LP) ancestor were isolated from the same chicken farm. In addition to the polybasic HA CS, mutations in all gene segments were observed. The aim of this study was to investigate the pathobiology and the genetic determinants for virulence and transmission in chickens, turkeys and ducks. Using reverse genetics different virus reassortants with an LP backbone carrying the polybasic CS (designated Lp\_poly) and single or multiple HP segments were generated. In contrast to galliformes, LP and HP viruses were avirulent in pekin, muscovy and mallard ducks, were restricted to the aerodigestive tracts and were excreted at considerable levels from oral and cloacal swabs. Interestingly, in turkeys and chickens, Lp\_poly viruses carrying the HP NS or M gene segments or NS segment were as virulent and transmissible as the HPAIV, respectively. All Lp\_poly viruses were detected in the brain of chickens and ducks, although the endotheliotropism was exclusively found in chickens, where the HACS and to lesser extent NA are major determinants for the endotheliotropism. In vitro, HP exhibited lower NA and higher polymerase activities compared to LP. Together, ducks are clinically resistant to this H7N7 virus and excreted high amounts of viruses in the oral and cloacal swabs. This study showed two major differences between chickens and turkeys: more genetic constellations to confer high virulence and transmission in turkeys than in chickens and neurotropism in turkeys vs. endothelial tropism in chickens.

**Keywords:** Avian Influenza; Chickens; Turkeys; Ducks; Virulence; High Pathogenicity

## **Efficacy of an Inactivated Monovalent AIV-H5 Vaccine against early challenge with HPAI-H5N8 clade 2.3.4.4b**

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Avian influenza viruses type A are members of the family Orthomyxoviridae, which are structured with a negative-sense, single-strand, segmented genomic RNA, associated to the emergence of new variants compromising poultry health and food security worldwide. Along with biosecurity and systematic surveillance, vaccination can play a major role in controlling HPAI outbreaks by mitigating viral shedding, prevent transmission and reducing mortality rates in those affected flocks. The objective of this study was to assess the efficacy of a monovalent inactivated oil-emulsion vaccine containing a reassortant virus with HA gene of the Chicken/ME-2018/H5N8 strain, having close relatedness to the clade 2.3.4.4b of H5N8 viruses currently circulating across Middle East, Europe, Asia and the Americas. To evaluate the efficacy of a single-dose regime, two-week-old SPF chickens were subcutaneously immunized with 0.5 mL of a vaccine formula containing 108/EID50 /dose. Three weeks post-vaccination experimental birds were challenged with a wild-type HPAI-H5N8 2.3.4.4b virus having 96.1% homology with the vaccine strain (nucleotide identity). Results demonstrated that the vaccine induced protective hemagglutination-inhibiting (HI) antibody titers as early as 2 weeks PV ( $\geq 5.0 \log_2$ ). Vaccinated birds were protected against clinical signs and mortality (100 % survival rate) and virus shedding was completely neutralized in both tracheal and cloacal swabs at 3 days post-infection. In this context, the monovalent vaccine has demonstrated to be an effective tool to control HPAI challenges in chickens infected with the circulating clade 2.3.4.4b. Results in vaccinated birds concisely show that i) protective HI antibody titers are triggered as early as 2 weeks post-vaccination; ii) birds were protected against clinical signs and mortality, and iii) viral shedding was consistently reduced.

**Keywords:** HPAI; H5N8; Clade; Challenge; Outbreak; Vaccine

## **A comparative study of epigenetic and pathogenesis mechanisms between the virulent and avirulent strains of Newcastle disease viruses using bioinformatics analysis**

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Avian orthoavulavirus 1 or Newcastle disease virus (NDV) is a disease affecting poultry and many bird species globally. While avirulent NDV strains can induce subclinical disease with minimal morbidity or asymptomatic infections, virulent NDV isolates usually produce significant systemic pathological alterations and high mortality. Little is known about the host's innate immune response to infection with a virulent or avirulent strain, and how this reaction may lead to serious pathological damage or even death in the case of a virulent strain infection. Thus, using transcriptional profiling with RNA sequencing of chicken embryonic visceral tissues (CEVT) infected with either the virulent NA-1 strain (genotype VII) or the avirulent vaccine LaSota strain (genotype II), the differences in epigenetic and pathogenesis mechanisms between these strains were investigated. In our investigation, we found that only chicken embryos infected with the virulent NA-1 strains showed signs of severe systemic pathological alterations and substantial mortality. Nevertheless, there were no variations between NA-1 and LaSota in the way of virus spread. Based on the results of the bioinformatics analysis for the differentially expressed genes virulent NA-1 infection generated strong innate immune responses and severe metabolic abnormalities in CEVT at 36 h post-infection. Notably, the host defense response to the CEVT infected with the NA-1 strain compared to the LaSota strain involved an acute hyperinflammatory response marked by upregulated inflammatory cytokines, an uncontrolled host immune defense with dysregulated innate immune response-related signaling pathways, as well as severe metabolic disorders with the reorganization of host-cell metabolism. These findings suggest that the pathophysiology of the virulent strain in ovo may be influenced by the host's uncontrolled immunological response as well as metabolic disorders caused by viruses that hijack host cell metabolism. In summary, our research unequivocally demonstrates that virulent NDV stimulates strong innate immune responses and serious metabolic abnormalities, which in turn enhance the immunopathological harm of NDV and, in severely afflicted animals, life-threatening symptoms. However, additional research on metabolic systems and the host's innate immune responses is required to fully understand the precise processes behind the interaction between the host and virulent NDV in contrast to avirulent NDV.

Keywords: Newcastle disease virus; host-pathogen interaction; RNA-seq; gene expression

## **First report of molecular detection of aMPV circulation in domestic and wild birds in Colombia**

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Avian Metapneumovirus (aMPV) is a member of the Avian Respiratory Complex (ARP). This is a RNA virus that causes both respiratory and reproductive diseases. Six subtypes have been reported worldwide: A, B, C, D, GuMPV-B29, and MK491499, which can affect poultry, domestic ducks, pheasants, as well as local and migratory wild birds. Migratory wild birds particularly act as reservoirs and can facilitate the spread of the virus across productive regions. There are no reports of the virus prevalence or circulation in Colombia so far, and no live vaccines have been approved to be used in poultry. This is a sub-diagnosed disease, due to a combination of mild symptomatology in infected birds and the bias towards other diseases of the ARP. Based on this, the purpose of this project was to conduct a cross-sectional study aimed at determining the presence of aMPV and its molecular characteristics in commercial and wild birds across different regions of Colombia. Samples were collected through convenience sampling from poultry farms, backyard poultry and from wild birds admitted to the Wildlife Rescue and Rehabilitation Unit of the Faculty of Veterinary Medicine and Animal Science at the Universidad Nacional de Colombia. 200 swabs from the respiratory and/or reproductive tract from poultry birds and 73 from wild birds were taken. Viral detection was carried out by amplification of the Nucleocapsid (N) and Glicoprotein (G) genes through PCR. Positive samples were sequenced and analyzed phylogenetic relationships and recombination events. So far, aMPV subtype B has been detected in 15 samples from poultry farms and one from a Rock Pidgeon (*Columba livia*). These results demonstrate for the first-time molecular evidence of aMPV circulation in domestic and wild birds in Colombia, contributing to the understanding of viral dynamics, ecology, and preservation of wild bird populations as well as assuring food safety associated with poultry production. Also provide relevant epidemiological data to guide disease monitoring and control strategies in the country.

**Keywords:** Poultry diseases; Viruses; RNA; Pathogens; Molecular Biology; Molecular detection; PCR; Sequencing; Poultry; Wild birds

## **Avian metapneumovirus circulation in broiler farms in Italy**

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The avian Metapneumovirus (aMPV)-induced disease is a concerning threat to poultry production worldwide. While this disease has historically taken a backseat to other respiratory ailments like infectious bronchitis and Newcastle disease in broiler chickens, the landscape is evolving in Europe. This shift is attributed to a reduction in antibiotic consumption, the success of existing respiratory disease control programs, and the extension of broiler chicken slaughter ages. Against this backdrop, it is increasingly apparent, particularly in Europe, that avian metapneumovirus, mainly subtype B, may gain prevalence in broiler chickens. Consequently, the primary aim of this study was to assess the circulation of aMPV in broiler farms situated in high poultry density areas in Italy, where no specific control strategies are implemented in broiler farming and broiler farms are frequently located in proximity of layer or turkey farms. To achieve this goal, a total of seven farms (A-G) non-vaccinated against aMPV were selected in Northern Italy. These farms were subjected to an aMPV serological survey involving the collection of a minimum of 15 blood samples per farm in two different periods (summer and winter) during 2022 and 2023. The samples were then tested to detect aMPV antibody presence using Biocheck ELISA kit. The statistical analysis was done using the R software v3.1. A p-value < 0.05 was chosen as the limit for statistical significance. A Pearson correlation test was performed to establish the correlation between the antibody titres and the age. And a linear regression model for the log of antibody titres and age as covariate was utilized to evaluate the season effect. The sampled birds varied in age across farms, ranging from 41 to 61 days (mean 46.5 days). All farms tested positive in both analyzed periods, except for one negative farm sampled at 41 days of age in summer 2022. The percentage of seropositive animals within farm ranged from 33.3% to 100%, with different CV values ranging from 55.6-130.1%, revealing a heterogeneous situation. No seasonality effect was detected, however a possible effect of the age at sampling might have affected prevalence and breadth of the titres, suggesting a late viral entrance. These results unequivocally confirm the circulation of aMPV in non-vaccinated broiler farms in Italy. Further studies are warranted to comprehend the impact of this prevalence on the productive parameters of the birds.

Keywords: Avian metapneumovirus (aMPV), broilers, prevalence



## **Infectious bronchitis virus surveillance in layers and broiler breeders in Spain from 2020 to 2023**

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The avian infectious bronchitis virus (IBV) is considered one of the major economic burdens worldwide. Detected for the first time in Spain in 1970s, a competition of different strains to be the prevalent one began. Until 2020 the circulating strains corresponded to Genotype I, concretely GI-1, GI-12, GI-13, GI-19 and GI-21 lineages. Nonetheless, in 2020 two new genotypes, Genotype II (GII) and Genotype VIII (GVIII), were detected for the first time coinciding with reports of severe egg drop. In order to gain more insight into the molecular epidemiology of IBV in Spain, 1321 samples from layers and 284 samples from broiler breeders, collected between January of 2020 and October 2023, were analysed in the Centre de Sanitat Avícola de Catalunya i Aragó (CESAC). In the case of multi-age layer farms, several flocks of distinct ages were sampled at the same time and minimum once per year. Tracheal and cloacal swabs were independently screened for IBV using quantitative reverse transcriptase – polymerase chain reaction (RT-qPCR), and subsequently characterized by means of specific genotype RT-PCRs and Sanger sequencing. The obtained results placed these new genotypes as the more prevalent field strains since its appearance in Spain representing the 57% for layers (GII 33% and GVIII 24%) and the 36% for breeders (GII 18% and GVIII 18%) of positive IBV samples detected in this study. Very interestingly, IBV field virus in layers was detected in almost all flocks tested independently of age and sampling, which could indicate a continuous circulation of strains in multi-age layer farms. Regarding symptomatology, in the case of broiler breeders it matched with GII and GVIII European reports, presenting high avidity for the reproductive system while the respiratory tract is barely affected. In contrast there is a lack of clinical reports in layers. As the detection of these new genotypes is technique dependent, further studies have to be done in order to know if these new genotypes were circulating low profile in Spain before the introduction of genotype specific RT-PCRs.

**Keywords:** Broiler breeders; egg drop; Genotype II; Genotype VIII; Infectious bronchitis virus; multi-age layer farms

**Effects of polih herbal based product supplementation on growth performance, and gut health in broiler chickens challenged with Eimeria spp**C. Sol<sup>1</sup>, G. Villalobos<sup>1</sup>, F. Horta<sup>1</sup>, J. Lee<sup>2</sup>, W. Kim<sup>2</sup><sup>1</sup>Nuproxa Switzerland Ltd., Etoy, Switzerland, <sup>2</sup>Department of Poultry Science. University of Georgia, Athens, United StatesPresenting author: [cinta.sol@nuproxa.ch](mailto:cinta.sol@nuproxa.ch)

Growing concerns about drug use, synthetic additives, and resistance in poultry production have increased interest in natural phytoGenic supplements. This trend aligns with the rising demand for organic products in the market. This study was conducted to investigate the effects of supplementation of a natural polih herbal product based on *Acacia concinna* and *Balanites roxburghii* (NuxaSan 500™, Nuproxa Switzerland Ltd.) on growth performance, gut permeability, intestinal lesion score, oocyst shedding count, tight junction, and pro-inflammatory cytokine in broiler chickens challenged with *Eimeria* spp. A total of 288 one-day-old Cobb 500 male broilers were randomly distributed into 3 treatments with 8 replicates per treatment and 12 birds per cage for 28 days. Treatments were: Negative control, NC (basal diet, non-challenged), Positive Control, PC (basal diet challenged with *Eimeria* spp. inoculation) and PC + 500 ppm NuxaSan 500™ (NUX). Birds in PC and NUX groups were inoculated with 62.500 oocyst of *E. acervuline*, 12.500 oocyst of *E. maxima*, and 12.500 oocyst of *E. tenella* on d14, and NC group was orally inoculated with the same amount of PBS. Performance results showed that at 28d, birds in NUX group were the heaviest (1648a, 1495b and 1704a, expressed in grams, for NC, PC and NUX, respectively,  $P=0.0002$ ), with the lowest FCR (1.40b, 1.45a and 1.39b, for NC, PC and NUX, respectively,  $P=0.0014$ ) and lowest mortality (1.04%, 2.08% and 0.00%, respectively). The use of NuxaSan achieved a significant oocyst excretion reduction ( $P<0.05$ ) of *E. tenella* at 6-7-8-9 days post-inoculation (DPI), *E. maxima* at 7 and 9 DPI and *E. acervuline* at 6DPI ( $P=0.055$ ). Moreover, NUX supplementation decreased ( $P<0.0001$ ) the score of incidence of severe lesions in the duodenum (0.00c, 2.63a, 2.00b) and ceca (0.00c, 2.13a and 1.38b) for NC, PC and NUX. For gene expression, *Eimeria* spp. challenge increased ( $P<0.05$ ) the gene expression of claudin 1 (CLDN-1) and pro-inflammatory cytokine including interleukins IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  in jejunum compared to NC group; in contrast, for NUX group the gene expression of CLDN-1 and IL-1 $\beta$ , TNF- $\alpha$  in jejunum were reduced ( $P<0.0001$ ) but numerically increased GPX in jejunum compared to PC. The supplementation with 500 ppm of NuxaSan 500™ effectively mitigated negative effects caused by the challenge with *Eimeria* spp., suggesting that it could be a dietary strategy to improve performance and gut health in broilers challenged with coccidiosis.

Keywords: Broiler; Coccidiosis; *Eimeria* spp.; PhytoGenics; Intestinal health

## **Dietary phytogetic inclusion level affects performance and expression of heat shock and inflammatory genes in the whole blood of cyclic heat-stressed broilers**

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Heat stress is among the most challenging stressors for poultry production. The aim of this study was twofold: a) to investigate the inclusion level effects of a natural phytogetic blend (PB) on broiler growth performance, and b) to determine heat shock (heat shock factor-1) and inflammatory (nuclear factor-kappa B; NF-κB) responses in broilers blood following a period of cyclic heat stress at the end of the finisher phase. One day-old, Ross 308 (n=490) broilers were assigned into 5 dietary treatments, with 7 replicates of 14 chickens each, for a 40-d feeding trial. Experimental treatments received corn-soybean meal basal diets with no PB (CON) or supplementation with PB at 500 (PB500), 1000 (PB1000), 1500 (PB1500) and 2000 mg/kg diet (PB2000), respectively. The PB had olive oil polyphenols, carvacrol and thymol among its main bioactive components (NuPhoria®; Nuevo SA, Greece). Birds were reared for 35 days under thermoneutral conditions and then subjected to 7h cyclic heat stress ( $32 \pm 1$  °C; RH  $55 \pm 5$  %) for 4 consecutive days (d 36-40). Performance responses were monitored per growth phase and results reported as overall. Broiler blood samples at d 40 were collected for qPCR analysis. Data were analyzed by ANOVA, and statistical significance was at  $P < 0.05$ . Linear and quadratic patterns of biological responses to PB inclusion levels were studied via polynomial contrast analysis. Overall FCR, protein efficiency ratio, energy efficiency ratio and nutritional efficiency were improved ( $P < 0.05$ ) with PB inclusion, primarily in PB1000 and secondarily in PB1500, compared to CON. The heat challenge had a significant impact on the majority (8/9) of the HSF1 pathway genes assessed in blood. In particular, increasing PB inclusion level down-regulated ( $P < 0.05$ ) the HSF1 pathway genes in a linear and quadratic manner. Moreover, PB inclusion down-regulated the expression of the inflammatory response genes with PB1000 displaying the strongest effect (8/14) compared to CON. As a conclusion, PB1000 beneficially affected overall broiler performance indices including the 4 days cyclic heat stress. Furthermore, blood nutrigenomic analysis at the end of the heat challenge, highlighted PB beneficial modulatory effects in the expression of heat shock and inflammatory genes, with PB inclusion levels from 1000 to 2000 mg/kg diet being the most effective. Considering overall performance and nutrigenomic findings together, PB1000 could be considered as the most prominent.

Keywords: broilers; phytoGenics; heat stress; performance; nutrigenomics

## **Growth performance and antioxidant response of broiler chicken fed oxidized lipids with or without phytogetic feed additives**

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The *in vivo* antioxidant effects of two phytogetic feed additives, included alone or in combination, were tested in a broiler oxidative distress challenge model using oxidized lipids in feed. The two feed additives were an encapsulated product based on capsicum, black pepper, and ginger (Spicy) and a liquid rich in hydroxytyrosol (HT) obtained from by-products of olive oil production. A total of 720 day-old male Cobb 500 were allocated to 72 pens with 8 treatments and 9 replicates, with 10 birds per replicate. The treatments were arranged in a 2 x 2 x 2 factorial, the factors being: fat source (4% soybean oil or 4% peroxidized soybean oil); spicy (0 or 250 ppm); and HT (0 or 20 ppm). Peroxidation of oil was achieved at 95°C bubbling air continuously at a rate of 80 L/min during 72 hours, which significantly increased peroxide values (3.6 vs. 170 mEq/kg) and hexanal (0.4 vs. 30.4 ppm) for fresh and peroxidised oils respectively. Birds were fed their respective diets for 21 days, and at the end of the trial, plasma from one bird per pen was sampled. Animal performance and the activity of the endogenous plasmatic antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) were analyzed. No significant differences were observed in growth performance. The activity of CAT was significantly higher ( $P < 0.05$ ) in the plasma of birds fed the peroxidized oil diets indicating antioxidant system activation. A significant interaction ( $P < 0.05$ ) between fat source and HT was observed, with HT decreasing the CAT activity in birds fed the peroxidized oil. A similar interaction between fat source and HT tended ( $P=0.07$ ) to occur in GPX activity. No significant effects or interactions with HT were observed with the Spicy additive. In conclusion, feeding 4% peroxidized soybean oil to broilers for 21 days did not affect animal performance but increased the need of extra antioxidant response measured as CAT activity. The inclusion of an olive oil by-product rich in HT showed lower CAT activity in birds fed the peroxidized oils which might be indicative of an increased plasma antioxidant level and hence a lower need of activation of this antioxidant enzyme. However, this effect of HT needs to be confirmed.

**Keywords:** Phytogetic feed additives, peroxidised soybean oil, antioxidant activity

## **Effect of dietary supplementation and in ovo feeding of menthol on the immunocompetence traits, mineral composition and fatty acid profile of breast and thigh muscle of commercial broilers**

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Menthol is an organic compound, more specifically a monoterpenoid, made synthetically or obtained from the oils of peppermint or other mints. Comprehensive studies on dietary supplementation vis-à-vis in ovo feeding of menthol in commercial broilers is scanty. Hence, a study was carried out to evaluate the effect of dietary supplementation of menthol vis-à-vis in ovo feeding of menthol on the immunocompetence traits, mineral composition and fatty acid profile of breast and thigh muscle of commercial broilers. 375 fertile eggs of commercial broiler breeder birds were set for incubation. On 18th day of incubation, in ovo feeding was done and eggs were divided into three groups: un-injected control, sham control (injected with 0.5 ml of 5% ethanol) and in ovo injected with menthol (0.5 ml of 1% menthol solution in 5% ethanol). After hatching, 180-day-old chicks were reared for feeding trials. Chicks from these three groups were further divided into two subgroups, each with three replicates and ten chicks per replicate. One subgroup received a basal diet, while the other received a basal diet with menthol supplementation at a rate of 250 mg/kg diet. Birds were reared for 42 days (6 weeks) and kept on a Basal or Control diet [broiler starter diet, 22% CP and 3100 ME till 3 weeks and thereafter broiler finisher diet, 20% CP and 3200 ME till 6 weeks]. After 6 weeks, HA titre ( $P<0.001$ ), IgM titre ( $P<0.001$ ), IgM concentration ( $P<0.05$ ) in in ovo menthol group were significantly higher than sham control and un-injected control group. Further, HA titre ( $P<0.01$ ), IgM titre ( $P<0.01$ ), serum IgY ( $P<0.01$ ), IgM concentration ( $P<0.001$ ), FWI ( $P<0.002$ ) were significantly higher in dietary menthol supplemented group birds as compared to basal diet fed group birds. In addition, in ovo feeding along with dietary supplementation of menthol resulted in significantly higher ( $P<0.05$ ) HA titre. Dietary supplementation of menthol resulted in significantly higher concentration of sodium ( $P<0.04$ ) in breast muscle and significantly higher concentration of copper and iron ( $P<0.04$ ,  $P<0.01$ ) in thigh muscle. In ovo feeding or dietary supplementation of menthol resulted in significant decrease in saturated fatty acids and significant increase in omega-6 fatty acids in breast and thigh muscle. Further, in ovo feeding along with dietary supplementation of menthol resulted in significant increase in polyunsaturated fatty acids ( $P<0.003$ ) and omega 6 fatty acids ( $P<0.001$ ) in breast muscle.

Keywords: Menthol; Broilers; Immunity; Fatty acid profile; Meat

## **Quantitative and qualitative evaluation of plant intake in laying hens: n-alkanes as predictive fecal markers for dietary composition assessment**

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The shift in animal welfare standards towards free-range housing for laying hens in the EU has raised questions about changes in dietary composition. Accurate assessment of outdoor plant material intake is crucial for effective feeding strategies. This study introduces an approach using n-alkanes as markers to determine plant intake in laying hens, involving n-alkane recovery rate assessment, discriminant analysis and linear equation-solving for both qualitative and quantitative assessment, respectively, considering systematic n-alkane combinations. Two diets: a standard commercial diet and a diet incorporating 1% alfalfa were tested. Chemical analyses showed an altered n-alkane profile due to alfalfa inclusion, resulting a recovery rates ranging from 30-44% depending on the n-alkane type and diet. Statistical analysis revealed significant differences in recovery rates among the different alkanes for the same diets and between the diets for the same alkane, together with an interaction between n-alkane carbon chain length and initial concentration in the diet. The method accurately predicted plant inclusion, with a slight overestimation (2.80%) using the combination C25-C29-C33. This error, can be attributed to the minimal incorporation of alfalfa (1%) and the variability among animals, leading to an unexplained committed error. Accurate qualitative classification of the animals based on fecal n-alkanes profiles was observed. The study successfully demonstrated the utility of n-alkanes for estimating dietary composition, providing a non-invasive approach for future free-range studies. Ongoing projects are being conducted to validate the applicability of this methodology under real free-range conditions.

**Keywords:** n-alkanes; laying hens; recovery rate; alfalfa; outdoor consumption; free-range

## Effects of dietary supplementation of essential oils, lysozyme and vitamins blend on layer hen performance, viral vaccinal response and egg quality characteristics

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Health of respiratory tract is of paramount importance for layers as it may affect gut health, laying performance and egg quality. Viral diseases or even standard vaccination may impair trachea epithelium functioning and trigger oxidative stress. In the present study, we evaluated the effects of the dietary supplementation of a blend of feed additives (BFA) containing dominantly lysozyme, essential oils and vitamins on the egg quality and production as well as laying hens' health during oral vaccination schedule (25-33 wk of age). 320 Lohmann-type hens, aged 24 weeks, were housed in 32 cages. After a week of adaptation, they were divided into two groups. Half received oral supplementation of a 1 g/L BFA blend for the subsequent 8 weeks. Hen body weight was measured at the start and end of the trial and egg production was recorded daily. At week 25, a half of the layers were orally vaccinated for Infectious bronchitis (IBV) and Newcastle disease (NDV). At week 32, 8 eggs per cage were collected to determine egg quality parameters, rate of lipid oxidation in fresh and refrigerated shell eggs, and albumen protein oxidation. Egg yolks were also tested for total phenolic content (TPC) and total antioxidant capacity (TAC) after challenge with iron solution. At the end of the trial 2 hens per pen underwent blood sampling, and tissue collection from the trachea, liver, lung, and intestine for histopathological analysis. Water supplementation with the BFA significantly improved tracheal health after vaccinal application ( $p=0.009$ ) and increased egg production rate at 92.93% in 3rd week compared to the control group which had a 83.24% egg production rate ( $p<0.001$ ). Egg quality parameters were consistent across experimental groups. However, BFA supplementation notably increased TPC to 245.1 $\mu$ gGAE/g, compared to the control's 173.5 $\mu$ g. The treatment also enhanced total antioxidant capacity TAC in the egg yolk to 12.3% of ascorbic acid, contrasting with the control's 5.5%. BFA in drinking water reduced yolk lipid peroxidation (20.8 ng/mL vs. 33.5 ng/mL in the control) and albumen protein carbonyls (6.2 nmol/mL vs. 13.7 in the control). All antioxidant parameters exhibited a significant  $p$ -value below 0.001. In conclusion, water supplementation with the BFA enhanced IBV and NDV humoral immune response after vaccination; it also promoted hen egg productivity and lipid oxidative stability of the yolk and protein oxidation of the egg albumen.

Keywords: laying hens; oral vaccination; plant extracts; lysozyme; egg quality; antioxidant activity; trachea evaluation

## Application of cannabis-originated products in turkey nutrition

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In recent years, due to the growing interest in products derived from hemp, their production has been increasing dynamically around the world. Another product obtained from hemp that may have practical application in turkey nutrition is cannabidiol (CBD), which has an effect that supports the functions of the digestive tract (impacting mechanically, humorally, neurologically and immunologically on the digestive system, including the microflora modification). However, there is lack of such research in turkeys. In the present study, the performance response of turkeys to the dietary hemp seed cake and CBD inclusion was investigated. In feeding trials (140 days of rearing period) turkeys were feed diets: Control (T1) with no supplementation of hemp seed cake and CBD, (T2) with inclusion level of 10% of hemp seed cake, (T3) with 20% hemp seed cake inclusion or either of 0.01% or 0.02% CBD in treatments T4 and T5, respectively. It was found that both, hempseed cake and CBD extract significantly affected selected indices of bird's performance. Body weight (BW) at day 56 was significantly higher in treatment T3 comparing to T4 ( $P=0.007$ ), the same was true for average daily weight gain (ADWG) for this period ( $P=0.007$ ) however, feed conversion ratio (FCR) was significantly improved in treatment T4 comparing to T2 ( $P=0.012$ ). BW at day 56 was significantly higher in treatments T1 and T3 comparing to T4 ( $P=0.007$ ), ADWG for the period 1-56 days was significantly higher in treatment T3 comparing to T1 and T4 ( $P=0.007$ ), average daily feed intake (ADFI) was significantly higher in T3 comparing to T4 ( $P=0.016$ ), and FCR in this period was significantly improved in T5 comparing to T2 ( $P=0.037$ ). BW at day 84 was significantly higher in T3 vs. T4 ( $P=0.031$ ) while, ADWG calculated for the period 1-84 days was higher in T3 vs. T4 ( $P=0.031$ ). BW at day 140 was significantly higher in T3 comparing to T4 ( $P=0.019$ ) and the same was true for ADFI calculate for the period between days 113-140 ( $P<0.001$ ). ADWG calculated for the total experimental period (days 1-140) was significantly higher in T3 comparing to T4 ( $P=0.019$ ). This data indicates that both investigated additives (hempseed cake and CBD) are well tolerated by the birds because they did not compromise performance results however, the most effective response regarding turkey performance was found in group received 20% hempseed cake or 0.02% CBD extract in the diet.

Keywords: cannabidiol; turkeys; performance results



## **In ovo delivery of carvacrol triggered the expression of antimicrobial peptides in the yolk sac of broiler embryos**

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During broiler embryonic development, the yolk sac (YS) is known to play an important role in early immune defence. The bioactive compound carvacrol has the potential to enhance immune function, thereby reducing disease impact. After in ovo delivery, it was observed that carvacrol migrates primarily to the yolk. However, it is unknown whether the presence of carvacrol in the yolk could impact defence responses in the YS. Therefore, the aim of this experiment was to study the impact of in ovo delivery of carvacrol on YS immune defence mechanisms. It was hypothesized that in ovo carvacrol delivery would stimulate anti-pathogenic defence pathways in the YS. Two solutions were injected into the amniotic fluid of fertile Ross 308 eggs at embryonic day 17.5: either 1mL of (1) 0.9% saline or (2) carvacrol (0.5% v/v) with polysorbate 80 (1:1 v/v) in 0.9% saline. Transcriptomic analyses (RNAseq) of YS samples collected at E19.5 (n = 6) were performed, comparing the control (saline) and the carvacrol-injected group. The results showed 268 genes upregulated and 174 downregulated in the carvacrol group compared to the control ( $-0.5 > \log \text{ fold change} > 0.5$  and  $P < 0.05$ ). Functional analyses of these differentially expressed genes (DEG), using KEGG, REACTOME, and Gene Ontology databases showed that the 'NOD-like receptor signalling pathway' was significantly enriched ( $P = 0.002$ ) by 9 upregulated (TRPM2, CATH1, CATH2, IL18, TMEM173, DEFB4A, PLCB2, IKBKE and CASP18) and 2 downregulated DEG (CCL5 and NEK7). NOD-like receptors are intracellular pathogen sensors, which regulate anti-pathogenic immune responses. The pathway 'Antimicrobial peptides' was also significantly enriched ( $P = 0.001$ ) with 6 upregulated DEG (CATH1, CATH2, AVBD1, AVBD6, AVBD7 and RSFR). Antimicrobial peptides are part of the innate immune defence and are amongst the molecules produced after NOD-like receptor pathway activation. This indicates that in ovo delivery of carvacrol could prepare the newly hatched chick against bacterial pathogens by promoting antimicrobial peptide production through activation of NOD-like receptor signalling in the YS. In conclusion, in ovo delivery of carvacrol has the potential to enhance anti-pathogenic responses in the YS via upregulation of antimicrobial peptides expression. This work was funded by AgriFutures Australia and Delacon Biotechnik.

Keywords: In ovo; Carvacrol; Yolk sac; Antimicrobial peptides; Broiler; Embryo;

## **A sustainable and efficient strategy to enrich eggs with n-3 LC-PUFA**

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The health benefits of n-3 LC-PUFA such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) for humans are well established. Although eggs are not a natural source of these fatty acids, it is possible to enrich their contents in egg yolk through changes in the fatty acid profile of hens' diet. The use of fish oils containing EPA and DHA or vegetable oils rich in alpha-linolenic acid (ALA, 18:3n-3) are the most common strategies to this purpose. In this experiment, we hypothesized a more efficient and sustainable approach based on the use of Echium plantagineum oil, which contains a well-balanced linoleic acid (LA, 18:2n-6) to ALA ratio as well as relevant quantities of stearidonic acid (SDA, 18:4n-3), the first metabolic intermediate fatty acid in the conversion of ALA to EPA and DHA. To this aim, 3 different oils were supplemented to feed 48 Canarian laying hens for 30 days: Soy group (1.25% soy oil) mainly containing LA; Linseed group (1.1% linseed oil + 0.15% animal fat) particularly rich in ALA; Echium group (1% E. plantagineum oil + 0.25% linseed oil) richer in SDA and ALA. No significant differences were found neither in physiochemical properties nor in sensory analysis of eggs ( $P > 0.05$ ). However, the significant increments ( $P < 0.05$ ) in n-3 LC-PUFA specially EPA, docosapentaenoic acid (DPA, 22:5n-3) and DHA ( $0.14 \pm 0.05\%$ ,  $0.31 \pm 0.13\%$  and  $1.95 \pm 0.38\%$  respectively), in egg yolks from the Echium group, confirmed the importance of the n-6/n-3 dietary balance together with the presence of SDA, and verified the efficiency of this strategy to better modulate the endogenous production of n-3 LC-PUFA

**Keywords:** Egg; Canarian laying hen; Omega-3; Polyunsaturated fatty acids; Echium plantagineum

## **Effect of activated vitamin D3 on eggshell quality and performance in brown laying hens to 100 weeks of age**

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There are a wide range of benefits in extending the laying hen production to 100 weeks of age. The potential financial and environmental benefits that could be gained through this longer lay cycle are restricted by persistency of egg production and consistency in eggshell quality. The formation of eggshells is an extremely physiologically demanding process which requires immense calcium requirements. The utilization of vitamin D during this process is essential. Calcitriol, the active form of vitamin D plays a vital role in calcium and phosphorus metabolism and may have beneficial effects on calcium utilization for eggshell quality in older laying hens. Calcitriol is an essential nutrient for optimal bone health, eggshell quality, and overall productivity in laying hens. An herbal source of vitamin D containing calcitriol and natural triterpenoids has been sourced as an active form of vitamin D. This active form of vitamin D was added on top of a standard layer ration containing vitamin D. This herbal vitamin D is readily available to the layer, no further metabolic steps occur in the liver and kidneys. The availability of this special form of vitamin D is not compromised in critical phases. For this trial commercial brown layer hens were acquired at 55 weeks of age. The ration consisted of a diet typical to Australian production. Two different levels of herbal vitamin D were tested. At 60-100 weeks of age, egg production, egg weight and feed intake were measured weekly. Egg mass and feed conversion ratios were calculated. Quality testing occurred throughout the trial including: Haugh unit, yolk color, eggshell breaking strength, eggshell thickness, and relative eggshell weight. Cumulative data showing that egg weight, eggshell weight and shell breaking strength between the groups did not differ. Both herbal vitamin D treatment groups had significantly higher shell thickness compared to the control. Layer egg production was higher in the two herbal vitamin D treatment groups. The conclusion of this study will show 100 week of age measurements for bone strength. We will show the benefits of the addition an herbal activated vitamin D to a long layer cycle benefits egg production, eggshell quality and supports layers in periods of high calcium demands.

Keywords: eggshell quality; long layer cycle; 100 weeks; herbal vitamin D; calcitriol

## Effects of hen age and egg storage conditions on the quality and protein composition of the vitelline membrane

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The egg vitelline membrane (VM) is a protein-based structure surrounding the yolk. It contributes to protect the egg from bacterial contaminations, and it enables the separation of white and yolk after egg breaking. Various factors, such as egg storage conditions and the age of laying hens, can affect the quality of this membrane. Considering the lengthening of the production period for laying hens, the aim of this study was to investigate the effects of both hen age and storage conditions on the physical and biochemical properties of the VM. Eggs from hens aged 32-34 (peak of lay), 71-73 (late laying period) and 94-99 (very late laying period) weeks were stored for up to 28 days at 4°C or 20°C. Several egg quality parameters were measured (n≥20 eggs per condition): yolk mass, yolk index, deformation and breaking strength of the VM. In parallel, VM samples were collected to analyze their protein composition by SDS-PAGE and mass spectrometry (6 pools of 4 egg samples per condition). As expected, the four quality parameters studied were significantly altered by both hen age and storage time. In particular, VM deformation and rupture strength in eggs produced by 94-99 week-old hens decreased by about 6% and 11-13%, respectively, compared with younger hens. A higher diminution of these two parameters was observed with storage time (average decrease of 24% and 28% in eggs stored for 28 days), regardless of hen age. High storage temperature also had a significant negative effect on these parameters, with the exception of yolk mass, which did not change significantly between 4°C and 20°C. Importantly, no interaction between hen age and storage conditions was observed in our conditions. The protein profile of solubilized VM was also altered, especially in eggs stored for 28 days at 20°C. At least 219 proteins were identified by the proteomic approach, and the quantification revealed differential relative abundances (Log<sub>2</sub> Fold Change ≥ 1 or ≤ -1 with p-value ≤ 0.05) for half of these proteins as affected by hen age and/or storage conditions. In conclusion, increasing the production period of laying hens has a negative impact on the mechanical properties of the VM, but it does not seem to worsen its degradation kinetics during egg storage. In addition, our proteomic analyses allowed the identification of proteins potentially involved in alterations of the VM induced by hen age and egg storage.

Keywords: Egg; vitelline membrane; hen age; storage; quality; proteomics

## **Nutritional value and sensory characteristics of eggs from laying hens fed hempseed**

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In recent years, the poultry industry has been focused on feed ingredients to produce table eggs with improved nutritional quality by enhancing n-3 fatty acid (FA) content. The present study evaluated the effect of hempseed supplementation (as environmental and nutritional enrichments) over the diet on the chemical and mineral composition of eggs, cholesterol content, and the FA profile of the yolk, besides the egg sensory characteristics. To this purpose, 900 Hy-line Brown laying hens were randomly allocated in 4 pens of an experimental aviary and fed a corn and soybean meal-based diet. From 37 to 40 weeks of age, in two pens, whole hempseeds were distributed (10% of the daily feed intake) in two circular feeders per pen once a day. A total of 480 eggs (60 per pen per sampling time) were collected after 14 and 28 days of feeding laying hens with hempseeds. The chemical composition and cholesterol content of egg yolk were not affected by either the diet or the sampling time ( $P > 0.05$ ), whereas FA profile (expressed as % of total fatty acids) was largely affected by hempseed supplementation. Namely, total saturated FA (SFA;  $P < 0.001$ ), in particular myristic and palmitic acids, decreased in the yolks from hens fed hempseeds compared to control hens, as for total monounsaturated FA (MUFA;  $P < 0.05$ ). Both n-3 (+30%;  $P < 0.001$ ) and n-6 PUFAs (+11.4%;  $P < 0.05$ ) increased, whereas n6/n3 PUFA ratio (-20.17%;  $P < 0.001$ ) decreased in eggs from hens fed hempseed compared to control hens. Dietary treatment did not influence the mineral composition of yolk or albumen ( $P > 0.05$ ). The sampling time did not affect the total SFA, MUFA, and PUFA of yolk lipids ( $P > 0.05$ ), whereas the yolk and albumen of the eggs collected on day 14 had higher levels of sodium, magnesium, and phosphorus ( $P < 0.05$ ) than eggs collected on day 28. At the sensory evaluation, in the ranking test, 46% of the participants found out the difference between boiled eggs from hens receiving or not hempseed supplementation. Moreover, the ranking of the eggs did not indicate the preferences of the participants for one type of egg over another ( $P > 0.05$ ). In conclusion, the supplementation with hempseed at 10% of daily feed intake in laying hens improved the FA composition of yolk lipids without impacting the mineral content or the sensory profiles of the boiled eggs.

Keywords: *Cannabis sativa*; yolk; fatty acids; minerals

## **Determination of elemental composition of chicken eggs using X-ray fluorescence spectroscopy**

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Eggs provide a wide range of minerals, vitamins and protein for humans. Due to their low production cost, nutritional advantages, and lack of religious and cultural restrictions among animal-based food sources, eggs contribute an important part in the human diet. Elemental analysis is used as one of the methods to evaluate the nutritional advantages of chicken eggs. X-ray Fluorescence (XRF) spectroscopy can be applied as a potential technique to trace elements and minerals in chicken eggs. This method allows the simultaneous detection of elements with an atomic number of 11 (Sodium) and higher and has been widely applied in elemental analysis of various materials. While benchtop spectrometers offer extremely low limits of detection down to ppb and can thus be used for fingerprinting, handheld devices can be used in situ, offering the opportunity for rapid, low-cost and green quantification of the elemental composition of solid and liquid samples. Thus, this method is advantageous compared to standard analytical methods such as inductively coupled plasma mass spectrometry (ICP-MS). The study has been carried out using X-ray Fluorescence (XRF) spectroscopy of chicken eggs from two housing systems of “free range” & “barn”. Samples were collected from the local supermarket. After separating the edible and non-edible parts, egg whites and egg yolks were dried separately for further analysis. In total 27 elements, ranging from Magnesium (atomic number 12) to lead (atomic number 82) have been analysed. In egg white, Mg, Al, Sc, V and Ce could not be detected. Traces of Ti, Mn, Co, Cu, Zn, Ge, Se, Br, Rb, Sr, Ba, and Pb were identified in the range of 0.4 – 17.7 mg/kg. The elements P, S, Cl, K and Ca contributed the main elements ranging from 283.7 mg/kg for phosphorous to 10326.3 mg/kg for calcium. The results of elemental composition provide not only the nutritional value of different parts of table eggs but will also be used for fingerprinting of eggs from different origin.

Keywords: Elemental composition, Chicken eggs, XRF, Free range, Barn

## **Incidence of internal spots in commercial eggs in Spain**

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Consumers demand homogenous and quality products. Although they cannot be perceived at the time of purchase, internal specks are rejected and lead to complains at the supermarket. In this study, we have focused on the incidence of these quality problems in Spain, assessing different factors that could affect their presence. From January 2020 till December 2022, a total of 215346 eggs from brown laying lines and 5829 from white laying lines have been analysed as part of the quality control protocol of the main Spanish egg producer. They were obtained in 14 production farms disseminated throughout the country, with hens in cage-, barn-, free-range- and organic systems (corresponding to 53.4%, 10.2%, 33.4% and 3.0% of the eggs, respectively in brown lines, and 100% in cages in white lines). At the packing centre next to each farm, once a week 30 eggs per house were analysed in the morning of the same day of laying. Meat and blood spots were assessed as internal spots after opening the eggs, classifying them into one of three categories: clean eggs (no spots), spots smaller than 2mm and spots bigger than 2mm. Data were analysed with SPSS 26.0 by a X2 test assessing the effect of the housing system, egg weight, season, animal age and Haugh Unit (HU) on the frequency of internal spots occurrence. As expected, white eggs showed a higher incidence of clean eggs (99.4%) than brown eggs. In this case, the housing system highly influences the presence of internal spots ( $p < 0.001$ ), since cage-, barn-, free-range- and organic systems had 75.5%, 71.4%, 86.6% and 86.8% of clean eggs, and 8.8%, 9.9%, 2.6% and 2.5% of eggs with spots  $> 2$ mm, respectively. Hens younger than 40 weeks old presented the lowest incidence of eggs with spots  $> 2$ mm (5.9%, 7.4%, 1.7% and 1.6%, respectively for the previously mentioned systems), increasing with age in each system. This relates to the egg's weight, since eggs between 43-53 g had the highest rate of clean eggs, and eggs heavier than 73 g, the lowest. In outdoor systems, the lowest percentage of clean eggs occurred in spring (84.3% and 83.3% in free-range and organic, respectively). Those eggs with HU over 90 also showed higher percentage of clean eggs (79.9%) than eggs with less than 85 HU (72.3%). In conclusion, although the majority of eggs commercialised in Spain have no internal spots, the occurrence increases in indoor housing systems, heavier eggs from older hens, eggs with lower HU or in spring in outdoor housings.

**Keywords:** Blood spots, meat spots, housing system, age, season, Haugh unit

## **Cut-off rate of floor eggs in the cage-free system: exploring possible contributing factors**

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Australian supermarkets have changed from a predominant supply of eggs sourced from hens housed in cages to cage-free systems. However, a common occurrence inside cage-free systems is the eggs laid outside the nest boxes, (i.e. floor eggs). As initial research into variables that may contribute to the incidence of floor eggs within Australian farms, questions about floor eggs were included in a larger study on Analytical epidemiological of Spotty Liver Disease, that was funded by industry body – Australian Eggs. Sixty-nine flocks housed in either conventional or free-range systems from 5 of the 6 Australian states were enrolled in the study. For each flock, the percentage of floor eggs was recorded, and all variables classified as either categorical or continuous were collected. Categorical variables included: farm location, farm system and indoor housing, additional perches, platforms, light colour, strain of hen, and incidence of feather pecking. Continuous variables were pullet age and number at transfer to the laying shed, shed floor area, stocking density, nest space and density, first-age access to nests, and outside area in free-range. The impact of each variable at nominated 2% incidence of floor eggs as being a concern to produce saleable eggs was analyzed. Floor egg levels ranged from 1 to 28%, with  $\geq 2\%$  being identified as a problem and values  $< 2\%$  being an acceptable level of laying eggs on the floor. Categorical variables were assessed using contingency tables and Pearsons Chi-square, or when the expected value was  $< 5$ , Fisher's two tailed exact test was used. Analysis of continuous variables was via Student t-test. None of the continuous variables analysed were found to contribute significantly to the incidence of floor eggs. However, the categorical variable of strain of hen was significant on the percentage of eggs laid on the floor ( $P = 0.035$ ). Among 69 flocks, 14 flocks involved strain ISA-Brown, 47 flocks used strain Hyline Brown, and 7 used strain Lohmann. The use of strain ISA-Brown (8 of 14 farms) reported the problem of eggs on the floor compared to strain Hyline Brown (10 of 37 farms) and strain Lhomann (2 of 7 farms). This survey was not designed specifically for floor eggs, and therefore these preliminary results will be used in a larger survey aimed to identify factors associated with floor eggs in Australian cage-free systems.

Keywords: laying hens; egg production; floor eggs; ground eggs; cage free



## **Impact of eggshell translucency and trace mineral supplementation on eggshell quality parameters**

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The poultry industry is facing a problem with shell quality of broiler breeder eggs. The use of elevated amounts of trace minerals such as zinc (Zn), manganese (Mn), and copper (Cu) within a broiler breeder diet has been previously linked to an increase in eggshell quality, which in turn may lead to an increased hatchability. A parameter that has been linked to increased eggshell quality is shell translucency. The translucency level of the eggshell can be affected by the mineral levels within the diet. In this study, Zn, Mn and Cu were added to the water of commercial broiler breeder houses at the level of 40, 40, 7 ppm, respectively. This was conducted on 4 farms with the second adjacent house not receiving any additional trace mineral supplementation. The objectives of this study were to explore the effects of added amino-acid complexed minerals supplementation on shell translucency scores, coloration lightness, thickness, and breaking strength. In addition, the effect of eggshell translucency score on coloration lightness, thickness and breaking strength was also determined. Translucency was completed with Zinpro® BlueBox™ using a 3-point score system: TS1=none or few small translucent spots; TS2=several small translucent spots; TS3=many large translucent spots. Data was analyzed using PROC GLM procedures in SAS 9.4 with alpha set at 0.05; means were separated by Tukey's Studentized Range Test when appropriate. The results showed that mineral supplementation increased coloration lightness from 74.9 to 75.1 ( $P=0.0245$ ), shell thickness from 0.451mm to 0.455mm ( $P<0.0402$ ), TS1 eggs by 1.05%, TS2 eggs by 5.86%, and decreased TS3 eggs by 6.91% ( $P<0.0001$ ). Significance was found in shell thickness ( $P<0.0001$ ) between all levels of translucency: TS1 eggs (0.440mm), TS2 eggs (0.452mm), and TS3 eggs (0.462mm). Significance was also found for coloration lightness ( $P<0.0001$ ) between TS3 eggs (74.2) when compared to TS1 (74.8) and TS2 (75.2) eggs. Furthermore, mineral supplementation increased egg hatchability from 74.1% to 75.4%. This study suggests that translucency score is an effective predicting parameter for other eggshell quality characteristics and hatchability. It also suggests that amino-acid complexed minerals supplementation to broiler breeders can positively impact eggshell quality and hatchability.

Keywords: eggshell translucency; trace mineral supplementation; eggshell quality; broiler breeders

## **Non-invasive early assessment of chicken egg fertilization through VOC profiling using HSSE-GC-MS**

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Determining chicken egg fertilization status non-invasively before or during early incubation holds significant potential for optimizing the efficiency of the poultry industry. So far, no robust method has been established to assess fertilization earlier than incubation days 3 or 4. Over the past decade, only two studies reported the potential of volatile organic compounds (VOCs) to carry crucial biological insights into egg fertility. However, further research was said to be required to gain a deeper understanding and utilize this information to predict fertility and evaluate embryonic development. To address this gap, we incubated 45 Isa Brown eggs under standard conditions and sampled VOCs on days 0, 2, 4, 6, 8, 10, and 12. For each measurement day, eggs were individually enclosed in airtight glass jars together with PDMS-coated stir bars for VOC extraction at 37.7 °C. After 2 h of accumulation, VOCs adsorbed to the stir bars were identified using headspace sorptive extraction-gas chromatography-mass spectrometry (HSSE-GC-MS). Multiple logistic regression and PLS-DA models for fertility prediction were built and hierarchical cluster analysis was performed to assess VOC emission dynamics. A total of 162 VOCs were identified with alcohols and carboxylic acids being the most abundant. Hexanal levels were elevated in unfertilized eggs, whereas compounds such as propan-2-ol, propan-2-one, hydrazine, and carboxylic acids were higher in fertilized eggs. The fertility prediction model obtained through multiple logistic regression was preferred given its lower complexity and better validation performance. Prediction accuracies ranged from 68% to 75% during the initial 4 days, with an accuracy of 75% on day 0, and subsequently reaching 85% and 100% on days 6 and 8. Concerning the emission dynamics, VOC levels peaked on days 0 and 2, followed by a decline in most chemical classes, except for carboxylic acids. Plausibly, the increased carboxylic acid abundance was linked to rising embryonic metabolism and enhanced CO<sub>2</sub> production. In conclusion, egg fertilization status can be non-invasively assessed through VOCs during early incubation. Future studies targeting specific VOC biomarkers could improve VOC quantification and, consequently, fertility predictions. Finally, these new insights into the emission dynamics provide additional support for comprehending the biological information encoded in VOCs and their connection to biochemical processes in developing embryos.

**Keywords:** Volatile Organic Compounds (VOCs), Chicken hatching eggs, Fertility, HSSE-GC-MS, Multiple logistic regression, PLS-DA, Hierarchical cluster analysis

**Non-invasive embryonic blood vessel detection during automated in-ovo sexing****C. Van Der Pol<sup>1</sup>, J. Snijders<sup>1</sup>, L. Van Den Tweel<sup>1</sup>, A. Pennings<sup>1</sup>, J. Wijnen<sup>1</sup>**<sup>1</sup>HatchTech, De Klomp, NetherlandsPresenting author: [cvdpol@hatchtech.com](mailto:cvdpol@hatchtech.com)

The common industry practice of culling newly hatched male layer chicks faces increasing ethical concerns and legislative limitations. A practical solution to this problem is determining the sex of the chicken embryo while it is still in the egg, so called “in-ovo sexing”. Embryonic allantoic fluid can be used for in-ovo sexing through biomarkers such as hormones and DNA. The Circuit is an automated sampling system that collects allantoic fluid from hatching eggs between embryonic day (E)9 and E12, before the embryo develops consciousness. The Circuit lasers a 0.3 mm hole in the eggshell, after which fluid is sampled from the egg without penetrating the egg using suction pipettes. To optimize this process, the effect of hitting or avoiding extra-embryonic blood vessels directly underneath the eggshell on sampling success and embryonic survival was investigated. In a first experiment, 833 LSL Lite hatching eggs were lasered on E9 and then candled to see if any blood vessels had been hit by the laser. The eggs were divided into three groups: arteries and veins hit, arterioles and venules hit, or no blood vessels hit. On E11, embryonic mortality was checked through candling. When arterioles and venules were accidentally hit, percentage of alive embryos was 3.6% lower than for eggs where the laser did not hit any blood vessels. When arteries and veins were hit, percentage of alive embryos was 11.8% lower than when no vessels were hit (all  $P < 0.001$ ). In a second experiment, the effect of avoiding blood vessels on sampling success was studied. A 30 $\mu$ l allantois sample without blood contamination was categorized as a successful sample for sex analysis. 5,280 LSL Classic hatching eggs were either lasered randomly (meaning that in some cases, blood vessels were hit) or vessels were actively avoided by aiming for an area on the eggshell without blood vessels. More successful allantois samples were obtained from eggs in which the blood vessels were avoided (8.1%;  $P < 0.001$ ) compared to eggs that were lasered randomly. It was concluded that avoiding blood vessels when lasering a hole for in-ovo sexing greatly contributes to successful allantois sampling and embryonic survivability. Therefore, vision hardware and software have been developed on the Circuit to avoid lasering on extra-embryonic blood vessels during the sampling process.

Keywords: in-ovo sexing; embryonic development; incubation

## **Epigenetic programming of avian hypothalamus by in-ovo green monochromatic illumination and its effect on growth, appetite and metabolism in broilers**

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The embryonic period is widely acknowledged as a pivotal phase in the development of broilers. It is during this phase that the avian brain is highly sensitive to a wide array of stimuli or environmental changes. Recent studies have shown that in-ovo monochromatic green illumination (GMI) improves broiler's growth at 35 days of age, through elevation of the somatotrophic axis activity. This study aims to investigate whether exposure to GMI during chicken embryonic development can induce epigenetic "programming" in the hypothalamus, a brain region that play pivotal roles in regulating growth, appetite and metabolism. 150 Ross 308 broiler eggs were incubated under four different lighting regimes: no lighting (Control), green light illumination throughout the 21-day incubation period (Green), white light illumination throughout the 21-day incubation period (White) and green light illumination during the last three days of incubation (Green 3 Days). At day of hatch, hypothalami from all chicks along with full brains were collected. Quantitative real-time PCR (qPCR) analysis was used to measure mRNA expression levels of growth, development, photoreception, and appetite-controlling genes. Additionally, chromatin immunoprecipitation (ChIP)-qPCR analysis was performed to establish the binding levels of H3K27AC on the transcriptional sites of targeted genes. A significant elevation was shown in key hypothalamic genes related to growth (GHRH), appetite (AgRP) and metabolism (TRH), in chicks from the Green 3 Days treatment group, in comparison to control. Moreover, we identified in the same treatment group increased acetylation binding levels on histone 3 Lysine 27 (H3K27ac), which are key epigenetic modification that promotes transcription. Notably, exposure to GMI during the embryonic period heightened the responsiveness of the hypothalamus to green light, even persisting after hatching, as we witnessed a higher hypothalamic responsiveness from the same treatment group in immunofluorescent stainings. This observation hints at the presence of a molecular memory effect. Collectively, these results indicate that in-ovo exposure to GMI, specifically in the last three days of incubation, induce stable epigenetic modifications that can alter gene expression and ultimately improve broiler's growth, as well as suggesting that embryonic environmental factors may have a trans-generational effect that can be used to enhance production efficiency and sustainability.

**Keywords:** green monochromatic illumination, neuro-epigenetics, embryonic photostimulation

## **Effect of thermal manipulation of slow-growing broiler chickens on chick quality and physiology**

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The ability to absorb changes and to anticipate future perturbations through adaptive capacity has been shown to be affected by early life experiences that can already be applied during the perinatal period. Adjusting the incubation temperature profile can f.e. program chickens during the embryonic phase to cope better with various challenges in later life, such as fluctuations in temperatures and/or exposure to pathogenic challenges. Especially slow-growing broiler chickens, that are often used in organic rearing systems with outdoor access, may benefit from this so-called embryonic thermal manipulation as they may experience more extreme temperatures and higher pathogen exposure than indoor reared animals. However, most studies about thermal manipulation have been performed in fast-growing broiler chickens. This study investigated the effects of three different embryonic temperature profiles on chick quality and physiology at hatch of slow-growing broiler chickens, often used in organic rearing systems with outdoor access. Slow-growing broiler chicken embryos (Hubbard, JA657) were exposed to one of 3 eggshell temperature profiles during embryonic day (E)8 until E16: (1) constant eggshell temperature (CON; 37.8°C), (2) thermal manipulation (TM) with daily 12 h of a high eggshell temperature (HIGH; 38.9°C), or (3) TM with daily 12 h of a high or low eggshell temperature (HIGH/LOW; 38.9°C/37.6°C). Several chick quality characteristics per sex were measured at 6 h after emergence from the eggshell and blood was collected to assess the metabolic, antioxidant and inflammation status. Additionally, samples of the skin from the breast and upper thigh were collected to assess the epidermis and dermis thickness and blood vessel perimeter. Samples of the jejunum and bursa of Fabricius were collected to assess villi characteristics. Results showed no effect of thermal manipulation on chick quality parameters such as yolk-free body mass, chick length, navel quality or relative organ weights in slower-growing broiler chickens ( $P \geq 0.10$ ). Data of skin, jejunum, and bursa of Fabricius measurements are currently processed and will be presented during the conference. No negative effects of TM were found on chick quality. In a subsequent study, effects of these TM treatments on resilience in later life will be evaluated by measuring physiological and behavioral parameters.

**Keywords:** incubation, thermal manipulation, chick quality, slow-growing chickens

## **Impact of feed restriction on testicular parameters in broiler breeder roosters during the production period**

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There are many concerns regarding broiler breeder roosters, mainly due to extensive genetic selection for improved meat production at the expense of breeding capacity. Numerous studies focus on the relationship between reproductive system and different feeding aspects (quality, quantity, and rationing). Understanding fertility decline in commercial farm conditions, where natural mating occurs, is crucial. Our objective was to replicate an accidental event in field conditions, testing hatchability percentage recovery after severe feed restriction and underlying changes in reproductive parameters. We monitored a control group of roosters and compared them to those subjected to severe feed restriction and subsequent recovery. Data included body weight, fleshing condition, testes weight, testosterone levels, comb area, footpad condition, testes histology and hatchability percentage. Roosters were studied from 35-45 weeks of age, divided into four phases: pre-restriction, post-restriction coinciding with maximum weight loss, and two recovery phases. Testes weight reduction coincided with body weight loss, testosterone decline, seminiferous tubular diameter reduction, and testicular regression with halted spermatogenesis. Comb area decreased two weeks later, recovering two weeks after body weight recovery. Hatchability experienced two declines, the first coinciding with maximum weight loss and the second occurring 49 days post-restriction. Hatchability percentage recovery was observed 63 days after the restriction began. Despite severe food restriction causing significant reproductive alterations, it was proven that these could be reversed. Testicular dynamics resembled those of wild birds influenced by seasonal reproductive cycles, indicating domestic roosters' preserved sexual adaptability under moderate stress.

**Keywords:** Roosters; fertility; food restriction; testicular regression; recovery

**Incubation of chicken eggs should take 24 days instead of 21 days****J. Wijnen<sup>1</sup>, A. Pennings<sup>1</sup>, J. Snijders<sup>1</sup>, C. Van Der Pol<sup>1</sup>**<sup>1</sup>HatchTech B.V., De Klomp, NetherlandsPresenting author: [jwijnen@hatchtech.com](mailto:jwijnen@hatchtech.com)

In commercial poultry industry over half of total embryo mortality occurs at the start of incubation. Roughly 4.5 % mortality during the first 3 days of incubation is generally accepted, whereas the target for the total incubation period (21 days) is only 8.9 %. This has always been deemed unavoidable due to the vulnerability of young embryos. However, literature showed that early embryo mortality can be dependent on the rate with which eggs are warmed from storage- to incubation temperature ( $\leq 18-37.8^{\circ}\text{C}$ ). A gradual linear increase in egg temperature during 24 hours lowered early embryo mortality in long stored broiler eggs compared to rapid warming (4 hours). This was the first and only study until now that investigated gradual warming of eggs at the start of incubation and, therefore, the effect of warming durations longer than 24 hours remained unknown. The current study investigated the effect of slowly ( $+0.06^{\circ}\text{C} / \text{hour}$ ) and linearly warming eggs during 6 days (Slow) from storage to incubation temperature compared to a warming duration of 22 hours (Control). Thereafter, all eggs were incubated at  $37.8^{\circ}\text{C}$  eggshell temperature till hatch. In total 80,640 eggs were incubated in 11 consecutive experiments. Eggs originated from either broiler (Ross308) or layer (DekalbWhite) flocks of various ages (26–58 wk) and were stored between 0-22 days prior to incubation. Embryo mortality through candling and break-out was always determined, hatchability was often calculated, and sometimes chick quality was measured at hatch moment. Early embryo mortality was lower for Slow compared to Control in 9 out of 11 experiments ( $P \leq 0.02$ ) with an average difference of 7.4 %. Hatchability was higher for Slow compared to Control in 3 out of 5 experiments ( $P \leq 0.04$ ) with an average difference of 9.1 %. Chick quality was improved for Slow compared to Control in 2 out of 3 experiments ( $P = 0.01$ ), expressed by longer length whereas navel score did not differ between treatments ( $P \geq 0.21$ ). The Slow treatment prolonged the duration from set till pull with 3 days compared to Control, resulting in a total incubation duration of 24 days. It can be concluded that early embryo mortality is affected by the heating profile from storage to incubation temperature. Incubating chicken eggs during 24 days including a very gradual egg warming process at the start significantly reduces early embryo mortality and it could be considered as a strategy to enhance hatchability and to improve chick quality.

Keywords: Incubation temperature, embryonic development, broiler

## Rediscovering Italian poultry treasures: reviving the Millefiori Piemontese breed

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In Italy, 53 local chicken breeds are officially recognized, with most of them being endangered or extinct. Three breeds originate from the Piedmont region in northwest Italy: Bionda Piemontese (BP), Bianca di Saluzzo (BS), and Millefiori Piemontese (MP). The MP was considered extinct in the 1990s, but a few birds were recently found in the region's rural areas. Since 2015, a conservation and selection program for BP and BS has been conducted at the Avian Conservation Center (ACC) of the University of Turin. The aim of this research was to reconstruct a population of MP to ensure the conservation of the breed. As a first step, 53 birds phenotypically considered MP were sampled from five rural farms. By microsatellite analysis 98 alleles were observed; of these, 17 were private alleles of two farms. The average number of alleles per locus ( $N_a$ ) was 3.77, and the effective number of alleles ( $N_e$ ) was 2.47. Observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) were 0.56 and 0.53, indicating a low level of inbreeding. From both phenotypic and genetic perspectives, MP was clearly different from BP, BS, and other Italian breeds. For this study, the first MP generation was created by collecting 150 eggs from three small rural farms. These eggs were incubated simultaneously with approximately 500 eggs from BP and BS obtained from the ACC. All birds received the same feed and were raised under the same conditions throughout the entire experiment. At 277 days old, 6 (BP and BS) or 4 (MP) families were established, each consisting of 1 male and 10 females. Egg production was recorded daily for each family. Two simultaneous incubation tests were conducted at 300 and 368 days of age for each breed. Eggs collected over 10 days were incubated, resulting in the following totals: MP=253, BP=804, and BS=683. Eggs were stored in a controlled environment at 18-20 °C and 80% RH, and then incubated at 37.8 °C and 55% RH (Victoria I-9, Italy). The average incubation outcomes were as follows: a) Fertility: MP: 78.9%; BP: 92.7%; BS: 90.8% (MP vs BP:  $p < 0.001$ , chi-square=25.22; MP vs BS:  $p < 0.001$ , chi-square=32.56; BP vs BS:  $p > 0.05$ ); b) Hatchability: MP: 78.7%; BP: 84.3%; BS: 78.9% ( $p > 0.05$ ). MP had a lower fertility rate compared to BP and BS, but hatchability was similar across all breeds, indicating that MP eggs can adapt to standard incubation parameters. These results suggest that a more effective breeding and mating program is necessary to improve the fertility rate of MP.

Keywords: biodiversity; autochthonous chicken breeds; productive performance



## **Carvacrol fed to broiler breeder hens was transferred to fertile eggs enhancing embryonic gut development**

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Plant essential oils (EO) have the potential to improve gut health and performance in broiler chickens. Carvacrol is a bioactive compound present in several EOs. The objective of this study was to investigate the possibility of improving the embryonic development of broiler chickens by feeding carvacrol to female breeders. We hypothesized that carvacrol fed to broiler breeder hens can be transferred to the fertile eggs and enhance embryonic development. A total of 96 female and 12 male broiler breeders Ross 308 (30 weeks old) were randomly distributed across 12 floor pens, one male and eight females in each. Birds were divided into two treatment groups with six pens in each group. Each group was fed either a control diet (CP 15%, ME 2800 kcal/kg) or a diet supplemented with 3.2mL/kg food grade  $\geq 98\%$  carvacrol. Feed was provided at 167 and 128 g/bird/day for females and males, respectively. The feeding trial was conducted over seven days. Birds were weighed at the beginning and the end of the experiment, and egg number and egg weight were recorded daily. Eggs from the last two days of the trial were used for yolk sampling (6 eggs /treatment) and incubation (32 eggs/ treatment). Blood samples from one hen per pen were collected at the end of the feeding trial. Yolk from eggs and chicks and blood samples were analyzed using GC-MS to measure relative carvacrol concentration. Body weight, organ weight, and chick quality at hatch were recorded. The results showed that carvacrol supplementation did not affect weight gain in the female breeders over the experimental period (90.8g vs 99.5g;  $P > 0.05$ ). Egg number and egg weight were not different between the control and carvacrol groups ( $P > 0.05$ ). FCR tended to be lower in the carvacrol compared to the control group (3.25 vs 3.64,  $P = 0.09$ ). Analyses of blood and yolk samples showed that the carvacrol group had significantly ( $P < 0.001$ ) higher amounts of carvacrol compared to the control (blood:  $0.15\mu\text{M}$  vs  $0.0\mu\text{M}$ ; egg yolk:  $0.94\mu\text{M}$  vs  $0.0\mu\text{M}$ , and chick yolk:  $0.46\mu\text{M}$  vs  $0.0\mu\text{M}$ , respectively). Hatchability between the carvacrol and the control treatment was not different ( $P > 0.05$ ), 94.8% and 96%, respectively. Intestinal weight, as well as combined gizzard and proventriculus weight relative to body weight at hatch was significantly ( $P < 0.05$ ) higher in the carvacrol group compared to the control (0.058 vs 0.054 and 0.065 vs 0.061, respectively). In conclusion, dietary carvacrol administered to broiler breeder hens was transferred to their blood and the yolk of the fertile eggs and resulted in improved development of the GIT in the chicks. This work was funded by AgriFutures Australia and Delacon Biotechnik.

Keywords: essential oil, broiler breeder, carvacrol, gut health, intestinal development,

## **The influence of nutrition on body composition and allometric growth patterns in laying hen pullets**

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Nutrition during rearing influences the growth curve and body composition of laying hen pullets, potentially influencing long-term lay. Therefore, we aimed to study the influence of rearing diet nutrient density (low -10%, Hy-Line breed standard and high +10% ME and essential amino acids, CVB formulated) on allometric growth curves. Per treatment, 360 one-day-old Hy-Line (W80) pullets were randomly divided over 8 pens, with 45 pullets per pen. The experiment lasted until 35 weeks of age and all hens were fed a breed standard diet from week 17 onwards. BW, feed intake and egg production were measured weekly. One pullet per pen was dissected weekly until week 17 and in week 20 and 35, and full body composition analysis was determined, including dry matter (DM), crude protein (CP), crude fat (CF) and crude ash. Data were subjected to mixed model analyses. To determine a multiphasic allometric relationship between body components, an overall growth curve was established and inflection points were determined. Egg production parameters until week 35 were not affected by rearing diet. From week 2 until 35, linearly higher BW was obtained by increasing diet density (except in week 18 to 20). Body CP grew in 2 phases of growth, initially at a similar rate as body DM with an inflection point around week 12, after which body CP grew slower than body DM. Body CP growth was not influenced by diet density. Body CF grew in 3 phases of growth, increasing relative to body DM at every inflection point. Feeding higher density diets increased the CF growth curve already from an early age, with a detectable impact starting at 2 weeks. The segmented regression analysis between body CP weight and breast weight showed an almost linear fit until 260g of breast (week 17). After this inflection point, the slope was much lower, indicating that body CP levels influenced other growth than breast weight, such as organs. Feeding a higher density diet significantly increased the breast weight at the same body CP level. Segmented regression showed that until approximately week 12, the fat pad weight growth was much lower than the body CF growth. This indicates that during this developmental phase, more fat was deposited into other body parts, such as intermuscular fat. After this first phase, the slope was twice as high, indicating that more body CF was stored in the fat pad. In conclusion, these results showed that rearing diet density influences body composition and growth curves of body CF more than body CP, starting at an early age.

Keywords: pullet development; rearing; nutrition; diet density

**Effect of dietary balanced protein levels during rearing on early egg production and egg quality of white and brown egg-type strains****J. Chew<sup>1</sup>, M. Zuidhof<sup>1</sup>, L. Star<sup>2</sup>**<sup>1</sup>University of Alberta, Edmonton, Canada, <sup>2</sup>Schothorst Feed Research, Lelystad, NetherlandsPresenting author: [chew1@ualberta.ca](mailto:chew1@ualberta.ca)

This study aimed to determine the impact of dietary balanced protein levels during rearing on early egg production and egg quality of Bovans Brown (Brown) and Babcock White (White) hens. This experiment consisted of a dose response study using six isocaloric diets containing varying levels of the recommended dietary balanced amino acid profile. These levels were 60, 70, 80, 90, 100 (control), and 110% of the breeder recommended dietary balanced protein level. Two additional choice treatments enabled birds to select from either 60, 80, and 100%, or 70, 90, and 110%. Brown and White pullets (n=128; 8 birds per strain × dose level) were floor reared in pens. All birds were fed with multi-feeder precision feeding stations that allocated the assigned diet to each individual bird, thus each bird was an experimental unit. Treatments were applied from 0 to 18 weeks of age, after which all birds received common commercial pre-lay and layer diets until the end of the trial at 30 weeks of age. Birds were photostimulated at 17 weeks of age. Data collection included pedigree egg production and egg quality parameters. The effect of dietary treatments, strain, and their interactions were analyzed using ANOVA with Tukey's multiple range test for mean separation. No differences were observed between dietary treatments in hen day egg production and total eggs per hen, and no interactions were found. To 28 weeks of age, White hens laid more eggs than Brown (50 versus 48 eggs per hen, respectively, P=0.02). There was no effect of dietary treatments on any egg quality parameters. Brown hens laid heavier eggs than White hens (57.6 versus 55.9 g, respectively, P=0.03). Relative to the total egg weight, the Brown birds laid eggs with greater albumen weights (69.1 versus 67.8% of egg, respectively, P<0.01) and White birds' eggs had greater yolk weights than the Brown birds (22.3 versus 21.1 % of egg, respectively, P<0.01). Eggs from the Brown hens had greater eggshell strength than eggs from the White hens (45.3 versus 39.7 N, respectively, P<0.01). At 26 weeks of age, Brown hens had greater egg mass than White hens (60 versus 58 g/d, respectively, P=0.01). In summary, in the current study, pullet diets ranging from 60 to 110% of the breeder-recommended dietary balanced protein levels did not affect egg production or egg quality to 30 weeks of age. The results suggest the potential for optimizing pullet nutrition strategies which may yield economic and environmental benefits.

Keywords: dietary balanced protein; precision feeding; laying hens; egg production; egg quality

## **Adequate dietary amino acid supply allows to moderately reduce dietary crude protein at the onset of lay without affecting laying performance of Isabrown layers**

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Knowledge of the reduction of dietary crude protein (CP) level in layer diets is quite poor and inconsistent, especially in the laying peak period. On the other hand, clear benefits in terms of environmental performance and health could be achieved if laying performance is maintained. In total, 960 Isabrown layers were fed 5 dietary treatments with 9 or 10 replicates of 20 layers, from 18 to 30 weeks of age. Treatments were iso-energetic, iso-digestible lysine and consisted of a standard CP diet (SCP; 17.0% CP, 2700 kcal AMEn/kg and 0.70% dig Lys); a lower CP diet reduced by 1.5%pt without (CP-1.5/AA-) or with (CP-1.5/AA+) controlling dietary amino acids (AA) beyond Thr and a lower CP diet reduced by 3.0%pt without (CP-3.0/AA-) or with (CP-3.0/AA+) controlling dietary AA beyond Thr. The reduction of dietary CP level was achieved by replacing soybean meal by sunflower meal, wheat and L-AA. Due to a high variability inter and intra-treatment in the beginning of the trial period, the statistical analysis was only carried out from 22 to 30 weeks of age. An interaction was observed between week of age and treatment for laying rate, egg mass, feed intake and egg weight ( $p < 0.05$ ), indicating stronger differences were observed between treatments after week 26. Reducing dietary CP level by 1.5% and 3.0%pt without controlling dietary AA reduced feed intake by 3.7g/d and 5.9g/d, but addition of dietary AA increased feed intake (+1.2g/d) in the -1.5%pt CP treatment. Similarly, reducing dietary CP level without controlling dietary AA supply reduced egg mass by 1.5 and 3.2g/d at -1.5 and -3.0%pt, but dietary AA supply reversed egg mass at least at -1.5%pt of CP (+1.1g/d). Interestingly, egg weight responded both to dietary CP and AA supply, as it was reduced by 1.6 and 3.0g at -1.5 and 3.0%pt of CP, respectively, and was increased by dietary AA supply by 0.7g at both levels of CP. Feed conversion ratio was not affected by dietary treatment. A stepwise decrease in broken, dirty and uncompliant eggs was observed due to dietary CP reduction, without any influence of dietary AA. Finally, carbon footprint was calculated thanks to Matriciel® tool and was reduced stepwise by reducing dietary CP (-4.2% with -1.5%pt CP, -6.2% with -3.0%pt CP). Dietary CP reduction at the onset of lay is achievable when reducing by 1.5%pt and controlling AA beyond Thr with benefits in terms of egg quality and environmental performance. However, further reduction beyond -1.5%pt does not allow to maintain performance and needs to be further explored.

Keywords: crude protein ; amino acid ; layer ; carbon footprint

## **Impact of soybean origin and harvest year on chemical composition, amino acid profile, and protein quality indicators of soybean meals collected from 2007 to 2023**

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Numerous factors influence the chemical composition and protein quality of the soybeans and thus, the nutritive value of the corresponding soybean meals (SBM). The objective of this research was to evaluate the effects of the country of origin of the original beans on the chemical composition, amino acid (AA) profile, and protein quality indicators of commercial SBM collected at random from 2007 to 2023. In total, 876 samples of SBM from Argentina (ARG; n = 279), Brazil (BRA; n = 292), and United States of America (USA; n = 305) were collected at crushing plants and feed mills at different locations of the European Union by specialized personnel. Samples were analyzed by wet chemistry for all the variables, except the AA that were determined by NIRS. The data were analyzed as a completely randomized design using the mixed procedure of SAS. The main effect of the model was the country of origin of the beans with harvest year considered as a random effect. The variance (%) due to harvest year was determined for all the variables studied. Moisture content was lower for the ARG meals than that for the BRA and the USA meals (11.1 vs. 11.8 and 11.6%;  $P < 0.01$ ). On 88% DM bases, BRA meals had more CP (47.3 vs. 45.8 and 46.5%), CF (4.79 vs. 4.13 and 4.10%), NDF (10.1 vs. 8.91 and 8.80%), and raffinose (1.35 vs. 1.28 and 1.12%) but less sucrose (5.71 vs. 7.22 and 7.15%) and stachyose (4.35 vs. 4.90 and 5.23%) than the ARG and USA meals ( $P < 0.001$ ). No differences among soybean origins were detected for ash or ether extract contents. PDI, KOH, and TIA values were higher for the USA than for the South American meals ( $P < 0.05$ ) but no differences were detected for urease activity or reactive Lys. The abundance and the profile of the AA varied with the country of origin of the beans. The profile (% CP) of Lys (6.15 and 6.11 vs. 6.07%) and TSAA (2.82 and 2.82 vs. 2.77%) were better for the USA and ARG meals than for the BRA meals ( $P < 0.001$ ). Harvest year explains part of the variability observed for most of the variables studied. For sucrose, stachyose, and raffinose, crop year justifies over 35% of the variability detected (42.7, 35.2, and 36.8%, respectively). Similarly, most of the variability among SBM for AA profile and protein quality indicators was aimed by harvest year. It is concluded that both harvest year and country of origin of the beans are relevant factors to be considered when studying the chemical composition, AA profile, and protein quality of SBM.

**Keywords:** amino acid profile; chemical composition; harvest year; protein quality indicator; soybean meal origin; variability

## **The impact of digestible lysine on egg weight control and eggshell quality in old Lohmann Brown hens during 82 to 92 weeks**

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Adjusting dietary amino acids has been proposed as a potential strategy for improving eggshell quality by slowing down increases in egg weight. This study investigated the effects of varying digestible lysine (dLYS) levels on production performance and eggshell quality in Lohmann Brown hens aged 82 to 92 weeks. A total of 240 hens were housed in individual cages and assigned to six treatments (40 replications per treatment). One of the treatments adhered to the recommended dLYS level (6.40 g/kg or 768 mg/bird/day), based on breeder guidelines. The remaining treatments were formulated to meet all nutrient recommendations except for dLYS, which was adjusted at varying percentages: +5% (6.72 g/kg), -5% (6.08 g/kg), -10% (5.76 g/kg), -15% (5.44 g/kg), -20% (5.12 g/kg) of the recommended dLYS level. The ratio of other amino acids to dLYS was kept constant among the treatments by using crystalline amino acids. For eggshell quality, ten eggs per hen were collected to measure eggshell-breaking strength (ESBS) during the last two weeks of the study. According to industry standards, eggs with ESBS lower than 3.0 kg.f were considered eggs with low shell quality (EWLSQ). The results showed that increasing dLYS from 5.12 to 5.76 g/kg (599 to 683 mg/bird/day) significantly increased hen-day egg production (HDEP) by 3.26%, whereas further increases in dLYS did not result in higher HDEP. On the other hand, body weight, egg weight, and feed conversion ratio were not affected by the dLYS levels ( $P > 0.05$ ). Varying dLYS levels influenced shell-less egg occurrence, with an initial decrease in shell-less eggs by increasing dLYS level from 5.12 to 5.44 g/kg (599 to 660 mg/bird/day), followed by an increase in the percentage of shell-less eggs by increasing dLYS level beyond 6.08 g/kg (731 mg/bird/day). The ESBS was not affected by the dietary dLYS levels ( $P > 0.05$ ); however, the percentage of EWLSQ was affected by dLYS levels ( $P < 0.05$ ). Increasing the dLYS level from 5.12 to 5.76 g/kg (599 to 683 mg/bird/day) did not affect the percentage of EWLSQ. This value was higher at 6.08 and 6.40 g/kg (731 and 752 mg/bird/day) compared to 5.76 g/kg (683 mg/bird/day) dLYS. The highest percentage of EWLSQ was observed at 6.72 g/kg dLYS (776 mg/bird/day), surpassing all other levels. In conclusion, optimal HDEP was observed at dLYS intake ranging from 683 to 731 mg/bird/day, with a lower percentage of EWLSQ at 683 mg/bird/day of dLYS.

**Keywords:** amino acids; eggshell quality; long-life laying hens; nutrient requirement

## **Cytokine expression during a mixed *Eimeria* infection in broilers given low protein diets supplemented with methionine or threonine**

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Given that most of the effector mechanisms of the immune response are proteinaceous in nature, amino acid intake affects the immune response of a host. This includes gene expression of pro- and anti-inflammatory cytokines which have important implications on the outcomes of an infection. This study investigated the effect of reduced CP diets supplemented with Met or Thr on cytokine gene expression during a mixed *Eimeria* infection. 768 day old Cobb 500 chicks were allocated to 1 of 4 experimental diets from d9-28; a high protein (19% CP), a low protein (LP) (16% CP) and the LP diet supplemented with Met or Thr at 50% above breeder recommendations. On d14 of age, half of the birds were inoculated with either 1ml of H<sub>2</sub>O and half were inoculated with a solution of sporulated oocysts (12,500 *E. maxima*, 12,500 *E. tenella*, 62,500 *E. acervulina*). The study was a 2 x 4 factorial design with 8 replicates per treatment. On d21 and 28, spleen and caecal tonsils (CT) from one bird per pen were taken to quantify cytokine gene expression. On d21, there was an interaction between diet and infection on IL21 expression in the spleen and CT due to increased gene expression in infected Met birds ( $P < 0.01$ ) vs all other treatments. An interaction was also observed on IL10 expression ( $P < 0.05$ ) due to a reduced expression in infected Thr birds vs all other treatments. Infection significantly increased the expression of iNOS, IL10, IL8 and IL21 in the spleen ( $P < 0.01$ ). In the CT, iNOS expression was significantly lower in infected birds ( $P < 0.05$ ), whereas IFN $\gamma$ , IL1, IL10, IL17 and IL21 expressions were greater in infected birds ( $P < 0.05$ ). On d28, an interaction between diet and infection was observed on the expression of IL17 in the spleen on d14pi ( $P < 0.05$ ), as there was a significant increase in expression in the infected 19% birds vs the uninfected 19% birds, with no other significant differences between treatments. Expression of iNOS, IL10 and IL17 in the spleen, and iNOS, IL1, IL17 and IL21 in the CT were significantly greater in the infected birds ( $P < 0.01$ ). Expression of IFN $\gamma$  in the CT was significantly lower in infected compared to uninfected birds ( $P < 0.01$ ). It has been suggested that early activation of cytokines such as IL21 and IL10 is key to the development of resistance to coccidiosis. Providing additional Met and/or Thr during infection may help to establish resistance and reduce the impact of coccidiosis, although this requires further investigation.

Keywords: *Eimeria*; methionine; threonine; cytokines; IL10; IL21

## **Is the expression of markers for breast muscle development in broiler chickens affected by breed, sex, age, and dietary energy level?**

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We hypothesized that breed (Ross 308, Hubbard JA757, RedBro, and Ranger Gold), age, sex, and dietary energy (standard vs low energy (LE; -150 kcal/kg)) affect mRNA expression of markers of breast muscle development. There were 16 treatments (4 breeds, 2 sexes, and 2 dietary energy levels) with 8 replicates per treatment and the bird as the experimental unit. Breast muscle samples from Ross 308 chickens were collected at 1, 20, and 37 d of age, and from slow-growing breeds at 1, 24, 37, and 56 d of age. A factorial design (3×2×2; breed × sex × diet) was applied to the slow-growing breeds at the same age. The age effect was compared within each breed. The assessed markers were: Atrogin-1 (ATRG1), with a negative correlation with muscle cell proliferation; Myostatin (MSTN), with a positive correlation with breast muscle weight; Muscular isoform of carnitine palmitoyltransferase 1 (M-CPT1), which regulates lipid metabolism; Acetyl-CoA carboxylase (ACC), a lipogenic enzyme; and Carbohydrate sulfotransferase 1 (CSRP3), with a positive correlation with white striping (WS). In Ross 308, ATRG1 expression was 2-fold higher ( $P<0.05$ ) at 37 d of age than at 20 d of age. An interaction sex × diet was observed in slow-growing breeds. The ATRG1 expression was 2-fold increased ( $P<0.05$ ) in female Hubbard JA757 fed the LE diet. ATRG1 expression decreased ( $P<0.01$ ) with time. Ross 308 birds fed the LE diet had a 2-fold increase ( $P<0.05$ ) of MSTN at 37 d of age, suggesting a compensatory mechanism to support muscle breast weight gain. No interactions were observed for MSTN in slow-growing birds, and the expression of MSTN increased ( $P<0.01$ ) with time in RedBro and Ranger Gold. An interaction breed × sex × diet was observed for CSRP3. Male Hubbard JA757 fed the standard diet had a 2-fold decrease ( $P<0.05$ ) of CSRP3 when compared with the other groups. Male RedBro fed the standard diet had a 2-fold increase ( $P<0.05$ ) of CSRP3 from 20 to 37 d of age, followed by a 3-fold decrease ( $P<0.05$ ) from 37 to 56 d of age. However, no signs of WS were observed in the sampled tissues. All slow-growing breeds showed a decreased expression ( $P<0.01$ ) of M-CPT1 and ACC with time. This indicates that decreasing dietary energy levels, intramuscular fat, and breast muscle lipogenesis were not affected by the dietary intervention. In summary, the expression of breast muscle development markers is breed-related and some of them (e.g. MSTN and CSRP3) can be affected by the breed, diet, and sex.

**Keywords:** Ross 308; Hubbard JA757; RedBro; Ranger Gold; Gene Expression; Breast Muscle



**Comparative effects of low-protein soybean meal or high-protein soybean meal with wheat bran or prebiotics in broilers challenged with Eimeria using immune response and bacterial antimicrobial resistance gene abundance****S. Veluri<sup>1</sup>, R. Shanmugasundaram<sup>2</sup>, A. Oladeinde<sup>3</sup>, R. Selvaraj<sup>1</sup>, J. Lawrence<sup>3</sup>, D. Cudnik<sup>3</sup>, G. Zock<sup>3</sup>, O. Olukosi<sup>1</sup>**<sup>1</sup>Department of Poultry Science, University of Georgia, Athens, United States, <sup>2</sup>Toxicology and Mycotoxin Research Unit, USDA-ARS, Athens, United States, <sup>3</sup>Egg and Poultry Production Safety Research Unit, USDA-ARS, Athens, United States

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A 42-day floor pen trial with 1,200 Cobb 500 broiler chicks was used to study the effect soy hull (SH) and wheat bran (WB) fibers compared to prebiotics on antimicrobial resistance (AMR) genes in cecal bacterial and immune parameters in Eimeria-challenged broilers. Ten treatments were arranged in a 5 (diets) × 2 (challenge; CH vs. no challenge; NCH) factorial with 6 reps and 20 birds/rep. Diets included 1. high protein soybean meal (HPSBM), 2. HPSBM + prebiotics, 3. HPSBM + WB, 4. low-protein SBM (LPSBM), and 5. LPSBM + xylanase. Soyhulls were added to HPSBM (48% protein) to produce LPSBM (43% protein). All the diets (maize-based) were isocaloric and isonitrogenous. On d15, birds in the CH group were challenged with Eimeria oocysts. High throughput TaqMan PCR was used to determine the abundance of genes associated with commensal and pathogenic bacteria and AMR determinants for tetracycline (e.g., tetL, tetM, and tetB) in cecal samples collected on d 14 and 21. Spleen and ceca tonsils were used to determine immunological responses on d21. On d14 (prior to the Eimeria challenge), the HPSBM group had higher levels of *C. perfringens* ( $P < 0.05$ ), *E. coli* ( $P < 0.05$ ), tetM ( $P < 0.05$ ), and strB ( $P < 0.05$ ) than birds fed LPSBM. Higher levels of *C. perfringens* were observed for LPSBM + xylanase than the LPSBM treatment group ( $P = 0.03$ ). On d 21, there was only a significant effect of diet on tetM and strB, where birds receiving HPSBM+ prebiotics had lower levels of tetM and strB ( $P < 0.05$ ). On d 21, splenocyte nitric oxide (NO) production was low in the NCH group. In the CH group, NO was lower in HPSBM and HPSBM + WB than HPSBM + prebiotics, and LPSBM + xylanase, but the LPSBM treatment had the greatest NO production. Spleen and ceca tonsil IL-10 mRNA expression was low in NCH group. In the CH group, IL-10 mRNA expression was highest for LPSBM, followed by HPSBM + WB ( $P < 0.05$ ) compared to the other diets. In conclusion, the LPSBM diet reduced the levels of *C. perfringens*, *E. coli*, and tetracycline and streptomycin resistance genes. In addition, LPSBM enhanced the anti-inflammatory response during the challenge. However, prebiotic supplementation to HPSBM reduced the levels of AMR genes in broilers challenged with Eimeria. Therefore, LPSBM is advantageous in enhancing the gut health of broiler chickens challenged with Eimeria, using a mode of action different from the effect of prebiotics addition to HPSBM.

Keywords: Low-protein soybean meal, high-protein soybean meal, microbiota, immune response

## Caecal microbiota composition in chickens of different genotypes fed low-input diets

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Due to the key role of gut microbiota on chicken health, its management can contribute to the improvement in the resiliency of poultry production based on alternative genotypes and low-input diets. Thus, the current study assessed the changes in the caecal microbiota composition in chickens belonging to a standard fast-growing genotype (Ross 308), two local breeds (Bionda Piemontese, BP; Robusta Maculata, RM), and their crosses (BP×S and RM×S) with a moderate-growth genotype (Sasso, S) fed standard and low-input diets. A total of 441 chickens were housed in 40 pens, half of the pens were fed a low-input diet (diet LI: ME 2,921 kcal/kg; CP 17.5%; lysine: 0.95%) and half a standard diet (diet S: ME 3,050 kcal/kg; CP 18.5%; lysine: 1.07%) from 20 days of age until slaughtering (47 days for Ross and 105 days for the other genotypes), when caecal contents were sampled from 60 chickens (12 for each genotype), and analysed using a 16S rDNA multi-amplicon sequencing approach. Within Ross chickens, sequencing results did not evidence any differences in the microbiota composition between chickens fed the two diets (False Discovery Rate, FDR,  $P > 0.05$ ). As for the other genotypes, differences in the beta diversity were found between chickens fed the standard and low-input diets in BP, BP×S, and RM×S chickens (PERMANOVA, FDR  $P \leq 0.05$ ) without significant differences as for the Shannon index for the alpha diversity measure and the Wilcoxon test for the differential abundance analysis. Then, as for the effect of the genotype, the beta diversity of caecal microbiota significantly changed between BP vs. RM and BP×S vs. RM (PERMANOVA, FDR  $P \leq 0.05$ ). In conclusion, under the conditions of the present study, moving from a standard to a low-input diet had no main effects on gut microbiota composition, whereas differences in gut microbiota composition according to genotype were not so relevant to be expected to modify poultry health, and welfare. Acknowledgement: This work was supported by Italian Ministry of Research and University funding project PRIN2017, grant number: 2017S229WC.

Keywords: Microbiota; Feed efficiency; Low-Input Dietary; NGS

## **Effects of in ovo feeding of sericin on hatching performances and hepatic gene expression of broilers**

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In ovo feeding (IOF) has shown promising results in improving the growth and immunity of broilers. A preliminary trial was conducted to evaluate the effects of IOF of sericin (SER) a silk protein, composed of at least 17 amino acids, on the hatchability parameters and hepatic gene expression of antioxidant enzymes, stress, and DNA-methylation-related genes on the day of hatch. A total of 210 fertile eggs from 29-week-old Ross 308 breeders were divided into 5 treatments of 42 eggs each. At Embryonic day 17.5, IOF of 0% (0SER), 1.5% (1.5SER), 3.0% (3.0SER), and 4.5% SER (4.5SER) solutions dissolved in distilled water was performed. The highest hatchability was recorded in the 0SER group (67.9%), followed by no-injection (UCON), 3.0SER, 4.5SER (66.7%) and lowest in 1.5SER (64.3%). UCON treatment was removed from further analysis after comparing the hatchability percentages numerically. Various organ weights (liver, yolk sac, gizzard, proventriculus, and heart) showed no differences among treatments. Hepatic gene expression of superoxide dismutase (SOD) was highest in 3.0SER and lowest in 4.5SER ( $p=0.044$ ) but showed no significant difference with 0SER. The relative expression of glutathione peroxidase (GPX) 1 and catalase (CAT) showed a linear ( $p=0.012$ ) and quadratic ( $p=0.023$ ) relation, respectively, while that of nuclear erythroid factor-2 (NRF2), NADPH oxidase (NOX)1, NOX4, heat shock protein (HSP) 70, and HSP90 remained unaffected. Among the DNA methylation-related genes, ten-eleven translocation (TET) 3 ( $p=0.028$ ) and Methyl-CpG Binding Domain (MBD) 4 ( $p=0.007$ ) were significantly downregulated in the treatment that received SER injection. Further, Pearson's correlation analysis revealed that the expression of NOX1, NOX4, HSP70, TET1, TET2, TET3, growth arrest and DNA damage inducible alpha (GADD45A) and MBD4 were highly correlated with each other. A weak correlation of DNA methyl transferase (DNMT) 3A with GPX1 and DNMT1 was also noticed. In conclusion, IOF of SER did not negatively affect hatchability characteristics in broiler chicks. However, it downregulated the hepatic expression of TET3 and MBD4 genes which were also found to correlate with HSPs and NOX genes, indicating a possibility of reduced free radical production due to reduced DNA-demethylation in the present study. There existed a weak correlation found between DNA-methylation-related genes (DNMT1, DNMT3A) and antioxidant enzyme (GPX1) which further strengthens our hypothesis.

Keywords: in ovo feeding; sericin; DNA methylation; hepatic gene expression

## **Dietary amino acid density affects growth rate and body composition of fast- and slow-growing broiler chickens**

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Knowledge around body composition and allometric growth can be used to define nutrient requirement to support optimal growth in broiler chickens. The objective of the present study was to evaluate growth rate and nutrient deposition of both fast- (FG) and slow-growing (SG) broiler chickens in response to dietary amino acid (AA) density. A 2 x 3 factorial design was applied, with two breeds (Ross 308 or Hubbard JA757) and three dietary amino acid levels (low, medium or high). A total of 540 broiler chickens were randomly divided within genetics breeds over 36 pens, with 15 chickens per pen. The study was divided into three phases: starter (day 0-14), grower (day 14-35), and finisher (day 35-42). Digestible lysine content of the medium AA diet of each phase was 1.22, 1.13 and 1.05%, respectively. Ideal protein profile was maintained over experimental diets. For the low and high AA diets, digestible lysine content was reduced and increased by 15 %. Average body weight gain (ADG) and average daily feed intake (ADFI) were determined weekly. Two chickens per pen were dissected on day 7, 21, 28, 35 and 42 and body chemical composition was determined. Data were analysed using mixed model in R. Incremental increase of dietary AA density linearly increased ADG of FG chickens but not of SG chickens, suggesting a lower AA requirement of SG chickens. Growth rate of FG chickens obtained from Gompertz equation increased from 0.031 to 0.049 with higher dietary AA densities, with an earlier inflection point from 51 to 36 d. No difference in growth rate was observed in SG chickens among experimental diets. Decreasing dietary AA density by 15% reduced breast meat yield while increasing dietary AA density by 15% did not further increase breast meat yield in both breeds. SG chickens fed reduced dietary AA diet showed a higher body fat growth over body protein growth probably due to an oversupply of energy. No differences in allometric growth were observed in FG chickens in response to dietary AA density. In conclusions, dietary AA density affects growth rate but not body composition of FG chickens. On the contrary, SG chickens show differences in body composition but not in growth rate when fed incremental AA densities.

**Keywords:** Body composition; Broiler chickens; Amino acid density

## Nutritional needs of slow-growing broilers

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In recent years, production methods for broilers have emerged. The retail sector in Belgium wants to implement higher animal welfare standards by 2026. To achieve this, it is suggested to shift to slow-growing breeds kept among other measures. The production of slow-growing broilers requires an adaptation of management measures, especially concerning the feeding strategy. Knowledge in this area remains limited today, hence a trial was conducted at the ILVO research farm. The objective was to gain insight on the nutritional needs of these slow-growing broilers while optimizing technical performance, meat quality, supporting the health of the animals at the same time. Fast growing (ROSS 308) and slow-growing broilers (Hubbard Redbro) were fed 1 out of 3 dietary treatments differing in energy, crude protein (CP) and amino acid levels until slaughter weight (d38 and d49). A standard diet was fed to the fast growing broilers (T1) as well as to one group of the slow-growing broilers (T2). Energy and CP values were 11.75MJ/kg and 20.5% (starter), 11.94 MJ/kg and 19.5% (grower) and 12.2MJ/kg and 18.5% (finisher), respectively. For the other 2 groups of slow-growing broilers, energy was reduced by 2.5% and 5% and CP by 7% and on average 10% for T3 and T4, respectively. Each treatment was repeated 8 times resulting in 240 male broilers per treatment. Performance, litter quality, food pad lesions, slaughter yield and meat quality was assessed. No significant differences in foot pad lesions and litter conditions were observed. Highest growth rate, feed intake and slaughter weight accompanied by the lowest feed conversion was observed for the fast growing broilers. A significant reduced performance was seen for their slow-growing counterparts (T2); that can be explained by different genetics. Further, a reduction of the energy as well as of the CP content for the slow-growing broilers negatively affected growth ( $p < 0.01$ ) and slaughter weight ( $p < 0.01$ ). Feed conversion only increased for the T4 group ( $p < 0.01$ ). Although slaughter yield and carcass characteristics were comparable between treatments, water holding capacity and shear force were lower for the fast growing broilers compared with the slow-growing. Within this genotype, broilers given T3 obtained highest meat quality. To conclude, a reduction of energy as well as of CP content for slow-growing broilers is feasible but limited as excessively low nutrient density negatively impacted the broiler performance.

Keywords: slow-growing; broilers; nutritional needs:

## **Association between genotypic and phenotypic characteristics of *E. coli* strains isolated from broiler breeder flocks**

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*E. coli* is characterized by high genomic diversity which complicates establishing an efficient immunoprophylaxis programme for protection of poultry in intensive production. Due to high heterogeneity and different classification systems, it is difficult to find a clear correlation between various genotypic and phenotypic characteristics of the field strains. In this study, 52 *E. coli* strains isolated from four flocks on two broiler breeder farms were analysed. The aim was to investigate a possible association between the genotypic and phenotypic characteristics of the strains. The investigated features included phylogenetic groups, sequence types (STs), biofilm-formation ability and antimicrobial susceptibility. Phylogenetic group determination was done through PCR using a protocol as described by Clermont et al. (2013), while ST identification was done based on whole-genome sequences analysed using the CGE online tools. Biofilm-forming ability assays were performed twice in triplicates using a quantitative adherence test on microtitre plates, and antimicrobial susceptibility was tested using microdilution method against 23 antibiotics. In total, six different phylogenetic groups and 12 STs were detected. Microdilution assay revealed that all strains were resistant to oxacillin and tylosin, but high resistance rates were also detected to tetracycline (28.85%), ceftiofur (26.92%) and nalidixic acid (15.38%). In total, 27 strains were multidrug resistant (51.92%). Interestingly, only four strains showed weak or moderate biofilm-forming ability. Statistical analyses of the results showed a significant association of the total number of antibiotics to which the strains were resistant and phylogenetic groups ( $p=0.013$ ), as well as STs ( $p<0.0001$ ). Logistic regression analyses revealed that strains belonging to phylogenetic groups which are considered commensal are significantly more likely to be multidrug resistant compared to pathogenic strains (OR 11.5,  $p=0.003$ ). When STs were analysed, the results showed that strains ST23 and ST135 are significantly more likely to be multidrug resistant compared to ST95 (respectively OR 713,  $p<0.0001$ ; OR 217,  $p=0.0003$ ). Overall results indicated a link between some phenotypic characteristics, specifically antimicrobial resistance, and individual phylogenetic groups and STs.

Keywords: *E. coli*; poultry; broiler breeder; ST; phylogenetic group; microdilution; AMR; MDR

## **Full characterization of 23 salmonella wild type bacteriophages suitable for the eradication of the most relevant salmonella zoonotic serovars**

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Despite the efforts carried both by the European Union (EU) and the poultry farmers, *Salmonella* spp. is still the second most frequent etiologic agent responsible for foodborne diseases in the EU. The poultry products and its derivatives stand as one of the main sources of *Salmonella* to humans. Added to the threat *Salmonella* is by itself, the uprise of drug resistant bacteria and multidrug resistant bacteria worldwide only worsen the prospects for conventional measures such as antibiotic therapy or disinfectants as prophylactic tools. In that sense, bacteriophages or phages arise a promising tool to both treat infections and recondition facilities. The aim of this study was to fully characterize *Salmonella* wild type phages isolated from poultry samples to assess which ones are more suitable to eradicate *Salmonella*. To this end, first, a phenotypic characterization was carried out based on Host-range Analysis and Transmission Electron Microscopy. The host-range analysis was performed through the spot-test against 48 different *Salmonella* field strains belonging to 18 serovars, including *S. Typhimurium*, *S. Typhimurium* monophasic variant, *S. Enteritidis* and *S. Infantis*. Hereafter, the bacteriophages that i) showed wider ranges of lysis serovar-wise, ii) did not resemble each other by specter of lysis and iii) morphologically differed from one another on the TEM images (N=23), were selected for further characterization. In vitro studies regarding their stability to different ranges of environmental conditions were carried with the selected phages; pH (2 to 12), different temperatures (-80 to 80°C) and ultraviolet radiation. Furthermore, extractions of DNA matter from high titer aliquots (10<sup>9</sup>-10<sup>10</sup> CFU/mL) were carried and sent to be sequenced with Illumina MiSeq. The sequencing results showed that the bacteriophage selection contained viruses from 4 different families and 10 different genus. The results also enlightened that out of the 23 phages selected for the study, 21 were genetically different from one another. Besides, out of the 48 *Salmonella* strains 47 (97,92%) were lysated by the 23 bacteriophages, lysing 100% of the *S. Typhimurium*, *S. Typhimurium* monophasic variant, *S. Enteritidis* and *S. Infantis* strains. The collection of natural wild-type bacteriophages, which infect a wide range of *Salmonella* spp. strains, offers the poultry sector a novel alternative for *Salmonella* control, serving as both therapeutic and prophylactic agents.

Keywords: *Salmonella*; Bacteriophage; Sequencing; Characterization; One-Health

[008-06]: Parallel B(6)-S08-Poultry Health: Antibiotic Resistance and Alternatives products (ID: 130722) - Australia

## **In ovo carvacrol delivery downregulated splenic inflammatory responses after a post-hatch lipopolysaccharide challenge in broiler chicks**

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The phytochemical carvacrol impacts immune function in broilers, potentially mitigating disease impact. But, effects of in ovo carvacrol delivery on early post-hatch disease resistance remain unknown. Hence, this study explored its impact on early immune responses, hypothesizing that in ovo carvacrol would lower inflammatory responses during an early lipopolysaccharide (LPS) challenge. The experiment included four groups in a 2x2 factorial arrangement: (1) in ovo saline + no challenge, (2) in ovo saline + LPS, (3) in ovo carvacrol + no challenge, (4) in ovo carvacrol + LPS. At embryonic day 17.5, 0.9% saline or carvacrol (0.5% v/v) with polysorbate 80 (1:1 v/v) in saline was injected in the amniotic fluid of fertile Ross 308 eggs. At d7 post-hatch, chicks were intraperitoneally injected with saline or 2 mg/kg LPS in saline, and spleens were collected 6h later (n=10). Relative mRNA expressions of IL1 $\beta$ , IL2, IL6, IL8, IL10, IFN $\gamma$ , TNF $\alpha$ , NF $\kappa$ B, I $\kappa$ B, TLR2, TLR4, AvBD6, IgM, IgY and IgA were measured by qRT-PCR. Data were analysed using two-way ANOVA. Interactions between in ovo delivery and challenge were found. For carvacrol-treated chicks, LPS challenge did not affect IL1 $\beta$  expression whereas saline-treated chicks had higher values after challenge compared to the control (P=0.03). IFN $\gamma$  expression was highest for carvacrol-treated, non-challenged chicks, compared to the control (P=0.02). For saline-treated challenged chicks, TNF $\alpha$  expression was higher than for both non-challenged groups (P=0.02). For NF $\kappa$ B and TLR4, no challenge effect was found for saline-treated chicks, whereas LPS challenge resulted in lower values for carvacrol-treated chicks than for the control (P=0.03; P=0.02). No main effects were found for in ovo treatment, however, LPS challenge increased expressions of IL8, IL6 and IL10 (P<0.001), and decreased I $\kappa$ B, AvBD6, IgM (P<0.001), IgY and IgA (P<0.05). These results suggest that in ovo carvacrol delivery has anti-inflammatory effects by inhibiting LPS-induced TLR4 activation, thereby downregulating NF $\kappa$ B, which mitigates IL1 $\beta$  and TNF $\alpha$  production. In contrast, carvacrol triggers IFN $\gamma$  which is involved in T-cell stimulation, highlighting the potential of carvacrol to modulate adaptive immune responses. In conclusion, based on splenic gene expression, in ovo carvacrol delivery has the potential to mitigate release of key inflammatory cytokines, but increases IFN $\gamma$ . This work was funded by AgriFutures Australia and Delacon Biotechnik.

Keywords: In ovo; Carvacrol; Immune system; Challenge; Inflammatory; Broiler;



## Understanding the mode of action of *Bacillus subtilis* 29784 poultry probiotic through its secreted metabolites: A resilient approach

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The probiotic *Bacillus subtilis* 29784 (Bs29784) maintains intestinal health and improves animal resilience and performance. It produces several bioactive metabolites such as hypoxanthine (HPX), Niacin (NIA), and pantothenate (PTH) which have been detected in vitro and in vivo. The purpose of this study was to determine the functional link between these metabolites and animal's intestinal health by assessing their influence on the three pillars of animal resilience: immune response, intestinal barrier, and microbiota. For this purpose, we evaluated in vitro Bs29784 vegetative cells (metabolic active form), spores and metabolites capacity to modulate global immune regulators and intestinal integrity using HT-29 reporter cell lines both in the presence and absence of a proinflammatory challenge. Finally, we simulated chickens' ileal and cecal fermentations to determine the effect of Bs29784 metabolites on the microbiota and their fermentation profile. Bs29784 vegetative cells downregulated inflammatory response to a higher extent than the spores ( $p < 0.0001$ ), indicating that its beneficial effects are linked to its metabolic activity. To pursue this hypothesis, we studied individually Bs29784 metabolites. The findings indicate that each metabolite produced distinct positive effects. Specifically, PTH and NIA reduced inflammation (23.2% and 9.7% respectively;  $p < 0.0001$ ) and HPX enhanced mucin production by increasing 19.3% MUC2 expression ( $p = 0.011$ ). Furthermore, PTH and HPX increased epithelial resilience to an inflammatory challenge by limiting permeability increase ( $p = 0.024$ ). In ileal fermentations, PTH increased butyrate levels to 0.31mM ( $p = 0.076$ ). In cecal fermentations NIA increased propionate production by 6.7% ( $p = 0.016$ ), HPX increased 5.7% butyrate ( $p = 0.019$ ) while PTH increased 5.1%, 6.8% and 16.1% the levels of acetate, butyrate, and propionate respectively ( $p = 0.087$ ). All molecules lead changes to microbiota explaining the different fermentation patterns such as the increase of *Ruminococcus* and *Clostridium\_XIVb* in presence of NIA or PTH and the increase of *Ruminococcus*, *Anaerotruncus* with HPX ( $p \text{ adj.} = 0.040$ ) in the cecal fermentation. Altogether, we show that Bs29784 modulates intestinal health by acting on the three lines of resilience via its secreted metabolites.

Keywords: *Bacillus subtilis* 29784; niacin; hypoxanthine; pantothenate; probiotic metabolites; intestinal health; inflammation; animal resilience

## **Microbiome Modulation by a precision biotic lead to increased production of short-chain fatty acids in the intestine of broiler chickens**

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The objective of the present studies was to evaluate: (1) the impact of the supplementation of a Precision Biotic (PB) to broiler diets on the modulation of the cecal microbiome collected from broilers subjected to a necrotic enteritis challenge model (Experiment 1); (2) to measure the cecal production of short-chain fatty acids (SCFA; Exp. 2). In Exp. 1, day-old chicks were placed on a completely randomized block design with 3 treatments, 10 replicates, and 25 birds/replicate. The treatments consisted of a control group, a challenged control, and a challenged group supplemented with PB at 0.9 kg/MT. All treatments were vaccinated against *Eimeria* (Coccivac®-B52, Merk Animal Health USA) at d 0. The challenge groups were given *Clostridium perfringens* via drinking water (106 CFU/bird) on d 15, 21, and 28. On d 22 and 46, 1 bird/pen was sacrificed, and the cecal content was collected for microbiome analysis. In Exp. 1, it was observed that the supplementation of PB significantly ( $P < 0.05$ ) improved the body weight gain and feed conversion ratio by 2.5 and 2.7%, respectively vs. the challenged birds. In the microbiome, an increased relative abundance of several species related to SCFA production was observed at both d 21 and d 42, including an increased relative abundance of several *Faecalibacterium* species (a known butyrate producer) on day 42 ( $P < 0.05$ ). This was paired with an increased relative abundance of both the propionate ( $P < 0.05$ ) and butyrate pathways in birds with PB supplementation at both d 21 and d 42. In Exp. 2, day-old chicks were placed on a completely randomized block design with 2 treatments, 8 replicates, and 25 birds/replicate. The treatments consisted of a control group, and a group supplemented with PB at 0.9 kg/MT. At d 14, all the birds were challenged with 30 x of a coccidiosis vaccine (Zhengdian, Fushan, China) via oral gavage. On d 21 and 43, 2 birds/pen were sacrificed, and the cecal content was collected for SCFA analysis. PB led to an increase in the production of propionate ( $P=0.02$ ; 47%) and valerate ( $P=0.08$ ; 29%) on d 21, and numerically increased acetate, propionate, and butyrate on d 42. Taken together, the microbiome metabolic shift observed with the supplementation of PB, plus the observations with increased SCFA production, may explain the improvement in growth performance obtained with the supplementation of PB.

Keywords: Broilers, microbiome metabolism, precision biotic, metagenome

[008-09]: *Parallel B(6)-S08-Poultry Health: Antibiotic Resistance and Alternatives products (ID: 131013) - Poland*

## **Probiotics or Prophybiotics? Validation of effects of in-ovo administration on gut health and production of broiler chickens**

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In-ovo administration of probiotics and phytobiotics have shown promising potential in improving the post hatch gut health of broiler chickens when administered individually. However, the effects of their combined application has yet to be elucidated. We coined the term prophybiotic (probiotic + phytobiotic) to describe such a combination. The current study to the best of our knowledge, elucidates the effects of the combined use of a selected probiotic and a phytobiotic in-ovo, on broiler gut health and production for the first time as opposed to the use of probiotic alone. On 12th day of incubation, ROSS 308 hatching eggs were injected with either *Leuconostoc mesenteroides* (B/00288) (probiotic: PB) or *Leuconostoc mesenteroides* with garlic aqueous extract (prophybiotic: PPB). The relative abundance of bacterial species in the feces and cecal content and immune related gene expression in the cecal mucosa were determined using qPCR. Production parameters such as hatch quality, body weight, feed efficiency and carcass parameters were also assessed. In PPB treated chickens, increases in the abundance of beneficial bacteria in the feces (day 7: *Faecalibacteria* and *Bifidobacteria*) and cecal content (*Akkermansia*), and decreased abundance of *E. coli* in both feces (day 34) and cecal content were observed. Up-regulation in the expression of immune related genes (*Avian beta defensin 1*, *Free fatty acid receptor 2* and *Mucin 6*) in cecal mucosa was also observed. Conversely, PB treatment only resulted in an increase in *Faecalibacteria* in feces (day 7) and *Akkermansia* in the cecal content with a tendency ( $p$  value  $<0.1$ ) for a higher *Mucin 6* gene expression in cecal mucosa. Both treatments did not impair the production parameters studied. In conclusion, our results suggest that prophybiotics may have enhanced potential in optimizing gut health of broiler chickens without compromising the production and efficiency, when compared to using probiotics alone. Our study highlights the potential of carefully selected prophybiotic combinations for better results in improving gut health of broiler chickens.

Keywords: ceca; garlic; immune gene expression; *Leuconostoc mesenteroides*; microbiome; phytobiotics; probiotics

[008-10]: Parallel B(6)-S08-Poultry Health: Antibiotic Resistance and Alternatives products (ID: 131182) - Sri Lanka

## **An assessment of the antibacterial properties of black soldier fly larvae (*Hermetia illucens*) meal against several pathogenic bacteria found in poultry**

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Increasing feed costs may deprive the growth and production goals of commercial broilers. Meanwhile, synthetic antibiotics pose global health and environmental risks due to resistance in poultry industries, causing poor absorption and excretion. The use of black soldier fly larvae (*Hermetia illucens* L.; BSFL) is an environmentally conscious and sustainable substitute for costlier feed ingredients as a protein source. They are also a source of natural antibiotics and compounds that strengthen the immune system, changing the microbiome of chickens' guts while eliminating pathogenic bacteria. The study involves in evaluating the in-vitro antibacterial effect of ethanolic extract of BSFL de-fatted (DF) larvae against selected bacterial isolates (*E. coli*, *Proteus vulgaris*, *Proteus mirabilis* and *Salmonella* spp.) isolated from broiler chicken meat. The antibacterial effect of the ethanolic extract was measured using the well diffusion method. The bacterial isolates were grown in LB broth and  $1 \times 10^5$  CFU/ml cell culture lawns were made on Muller Hinton agar plates. The ethanolic extract of BSFL DF meal was added to the wells made on culture plates. They were incubated overnight at 37°C and inhibition zones (IZ) were measured. In the present study, the antibacterial effect of BSFL DF meal extraction effectively inhibited ( $p < 0.05$ ) some pathogenic bacterial strains tested; *E. coli* ( $18.51 \pm 0.42$  mm), *Proteus mirabilis* ( $15.80 \pm 0.67$  mm) and *Salmonella* ( $11.37 \pm 0.36$  mm and  $12.42 \pm 0.91$  mm) prevail in poultry gut compared to the commonly used synthetic antibiotic, Trimethoprim. The presence of lauric acid is a potent natural antibacterial agent and the high degree of fatty acid in BSFL meal would be the reason for the antibacterial effect revealed. The results of the present research revealed the potential antibacterial activity of the ethanol extract from BSF DF larvae against some pathogenic bacteria.

**Keywords:** Antibiotics, Antimicrobial properties, Black soldier fly larvae, Poultry

## **Butyric acid and medium-chain fatty acids improve vaccine efficacy in broiler chickens by modulating the adaptive immune system**

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Medium chain fatty acids (MCFAs) and butyric acid may contribute to better disease resistance of broiler chickens by modulating immune cells, thereby leading to increased vaccine efficacy. To check our hypothesis, first we tested immunomodulatory effects of a specific blend of butyric acid and MCFA (C-prove 80) on primary cultured peripheral blood mononuclear cells (PBMCs). PBMCs were isolated according to Larsberg et al. (2021) and seeded in RPMI with 5% of the test component. Flow cytometry analyses showed an increase in cytotoxic T cells after treatment with C-prove 80. Activation by CD25-FITC fluorescence and proliferation was measured with a Incucyte S3 live cell imaging device. Treatment with C-prove 80 led to a higher proliferation capacity and an increased activation rate of PBMCs. Thus, a faster and more efficient immune response towards infectious diseases could be assumed. Next, we investigated whether the observed immunomodulatory effects could result in an induced vaccination efficiency against Newcastle Disease virus (NDV) and Infectious Bursal virus (IBV) in broiler chickens. A total of 380 male Ross 308 birds was used for this study and divided in two treatment groups, each consisting of 10 repetitions of 19 birds. Animals received drinking water and a standard commercial feed ad libitum throughout the entire study. Birds were vaccinated against IBV on D17 (Bursa Plus). C-prove 80 was added to the drinking water of half the birds (T02) at 1L/1000L from D0-D10 and D18-D21, while chickens from group T01 received unsupplemented water. On D34 the humoral immune response against IBV was quantified with ELISA by taking 2 blood samples per pen. IBV antibody titers were significantly ( $p < 0.05$ ) increased in birds receiving C-prove 80 in the drinking water (9176 vs 7814), while NDV titers were only increased numerically (4155 vs 1948). To conclude, this study shows that adding a combination of butyric acid and MCFA in the drinking water of broilers may induce vaccine efficiency through an immunomodulatory effect on lymphocytes. An increased cytotoxic T cell count may further increase the bird's disease resistance through an increased clearance capacity of virus-infected cells. We hypothesize that these effects are mediated through a direct binding and activation of free fatty acid receptor 2 (FFAR2) - known to be expressed on PBMCs - as we observed in a receptor activation assay using HEK293 cells.

Keywords: Butyric acid, medium chain fatty acids, broiler chicken, PBMC, vaccination

## Use of artificial intelligence tools to simplify broiler welfare assessment protocols

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We used Chaid decision trees on a dataset of broiler flocks, which welfare had been assessed with the AWIN protocol, as a proof of concept to test the suitability of artificial intelligence to simplify welfare assessment protocols. Our aim was to identify a sub-set of key welfare indicators capable of discriminating flocks passing or failing welfare audits in the same way than by applying the complete protocol. A dataset was constructed with data collected during the welfare assessments done on 57 fast growth broiler flocks during previous research projects. The final welfare audit score was used to classify the flock as passing or failing the audit. Then a decision tree-based statistical model for flock classification was built using the CHAID criterion, applying cost-complexity pruning to prevent overfitting, and using 10-fold cross validation for model assessment. The final decision tree included cumulative mortality (%), the incidence of lame and immobile birds (%) as indicators of mobility problems, and the incidence of birds with back wounds (%) as indicator of skin lesions, all of them acknowledged to be critical on-farm welfare indicators. The mean prevalence of cumulative mortality, immobile birds, lame birds, and birds with back wounds in flocks passing the audit were respectively (mean  $\pm$  se)  $2.77 \pm 0.14\%$ ,  $0.16 \pm 0.02\%$ ,  $0.25 \pm 0.02\%$ , and  $0.003 \pm 0.001\%$ . Values for flocks failing the audit for the same parameters were  $4.39 \pm 0.49\%$ ,  $0.24 \pm 0.05\%$ ,  $0.49 \pm 0.09\%$ , and  $0.015 \pm 0.006\%$ , respectively. Cumulative mortality was the first partitioning variable and had the highest relative importance. Additional variables contributing to the decision tree were assessed using transects, suggesting that the welfare status of flocks can be satisfactorily predicted by doing transect walks and checking mortality records the day of the assessment. The 10-fold validated model was overall efficient, being able to correctly predict assessment outcomes in 80.70 % of cases. Model specificity was very good (0.8696), but sensitivity was only average (0.5455). Results are promising and suggest that simple but scientifically sound tools can be offered to the industry for animal welfare self-assessment. Model readjustments and improvement will be possible once more information is available and predictions can therefore be based on larger sample sizes.

Keywords: artificial intelligence; assessment; chicken; decision tree; welfare

## **Welfare evaluation in the main Italian rearing system for poultry meat production by ebene app**

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The aim of this study was to evaluate the welfare status of broiler chickens in the main Italian rearing systems through the EBENE App. This application allows the user to collect several environmental and animal-based parameters and, according to these variables, it provides scores following the four behavioral principles (i.e., Welfare Quality): “Good Feeding”, “Good Housing”, “Good Health”, and “Appropriate Behavior”. A total of 25 farms representative of 5 broiler production categories were involved: males fast-growing genotype raised in conventional conditions (MC), males and females fast-growing genotype kept in semi-intensive rearing system and fed vegetable diets (FV and MV), medium-growing genotype raised in free range system (FR) and females fast-growing genotype raised in organic system (O). Out of the 29 welfare indicators provided by the EBENE App, 3 were included in the Good Feeding category, 9 in the Good Housing, 7 in the Good Health, and 10 in the Appropriate Behavior. The Kruskal-Wallis test highlighted that the rearing system influenced 100%, 56% and 70% of the indicators for Good Feeding, Good Housing and Appropriate Behavior, respectively ( $p < 0.05$ ). Results evidenced that O and FR systems showed similar scores, exhibiting a higher evaluation for Drinker Availability (Good Feeding), Available Space (Good Housing), Injured animals (Good health), Foraging and Human Approach (Appropriate Behavior) indicators compared to MC, FV and MV ( $p < 0.05$ ). Interestingly, the Human Approach indicator showed that in FR and O systems the farmer spent more time with the animals than in the other rearing systems. Conversely, the score for Mortality (Good Health) was lower in FR and O than in MC, FV, and MV ( $p < 0.05$ ). This is probably due to the higher slaughtering age of animals in FR and O systems. Among the outdoor rearing systems, FR showed a better score compared to O for Other Health Problems category (Good Health) and Social Interaction (Appropriate Behavior;  $p < 0.05$ ). The main difference between the FR and O systems was related to the genotype used, which can influence the Good Health and Appropriate Behavior indicators in both systems. In general, from this survey emerged that all investigated rearing systems can ensure adequate animal welfare conditions. Moreover, the chicken genotype and the farmer management are the main factors affecting the Appropriate Behavior indicators.

**Keywords:** welfare status, behavior indicators, broiler chickens, rearing systems

## **Broiler welfare assessment: footpad and hock health analysis in Austrian abattoirs, 2020-2022**

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In Austrian abattoirs, official veterinarians systematically assess foot pad and hock health in broilers for welfare monitoring using a 10-level-scoring (1 best, 10 worst). This study investigates risk factors in Austrian broiler flocks, considering variables such as housing type (organic/conventional), flock size, slaughter age, veterinary intervention, thinning, year, season, and assessor. The dataset comprises 20,836 batches from 13,670 broiler flocks on 709 Austrian farms (slaughtered 01.01.2020–31.12.2022). The statistical analysis involves univariate evaluations between the target variable (scoring) and potential influencing factors. Subsequent multivariate analyses encompass decision trees and generalized linear mixed models. The most common score was 1 (27% batches) and the least common 10 (0.04% batches), with intermediate scores decreasing exponentially. Lesion severity was lower in 2020 (median = 2) than in other years (median = 3) and in summer (median = 2) than winter (median = 4). Despite a higher median score in organic (median = 4) than conventional flocks (median = 3), highest-scoring batches were more common in conventional housing (organic: 0.02%, n=1; conventional: 0.04%, n=7). Slaughter age interquartile range was 54-57 days for organic and 31-38 days for conventional broilers. Older batches had negligibly higher scores than younger (organic:  $\rho = 0.186$ ,  $p < 0.001$ ; conventional:  $\rho = 0.145$ ,  $p < 0.001$ ). Batches that underwent veterinary intervention had a lower median score of 2 compared to untreated batches (score 3). Negligible negative Spearman rank-correlations between flock size and health score were identified in both housing types, but were stronger in organic ( $\rho = -0.118$ ,  $p < 0.001$ ) than conventional flocks ( $\rho = -0.0834$ ,  $p < 0.001$ ). Single-batch slaughter was used in 31% flocks with available scoring values. Single-batch slaughters had a median score of 3, while multiple partial slaughters started with a median score of 2, with subsequent batches scoring 3 or higher. Negligible correlation ( $\rho = 0.085$ ,  $p < 0.001$ ) between the score difference between two consecutive batches and the number of days between the slaughter batches was found. Median scores ranged between 2 and 5 between assessors. We acknowledge the necessity of additional risk factors, such as litter quality, feed composition, management and stocking density, to enhance the effectiveness of official assessments in broiler welfare monitoring.

**Keywords:** footpad dermatitis, hock burns, health, welfare monitoring, slaughter house, meat chickens



## **Interobserver reliability of a scoring system to evaluate bruises in turkey carcasses**

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Traumatic injuries such as bruises have been considered as an important indicator to assess animal welfare. The possibility to assign the injury to a particular stage or moment in the production process may allow judgements on possible causes and thus reduce its prevalence. Currently, there is no consistent definition or scoring system for bruised poultry carcasses in German meat-inspection and the prevalence is affected by the variability of scoring systems as well as the observer bias. Therefore, the objective of this study was to determine the inter-observer reliability of bruise characteristics at the slaughter line and to validate the findings with measurements of bruises and photographed carcasses. Inter-observer reliability was assessed with two observers who each scored 10,880 turkey carcasses simultaneously at a running slaughter line (1.2 – 0.9 birds / s) after a short training. The strength of agreement, calculated as intraclass correlation coefficient (ICC) was “good” for the total number of detected bruises and number of bruises on breast, wings and legs per flock (0.81 – 0.88). Agreement (ICC) on the number of small, medium and large bruises ranged between “good” and “moderate” values (0.70 – 0.84), whereas number of bruises in the different colors (red, violet, green-violet, green-yellow, yellow-orange) showed “moderate to poor” reliability (0.04 – 0.64). Observer 1 showed a “good” and observer 2 a “moderate” agreement with the photographs. Additionally, agreement on bruise characteristics was tested on photographs (N= 513 carcasses) without time limit. Highest agreement (weighted Kappa, Kw) between observers was found for location of bruises (Kw = 0.98). Again, color of the bruises showed the lowest agreement (Kw = 0.36), whereas it was “moderate” for the size of bruises (Kw = 0.45). When comparing each observers scoring values for size with size measurements (digital analysis) of bruises, level of agreement was “substantial” (Kw = 0.63) for observer 1 and “fair” (Kw = 0.25) for observer 2. Overall, the total number of detected bruises and the location of bruises showed the highest agreement between observers at the slaughter line and on photographs indicating a reliable scoring system. However, as the variable color showed a low agreement, a standardized objective method should be developed to objectively assess bruise prevalence and characteristics.

Keywords: animal welfare; turkeys; observer agreement; bruises; scoring system

## **LEGMONI, a ready-to-use tool for evaluating laying hen welfare at the slaughterhouse**

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Laying hens have a relatively long lifespan which is influenced by various factors (hatchery, rearing, egg production, transport and slaughterhouse). Their welfare can therefore be affected in many ways. In Flanders, all spent laying hens are slaughtered. Hence, the slaughterhouse seems to be a good location for welfare monitoring, both in terms of biosecurity and feasibility. The aim of this project was to develop a tool to evaluate laying hen welfare at the slaughterhouse. Therefore, a mobile application (LEGMONI) has been developed to assess the welfare of each flock with instant access to the results. The main animal welfare indicators (AWI) and the stage of production to which they can be associated, were determined through an experimental phase where assessments on 20 flocks were done during several stages (during production and catching, in the slaughterhouse). After statistical analyses and consultations with the expert panel, a list of final AWI was compiled, taking into account assessment time and the most appropriate location in the slaughterhouse to measure. The final assessment is conducted in four questionnaires according to slaughterhouse location. The first questionnaire is taken in the lairage, focusing on climate and behavior (panting or huddling) in the crates. Those indicators mainly relate to the slaughterhouse, but the catching team can also have an impact. The next step is the assessment of individual stunned hens for different AWI (feather score, injuries, footpad dermatitis, keel bone damage) which provide information on the production period. Afterwards, the hens are evaluated at the evisceration line during 2x3 minutes for bruises and fractures on breast, wings and legs. This mainly provides information on the catching process, but influences from farm management and the slaughterhouse can also be involved. Finally, the number of rejected hens, deaths on arrival and general information (farm management, transport time) is collected. The completed questionnaires can be uploaded and a report is immediately generated with calculated scores for the AWI and also benchmarking (as percentile) is displayed. A digital report is shared with all actors for a specific flock. The results could lead to public statistics and the setting of thresholds for minimum scores. Assessing and benchmarking hen welfare encourages the whole chain to address points of attention, and attempts to increase chicken welfare over time.

**Keywords:** laying hens; monitoring; welfare; slaughterhouse; mobile application

**Determination of ideal perch space allowance for pullets in Canada**C. Adler<sup>1</sup>, T. Shynkaruk<sup>1</sup>, K. Buchynski<sup>1</sup>, S. McPhee<sup>1</sup>, A. Herr<sup>1</sup>, E. Herwig<sup>1</sup>, K. Schwean-Lardner<sup>1</sup><sup>1</sup>University of Saskatchewan, Saskatoon, Canada

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Perch access during rearing is known to prepare pullets for later life in single- or multi-tier laying systems. However, recommendations for pullet perch space allowance in Canada are based on laying hen data. This study aimed to find the ideal perch space allowance for pullets up to 18 wk of age, examining performance, health, and behaviour indicators. Two trials were conducted with 1,232 Lohmann LSL-Lite (LW) and 1,232 Lohmann Brown-Lite (LB) pullets. The study was a randomized complete block design (trial as block) with a 2 (strain) x 4 (perch space) factorial arrangement. Birds were raised in 16 floor pens, measuring 3x3 m each (897.67 cm<sup>2</sup> per bird). Wooden perches were provided from d 1, allowing 6, 9, 12, or 15 cm perch space per pullet (4 replicates per treatment combination). Data were tested for normality before analyses, and log+1 transformed when not normally distributed. Significance was set at  $P \leq 0.05$ . Data collected included: body weight (wk 0, 4, 8, 12, 16, and 18), pullet width (wk 4, 8, 12, 16, and 18), comb damage (wk 12 and 18), heterophil-to-lymphocyte ratio (12 and 18 wk), keel bone deviations (wk 18), bone breaking strength (wk 18), perch use (wk 3, 9, 15, and 18), and behaviour (wk 3, 6, 9, 12, 15, and 18). On wk 16 and 18, the LB body weight slightly increased while the LW body weight slightly decreased with increasing perch space. At 9 wk, birds perched a higher percentage of time during the photoperiod when provided 12 or 15 cm compared to 6 cm perch space. At wk 15, birds perched a higher percentage of time during the photoperiod when given 15 cm compared to 6 or 9 cm. During the scotoperiod at wk 18, birds perched a higher percentage of time with 12 and 15 cm perch space compared to 6 cm. Birds spent a higher percentage of time wing flapping on the perch at 3 wk when provided 15 cm compared to 6 cm. At 15 wk, the percentage of time LB pullets performed active behaviours and standing on the perch decreased with increasing perch space, while the opposite was found in LW pullets. Pullet width at 18 wk was 13.85 cm (sitting) and 12.94 cm (standing) for the LB and 12.52 cm (sitting) and 11.66 cm (standing) for the LW pullets. Perch space did not affect comb damage, heterophil-to-lymphocyte ratio, keel bone deviations, or bone breaking strength. Overall, a higher percentage of pullets were on the perches when 12 or 15 cm were given, while perch space allowance had minimal effects on body weight and no impact on health indicators.

Keywords: perch use; pullet behaviour; pullet health; pullet management; laying systems

## Direct and carry-over effects of feeding pullets during sensitive periods in early rearing

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Adequate nutritional strategies during the rearing period not only support egg production but are also important to cope with challenges in early and later life. Previous research suggested that pullets might be more sensitive to challenges in early rearing, leading to stress, which could affect layer's performance and coping abilities. Therefore, this study aimed to test four diets during two sensitive periods in early rearing on performance, endocrinology, and behaviour of layers until 35 weeks of age (woa). In total, 1248 one-day old pullets were divided over 48 identical pens. Treatments were arranged according a 2x4 factorial design with two sensitive periods (2-4 or 5-7 woa) in which stress was induced by disruption and in which four diets were provided. The disruption consisted of removal of litter material, perches and pecking blocks, and housing on only slats. The diets were provided during and two weeks after the sensitive periods. There was a control diet (CON), and three diets with either increased fat/fibre (low starch; FAT), energy (+100 Kcal; AME), or essential amino acids (+10%; AA). No interaction effects between the treatments were found on performance. During both sensitive periods, the ADFI of the pullets receiving stress was increased ( $p < 0.0001$ ). At 2-4 woa, the pullets which were stressed ate on average 1.7g/d more than the pullets without stress. The same effect was seen at 5-7 woa, in which the stressed pullets ate on average 1.2g/d more than pullets without stress. Also, throughout early rearing (1-10 woa), pullets receiving the AME diet during the sensitive periods ate less compared to the other diets (-0.8g/d;  $p = 0.003$ ). During laying no effects were found on laying rate or egg mass. However, egg weight of layers that received the FAT (56.0) and AA (56.3) diets throughout either sensitive period was lower between 26-30 woa compared to the AME (56.9) diet, but not as compared to CON (56.3;  $p = 0.046$ ). Similar effects were found for the total laying phase (21-35 woa), with the lowest egg weight for the FAT and AA diets ( $p = 0.035$ ). Further analyses of endocrinology and behaviour are pending. In conclusion, stress during early rearing only had direct effects on ADFI during the period in which the pullets were stressed, and diet influenced ADFI during early rearing. Also, diets during the sensitive periods affected egg weight during early lay but stress did not affect performance during lay/did not result in carry over effects into lay.

Keywords: Rearing period; laying hens; sensitive period; feed intake; egg weight

## **Status quo of light conditions in turkey barns to identify light-associated risk factors of behavioral disorders in turkey flocks**

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Insufficient light conditions are discussed in reference to the occurrence of behavioral disorders, particularly feather pecking in turkeys. In order to ban the beak trimming in turkey husbandry, the light conditions for turkeys need to be improved. In a first step, the current state of lighting conditions in turkey barns were determined in north-east Germany. This included e.g. recording installed light sources with light spectrum, light intensity, and frequency. Additionally, the possibilities for influencing the lighting conditions in the barns were investigated, e.g. dimming of lamps and daylight exposure. Light measurements were conducted in 17 barns, each with a size of about 1,600m<sup>2</sup>, including both naturally ventilated and mechanically ventilated barns, with 2 different spectroradiometers (BTS2048-VL and BTS256-EF, Gigahertz Optic). About 75 different measuring points were recorded for each barn about 60cm above the ground. Each measurement was the mean of 10 measured values, integration time was 100ms. In the examined barns, fluorescent lamps (10x) and LEDs (7x) were installed and three of each were dimmable. In barns with LED lighting, measured wavelengths were between 400-780nm, so the artificial light spectrum of the LEDs did not contain a UV-A component. With fluorescent lamps, visible light is generated, inter alia, by the conversion of UV light in a fluorescent layer of the lamp, so a small amount of UV radiation (around 360nm) was measurable in the light spectrum. However, fluorescent lamps cannot achieve a daylight-like spectrum, as they emit a narrowband light spectrum characterized by peaks in three color ranges, and thus do not fulfill the light requirements of turkeys. When green blinds were used, the transmission of green wavelengths was enhanced, leading to a spectral shift and, consequently, a potential alteration in color perception by the turkeys. The measured fluorescent lamps may have been perceived as flickering by turkeys because the measured frequency was 100 Hz, thus below the flicker fusion frequency (FFF) of poultry. Light intensities varied significantly at the same dimming setting, making it challenging to make recommendations for light reductions based on dimming settings. For turkeys, the low frequencies (100 Hz) as well as the limited spectrum in artificial light and partially monochromatic light caused by green blinds, which supports false color vision, can trigger stress and be risk factors for behavioral disorders.

**Keywords:** Behavioral disorder, turkey, lighting, flicker fusion frequency, UV-light

## Will turkey hens utilize a perch when given the opportunity?

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Turkeys, like most poultry species, exhibit a natural desire to perch; however, are not provided with this opportunity in most commercial floor barns. The use of perches has been well documented in laying hens, however species differences including size, weight, and postural differences make comparisons difficult. With limited data on perch use in turkeys, the objectives of this study were to examine the impact of perch design on perch use, performance, and health of turkey hens reared to 11 weeks of age. Design treatments included: control (no perch), round (5 cm diameter round wooden dowel), 5 cm, 10 cm, or 15 cm wide (flat wooden boards, 5 cm high, with rounded edges). Each perch was placed in the pen in an "L" shape, measuring 2.4 m x 2.4 m long and 42 cm high, allowing for 18.5 cm of perch space per bird. A total of 620 Nicholas Select turkey hens were randomly allocated to 20 pens, with 1 of 5 perch design treatments at 11 d of age (4 replicates per treatment). The experiment was a complete randomized design with a one-way factorial arrangement. Data were checked for normality and log+1 transformed when necessary. An analysis of variance (Proc Mixed, SAS 9.4) was performed and means separation was conducted using Tukey's range test. Significance was declared at  $P \leq 0.05$ . At both 9 and 11 wk of age, there was a higher percentage of birds using the perch as perch width increased ( $P < 0.01$ ). When evaluating the scotoperiod only (9 and 11 wk), there was a higher percentage of birds on the perch in the wider treatments (10 and 15 cm) compared to the round, with 5 cm being intermediate ( $P < 0.01$ ). Hen performance (body weight, mortality corrected feed-to-gain ratio, and mortality) was unaffected by perch design. Mobility (subjective gait score;  $P = 0.23$ ) and footpad lesion scores ( $P = 0.28$ ) were unaffected by perch design at wk 7. Hens in the control treatment had poorer mobility at wk 11 compared with round ( $P = 0.04$ ). Footpad lesion scores at 11 wk were poorer in control hens compared with 15 cm ( $P = 0.02$ ). Perch design did not impact keel bone deviations ( $P = 0.54$ ) and there were no keel bone fractures found in any treatment. This study demonstrates that turkey hens will utilize a perch, with more hens utilizing the wider flat perches, emphasizing the importance of proper design when providing birds with enrichment. In addition, this study demonstrated no negative impact of perch design on turkey hen performance and health.

Keywords: perching; environmental enrichment; mobility; footpad score; welfare

## Genetics of feeding behavior in medium-growing broiler chickens

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Feed efficiency is the first element of economic and environmental performances of poultry production. Phenotypic and genetic correlations between feed efficiency and feed intake, growth rate, and body composition have been described, but little is known about how it associates with feeding behavior. Thanks to the development of electronic feed stations, it is now possible to study these traits on numerous individuals in rearing conditions. Our objectives were to establish how feeding behavior associates with feed efficiency and to estimate their heritability, in order to check whether they could be used in genetic selection. We used 317 birds from two lines divergently selected for high or low digestive efficiency, usually showing a 30% difference at 3 weeks. Birds were identified at hatch with a RFID chip. Each time a chicken ate, its chip was read by antennae placed on three electronic feed stations, allowing 24 birds to eat simultaneously. We recorded time at beginning and end of each visit from 3 to 47 days. From these raw data, we calculated the daily number of meals and their duration, the intervals between meals, the total duration of feed intake, and the preference of animals for a given access to feed. We performed an analysis of variance including sex and line as fixed effect, body weight and age as covariates and animal as random effect with R to estimate the link between feed efficiency (line) and feeding behavior. The VCE software was used to estimate heritability of feeding behavior traits with an animal model, including all available pedigree data on the lines (N=12316). We found a difference between lines in meal duration and total duration of feed intake, poorly efficient birds eating for longer than efficient birds. At older ages, birds took fewer but longer meals, meals were more distant in time from each other and birds showed more marked preferences for a given place to feed. Heritability of feeding behavior varied largely with age. Preference for some places to feed was poorly heritable ( $0.10 < h^2 < 0.20$ ) but the other traits showed high heritability ( $h^2 > 0.40$ ). Our results thus suggest that there is an association between feed efficiency and feeding behavior that could be exploited in genetic selection. Estimation of genetic correlations between feeding behavior traits and feed efficiency is now needed to confirm these first results.

Keywords: animal feed efficiency, genetic parameters, behaviour, chicken

## **Evaluation of selection indices for adoption in dual-purpose and specialized breeding goals in indigenous chicken breeding programmes**

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This study aimed to evaluate various selection indices for adoption in dual-purpose (ICD), meat (ICM) and layer (ICL) breeding goals based on economic response, index accuracy and rate of inbreeding per generation, and number of years required to attain pre-defined gains. In the ICM goal, the objective was to improve live weight at 12 weeks of age (LW12), daily gain (ADG) between 9 and 20 weeks of age and average daily egg weight for 12 weeks post onset of lay (EW12) or together with feed efficiency between 9 and 20 weeks of age and antibody traits at 16 weeks of age. For the ICL goal, the objective was to improve age at first egg (AFE) and cumulative egg number at 12 weeks from onset of lay (EN12) or together with feed efficiency measured for 12 weeks' post onset of lay and antibody traits at 28 weeks of age. In the ICD goal, the objective was to improve LW12, ADG, AFE and EN12 or together with feed efficiency from 9 weeks of age to 12 weeks post onset of lay and average antibody titer levels at 16 and 28 weeks of age. Across the goals, highest genetic gains in targeted production traits were observed in indices with only production traits while addition of feed efficiency and antibody traits in the goals resulted to the lowest genetic gains in targeted production traits. Highest economic responses of US\$ 49.83, US\$ 65.71 and US\$ 37.90 were estimated in indices with only production traits in the ICD, ICM and ICL goals, respectively. With respect to accuracy, highest estimates of 0.77 and 0.70 were observed in indices that considered production traits together with feed efficiency in the ICD and ICL goals, respectively. However, in the ICM goal, the highest index accuracy of 0.96 was estimated in an index with only production traits in the goal. The lowest inbreeding level of 0.62 % in the ICD goal was observed in an index with only production traits while in the ICM and ICL goals least estimates of 0.96 % and 0.62 %, respectively, were estimated in indices with both production and feed efficiency traits. Targeting only production traits in the ICD, ICM and ICL goals required the least number of years of 7.46, 4.88 and 8.52, respectively, to achieve pre-defined gains in these goals. Results from this study demonstrate that production, feed efficiency and antibody traits can optimally be combined in an index, however, the choice of index to adopt should depend on the production environment in which resulting terminal progenies from each of the goals are expected to perform in.

**Keywords:** economic response, genetic gain, inbreeding, index accuracy, indigenous chicken



## **Morphological characteristics of breeder males at 60 weeks of age and their relationship with fertility and testicular development**

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Optimal male management is crucial for production success. As breeder males age, they reduce their fertilization capacity, due to factors such as libido, semen quality, and successful matings decline. In this situation, the introduction of young males, around 40 weeks is recommended to maintain the fertility. To avoid an increase in the male ratio when introducing young males, it is crucial to discard older males at the same time and maintain a proportion, between 9-10%. The selection of old males to discard is key to the success of the introduction of young males, initially eliminating those with obvious lameness and clear signs of low activity. However, once the most obvious cases are removed, it may be more challenging to determine specific characteristics for discarding. The objective of this study was to determine the external characteristics of old males, most related to fertility. For this study, 240 males from 4 different strains were evaluated and selecting departments according to the fertility shown: maximum, minimum, and medium at 56 weeks of age, which were housed in the Trial Farm of Cobb Española. Body weight, tarsus length, fleshing, cloaca condition, leg condition and comb size were recorded. Subsequently, they were sacrificed following the animal welfare regulations (RD 1099/2099), and the testicles were weighed, and samples were histologically studied. The results revealed that males with poorer fertility showed fleshing values slightly higher than those with good and average fertility, possibly affecting mating stability. Worse fertility correlated with lower comb scores and smaller comb size. Males with better fertility showed greater testicular weight. Histologically, seminiferous tubules' diameter was smaller in males with poor fertility. Fleshing and comb condition/size are indicative for discarding males with low sexual activity/fertility.

**Keywords:** breeder males; fertility; testicular development

## **Genetic evaluation of clutch traits in laying hens**

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Continuous genetic improvement of laying hens has contributed to substantial increase in the number of eggs laid per hen during its productive cycle. Modern layer hens lay an egg a day for long periods of time in oviposition intervals very close to 24 hours. This study aimed to unravel the aspects of clutch length in laying hens to explain the individual laying pattern as an alternative to more traditional egg production traits. Data from two pure line laying hens of the H&N commercial breeding program was used. In total, 2,141 White Leghorn (WL) and 2,298 Red Island Red (RIR) hens were used in this study. The data was split into two datasets from 20 to 52 weeks and from 56 weeks until 100 weeks of age. Shorter average clutch lengths were observed at older ages (17.3 and 20.5 for RIR and WL respectively) compared to younger ages (42.9 and 35.2 for RIR and WL respectively). Genetic parameters were estimated for different egg production traits such as clutch number, and average and maximum clutch length. Moderate heritabilities were estimated for clutch traits ranging between 0.08 and 0.23, which were at a similar or higher level than the heritability for egg production. As expected, there was a negative correlation between the number of clutches and the maximum and average clutch size ( $rg=-0.89$  to  $0.94$ ). Egg production was positively genetic correlated with average and maximum clutch size (ranging between  $rg=0.44$  and  $0.67$ ). The results from this study indicate that average clutch size could be used in breeding programs to increase the persistency of the laying hens. Furthermore, birds that show constant egg production for longer periods of time might also reflect better resilience, birds that can cope better with the environment and can keep their production more stable.

**Keywords:** layers; egg production; oviposition pattern; clutch length

**Bone quality evaluation by keel bone palpation and live tibial X-ray density measurement in a White Leghorn pure line population****B. Andersson<sup>1,2</sup>, M. Schmutz<sup>1</sup>, D. Caverio<sup>3</sup>, I. Dunn<sup>4</sup>, S. Struthers<sup>4</sup>, P. Wilson<sup>4</sup>, H. McCormack<sup>4</sup>, R. Preisinger<sup>5</sup>, J. Tetens<sup>2</sup>**<sup>1</sup>Lohmann Breeders GmbH, Cuxhaven, Germany, <sup>2</sup>Universität Göttingen, Göttingen, Germany, <sup>3</sup>H&N International, Cuxhaven, Germany, <sup>4</sup>The Roslin Institute, Edinburgh, United Kingdom, <sup>5</sup>EW Group, Visbek, Germany*Presenting author: b.andersson@lohmnn-breeders.com*

Bone quality issues and in particular keel bone damage are frequently reported in laying hens. The assessment of bone quality is often made by keel bone palpation. The keel bone palpation is easy to conduct on live birds but has limits in aspects of repeatability and is subjective. The aim of this study was to estimate and analyse the genetic parameters for the bone quality traits of keel bone palpation score, live bird x-ray, post-mortem breaking strength and post-mortem bone density. All measurements were performed on 970 white leghorn pure line hens housed in single cages. The bone evaluation on live birds were made at 72 weeks of age and post-mortem analysis at 98 weeks of age. The x-ray density measurement was performed by a portable x-ray generator and the FIJI (ImageJ) image analysis program. Heritabilities, genetic and phenotypic correlations were estimated by VCE 6 software. The heritability for the palpation score was moderate ( $h^2 = 0.17$ ) while it was high for the live tibial x-ray density ( $h^2 = 0.6$ ). The phenotypical correlation between palpation score and tibial density was low ( $r_p = 0.08$ ) but higher for the genetic correlation ( $r_g = 0.22$ ). For the post-mortem traits tibia breaking strength and tibia density the phenotypic correlation was low for the palpation score ( $r_p = 0.14$  and  $0.12$ ) and moderate for the live tibial density ( $r_p = 0.51$  and  $0.59$ ). Accordingly, the genetic correlations were moderate for the palpation score ( $r_g = 0.54$  and  $0.43$ ) and moderate to high for the live tibial density ( $r_g = 0.58$  and  $0.85$ ). The moderate heritability as well as significantly higher correlations between live tibial x-ray density and the post-mortem measurements show, the live tibial x-ray density measurement is a more reliable trait than the subjective keel bone palpation score. This suggests the live tibial x-ray density measurement should be preferred over the keel bone palpation score in a layer breeding program to increase accuracy. This study is part of a project funded by the Foundation for Food and Agricultural Research and BBSRC.

Keywords: Keel Bone, X-ray, Bone quality, Layers

## **Genome-wide association study on egg quality, laying performance and body weight in a backcrossing scheme between Araucana chicken and white layers**

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**Background** It is of foremost interest in evolutionary studies and animal breeding to decipher the genetic background of chicken traits that are either economically important or fancied by breeders. The upcoming of technologies for genomic characterization, such as SNP arrays has enabled the precise detection of genome regions and candidate genes associated with the traits of interest. Genome wide association studies (GWAS) can be even more powerful when applied to a coordinated crossing scheme of phylogenetically distant breeds. In this study, we focus on a back-crossing scheme between Araucana chicken and commercial white layers from the EU-Horizon 2020 IMAGE project which was initially designed to demonstrate the introgression of blue egg shell colour, caused by a retroviral insertion on chromosome 1, into a white layer line. **Animals and methods** As founders of the F1, six males of Araucana chicken were crossed with ten females of high-performing white layers. Followed by two back-cross generations with white layer hens (BC1 and 2) and a final intercross (IC). All animals were genomically characterized by a filtered marker panel of 44k biallelic SNPs. Egg number, egg weight, shell coloration and body weight were measured at multiple timepoints in the BC1, BC2 and IC and the white layer founders of the BC cohorts. GWAS was conducted as single-marker regression with polygenic correction using GCTA-MLMA (Yang et al., 2011). Loci being significant after multiple testing correction with Benjamini-Hochberg were annotated with the Ensembl Genes, version 105. **Results and discussion** The association of the Araucana derived insertion with egg shell colour led to a pronounced signal on chromosome 1, demonstrating the power to detect trait associations in our experimental setting. While egg number showed numerous lower signals across most chromosomes, few became significant, indicating a polygenic background of laying performance. Oppositely, egg weight and body weight, were both highly associated with only few regions. For body weight, chromosomes 1, 4, and 27 had outstanding peak signals directly next to genes such as LCORL and IGF2BP1, both reported separately before to influence body measures. Egg weight at 40 weeks of age was associated with two signals on chromosome 1 and 4 that coincided with the body weight signals, while the signal on chromosome 1 vanished at older age, which suggests changes in the physiology of egg weight with increasing age.

**Keywords:** GWAS; laying traits; body weight

**Studying glycerol toxicity on frozen chicken sperm and strategies to mitigate it****H. Lin<sup>2,1</sup>, P. Mermillod<sup>2</sup>, I. Grasseau<sup>2</sup>, E. Blesbois<sup>2</sup>, A. Vitorino Carvalho<sup>2</sup>**<sup>1</sup>Division of Physiology, TLRI, MOA, Tainan, Taiwan, <sup>2</sup>INRAe, CNRS, IFCE, Université de Tours, UMR PRC, Nouzilly, FrancePresenting author: [linherbie@gmail.com](mailto:linherbie@gmail.com)

Glycerol (GLY) is a common cryoprotectant for sperm cryopreservation in various species. However, its use has adverse effects, reducing sperm fertility. This is particularly evident in chickens, where the optimal sperm freezing protocol uses 11% GLY, while only 6% GLY in inseminated semen causes complete infertility. GLY toxic effects can manifest at two distinct stages: during freezing and thawing at 4°C, and in the oviducts at 41°C following insemination. Varying temperatures may contribute to different levels of GLY toxicity. Therefore, a better understanding of GLY cytotoxicity on sperm needs considering temperature variations. This study was first to investigate GLY adverse effects on the functional capacity of chicken sperm and their impact on sperm storage in the female genital tract. Sperm parameters, such as motility, membrane integrity, apoptosis, mitochondrial activity, ATP concentration, and perivitelline membrane penetration ability were examined. These evaluations were conducted after a 60-min incubation with various concentrations of GLY (0, 1, 2, 6, and 11%) at two temperatures (4 and 41°C). Furthermore, the ability of sperm to colonize sperm storage tubules (SST) was assessed through in vivo insemination and in vitro co-incubation in the presence of 0, 2, or 6% GLY. The results showed that only 6% and 11% GLY had an impact on sperm parameters at 4°C, while greater changes were observed at 41°C, especially regarding sperm motility. In vivo, 2% GLY reduced sperm arrival in SST by half, and a total absence of sperm was observed with 6% GLY. Conversely, 2% GLY during in vitro co-incubation did not decrease the number of SST filled with sperm, whereas a reduction was observed with 6% GLY. These findings highlight a greater impact of GLY at 41°C compared to 4°C and underscore the significant influence of GLY on sperm functionalities, particularly their motility and interaction with the female reproductive tract, including sperm storage in SST. In addition, to mitigate GLY negative impact during insemination, this study then aimed to design a new protocol for GLY removal after thawing by centrifugation on Percoll supplemented with sucrose (SP). This new technique was compared to the conventional serial dilution (SD) method and found to be more effective in removing GLY. Indeed, it can enhance sperm storage in SST, improve fertility rates and duration, and simplify sample processing, making sperm cryopreservation practical in poultry farms.

Keywords: sperm, glycerol, toxicity, sperm storage tubules (SST), fertility

## **The antibody response to experimental *Ascaridia galli* infection in three local chicken breeds**

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*Ascaridia galli* is, together with *Heterakis gallinarum*, among the most common helminths found in chickens. Prevalence's, especially in free-range flocks, are very high and not only cause significant economic losses, but also seriously impact animal welfare with increasing mortality rates. As resistance to anthelmintics is increasing and deworming is complicated by restrictions in organic farming and withdrawal periods, the interest in naturally resistant or resilient birds is growing. Frequently, such characteristics are attributed to local breeds without convincing evidence. It is largely unclear to what extent resistance or robustness is interrelated with production traits, so a perceived high resistance might just be a result of a low performance level. Furthermore, measurable traits are needed to account for these traits in breeding programs. In the study presented here, we analyzed the antibody response to experimental *Ascaridia galli* infection in the three local chicken breeds Altsteirer, Bielefelder, and Ramelsloher as a proxy for immune response to the parasites, and as a possible indicator trait for selection. We tested a total of 360 young male chickens, 120 of each breed, which were divided into control and treatment groups, with a controlled infection trial starting at 7 weeks of age. Birds were infected with 1000 embryonated eggs, while the control received the same volume of saline. We collected serum samples from 60 chickens per breed 3 weeks after the infection, and secondly at the time of slaughter at 14 weeks of age. Ascarid-specific IgY antibody levels were measured using an ELISA assay. All control animals were serologically negative, i.e. showing antibody levels below the infection threshold (50 mU/ml). All infected animals had elevated antibody levels, but also showed breed-specific variation. The antibody levels in the infected groups were analyzed using ANOVA. Altsteirer showed the highest mean antibody levels ( $\mu=371$  mU/ml), while the Bielefelder breed showed the lowest average antibody response to *A. galli* infection ( $\mu=226$  mU/ml). Also, the number of worms in Bielefelder was the highest, whereas it was lowest in the Altsteirer. Our results indicate that different breeds show variation in their response to the infection as measured by worm-specific antibody response.

Keywords: Antibody, *Ascaridia galli*, Chicken, infection, local breed

## **New insight into Wooden breast myopathy by physics behind type III collagen structure**

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In the past fifteen years, the poultry industry has faced the occurrence of muscular abnormalities (and among them, the Wooden Breast – WB – defect) mainly affecting the Pectoralis major muscles (PMs) belonging to fast-growing and high breast yield genotypes selected for meat production purposes. Although in the past few years several studies have been conducted to elucidate the mechanisms likely responsible for the onset of WB, the causative factor triggering the development of this condition has not been identified. However, the extensive proliferation of connective tissue observed in WB, along with the strong association existing between the occurrence of muscle regeneration and collagen type III (COL3), was demonstrated. Within this context, the present study aimed to elucidate the effect of WB on collagen characteristics to deepen the knowledge concerning the role of COL3 in the pathogenesis of this condition. For this purpose, a total of 10 PMs were selected 3 h post-mortem and classified according to their phenotype as normal (N) or WB. Then, COL3 was extracted, purified and characterized in order to study the water and macromolecular physical state of COL3 extracted from N and WB by Differential Scanning Calorimetry (DSC), Fourier Transform InfraRed (FT-IR) spectroscopy, and Scanning Electron Microscope (SEM), after sorption isotherms determinations. Data were analyzed by Student's t-test and considered significant at  $p < 0.05$ . Overall, the findings of the present study evidenced that COL3 extracted from N and WB muscles exhibited a different sorption behavior, with a coefficient of determination of up to  $R^2 = 0.99$  concerning Guggenheim, Anderson, de Boer (GAB) equation, a standard equation used for sorption data fitting. Glass transition and degradation temperatures of COL3 were found to differ between N and WB ( $p \text{ level} < 0.05$ ). Moreover, when comparing the data obtained by FT-IR of COL3 obtained from N and WB, different peaks were identified in several wavelengths, 3300-3200, 1650-1630, 1460-1380, and 1330-1220  $\text{cm}^{-1}$ , thus denoting a different secondary structure. This outcome was even corroborated by SEM further suggesting the existence of a different molecular arrangements of COL3 which is associated with the onset and progression of WB defect. Overall, all the analyses give effort to the hypothesis that the COL3, of N and WB muscles, have different solid structures.

**Keywords:** collagen of type III; wooden breast anomaly; secondary structure; Mid-Infrared spectroscopy; Differential Scanning calorimetry;

## **Adenoviral gizzard erosion complicated with Clostridium Perfringens ventriculitis in a laying hen flock**

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Fowl aviadenoviruses (FAdV) are a well-recognized cause of morbidity and mortality in poultry flocks. Three major clinicopathological conditions following its infection are: Inclusion body hepatitis, Hepatitis-hydropericardium syndrome and Adenoviral gizzard erosion (AGE). The latter is most often caused by FAdV serotype 1 (FAdV-1), and its occurrence in laying hens is clinically characterized by mild increase in weekly mortality and reduction in egg production. On the other hand, more severe gizzard pseudomembranous lesions have been described associated with Clostridium perfringens in broilers associated to higher mortality. Previous injury to gizzard mucosa is thought to enable growth and toxin production by C. perfringens, which is otherwise unable to cause disease in healthy gizzards. We present an outbreak in 23-week-old laying hens which presented a moderate increase in weekly mortality and reduction in egg production. The animals displayed pale comb, and at necropsy they revealed severe ulcerative ventriculitis with digested blood found along the gastrointestinal tract. Tissue samples were fixed for histopathological assessment, and fresh tissue samples and swabs from gizzard lesions were taken for microbiological cultures. At histopathology, lesions were seen in the gizzards, consisting of a necrotizing and lymphoplasmacytic ventriculitis with intranuclear inclusion bodies characteristic of AGE by FAdV-1. Moreover, some animals had more severe lesions due to the addition of a rim of necrotic tissue covering the mucosa, with presence of bacillary gram-positive bacteria. Fresh gizzard samples were used for molecular detection by PCR and sequencing, targeting a hexon gene region of FAdV. The phylogenetic analysis confirmed the presence of FAdV-1. Swab samples from gizzard mucosa resulted in C. perfringens growth, also confirming its presence within the lesions. This is the first case reporting gizzard lesions produced by FAdV and C. perfringens coinfection, which resulted in higher mortality rates. Moreover, we suggest that FAdV produces gizzard mucosal damage, creating an adequate microenvironment within ventricular mucosa that permits C. perfringens growth and damage caused by its toxins.

**Keywords:** Fowl aviadenovirus serotype 1 (FAdV-1); Adenoviral gizzard erosion (AGE); C. perfringens; laying hens.



## **Evaluating the impact of intermittent multi-mycotoxin exposure on layer breeder performance, egg quality, and hatchability: insights from an intervention study**

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Mycotoxins, harmful fungal byproducts found in cereals, pose global threats to animal health. While in-feed interventions are known to effectively prevent mycotoxicosis, there is limited data on their performance during intermittent challenges, commonly encountered in commercial animal production. This study aimed to assess the impact of a natural intermittent challenge (100 ppb Aflatoxin B1 (AFB1) + 100 ppb Ochratoxin A (OTA)) on the performance and health of HyLine W36 layer breeders, including hatchery parameters. The study evaluated the effectiveness of an in-feed intervention comprising bentonite, yeast cell wall components, and a blend of phytomolecules (IFI, EW Nutrition GmbH, Visbek, Germany). A total of 576 hens (18 replicates per diet, 8 hens each) and 58 roosters were randomly assigned to four diets at 28 weeks of age: 1-control (C), 2-control+IFI at 2 kg/ton of feed (CIFI), 3-intermittent mycotoxin challenge (IMC), and 4-IMC+IFI (IMCIFI). The 72-day experimental period included alternating 10-day challenges (ChI) and 21-day non-challenge intervals (NChI), with a total of three challenge and two non-challenge periods (IMC, IMCIFI diets only). IMC tended to decrease overall egg production, egg mass, shell weight, and shell thickness. The initial 10-day challenge interval (ChI) did not impact production parameters, but from the first NChI, all parameters were lower for the IMC group. Productivity declines persisted after the initial 21-day NChI period, continuing through subsequent ChI and NChI periods. Two incubation trials were conducted during the first ChI and third NChI periods, revealing a significant decrease in fertility and hatchability for IMC in both trials. Toward the study's conclusion, oxidative stress biomarkers in the blood serum of 15 hens per treatment indicated higher Malondialdehyde (MDA) and lower Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) in the IMC group compared to group C. The IFI effectively mitigated all adverse effects of IMC on productivity and biomarkers. Intermittent exposure to AFB1 and OTA detrimentally affected layer breeder productivity, egg quality, hatchability, and induced oxidative stress. These negative impacts persisted even after the withdrawal of contamination. This study underscores the effectiveness of in-feed interventions in mitigating the adverse effects of intermittent mycotoxin challenges on layer breeder health and productivity.

Keywords: breeder; hatchability; mycotoxins; oxidative stress

**Impact of moulting on the immune system of commercial layers****S. Rautenschlein<sup>1</sup>, M. Auerbach<sup>1</sup>, A. Grund<sup>1</sup>, R. Lindenwald<sup>1</sup>, H. Hufen<sup>2</sup>, R. Preisinger<sup>3</sup>, C. Sürrie<sup>4</sup>**<sup>1</sup>Clinic for Poultry, Hannover, Germany, <sup>2</sup>Boehringer Vetmedica GmbH, Ingelheim, Germany, <sup>3</sup>EW Group GmbH, Visbek, Germany, <sup>4</sup>Lehr- und Forschungsgut Ruthe, Ruthe, Germany*Presenting author: silke.rautenschlein@tiho-hannover.de*

Moulting is a physiological process for poultry species. It may also be induced in commercial layers to extend production time, improve egg shell quality as well as feather integrity and animal health. Only little is known about the impact of moulting on the consistency of immunity and on immune cell populations. It was speculated that due to changes in light program and feed composition associated with stress of the birds during the moulting period the immune system may be transiently affected and therefore prophylactic strategies may have to be adjusted accordingly. Two commercial layer flocks were monitored during their first laying period, the induced moulting phase, and thereafter with respect to general health parameters including mortality rates with laboratory investigations on possible causes, as well as serum antibody levels for selected pathogens. Furthermore, blood cell counts were evaluated and the impact of moulting on different cells of the innate and acquired immune system was investigated. The moulting did not induce an increase in the mortality rate in either flock. The antibody levels against infectious bursal disease virus and avian Metapneumovirus, against which birds were vaccinated, were stable or slightly increased throughout the whole observation time, respectively. Interestingly, blood cell counts of thrombocytes as well as granulocytes were reduced after the moulting period compared to the laying period before suggesting an effect on the innate immunity of the birds. The T and B lymphocyte number were not affected supporting the serology data. Overall our study confirms that moulting may impact especially innate immune cell populations. Therefore, only clinically healthy birds are recommended to undergo induced moulting, and measures supporting immune cell functions as well as hygiene may be undertaken to maintain flock health during that period.

**Keywords:** layer chicken; moulting; immune system

## **Protection against experimental infection by *Salmonella Gallinarum* biovar *Gallinarum* using different live vaccination programs**

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Correct control of *Salmonella* spp in long-living birds requires strict biosecurity measures, cleaning and disinfection and vaccination program implementation. Herein, the efficacy of five vaccination programs was evaluated. Two live attenuated vaccines, Cevac® *S. Gallinarum* (SG9R strain; IM injection) and Cevac® Salmovac (*Salmonella* Enteritidis auxotrophic strain, oral gavage) were used as described. Group 1 (G1) was vaccinated with *Salmonella* Enteritidis auxotrophic strain 1 week-of-age (woa) and with *Salmonella* *Gallinarum* vaccine at 4 and 8 woa; G2 received *Salmonella* Enteritidis auxotrophic strain at 1 and 5 woa and SG9R at 8 and 12 woa; G3 *Salmonella* Enteritidis auxotrophic strain at 1 and 5 woa; G4 SG9R at 4 and 8 woa and G5 received *Salmonella* Enteritidis auxotrophic strain at 8 and 12 woa. G6 was not vaccinated (control). Each group was composed by 72 commercial brown layer-hens, tested negative for *Salmonella* before challenge. Groups were challenged with SG at 14 WOA. SG protection against mortality was evident in vaccinated birds. No mortality caused by fowl typhoid was recorded in vaccinated groups G1, G2, G4 and G5 birds. In G3 (vaccinated only with *Salmonella* enteritidis auxotrophic strain) the onset of the disease and mortality was delayed, and mortality rate reached 42% during the 2 weeks observation period but was still significantly lower ( $p < 0.05$ ) than in control group, in which was recorded 87.5% mortality rate. As evaluated, primary vaccination with SE auxotrophic vaccine followed by booster dose with SG9R vaccine showed satisfactory efficacy against SG. With the results obtained, it can be ensured that there is no negative interference between the vaccination of *Salmonella* Enteritidis auxotrophic strain and *Salmonella* *Gallinarum* vaccine, showing 100% protection against the challenge of *Salmonella* *Gallinarum* when both vaccines were applied at different ages of life.

Keywords: vaccination, salmonellosis, layer-hens, fowl typhoid, biosecurity

## **Combination of live and inactivated Salmonella vaccines to control Fowl Typhoid in laying hens**

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Fowl typhoid (FT) caused by *Salmonella* ser. Gallinarum biovar Gallinarum (SG) is a poultry disease distributed worldwide. Although it has been eradicated in commercial production of many developed countries, it still persists in developing countries of Latin American, Africa, and Asia as well as in some European countries. There are many products and strategies aimed to control the pathogen in the farms, and vaccines are frequently used to reduce mortality, clinical signs as well as vertical or horizontal transmission. There are several types of *Salmonella* vaccines such as inactivated bacterins and live attenuated strains, which may be administered during the first days of life of the chicks. New trends in the use of vaccines for the control of *Salmonella* include the combination of inactivated and live vaccines, in order to improve the protection of birds and to explore their potential for heterologous protection. In this context, the aim of this work was to assess the protection against FT conferred by vaccines based on *S. Enteritidis* (SE) or SG and their combination. Five experimental groups of 22 birds each were included: 1) inactivated vaccine formulated with SG and SE, 2) a live vaccine based on a live attenuated strain of SE, 3) a plan combining the administration of both live and inactivated vaccines, 4) a plan using the attenuated SG 9R strain, and 5) control unvaccinated birds. At 30 weeks of age, all hens were challenged with a 10<sup>6</sup> colony-forming unit of a virulent strain of SG and mortality was recorded during the subsequent 15 days. The results showed that the plan that included only the inactivated vaccine did not show significant protection ( $p=1$ ) while the plan based on the administration of the attenuated strain of SE significantly reduced mortality in the group of birds ( $p=0.0309$ ). However, highest levels of protection were obtained in the group of hens immunized with the combination of the inactivated vaccine and the live attenuated SE strain ( $p < 0.0001$ ), which was statistically similar to the homologous protection conferred by the SG 9R strain, a vaccine used in many countries to control FT. These results demonstrate that the combination of existing vaccines together with strict biosecurity measures in the farms may help to improve the control of the pathogen in countries where FT is an emerging or re-emerging disease.

**Keywords:** *Salmonella*, Fowl Typhoid, vaccine

**Avian intestinal spirochaetosis in poultry in Ireland – preliminary results**S. Mignacca<sup>1</sup>, D. Bochynska<sup>1</sup>, D. Gudynaite<sup>1</sup>, S. Omerovic<sup>1</sup>, D. Petzoldt<sup>2</sup>, J. Moriarty<sup>1</sup>, A. Sharp<sup>1</sup><sup>1</sup>Department of Agriculture, Food and the Marine - Pathology Division, Celbridge, Ireland, <sup>2</sup>SAN Group Biotech Germany GmbH, Cloppenburg, Lower Saxony, GermanyPresenting author: [sebastian.mignacca@agriculture.gov.ie](mailto:sebastian.mignacca@agriculture.gov.ie)

The genus *Brachyspira* includes nine recognised species, and avian intestinal spirochaetosis (AIS) is a gastrointestinal disease in poultry caused by the colonisation of the large intestine by some of them. AIS mainly affects poultry over the age of 15 weeks, and clinical signs can range from asymptomatic to severe. Wild birds generally represent the source of infection and are usually asymptomatic. This study aims to report AIS through the routine surveillance within the State Vet Laboratory in Ireland during September 2022 - December 2023. Study included only whole avian carcass submissions. After post-mortem examination (PME), faecal pools (up to 5 adult animals for each submission) were sent to SAN Group Biotech Germany and tested for *Brachyspira* spp, *Brachyspira intermedia*, *B. intermedia* & *B. pilosicoli*, and *B. hyodysenteriae* PCR using Kylt® products for DNA extraction and qPCR detection. Out of 155 avian submissions (438 birds in total), 47 faecal pools [17 commercial layers (CL); 10 broiler breeders (BB); 10 backyards (BY); 6 exotics (E); 4 others] were tested. Thirteen samples were positive for *B. intermedia* (4 CL; 2 BY), *B. intermedia* & *pilosicoli* (2 CL), and *Brachyspira* spp\* (1 CL; 1 BY; 3 E). CL and BY were layers with age ranged from 30-w-o till 2.5-y-o (~48-w-o), whilst the E were unspecified adult peacock, flamingo, and ornamental hens, respectively. Animal's history reported weight loss, increased mortality, decreased production, increasing in pale eggshells, dehydration, and changes of the dropping. On PME, intestinal content ranged from normal to dark or, alternatively, pale and mucoid, and mild to severe segmental gassy intestine, mainly of ceca and colon, was also seen. Histological changes ranged from mild to multifocal lymphoplasmacytic typhilitis (layers), or multifocal severe necrotising typhilitis associated with mixed bacterial aggregates (peacock). In some cases, numerous slender filamentous bacteria were clearly seen within the lamina propria of the caecal mucosa in sections stained with Warthin-Starry. However, this technique was often unrewarding. Ten positive submissions had intercurrent diseases (bacteriemia, parasitism, gout), and it is uncertain if *Brachyspira* spp represented either a primary or secondary agent. *B. intermedia* was the common species identified. BB samples tested all negative. *B. hyodysenteriae* was not detected. These data represent preliminary results of AIS in Ireland. \*Other non-identified *Brachyspira* species.

Keywords: *Brachyspira* spp; PCR; poultry; spirochaetosis

**Compatibility of mixing of several respiratory vaccines in spf chickens**

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The imperative to reduce vaccination days in all types of chicken (broilers, layers and breeders) has prompted studies exploring the joint administration of various respiratory vaccines. This trial aimed to assess the compatibility of mixing Newcastle disease (ND) with infectious bronchitis (IB), variant IB, and swollen head syndrome (SHS) vaccines in specific pathogen-free (SPF) chickens, focusing on serological response. One hundred and six SPF chickens were randomly assigned to six groups at 1-day-old (18 chickens in Groups 1, 2, 3, 4, and 6, and 16 chickens in Group 5). Chickens in Groups 1-5 received different vaccines: G1 (IB var 793/B), G2 (IB var QX), G3 (ND CLON/H120 + SHS), G4 (ND CLON/H120 + SHS + IB var 793/B), and G5 (ND CLON/H120 + SHS + IB var QX). The vaccines were mixed, diluted in the same diluent, and administered as a single inoculation by eye drop (I/O). Group 6 served as the non-vaccinated control group. Each group was housed separately, provided with ad libitum feed and water. Body weight (at 1, 21, and 42 days of age) and blood samples (at 1, 14, 21, 28, 35, and 42 days of age) were collected. Serum samples were tested for NDV antibodies (HI test and BioChek ELISA test kit), IBV antibodies (BioChek ELISA test kit), and aMPV antibodies (BioChek and Civtest ELISA test kits). Antibody titers were analyzed and compared using ANOVA and the least significant difference (LSD) test, with significance set at  $p < 0.05$ . As expected, the serological response to the SHS vaccine (Groups 3, 4, and 5) was not detected by the BioChek test kit, while Civtest showed low titers. Antibodies against SHS vaccine in G3 had the highest titer compared to other vaccinated groups. The IB titers in G1 and G2 were lower than expected, while G3, G4, and G5 had normal titers for classic IBV and variant virus, respectively. ND vaccine titers in G4 and G5 were lower than those in G3 at all time points. No differences in body weight between vaccinated groups and the control were detected at 42 days of age. In light of these results, it is suggested that mixing ND, IB, and SHS vaccines is safe and does not adversely affect the performance of common respiratory vaccines for all types of chicken. However, some interference may be observed when variant IB vaccines are included.

Keywords: respiratory vaccines; SPF chickens; mixing; serologies

## **Molecular characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in clinical samples from Spanish poultry flocks**

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*Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) cause considerable economic losses in the poultry industry worldwide. Prevention and control of these agents is based on the use of antimicrobials and vaccination, the latter being the best long-term solution. Utilization of live attenuated vaccines also requires the use of accurate diagnostic tools that differentiate between vaccine and field strains to effectively monitor control programs. Molecular identification of MG and MS was evaluated in 575 and 522 clinical cases respectively, received in our laboratory during the period 2020-2023. Using two qPCR panels targeting specific SNPs, differentiation of field isolates from mycoplasma vaccine strains (MG 6-85 and MSH) was performed on 39 MG and 26 MS positive samples. Additional genotyping of previously characterized samples by qPCR panels (MG=11, MS=14) was also performed using already described MLST protocols (Beko et al. 2019; El-Gazzar et al. 2017). MG was identified in 33% (190/575) of evaluated cases, in samples from chickens (38%), turkeys (20%), partridges (25%) and others (31%). The qPCR characterization identified 56% (22/39) of samples as MG field isolates, 41% (16/39) as MG 6-85 vaccine strain and one sample with co-infection of both types of strain. The MLST analysis of eleven MG samples allowed the identification of eight different sequence types (ST), including ST14 of the vaccine strain and seven ST for field strains (chicken=5, turkey=1, partridges=1). On the other hand, the 53% (278/522) of cases were positive for MS, mainly in chicken (72%) but also in turkey (4%) samples. Twenty-three (88%) of the characterized MS samples were identified as field isolates and the remaining three (12%) as MSH vaccine strain. Genotyping of fourteen MS samples allowed the identification of eleven different ST, including the ST43 of the vaccine strain and ten ST for field strains (chicken=9, turkey=1). Results of the MLST analysis for MG and MS samples corroborated the previous characterization performed by the qPCR panels. In this study, we described novel diagnostic tools for accurate differentiation of vaccine and field strains of MG and MS on clinical samples. Genotyping results indicated a remarkable genetic diversity of MG and MS strains circulating in our poultry industry, as reported by previous studies for these agents in other countries. We recommend the use of these tools for better monitor vaccination and improve control measures

**Keywords:** *Mycoplasma gallisepticum*; *Mycoplasma synoviae*; genotyping MLST; vaccine differentiation; Spain

## **Comparison of fowl adenovirus ELISA and virus neutralization titers in commercial broiler breeder flocks with distinct vaccination schemes against avian adenovirus**

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Inclusion body hepatitis (IBH) outbreaks are currently a growing concern in the meat-poultry industry. In Spain, since 2011, recurrent cases of IBH, mostly involving fowl adenovirus (FAdV) 8b and -11, are observed and linked in most cases to vertical transmission from breeders to progeny. Hence, most preventive actions are around broiler breeder flocks. In this sense, two inactivated vaccines have been used in our country to induce humoral immunity against FAdV in breeders. However, few information exists regarding the interpretation of serological titers in breeders around the transfer age from rearing to lay. To address this, serological monitoring of BB flocks around the transfer age, between 17 and 30 woa, was done using both, a commercial FAdV-ELISA and virus neutralization test (VNT) against FAdV-8b and -11. A total of 43 flocks were included, from which 24 were unvaccinated; 9 were vaccinated once around 10 weeks of age (woa) with vaccine A (inactivated vaccine including FAdV-8b and -11); and 10 were vaccinated once around 10 woa with vaccine B (inactivated vaccine including FAdV-4, -8b and -11). Ten sera per flock were analyzed with both techniques. No differences were observed in the mean ELISA titers among unvaccinated and vaccinated flocks. However, VNT clearly showed distinct seroconversion patterns among the three groups. The lowest FAdV-8b and -11 VNT titers were observed in flocks vaccinated with vaccine B, where only 9 % and 28% of sera samples showed titers above 1:256. Similarly, the percentage of sera samples with FAdV-8b and -11 VNT titers over 1:256 were 24% and 53% respectively in unvaccinated. In contrast, 98% and 100% of sera samples from flocks vaccinated with vaccine A showed FAdV-8b and -11 VNT titers above 1:256. In general, although flocks showed seroconversion based on commercial ELISA at the onset of lay, poor specific FAdV-8b and -11 VNT titers were present in unvaccinated and flocks vaccinated with vaccine B. In addition, poor correlation was observed between commercial ELISA titers and FAdV-8b and -11 VNT titers in any of these groups, which indicates that current commercial ELISA tests effectively detect antibody titers against any group 1 FAdV but could not predict titers against specific serotypes. Also, it is demonstrated that not all commercially available inactivated vaccines are equally effective to induce high antibody titers against FAdV. Finally, the present study contributes to establish a reference to better understand FAdV ELISA and VNT titers.

**Keywords:** ELISA, Fowl adenovirus, inclusion body hepatitis, serology, virus neutralization



**IBDV detection in Northwestern Germany: Epidemiology and correlation to broiler performance****V. Naccache<sup>1</sup>, F. Kloska<sup>2</sup>, W. Stertenbrink<sup>1</sup>, D. Klosterhalfen<sup>1</sup>, M. Thielke<sup>1</sup>, L. Kalvelage<sup>1</sup>, S. Rautenschlein<sup>3</sup>**<sup>1</sup>Tierarztpraxis WEK, Visbek, Germany, <sup>2</sup>Ceva Tiergesundheit GmbH, Düsseldorf, Germany, <sup>3</sup>Klinik für Geflügel, Stiftung Tierärztliche Hochschule, Hannover, GermanyPresenting author: [v.naccache@outlook.de](mailto:v.naccache@outlook.de)

Infectious bursal disease virus (IBDV) leads to immunosuppression in young chickens and thus to high economic losses worldwide. Since recently, a comprehensive study proved the high prevalence of reassortant field IBD viruses in Europe. The aim of this study was to conduct an epidemiological and phylogenetic investigation of the virus in broiler flocks in Northwest Germany and to connect these data to broiler performance. Samples were collected during routine veterinary monitoring from 53 broiler flocks. Flocks were vaccinated with live intermediate or intermediate plus vaccines between 12 to 18 days of life via drinking water. Animals have been sampled between day 24 and 43 being implemented in routine veterinary diagnostic work. Sampling included blood samples (n=20/flock) and bursae (at least 5/flock). Blood samples were analysed by using a commercial ELISA kit. Bursae were pooled and analysed by RT-PCR followed by sequencing. Results from the various screenings were compared with the performance data (weight at slaughter, daily weight gain, feed conversion ratio [FCR], condemnation rate, European Poultry Efficacy Factor [EPEF]) from the sampled cycles. RT-PCR findings revealed about 53% negative bursa samples. Consequently, there are 47% positive samples, of which 48% were confirmed as field strains by sequencing. Vaccine strain was found in 25 % of total flocks. All field viruses are reassortant-strains belonging to genogroup A3B1 with 98% similarity to the reassortant genotype circulating in the Netherlands (reassortant strain D4320/6, genbank accession no. MN786768). Daily weight gain and weights at slaughter were lowest in the reassortant group compared to the vaccine group (66.5 g vs. 69.2 g and 2.59 kg vs. 2.71 kg). Additionally, the EPEF is lowest in the reassortant group, about 10 points compared to the vaccine group ( $p < 0.05$ ). A recent study about the infections kinetics of the reassortant strain found the highest detection rate before 32 days of age. Taking the wide sampling window into consideration we can assume a higher prevalence of A3B1 virus infected flocks in the negative tested group (53% of flocks). Strains which belong to the reassortant cluster lead to a subclinical form of Gumboro disease with broilers showing immunosuppression and loss of performance. Future vaccination programs should be adapted to the current epidemiological situation and might switch to in-ovo vaccination to improve animal welfare and health.

Keywords: IBDV, reassortant, broiler, performance, epidemiology

## **Pathology of colibacillosis and its molecular detection from recent outbreaks in Chicks**

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Colibacillosis is caused by avian pathogenic *Escherichia coli* (APEC) that affects poultry. Prevention of colibacillosis is becoming challenging due to the emergence of virulent and resistant strain and hence, limited availability of drugs. Early detection is essential to prevent colibacillosis. Because of the strain diversity and economic significance, this study investigated the pathology of colibacillosis and its molecular detection from clinically sick birds from the recent outbreak. Dead birds and cloacal swabs from sick live birds were collected and enriched in tetrathionate broth. Then the bacteria were characterized by different bacteriological media and biochemical test and confirmed by PCR and histological studies. The organisms showed round and white colonies on agar media with metallic sheen on EMB agar. It revealed pink colored, short rod to coccoid shaped, single or paired Gram-negative bacilli on Gram's staining. Active motility was observed on hanging drop slide. It fermented basic sugars with production of acid and gas. Postmortem findings showed congested and consolidated lungs and liver. The intestine showed mucus, congestion, haemorrhage and airsacculitis. Histologically, sections of heart showed pericarditis with thickening of pericardium. Livers exhibited coagulation type of focal necrosis with infiltration of heterophils and lymphocytes densely located in the portal area. The lungs displayed severe congestion, infiltration of heterophils and lymphocytes in the wall of the bronchus as well as in the peribronchial alveoli. These findings will help field veterinarian as well as poultry farmers for effective management. However, further studies are warranted to identify the genes responsible for emergences of *E. coli*.

**Keywords:** *E. coli*, colibacillosis, chicks, pathology, PCR

## **Morphological and immunological effect of in ovo administration in chicken of two different compound with probiotic and postbiotic feature**

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The use of the “in ovo” technique for the administration of substances to the chicken before hatching has been widely demonstrated to be effective, with multiple studies observing how this early interaction with the chicken allows an improvement in growth performance and functionality of the immune and gastrointestinal systems. The administration into the amniotic fluid permits a direct contact with gastroenteric and respiratory apparatus during hatching, when it ingests the residue of the amniotic fluid. Aim of this study is to evaluate the effect of in ovo administration of two different mixture. 150 embryonated eggs were divided into three treatment groups of 50 eggs with 3 replicate cages of 15 birds each. At day 18 of incubation eggs were subjected to in ovo administration into the amniotic fluid of the following mixture:  $1 \times 10^5$  of a probiotic bacteria blend (FSG68/22® : Bifidobacterium longum subsp longum, Bifidobacterium longum subsp infantis, Bifidobacterium animalis subsp. lactis., Bifidobacterium breve, Bifidobacterium bifidum , Lactobacillus reuteri, Saccharomyces boulardii) in 0,05 ml of reconstituted Marek disease Vaccine (P group);  $50$  of freeze dried and tyndallized product obtained from the intestinal contents of healthy adult chickens (In Ovo patent ®) enriched with live probiotic bacteria  $1 \times 10^5$  (FSG 68/22 ®) in 0,05 ml of reconstituted Marek disease Vaccine (T group); 0,05ml of reconstituted Marek disease Vaccine (C group). At 42 days of age, 5 chickens per replicate were sacrificed by cervical dislocation to collect organs samples for histopathological evaluation. When compared to group C, the parameters of villus height and crypt depth are both increased in treated groups, indicators of an increased absorbent surface. In treated groups, and especially in group T, is also observed a constant functional increase of the lymphopoietic system, resulting from the evaluation of thickness of the lamina propria, area of the splenic, bursal and intestinal lymphoid tissue, parameters related to a greater cellular mitotic index. Also coccidian lesion and oocyst elimination in stool are reduced in group T. Preliminary results demonstrate the benefits of the treatment in all observed parameters, confirming the safety of the in ovo technique and its potential in preventing perinatal and early rearing period bacterial and parasitic disease.

Keywords: in ovo, probiotic, postbiotic, fecal transplant

**Lipids: Consistently inconsistent – Variability in commercial fats and oils****K. Bierinckx<sup>1</sup>, D. Gonzalez Sanchez<sup>1</sup>**<sup>1</sup>Kemin Europa N.V., Herentals, Belgium

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Lipids are the feed ingredients with the highest energy as well as the highest cost per unit of energy. Therefore, it is key to monitor and assess conscientiously their cost per kcal, with the final goal of making the best purchasing decision and improving feed formulation accuracy. As progressively more industrial by-products become available as alternatives to lipid sources, nutritionists are often faced with an increased variation of lipid quality, which requires a more precise, both nutritional and economic evaluation. The Lipid Evaluation Test (LET) assesses in total ten different chemical characteristics in fats and oils. Three parameters define their oxidative quality: peroxide value, thiobarbituric acid value, and oil stability index. Three nutritional parameters are evaluated: the level of free fatty acids, the unsaturated:saturated ratio, and the fatty acid profile. These nutritional parameters are used to estimate the dietary energy contribution of lipids in feed for broilers through the equation empirically developed by Wiseman et al, 1991. LET also incorporates the assessment of three energy-diluting factors: moisture, impurities, and unsaponifiable compounds. Since its launch in 2014, more than 2140 lipid samples have been analyzed by the LET. Of this extensive dataset, 73% of samples are represented within five groups: soybean oil, animal fat, acid oils, sunflower oil, and poultry oil. Findings show that in almost two out of three samples, signs of oxidation are detected. Primary oxidation was noted in all samples, yet more pronounced in vegetable oils. Higher levels of secondary oxidation compounds were detected typically in processed lipids like animal fats and acid oils. Most oils and fats analyzed showed mainly oxidation in its initial phase, except sunflower oil where a more evolved oxidation was registered. LET also reveals the presence of a large variation in dietary energy values, even between identical fat sources. The inclusion of energy-diluting factors demonstrated a more excessive variation in the nutritional value of lipids. In extreme cases, even up to 100%. This study confirms that a correct estimation of the metabolizable energy content and oxidative quality of a lipid is only possible when a proper and full chemical evaluation is done. The LET therefore offers a suitable, analytical platform to gain in-depth insights into the nutritional and oxidative quality of fats and oils.

Keywords: lipid quality, oxidation, nutritional value, broilers, metabolizable energy

## **Evaluation of the simple and interactive effect of corn and oil quality in 21-day-old broiler chickens**

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The effects of climatic conditions on poultry and livestock production go beyond heat stress as it extends to nutrition and the health of the animal. The objective of this study was to evaluate the effect of corn quality (regular corn vs. naturally contaminated corn fines [CF] with mycotoxins) and oil quality (fresh vs. oxidized) on bird performance, relative liver weight, nutrient and energy utilization, and ileum histology of broiler chickens. The experiment used 256-day-old male by-product Cobb breeder chicks in a RCBD consisting of 4 treatments (2 x 2 factorial arrangement) with 8 replicate cages of 8 birds per cage. Fresh soy oil (peroxidized value [PV] = 8.5 meqO<sub>2</sub>/kg) was oxidized by continuous heating at 95°C for 8 days resulting in a PV of 167 meqO<sub>2</sub>/kg. Diets were fed from day 0 to 21. Birds and feed were weighed on days 0, 7, 14, and 21 for performance determination while the liver and section of the ileum (mid-ileum) were taken from the same bird for the determination of relative liver weight and histology measurements. The predominant mycotoxins in the regular corn and CF were deoxynivalenol (531 vs. 7,959 ppb), aflatoxin B1 (<1.3 vs. 2.1 ppb), total fumonisin B1, B2, and B3 (700 vs. 23,200 ppb), and zearalenone (109 vs. 1,403 ppb). Each diet contained 3.5 and 60.5% of the respective oil and corn quality sources. There was no significant 2-way interaction on performance. Corn fines showed a tendency ( $P = 0.06$ ) to increase relative liver weight (6.8%). The overall body weight gain (15.6%), feed intake (5.2%), and feed efficiency (9.8%) were lower ( $P < 0.05$ ) in birds fed diets containing CF. The main effect of oil quality showed lower ( $P < 0.05$ ) Ca retention in birds fed diets containing oxidized oil compared to birds fed diets containing fresh oil. Interaction between mycotoxin-containing CF and oxidized oil resulted in the lowest ( $P < 0.05$ ) AMEn, DM, energy, N, and P utilization. Corn fines resulted in a lower ( $P < 0.05$ ) villi height-to-crypt depth ratio compared to that of the birds on regular corn. Interaction between CF and fresh oil resulted in deeper ( $P < 0.05$ ) crypt depth compared to the remaining treatments. Results from this study showed that both the mycotoxin-contaminated CF and oxidized oil singly or in combination reduced performance, nutrient utilization, and modified ileum intestinal morphology of 21-d-old broiler chickens.

**Keywords:** broiler; corn fines; nutrient retention; performance; oil quality

**Inclusion of a stabilized emulsifier in low-energy diet for broilers: effects on nutrients retention, excreta trait and growth performances****L. Marchetti<sup>1</sup>, R. Rebucci<sup>1</sup>, C. Piantoni<sup>1</sup>, V. Perricone<sup>1</sup>, V. Bontempo<sup>1</sup>**<sup>1</sup>Department of Veterinary and Animal Science (DIVAS), Università degli Studi di Milano, Lodi, ItalyPresenting author: [luca.marchetti1@unimi.it](mailto:luca.marchetti1@unimi.it)

Increasing sustainability of feed production chain is crucial according to current European legislation. Broilers generally require high energy diets and vegetal oils are included in feed to rise the caloric content. Minimizing the inclusion of complex energy sources, may represent an optimal approach to enhance the sustainability of the feed production chain. This study focused on the effects of the inclusion of an emulsifier composed by polyethylene-glycol ricinoleate and bi-distilled oleic acid stabilized on silica (Nutriemul P, Sevecom part of Barentz) in low-energy diets on performances and apparent total tract retention (ATTR) of broiler. Immediately after hatching, 720 male Broilers ROSS 308 were divided into 4 homogeneous groups (9 replicates per group / 20 chicks per replicate): positive control (PC) fed with a standard diet, negative control (NC) fed a basal diet reduced in energy content (-70 kcal/kg) through soybean oil modulation, T1 and T2 fed NC diet + 250 and 500 g/ton of emulsifier, respectively. Performances were evaluated at 0, 10, 21, and 42 d on trial. Excreta were collected at 24 and 42 d directly from polyethylene trays and pooled to determine dry matter (DM), crude protein (CP), ether extract (EE), gross energy (GE), apparent metabolizable energy (AME), and calculate their ATTR. Nitrogen ammonia was assessed on excreta samples. Performance analysis was executed with a MIXED procedure of SAS, while ATTR and nitrogen ammonia were analysed using a GLM procedure of SAS. The inclusion of emulsifier in T1 and T2 ameliorated ATTR of DM compared to NC at 42 d ( $P < 0.05$ ). CP and EE retention rate ameliorated in T1 and T2 vs NC at 42 d ( $P < 0.05$ ). GE retention, AME and AME/GE ratio were enhanced in T1 and T2 vs NC at 42 d ( $P < 0.05$ ). Nitrogen ammonia decreased in T2 samples at 42 d compared to NC ( $P < 0.05$ ). Body weight was higher in T1 vs NC ( $2845.67 \pm 90.69$  g vs  $2557.52 \pm 83.68$  g;  $P < 0.01$ ) and in T2 vs NC ( $2773.83 \pm 93.56$  g vs  $2557.52 \pm 83.68$  g;  $P < 0.01$ ) at 42 d. Average daily gain improved in T1 vs NC ( $68.37 \pm 2.21$  g vs  $61.34 \pm 2.05$  g;  $P < 0.01$ ) and in T2 vs NC ( $66.63 \pm 2.29$  g vs  $61.34 \pm 2.05$  g;  $P < 0.01$ ) considering the overall trial period. Improving retention of dietary nutrients through emulsifier revealed promising results towards an optimization of complex energy sources inclusion, showing positive reflexes in performances, sustainability, and economic terms.

Keywords: "emulsifier"; "nutrient retention"; "broiler"; "performance"; "low-energy diet"

**Intelligent emulsifier addition to broiler feed formulations to improve FCR****D. Michels<sup>1</sup>, S. Verkempinck<sup>1</sup>, K. Vermeulen<sup>2</sup>, R. Spaepen<sup>2</sup>, E. Burton<sup>3</sup>, D. Scholey<sup>3</sup>, A. Wealleans<sup>2</sup>, T. Grauwet<sup>1</sup>**

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Increasing awareness of poultry production's environmental impact implies the industry must look for strategies to improve its sustainability, including reducing raw material use through improved feed conversion (FCR). One established approach is the use of absorption enhancers based on lysolecithins to improve lipid digestion and nutrient absorption, improving the rate and extent of emulsification in the gizzard of birds. However, *in vitro* digestion research in humans showed significant improvements in fat digestibility when preparing oil-in-water (O/W) emulsions prior to ingestion. Therefore, the present research aimed to evaluate the impact of a pre-prepared O/W emulsion versus separate oil and absorption enhancer additions to broiler feed on fat digestibility and FCR. Diets were prepared containing oil and a commercially available absorption enhancer (LEX) separately (SEP) or prepared as an O/W emulsion (O/W). The *in vivo* performance trial showed a significant improvement in FCR from 1.59-1.64 to 1.34-1.40 over the course of three weeks from feed formulations containing oil and LEX separately (SEP) to feed containing the O/W emulsion (O/W). The largest improvements in FCR were gained in the first week from 2.83-2.88 to 1.56-1.67, respectively. However, the digestibility trials did not show significant differences in fat digestibility. Additionally, complementary *in vitro* digestion of the diets was performed using an adapted *in vitro* digestion protocol simulating the grower broiler gastrointestinal tract. These *in vitro* digestions enable mechanistic insights into the digestion process by assessing the digestion kinetics (i.e. rate and extent). While no significant differences were present between the extent of fat digestibility of the diets, digestion kinetics showed significant differences. Their sigmoidal behavior showed significantly shorter lag phases in diets containing more LEX and/or an emulsion instead of the separate ingredients. Alongside fat digestibility, also *in vitro* protein and starch digestibility were assessed. Results showed different digestion behaviors in both fat and protein digestion in diets containing no emulsifiers, thereby indicating interactions between these two macronutrients, which were thought to occur less as the result of emulsifiers being present. Therefore, this study showed that the addition of O/W emulsions to feed formulations affects macronutrient digestion kinetics, thereby presumably improving the FCR.

**Keywords:** Emulsifiers; Nutrient Digestion; Broiler; Performance; Fat

## **The effect of a combination of organic acids and medium chain fatty acid mono esters administered via water on broiler performance, mortality rate, and foot pad score**

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A total of 630,000 1d old Ross-308 broilers, males and females, from 3 consecutive batches, were used to evaluate the effect of administration of medium chain fatty acid (MCFA) monoesters and organic acids (formic acid, propionic acid, sodium formate; Eastman Protaq Bond™ LF5Na, Protaq) via water on performance, mortality rate, foot pad score and Salmonella incidence in broilers. The trial was carried out at Nibe (Denmark) in commercial conditions in two different 3-barn farms. There were 3 experimental treatments: a Control (T1) based on the habitual management on the farm; T2: consisting in the application of 1L of Protaq per 1,000L of water in two passes (from 30-35d and from 40-42d); and T3: consisting in the application of 1L of Protaq per 1,000L of water from 30-42d. Each treatment was allocated to a different house in three consecutive batches, and there were 6 replicates per treatment arranged in a (2x3x3) factorial design based on two locations, 3 houses and 3 rounds. A partial thinning was done in each house according to standard procedures. The experimental unit was a house with 35,000 birds housed together. All diets were formulated to meet or exceed the nutrient requirements recommended by NRC for broilers at each age. The diets were presented in pellet form, feed and water were ad libitum available. Weight gain, feed intake and feed conversion ratios of each batch were determined, and mortality percentage was calculated. Salmonella was analysed through 2 pairs of boot swabs before slaughtering. Performance data were analysed by ANOVA as a completely randomised design by GLM of SPSS, including the experimental treatment, the round, and their interaction in the model. Mortality rate was analysed by X2. Protaq administration via water (T3) from 30-42d increased the average daily gain of birds, corrected for day 34, by 5% in comparison to non-supplemented animals, whereas animals fed T2 presented intermediate values (63.6, 65.7 and 66.7 g/d for T1, T2 and T3, respectively,  $P=0.041$ ). Also, the Protaq application increased the - at 34 days corrected - body weight by 4% and 6%, respectively (2,004, 2,087 and 2,120g for T1, T2 and T3, respectively,  $P=0.025$ ). No significant effects on feed intake ( $P=0.140$ ), feed conversion ( $P=0.410$ ), or mortality rate ( $P=0.660$ ) were found, and Salmonella incidence was not analyzed since all the samples were negative. The results of the current trial showed that a combination of organic acids and MCFA monoesters administered via water from 30-42d is effective to improve broiler performance.

Keywords: broilers; water; MCFA monoesters



## **Influence of free fatty acid content and degree of fat saturation in laying hen diets on bone quality**

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Shortages and price fluctuations have driven the use of unconventional ingredients in poultry nutrition, such as acid oils (AO) and fatty acid distillates (FAD). These fat by-products are characterized by their high levels of free fatty acids (FFA), which have been reported to reduce calcium (Ca) absorption, particularly when originating from fats abundant in saturated fatty acids (SFA). Given the crucial role of Ca mobilization from the medullary bone in supporting the eggshell formation, any disruption in Ca metabolism can adversely affect bone mineralization. Thus, a trial was conducted to assess the impact of dietary FFA and degree of fat saturation on bone quality. In a 15-week study, 144 laying hens (19 weeks old) were randomly assigned to 8 dietary treatments (6% added fat) formulated by progressively replacing soybean oil (rich in unsaturated fatty acids; UFA) with soybean AO, or palm oil (rich in SFA) with palm FAD. This resulted in 4 UFA-rich and 4 SFA-rich diets with varying FFA content: 10, 20, 30, and 45%. Tibias (6 birds/treatment) were collected at the end of the trial for the assessment of mineral composition, morphological properties (length, weight, diameter, and cortical bone thickness), and mechanical characteristics (breaking strength and elasticity). The data was analysed using two-way ANOVA with the GLM procedure. Orthogonal polynomial contrasts were employed to determine the linear effect of increasing %FFA. The degree of saturation impacted on Ca and phosphorus (P) bone content, with higher levels found in soybean-based diets ( $P < 0.001$ ). A significant interaction was observed for medullary bone mineral content, showing a linear decrease in palm diets as the dietary %FFA increased ( $P < 0.05$ ). This effect was not observed in soybean diets. In contrast, the total ash content, mineral composition of cortical bone, and morphological and mechanical traits remained unaffected. These results suggest that the degree of fat saturation exerts a greater impact on calcium metabolism than the FFA content. Despite differences in bone mineral content among treatments, key morphological and mechanical properties assessed in this study were not affected. However, hens were fed these experimental diets up to 34 weeks of age, and differences might become more apparent at a later stage. Therefore, examining the long-term effects of feeding laying hens with AO and FAD in terms of lay persistency merits further investigation.

**Keywords:** Acid oil; bone quality; calcium; fat by-product; fatty acid distillate

## **Apparent fatty acid digestibility in laying hens fed medium-chain fatty acid-rich diets**

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In recent years, new ingredients and by-products have been studied for their properties as alternative raw materials in poultry diets. Moreover, the use of by-products in feeding contributes to the circular economy. To introduce these new sources into feed composition, it is necessary to study their digestibility. An experiment was conducted to assess the digestibility in layers of three different fat sources included in diets. Fifty-four Lohmann Brown laying hens (23 weeks old) were randomly assigned to 18 experimental cages, with 3 hens per cage (each cage serving as a replicate). The cages were divided into 3 different diet groups (6 replicates/treatment). Each diet was supplemented at 5% with one of these different oils: palm kernel oil (PKO) and palm kernel fatty acid distillate (PKFAD) as medium chain fatty acid (MCFA)-rich fat sources (around 40% of its composition are MCFA), and soybean oil (SO) as control. Lauric acid (C12:0) was much more abundant in PKO (22.14 mg/g feed) and PKFAD (23.76 mg/g feed) diets than in SO (1.93 mg/g feed) diet. SO diet presented a higher proportion of long-chain fatty acid (LCFA) than the other two fat sources. At the end of a 12-week feeding period, hens' excreta were collected for three consecutive days. The fatty acid content of the diet and the excreta was determined by gas chromatography. Using an inert marker (TiO<sub>2</sub>), apparent total tract digestibility (ATTD) coefficients of the different fatty acids were assessed. Results showed that generally, PKO presented lower ATTD coefficients in most of its fatty acids, while SO had the best LCFA values, with an average of 0.87. In relation to ATTD of MCFA, C12:0 was more digestible ( $P < 0.05$ ) in SO (1.00) than PKO (0.97), and both than PKFAD (0.94). The ATTD coefficient of myristic acid (C14:0) presented the highest value for PKO (0.96) and PKFAD (0.89). Regarding the LCFA, within monounsaturated fatty acid (MUFA) oleic acid (C18:1n9c) revealed high ATTD values in the three diets (SO:0.88, PKFAD:0.84 and PKO:0.83;  $P = 0.06$ ). About polyunsaturated fatty acid (PUFA), linoleic acid (C18:2n6c) presented differences in ATTD ( $P < 0.05$ ) between PKO diet (0.65) and the other two diets (PKFAD:0.81, SO:0.88). Alfa-linolenic acid (C18:3n3) presented the same model, where PKO had the lowest digestibility value (PKO:0.69 vs PKFAD:0.82 and SO:0.92;  $P < 0.05$ ). These data are promising to introduce new fat sources in feeding as alternative to conventional oils, even for their combined use.

Keywords: fatty acid; palm kernel; laying hen; digestibility.

## **Dietary inclusion of Echium plantagineum oil and metabolism modulation of n-3 LC-PUFA in Canarian chicken**

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It has been demonstrated that poultry could become a sustainable source of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) through desaturation and elongation of the dietary C18 precursor  $\alpha$ -linolenic acid (ALA, 18:3n-3). However, the high n-6/n-3 ratio in poultry diets, the competition between n-6 and n-3 fatty acids for the same enzymes, and the rate-limiting  $\Delta 6$  desaturase in LC-PUFA production suggest that the use of dietary vegetable oils rich in ALA might not be the unique and most efficient strategy to obtain poultry products containing high n-3 LC-PUFA levels. As an alternative, we propose the use of Echium plantagineum oil which contains not only a balanced linoleic acid (LA, 18:2n-6)/ALA ratio, but also stearidonic acid (SDA, 18:4n-3), the first metabolic intermediate fatty acid in the conversion of ALA to EPA and DHA. To that purpose, 60 Canarian chickens (local breed from the Canary Islands, Spain) at the age of 18 weeks were divided into 3 groups and fed with a commercial cereal-based feed supplemented with soy oil rich in LA; linseed oil rich in ALA; or E. plantagineum oil, a balanced LA/ALA oil also rich in SDA and  $\gamma$ -linolenic acid (GLA, 18:3n-6). After 45 days of trial, brain, liver, intestine and muscle (breast) were collected to determine their fatty acid composition and to evaluate the gene expression of fatty acid elongases and desaturases (*fads1*, *fads2*, *elovl2* and *elovl5*). Total n-3 LC-PUFA contents in liver and muscle ( $10.68 \pm 0.70\%$  and  $7.55 \pm 0.41\%$ , respectively) were higher ( $P < 0.05$ ) in the Echium group compared with those values of linseed group ( $9.53 \pm 1.09\%$  and  $5.14 \pm 1.60\%$ ) and soy group ( $5.25 \pm 0.41\%$  and  $4.25 \pm 0.82\%$ ). Although significant differences were not found in total n-3 LC-PUFA in brain and intestine among groups ( $P > 0.05$ ), an upgrade tendency was observed in the Echium and linseed groups compared with the soy group. Furthermore, a higher relative gene expression of *fads1*, *fads2*, *elovl2* and *elovl5* was obtained in the individuals from the Echium group ( $P < 0.05$ ), confirming the capability of this oil to induce upregulation of the n-3 LC-PUFA synthesis. These results showed that a balanced C18 n-6/n-3 ratio and higher SDA dietary contents, can positively modulate the endogenous production of n-3 LC-PUFAs in the liver and muscle tissue of canarian chickens.

**Keywords:** Canarian chicken; Echium plantagineum; n3 LC-PUFA; metabolism; gene expression

**Effect of a high-energy diet on liver stress and lipid metabolisms, meat and carcass traits in a slow-growing chicken breed**N. Stoppani<sup>1</sup>, F. Raspa<sup>1</sup>, E. Fiorilla<sup>1</sup>, C. Mugnai<sup>1</sup>, V. Zambotto<sup>1</sup>, S. Nurisso<sup>1</sup>, A. Schiavone<sup>1</sup>, D. Soglia<sup>1</sup><sup>1</sup>Università degli studi di Torino, Dipartimento di Scienze Veterinarie, TORINO, ItalyPresenting author: [nadia.stoppani@unito.it](mailto:nadia.stoppani@unito.it)

Bionda Piemontese (BP) is a slow-growing dual-purpose autochthonous chicken breed reared in Piemonte region (North-west Italy). In this study, 60 BP were fed with two isoproteic diets [15 males (M) and 15 females (F)/dietary treatment], which differ for the lipid content: a low lipid diet (LL, ether extract EE=3.6%) and a high lipid diet (HL, EE=9.3%). The experimental diets were administered during the finishing period (120-150 days of age), to evaluate the effects on slaughter traits and liver gene expression. At 150 days of age, birds were slaughtered and the following parameters evaluated: live weight, carcass yields (carcass, breast, thigh and abdominal fat) and meat quality traits (pH and colour). Thirty-six liver samples (9 samples/group) were collected in RNAlater for gene expression analysis. Genes linked to stress and lipid metabolisms were analysed: CAT, CASP6, FADS2, ACOX1, LPL, SREBF2, HSP2, SOD1, FABP1, ACTB and GAPDH was used as housekeeping. Multiplex Digital Expression Gene Analysis (MuDEGA) was used by NGS sequencing (MiSeq Illumina). Total RNA was extracted with FastGene® RNA Premium Kit and evaluated for its quantity (Qbit®, RNA Broad-Range Assay Kit) and integrity (RIN, Agilent 2100 Bioanalyzer). Reverse-transcription, multiplex PCR, library preparation, and digital count by NGS sequencing were performed. Differential expression gene analysis was performed using DESeq2 R package. P-adjusted value<0.05 was used as threshold. The statistical analysis showed no significant differences according to the dietary treatments on both slaughtering traits and liver gene expression. Interesting significant differences (p<0.001) were found between sex. In particular, abdominal fat weight (M= 1.2%; F= 4.4%); pH thigh (M=5.9; F=6.0); breast redness a\* (M=-0.4; F=-2.2), breast yellowness b\* (M=5.2; F=9.3); breast lightness L\* (M=55.5; F=57.7); thigh yellowness b\* (M=10.4; F=11.4) and in LPL liver expression. LPL resulted upregulated in M compared to F (p<0.001; Fold Change =2.57). This gene is involved in liver fatty acid metabolism and its overexpression could be linked to an increase in lipid synthesis and accelerate lipolysis. In conclusion, HL diet did not influence the slaughtering traits and the expression of liver genes linked to stress and lipid metabolism in Bionda Piemontese breed. However, some quality parameters and LPL expression were influenced by the sex of the animals.

Keywords: local breeds; chicken; diet; gene expression; meat

## **Computed tomography as a non-invasive tool to characterize and advance on chicken breast myopathies**

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Computed tomography (CT) is a non-invasive technology based on X-rays that allows the evaluation of the internal body characteristics both in vivo and postmortem. Breast myopathies are muscle defects mainly associated with fast-growing broilers. Industry and scientists are making considerable efforts to understand the mechanisms behind these abnormalities and to find solutions to reduce them, but robust technological tools are still scarce. This study evaluates the feasibility of CT to identify breast myopathies (i.e., white striping, WS; wooden breast, WB; and spaghetti meat, SM) based on carcass and muscle characteristics by scanning the whole carcass. A total of 240 chicken carcasses were selected in a commercial slaughterhouse for myopathy presence (60 WS, WB, SM, and control (CO)). The refrigerated carcasses were scanned with a Philips Brilliance 16 CT (120 kV, 200 mA, 3-mm thickness images). Three images of different sections along the breast (i.e., cranial, central and caudal) were chosen. For each image, a region of interest (ROI) including the entire breast area was selected. Different morphological and density measurements were calculated from this ROI. Statistical comparisons were made with LSMEANS using R software. The area (mm<sup>2</sup>) and perimeter (mm) depended on myopathy in the 3 breast sections ( $P < 0.001$ ), with the highest values for WB, the lowest for CO, and WS and SM in between. In terms of density (Hounsfield values, HU), WB had a lower average compared to the other 3 types in the cranial region. In the central and caudal regions, the average density was the lowest in WB, followed by WS and SM, and the highest in CO ( $P < 0.001$ ). When representing the distribution of the relative volume associated with each HU (density histogram), the WB's curve was more shifted towards lower HU than the other types, indicating that more volume had less density. Within a specific muscle density range (60-80 HU), the WS's curve was placed on the lowest values, CO on the highest ones, and SM in between. These findings can be related to the changes in the muscle chemical composition (reduced protein and increased water, fat and collagen) and the macroscopic and histological lesions caused by myopathies. In conclusion, CT shows potential to discriminate myopathies and could be a useful technology for industry and researchers to characterise them in a non-invasive way as well as to assess the effectiveness of strategies applied pre- and post-mortem to reduce them.

**Keywords:** non-invasive; morphology; density; white striping; wooden breast; spaghetti meat

## Use of shear-wave elastography to predict in vivo the presence and severity of the wooden breast defect in chickens

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The standard and heavy chicken production sector is faced with breast meat quality defects such as Wooden Breast (WB). It is characterized by abnormal rigidity of the skeletal muscle due in particular to the progressive installation of connective tissue to the detriment of muscle fibers. Despite intensive research worldwide, professionals are still waiting for solutions to reduce these defects, which appeared around 10 years ago. Having a sufficiently accurate method for estimating the presence and severity of the WB defect in live animals would be an asset in the search for solutions, particularly genetic ones. Our aim was to demonstrate that ultra-fast shear-wave elastography, which enables the rigidity of a tissue to be quantified in real time and in a minimally invasive way, could be effective in detecting the presence of the WB defect in live animals. An initial experimental phase was carried out on 200 individuals (males and females) of the same genetic origin but affected in different ways by defects in muscular integrity. It demonstrated the possibility of discriminating between groups according to their degree of WB (noted visually after slaughter), on the basis of the value of the shear modulus measured at the pectoral level of live chickens. A second phase was then carried out on 300 pedigree animals from the same line to validate the possibility of using elastography under selection conditions to predict the WB defect in vivo. We defined the age at which the measurement should be taken, the position and orientation of the probe, the number of measurements to be taken per individual and the number of operators required. The procedure put in place allows 3 operators to measure around 30 individuals per hour, and its application would be therefore compatible with the commercial selection conditions. During the two experimental phases, we observed a gradual increase in elastography values with the intensity of the WB defect measured post-mortem, but also the possibility of discriminating the animals most affected by WB (score 2 and 3) from the others. Initial estimates of genetic parameters reveal a heritability of 0.2 for the elastography measurement and a positive genetic correlation of 0.69 with the WB score. These initial results therefore suggest that elastography could be a diagnostic tool that could be applied in selection, with beneficial spin-offs for the entire industry.

Keywords: meat; wooden breast; shear-wave elastography; in vivo diagnosis

## **Chicken breast myopathies affect colour acceptance and willingness to purchase during shelf life**

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Myopathies are muscle disorders that affect poultry and result in abnormal texture and reduced meat quality. Wooden breast (WB) and white stripping also negatively affect consumer acceptance and purchase intention, but little is known about the impact of spaghetti meat (SM). Also, to our knowledge, the evolution of sensory quality of meat with myopathies during shelf-life has never been assessed. The aims of this work were (1) to compare sensory attributes (i.e. colour, odour, and willingness to purchase) of breasts with or without myopathies, and (2) to assess their change during refrigerated storage. A total of 75 chicken carcasses were selected for myopathy presence (25 WB, SM, and control) in a commercial slaughterhouse. Breasts were deboned and packaged in pairs from the same carcass in MAP (30% O<sub>2</sub>, 40% CO<sub>2</sub>, and 30% N<sub>2</sub>) and stored at 4°C and 12h light regime to simulate commercial storage conditions. After 4, 7, 11 and 14 days of packaging, 15 breasts (5 per myopathy group) were evaluated by 2 trained panellists for colour and odour acceptance, and willingness to purchase. A 5-point scale was used, where 5 indicated the best (“highly desirable colour”, “highly desirable odour”, and “would definitely buy”) and 1 the worst punctuation (“highly undesirable colour”, “highly undesirable odour”, and “would definitely not buy”). Data was analysed with R, using ANOVA and pairwise t-test with Bonferroni correction. No significant interaction was found between myopathy presence and storage time ( $P>0.05$ ) for any attribute. However, both effects were significant for colour acceptance and willingness to purchase ( $P<0.05$ ). Control breasts showed the highest colour preference, SM intermediate (0.55 points below control), and WB the lowest one (0.96 points below control). These differences increased for willingness to purchase, as SM punctuations were 0.94 and WB 1.73 points below control. Odour acceptance was only affected by storage time ( $P<0.05$ ). For all attributes, the lowest scores were recorded after 14 days (average values below 2). In 7 and 11 days, breasts had lower punctuations than those in 4 days for odour and colour acceptance. These results confirmed that breasts with myopathies fail to appear visually desirable and would not be bought by consumers. Odour, however, is less affected by myopathies. These attributes worsen with storage time, reaching punctuations close to the acceptance limit after 7 days and unacceptable after 14 days of storage.

Keywords: wooden breast, spaghetti meat, sensory quality, refrigerated storage

## Gene expression patterns associated with processing yield of marinated breast meat in broilers affected by white striping and wooden breast defects

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White striping (WS) and wooden breast (WB) severely affect the technological properties of chicken meat. Several studies investigated their effects on both marinated and non-marinated meat, showing a reduction in marination uptake and retention of the affected fillets. In the present study, we performed a weighted gene co-expression network analysis (WGCNA) to exploit relationships between genes and the reduced processing yields in marinated meat affected by WS and WB. The analysis was performed using the microarray profiles and phenotypic evaluations referring to the marination process (uptake %; cook losses %; and overall processing yield %) of 12 broiler Pectoralis major muscles (6 normal vs. 6 affected by both WS/WB). In particular, a parallelepiped meat cut (8×4×2 cm) weighing about 60 g was excised from the middle part of each fillet and individually labeled and tumbled with a 20% (wt/wt) sodium chloride (6%) and sodium tripolyphosphate (1.8%) marinade solution, and subsequently cooked in a water bath at 80°C for 25 min. Marinade uptake, cook losses and processing yield (calculated as ratio between weights of raw meat before tumbling and after cooking) were calculated for each sample. WGCNA identified 212 clusters of co-expressed genes (i.e., modules) associated with the phenotype “processing yield” ( $P < .0001$ ). Among them, the modules most significantly correlated ( $r > |0.8|$ ;  $P < .0001$ ) with this phenotype were selected for further analyses: darkgreen, khaki4, and midnightblue. Functional analyses were carried out using both DAVID tool and ClueGO Cytoscape plug-in to explore the biological function of selected modules. DAVID analysis identified terms related to the spliceosome and extracellular exosome ( $P = .07$ ). ClueGO analysis evidenced terms related to oxidative phosphorylation; proteoglycan metabolic process; fatty acid oxidation; and targeted protein degradation ( $P < .05$ ). Also, hub genes were identified as those with the highest gene significance (GS) and module membership (MM) values ( $> |0.8|$ ). Genes encoding a specific type of fibroblast growth factor (FGF16), myosin (MYO18B), and collagen (COL12A1), as well as genes related to obesity in humans (PPP1CB and GPD1L) were found as hubs. In conclusion, our results evidenced that gene expression patterns significantly related to the variability of processing yield of marinated meat affected and not affected by WB/WS mainly resemble molecular pathways related to the onset and progression of these defects.

Keywords: Broiler myopathies, marination, WGCNA, functional analysis, hub gene



**Dietary specification on meat quality of fast and slow growing broilers****H. Scott-Cook<sup>1</sup>, S. Mansbridge<sup>1</sup>, A. Mackenzie<sup>1</sup>, V. Pirgozliev<sup>1</sup>**<sup>1</sup>National Institute of Poultry Husbandry, Harper Adams University, Shropshire, United KingdomPresenting author: [hscott-cook@live.harper.ac.uk](mailto:hscott-cook@live.harper.ac.uk)

The poultry industry is under sustained pressure from certain NGOs to improve broiler welfare. Breed selection and dietary specifications are important factors for ensuring sustainability of the poultry industry. Ross 308 (RS) is a well-established fast-growing broiler, however slow growing broiler breeds, such as Hubbard Redbro (HR), are coming onto the market under initiatives including the Better Chicken Commitment (BCC). Two commonly used diets for slow growing broilers are RS and BCC spec, the latter of which contains less metabolizable energy and crude protein. However, research on the interaction between dietary spec and slow or fast-growing broilers is limited. This study evaluated the effects of feeding a standard RS diet vs BCC diet on breast meat quality variables when fed as meal to as-hatched RS and HR broiler chickens from 0 to 42 d age. The overall body weight (BW) for the period of the study was also determined. Each diet was fed to 7 floor pens, 15 birds in a pen, over 4 growing phases, following randomisation. At the end of the study, one bird per pen was electrically stunned and killed by exsanguination, and both breasts were carefully dissected. The breast meat quality variables, including colour (L\* indicated lightness, a\* redness, b\* yellowness), pH, drip loss (DL), toughness (TM), firmness (FM), white striping (WS), woody breast (WB) and cohesion defects, were then assessed. Data were statistically analysed by two-way ANOVA following a 2 x 2 factorial structure (breed x dietary spec). Ross 308 birds and the birds fed RS diet were heavier at 42d, compared to the other respective groups ( $P < 0.001$ ). Ross birds fed BCC spec maintained 46 g daily growth, which was comparable to the growth of slow growing broilers in general. Ross birds had higher L\* but lower a\* score than HR birds ( $P < 0.001$ ) and there was no difference in b\* score ( $P > 0.05$ ). Compared to HR, an increase in WS was observed in RS birds ( $P < 0.001$ ), but no breed by diet interaction observed. There was a breed by diet interaction for DL as it was higher ( $P < 0.05$ ) in RS birds fed with RS spec diet. No differences were detected ( $P > 0.05$ ) for the other studied breast meat quality variables. The results confirm that dietary specification and breed choice are important factors to consider when optimising slow growing broiler systems. Further research is needed to improve understanding of the impact of dietary specs on meat quality of rapid and slow growing broilers.

Keywords: slow growing broilers; poultry; diet specification; meat quality

## **Meat quality: electrical water bath stunning vs. CO<sub>2</sub> gas stunning in broiler welfare assessment**

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In the CasStunn project (Implementation of a controlled atmosphere stunning as an alternative of an electrical bath stun for broilers) we conducted a comparison between electrical water bath stunning and CO<sub>2</sub> stunning of broilers of 4 different fattening methods (light, heavy, outdoor climate, organic). From 2021-2022 1668 carcasses of broilers stunned by electrical water bath stunning and in 2023 1410 carcasses of broilers stunned by CO<sub>2</sub> were examined. The examination for both stunning methods was the same. In this examination, visual inspection determined the parameters of hemorrhages and petechiae, considering any hemorrhage  $\geq 0.5$  cm as positive (present). The assessment of fractures involved both visual inspection and palpation, categorizing bones with a separation between ends and/or crepitation as positive for fractures. All parameters received classification as either "present" or "absent," with the further specification of "left" or "right" for fractures. In the electrical water bath stunning the muscle and fat base of the leg exhibited the highest prevalence of petechiae, while the wing, wing tips, fillets, and tenderloins were the most common sites for hemorrhages. Bones of the anterior wing tip and the furcula were the primary locations for fractures, with organically reared broilers showing an overall lower frequency of fractures compared to their conventionally raised counterparts. At this moment the analysis is not yet complete, so no final statement can be given about the results of the CO<sub>2</sub> stunning. In general stunning may cause hemorrhages and fractures, but they are not necessarily a direct result of it. In our project, we could not examine the animals before stunning. Therefore, the results will present a survey of the entire production process, but the problem of (stunning-related) carcass damage existed in both stunning methods and all fattening methods investigated.

**Keywords:** stunning; broiler chickens; slaughter; animal welfare; meat quality; fractures; hemorrhages

## Effects of provisioning black soldier fly whole larvae or larvae meal on carcass and meat quality traits in fast-growing broilers

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Black soldier fly larvae (BSFL) represent a promising protein source for poultry. The aim of this study was to assess in Ross 308 male broilers, the effects on carcass and meat quality traits of feeding strategies using products derived from BSFL. Birds were reared from D1 in floor pens (402 birds; 6 pens). Six strategies were tested: a control (C) vs. the inclusion of 10% defatted BSFL meal in the feed (M) vs. the provision of live (L) or dry (D) BSFL either in starter phase (1-13d; LLS vs. DLS) or during the entire trial (LL vs. DL). For strategies with BSFL, a pelleted diet with adjusted composition was provided ad libitum and larvae were distributed twice a day to represent 15% of daily DM intake. The growth performances were registered. At D36, 34 birds per strategy were slaughtered. Abdominal fat pad percentage and meat yields were measured, as well as, the ultimate pH and color of breast and leg meat. For breast meat, structural defects (white striping, wooden breast and spaghetti meat), drip loss, and tenderness were also evaluated. Finally, the sensory traits of roasted leg meat from C, M, and LL strategies were assessed with a trained jury. At D36, DL birds were lighter than C ones (2056 vs. 2272 g;  $P<0.001$ ). Abdominal fat pad percentage was higher in DL and M compared to C (2.0 and 1.6%, resp. vs. 1.4% BW;  $P<0.001$ ). Comparatively to C, breast meat yield was increased in LL and lowered in DL (LL: +1.1 % pts; DL: -2.6 % pts;  $P<0.001$ ). The ultimate pH of breast meat in DL was lower compared to C (5.64 vs. 5.79;  $P<0.001$ ). This difference could explain the significantly lighter meat (L\*;  $P<0.001$ ) and the higher drip loss (5.1% vs. 3.3% of initial breast weight;  $P<0.001$ ) observed in DL comparatively to C. In LD, compared to C, the proportion of breasts without any defect was higher both for white striping (91 vs. 56%;  $P<0.05$ ) and wooden breast (91 vs. 50%;  $P<0.01$ ), probably because of smaller breast muscles. Yellowness of leg meat was found to be lower in LL compared to C (b\*;  $P<0.05$ ), probably because of a lower feed (and therefore maize) intake. Finally, no effect on sensory traits for leg meat was found, except for meat tenderness which was found slightly lower in LL compared to C ( $P<0.01$ ). These results suggest that feeding broilers with BSFL meal or live larvae are two relevant strategies to improve protein self-sufficiency in Europe without significant detrimental effect on the quality of chicken meat.

Keywords: insect larvae; nutrition; broiler; carcass quality; meat quality

**Palm kernel fatty acid distillates: revalorizing this by-product as feeding fat. Effects on lipid composition, oxidation and sensory acceptance of chicken meat.****L. Parro<sup>1</sup>, F. Guardiola<sup>1</sup>, S. Vichi<sup>1</sup>, M. Rafecas<sup>2</sup>, R. Sala<sup>3</sup>, A. Caralt<sup>1</sup>, A. Tres<sup>1</sup>**

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Palm kernel fatty acid distillates (PKFAD) are refining by-products of the physical refining of palm kernel oil that are rich in medium-chain fatty acids (MCFA), most of them in free form. Fats and oils are added to broiler feed to increase energy, but there is a need of using available, cheaper, sustainable and reliable alternatives to conventional fats. This study aimed to assess the impact of PKFAD as the primary fat source in broiler feed compared to crude palm kernel oil (PKO) or to a commercial control (crude soybean oil from 0 to 21 days or crude palm oil from 22 to 35 days). A total of 3264 chickens were distributed in 24 pens (8 pens/treatment). At slaughter, the thighs and drumsticks with skin of four chickens from each pen, were deboned and placed in RPET/PE trays. Trays were stored under commercial refrigeration conditions (at 3-5°C, packed in a modified atmosphere of O<sub>2</sub>/CO<sub>2</sub>; >70/>20) for about 12 hours (fresh meat samples) or for 7 days (refrigerated meat). The determinations carried out in dark chicken meat with skin were: i) the fatty acid (FA) composition, determined after lipid extraction and methylation by gas chromatography using a flame ionization detector (GC-FID); ii) TBA values, measured through a third derivative spectrophotometry method after acid aqueous extraction; iii) the volatile compound content, determined by headspace solid-phase microextraction coupled with gas chromatography and mass spectrometry (HS-SPME-GC-MS); iv) the sensory acceptance test, carried out using a nine-point hedonic scale. Chicken meat FA composition showed significant differences between dietary treatments for some FA. Compared to the commercial control, PKFAD and PKO presented an increase in the proportion of MCFA (10% more compared with the control) and a reduction of monounsaturated and polyunsaturated FA (P<0,01). Regarding lipid oxidation in fresh meat, TBA values (≈20 µg/kg) did not differ between dietary treatments (P>0,05). After refrigeration meat TBA values increased in all treatments, but the increase was lower when PKFAD was used (TBA ≈70 µg/kg in PKFAD and ≈ 100 µg/kg in the control and PKO)(P<0,01). Using PKFAD instead of the commercial control led to an increase in nonanal (P<0,05). Although significant, these differences between treatments were not perceived by the regular consumers as no significant differences were found on consumer acceptance of fresh and refrigerated chicken meat (P>0,05).

**Keywords:** Animal feeding; Fat by-products; Medium-chain fatty acids; dark chicken meat quality.

## **Whole dehydrated and live black soldier fly larvae in autochthonous chicken breeds, effects on slaughtering performance and meat quality**

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Poultry biodiversity is essential for sustainable farming, and autochthonous chicken breeds could benefit from dietary inclusion of insect larvae. This study aims at evaluating meat yields, quality, and color of a local chicken breed, Bianca di Saluzzo (BS), fed with alternative ingredients and black soldier fly larvae (BSFL), evaluating potential differences in the consumption of whole dehydrated and live BSFL. 192 male BS of 39 days of age were allotted in 24 pens (6 replicates/treatment) and assigned to 4 experimental groups. The groups were categorized as follows: 1) Control Group (C) fed a commercial diet; 2) Alternative Group (ALT) fed an Alternative Basal Diet (ABD) (0% soybean meal); 3) DL group fed the ABD diet supplemented with dehydrated BSFL, 4) LL group fed the ABD diet supplemented with live BSFL (BSFL inclusion was 4% of expected daily feed intake on dry matter for both DL and LL). The larvae were provided to the animals every day at 11 a.m. and larvae consumption time was recorded three times a week using a stopwatch. Every 14 days the birds were weighed, and the feed conversion ratio was calculated. At 147 and 174 days of age, 12 birds/treatments were slaughtered. Hot and chilled carcass and breast/thigh muscles weights were recorded, and the corresponding yields (% live weight) were calculated. Meat color and pHu were recorded on breast and thigh. Breasts were put through a Near-Infrared scanner to screen for muscle abnormalities (wooden breast) and estimate the protein content based on a calibration for chemical composition. All data were analyzed using a generalized linear mixed model (SPSS,  $P < 0.05$ ). The larvae consumption time was influenced by the age of the birds ( $P < 0.05$ ), the time spent eating both dehydrated and live larvae was the highest between 39-59, 102-122 and 123-146 days of age ( $P < 0.05$ ). No differences were found in breast and leg meat yields and pHu. Higher levels of yellowness in both breast and thigh meat were found for the ALT, DL and LL treatments compared to the C group. Finally, protein content in breast meat was higher in DL compared to the other groups ( $P = 0.035$ ). In conclusion, the study highlighted how the ABD diet impacted on meat color and how the supplementation of dehydrated and live BSFL had no overall negative impact on meat quality. Moreover, the provision of dehydrated BSFL did not differ from live BSFL opening future possibilities as safer and easier to manage compared to their live counterpart.

**Keywords:** local chicken breed; insect larvae; black soldier fly; meat quality; dehydrated insect larvae

## **Economic effects of EFSA's welfare recommendations**

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Solicited by the European Commission, the EFSA (European Food Safety Authority) has issued numerous recommendations to improve animal welfare in farming. In 2023, three reports related to poultry were published by EFSA's scientific experts: "Welfare of laying hens on farm," "Welfare of ducks, geese, and quail on farm," and "Welfare of broilers on farm." Highlighting some key measures, the EFSA suggests, for instance, reducing the maximum density for broilers to 11 kg/m<sup>2</sup>, prohibiting fast-growing strains, and incorporating winter gardens to enhance broiler welfare. It is noteworthy that the EFSA's reports deliberately focus on animal welfare considerations, emphasizing the ethical treatment of animals. However, this approach raises questions about the potential economic repercussions for the poultry industry in France. As these recommendations may necessitate changes in infrastructure, management practices, and breeding methods, it becomes imperative to evaluate the economic feasibility and impacts on the industry stakeholders. Therefore, the core objective of this presentation is to conduct a comprehensive assessment of the economic implications associated with the implementation of EFSA's recommendations within the specific landscape of the French poultry industry. By exploring both the potential challenges and benefits, this analysis aims to contribute valuable insights for industry stakeholders, policymakers, and the broader community invested in the sustainable evolution of poultry farming practices.

Keywords: welfare; EFSA; broiler; laying hen;

## **HPAI vaccination in France: Concept, Experiences during the first stages and economic effects of the choice for vaccination**

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France has been the European country most affected by HPAI in recent years, particularly peaking in 2021/2022 with over 1300 infection outbreaks. HPAI is a viral disease with enormous economic consequences for the country, including preemptive culling, reduced production, and compensations. Besides the direct implications for animal health, HPAI also poses a risk to human health and the economy, underscoring the need for preventive action. The French government has therefore decided to launch a vaccination campaign to combat the virus. In the strategy chosen by France, only duck farms with over 250 heads will be vaccinated, while other poultry species are not affected. France faces a significant logistical challenge given the number of involved farms (2,700) and animals to be vaccinated (64 million), requiring two vaccinations a few days apart. The vaccination campaign commenced on October 1, 2023. This large-scale vaccination campaign is unprecedented in Europe but deemed necessary for securing the French poultry meat industry. Drawing lessons from this initiative is crucial, and that is the focus of this abstract.

**Keywords:** HPAI ; vaccination ; France ; ducks

**economic perspectives of the dutch broiler sector****L. Vissers<sup>1</sup>**<sup>1</sup>Wageningen Economic Research, Wageningen, Netherlands*Presenting author: luuk.vissers@wur.nl*

In the Netherlands, criticism grew about the fast growth rate and high stocking density of broilers reared in conventional systems. In response to the societal critique, more extensive systems were introduced containing slower-growing breeds. These broilers have a higher level of animal welfare compared to fast-growing broilers reared in conventional systems. However, it is expected that slower-growing broilers have higher ammonia emissions and particulate matter emissions per animal. To improve the environmental sustainability of poultry production, the Dutch government aims to mitigate ammonia emissions and particulate emissions from poultry housing. It is currently unclear to what extent measures can contribute to the sustainability of broiler production and how they affect the farm profitability. To fill this gap, the aim of this study was two-fold: 1) to explore development pathways that enhance the environmental sustainability of broiler production 2) to analyse the effect of the measures on the environmental and economic performance. Farm income was used as an indicator for economic performance. The environmental performance was indicated by the ammonia emissions and particulate matter emissions. Only emissions at farm level were considered. The development pathways were created in consultation with stakeholders. In these sessions, the following development pathways were identified: 1) high-tech 2) distinctive market concept with a focus on circularity and 3) organic. A deterministic, spread-sheet model was developed to calculate the environmental and economic effect of measures associated with these pathways. The effects were compared to the reference situation, which was the average environmental and economic performance of the conventional and 1-star Better Life system from 2018 to 2021. Results show that compared to this reference situation, ammonia emissions can be reduced up to 70% compared to the reference situation. Reduction in particulate matter emissions ranged from 21% to 81%. Although these emissions can be reduced substantially, it also affects farm income considerably. Farm income decreased by 22 thousand to 183 thousand euros. Our study shows that adequate policy and private instruments are needed to facilitate the transition towards more environmentally friendly broiler production systems.

Keywords: broiler production, economics, environment



## **Additional costs of turkey hens with untrimmed beaks in enriched housing conditions**

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Injurious pecking is a serious welfare problem in turkey production. Therefore, beak trimming is an intervention that aims to minimise severe pecking injuries but is likely to be associated with pain due to amputation. The objective of this experimental study was to keep beak-intact turkey hens under enriched housing conditions and also to evaluate the economic impact. A farm-specific package of measures was developed on four conventional farms. These included the use of enrichment material (1 item per 500 birds), structural elements (1 item per 1000 birds), increasing the crude fibre content of the diet by using oats and optimising management (animal health/light). In the first of three phases, enrichment materials (variable cost), structural elements (short-term depreciation) and lighting management (medium-term depreciation) were implemented in beak-trimmed flocks. Subsequent runs were carried out with optimised housing and intact beaks. Accounting data were collected in the farm's baseline situation for further processing and evaluation in the TIPI-CAL model and for comparison with quantified trial data (ex-post analysis). The valuation of animal losses due to intact beaks was calculated in monetary terms and allocated to farm cost. The results show that the enrichment materials resulted in additional costs of 0.11 to 1.11 € per animal place and cycle (16 weeks). The additional labour required amounts up to 8 hours per 1000 animal places and cycle and was calculated as family labour input. Investments range from 26.50 € per animal place and vary due to the different farm specific conditions. The occurrence of losses due to injurious pecking costs up to 0.94 € per animal place. The cumulative additional costs on the farms range from 8.15 € to 18.58 € per 100 kg LW. The largest share of the additional costs is accounted for by farm expenses, followed by depreciation and opportunity cost of additional family labour. In conclusion, the results of the study show that keeping turkey hens with intact beaks in combination with optimised housing require additional labour input, which, together with higher losses, is reflected in additional costs. If these are not covered by higher product prices, the profitability of the farm will deteriorate. This project (Model- and Demonstration Project for animal welfare: #Pute@Praxis) was financially supported by the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food.

**Keywords:** Turkey hens, intact beaks, welfare, enrichment, production costs, economic efficiency

**BroilerNet- Practice and Science Broiler production innovation network****N. Majó<sup>2,1</sup>, L. Morgans<sup>3</sup>, P. Ferrari<sup>4</sup>, J. Malchow<sup>5</sup>, M. Gebśka<sup>6</sup>, I. De Jong<sup>7</sup>, S. Gunnarsson<sup>8</sup>**

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The global consumption of poultry meat is anticipated to increase until becoming the most consumed meat in the coming years. The European broiler industry faces the challenge of furnishing safe, high-quality products at affordable prices, meeting society's call for sustainability, improved animal welfare, and enhanced approaches to managing animal health concerns like antimicrobial resistance. This requires a substantial overhaul in broiler production, requiring the poultry industry and, particularly, farmers, to develop greater adaptability in their day-to-day practices. Merely providing access to information does not seem enough for generating innovation among farmers, because their capacity to innovate is influenced by various factors such as policies and market dynamics. The BroilerNet project, funded by Horizon Europe, seeks to bridge this gap by fostering research and innovation in the broiler sector via an interactive innovation model. Broilernet has established 13 national-level innovation networks (BINs) involving broiler farmers and stakeholders in diverse European countries (SE, SI, DE, EL, IT, SP, GR, SL, GE, PT, FR, PL, IE) to collect/define specific challenges currently encountered by farmers in three specific areas of the poultry production: environmental sustainability, animal welfare, and health management. BINs will also be responsible for suggesting specific good practices already in place to tackle the selected challenges. Moreover, three EU-level Thematic Expert Networks comprising experts in these three specific areas will be in charge to evaluate and rank the good practices proposed by the BINs and to identify the best ones. With such a multi-stakeholder, bottom-up approach, the network aims to identify the sector's most pressing needs and gather and assess effective practices to address them. BroilerNet will collaborate with existing and new EIP-AGRI Operational Groups to amplify their impact. The best practices selected will be awarded by the BroilerNet Championship and disseminated through many outreach initiatives to benefit broiler farmers across Europe.

**Keywords:** Broiler, innovation, network, welfare, sustainability, health

## **Animal health law: Qualification and current skills regarding biosecurity in commercial poultry farms**

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According to the requirements for biosecurity in livestock farming of the European Animal Health Law (Regulation (EU) 2016/429), livestock farmers are obligated to minimize the risk of spreading diseases as well as zoonoses and are therefore required to have knowledge about biosecurity principles. In Germany, the current risk of an outbreak of highly pathogenic avian influenza (HPAI) in commercial poultry farms is threatening. Therefore, knowledge about biosecurity is exceedingly required. A project funded by the North Rhine-Westphalia Chamber of Agriculture/Animal Disease Insurance Fund was conducted to identify biosecurity-related weaknesses and risks on poultry farms in North Rhine-Westphalia, Germany. A total of 16 poultry farms (layers, broilers, and turkeys) were visited for this purpose. Farm size differed between 4,000 - 269,000 animals. In addition, the farmers' skills in terms of knowledge and implementation of the legal framework on the farm, the current status quo of biosecurity measures as well as the outcome of a self-assessment of the farmers were evaluated. As one result, it could be determined that the farmers with higher level of qualification and training, had more competence in the field of biosecurity and tended to apply more effective biosecurity measures on the farm. Competences of farmers regarding on farm traffic and movement, cadaver management and salmonella prevention were rated worst when compared to competences in other risk-categories. Further deficiencies could be detected regarding cadaver management, enclosed feed and litter storage as well as the general hygiene management on the farm. Species-related, turkey farmers had higher competence rating in terms of biosecurity measures compared to layer and broiler farmers. Based on the farm visits and the derived findings and results, a pilot lecture course was assembled which should enable farmers to systematically analyze farm-specific biosecurity weaknesses, implement specific measures and monitor their success. This includes skills to prioritize certain biosecurity measures on the farm according to the probability of the occurrence, extent, and controllability of biosecurity-related events on the farm. Re-evaluating the success of taken measures were also trained as farmers often underestimate the risk of biological hazards

**Keywords:** Academy; Education; Training; Risk Management

## **Diet composition influences probiotic and postbiotic effects on broiler growth, physiology and microbiota**

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Feed ingredients can affect dietary additive efficacy. Moreover, lower-quality ingredient use can represent challenging conditions for broilers' growth and health, which might be alleviated by dietary probiotics and their non-viable forms (postbiotics). This study assessed the effects of two diets (standard [SD], challenge [CD]) on the effects of a Lactobacilli probiotic (Pro) and postbiotic (Post). A completely randomised block study with two diets (SD, CD) and three additive treatments (Control, Pro, Post) involving 1,368 day-old Ross 308 male broilers, equally distributed among 36 pens, from d1 to 42 was conducted. Both diets were formulated to contain identical nutrient levels, with CD formulated to be higher than SD in non-starch polysaccharide content by including rye and barley. Body weight (BW) and feed intake were measured and readout parameters related to tibia health, strength, composition and growth at d35 and 17 plasma parameters related to health and nutrition at d21 and 35. Caecal microbiota composition was assessed by 16S rRNA gene sequencing at d14 and 35, and  $\alpha$ - and  $\beta$ -diversity indexes (Observed, Chao1, Bray, Jaccard) were calculated. Caecal short-chain fatty acids and semi-polar metabolome were determined in the Control SD and all CD groups. ANOVA, ANCOVA, PERMANOVA and logistic regression were applied, with p or adjusted-p < 0.05 considered significant. Compared to SD, CD decreased body weight (1936 vs 2033 g), increased feed conversion ratio (FCR) and impaired tibia health and strength at d35, thereby, confirming the challenging effect of CD. At d35, within SD, Pro and Post did not affect BW, FCR, operational taxonomic unit (OTU) abundance and diversity indexes. Oppositely, within CD, Pro and Post increased BW (4.7 and 3.2%, respectively) and affected OTU abundance (37 and 44 OTU, respectively), with Post but not Pro affecting  $\beta$ -diversity indexes. Within CD, Post increased caecal acetate (21%) and butyrate (41%) concentration. Compared to the control CD, Pro and Post had 240 and 2 differentially abundant metabolites, respectively. Plasma biochemical parameters showed diet-dependent and independent effects for Pro and Post. The results highlight that diet composition can affect pro- and postbiotic effects and thus that diet formulas must be considered to evaluate the effects of microbe-based additives. Furthermore, our results show the potential of dietary challenging conditions to assess recovery effects of pro- and postbiotics.

**Keywords:** Diet composition; challenge; probiotic; postbiotic; microbiota; metabolome

## **Transcriptomics reveals different modes of action of three probiotic strains compared with an antibiotic growth promoter in broiler chickens**

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Probiotics are considered potential alternatives to antibiotics although their mechanisms of action remain relatively unexplored. Based on 2 trials, we investigated the effects of 3 probiotic strains (*E. faecalis* [EntF], *B. fragilis* [BacF], and *L. salivarius* [LigS]) and an antibiotic growth promoter (AGP), Zinc Bacitracin, on broiler gene expression. Wheat-rye-based diets without exogenous enzymes were used to induce an intestinal medium (Trial 1) or low-grade (Trial 2) inflammation. mRNA from cecum and jejunum mucosa was analysed by RNAseq (DESeq2; Wald test,  $P < 0.05$ ; absolute fold-change  $> 1.5$ ). Trial 1 had 4 treatments (TR) [TR1=Basal diet (BD), TR2=BD+EntF, TR3=BD+BacF, and TR4=BD+LigS (all probiotics at  $5 \cdot 10^7$  CFU/bird·d)], and lasted 35d (6 pens/TR, 36 birds/pen). Two animals/pen were euthanized at 7 and 21d for sampling. Compared with TR1, all probiotics improved FCR at 0-8d. Between 7 and 21d, TR1 upregulated (up) 2742 genes and downregulated 1985 (down) in jejunum, while upregulated 370 genes and downregulated 228 in cecum. Animals treated with probiotics had similar results but only in jejunum (TR2 up 1112/down 1062 genes; TR3 up 130/down 116 genes; TR4 up 2164/down 2159 genes). However, the number of genes up or downregulated in cecum was very low in all groups compared to TR1 (TR2 up 16/down 73 genes; TR3 up 2/down 28 genes; TR4 up 1/down 27 genes). Trial 2 had 4 TR [TR1=BD, TR2=BD+Zn Bac (55ppm), TR3=BD+(EntF+BacF+LigS) [ $1.6+1.6+0.1 \cdot 10^8$ CFU/bird·d (0-10d)], and TR4 same as TR3 but 0-35d] and lasted 35d (13 pens/TR, 20 birds/pen). One bird/pen was euthanized at 7 and 21d for sampling. TR3 and TR4 had similar FCR improvements than AGP for the whole trial, compared with TR1. TR2 clearly modulated caecal genes between 7 and 21d, in contrast with the birds fed the probiotic blends, in which the jejunal modulation was prevailed (Jejunum: TR1: up 23/down 136 genes; TR2 up 37/down 199 genes; TR3 up 1576/down 843 genes; TR4 up 166/down 284 genes. Cecum: TR1: up 0/down 4 genes; TR2 up 371/down 433 genes; TR3 up 2/down 2 genes; TR4 up 0/down 25 genes). Our results suggest different modes of action for AGP and probiotics despite resulting in similar improvements in performance. While AGP clearly modulated gene expression in cecum, probiotics modified gene expression in jejunum. Functional categorization of genes revealed the regulation of different immunity pathways, but further analyses are required to understand how these changes could improve animal health.

**Keywords:** Probiotics; Antibiotic Growth Promoters; Transcriptomics; Poultry Nutrition

## **Dietary muramidase improves apparent ileal energy digestibility, gut functionality and increases broiler growth performance independent of diet nutrient density**

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Muramidases (MUR) are enzyme hydrolyzing peptidoglycan (PGN) from bacterial cell walls and demonstrate a positive effect on broiler performance by reducing the antinutritional effects of PGN in the gut. Two studies were conducted to investigate the efficacy of MUR on broiler growth performance fed diets divergent in nutrient density. In both studies male broilers (Ross 308) were housed in floor pens and randomly assigned to a 2x2x2 factorial arrangement of treatments with two levels of nutrient density (low (LD) or high (HD) diets; formulated to contain 5% of dietary difference), two reductions of nutrient density (PC and NC, containing 2% of dietary differences between PC and NC in each nutrient density group) with or without addition of 35000 LSU/kg of MUR. Pelleted basal diets (mainly corn, wheat and SBM), were fed for 35 days, during starter, grower and finisher periods. All diets contained a combination of carbohydrases and phytase enzymes. Each treatment was replicated with 6 pens or 5 pens of 16 birds in study 1 and 2 respectively. Apparent ileal digestibility (AID) of DM, CP, GE were determined, organ weights were assessed, muramic acid concentration, as a biomarker for PGN, was evaluated in jejunal content and excreta. Collected data was subjected to ANOVA and significant differences separated using the Tukey test. MUR inclusion increased body weight (BW) gain and BW-corrected feed conversion ratio (FCR) from hatch to day 35 independent of diet density ( $P < 0.05$ ). Significant reduction of FCR was observed in the starter phase in animals fed MUR compared to control. In study 1, MUR generated a 4% increase in the AID of energy ( $P < 0.05$ ) and this effect was larger in the LD diets (+7%,  $P < 0.05$ ), resulting in a significant diet density\*MUR interaction. A significant increase of soluble muramic acid was observed in jejunal content and excreta of animals fed MUR compared to PC of LD diet. A significant decrease of total digestive tract weight relative to BW was observed in animals fed MUR (-11%,  $P < 0.05$ ). This effect was changing from duodenum to jejunum. These results confirm that MUR can positively influence broiler growth performance and gut functionality in diets containing adjacent feed enzymes and divergent in nutrient density. The effects observed on nutrient digestibility, live performance and organ weight may be associated with solubilization of PGN in the intestine and a putative reduction in the antinutritional effects and inflammation from PGN.

**Keywords:** zootechnical performance; nutrient density; digestibility; gastrointestinal functionality; energy; muramidase; carbohydrases

## **The effect of NSPase and feeding regimen on performance of broilers fed diets based on high and low viscosity barley**

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The effect of NSPase and feeding regimen on performance of broiler chickens given high and low viscosity barley diets was studied in a 2x2x2 experimental design. A total of 1936 one-day-old Ross 308 female broiler chicks were randomly placed in 88 floor pens bedded with wood shavings, and with 22 birds in each pen and 11 pens per treatment. From d 1 to 10, all birds were offered a commercial starter diet. From d 11, the birds were given diets containing 54% of either a high viscosity barley variety (18.9 centipoise) or a low viscosity variety barley variety (3.3 centipoise), with or without NSPase (xylanase and  $\beta$ -glucanase) and fed ad libitum (ADL) or intermittently (INT). Throughout the experimental period, all birds had 18 h of lighting and 6 h of darkness per day. ADL-fed birds were offered feed continuously during the light period. From d 11 to 17, the INT-fed birds had feed access from 06:00 to 08:00, 10:00 to 11:00, 13:00 to 14:00, 16:00 to 17:00, 19:00 to 20:00 and 22:00 to 24:00. From d 18 to 32, the INT-fed birds had feed access from 06:00 to 08:00, 10:00 to 11:00, 14:00 to 15:00, 18:00 to 19:00 and 22:00 to 24:00. Weight gain, feed intake, water intake and mortality were recorded from d 11 to 33. The data were analysed using three-way analysis of variance. High viscosity barley resulted in worse performance than low viscosity barley. NSPase increased ( $P=0.003$ ) weight gain and improved ( $P=0.027$ ) FCR, and the two-way interactions between NSPase and barley variety indicated that the extent of improvement was higher in high viscosity barley diet. Despite a reduced weight gain, FCR was significantly better ( $P<0.0001$ ) with INT feeding. However, as hypothesized, no interaction between NSPase supplementation and feeding regimen was observed in this regard. Feed intake ( $P=0.026$ ) and weight gain ( $P=0.016$ ) were higher in the ADL group as compared to the INT group when diets were supplemented with NSPase, resulting in two-way interactions between NSPase and feeding regimen. NSPase interacted in a two-way interaction with barley variety, and reduced water intake ( $P=0.001$ ) and litter caking ( $P=0.0001$ ) in birds fed with high viscosity barley diets. Overall, this work demonstrated that NSPase would reduce the difference in the nutritive value of barley varieties differing on the basis of viscosity. While INT feeding showcased superior feed conversion efficiency, it did not align with the expectation that it would enhance NSPase efficacy.

**Keywords:** NSPase; Barley; Feeding Regimen; Broiler Performance

## **Differences in origin and dose of dietary endoxylanases result in distinct arabinoxylan degradation patterns in the gi tract of broilers fed wheat-based diets**

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Various endoxylanases are abundantly used in poultry feed nowadays. They originate from different microorganisms, each having unique biochemical properties that determine the hydrolysis of wheat arabinoxylan (AX). This enzyme-related factor might contribute largely to the variable response in the field and complicates a clear understanding of the functionality of endoxylanase *in vivo*. In this study, we hypothesised that AX hydrolysis and microbial fermentation in broilers fed a wheat-based diet are different for endoxylanases of different origins and at different doses. A digestibility trial was designed to provide further insights into the contribution of origin (*Bacillus subtilis* vs. *Nonomuraea flexuosa*) and dose (0, 10, 100, 1000 ppm) of endoxylanases on the structure-function relationship of AX hydrolysis products formed in the hindgut of broilers (Ross308) at the end of the starter (d11) and slaughter age (d36). The residual AX population and the fermentation metabolites were analysed on ileal, caecal and excreta samples (n = 5). Data were statistically analysed using a 3-way ANOVA with enzyme, dose and age and their second-order interactions as model effects. Endoxylanase supplementation resulted in more extensive degradation of wheat AX and reduction in intestinal viscosity compared to the control (P < 0.05). Despite their difference in substrate preference, solubilising the wheat AX was the main activity of both endoxylanases. Endoxylanases produced from *B. subtilis* stimulated precaecal digestion of dietary AX by creating a large pool of solubles, much of which were of higher molecular weight as they increased ileal viscosity compared to broilers fed an endoxylanase from *N. flexuosa* (P < 0.05). The latter endoxylanase mainly triggered caecal AX fermentation in the young broiler by delivering easily fermentable AX substrates with a low degree of polymerisation (P = 0.03). At least 100 ppm was required for both endoxylanases to obtain improved hydrolysis of the dietary AX along the GI tract against the control (P < 0.05). The effects observed were particularly present in young broilers as they are more prone to anti-nutritional factors and dependent on the fibre-degrading capacity of the young intestinal microbiome. From this study, it is clear that the origin and dose of endoxylanases added to a wheat-based diet determine the nature of the heterogeneous pool of AX molecules formed, which dictate intestinal viscosity and AX hydrolysis at a young age.

Keywords: Endoxylanase; Fibre; Broiler; Fermentation



**Assessment of in vitro fermentation efficacy of yeast-derived  $\beta$ -glucans with different molecular mass and solubility using broilers' caecal content**Y. Gu<sup>1</sup>, A. Bautil<sup>1</sup>, D. Croonen<sup>1</sup>, C. De Schepper<sup>1</sup>, K. Brijs<sup>1</sup>, N. Everaert<sup>2</sup>, C. Courtin<sup>1</sup><sup>1</sup>Laboratory of Food Chemistry and Biochemistry, KU Leuven, Leuven, Belgium, <sup>2</sup>Nutrition & Animal Microbiota EcoSystems Lab, KU Leuven, Leuven, Belgium

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In recent years, in poultry nutrition, finding effective alternatives to in-feed antibiotics to maintain high efficiency and sustainable production has been a focus. 1,3-1,6  $\beta$ -glucan, a polysaccharide found in yeast cell walls, is investigated as a potential antibiotic substitute due to its immunomodulatory properties and ability to improve intestinal health. However, very few studies have investigated the structure-function relationship of yeast  $\beta$ -glucans, especially concerning its prebiotic potential. This study aims to narrow this gap by investigating the in vitro fermentation of a total of 6 yeast  $\beta$ -glucan samples (purity > 75%) with different molecular weight (MW) distribution and water solubilities. Using 21-day-old broiler caecal contents as inoculum (5%), 48 h in vitro fermentations with 100 mg/mL  $\beta$ -glucan samples were conducted to assess their fermentation propensity. Gas production was measured every 2 hours for the first 8 hours, followed by measurements every 4 hours until the end. Samples (3 replicates) were collected at 12 and 48 h for short-chain fatty acid analysis. The results showed that yeast  $\beta$ -glucan samples exhibited increased gas and short-chain fatty acid ( $P < 0.05$ ) production compared to a non-supplemented sample. The water-soluble  $\beta$ -glucans with low MW showed more and more rapid gas and butyric acid ( $P < 0.05$ ) production compared to the water-insoluble  $\beta$ -glucans with high MW at 12 and 48 h. The high MW  $\beta$ -glucans produced higher levels of acetic and propionic acids at 48 h ( $P < 0.05$ ). Less production of branched-chain fatty acids was observed with low MW water-soluble  $\beta$ -glucan compared to the high MW water-insoluble  $\beta$ -glucan samples. These findings suggest that MW and solubility would dictate the potential prebiotic activity of yeast-derived  $\beta$ -glucans. Microbiome analysis is used to explore the relationship between  $\beta$ -glucan characteristics and functionality, aiming for a better understanding of their full potential in broiler feeding.

Keywords: yeast  $\beta$ -glucan; in vitro fermentation; structure-function; prebiotic

**Impacts of early nutritional strategies on digestive homeostasis of chicks**

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The plasticity of the digestive tract in the first days of life offers a unique window to early orientate digestive functions. Different feeding strategies can be considered to early modulate and improve chicken health and welfare while ensuring feed efficiency. In this trial, the effects of five additives (vitamin E, carvacrol, butyrate, capsaicin, xylo-oligosaccharides), targeting specific biological functions, on digestive homeostasis were studied in diets based on maize and soybean meal (MS) or on wheat and faba bean (ALTER). A total of 432 chickens were distributed in 72 pens (6 pens of 6 animals/treatment) and reared for 10 days. Data were analysed using ANOVA to identify the effects of the additives, the diet and the interaction of both on growth performance, intestinal enzymatic activities and indicators of redox balance in the gut and the blood analysed at 10 days of rearing. In animals fed the ALTER diet, feed conversion ratio was deteriorated but litter moisture reduced compared to the MS diet. In both diets, lipid peroxidation in the jejunum was decreased by the addition of vitamin E, illustrated an antioxidant activity (-40%, p-value=7.10-10). Total plasma antioxidant status was improved in the ALTER diet (ALTER +40.2%, p-value=1.0-2). It also induced a reduction of alkaline phosphatase activity (-46%, p-value=8.10-6) in the jejunum, illustrating lower inflammation. Supplementation of the ALTER diet with carvacrol also led to a decrease of alkaline phosphatase activity (-44%; p-value=1.10-6) and, in the ALTER diet compared to the MS diet, to an increase of glutathione reductase activity (+32%, p-value=3.10-2), enzyme involved in the regulation of oxidative stress. Finally, capsaicin supplementation in both diets induced a reduction of plasmatic haptoglobin (-20%, p-value=4.10-3), suggesting a systemic anti-inflammatory activity. Two molecules, butyrate and xylo-oligosaccharides, did not show any detectable effect on the measured parameters. Overall, digestive homeostasis can be modulated early in life depending on the type of diet and feed additives. The long-term effects of such strategies remain to be tested.

**Keywords:** Early nutrition, digestive homeostasis, chicks

## Effect of the dietary supplementation with sodium butyrate on gut morphology and caecal microbiota of broiler chickens

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A total of 900 chicks (Ross 308) were housed in 36 pens (25 animals/pen) according to a 3 × 2 × 2 factorial design encompassing three dietary supplementation rates of microencapsulated sodium butyrate (0 mg/kg, 150 mg/kg, and 300 mg/kg), two levels of ether extract (grower diet 6.7% vs. 7.7%; finisher diet 7.7% vs. 8.9%), and two sexes (males vs. females). The grower and finisher diets were based on maize (56-62%), soybean meal (25-31%), and full fat soybean (5.5-9.0%), besides animal fat (2.5-5.0%). Health and growth performance were monitored from hatching until commercial slaughtering at 42 d of age. At 39 d of age, 36 chickens (1 per pen, 6 per experimental diet) were slaughtered and jejunum tissue and caecal content sampled. The jejunum was stained with hematoxylin/eosin for morphometric evaluation. The intestinal microbiota was evaluated by sequencing of V2, V4, V8 and V3, V6-V7, V9 regions of the rRNA 16S gene. The dietary supplementation with sodium butyrate did not affect growth performance: final live weight averaged 2,733 g, with an average feed intake of 182 g/d and a feed conversion ratio of 1.53. Similarly, nor the dietary supplementation with sodium butyrate nor the fat level affected gut morphology or microbiota composition at 39 d of age. As for sex, performance was higher in males compared to females. The latter also showed greater ratio villi/crypt compared to males (4.63 vs. 4.12; P=0.02) without differences in villi length or crypt depth. Both alpha and beta diversity significantly changed between the bacterial community of females and males. Regardless from the dietary treatment and sex, at a phylum level, Firmicutes was the most represented and Bacteroidetes was the least; at a genus level, Faecalibacterium dominated, followed by an Uncultured genus of the Lachnospiraceae family, Parabacteroides, Lactobacillus, and Bacteroides. In conclusion, under the good health conditions of the present trial (mortality <1%), the different dietary treatments, with regard to sodium butyrate supplementation and fat content, did not affect gut health and microbiota of broiler chickens.

Keywords: Sodium-butyrate;gut morphometry;gut microbiome

## **In-ovo injection of sodium butyrate improves growth performance in underweight broilers by modulating microbiota and gut barrier gene expression**

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The hatchling body weight (BW) significantly impacts subsequent broiler performance. Underweight broiler chicks often fail to reach their genetic potential, contributing to poor flock uniformity. This study investigates the response of broiler chickens with different BW to in-ovo injection of sodium butyrate (SB). On day (d) 12, fertile eggs (Ross 308, n=1000) received injection (0.2 mL) of physiological saline (control) or three SB doses [0.1% (SB1), 0.3% (SB3), and 0.5% (SB5)]. Post-hatch, 96 male chicks per treatment, categorized as high (HBW:  $55.1 \pm 2.31$  g) or low (LBW:  $45.6 \pm 2.82$  g) BW, were assigned to 8 groups (2 BW  $\times$  4 SB) with 4 replicate pens (12 chicks/pen). Production parameters, intestinal length (d 14 and 42), caecal Bifidobacterium and Lactobacillus spp. abundances (d 14), and expression of gut barrier (MUC6, CLDN1, TJAP1) and immune response (IL-1 $\beta$ , IL-10, IL-12p40) genes in ileum (d 14 and 42) were assessed. Data were analyzed by 2-way ANOVA. HBW chicks consistently upheld their BW advantage and outperformed LBW counterparts when both received the control dose. SB consistently affected BW during the entire grow-out period, with 0.3% SB demonstrating higher chick performance than others. The interaction between BW and SB was noted, and LBW chicks receiving either 0.1% or 0.3% SB showed highest BW on d 7 and 14 among all groups. After d 14, LBW-SB3 group attained a higher BW compared to both LBW and HBW control groups, but not significantly different from HBW-SB3 and LBW-SB1 groups. The trend was similar for other performance parameters. LBW-SB3 showed shortest jejunum, ileum, and caecum lengths on d 14, and lower jejunum and ileum lengths on d 42. The 0.1% SB showed a bifidogenic effect in LBW birds and LBW-SB1 group showing a tendency for higher Bifidobacterium levels. On d 14, LBW-SB3 group showed higher CLDN1 gene expression and increased TJAP1 gene expression than all other groups. On d 42, LBW-SB1 group had up-regulation of CLDN1, and LBW-SB3 group showed increased MUC6 levels compared to SB-treated low and high BW chicken groups. In conclusion, LBW chicks seemed to benefit more from SB injection, while the response in HBW birds was less pronounced. The 0.1% SB modulates gut microbiota, while 0.3% SB enhances growth performance and modulates gene expression for improved intestinal barrier function, presenting a targeted approach to optimize underweight broiler performance. Acknowledgment: MonoGutHealth MSCA – ITN (N° 955374).

Keywords: in ovo injection; microbiota; underweight; flock uniformity

## **A walk through the broiler breeder life**

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The two most common animal-based indicators used to evaluate leg health in broiler chickens are footpad dermatitis (FPD) and gait scoring (GS), but these indicators are less explored in broiler breeders. This study is the first to investigate FPD and GS in broiler breeders during their life span from rearing to end of life. In total, 8 flocks were monitored at five different time points, in rearing (5 and 15 weeks of age), during the production period (25 and 45 weeks of age) and at the end of the production period (approximately 60 weeks of age). At each visit, 50 hens and 25 roosters were gait scored (6-point scale) and from another 50 hens and 25 roosters' footpads (5-point scale) were evaluated (total n = 3000 breeders, 2000 hens and 1000 roosters). Litter quality and air quality were measured at each visit. The results showed that the overall prevalence of FPD in rearing was low and that it increased towards the end of the production, with mean FPD in the hens at the last investigation was a score 2, and for the roosters the score was 1.5. In all houses, the litter was dry and loose. FPD were not related to the litter quality, but to air quality, especially the ammonia concentration ( $P < 0.001$ ). Overall, the GS were low, and increased with age in both hens ( $P < 0.001$ ) and roosters of both hybrids ( $P < 0.001$ ).

**Keywords:** broiler breeders; breeders; parent stock; footpad lesions; footpad dermatitis; gait score

**Contact with an adult hen after hatching promotes maturation of the chick caecal microbiota and has a greater effect than hatching systems**

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On-farm hatching (OFH) with an enriched microbial and stimulating environment by the presence of an adult hen is a promising solution to improve the early perinatal conditions of chicks. The effects of hatching systems (conventional hatchery (CH) or OFH) and the presence of an adult hen were analysed on the chick caecal microbiota and IgA production. The combination of CH and post-hatching treatment with antibiotics (CH + AB) was added as an experimental control group. Day-old certified JA 757 chicks were allotted within five hatching and rearing conditions: OFH, CH, CH + AB, as well as both hatching systems with an adult hen at hatching (OFH + H, CH + H). Caecal bacterial communities were assessed at 20 (n= 7 to 14 per group) and 56 days (n = 16 per group) of age by 16S rDNA sequencing. Caecal IgA were analysed at both times by ELISA. At 20 days of age, post-hatching contact with an adult hen and antibiotics treatment shifted the community structure in the opposite direction (beta-diversity) with the first being the closest to the hen bacterial community, whereas there was no difference between hatching systems. Shannon diversity increased with the presence of an adult hen in on farm hatchery system ( $4.27 \pm 0.18$  and  $3.66 \pm 0.42$  in the OFH + H and OFH groups respectively  $p < 0.05$ ) and decreased in antibiotic treated chickens ( $3.16 \pm 0.38$  and  $3.85 \pm 0.46$  in CH + AB and CH groups respectively). Contact with an adult hen, whatever the hatching system, increased the caecal relative abundance of numerous genera including *Bacteroides*, *Olsenella*, *Mucispirillum*, *Desulfovibrio* and *Succinatimonas*, whereas antibiotic treatment of CH chicks has induced the dominance of *Escherichia-Shigella* group. At 56 days of age, caecal microbiota composition was similar between chicken groups. At D20, post-hatching contact with an adult hen decreased caecal IgA concentration of OFH chickens. In CH chickens, antibiotic treatment increased IgA concentration in comparison with post-hatching contact with an adult hen. These effects were no significant anymore at D56. Results of this study show that hatching and rearing conditions, at least during the first 20 days of life, have a strong impact on the dynamics of the caecal microbiota implantation and on mucosal immunoglobulin IgA production in chicks. The study of these effects on the intestinal immune molecular effectors remains to be determined.

Keywords: On Farm hatching; Hen; Chick; Postnatal; Microbiota, IgA

**Microbiome diversity along the GIT of indigenous chicken**

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The gut microbiome modulates host immune system, metabolism, adaptability and in the case of poultry production, food safety. Unfortunately, information on the gut microbiome of African indigenous chicken is still scanty, despite the contribution of this animal to human nutrition and biomedical research. Therefore, our capacity to accurately quantify gut microbiome diversity is extremely relevant to shaping host-specific nutrition, novel therapy, adaptability and productivity. The current study used 16S rRNA gene high-throughput Illumina sequencing to shed light on the metagenomic diversity along four different gastrointestinal tract regions (crop, gizzard, jejunum, cecum) and liver. 80 biopsy tissue samples were collected from sixteen individuals of local chickens fed on the same diet and reared under an intensive farming system for 14 weeks. Alpha diversity index exhibited similar pattern distribution within-sample and tended to increase along the gastrointestinal tract with a pic noted within Jejunum organ parts. The beta diversity and hierarchical clustering analysis showed that the cecum sample organ tends to cluster separately from other sample organ locations. Analysis of the four GIT content and liver regions of the local chicken revealed heterogenous taxonomy dynamic along the GIT organ and liver. Venn diagram and heatmap further demonstrate that each organ section forms a unique ecosystem on its own, each associated with a particular physiological function. Before making, any concrete recommendation regarding indigenous chicken breeding, further study is needed to demonstrate the specific contribution of each microbiome community to the metabolic function of each organ location in indigenous chicken for precision breeding and genetic improvement of local chicken.

**Keywords:** Keys words: Gut biogeography, Comparative metagenomic, Indigenous chicken, 16S rRNA gene, illumina Miseq.

**The microbial evaluation of the Boschveld chickens fed Sorghum-based diets**N. Nemukondeni<sup>1</sup>, C. Mbajjorgu<sup>1</sup>, T. Mafuna<sup>2</sup>, M. Mabelebele<sup>1</sup><sup>1</sup>University of South Africa, Florida, Johannesburg, South Africa, <sup>2</sup>University of Johannesburg, Auckland Park, South Africa*Presenting author: Mabelm@unisa.ac.za*

The use of metagenomics techniques has become a reality in the poultry industry assessing the effects of diet formulation on their gut health. Hence, the objective of this study was to evaluate the effects of feeding sorghum-based diets on the gut microbes of Boschveld indigenous chickens. A total of 420 unsexed day-old Boschveld indigenous chickens were assigned into a completely randomized design with 3 sorghum varieties (red, brown and white) x 5 inclusion levels (0, 25, 50, 75 and 100%) x 4 replicate with 7 chicks per replicate for 90 days. Two chickens per replicate were slaughtered at days 60 and 90, and caeca were collected and stored in the tubes filled with 70% ethanol and immediately kept in ice. Samples collected were further processed using the 16s targeted rRNA metagenomics sequencing on Illumina's MiSeq platform. The resulting amplicons were purified, end-repaired and Illumina-specific adapter sequences were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). The amplicons were further sequenced on Illumina's MiSeq platform using a MiSeq v3 (600 cycles) kit. Analysis was done using in-house python scripts version 3.6.1. kronaTools and the Rstudio software following phyloseq package R version 3.5.0. The bacterial communities at alpha diversity indices Observed, Shannon and Simpson were more abundant at day 90 of sample collection than day 60. While the group of chickens offered diets with the inclusion level 3 (50%) of sorghum varieties had more bacteria than other inclusion levels. The bacteria communities dominated at phylum level were Firmicutes, Proteobacteria, Bacteroidia, while Lactobacillus - Aviarium, Comamoducae and Methylobacterium were dominating at genus levels. It may be concluded that the bacterial diversity and its patterns changed as chickens were getting older. The findings of this research shed an understanding of the bacterial diversity influenced by feeding different diets dominating chickens' gut microbiome.

Keywords: microbiota; unisex; amplified; indigenous chickens; sorghum



**The effect of non-starch polysaccharide degrading enzymes (NSPase) on gut leakage and intestinal histology in young broilers fed high-fiber, viscous, diets****E. Vinyeta<sup>1</sup>, Y. Dersjant-Li<sup>1</sup>, A. Bello<sup>1</sup>, K. Gibbs<sup>1</sup>, R. Santos<sup>2</sup>**<sup>1</sup>Danisco Animal Nutrition & Health (IFF), Oegstgeest, Netherlands, <sup>2</sup>Schothorst Feed Research B.V., Lelystad, Netherlands*Presenting author: ester.vinyeta@iff.com*

The effect of supplemental phytase and NSPase on growth performance and gut health in young broilers fed high-fiber diets was evaluated. Five treatments were tested (6 replicate pens/treatment, 6 male Ross 308 broilers/pen): 1) a commercially representative corn-wheat-soybean meal-based positive control (PC); 2) a negative control (NC) containing 15% rye and 5% barley, reduced in Ca and retainable P (-0.2% and -0.17%, respectively, vs. PC); 3) NC+1000 FTU/kg of a novel consensus bacterial 6-phytase variant (PhyG); 4), as 3) plus 1220 U/kg xylanase and 152 U/kg  $\beta$ -glucanase (XB100), and; 5), as 3) plus 2440 U xylanase and 304 U/kg  $\beta$ -glucanase (XB200). Diets were fed ad libitum as a mash from 0–14 days (d) of age. Body weight gain (BWG) feed intake (FI) and feed conversion ratio (FCR) were recorded each week. On d 14, 4 birds/pen received FITC-d by oral gavage; blood was collected for the determination of gut permeability. Jejunal and ileal digesta were collected on d14 from all birds for viscosity determination and sections of jejunum and ileum were collected from 2 birds/pen for histological analysis. Data were analyzed by ANOVA and Tukey's test was used for means separation; pen was the experimental unit. Compared to PC, NC reduced d7 and 14 BW, overall BWG and FI, and increased ileal and jejunal digesta viscosity (+45- 64-fold), gut permeability (+93%) and ileal crypt depth (CD;  $P < 0.05$ ). Both PhyG and XB100 increased ( $P < 0.05$ ) d14 BW vs. NC but not to the level of the PC, whereas XB200 further increased ( $P < 0.05$ ) d14 BW vs. PhyG and XB100, to a level not different from the PC. In addition, XB200 maintained overall (d 0–14) BWG, FI and FCR vs. PC, whereas XB100 maintained only FI and FCR vs. PC. Ileal digesta viscosity was reduced in all enzyme treatments vs. NC ( $P < 0.05$ ), to a level not different from the PC. Diet XB200 reduced ( $P < 0.05$ ) gut leakage vs. NC, equivalent to PC, while PhyG and XB100 numerically reduced gut leakage vs. NC. Both NSPase treatments maintained jejunal and ileal villus height, CD and VH:CD ratio equivalent to PC. In summary, in a high-fiber, mixed-cereal diet, supplementation of XB100 on top of phytase partially recovered performance back to the level achieved by a low fiber unsupplemented, diet, whereas XB200 fully recovered performance and was most effective at reducing digesta viscosity and gut leakage. Increasing the dose of XB can improve gut health and performance of young broilers fed high-fiber diets.

Keywords: enzymes; gut health; NSPase; phytase; probiotic

## Effects of electrolyzed-reduced water and functional amino acid supplementation in drinking water to alleviate heat stress in broilers

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Heat stress (HS) can adversely impact broilers, leading to decreased animal health, meat quality and performance. However, these effects can be mitigated by supplementing drinking water additives. In this study, we used electrolyzed-reduced water (ERW), characterized by high pH, low redox potential (ORP), high active hydrogen and low dissolved oxygen concentration. ERW is produced by passing water containing dissolved sodium chloride through an electrolysis chamber. Additionally, we tested a mixture of functional amino acids to outweigh a potential deficiency caused by reduced feed intake due to HS. In total, 720 male broilers (Ross 308) were distributed over 4 treatments (9 replicates/treatment, 20 birds/replicate). All animals were subjected to HS conditions in the finisher phase (32°C ± 2°C and RH 55–65% for 6h daily, d27–40). A three-phase feeding scheme was applied using a standard diet. Drinking water treatments were provided during the finisher period (d26–d40). Two treatment groups (i.e. EWR10 and EWR100) received ERW concentrated at 10% (pH 7.8–9; ORP 55.8) and 100% (pH 10–10.7; ORP -200.2), respectively. A third treatment group (i.e. AA) received an amino acid mixture (L-arginine (4.5 g/L), L-monosodiumglutamate (3 g/L), L-threonine (2.5 g/L)) via the drinking water (pH 8.3–9.3). The control treatment (CON) was given normal drinking water (pH 7.4–8.2; ORP 91.9). Statistical analysis was done with a linear mixed model with ‘treatment’ as independent factor. Both EWR10 and EWR100 had no significant effects on performance during HS as compared to CON. However, AA reduced feed intake with 5.2% (d25–39; p<0.01), growth with 8.2% (d32–39; p<0.01) and decreased feed conversion with 6.1% (d25–39; p<0.01) as compared to CON. Although, slaughter weight was not affected here. Furthermore, the treatments could not reduce rectal temperature or panting frequency. Both ERW treatments had no significant effects on meat quality. However, AA caused increased thawing- (p<0.01) and cooking loss (p<0.01) and increased shear force (p<0.01) as compared to CON. Additionally, pectoral muscle pH was lower (p=0.01) which could be associated with a lighter meat colour (p<0.01). In summary, none of the treatments enhanced the broilers’ performance and meat quality during HS in this experiment. Contrary to expectations, AA caused an increased thawing and cooking loss, decreased feed intake, growth and feed conversion during HS.

Keywords: heat stress;broilers;electrolyzed water;amino acids;performance;meat quality

## Exploring the avian breath: a comprehensive meta-analysis of factors shaping chicken respiratory microbiota

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Among avian species, chickens are the most extensively studied in terms of respiratory microbiota. While several studies have individually explored the airway poultry microbiota, a comparative analysis has not been conducted yet. Therefore, the factors influencing the chicken respiratory microbiota and its core composition have not been described yet. In our research, we have collected raw data of 16S rRNA sequencing from diverse studies, including samples from nasal turbinate, trachea, and lung. All the data was acquired from NCBI's SRA database using the SRA toolkit and processed using de DADA2 pipeline from the Qiime2-2023.2 environment. Before this, primer sequences were trimmed using the cutadapt software. The samples were classified using the SILVA 132 full-length classifier, pre-trained and provided by Qiime2 resources. We collected as much metadata as possible from the original works and normalised it for our study. The metadata gathered included information about several factors that could influence microbiome composition, such as breed, SPF status, weight, age, rearing conditions, etc. In our meta-analysis, we found that different factors exert a notable influence over microbiota composition, being SPF status one of the most relevant. Across the three respiratory tissues, SPF chickens displayed a higher diversity in microbiota compared to non-SPF ones. The median  $\alpha$ -diversities of these three tissues showed significant differences, with the trachea being the least diverse and the nasal tissue being the most, as expected due to its different environmental exposure. Another significant factor is the chicken breed. Our results indicate that the microbiota of chickens within the same breed exhibited greater similarity among themselves and more notable differences compared to other breeds. Furthermore, we defined the core microbiota of the respiratory tract in chickens. Our results show a new perspective on poultry respiratory microbiota, showing the most important factors that influence its composition. Also, we provide a comprehensive and integrated look at the airway respiratory microbiota for the first time. These findings could be relevant for future studies, providing important knowledge about avian respiratory microbiota.

Keywords: Chicken; Respiratory; Microbiota; Meta-analysis;

## **Potential biosecurity breaches in laying hen farms: characterization of the poultry-wildlife interface through a camera trap study**

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The diligent application and enforcement of biosecurity measures stand as one of the most effective tools for preventing disease transmission at the poultry-wildlife interface. Uncontrolled direct or indirect interactions between sympatric wild animals and domestic birds can indeed mediate the introduction of pathogens into poultry holdings. The study hereby presented aimed to characterize the spatiotemporal patterns of wild birds and mammals visiting the surroundings of poultry houses. Eight camera traps were deployed for a year (January to December 2021) in three commercial chicken-layer farms, including free-range and barn-type setups, located in a densely populated poultry area in Northern Italy. The positions of the camera traps were selected based on wildlife signs identified during preliminary visits to the establishments studied. Various methods, including time series analysis, correspondence analysis, and generalized linear models, were employed to analyze the daily animal visits. As a result, seven different wild mammals, 27 wild bird species, and domestic pets (cats and dogs) were identified. The time series analysis revealed a general trend of increased wildlife visits during spring and winter months. With respect to mammals, coypus (*Myocastor coypus*) and domestic cats (*Felis catus*) were more frequently observed. Among birds, Eurasian magpies (*Pica pica*), ring-necked pheasants (*Phasianus colchicus*) and Eurasian collared doves (*Streptopelia decaocto*) were the most frequent visitors. These animals were predominantly engaged in foraging activities near poultry houses, emphasizing the importance of reducing the attractiveness of the monitored sites to wild animals. To achieve this, useful tools may include regular inspections of the farm area, swift removal of feed spills and eggs fallen from automated egg-transport system, and the implementation of measures to discourage wildlife habituation. Overall, the data presented lay the groundwork for designing novel surveillance and intervention strategies to prevent cross-species disease transmission. The use of visual evidence depicting wild animals approaching poultry houses could also assist health authorities in educating and raising awareness among stakeholders about the presence of wildlife on farms and the potential risks of pathogen spillover.

**Keywords:** Poultry-wildlife interface; disease transmission; biosecurity; laying hen farms; camera-traps.

## **The biofilm removing capacity of drinking water disinfectants in an in vitro model**

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A major, and invisible, risk factor for disease transmission in the poultry industry is the presence of biofilm in the drinking water system. Protecting pathogens from environmental, physical and chemical exposure, the biofilm creates optimal conditions for pathogens to flourish. The process is accelerated by the relatively high temperatures and low water flow at the start of a new round. Previous studies have confirmed the ability of bacteria and viruses to become part of the biofilm network, resulting in subsequent spreading of these pathogens to susceptible birds. It is essential for optimal poultry health and productivity, that besides drinking water disinfection, the biofilm is completely removed from the drinking water lines. A biofilm generator is used to test the effectiveness of 2 drinking water disinfectants on biofilm removal: Intra Hydrocare (IH), an ECHA authorized drinking water disinfectant based on hydrogen peroxide and chlorine dioxide (CD). In the biofilm generator, consisting of transparent poultry drinking water lines which are connected to one water reservoir, a uniform biofilm is created. Once the biofilm was clearly visible in the transparent lines, swabs were taken to analyse the presence of micro-organisms. Applied concentrations of the disinfectants were 1.5 % CD solution and 2% IH solution. After a contact time of 10 hours, simulating practical circumstances, the water line disinfected with CD clearly still contained biofilm. The water line disinfected with IH was visually clear from any biofilm. This was confirmed by microbiological analyses from swabs taken from the inside of the water lines in both groups and by adenosine triphosphate (ATP) measurements, a procedure which measures cell viability based on the detection of ATP, expressed in relative light units (RLU). Swabs from the water line disinfected with CD contained 14291 RLU and >300 CFU/cm<sup>2</sup>, which falls in the unsatisfactory category. Swabs from the water line disinfected with IH contained 14 RLU and 0 CFU/cm<sup>2</sup>, falling in the cleanest classification. These results demonstrate that IH is able to remove all the complete biofilm, while chlorine dioxide was not able to clean out the biofilm.

Keywords: Biofilm; drinking water; cleaning

## **A comprehensive review on backyard poultry: exploring non intensive production systems**

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Poultry sector, since its beginnings, has been differentiated according to production system (intensive, sub-intensive, and extensive), and adapted to the needs of the population. Within them, it is important to highlight the evolution of backyard production. The historical perception of backyard poultry primarily revolved around animals raised for sustenance. However, developed countries' livestock practices are evolving considerably in response to new societal concerns. Consequently, backyard poultry enthusiasts now rear their birds for multifaceted purposes, including emotional bonds, and non-commercial utilization. The private ownership of these animals and their non-commercial outputs result in a dearth of data concerning backyard owners' demographics, flock specifics, husbandry, and welfare. Moreover, each poultry production system varies in conditions, demands, and outcomes influenced by breed types, feeding methods, disease prevalence, management practices, and their intricate interplay. It is therefore necessary to collect the scattered information on backyard poultry farming, allowing for a wide overview of the risks facing this production system. Consequently, this comprehensive review aims to delineate the traits of backyard poultry, emphasizing prevalent infectious diseases and the zoonotic risks faced by farmers. It is widely known that infectious diseases in avian species pose significant threats to both animal welfare and public health, especially in production systems with outdoor access. Pathogens such as bacterial strains (*E. coli*, *Salmonella*, *Campylobacter*, and *Mycoplasma*), parasites (Helminths, lice, and mites), and viruses (Avian Influenza, Newcastle, Marek's disease, Infectious Bronchitis, Gumboro, Infectious Laryngotracheitis, and Fowlpox) prominently affect the health of backyard poultry. Additionally, certain pathogens like Avian Influenza, *Salmonella*, *Campylobacter*, and *E. coli*, pose risks to backyard farmers and consumers of their products. Hence, stringent biosecurity measures are essential for disease control. While commercial farmers are well-versed in on-farm biosecurity practices, hobbyists and backyard farmers might lack the knowledge necessary to safeguard their flocks from infectious diseases and their spread. Consequently, addressing the legal status of backyard poultry, educating owners on biosecurity protocols, and advocating proper veterinary care and disease management become imperative.

**Keywords:** Backyard, zoonoses, infectious disease, biosecurity, legislation.

## **Light as an enrichment tool: effects on animal health and behavior in commercial turkey production**

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Injurious pecking is a major welfare concern in commercial turkey production. Enrichment is considered to reduce aggressive pecking. Perches and plateaus, pecking blocks or straw bales and other pecking objects were demonstrated to be suitable to reduce the problem. More tools need to be investigated to contribute to the bundle of measures, which can be used by farmers to control injurious pecking. Using different light zones within the barn may also provide a more favorable environment for birds, as they can choose different light intensities or qualities depending on their needs. We hypothesized that a zone with reduced light intensity (resting zone, RZ) within the barn may allow turkeys to choose between an activity zone (AZ) with higher light intensity, water and feeder access and a RZ. In three commercial turkey hen farms with trimmed beaks one barn (project barn, PB) was provided with a RZ covering at least one-eighth of the barn with a more than 30 % light reduction, compared to a control barn (CB) with similar management but without RZ. Over a period of 2 years, flocks were observed for general health parameters, integument integrity (n=30 birds/barn on consecutive time points between week 9 and 15 of age), weight development, use of RZ by transponder tracking of n = 200 birds/barn as well as animal behavior by video recording. Our observations indicate that neither animal health nor bird weight development was affected by the RZ. Birds used the RZ between week nine and slaughter intensively, and almost all transponder tagged birds used it but with individual frequency. The RZ was used significantly more to rest than the AZ on two of three farms ( $P < 0.05$ ). Furthermore, birds demonstrated significantly less pecking activity at various body regions in the RZ compared to the AZ ( $P < 0.05$ ). While head pecking was observed in both zones at very low frequency, it was still lower in the RZ compared to the AZ. Overall, our field study demonstrates that turkeys used a RZ frequently, and this retreat zone may contribute to reduce injurious pecking in commercial production.

Keywords: injurious pecking; turkey; enrichment; light

## **Effects of Aflatoxin B1 and Ochratoxin on the Fitness and Neonatal Growth of White Pekin Ducks: An In Ovo Injection Study with and without Ameliorants, Investigating Mycotoxin-Threshold and Toxin-Toxin Interaction**

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Atmospheric vagaries triggering emergence of prominent Food-origin Mycotoxins such as, Aflatoxin-B1 (AFB1), Ochratoxin-A (OTA) continue to be researched worldwide, for their crippling impacts on fitness, neonatal growth and feed conversion, in meat-type ducks. Eying such a scenario, in a coastal climate, a study at ICAR-DPR, RS Bhubaneswar, explored the impact of Aflatoxin-B1 (AFB1) and Ochratoxin-A (OTA) on White Pekin duck embryos. Mycotoxins were injected in ovo, on day-23 of incubation, with groups receiving varying doses: [A] AFB1 @ 2ng/egg, [B] AFB1 @ 4ng/egg, [C] AFB1 @ 6ng/egg, [D] OTA @50ng/egg, [E] OTA @50ng/egg + AFB1 @ 1ng/egg, and [F] OTA @50ng/egg + Amino acids (Lysine & Methionine @10mg each), along with a Sham-control. Each of these groups was comprised of 90 fertile eggs/treatment, including 3 replicates within each group. The ducklings, which hatched by end of incubation period, were reared under uniform management and raised till 5th week of age. These were grown on a standard duck diet containing 2900KCal ME energy and 21% Crude Protein all-through and the feed efficiency evaluated every week. Results indicated a linear decrease in hatchability with increasing AFB1 doses. Group C (6ng/embryo) exhibited the highest growth retardation, while the OTA+AFB1 group showed the harshest impact on fitness. Hatchability dropped significantly for groups D and C, ranging from 15 to 32%. Weekly neonatal growth efficiency varied across groups, with OTA, individually or in combination, negatively affecting growth up to the 4th week. Post-natal mortalities ranged from 14% to 31% over the 4-week period in AFB1 and OTA interaction groups. Growth depression and stressed survival were consistently associated with OTA groups. Necropsies revealed nephropathic lesions and necrotic hepatitis. Administering lysine and methionine in ovo (Group D) did not significantly reduce mortalities but showed compensated growth up to the 3rd week. Morbid ducklings in groups A through C exhibited inefficient juvenile growth with toxic lesions in the liver, kidney, and spleen. It was concluded that Inter-toxin interaction created more severe morbidity in Pekin ducks, comparable to or higher than Group C which had received the highest amount of AFB1 in ovo. It was also proven that Lysine and methionine administered in ovo, tended to ameliorate duckling growth and feed efficiency, improving fitness from 0 to 5 weeks of age.

Keywords: Aflatoxin-B1; Duck; Fitness, In-Ovo; Lysine, Methionine, Ochratoxin-A, Pekins



## **Spatial patterns in the use of aviaries in relation to body condition and production – A study on individual laying hens**

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Modern technology of tracking individual laying hens in semi-commercial non-cage housing systems have revealed profound individual variation in the spatial use of aviaries. While spatial use has been linked to keel bone fractures, further connections with plumage, injuries, and production on the level of the individual hen remain unclear. Hence, the aim of this study was to correlate the individual presence on the aviary tiers, the litter, and the covered veranda with the plumage, toe injuries, and estimated individual egg production until 60 weeks of age. For that, we tracked the location and space use of 1125 hens across 5 pens (225/pen) representing 25 sires. Time and length of visits to the nest tier within the first 6 hours of light were used to estimate egg laying and the number of eggs was validated with the data of 2 field tests of the breeding company involving the same sires. Use of the areas of the aviary tiers, the litter, and the veranda was reduced to 4 factors in a Principal Component Analysis (PCA) explaining 56% of the variance. Toe injuries and plumage were scored with a tagged visual analogue scale when the birds were 40 and 60 weeks old (WOA). Generalized mixed linear models of egg production until 60 WOA as a function of the median of the factors of the PCA of the hens, sire, and pen during rear were conducted with pen during lay as the (random) subject factor. Among the factors from the PCA, only Factor 4 (explaining 8% of the variation) with positive loadings of the duration on the first and top tiers was positively linked to total egg production ( $F_{1,786} = 8.92$ ,  $P = 0.003$ ). Factor 1, with positive loadings of number of visits to the litter and the veranda, was associated with the interaction between plumage at the breast and toe injuries at 40 WOA ( $F_{1,935} = 4.73$ ,  $P = 0.03$ ) but, at 60 WOA, this factor was associated with the interaction between breast plumage and the sire ( $F_{24,954} = 1.86$ ,  $P = 0.008$ ). Irrespective of spatial patterns, birds with better plumage on the breast at 60 WOA had a higher total egg production (estimate =  $0.27 \pm 0.08$ ,  $t_{836} = 3.26$ ,  $P = 0.001$ ) and hens with serious toe damage at 60 WOA produced fewer eggs ( $F_{1,483} = 7.86$ ,  $P = 0.005$ ). We conclude that spatial patterns in the use of aviaries were barely linked with egg production, but poor plumage or toe injuries were linked with the number of daily visits to the litter and the veranda and decreased egg production.

**Keywords:** Laying hen, aviary, tracking, welfare, egg production

## **Influence of light exposure and early feed access on the stress response during the developmental and early posthatch period in laying hen chicks**

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The early life environment has been found to influence performance and behavior in chicken, though the importance of perinatal stimuli and their interactions are poorly understood, including effects on physiological stress responsivity. We investigated the influences of light exposure during the last 3 days of incubation and immediate posthatch feed and water access on stress responsivity in a 2x2-factorial design with two replicated trials. Hatching eggs (n=1280 eggs) were exposed to one of the following treatment combinations from 18 days of development to hatch (4 pens/treatment): feed and water, and continuous light exposure (FW+/L+), light only (FW-/L+), feed and water only (FW+/L-), as well as fully deprived (FW-/L). Chick processing, comprised of counting, sorting, grading, sexing, and vaccinating, was chosen as a stressful early life event. Blood samples (n=32-36/timepoint) were collected postmortem at two timepoints around chick processing: 1) prehandling as baseline (PRE), and 2) posthandling (PO). The blood samples were analyzed for serum corticosterone (CORT) concentrations to examine short-term treatment effects. Data were analyzed using linear mixed models with treatment and timepoint as fixed factors, with chick nested in pen nested in trial. Serum CORT was affected by the interaction of treatment and timepoint ( $X^2 = 536.92$ ,  $df = 6$ ,  $P < 0.001$ ). FW-/L- chicks had higher CORT at PRE than FW+/L+ chicks ( $P = 0.03$ ), a finding that could have been affected by the lights being switched on shortly before PRE. Switching on the lights, brings on a rapid environmental change and consequent novel visual stimuli for light deprived chicks, which could have led to increased fearfulness at the sampling time. Chick processing was expected to result in elevated CORT (PO vs PRE), though this was found only in FW+/L+ chicks ( $P < 0.01$ ), which might reflect maximum HPA stimulation in deprived chicks. Contrary to our expectations, FW+/L- chicks had lower CORT at PO than FW+/L+ ( $P < 0.01$ ) and FW-/L+ chicks ( $P < 0.01$ ). Possibly, the circadian rhythms between individuals of different treatment groups were asynchronised, which could have affected their circadian HPA-activity and metabolism. In conclusion, our findings suggest that stress responsivity was differentially affected by both treatment factors independently, though specific mechanisms and long-term effects will need to be investigated. Light exposure and early feeding might be important in HPA development.

**Keywords:** on-farm hatching ; corticosterone ; acute stress ; chick processing

## Cost-benefit analysis of the upright versus conventional method for catching and loading end-of-lay hens

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An alternative method for catching and loading birds in which the animals are kept upright instead of upside down, has been shown to have some animal welfare benefits in broiler chicken but hasn't been studied yet for spent hens. We conducted a cost-benefit analysis of 2 manual methods (upside-down by 1 leg vs upright) for catching and loading spent hens. Animal welfare, ergonomic and financial data were collected during the depopulation and slaughter of spent hens from 7 commercial flocks. Catchers caught  $\pm 3000$  hens/method/flock. Researchers timed and scored the catching and loading process at the farm and collected data on death-on-arrival and catching damage at the slaughterhouse. For ergonomic evaluations catchers were surveyed and the ergonomic load of simulated catching actions (experimental setting) was assessed from video-recordings by an expert. Observations during depopulation revealed that the handling was gentler ( $1.9\pm 0.5$  vs  $4.4\pm 0.5$ ,  $P<0.001$ ) and wing flapping frequency was lower ( $3.1\pm 0.6$  vs  $4.0\pm 0.5$ ,  $P<0.001$ ), both on a 7-point likert scale. This for upright versus conventional catching. More person-hours per 1000 hens were required for upright than conventional catching ( $8.2\pm 3.2$  vs  $4.8\pm 2.0$ h,  $P=0.011$ ). There was no difference of injuries scored at the slaughterline between both catching methods, except for wing bruises being less common for upright than conventional catching ( $1.1\pm 0.6$  vs  $1.7\pm 0.7\%$ ,  $P=0.04$ ). The additional cost for upright compared to conventional catching of 20,000 laying hens was €3478. This cost could be compensated by a price premium of  $\pm \text{€}0.0005$  per egg. According to the ergonomist both catching methods are ergonomically too demanding, but the catchers ( $n=29$ ) indicated that they preferred conventional catching. In conclusion, this study confirmed that from an animal welfare perspective the upright has some advantages to conventional. Unless the catching team is considerably larger, however, the entire process takes longer when catching upright, which likely is undesirable from an animal welfare perspective. The catchers' opinion about upright catching was predominantly negative, although the physical strain was not judged to be harder by an ergonomist. Upright catching was 1.8 times more expensive than conventional catching, but expressed per egg the price differential is almost negligible.

Keywords: laying hens; catching methods; injuries; costs; ergonomomy

## **Long-term effects of ramps provided during rearing and laying phases in laying hens**

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Ramps in aviaries, both during rearing or laying, have been shown to benefit laying hens in terms of their welfare. We investigated whether providing ramps in the rearing phase has long-term effects on bird health, production, and behaviour in the laying phase. Lohmann Selected Leghorn (LSL) pullets were raised in two treatments: four pens (600 birds/pen) with ramps to aid movement between rearing aviary tiers (RR) and four pens (600 birds/pen) without ramps (RO). At 17 weeks of age (WOA), birds were moved to the laying barn, in which 16 aviary pens (225 birds/pen) were populated. Half the pens (n=8) were supplemented with ramps (LR) and the other half were not (LO). Within each laying treatment group, four pens were populated with RR hens and four pens were populated with RN hens, creating four treatment combinations (RRLR, RRLO, ROLR, ROLO). From each pen, 15 focal hens were selected for radiographic imaging of their keel bones at 21, 36, 45, and 60 WOA and subsequently scored for fracture severity. Focal hens were also scored for feather condition and footpad quality at 36 and 60 WOA using the Welfare Quality® assessment protocol. The number of downward transitions and falls were assessed from video at 19-20 and 30-31 WOA at three times per day (i.e., lights on, midday and dusk phase) and from three aviary levels (i.e., first, nest and top tier) for 10 minutes each. Data on egg production (%), floor eggs (%) and feed consumption (kg) were collected from 18 to 60 WOA. Data were analyzed using (generalized) linear mixed models in R. When ramps were available, they were used in most of the observed downward transitions (79% in ROLR and 86% in RRLR). Hens who received ramps in lay (RRLR and ROLR) showed more transitions after lights on compared to midday or dusk phase ( $p<0.001$ ) and performed more transitions from the first and nest aviary tiers compared to the top tier ( $p=0.013$ ). Ramps reduced KBF severity ( $p<0.001$ ) with 21% of birds having a KBF score  $> 6$  in RRLR vs. 28% in ROLO. At 60 WOA, hens in the RRLR group had greater feather coverage than those in ROLR and RRLO ( $p<0.001$ ). Birds in the RRLR group had better foot health overall than those in treatments without ramps in lay ( $p=0.018$ ). No differences were found between treatments for any of the production data. Providing ramps, particularly during lay, has positive effects on welfare parameters and facilitates access to resources needed for important behaviors without negative impacts on production.

**Keywords:** Aviary; Ramps; Laying hen; Welfare

## **Exercise using wing-assisted incline running during rearing affects initial descent velocity in adult laying hens**

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Laying hens rely primarily on their hind legs to locomote as they are not efficient fliers. To scale steep inclines, chickens will flap their wings as they ascend to help propel their body upwards. This type of locomotion is termed wing-assisted incline running (WAIR) and involves muscles in both the hindlimb and breast. WAIR as an exercise to potentially help develop these muscles and improve the capacity of laying hens to modulate their body kinematics during aerial descent has not been extensively studied. In this study, we explored this further by training 82 birds from 5-21 weeks of age (WOA) in two different groups: 41 birds trained to run up an incline of 60° using WAIR and 41 untrained birds as the control. The trained group was required to exercise twice weekly, one session per day, alternating days of rest and exercise. Exercise sessions were only completed for the day when birds produced two consecutive runs with a 40% decrease in velocity from their fastest run following a minimum of 10 runs up the ramp. These birds were housed in 8 pens with one treatment and an approximately equal number of white- and brown-feathered birds per pen. At 37 WOA, a subsample of 23 trained birds (10 white- and 13 brown-feathered birds) and 25 untrained birds (12 white- and 13 brown-feathered birds) were used to measure body kinematic measurements during aerial descents from a 155 cm tall platform. A camera was used to record the descent, and the lower beak was tracked using MATLAB to calculate descent angle (°), take-off velocity and acceleration (m/s<sup>2</sup>). Generalized linear mixed models were used to analyze all outcome variables with training and strain as fixed effects and body weight as a covariate. Although descent angle and acceleration were not significantly affected by training in either strain, the take-off velocity was significantly higher in trained versus untrained birds (2.5 versus 2.3 m/s;  $F_{1,43} = 4.75$ ,  $p = 0.0348$ ) by about 0.2 m/s. Exercise during rearing using WAIR may then allow laying hens to strengthen their hindlimb muscles and potentially increase their capacity to control their flight during descent. This may be important for improving their welfare in adult life as greater initial flight velocities can be essential for securing resources, avoiding stressors/predators and reducing the power required to support body weight by the wings when taking off into flight.

Keywords: domestic birds; locomotion; flapping-flight; navigation; flight kinematics

## Access to outdoor space for laying hens: evaluating motivation and behavior of the animals

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Defining the importance of specific resources for farmed animals is a key element to develop sustainable and welfare-friendly farming systems. The present study was aimed at evaluating the motivation of laying hens to access to outdoor space with pasture resources. The behavior of hens with or without access to outdoor space was also assessed. A total of 54 laying hens (white Leghorns) at 38 weeks of age were housed in 6 shelters (3 with outdoor access: 10 m<sup>2</sup>/hen and 3 without outdoor access: 10 hens/m<sup>2</sup>). The behavioral evaluation was performed with the use of a video recording system. The motivation test was carried out on 9 hens with average body weight (BW) of 1.6 kg that were housed in a shelter with access to outdoor space. To access outdoor area, hens had to push through a one-way transparent weighted door. The door weight was 150 g and it was increased by 100 g every 2 days. The weight applied to the door represented the cost paid by the animals to obtain the resource. The test was closed when each animal did not pass the door for two consecutive days. The same protocol was applied to measure motivation towards feed (gold standard). Each animal was identified by a ring provided with a chip. A monitoring system (ChickenGate) with an antenna placed near the pushing door was used to record the animals which reach the resource. Hens reduced the number of visits for both tested resources as the door weight increased ( $P < 0.001$ ). Hens were willing to push a weight equal to 47% of their BW to access the outdoor space, while their willingness to pay for the access to feed stopped at a weight equal to 41% of their BW ( $P < 0.05$ ). However, a higher number of visits was observed towards feed compared to outdoor space ( $P < 0.05$ ). With the access to outdoor area, approx. 30% of the animals were observed pecking grass and 5% dust bathing; the percentage of animals walking and grooming increased while the percentage of animals resting decreased compared to the group without access to outdoor area ( $P < 0.001$ ). In conclusion, the present study demonstrates that laying hens are highly motivated to access outdoor space where they can find pasture resources and perform specie-specific behaviors. Acknowledgement: This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (Piano Nazionale di Ripresa e Resilienza (PNRR) – Missione 4 Componente 2, Investimento 1.4 – D.D. 1032 17/06/2022, CN00000022).

Keywords: motivation test; chickens; poultry welfare; poultry behavior

## **Using a validated x-ray method on individual living hens, bone density does not decline over their lifetime**

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In the laying hen industry bone quality has become a contentious issue. One question has centred on how bone density changes with age and with the production of eggs. We have often stated that hens are osteoporotic at the end of lay, this literally means that they lose bone over a period. However, there are very few studies with meaningful numbers of animals over time, probably because traditional bone density and quality measurements on poultry requires the bird to be sacrificed and would tend to be done towards the end of lay. Our development of a practical and validated x-ray method to measure bone quality in living laying hens has allowed an estimate of tibiotarsus density and keel damage from the prepubertal stage through a laying cycle. Hy-line brown hens (n=60) housed in 4 replicate pens furnished with perches and nest boxes were x-rayed at 10, 14, 18, 20, 24, 28, 36, 48 and 60 weeks of age and measurements made from the radiographs. Keel damage was estimated using a scoring system and the tibia tarsus density was measured as previously published. Repeated measures ANOVA was used to determine if there were significant differences over time. The results demonstrate that tibia bone density did not decrease significantly towards the end of lay. Rather, it rose significantly from 14 to 20 weeks of age and continued to slowly increase up to 60 weeks of age when the last measurement was made. The increase in bone density as the birds undergo puberty appears to be due to medullary bone deposition. Cortical bone stays relatively stable through life. Keel bone damage was first observed around the time of puberty at week 18 and slowly increased in incidence so that by 60 weeks of age around a third had some form of damage, mostly mild. No birds were severely affected and the majority recorded no damage. In conclusion, bone density in these laying hens did not decline with age and indeed appears to have increased. The incidence of keel bone damage started around puberty and did not show any obvious evidence of being concentrated at the end of the period although the small number of birds that had more damage were evident from the peak of lay. These results and other evidence accumulating on skeletal quality should help reappraise where interventions are made to ameliorate issues of bone health in laying hens. The work was funded by the Foundation for Food and Agricultural Research and a BBSRC Institute strategic program grant.

Keywords: Keel; Bone quality; x-ray; laying cycle

## Occurrence of keel bone lesions measured at the slaughterhouse in laying hens: analysis of risk factors for on farm animal welfare

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Laying hens in conventional farms are exposed to several risks which can challenge their welfare. Different animal-based indicators (ABMs), i.e. keel bone lesions, breast blisters, and footpad lesions, can be collected at slaughtering for information about welfare on farm. Nevertheless, risk factors for ABMs and relationships with housing systems have not been fully elucidated yet. The present study evaluated the risk factors for keel bone (KB) lesions by collecting data in a commercial slaughterhouse for 15 months on 51 flocks (200 hens per flock): floor systems (16 flocks), enriched cages (16 flocks), aviary systems (19 flocks). The effects of slaughtering season, genetic line (brown vs white feathered), housing system, flock size, and length of the rearing cycle were evaluated by a multivariate logistic regression analysis; the risk factors were identified through a forward stepwise selection; the regression coefficients were expressed as odds ratio (OR) with 95% confidence interval. As for the odds of showing KB deviations, a lower value (OR: 0.70;  $P < 0.001$ ) was found in hens slaughtered in winter compared to spring; a higher value (OR: 1.26;  $P < 0.001$ ) was recorded in white compared to brown hens; the risk was lower in floor systems (OR: 0.78;  $P < 0.001$ ) and higher in enriched cages (OR: 1.41;  $P < 0.001$ ) compared to the aviary system; a higher odds ratio (OR: 1.50;  $P < 0.001$ ) was recorded in medium compared to small flocks. The odd ratio of showing KB deviations was lower in hens from flocks reared for extended cycles compared to those from standard ones (OR: 0.84;  $P < 0.01$ ). As for KB fractures, a higher odds ratio (OR: 2.04;  $P < 0.001$ ) was recorded in winter compared to spring, whereas a lower odds ratio (OR: 0.69;  $P < 0.001$ ) was recorded in white compared to brown hens. The odds ratio of showing KB fractures was 1.66 for enriched cages and 0.99 for floor systems ( $P < 0.001$ ) compared to aviary system. Higher odd ratios were obtained for KB fractures occurrence of medium and big flocks (OR: 1.69 and 1.83;  $P < 0.001$ ) compared to small flocks, whereas OR was lower in flocks reared for an extended compared to a standard cycle (OR: 0.69;  $P < 0.001$ ). In conclusion, changes in KB lesions according to slaughtering season, flock size, and rearing length require further insights. Brown and white hens have a different risk for KB deviations or fractures. Finally, hens in enriched cages are more exposed to risk for KB fracture compared to hens kept in aviary systems.

Keywords: welfare on farm; genotype; housing system; slaughtering season; flock size



## The effect of hypocalcaemia on keel bone radiodensity and damage

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Laying hens can be affected by limb or keel bone damage which may affect their welfare. Finding an easy and reliable bone quality phenotype that can be measured on the living hen would be beneficial as current methods require the hens to be culled. Using diets with varying levels of calcium (Ca) and phosphorus (P) to alter bone quality, tibia and keel phenotypes were measured to detect differences in bone integrity. 26 week of age Hy-line brown laying hens (n=72) in two replicates were fed either a control diet (CON; 3.28% Ca, 0.46% P), a hypocalcaemic (HC; 1.9% Ca, 0.48% P) or hypocalcaemic and hypophosphataemic diet (HCHP; 1.39% Ca, 0.31% P) for 5 weeks. Whole-body radiographs were taken and body weights (BW) were measured at 0, 3 and 5 weeks of the experiment as well as egg number (EN), egg weight (EW) and eggshell breaking strength (EBS). From these radiographs, keel pila carinae (KPC) and tibia (AUC) densities were measured. After 5 weeks of treatment the keels were dissected and radiographed. Dissected keel radiographs were used to measure overall density and to score percentage of keel affected, deviation, deformity, number of fractures and location of fracture. Each keel factor was given a score between 1 and 4 depending on severity. ANOVA was used with diet as treatment and replicate as blocking factor for all data except keel factors which used Kruskal-Wallis analysis with post-hoc Mann-Whitney U. All analysis was performed using Genstat 22nd edition. The results show that BW, EN, EW and EBS in HC and HCHP groups declined over the experimental period and was significantly different from CON group ( $P \leq 0.001$ ). At weeks 3 and 6 of the experiment, tibia cortical AUC was lower ( $P \leq 0.001$ ) in the HC and HCHP groups than in CON group. There was no difference in the KPC density between the dietary groups at 3 and 6 weeks. For the dissected keels, there was a difference in the overall radiodensity ( $P=0.014$ ), percentage bone affected ( $H(2)=7.62$ ,  $P<0.01$ ) and a difference in deformity ( $H(2)=9.21$ ,  $P \leq 0.001$ ) between the dietary groups. There was no difference in deviation, number of fractures and location of fractures scores. In conclusion, as poorer quality tibia was associated with more keel damage, improving the overall skeletal quality would be beneficial for keel quality and reduce damage. Tibia and keel quality are related and although the keel may be more prone to damage, attempts to improve the keel may not need to be treated in isolation.

Keywords: keel bone; bone density; laying hens; live xrays

## **Analyzing attitudes and satisfaction level of digital on-farm technologies for climate change adaptation and mitigation in poultry farming**

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In the EU, agriculture contributes significantly to greenhouse gas (GHG) emissions. While the poultry sector has a comparatively modest environmental footprint within the livestock industry, this sector still faces its own environmental challenges. For instance, the poultry industry plays a substantial role in the current worldwide emissions of NH<sub>3</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, thereby contributing, either directly or indirectly, to climate change. To address the ongoing climate crisis and to comply to the European Green Deal requirements, GHG emissions must be significantly reduced. Digital solutions, particularly decision support systems (DSS), are promising tools to assist livestock farmers achieve the globally agreed GHG reduction goals. However, there is a lack of studies addressing poultry farmers' attitudes and the DSS requirements for GHG mitigation on farm level. Users' feedback on technologies can support identify areas for improvement. Therefore, the study explores poultry farmers' attitudes to adopt DSS for climate mitigation. It further categorizes fourteen DSS features for GHG reduction based on their impact on customer satisfaction. Using the Kano model, a quantitative online questionnaire surveyed the satisfaction or dissatisfaction levels of 53 German poultry farmers with these features. A significant majority (app. 762%) of the respondents acknowledged observing the effects of climate change. In alignment with this awareness, around 70% expressed concern about climate change and the associated mitigation measures. Notably, 96% of participants indicated a willingness to invest in software to save resources and/or working time. However, only 30% would purchase software for support in climate change mitigation. Interestingly, 96% of respondents expressed strong interest in receiving potential compensation for CO<sub>2</sub> savings. The results from the Kano model highlighted the significance of data authority and integrability in DSS, with their absence causing dissatisfaction. Multi-target optimization emerged as an attractive feature, positively impacting satisfaction. Connectivity and market perspective, however, appeared indifferent. The findings guide DSS developers in prioritizing attributes crucial for customer satisfaction. The results illustrate farmers' awareness of climate change and their acceptance to invest in DSS. They also help focusing on must-have attributes to preserve customer satisfaction and to ensure successful GHG reduction implementation.

**Keywords:** Poultry Farming, Decision Support System (DSS), Kano Model, Climate Change

## Comparing Soybean Meal Reduction on Performance of Fast and Slow-Growing Chickens

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Animal feed industry in the European Union heavily depends on soybean meal (SBM), contributing to the ecological and environmental issues associated with deforestation. Therefore, one of the important challenges in the poultry production is to minimize the use of SBM, in conventional and alternative production systems, promoting the use of other local sources with lower environmental impact. For this purpose, the current study aimed to investigate the effects of SBM reduction in fast and slow growing (SG) chicks. In total, 840 Ross 308 (Fast) and 840 Hubbard JA757 (SG) day-old-chicks were allocated to 80 pens, with males and females kept separated and randomly assigned to 5 dietary treatments, up to 42 or 56 days respectively. A feed program containing three feeds (starter, grower, and finisher) was used as per genetic guideline recommendation. Five dietary feeds were formulated by reducing the SBM about 0%, 50% or 100% for the whole study period (CT, T50 and T100) or after the starter phase (TC50 and TC100) by increasing the use of other protein sources such as peas, rapeseed, sunflower meal and hydrolyzed porcine protein, in different proportions depending on the growing phase. While both breeds were adversely affected by the reduction in SBM, the impact was less pronounced in SG. During the starter phases, T100 did not affect the growth of SG females but reduced animal weight (BW) by 9.6 % in SG males ( $P<0.005$ ) and 11.6 % in Fast birds, independently of sex, compared to CT ( $P<0.001$ ). At the end of the growing period, in Fast birds, all dietary treatments with SBM reduction impaired BW by 5.9%, feed conversion ratio (FCR) by 7.1% ( $P<0.001$ ), and carcass yield (CY) was only negatively affected by T100 and TC100 compared to CT (67 vs 69%). In SG birds, only T100 and TC100 negatively affected final BW compared to CT (2728 and 2807 vs 2924 g, respectively;  $P<0.001$ ) and FCR and CY were not significantly affected by SBM reduction. The elimination of 100% SBM increased the feed cost by an average of 4.4 and 2.0 €/100kg feed for Fast and SG diets, and the carbon footprint decreased from 2,8 to 1,6 or 1,5 kg CO<sub>2</sub>/kg of live weight, respectively. In conclusion, while reducing SBM decreases CO<sub>2</sub> footprint, it also has negative economic and productive effects. Therefore, further research is needed to identify alternative local sources with a lower environmental impact that can replace SBM and promote sustainable poultry production without compromising productivity.

Keywords: Fast-Growth; Slow-Growth; Soybean Meal Reduction; cCarbon Footprint

## **Performance and environmental implications of modulating the metabolizable energy content in broiler chicken diets**

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Providing diets with adequate concentrations is crucial for productive performance and to maximize the use of natural resources, which leads to mitigating environmental impacts. Therefore, this trial was carried out to assess the effects of feeding diets with different nitrogen-corrected apparent metabolizable energy (AMEn) content on: 1) the growth performance of broiler chickens, and 2) the environmental impact and economics associated with broiler production. A total of 1,800 one-day-old male Ross 308 chicks were divided into three experimental groups (12 replicates/group): OLD group, receiving commercial diets with AMEn levels similar to those previously recommended by the breeding company (3,025, 3,100, 3,175 and 3,225 kcal/kg, in starter, grower I, grower II and finisher phases); NEW group, fed diets with AMEn content comparable to the new nutritional specifications (2,975, 3,050, 3,075 and 3,125 kcal/kg, respectively); and ALT group, in which the AMEn levels were further reduced compared to previous groups up to grower II phase but maintained similar performance and environmental implications of modulating the metabolizable energy content in broiler chicken diets to that of NEW group for the finisher one (2,925, 3,000, 3,025 and 3,125 kcal/kg, respectively). All diets were iso-nitrogenous and with a similar amino acid profile. The same raw materials were used to formulate all experimental diets and the reduction of AMEn was obtained by modulating the dosages of selected ingredients, mainly vegetable oil and full-fat soybean. Growth performance parameters were recorded at the end of each feeding phase (i.e., 11, 21, 32 and 42 days) and in the overall trial period. Performance data were analysed by means of one-way ANOVA, while Life Cycle Assessment (LCA) methodology, using a cradle to farm gate approach, was applied to quantify the environmental effects of the nutritional approach related to broiler production. Considering the performance results in the overall rearing cycle (0-42 days), final body weight and feed conversion ratio were not significantly affected by the dietary treatments (3,116 vs. 3,168 vs. 3,125 g/bird, and 1.605 vs. 1.585 vs. 1.605 g feed/g gain, respectively;  $P=0.43$  and  $P=0.17$ ). Slaughter yields were similar among groups while LCA analysis is still ongoing. In conclusion, these outcomes suggest that the AMEn content of broiler diets could be further optimized compared to current standards, maintaining optimal growth performances while potentially enhancing some sustainability aspects of this production chain.

**Keywords:** broiler chickens; metabolizable energy; nutrition; performance; Life Cycle Assessment.

## **Ammonia emissions can be reduced through a quality-controlled bedding material in broiler housing systems**

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According to the National Emission Ceilings (NEC) Directive ammonia (NH<sub>3</sub>) emissions in Germany must be reduced by 29% by 2030 compared to 2005. In order to comply with this limit value, the revised German General Administrative Regulation to the Federal Immission Control Act (Technical instructions for air pollution control; TA-Luft) demands a 70% reduction in ammonia emissions for forced-ventilation poultry houses with a capacity of 40,000 animal places (AP) or more. Poultry houses with a capacity of 30,000 to 40,000 AP must reduce their ammonia emissions by 40%. Technical solutions are available but due to high costs and the poor carbon footprint of the technical solutions, other options are needed. As ammonia emissions emerge from the litter, affecting the animal welfare and health, the bedding material plays a key role in reducing ammonia emissions from the litter. Therefore, a new straw pellet including sodiumbisulfate (SBS) was tested on 2 commercial broiler farms during 3 cycles from January to August 2023 with a total of 234,900 Ross 308 broilers. Both farms had two identical poultry houses. The animals were kept for 42 days. Litter management followed standard recommendations with removing the bedding and cleaning the barn between each cycle. The international VERA test protocol for animal husbandry and management systems was used as the basis for the measurement planning (VERA 2018). Following a case and control method, one barn on each farm was littered with 1.2 kg/m<sup>2</sup> straw pellets (control) and the other barn was littered with 1.5 kg/m<sup>2</sup> straw pellets including about 20 % SBS. Case and control barns alternated between each run. Ammonia emissions were determined by FTIR analysis and by the volume flow rate of the poultry houses. Average mortality rate was 2 % over each production cycle. The average feed conversion ratio was 1.48 kg/kg in both, case and control. The use of straw pellets with SBS reduced ammonia emissions by 58.2 % compared to conventional straw pellets. The recovery rates for nitrogen of 106.4 % support these results. In relation to the TA-Luft the ammonia reduction target was achieved with 85 %. In order to complete the VERA test protocol, the tests are ongoing with an expected completion in March 2024.

**Keywords:** Ammonia emission, broiler, litter, VERA test protocol

## **Impact of slower growing broilers and reduced stocking density on ammonia emission and animal welfare**

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This study explores the impact of the adoption of slower-growing broiler breeds at reduced stocking densities on welfare and ammonia emissions. The experiment spans four production cycles in a semi-practical environment, utilizing eight compartments of 112,5m<sup>2</sup>, which are each divided into two pens of 56,25m<sup>2</sup>. Pens are categorized into four experimental groups, based on breed and stocking density. A conventional broiler at high-density (CH) serves as the control group, with further groups including a conventional broiler at lower-density (CL), a slower-growing broiler at high-density (SH) and a slower-growing broiler at lower-density (SL). The breeds used were Ross 308 (conventional) with a 40-day slaughter age and Hubbard Redbro (slower-growing) with a 49-day slaughter age. High-density compartments have a maximal density of 42 kg/m<sup>2</sup> with 2360 birds, while low-density compartments have 30 kg/m<sup>2</sup> with 1700 birds. All pens are identical and straw bales and perches are provided. Data on mortality, feed - and water intake is collected daily. Welfare is evaluated weekly using gait- and footpad scores, behavioral assessments related to regular - and comfort behaviors, novel object tests and the utilization of straw bales and perches. Ammonia concentrations are continuously measured using Fourier Transformed Infrared Spectroscopy. While the experiment is ongoing, results of the first production cycle show specific trends. No differences are observed in most general- and comfort behaviors, but SL-birds show more 'standing' (6,31% of the observed birds) compared to CH-groups (2,71%) ( $p < 0,005$ ;  $n = 32$ ). SL-birds rest more on straw bales than SH-birds ( $p < 0,005$ ;  $n = 32$ ) and react less fearfully to novel objects. Gait scores at the end of the production cycle show better gaits in the SL- ( $p < 0,05$ ;  $\chi^2 = 9,799$ ;  $df = 1$ ;  $n = 160$ ) and SH-groups ( $p < 0,05$ ;  $\chi^2 = 5,378$ ;  $df = 1$ ;  $n = 160$ ), compared to the CH-group. At the end of the production cycle, slower-growing birds show worse footpad lesions when compared to conventional birds at the same stocking density ( $p < 0,05$ ;  $\chi^2 = 14,407$ ;  $df = 4$ ;  $n = 240$ ). Ammonia emissions per bird per year are lowest in the CH-groups and increase by 121,61% in the SL-group and 85,32% in the SH-group. This study indicates increased ammonia emissions with lower stocking densities and slower-growing breeds, and highlights the interaction between welfare measures, broiler behavior and environmental impact in commercial production systems.

**Keywords:** Slower growing broilers; Stocking densities; Welfare; Gait score; Footpad lesions; Behavior; Ammonia emissions; Environmental impact; Technical performance

## Feasibility of purified rainwater as drinking water for broilers

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Climate change leads to extended dry periods, posing challenges to the sustainable extraction of groundwater for poultry drinking water. An alternative solution lies in rainwater use, provided it can be effectively collected, stored and purified. This study forms part of the initiative for sustainable alternative water sources, evaluating a water purification system to supply purified rainwater to broilers (ACLIMA-project - contract number LIFE 20 CCA\_BE\_001720). The study aimed to assess the impact of purified rainwater as a safe drinking water source on the health and technical outcomes of broilers. The system encompasses multiple treatment steps: drum filter, UV disinfection, hydrogen peroxide dosing with residence in a buffer tank, sand filtration, activated carbon filtration, and a second dosage of hydrogen peroxide and UV disinfection. The experiment involved eight identical pens (75m<sup>2</sup>), each housing 1530 Ross 308 birds (males and females). Half of the pens received tap water (TW), the other half purified rainwater (RW) from day 10 of the trial. Water samples (TW and RW) were analyzed at day 10 – 17 – 25 – 31 – 39 for biological parameters: total bacterial count at 22°C, total bacterial count at 36°C, Coliformi, E. coli, Enterococci, molds, yeasts, sulfite-reducing anaerobes and Clostridium perfringens. Throughout the trial, none of these parameters in purified rainwater or tap water exceeded Belgian standards for poultry drinking water. Technical results demonstrated comparability (n.s.) between the TW and RW groups. Mortality rates were 2,44% (TW) and 2,37% (RW). Total water use per bird was 6,426 liters (TW) and 6,477 liters (RW), while total feed intake was 3,818 kg (TW) and 3,841 kg (RW). Water/feed ratio was 1,683 (TP) and 1,686 (RW). Live weight at day 39 was 2,740 kg (TW) and 2,735 kg (RW), with feed conversion rates of 1,514 (TW) and 1,524 (RW). Litter quality (dry matter content, roughness) and animal welfare parameters (feather quality, hock and feet lesions) were also examined. No significant differences were observed between treatments, except for a numerically lower visual score of foot and hock lesions on day 40 in the group RW (p=0.074). In conclusion, purified rainwater, treated through various steps, is a viable alternative for broiler drinking water. The results of this trial suggest that purified rainwater has no adverse effects on technical performance and animal welfare parameters in Ross 308 broilers.

Keywords: Water; Drinking water; Water quality; Purified rainwater; Climate change; Climate resilience; Technical performance; Animal health; Environmental impact

## Reusing cleaning water from broiler farms: on-site biological treatment and membrane filtration

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Broiler farms necessitate the removal of litter and wet cleaning after each production cycle, consuming a significant amount of cleaning water (approximately 11 l/m<sup>2</sup>). In Flanders, ground- or tap water serves as the primary sources for cleaning. The used water, containing particles like excrements, feathers and dust, is classified as 'manure,' necessitating specific handling. When used as an organic fertilizer it may overload soil and water. Storing cleaning water is needed in autumn and winter. Biological purification and water reuse can be an alternative. Implementing an on-site biological water treatment system with membrane filtration (integrated permeate channel construction (IPC®) with a nominal pore size of 40 nm) can significantly reduce the water demand on a farm. In the current study (ACLIMA-project - contract LIFE 20 CCA\_BE\_001720) a prototype system was tested for 12 months. During the testing phase, the cleaning water of a broiler house (896 m<sup>2</sup> - 18.400 broilers) underwent purification. The purified water was stored and assessed for quality. The results exhibited notable improvements in various parameters between untreated (influent) and purified (effluent) water. The pH increased from 7,4 (influent) to 8,3 (effluent), while the total phosphorus (P<sub>tot</sub>) content decreased from 43,98 mg/l (influent) to 4,95 mg/l (effluent). Similarly, the total nitrogen (N<sub>tot</sub>) content dropped from 252,56 mg/l (influent) to 6,06 mg/l (effluent). Microbiological parameters were examined: the total bacterial count at 22°C in the effluent ranged from 900 cfu/ml to 12.000 cfu/ml, well within the 100.000 cfu/ml standard. The total bacterial count at 36°C (effluent) varied from 560 cfu/ml to 2.600 cfu/ml, also meeting the standard. E. coli presence in the effluent ranged from less than 1 cfu/100 ml to 8 cfu/100 ml, within the 10.000 cfu/100 ml standard. Enterococci count in the effluent ranged from less than 1 cfu/100 ml to 10 cfu/100 ml. Salmonella sp. were not detected in the influent or effluent. In conclusion, the purification of cleaning water from broiler farms through a biological water treatment system with membrane filtration, proves to be a valuable technique for reusing this water if the water quality complies with the regulatory regulations of each member state. This approach establishes a circular use of cleaning water, leading to decreased dependence on ground- or tap water and a reduction in nutrient input in soil or surface water.

Keywords: broilers, cleaning water, biological purification, membrane filtration, circular use of water



## **Comparative life-cycle assessment for the sustainable production of fermentation-based L-methionine (L-Met)**

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An environmentally improved process is developed to produce L-Met eco by eliminating the decolorization in the L-Met production line in CJ CheilJedang Corp. plant at Kerth (Malaysia). Herein, L-Met eco environmental impact (EI) through a life-cycle assessment (LCA) was compared to petro-based DL-Met using a functional unit of 1 kg Met. LCA was performed based on the ISO 14040 and 14044 in four steps: 1) goal and scope definition, 2) inventory analysis, 3) impact assessment, and 4) scenario (Sc) analysis. In step 1, L-Met and L-Met eco are compared to investigate the EI of an improved process. Moreover, the eco-friendliness of L-Met and L-Met eco is compared with the DL-Met. The system boundary was determined as cradle-to-factory gate without consideration of transportation, animal production, and manure treatment. In step 2, the production of L-Met, L-Met eco, and DL-Met were modelled in SimaPro 9.1 by using both primary and secondary databases, such as Ecoinvent 3.6, Agri-footprint and Global Feed LCA Institute (GFLI) databases. Agri-footprint and GFLI databases were utilized to identify the regional EI. For primary database, we obtained the emission factor for MeSH from Arkema Corp. because database for MeSH was not existing in the secondary database. In step 3, ReCiPe 2016(H) midpoint method was adopted to calculate the EI of direct and indirect effects. Among the eighteen impact categories, we focused on the climate change category with global warming potential (GWP, kg CO<sub>2</sub> equivalent), which was defined as the cumulative radiative forcing, both direct and indirect effects, over a specified time horizon resulting from the emission of a unit mass of gas related to some reference gas. In step 4, the scenario analysis was conducted to investigate the impact of the following three scenarios: the base case including avoided product (Sc1); including avoided product and uptake CO<sub>2</sub> (Sc2); and including avoided product, uptake CO<sub>2</sub>, and bioavailability (BE) (Sc3). Under Sc1, GWP of L-Met eco was lower than L-Met 100 (2.43 vs. 2.61 kg CO<sub>2</sub>/Kg Met) and both had a lower GWP compared to DL-Met (2.6-3.32 kg CO<sub>2</sub>/Kg Met). Under Sc2, the GWP of L-Met and L-Met eco were considerably reduced (0.97 and 0.79 kg CO<sub>2</sub>/Kg Met, respectively). Considering BE of L-Met (Sc3), the GWP of L-Met and L-Met eco were further reduced (0.92 and 0.75 kg CO<sub>2</sub>/Kg Met, respectively). To conclude, L-Met eco has the least EI among Met sources regardless of the considered scenarios.

**Keywords:** L-Met eco, L-Met100, DL-Met, LCA, Bioavailability

[O20-12]: Parallel D(16)-S20-Sustainability (ID: 131303) - Greece

## **Exploring BroilerNet stakeholders' attitudes towards the sustainability of broiler industry in Greece**

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BroilerNet, project funded by Horizon Europe, aims to enhance the resilience and sustainability of the European broiler sector by creating space for interaction between science and practice and co-creation of ready-to-use best practices on broiler farms in Europe. The project engages with the most important Agriculture Knowledge and Innovation Systems (AKIS) actors involved in the broiler sector within the 13 participating countries through establishing national level Broiler Innovation Networks (BINs). This study aims to examine the Greek stakeholders' attitudes towards the main challenges that broiler industry faces at national level. A first meeting of the Greek BIN was organized by the Veterinary Research Institute (research partner in Greece) in collaboration with Agricultural Poultry Cooperative – PINDOS (industrial partner in Greece), on 15th March 2023 in Ioannina, at PINDOS premises to collect stakeholders' ideas towards the three key thematic topics: environmental sustainability, animal welfare and animal health. 19 stakeholders participated, 9 experts and 10 farmers. Following discussion group methodologies, three groups (of 7, 6, 6 persons) were created to cover the thematic topics, coordinated by trained for the purpose facilitators. Results concluded can be summarized as following: (a) Main challenges for the environmental sustainability of Greek broiler industry are the dead bird's management, the protected areas-national parks and relevant land availability for establishing new enterprises as well as the water availability and groundwater protection issues. The need for training farmers on environmental sustainability was mentioned by most of participants. Moreover, stakeholders highlighted as important challenges the energy management and a broader farms' modernization and renovation related to their footprint; (b) Animal welfare challenges mainly focused on efficacy of birds' thermal comfort and ventilation systems, training of farmers and synchronized thinning at high farm/population density areas; (c) Most important health challenges are related to farm biosecurity, bird respiratory diseases and gut health and the need for farmer training on animal health management issues. Antimicrobial resistance, high farm density in some areas, farm health management and monitoring programs and quality of raw materials (feed, water, day old chicks) are considered important factors that influence broiler production at farm level.

Keywords: broiler; sustainability; stakeholders

**Antimicrobial resistance in eggs: the role of layer production system.****Preliminary results**

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Eggs, a primary global source of animal protein worldwide, offer a healthy, versatile, affordable, and culturally acceptable product for the whole population. Moreover, poultry production, since its beginnings, has demonstrated the ability to adapt to consumer's needs. In fact, layer production systems have diversified into caged, non-caged, free-range, and ecological systems, aiming for more sustainable and animal welfare-enhancing production, in line with current societal demands. However, these new management options are exposing the sector to new challenges. Therefore, to assess the pros and cons of the different systems comprehensively it is necessary to consider different aspects. One crucial aspect is the effect on the spread of antimicrobial resistance (AMR) throughout the food chain. Thus, the aim of this study was to assess the AMR occurrence in commercial eggs from the different layer production systems, using *Escherichia coli* as sentinel bacteria. For this end, 288 eggs were sampled (72/production system). Once in the laboratory, eggs were pooled (12 eggs/pool), and eggshells and egg content were analyzed for *E. coli* detection. Afterwards, minimum inhibitory concentration assays were performed to evaluate antimicrobial susceptibility according to the Decision 1729/2020. Finally, the statistical analysis was performed using a GLM with the probit link function (significant differences with a p-value < 0.05). Overall, 14/50 (28%) *E. coli* isolates were obtained, 13 from eggshell and 1 from egg content: 3 from caged, 4 from non-caged, 5 from free-range (1 of them from egg content), and 2 for ecological system. Moreover, all the isolates were AMR, and the 50% were multi-drug resistant (MDR). Regarding each antibiotic, the one with highest levels of AMR was Sulfamethoxazole (92.9%), followed by Colistin (50.0%), and Gentamicin (50.0%). Only isolates from ecological and free-range systems showed resistance to Nalidixic acid, Tetracycline and Chloramphenicol, possibly due to higher exposure to the environment and wildlife. In addition, these results focus on eggshells contamination, highlighting the importance of shell control through the production chain (from laying to packaging). These preliminary results evidence the importance of further epidemiological studies on the development and spread of AMR in layer production, mainly in outdoors systems, under a One Health strategy.

Keywords: Antimicrobial resistance, layer production, eggs

## **Salmonella Infantis rules the roost: a continuous cycle of contamination between broiler farms and slaughterhouses**

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Over the last decade, *Salmonella enterica* serovar *Infantis* has spread throughout the broiler sector worldwide, becoming a persistent problem that poses a critical threat to human health. *Infantis* is currently the fourth most common serovar in human cases, mainly caused by the consumption of contaminated poultry meat. The continuous circulation of *Salmonella* between broiler farms and slaughterhouses maintains the dominant presence of this emerging serovar. First, colonised broiler flocks contaminate the transport and slaughterhouse equipment, leading to cross-contamination, especially when logistic slaughter is not applied due to an incorrect *Salmonella* negative status. Secondly, if the cleaning and disinfection of the transport equipment is inadequate, this may lead to contamination of the broiler houses or the farm environment. Therefore, the correct application of logistic slaughter was studied by checking the association between the official *Salmonella* status determined at three weeks by overshoes with the status at slaughter by testing the contents of the ileum and cecum for *Salmonella* in 125 flocks. In addition, the frequency of use of contaminated material on farms during partial depopulation or thinning was determined by sampling the trucks, forklifts, crates, and the catching crew in 13 flocks in Belgium. To assess introduction of contamination, the broiler houses were sampled before and after thinning using overshoes. The studies showed that, despite the official negative *Salmonella* status of all sampled flocks, at slaughter 25% of the broiler flocks were positive for *Salmonella* of which 78% *S. Infantis*. In addition, *Salmonella* (*Infantis*) was regularly found on the transport material used during thinning. In particular, the crates and loading surfaces of the trucks were frequently contaminated in 62% and 54% of the sampled flocks, respectively. However, in case of thinning, no introduction of *Salmonella* in the broiler houses was observed. In conclusion, the official *Salmonella* status deviates from the status determined at slaughter which can complicate logistic slaughter. Furthermore, transport material from slaughterhouses is often contaminated with *Salmonella*. Although not observed in this study, this can lead to colonization of the remaining birds and broiler house environment after thinning. Our results show that both primary production and slaughterhouse have the responsibility to combat this pathogen together.

Keywords: *Salmonella Infantis*; thinning; crates; official *Salmonella* status; gut status

## **A double encapsulation of bioactives to reduce salmonella livingstone shedding in artificially infected broiler chickens**

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Reducing Salmonella contamination of broiler chickens at the farm is essential to limit the risks of zoonoses in humans. In this 35-day trial, the effects of a double-encapsulated solution (NUQO©SAFE, NUQO, France) on performance and Salmonella excretion were studied in Ross 308 females artificially infected at 14 days of age. The double encapsulation allows a sequential release of the active ingredients in the small intestine, namely an outer layer of 5 plant and algae extracts released first and a core of 6 encapsulated acids released secondly. On day 1, the chicks were placed in brooder rings and randomly allocated to 3 groups of 40: a group receiving a control diet (CO), a group receiving a diet supplemented with 400g/t of the encapsulated solution (NQ 400), and a group receiving a diet supplemented with 800g/t of the encapsulated solution (NQ 800). On day 12, 36 chicks per brooder ring were randomly distributed in 6 cages of 6. They were all inoculated on day 14 with 108 colony forming units (CFU) of *S.livingstone* by oral gavage. Zootechnical performance were measured per pen at days 0, 12, 21 and 35. The number of Salmonella positive samples and Salmonella counts (CFU/ml) were determined at days 21 and 35 by direct-plating or after overnight incubation using cloacal samples taken from 4 animals per cage. The data were analysed with the SAS software either with a Fisher's Exact test (presence/absence of Salmonella) or by one-way ANOVA (performance and log10 transformed CFU/ml), with a level of significance at  $P<0.05$ , and a tendency at  $P<0.1$ . During the overall period (d0-35), NQ 400 improved BWG by 5% as compared to CO ( $P<0,1$ ), most likely due to an improved feed intake (+4%) and FCR (-1,3%). On day 21 (7 days post-infection), both NQ groups had a similar number of salmonella positive samples as compared to CO. Among the salmonella positive samples, NQ 400 had a similar count of salmonella as CO whereas NQ 800 significantly reduced it by in average 4.1 log10 ( $P<0.05$ ). On day 35, NQ 400 had limited effects on the number of positive samples whereas NQ 800 reduced it by 66% as compared to CO ( $P<0.1$ ). NQ 800 also reduced the salmonella counts of the positive samples by 1,7log10 as compared to CO ( $P<0.1$ ). This study shows that a dose of 400g/t of the double encapsulated solution improved broiler growth performance while a dose of 800g/t was needed and efficient to reduce Salmonella Livingstone excretion of the artificially infected broiler chickens.

**Keywords:** Broiler performance, Salmonella Livingstone, Salmonella shedding, Phytochemicals, Organic acids,

## **Aerotolerance and haemolytic activity of invasive and non-invasive campylobacter coli strains from broiler chickens**

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Campylobacter is the most common cause of foodborne human gastroenteritis worldwide. The largest contributors of human campylobacteriosis in developed countries are poultry and poultry products. In most European countries a higher prevalence of *C. jejuni* is found in broilers, which has led to most research focused on this species. However, a similar proportion of *C. jejuni* and *C. coli* is found in broilers in Spain. Thus, we aimed to assess some pathogenicity traits (i.e. aerotolerance and haemolytic activity) of *C. coli* strains from broilers with potentially different pathogenic capacity. As an obligate microaerophilic organism that requires low oxygen concentrations (2–10%) for optimal growth, campylobacters are considered sensitive to O<sub>2</sub> concentrations in the ambient atmosphere of the extra-intestinal environment. Haemolytic activity has been described in Campylobacter and may contribute to its effects when causing gastroenteritis. Aerotolerance was tested in 81 strains, both invasive (isolated from chicken liver, n=31) and non-invasive (isolated from caeca ; n=50) by serial passages on blood agar plates, incubated at 37°C for 48h under aerobic conditions. Overall, 56% of the strains were aerotolerant, with a higher prevalence in non-invasive strains (48% invasive vs 60% non-invasive). We tested the haemolytic capacity of *C. coli* strains (membrane bound as well as secreted) with rabbit red blood cells. Both the bacteria grown in broth and the culture supernatants were mixed with the red blood cells in 96 well culture plates. After 1h of incubation, plates were centrifuged, supernatants were gently collected and subjected to optical absorption (540 nm). Two out of 6 *C. coli* strains tested showed membrane bound haemolytic activity (>10-25%) against rabbit red blood cells. One *C. coli* strain also manifested haemolytic activity (>25%) in the supernatant. Aerotolerant *C. coli* might survive longer in the external environment during oxidative stress conditions, increasing the chances of its transmission to humans. *C. coli* can also be more pathogenic if, as found, has haemolysis activity. These results highlight the impact that not only *C. jejuni*, but also *C. coli* carried by broiler chickens can have on public health. To complete the pathogenicity assessment, adhesion and invasion assays with the intestinal cell line from chicken 8E11 are ongoing together with haemolysis assays with additional strains, and results will also be presented

Keywords: Campylobacter coli, broilers, caeca, liver, pathogenicity

## Colonization by different *Campylobacter jejuni* strains produces distinct effects on broiler chickens performance

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Campylobacteriosis is the most prevalent intestinal infection in humans worldwide, being chicken meat considered the most important source of it. The prevalence of *Campylobacter* can reach over 75% of chicken batches at slaughter. Besides human health implications, it has been reported that *Campylobacter* may also impair chickens' intestinal integrity, compromising birds' health and welfare. We aimed to determine the effect on bird performance of different *Campylobacter jejuni* field strains selected from broiler flocks showing different performance (measured as differences in feed conversion rate, FCR). For this we experimentally infected broiler chickens ROSS 308. The study started at 1 day of age randomly distributing the birds in 4 experimental groups (100 birds each distributed in 5 floor pens, 20 chickens per pen). Birds were challenged by oral gavage at 21 days with 3 different *C. jejuni* strains as follows: Unchallenged (U), Low FCR (L), High FCR (H), and Invasive (I, isolated from internal tissue the liver). Ten birds per group were euthanised at different time points, and caecal and liver samples were collected for *C. jejuni* assessment. *C. jejuni* was recovered from caecal samples from all but one infected bird at 2 days post infection (dpi) and from all sampled birds at 7 dpi. *C. jejuni* was recovered from internal tissue liver in up to 3 birds per infected group at different time points. All U birds were negative at all time points. Before infection, non-significant differences in FCR were detected among groups (FCR within  $\pm 0.5\%$  of U = 1.314 kg/kg), while at 14 dpi FCR was significantly impaired by 3% in H and I groups (1.642 kg/kg and 1.643 kg/kg, respectively) compared to U (1.599 kg/kg), with no significant differences between U (1.599 kg/kg) and L (1.606 kg/kg). Results show that birds can be colonized with *C. jejuni* strains that behave differently, with ones being non-harmful that may act as commensals, while others are harmful, having negative effects on performance. We also studied immune response and changes in intestinal integrity that are presented in a separate abstract.

Keywords: *Campylobacter jejuni*; broiler; liver; performance; prevalence

## Host immune response of broiler chickens to campylobacter jejuni strains with different pathogenic potential

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Campylobacter jejuni is a leading cause of foodborne enteritis with different degrees of severity and several extra-intestinal manifestations in humans. Poultry meat is the major source of the disease. Although considered as a commensal microorganism in the avian gut, available reports indicate that Campylobacter spp. can induce a pro-inflammatory response, damaging intestinal integrity and affecting broilers' performance. In order to understand the implications of host immune response associated to Campylobacter, ROSS 308 male broiler chickens were experimentally infected with different C. jejuni strains selected from flocks showing differences in feed conversion rate (FCR). One day old birds were randomly divided into 4 experimental groups (100 birds each distributed in 5 floor pens, 20 chickens per pen). At 21 days of age, broilers challenged were infected by oral gavage with a different C. jejuni strain recovered from either a flock of low FCR (L), or high FCR (H) or a C. jejuni invasive (I) strain (isolated from internal tissue liver). An unchallenged (U) group was included as control. At 2-, 7- and 14-days post-infection (dpi) 2 birds per pen were euthanised and samples were collected for intestinal morphology (ileum) and immunological assessment (cytokine expression in caecal tonsils tissue by RT-qPCR, and Immunoglobulin A [IgA] levels in bile by ELISA). At 7 dpi a reduction in the villus height: crypt depth ratio was observed in I and H infected chickens compared to unchallenged ones, due to deeper intestinal crypts. Bile IgA levels were elevated in all three challenged groups at 7 dpi, with L group having significantly higher titers compared to other groups. Remarkable differences in cytokine gene expression were observed among different C. jejuni strains. RT-qPCR revealed significant differences in IFN $\gamma$  gene expression, particularly on 7 dpi, in chickens infected with I (14-fold higher) and H (9-fold higher) strains compared to U control group, while L infected chickens showed only a minor and non-significant increase (2-fold higher). In contrast, both I and H strain infected chicken showed numerically down regulation or marginal IL6 gene expression on 7 dpi. Our results indicate that IFN $\gamma$  may contribute to pathological alterations (impaired intestinal integrity) and performance observed with I and H infected chickens. It cannot be excluded that IgA in L infected chickens may play a role in the defence of the mucosal barrier by bacterial coating.

Keywords: Campylobacter jejuni; broiler; host mucosal response; intestinal health; cytokine; IgA; IFN $\gamma$ .



**Effect of a bacterial probiotic on *Campylobacter* spp. cecal carriage****E. Rondel<sup>1</sup>, M. Brunon<sup>2</sup>, J. Le Douce<sup>2</sup>, E. Pagot<sup>2</sup>, A. Riggi<sup>1</sup>**<sup>1</sup>Phileo by Lesaffre, Marcq-en-Baroeul, France, <sup>2</sup>INNOZH, Ploufragan, FrancePresenting author: *e.rondel@phileo.lesaffre.com*

Campylobacteriosis has been for a few years the most prevalent food-borne disease in humans. Studies have demonstrated that the highest risk for human *Campylobacter* infections is associated with the consumption of chicken meat. To reduce the exposure to this pathogen, reducing the colonization of *Campylobacter* in live broilers is one of the avenues being studied. The aim of this study was to evaluate the effect of a bacterial probiotic, mix of *Bacillus amyloliquefaciens*, *B. licheniformis* and *B. pumilus* (Microsaf®, called the mix) on cecal carriage of *Campylobacter* spp. in a conventional broiler farm with recurring detection problems at slaughter. The presence of *Campylobacter* spp. on the farm was confirmed by laboratory analysis before the beginning of the trial. 90 one-day old chicks were placed to 6 pens and allocated to 2 groups of 45 animals: Control, fed a commercial diet free of the tested additive, and Test, supplemented with the probiotic mix at 500g/t. Animals were weighed individually at D1, D21 and D34. At D21, 4 animals per pen were sacrificed and their ceca sampled for microbiota analysis (results not presented in this abstract). At D34, all the remaining animals were euthanized, and their ceca sampled for *Campylobacter* PCR analysis. Only 12.5% (7 positive from 56 samples) of the ceca samples were positive to *Campylobacter* spp. The comparison between the Control group and the Test group showed a reduction of the *Campylobacter* spp. positive samples in the ceca (6 positive from 26 samples in the Control group vs 1 from 30 samples in the Test group,  $p=0,05$ ). Nevertheless, no significant difference has been seen on the *Campylobacter* counts between the groups,  $p=0,096$ ). At the end of the study, at D34, all the birds have been weighed individually and a significant difference has been seen for the Body Weight (1,998 kg for the Control group vs 2,158 kg for the Test group,  $p<0,001$ ). In conclusion the bacterial probiotic mix seemed to help reduce the contamination of the birds with *Campylobacter* spp. and contributed also to the improvement of their body weight at D35.

Keywords: *Campylobacter*, Food safety, Probiotic, Broiler

## **Assessing the impact of a commercial water disinfectant, blend of hydrogen peroxide, acetic, and peracetic acid on the performance and *Campylobacter jejuni* counts in experimentally challenged broiler chicks**

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*Campylobacter jejuni* (*C. jejuni*) infection stands as the primary cause of human bacterial gastroenteritis. The principal origin of this infection lies in poultry products, especially broilers meat, necessitating targeted control measures within the broiler production chain. While the EFSA's scientific opinion highlights the potential of water disinfection during broiler production in reducing the evidence of human campylobacteriosis current commercially available products lack substantial data on their efficacy. Moreover, a definitive dosage scheme for pathogen control in practical farming scenarios remains unpublished. Thus, the aim of this study is to assess the impact of continuous application of a commercial water disinfectant (Cid 2000<sup>TM</sup>), blend of hydrogen peroxide, acetic, and peracetic acid on both performance and *C. jejuni* counts in experimentally challenged broiler chicks. One hundred and twenty-eight (128) 1-day-old broiler chicks (Ross@308) were randomly allocated to 4 treatment groups, with 4 replicates, according to the following experimental design: group A; the negative control, group B; birds received tap water treated by Cid 2000<sup>TM</sup>; group C; birds were challenged by *C. jejuni* and received tap water without treatment, and group D; birds were challenged by *C. jejuni* and received tap water treated by Cid 2000<sup>TM</sup>. The duration of the experiment was 36 days and birds infected by 10<sup>6</sup> CFU *C. jejuni* on the 18th day. The Body Weight (BW) of birds was measured weekly, while the Average Daily Feed Intake (ADFI) and the Feed Conversion Ratio (FCR) were calculated for different time periods. The *C. jejuni* counts in the crop and caeca of birds were evaluated, at 23rd and 36th day of age. The statistical analysis showed no significant variations in the BW among experimental groups at any measured time point. For the period from 24th to 36th day the FCR in group C was significantly increased compared to that in group B. In addition, the ADFI in group B was significantly lower, compared to the other groups for the period from 14th-23rd day, and cumulatively the entire study period. Furthermore, the *C. jejuni* counts in ceca were significantly lower in group D compared to those in group C at the 23rd day. The findings of this study support the potential effectiveness of water disinfection in a *Campylobacteriosis* control strategy in broiler farms. However, the overdose and overuse of water disinfectants may adversely affect the performance of broiler chicks.

**Keywords:** *Campylobacter jejuni* (*C. jejuni*), organic and inorganic acids, water acidification/disinfection, performance, *C. jejuni* counts, hydrogen peroxide, acetic acid

## **Effect of dietary supplementation and in ovo feeding of alpha ketoglutaric acid on the immunocompetence traits, mineral composition and fatty acid profile of breast and thigh muscle of commercial broilers**

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AKG (alpha ketoglutaric acid) is a precursor of amino acid glutamine. Comprehensive studies on dietary supplementation vis-à-vis in ovo feeding (IOF) of AKG in commercial broilers are scanty. Hence, a study was carried out to evaluate the effect of dietary supplementation of AKG vis-à-vis IOF of AKG on the immunocompetence traits, mineral composition and fatty acid profile of breast and thigh muscle of commercial broilers. 375 fertile eggs of commercial broiler breeder birds were set for incubation. On 18th day of incubation, IOF was done and eggs were divided into three groups: un-injected control, sham control (distilled water) and IOF with AKG (0.6 ml of 1.5% AKG solution). After hatching, 180-day-old chicks were further divided into two subgroups, each with three replicates and ten chicks per replicate. One subgroup received a basal diet, while the other received a basal diet with 1% AKG supplementation diet. Birds were reared for 42 days (6 weeks) and kept on a Basal or Control diet [broiler starter diet, 22% CP and 3100 ME till 3 weeks and thereafter broiler finisher diet, 20% CP and 3200 ME till 6 weeks; BIS, 2007]. After 6 weeks, serum HA and IgM titre (log<sub>2</sub>) values to 1% GRBC were significantly higher ( $P<0.001$ ) after IOF of AKG (6.45 and 6.05 vs. 4.75 and 4.25) and/or dietary supplementation of AKG. Na content of breast muscle of birds of IOF AKG group was significantly higher ( $P<0.001$ ) than birds of un-injected control (178.66 vs. 126.62 mg/100g). Further, Fe and Mn content of thigh muscle of birds of IOF AKG group was significantly higher ( $P<0.02$  and  $P<0.01$ ) than birds of un-injected control group (7.83 and 0.14 vs. 4.75 and 0.11 mg/ 100 g). In breast muscle, IOF or dietary supplementation of AKG resulted in significantly lower ( $P<0.05$ ) SFA (29.18% vs. 31.72% and 29.58% vs. 31.48%). Birds of IOF AKG group had significantly higher ( $P<0.02$ ) MUFA than birds of un-injected control group (41.03% vs. 36.21%). Further, dietary supplementation of AKG resulted in significantly higher ( $P<0.03$ ) omega 6 fatty acids in breast meat cuts as compared to birds in the control diet (26.07% vs. 24.81%). Omega 6 fatty acids were significantly higher ( $P<0.03$ ) in birds after dietary supplementation of AKG (26.07% vs. 24.81%). Thus, IOF or dietary supplementation of AKG resulted in significantly higher serum HA and IgM. In addition, saturated fatty acids were significantly lower in breast muscle of birds after IOF or dietary supplementation of AKG.

**Keywords:** AKG; Broilers; Immunity; Fatty acid profile; Mineral; Meat

## **Accessing the impact of feeding different, highly available sources of essential trace minerals delivered from a premix blend on broiler performance**

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This trial was conducted to measure the effect of replacing inorganic trace minerals (ITM) in-feed with a program that contained organic trace minerals (OTM) fed together with hydroxychloride (HTM) to improve growth performance in broilers, when fed at nutritional levels. Also the Total Area of Contact with nutrients (ATC) was calculated. Researchers allocated 1650 male chicks from the same batch of breeders. Ross 308 strain were used, which were kept in production until 42 days of age. They were distributed completely at random, in 3 treatments. T1 negative control (no trace mineral added). T2 inorganic trace mineral added: iodine calcium (I 2 ppm), copper sulfate (Cu 12 ppm), iron sulfate (Fe 10 ppm), manganese sulfate (Mn 80 ppm), zinc sulfate (Zn 80 ppm), sodium selenite (Se 0.3 ppm). T3 Combination of ITM, HTM and OTM added: iodine calcium (I 2 ppm), sodium selenite (Se 0.2 ppm), OTM (Cu 2 ppm, Mn 10 ppm, Zn 10 ppm, Fe 10 ppm, Se 0.1 ppm), HTM (Cu 10 ppm, Mn 70 ppm, Zn 70 ppm), with 11 repetitions and 50 birds each treatment. The ATC was calculated using 2 chicks per repetition (22 chicks per treatment). For the morphometry of the distal part of the duodenum, the length, width and thickness were evaluated, as well as the number of villi found in a surface of 1,000,000  $\mu\text{m}^2$ . Crypt depth was also measured, using the Motic Images Plus 2.0 program. Considering that the villi have the shape of a rectangular prism, the formula applied to obtain the ATC was:  $\text{ATC} = (\text{length} \times \text{width}) \times 2 + (\text{length} \times \text{thickness}) \times 2 + (\text{width} \times \text{thickness})$ . The result was multiplied by the number of villi found in 1,000,000  $\mu\text{m}^2$  and the value obtained was divided by the study area of 1,000,000  $\mu\text{m}^2$ . The result means that for each  $\mu\text{m}^2$  of the study area (duodenum), there is a certain number of  $\mu\text{m}^2$  of ATC. At the end of the study, it was observed that chicken fed with T3 showed higher body weight (3.154 kg,  $P \leq 0.05$ ) +122gm/bird and +62gm/bird and FCR (1.542,  $P \leq 0.05$ ) -6.4pts and -4.3pts compared respectively to T1 and T2. Morphometries of the duodenum at 11 days of age, showed significant differences ( $P \leq 0.01$ ) of higher value of the ATC ( $P \leq 0.01$ ) for T3; 32.9  $\mu\text{m}^2$ . For T1; 29.4  $\mu\text{m}^2$ . For T2; 29.4  $\mu\text{m}^2$ . It is conclude that feeding high quality, highly bioavailable trace minerals as HTM + OTM can be key to maximizing the growth potential of broilers. Also demonstrated the benefit of trace mineral supplementation, as the lack of supplementation negatively affected the zootechnical parameters.

**Keywords:** trace mineral; source; hydroxychloride, organic, bioavailable

## **A combination of 25-Hydroxycalciferol, carotenoid, organic copper and glycans improved broiler breeder performance, egg quality and hatchability traits**

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A 16-week study was conducted to investigate the effects of a combination of 25-OH D3, carotenoids, organic copper and glycans on broiler breeder performance, egg quality and hatchability traits. A total of 189, 35-wk-old broiler breeder hens and 21 roosters (Ross 308) were allocated to 3 dietary treatments with 7 replicates and an initial mating ratio of 9:1. All broiler breeders received a corn/wheat-soybean meal basal diet (BD) in mash form, formulated to meet Ross 308 parent stock nutrient requirements. The treatments were: control (T1) – BD with cholecalciferol as the main source of vitamin D, T2 (BD with a combination of 25-OH D3 and carotenoids), T3 (BD with a combination of 25-OH D3, carotenoids, organic copper and glycans). Birds feed supply was strictly controlled, to avoid excessive weight gain and water was available ad libitum. Performance responses were determined in a weekly basis and results were reported as overall. At the 34th, 40th and 50th wk of breeders age, eggs were collected for quality and hatchability traits determination. The experimental data were analyzed by ANOVA procedure and statistical significance was determined at  $P < 0.05$ . Overall, breeder laying rate, egg mass and FCR in treatment T3 were significantly improved ( $P < 0.001$ ) compared to T1. Egg yolk color score was lower ( $P < 0.001$ ) in treatment T1 compared to T2 and T3. In addition, treatments T2 and T3 had higher ( $P < 0.05$ ) albumen height and Haugh Unit compared to T1 at wk50. Regarding the hatchability traits, hens in treatment T3 produced ( $P < 0.05$ ) more chicks per hen housed compared to T1. In conclusion, the supplementation of broiler breeder diets with a combination of 25-OH D3, carotenoids, organic copper and glycans enhanced production and reproductive performance, egg quality and hatchability traits in broiler breeder hen.

Keywords: broiler breeders; bioactive nutrients; reproductive performance; egg quality; hatchability

**Broiler breeder hens significantly benefit by replacing inorganic trace minerals with glycines****S. Rothstein<sup>1</sup>, A. Schlagheck<sup>1</sup>, B. Landwehr<sup>1</sup>, S. Keller<sup>1</sup>**<sup>1</sup>Biochem Zusatzstoffe Handels- und Produktionsgesellschaft mbH, Lohne, GermanyPresenting author: [Srothstein@biochem.net](mailto:Srothstein@biochem.net)

Producing fertile eggs for gaining the highest hatchability is one of the important needs of having profitable chicken breeding. In poultry diets, trace minerals are essential as they play important roles on reproduction performance, required biochemical processes and embryo development. It is widely accepted that inorganic trace minerals (ITM) are generally less bioavailable than organically bound trace minerals (OTM), in particular as glycines. Despite many positive effects, OTMs are usually used more cautiously due to their higher costs. Thus, a partly replacement of ITM with OTM is more common practice. Therefore, the aim of this study was to investigate the effects of 50% replacement of ITM with glycines (GLY) on tissue accumulation, digestibility, and key production parameters in older broiler breeder hens. In total, 240 Arbor Acres broiler breeder hens (initial BW: 4.01±0.15kg) aged 50 weeks were divided into 2 treatments, with 12 replicates of 10 hens each, housed in cages. Experimental treatments were as follows: 1. ITM (premix with 80 ppm Zn, 10 ppm Cu, 80 ppm Mn and 50 ppm Fe-Sulfates), and 2. GLY (50% of ITM replaced with Zn, Cu, Mn and Fe-Glycines (E.C.O.Trace®, Biochem). During the 10-week trial the birds consumed all daily feed (160g/cage/day) and did not differ between treatments. Egg production, hatchability, mortality, digestibility and accumulation of Zn, Cu, Mn and Fe in different body tissues and organs were measured. The results show significantly higher fertility and hatchability in the GLY group at the end of the trial. However, there were no differences in laying rates and settable eggs. The highest apparent digestibility of Zn, Cu, Mn and Fe was visible in the GLY group ( $p < 0.05$ ). In addition, there were significantly lower animal losses with glycines. For example, Zn accumulation in tissue & organs demonstrates partly tendential, but mostly significant differences in favour of the GLY group. The study demonstrated that replacing 50% of the ITMs as sulfates in a feed formulation with glycines results in significantly improved production parameters and lower mortality in older breeder hens. This strong improvement arises due to the significantly higher bioavailability, which is shown by parameters such as apparent digestibility combined with mineral accumulation in different tissues and organs. Using glycines in diets of older breeders can, therefore, support the longevity of the hens.

Keywords: trace minerals; glycines; breeder; poultry nutrition

**Reducing breast myopathies with supplementation of *Solanum glaucophyllum*****K. Bühler<sup>1</sup>, K. Pedrosa<sup>1</sup>, R. Losa<sup>1</sup>, M. Petracci<sup>2</sup>, F. Sirri<sup>2</sup>**<sup>1</sup>Herbonis Animal Health GmbH, Augst BL, Switzerland, <sup>2</sup>Department of Agricultural and Food Sciences, University of Bologna, Ozzano dell'Emilia (BO), ItalyPresenting author: *k.pedrosa@herbonis.com*

Fast-growing broilers have an increased risk to develop breast muscle growth-related myopathies such as white striping (WS), wooden breast (WB) and spaghetti meat (SM). Although the conditions are more prevalent in heavy broilers, early stages can already be observed in broilers from 21 d of age, indicating that growth rate rather than body weight might be a contributing factor. The exact etiology is not yet fully understood but reduced vascularization, increased oxidative stress, impaired muscle repair and disturbed mitochondrial metabolism are considered the most likely causes. 1,25(OH)2D3 has been shown to improve muscle health through various mechanisms. Therefore, a trial was conducted to test the effects of supplementing broiler diets with *Solanum glaucophyllum* (SG, as Panbonis®, Herbonis Animal Health, Switzerland) on the incidence of breast myopathies. SG is a plant that naturally contains glycosylated 1,25(OH)2D3 (G-1,25(OH)2D3). A total of 945 male Ross308 broilers were separated in 7 pens and received one of three diets during 47 days: an unsupplemented control diet (CON), CON supplemented with 1 µg G-1,25(OH)2D3 (SG1) and CON supplemented with 2 µg G-1,25(OH)2D3 (SG2). The control diet was a corn soy diet with commercial Apparent Metabolizable Energy, dLys and Ca contents and 4,000 IU of vitamin D. There were no treatment differences neither on final body weight (global average: 3.2 ± 0.1 kg) nor on feed conversion ratio (1.857 ± 0.044) ( $p \geq 0.74$ ). Independent of the dosage, SG supplementation significantly reduced the incidence of WB and SM ( $p < 0.001$ ). In the SG diets, 54.0 and 52.1 % of the breast muscles were considered normal for WB compared to 36.3 % in the CON group. The values for SM were 84.3, 89.0, and 59.2 %, respectively. However, the incidence of white striping was higher in the supplemented groups ( $p < 0.001$ ). This trial clearly shows that the supplementation of SG reduces the incidence and severity of certain breast myopathies in broilers without reducing growth performance. Why the effects were different for WB and SM than for WS is currently unclear and might be based in the different etiology of the different myopathies. Further studies are planned to gain more information on the underlying mode of action and on potential influence of SG on mitochondrial physiology.

Keywords: broiler, 1,25(OH)2D3, Pectoralis major, white striping, wooden breast, spaghetti meat

## **Enhancing feed digestibility : Exploring the potential of calcium humophosphate as a novel source of phosphorus on broiler nutrition**

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Phosphorus (P) stands as a crucial element within animal nutrition, ranking as the second most abundant mineral in the body at 0.9%. It plays pivotal roles in skeletal structure, energy metabolism, and oxygen delivery. To enhance the economic and environmental sustainability of poultry production, a novel phosphate source known as calcium humophosphate (CHP) has been developed. A specific industrial process is used to obtain the CHP rather than a simple mixture of a phosphorus source and humic substances. The objective of this study was to provide a more detailed understanding of the contribution of CHP in broiler nutrition. A total of 200 one-day old male broilers were weighed and randomly distributed in groups of 10 into 20 cages in an environmentally controlled room. All diets were corn-soybean based and supplemented with phytase (1000 FTU/kg). During the initial phase (1 to 10 days), all birds were fed a standard diet. Subsequently, during the grower phase (11 to 21 days), two experimental diets were introduced, each replicated 10 times. These diets were formulated to contain 0.72% total calcium (Ca) and 0.49% total P: one utilizing monocalcium phosphate (MCP; 18% Ca, 22.4% P) and the other incorporating CHP (16.0% Ca, 21.4% P) as the unique phosphate source. Birds' growth performance during each feeding phase and the ileal digestibility of dry matter, organic matter, Ca, and P at 21 days old were determined. No significant differences in growth performance among the diets were found. Incorporating CHP instead of MCP as the unique phosphate source in the diet statistically ( $P < 0.001$ ) enhanced the ileal digestibility of P (60.3% vs 64.0%) as well as dry matter (61.8% vs 65.3%) and organic matter (63.3% vs 66.8%) digestibility. Calcium can act as an antinutrient, when present in excessive amount. Observed effects might be attributed to the specific mode of action of CHP, which involves chelation with excessive cations, notably Ca and consequently improving digestibility. Also, humic substances concentrated in CHP, absent in MCP, appear to play a significant role in improving ileal digestibility, as previously observed in both broilers and ruminants for dry matter digestibility. Additional investigation into the mode of action of CHP in broiler nutrition is necessary to further improve feed efficiency and establish strategies for optimizing nutrient utilization in commercial poultry farming.

Keywords: phosphorus; digestibility; broiler; calcium humophosphate



## Changes in the acid-base balance of broiler chickens after a substitution of peptide-bound with free amino acids in the diet

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Free amino acids (AA) in the diet are needed to meet the requirement of broiler chickens for individual AA when dietary crude protein (CP) is reduced. In a previous trial, the acid-base status of broilers was affected after 14 days (d) of feeding diets with high concentrations of free AA. The present study investigated whether there are adaptations in the acid-base status after changing to diets with high free AA concentrations. A starter diet with 164 g CP/kg and 80 g soy protein isolate (SPI)/kg was offered to birds up to d 7. From d 7 to 22, the birds were either fed a diet almost identical to the starter diet (0AA) or one of two other diets where 50% (50AA) or 100% (100AA) of digestible AA from SPI were substituted with a mix of all 20 proteinogenic AA. To assess ammonia (NH<sub>3</sub>) excretion and to collect blood samples, each diet was tested in 7 metabolism units with 10 birds each and another 7 units with 15 birds each, respectively. Total excreta were collected on d 0–1, 1–2, 2–3, 4–5, 7–8, and 14–15 after diet change in 12-h intervals. Blood acid-base status was determined from 2 birds per unit on d 1, 2, 4, 7, and 14 after diet change using the i-Stat®Alinity system. Significant differences ( $P < 0.05$ ) from 0AA were found at each d after diet change. Blood pH was decreased for 50AA on d 4 and for 100AA on d 4 and 7. Blood partial pressure of carbon dioxide was increased for 50AA on d 4. On d 4, 7, and 14, blood bicarbonate, base excess, and total carbon dioxide were lower for 100AA. Blood chloride was higher for 100AA on d 1, 2, 4, 7, and 14, and for 50AA on d 1, 2, and 4. Blood sodium was raised for 100AA on d 4. The anion gap was decreased for 50AA and 100AA on d 1, for 100AA on d 2, and for 50AA on d 4, respectively. Blood glucose was lower for 50AA and 100AA on d 1, 2, and 4, and on d 14 for 100AA. NH<sub>3</sub> excretion increased with AA substitution level at all days. Blood data indicated that the acid-base balance of birds was shifted towards acidity when SPI was substituted by free AA. A respiratory acidosis was indicated for 50AA on d 4 after diet change, which was compensated by d 7. For 100AA, data suggested a metabolic acidosis from 3–7 d after diet change because blood pH was reduced. The unaffected blood pH and the other blood data indicated that adaptive responses compensated the acidifying effects by d 14 after diet change. The increased NH<sub>3</sub> excretion for 50AA and 100AA may have been an adaptation to excrete acids.

Keywords: Broiler chickens; acid-base balance; free amino acids

## **Laying hens require a minimum supplementation of riboflavin to maintain body weight, egg weight, and optimize egg riboflavin content**

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Riboflavin, a B vitamin (B2), acts as a coenzyme in energetic metabolism. The recommended dietary riboflavin for laying hens varies in literature (ranging from 2.1 to 8 mg/kg of feed). Understanding how laying hens respond to riboflavin intake can assist nutritionists in deciding on dietary supplementation. This study aimed to evaluate the response of 600 white laying hens (300 from line A and 300 from line B), aged 60 weeks, to riboflavin intake. The hens were randomly assigned to five dietary treatments for 20 weeks. The diets met or exceeded the hens' needs, except for riboflavin, which was included at five levels: 0.0, 0.6, 1.4, 2.4, and 5.0 mg/kg of feed. Measurements were taken for body weight (BW, kg), egg weight (EW, g), hen day egg production (HDEP, %), daily feed intake (FI, g), egg mass (EM, g), feed conversion ratio (FCR, g/g), egg components, eggshell quality, total egg yolk riboflavin content (EYR, mg/100g). The data were collected every 28 days, and a three-factor repeated-measures analysis was conducted, incorporating riboflavin level, age, and the strain of the hens as factors. Since there was no triple interaction between these factors, the evaluation focused on understanding the effect of dietary riboflavin with age. To investigate the behavior of each treatment over time, polynomial contrasts were employed. To estimate the level of riboflavin to optimize a given response, a curvilinear plateau model was fitted to each response variable. For daily feed intake, the riboflavin concentration in the feed served as the independent variable, while the intake of riboflavin was used for other responses. The basal feed (without riboflavin supplementation) contained 0.63 mg/kg of intrinsic riboflavin, and the subsequent treatments contained 1.37, 2.26, 3.03, and 5.31 mg of riboflavin per kg of feed. Riboflavin supplementation significantly affected FI, EW, egg yolk weight (EYW), and EYR. An interaction was observed between riboflavin supplementation and the age of hens for FI, HDEP, EW, EM, and EYR. Hens not supplemented with riboflavin showed a quadratic response over time, with reduced responses initially that recovered by the trial's end. This may be attributed to the reduction in HDEP with age, reducing the hens' riboflavin requirements. Regression analysis revealed a daily intake of 0.513 mg of riboflavin is necessary to prevent weight loss, while an inclusion of 1.80 mg/kg in the feed prevents a reduction in FI. To maximize EW, EYW, and EYR for 50% of the population, a minimum daily intake of 2.58 mg, 0.871 mg, and 3.24 mg of riboflavin, respectively, was estimated. For hens consuming 100 g of feed per day, we recommend including 4.14 mg/kg of riboflavin in the feed to maximize the responses of the laying hen population.

Keywords: coenzyme; egg production; flavin mononucleotide; flavin dinucleotide.

**Breaking the beak conundrum: navigating laying hen welfare in beak-trimmed and non-trimmed flocks****N. Demaitre<sup>1</sup>, N. Sleenckx<sup>1</sup>, N. Van Den Broeck<sup>1</sup>, I. Kempen<sup>1</sup>**<sup>1</sup>Experimental Poultry Centre, Geel, BelgiumPresenting author: [niels.demaitre@provincieantwerpen.be](mailto:niels.demaitre@provincieantwerpen.be)

In the poultry industry, beak trimming has long been utilized to curb injurious pecking behaviors among laying hens. However, the current association of beak trimming with adverse welfare effects has prompted a shift in North-Western Europe towards prioritizing non-beak-trimmed flocks, despite acknowledged pecking risks. Addressing a gap in applied data, this study focuses on the management of intact-beak flocks, employing longitudinal monitoring to evaluate the impact of beak treatment on cumulative mortality as a key indicator of laying hen welfare. Data were systematically gathered from six flocks spanning multiple years (four Isa brown flocks, 2017-2022; two Dekalb white flocks, 2020-2022)., Both beak-trimmed and non-trimmed hens were housed across two distinct aviary systems in separated compartments (aviary 1: 1,920 hens/group, with 2 or 4 test groups; aviary 2: 2,650 hens/group, with 2 or 4 test groups, varying based on the experimental design). The length of the flocks, ranging from 77 to 91 weeks of hen age, was meticulously determined through ongoing assessments of the hens health and developmental progress. Cumulative mortality percentages were calculated for both beak-trimmed and non-beak-trimmed hens within specified age ranges for each test group. Non-beak-trimmed Isa Brown hen groups consistently exhibited elevated cumulative mortality rates (6.1% to 35.2%), contrasting with beak-trimmed groups showing lower rates (4.8% to 15.2%). Despite this general trend, significant flock-specific variations were observed, notably in one flock where mortality rates converged between beak-trimmed (4.8-6.3%) and non-trimmed (6.1%) conditions. Conversely, Dekalb White hen flocks demonstrated narrower ranges in cumulative mortality, with rates ranging from 4.7% to 4.8% for beak-trimmed and 5.5% to 7.3% for non-beak-trimmed conditions. While affirming the practical benefits of beak trimming in mitigating mortality rates among Isa brown hens, our findings also suggest promising outcomes with non-trimmed brown hens. Achieving success in non-trimmed flocks demands adaptive management practices at the pullet and laying hen stage, informed by accumulated experiences. The species-specific analysis underscores a distinct contrast, as Dekalb White hens exhibit lower susceptibility to potential issues and consistently maintain lower mortality rates, irrespective of beak treatment.

Keywords: beak trimming, mortality,

## Associations between the gut-brain axis and feather pecking in laying hens

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Feather pecking (FP) is a significant welfare concern affecting millions of laying hens worldwide. Our recent research uncovered emerging evidence supporting the role of the gut-microbiome-brain axis in laying hen behaviour related to FP. Firstly, bacterial cecal droppings that are representative of the overall chicken gut bacterial community were examined in White Leghorn birds using 16S rRNA gene sequencing. Laying hens prone to FP (n = 20) exhibited a reduced overall cecal bacterial diversity (beta diversity;  $P < 0.05$ ) and a decreased presence of Lactobacillaceae in their cecal droppings compared to birds exhibiting low levels of FP behaviour (n = 20). In mammals, gut bacteria are crucial in controlling the enteric nervous system, influencing intestinal motility and signalling via the vagus nerve, which subsequently impacts the brain. Secondly, we used an ex-vivo model to investigate intestinal motility in laying hens. Cecal tissue was examined in an organ bath and tested whether cecal tissues' motility was affected by an intraluminal bacterial Lactobacillus stimulus. The results demonstrated a lower frequency ( $p < 0.001$ ) and an increased amplitude ( $p = 0.058$ ) of cecal gut lumen contractions when the intraluminal Lactobacillus stimulus was applied, regardless of the FP phenotype (FP birds: n = 13; non-FP birds: n = 17). Additionally, positive correlations were observed between the number of FP bouts and cecal velocity ( $p < 0.05$ ) as well as amplitude ( $p < 0.05$ ) of contractions in FP phenotypes. These two findings combined suggested that physiological differences in the response to Lactobacillus could be a promising avenue to explore to manage FP behaviour. To do so, we investigated the impact of Lactobacillus on behaviour in-vivo. An oral Lactobacillaceae supplementation (Lacto, n = 6 pens, 42 birds) significantly attenuated FP behaviour in stressed birds compared to those receiving a placebo (n = 6 pens, 44 birds). Specifically, FP behaviour ( $P = 0.664$ ) and the feather cover score ( $P = 0.996$ ) in stressed Lacto birds were not significantly different from that of non-stressed Lacto birds. In summary, the current presentation will outline the understanding of FP behaviour, provide evidence that intestinal motility and FP behaviour may be linked, and likely support the development of nutraceutical FP control strategies to improve the welfare of laying hens worldwide.

Keywords: Feather pecking; Behaviour; Physiology; Gut-Brain-Axis

## **Toe injuries are linked to altered movement behavior in laying hens in a semi-commercial aviary system**

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Toe injuries in laying hens are an emerging welfare concern because these injuries can lead to cannibalism and death due to bleeding out from toes. The origin of the increasing number of toe injuries detected in commercial systems is unknown but a possible reason could be toe pecking. Little is known how toe injuries affect movement behavior of hens in cage-free housing systems, such as in stacked-tier aviary systems. Our goal was to investigate how movement behavior differed longitudinally between hens with varying degrees of toe injury. We used four pens in a semi-commercial aviary system with 225 Lohmann Selected Leghorn hens per pen. In each pen, a Radio Frequency Identification system was used to continuously track hen location in the five pen areas (lower, middle and top tiers; litter area; a covered outdoor area). The hens were equipped with an RFID transponder attached to one leg at 15 weeks of age (WoA) and then tracked until 58 WoA. At 40 WoA, a tagged visual analogue scale was used to classify hens into three categories: having toes with severe, mild or without injuries. Specific movement behaviors (e.g. duration of stay in the litter, total number of transitions between tiers per day) were analysed retrospectively during the last 60 days before the toe assessment. Mixed models were used with toe injury as a fixed factor, WoA as a covariate, and their two-way interaction with henID nested in pen as a random term. Birds with no or mild toe injuries had 2.33- and 1.68-fold higher number of transitions the week before the toe assessment and 1.57- and 1.48-fold higher number of transitions eight weeks before the assessment, respectively, than birds with severe toe injuries. Moreover, birds with no or mild toe injuries spent  $168.4 \pm 15.7$  min and  $105.2 \pm 15.7$  min more time in the litter, respectively, than birds with severe toe injuries one week before the assessment. Eight weeks before assessment, the effect was similar with birds with no or mild injuries spending  $130.5 \pm 15.8$  min and  $128.8 \pm 15.7$  min more time in the litter than those with severe injuries. The difference in movement behaviors between hens with different toe injury classification thus seems to originate more than eight weeks before our assessment. Our data confirms long term effects of toe injuries and provides a foundation for future efforts to identify causes and whether injuries originate from toe pecking with birds pecking their own toes or those of other hens.

Keywords: toe pecking; resource usage; individuality

## **Lower stocking densities support broiler welfare – A proof of concept of the behavioural space model**

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The impact of stocking density on broiler welfare has been the subject of many studies, but a threshold stocking density, below which there is no further increase in animal welfare has not yet been determined. Here we assessed the welfare of fast-growing broilers at four final stocking densities, 5 kg/m<sup>2</sup>, 10 kg/m<sup>2</sup>, 15 kg/m<sup>2</sup>, 30 kg/m<sup>2</sup> (four replicates of each density, slaughter age was 36 days). The clinical welfare indicators were assessed based on the Welfare Quality® Assessment Protocol (d28 and d35) and the behavioural time budget was determined using scan sampling (d6, d13, d20, d27, d34). Activity was assessed during a free space test (d15 and d35) inside the home pen and recorded individually 24/7 by sensor technologies (RFID, d14-34) and by monitoring the group by passive infrared detection (PID, d1-34). There was a moderate correlation between the two sensor techniques ( $r=0.36$ ,  $p\leq 0.001$ ). Results showed that broilers at 5 kg/m<sup>2</sup> gained more weight than broilers at 15 and 30 kg/m<sup>2</sup> ( $p=0.001$ ). Broilers kept at 5 and 10 kg/m<sup>2</sup> showed better plumage cleanliness than those kept at 35 kg/m<sup>2</sup> ( $p < 0.05$ ), but no effect on footpad and hock dermatitis was found. Broilers at 5 kg/m<sup>2</sup> were more active in the free space test (running  $p=0.043$ , frolicking  $p=0.021$ , wing flapping  $p=0.007$ ), as well as in their undisturbed behaviour assessed by scan sampling (e.g., walking  $p=0.002$ , stretching  $p=0.008$ ) than birds at 30 kg/m<sup>2</sup>. In turn, broilers at 30 kg/m<sup>2</sup> were more inactive than broilers at 5 and 10 kg/m<sup>2</sup> (standing  $p=0.010$ , sitting  $p=0.096$ ). Although all broilers were less active with increasing age ( $p\leq 0.001$ ), treatment groups tended to differ in their general activity ( $p=0.055$ ). The activity level of broilers from stocking densities of 5 and 10 kg/m<sup>2</sup> were similar and greater than those at 30 kg/m<sup>2</sup>. Broilers kept at 30 kg/m<sup>2</sup> showed the lowest individual activity (as measured by RFID), whereas broilers at 10 kg/m<sup>2</sup> were the most active (monitored by PID). In general, the results suggest that stocking densities below 15 kg/m<sup>2</sup> support better animal welfare. This study is a first proof of concept of the behavioural space model in the EFSA opinion on broiler welfare pointing towards a stocking density of 11 kg/m<sup>2</sup> or lower. Whether there are non-linear changes of activity levels between these stocking densities and stocking densities up to 42 kg/m<sup>2</sup> should be the subject of further research, particularly in relation to positive animal welfare indicators.

**Keywords:** activity, free space test, welfare indicator, Precision Livestock Farming

## **A slow-growing broiler strain has more beneficial effects on broiler welfare than providing environmental enrichment**

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In the EU mEATquality project, the relationship between extensive production factors, animal welfare and meat quality aspects will be determined. A slow-growing breed and provision of environmental enrichment are important factors in extensive broiler production systems, that are considered to have beneficial effects on welfare. The aim of this experiment was to determine the effect of both factors on broiler welfare, to determine which (combination) of factors has the greatest beneficial effect. The experiment was carried out under semi-commercial conditions with 184 broilers per pen and 5 pens per treatment combination (stocking density 30 kg/m<sup>2</sup>). Treatments were breed (fast- (Hubbard JA787 (F)) versus slow-growing (Hubbard S757N (S)) and presence (EE) or absence of environmental enrichment (NE) (a dustbathing area with peat, wooden barrier perch and lucerne bale in a net). Broilers were placed on the same day and housed until approximately 2.1 kg, which was 42 days for F and 63 days of age for S. All broilers received the same diet and management. Measurements included performance, home pen behaviour, fearfulness and play behaviour, litter quality (at 0.5 kg, 1.1 kg and 1.9 kg body weight), and welfare indicators (gait score, footpad dermatitis, hock burn, cleanliness and injuries) at 2 kg body weight. Only main effects of treatment were found. S broilers showed more locomotion, standing and foraging, and less inactive behaviour as compared to F broilers ( $P < 0.01$ ), while no effects of enrichment on home pen behaviour were found. Both factors had limited effects on responses to fearfulness tests. F showed more play behaviour in a test situation as compared to S ( $P < 0.02$ ), and EE broilers showed more play than NE ( $P < 0.05$ ). More S birds were perching, while more F broilers were interacting with the bale ( $P < 0.01$ ). Regarding welfare indicators, S had significantly better scores for cleanliness, gait, footpad dermatitis and hock burn as compared to F ( $P < 0.01$ ) and enrichment did not have any effect. Only breed affected performance which was better for F ( $P < 0.01$ ). In conclusion, the slow-growing strain had more beneficial effects on welfare as compared to environmental enrichment, which had few beneficial effects, although the enrichments were used by the broilers and thus likely met their behavioural needs. The experiment confirms that the use of slow-growing breeds are an important welfare-promoting factor in extensive broiler production systems.

Keywords: behaviour; broiler; strain; enrichment; welfare

## Multifactorial evaluation of poultry welfare in different genotypes and farming systems

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The aim of the study was to evaluate performance and welfare of autochthonous poultry genotypes (Bionda Piemontese - BP, Robusta Maculata - RM, BP x Sassò - S, RM x S and Ross as control), in two farming systems (standard - S, 33 Kg/mq vs free-range - F, 21Kg mq indoor + 1 mq/chicken outdoor), through a multifactorial analysis: (Live Weight – LW and mortality), behavioural (morning and afternoon for 7 consecutive days pre-machining on 3 chickens/genotype/system/machining age: first impact - FI, ethogram and tonic immobility - TI) and caecal corticosterone (pre-mac., Faecal Glucocorticoid Metabolites - FGM) and oxidative status (ANTIOX- umol HClO/ml and dROMs - UCARR) and leukocyte formula (E/L) analysis (at 44 and 84 days, on 3 chickens/genotype/system/machining age) blood samples were taken. LWS were used to evaluate the Gompertz curves. Ethogram, FI, TI, FGM, ANTIOX, dROMs and E/L were carried out considering genotype - G, system - S and age - A with ANOVA test and significance was set at  $P < 0.05$ . Spearman correlation was evaluated between all variables and each system was considered separately. t-Stochastic Neighbor Embedding (t-SNE) technique was used to visualize the data of resulting similarities. Significant differences ( $P < 0.0001$ ) were found in Gompertz grow curves, with Ross highest growth rhythm in both systems, even if in FR its loss of growth was more pronounced than in others genotypes. Mortality was found highest in Ross in F and then in S; the lower mortality values were found in autochthonous birds in S and null in F ( $P < 0.005$ ). Behavioural observations presented a graet effect of both S and G ( $P < 0.005$ ) for alimentary, kinetic, resting and comfort and significant interaction of S x G was found for alimentary and resting behaviours ( $P < 0.05$ ) and for the kinetic activities ( $P < 0.005$ ). Antioxidant status showed a significant ( $P < 0.05$ ) effect of both S and G, in autochthonous birds dROMs and ANTIOX increased in F, on the contrary Ross showed a reduction of ANTIOX in F and presented the highest value in S. Regarding E/L ratio values only in FR were found significant ( $P < 0.001$ ) differences between genotypes, with highest value in Ross and lowest in BP chickens In S, FCM was found positively (0.43;  $P < 0.05$ ) correlated with H/L and negatively (-0.40;  $P < 0.05$ ) with pecking behaviours; this last negative correlation (-0.42;  $P < 0.05$ ) was confirmed in F, moreover H/L was found negatively correlated with grass pecking (-0.43;  $P < 0.05$ ) and kinetic activities (-0.57;  $P < 0.05$ ). T-SNE analysis showed a that S, G and A are characterised by behaviours of birds. In particular, kinetic behaviours characterised autochthonous, young and F birds, on the contrary S is characterised by static and comfort behaviours. In conclusion, under a welfare point of view: Ross birds showed the worst values for all parameters except for the grow rhythm, on the contrary autochthonous birds showed the greater adaptability (physiological and behavioural) to F system.

Keywords: poultry, housing system, genotype, welfare



**The effect of on-farm hatching on spatial traits in a quasi-commercial barn****M. Petelle<sup>1</sup>, C. Montalcini<sup>1</sup>, M. Toscano<sup>1</sup>**<sup>1</sup>ZTHZ, Division of Animal Welfare, VPH Institute, University of Bern, Zollikofen, SwitzerlandPresenting author: [matthew.petelle@unibe.ch](mailto:matthew.petelle@unibe.ch)

Early environmental stress can significantly impact an individual's phenotype. One important early life stressor for laying hens is the hatchery processing and transport (up to 8h in Switzerland) to the rearing barn at one-day of age. An approach to mitigate the impact of the hatchery process and transportation is on-farm hatch, where eggs are transported to the rearing barn at 18 days of development and can have immediate access to food and water after hatching. Chicks hatched on-farm have displayed reduced cortisol levels, increased fearfulness in novelty tests, and greater body mass when compared to standard hatchery chicks. Previous studies, however, are often short in duration, focusing solely on the behavior and welfare of chicks or pullets resulting in a limited understanding of how on-farm hatch can influence adult spatial behavior. We conducted a study comparing the spatial behaviors of on-farm hatch and transported individuals after transfer to the laying barn at 17 weeks of age, a major stressor. Specifically, we tracked individuals throughout the multi-tier aviary system using a low-frequency tracking system and examined whether animals differed in their vertical travelled distance, time spent in various areas (top, nestbox, and the litter), the midpoint in time when animals used the nestbox in the morning, and whether individuals entered the wintergarden. To assess differences in these metrics between treatment groups, we compared differences in means as well as variance structure by assessing repeatability estimates over time. Our findings revealed long term effects, extending beyond two months of early life condition on spatial traits where on-farm hatch animals transitioned less between the aviary's tiers, spent more time in the top tier, less time in the litter, and had a slightly earlier midpoint duration in the nestbox. On-farm hatch animals became more similar to transported individuals over time. We found no clear effect of early life condition on the variance structure of these traits, with among- and within-individual variances being comparable across the entire production period. Our results suggest that transportation to the rearing barn may prime animals to be able to cope with stressors in adulthood.

Keywords: early environment, movement, behavior, phenotypic plasticity

## **Effects of providing uniform or gradient light intensity on behaviour and welfare of fast- and slower-growing broiler chickens**

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There are still many questions on how to provide light to broiler chickens. Previously, we found that light intensity preferences depended on age and breed, but that there were always broilers present at each light intensity. Therefore, we studied the effect of providing a uniform or gradient light distribution in the pen on behaviour and welfare of fast- and slower-growing broilers. Day-old chickens from a fast- (Ross 308) and slower-growing breed (Hubbard JA757) were housed in groups of 346 per pen (27.3m<sup>2</sup>) with 16 pens per breed. Per breed, 8 pens received uniform lighting ( $\pm 20$  lux) and 8 pens received a gradient lighting (range  $\pm 25 - 200$  lux from front to rear or vice versa). Behavioural observations were performed via scan sampling at four target weights, with a total of 16 scans per pen per target weight. The human approach and novel object tests were performed after each observation in the morning to assess fearfulness. Gait score, footpad dermatitis, hock burn, cleanliness and injuries were assessed at two target weights according to the Welfare Quality protocol. Analyses of images for activity and distribution are in progress. An interaction between light treatment and target weight was found for eating and drinking ( $P < 0.01$ ), with broilers showing more ingestion at 0.8, 1.3 and 2.4 kg when comparing uniform versus gradient lighting. Light treatment further affected comfort ( $P < 0.02$ ) and foraging behaviour ( $P < 0.01$ ), with broilers showing less comfort and foraging behaviour when comparing uniform versus gradient lighting. Light treatment did not affect other behaviours (i.e. standing, locomotion, inactive), nor did it affect responses to the human approach and novel object tests. For cleanliness, an interaction between light treatment, breed and target weight was found ( $P < 0.05$ ). At 2.6 kg, slower-growing broilers had better cleanliness compared to fast-growing broilers, but this was only found for the gradient lighting and not for the uniform lighting. Light treatment did not affect gait score, footpad dermatitis and hock burn. Thus, fast- and slower-growing breeds responded similarly to the light treatments with regard to their behaviour. Gradient lighting stimulated comfort and foraging behaviour, but reduced ingestion behaviour compared to uniform lighting. Light treatment did not affect fearfulness and had minimal effects on welfare measurements. These findings indicate that gradient lighting might promote species-specific behaviour.

Keywords: broiler chickens; light intensity; behaviour; welfare

## **Stunning effectiveness: electrical water bath stunning vs. CO<sub>2</sub> gas stunning in broiler welfare assessment**

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The objective of the "CasStunn" project (Implementation of a controlled atmosphere stunning as an alternative of an electrical bath stun for broilers) is to verify the stunning effectiveness of the electrical water bath stunning and a CO<sub>2</sub> gas stunning system of broilers in order to assess the current state of animal welfare at the slaughterhouse. For data collection, the stunning effectiveness of four distinct fattening methods of broilers was examined from 2021-2023: 54 flocks of conventional light fattening (average body weight: 1.8 kg), 29 flocks of conventional heavy fattening (average body weight: 2.5 kg), 18 flocks of outdoor climate reared broilers (average body weight: 2.3 kg), and 22 flocks of organically reared broilers (average body weight: 2.0 kg). The study was conducted at a slaughterhouse in Germany where 4,800 broilers are slaughtered per hour. The stunning system of the slaughterhouse was converted from electric water bath to CO<sub>2</sub> gas stunning for the project, allowing the comparison of the two stunning systems within the same slaughterhouse. The assessment of animal behavior took place at least twice per flock. Behavioral parameters such as wing-flapping, erratic movements, and respiration were recorded after stunning as "indications of mis-stunning" in at least 160 broilers and recorded as percentage of the assessed broilers. A total of 10 broilers at a minimum per flock were examined for reflexes (pupillary and corneal) after debleeding. If animals were not immersed in the electric water bath or showed clear signs of consciousness after stunning, such as raising the head, these were scored as "mis-stunning". For additional documentation, videos were recorded during the CO<sub>2</sub> gas stunning process to facilitate a precise assessment of the behavior in the stunning facility. In the electrical water bath stunning, organically reared broilers showed a significantly higher incidence of mis-stunning after neck cutting compared to other fattening methods ( $p < 0.05$ ). The mean results were as follows: organically reared: 0.75%, heavy: 0.20%, light: 0.21% and outdoor climate: 0.52% fattened ones. Overall, the results suggest that the fattening method may influence the stunning effectiveness of the system. The final analysis of the CO<sub>2</sub> stunning will be completed in spring 2024, the additional data can be presented at the conference.

**Keywords:** Stunning effectiveness, Animal Welfare, Broilers, Slaughter, Mis-stunning

**Exogenous supplementation of additional level of arginine (L\_Arg) in corn-soy based diets is required for broiler breeder hens during late production phase****G. Kolluri<sup>1</sup>, G. Khillare<sup>1</sup>, G. Marappan<sup>1,2</sup>, J. Mohan<sup>1</sup>, J. Tyagii<sup>1</sup>, R. Jaywant<sup>1</sup>**<sup>1</sup>ICAR-CENTRAL AVIAN RESEARCH INSTITUTE, BAREILLY, India, <sup>2</sup>ICAR-NATIONAL INSTITUTE OF ANIMAL NUTRITION AND PHYSIOLOGY, BENGALURU, India

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L-Arginine (Arg) is a conditionally essential amino acid for humans and animals and is also the fifth most limiting one in corn-soy diets. This investigation was carried out to assess the productive and reproductive performance of broiler breeders fed with different concentrations of Arg. The effect of 4 levels of Arg was evaluated on productive and reproductive performance in broiler breeder hens for 9 weeks (51-60 weeks of age) duration. Hens were fed with 0.90, 1.20, 1.35 and 1.60% in T1, T2, T3 and T4 group respectively. The Arg levels in T2, T3 and T4 were 25, 50 and 75% higher than the basal level of T1 group (0.9%). Data generated from linear regression analysis indicated a positive relationship of varied L-Arg doses with reproductive fitness traits significantly ( $p < 0.01$ ), while the negative relationship was observed with abdominal fat ( $p = 0.001$ ). There was an increase in egg production recorded in T2 (14%), T3 (13%) and T4 (13%) groups. Breeders fed 1.60% laid heavier eggs when compared to other groups (T1, T2 and T3). Increase ( $p = 0.005$ ) in the ovarian and follicular weights, were also observed at higher level of 1.6% than control. Abdominal fat was decreased in supplemented groups in a dose dependent manner. Birds revealed higher ( $p = 0.054$ ) number of large yellow follicles and post-ovulatory follicles in the extra level Arg supplemented groups. Estrogen increase was noticed during different time points in T4 and T3 groups. Progesterone (P4) showed variation between treatment and control group from 55-60 weeks. The higher levels of P4 concentration (pg/ml) were observed in T4 group at 60 weeks. The m-RNA relative expression of Follicle stimulating hormone receptor (FSHR) was up-regulated ( $p = 0.041$ ) in response to L-Arg supplementation in dose dependent manner. Increased Arg level in diets resulted in relatively higher fold expression of FSHR as 0.8, 2.1 and 1.4 in T2, T3, and T4 groups respectively. Similarly, the Leutinizing hormone receptor expression was up-regulated in all the higher Arg fed groups with a change of 0.3, 1 and 1.5 folds in low, medium and high dose groups respectively. Higher fertility and hatchability on fertile egg set basis were noticed in high dose group (1.6%). In conclusion, addition of 75% extra L-Arg (1.6%) to the basal level (0.9%) enhances productive and reproductive attributes of broiler breeder hens during late production phase by positively altering the gonadotropin hormone receptor gene expressions in ovarian tissues.

**Keywords:** L-Arginine, Broiler breeders, reproductive performance

## Expression of genes involved in calcium and phosphorus homeostasis in hen's kidney: effects of sexual maturity and PO stage

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Eggshell formation in laying hens takes place daily in the uterine gland, with an active phase of calcium (Ca) carbonate deposition from 9 to 22 h Post-Ovulation (PO), which requires large quantities of Ca<sup>2+</sup>, delivered from the blood stream to the uterus. This occurs during the night when feed intake is inhibited, so that bone resorption substantially contributes to Ca<sup>2+</sup> supply to the blood pool, and also releases inorganic P (iP). Fibroblast growth factor 23 (FGF23) is a key regulator of this physiological process. It is secreted by bone cells and acts on the kidney to increase P excretion. We previously observed that FGF23 expression was induced in the medullary bone of laying hens during the active phase of eggshell calcification and increased with age. The effects of this factor depend on the expression of specific FGF receptors (FGFR), of a co-receptor  $\kappa$ -Klotho (KL) and of Solute Carriers (SLC) involved in iP reabsorption. Moreover, FGF23 also increases the activity of the 24-hydroxylase enzyme (CYP24A1), which reduces the levels of 1,25(OH)D<sub>3</sub>. The aim of the present study was to provide an integrative description of these candidate genes' expression in the kidney, as a function of sexual maturity and during the ovulatory cycle. From hatch to sacrifice, the animals were fed standard balanced diets providing Ca, P and vitamin D<sub>3</sub> as appropriate for their physiological state. Kidney samples were collected from pullets at week 17 (W17, n = 12), and laying hens (n = 8 at W28 0-1 h PO or 10-11 h PO) to prepare RNA. PCR primers were selected from available data bases to develop qRT-PCR quantification. The data expressed as relative expression, in comparison to a set of 3 reference genes, were analysed by one way-ANOVA or otherwise by Kruskal Wallis (effect of age W17 vs WK28 at 0-1 h or 10-11 h PO stage at W28). All the candidate genes (FGFR-2 and-3, KL, SLC20A1, SLC20A2, SLC34A1, CYP24A1) were expressed in the kidney across ages and PO stages. Five candidates showed increased expression with age (FGFR-2, KL, SLC34A1, SLC20A1, CYP24A1, p < 0,05), while CYP24A1 and SLC20A1 expression increased significantly at 0-1 h PO (p < 0,05). The data show that several FGF23 target genes show increased expression with the onset of lay suggesting the importance of this factor in the complex regulation of P/Ca homeostasis at this stage.

Keywords: Laying hen; endocrinology; kidney; FGF23; Vitamin D; Phosphorus and Calcium metabolism

## Neural regulation of the stress response in chicken

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Stress disorders are a major problem in poultry and other farm animals, affecting their well-being and health, and having a negative impact on production and quality of derived products. To improve poultry welfare, it is critical to understand the neural mechanisms regulating emotions. This has been extensively studied in mammals, with the central extended amygdala (including central amygdala and lateral bed nucleus of the stria terminalis) playing key roles, due to their involvement in triggering endocrine, autonomic and behavioral responses upon exposure to a stressing stimulus. This regulation is complex, involving different cells and circuits. However, the central amygdala in birds is poorly understood, and its possible involvement in stress is unknown. As part of a European network to study the basis of stress in poultry, our aim was to investigate the central extended amygdala of Leghorn chicken at molecular, cellular, and circuit levels, and to analyze its involvement in stress. We first identified its major neuron subpopulations based on their molecular profile. To that aim, we combined multiple labeling of neuropeptides and developmental-regulatory transcription factors. We found major cell types similar to those present in mammals, containing enkephalin, somatostatin and corticotropin-releasing factor. To investigate their connections, we carried out tract-tracing experiments using biocytin, and combined it with phenotypic cell markers. As in mammals, we found that different cells and channels are involved in regulating the outputs of the chicken extended amygdala to endocrine and autonomic centers. Finally, to analyze their implication in stress, we carried out a behavioral experiment consisting in 30-minute social isolation of post-imprinted chickens (7/8 days posthatching), which induces distress vocalizations. Variations in expression of the immediate early gene cFos were analyzed in the brain using quantitative RT-PCR. We found significant increase in cFos expression in both the central amygdala and bed nucleus of the stria terminalis in experimental animals compared to their paired controls, thus supporting their involvement in stress response. Overall, our results help to better understand regulation of the stress response by the extended amygdala in chicken. Support: H2020-MSCA-ITN-2018-812777

Keywords: chicken, welfare, stress regulation, brain, amygdala

## **Long-term effects of prenatal temperature stimulation on mRNA expression of Bdnf, Npy, and Pomc in the chicken hypothalamus**

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In precocial birds, thermoregulation develops early in ontogeny, and perinatal thermal experiences can affect its long-term functionality. However, the effects of this adaptation process on other physiological systems and the underlying neural and molecular mechanisms are still not fully understood. The aim of our study was to elucidate whether these early adaptation processes effect molecular biomarkers related to developmental plasticity and energy metabolism. We investigated how a short-term prenatal temperature training (PTT, +1°C for 2 hrs, embryonic days 17-20) affects the basal mRNA expression levels of Bdnf, Npy, and Pomc at 35 days of age in the hypothalamic nucleus infundibular (IN) in broiler chickens. The IN plays an important role in the central coordination of different physiological systems and metabolic functions. We hypothesized that the PTT establishes long-term changes in basal Bdnf mRNA expression levels reflecting an increased adaptive plasticity, and long-term changes in basal Npy and Pomc mRNA expression levels reflecting shifts of the physiological feed-back cycles underlying feeding behaviour, feed conversion, and satiety. We conducted a winter and a summer trial with consistently higher ambient temperatures. Mean mRNA expression levels at 35 days of age were descriptively higher for Npy, Pomc, and Bdnf in the PTT group compared to the control group. The group difference for Bdnf was statistically significant when analyzed across both trials (N=72, padjust= 0.048). Further analysis revealed that this effect was mainly driven by differences in the summer trial. For all three target genes descriptively, we observed highest mean mRNA expression levels for the PTT group in the winter trial and lowest expression levels for the control group in the summer trial, with the PTT rescuing the dampening effect of the higher ambient temperature on the expression levels. For Bdnf, the difference was statistically significant even after adjusting for multiple testing. We did not observe any significant sex differences for any of the target genes in both trials. Our findings indicate that short-term PTT during a critical developmental period of the thermoregulatory system induces changes in mRNA in avian hypothalamic neurons that are associated with developmental plasticity. This effect may contribute to long-term physiological adaptations that improve resilience towards stressful environmental conditions, such as high ambient temperatures.

**Keywords:** temperature stimulation; thermoregulation; gene expression; energy metabolism; brain-derived neurotrophic factor; incubation methods

## **Comparative analysis of transcriptional profiles of chicken jejunum and - derived organoids collected early post-hatch**

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Organoids are cell cultures derived from stem cells than can self-organize and differentiate to recapitulate the morphology and functions of the initial tissue. In the literature, intestinal organoids of the chicken are already described at macroscopic levels. Here, we present an exhaustive transcriptomic characterization of such organoids derived from jejunal samples collected at various stages of digestive tract development. The data were finally compared to the ones of the initial tissue. The objectives of this study were i) to evaluate the degree of similarities between jejunums and derived organoids, ii) to assess whether the age features of the initial tissue can be reproduced after organoid culture. A series of organoids ( $n \geq 15$ ) were cultured over 8 days from jejunal samples collected at day 0, 2 or 7 post-hatch. Differential analysis revealed a sharp disparity in expressional profiles between jejunal tissues and organoids (approx. 4000 differentially expressed genes - DEGs). A principal component analysis was performed on expression data. The 1st component separated jejunal tissues from organoids and explained 34% of the total variance. The 2nd component (9% of total variance) distinguishes the different jejunums tissues according to the collection age and, to a lesser extent, different organoids. The functions associated with DEGs were explored using enrichment approaches (Gene Ontology, KEGG). They are associated with extracellular matrix and muscle fibres, consistent with expectations, as organoids consist of intestinal epithelial cells, while the jejunum also includes conjunctive and muscular layers. The presence of the different epithelial cell types was attested by genes specific to these populations. LGR5, an intestinal progenitor marker, showed similar expression levels between jejunums and organoids. Mucus cells (MUC2), Paneth cells (REG4), enterocytes (ALPI) and enteroendocrine cells (CHGA) were detected in organoids, but at overall lower levels than in the jejunum. The tight junction genes (TJP1-2-3, CLDN2, CDH1, OCLN) as well as intestinal transporters (ABCB1-C3-G2, SLC5A1, SLC9A3, SLCO2B1, SLC15A1, COT1) displayed similar levels of expression between tissues and organoids. This work demonstrates that organoids derived from jejunum present similar transcriptomic expression patterns regarding functions of intestinal epithelial cells and can be used for further functional studies.

Keywords: Organoids, jejunum, transcriptional profiles, development,



## **Comparative analysis of caeca microbiota composition in broiler chickens fed diets with alternative protein sources**

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In this research we compared the impact of the supplementation of three different alternative protein sources in broiler diets on the caeca microbiota. The results were obtained from three trials carried out on broiler chickens of the same strain and gender (male Ross 308), supplied by a commercial hatchery and reared in the same experimental farm, adopting analogous environmental and management conditions as well as feed formulation. In the first trial, the birds were divided in five groups fed a commercial basal diet (group CON-A) or the same diet supplemented with 3 or 6% of microalgae in the grower phase (groups G3A and G6A) or in both grower and finisher phases (groups GF3A and GF6A). In the second trial, the birds were allocated in three groups fed a commercial basal diet (CON-IM) or the same diet supplemented with either 9 or 18% black soldier fly meal (groups IM9 and IM18). In the third trial, the birds were split in four groups fed a basal diet (group CON-S) or the same diet with 2, 4 or 6% of single cell protein meal (groups SC2, SC4 and SC6). Caeca contents from representative birds (i.e., with a body weight similar to that of the corresponding group) were collected at approximately 21 and 42 days in each experiment, submitted to DNA extraction and then tested by 16S metagenomic sequencing. The results of the genus beta diversity showed that microalgae were effective in modulating the caeca microbiota at 21 days in groups G3A and mostly in G6A ( $p=0.048$  and  $0.004$ , respectively). At 42 days, only the dietary treatments G6A and GF6A were effective ( $p=0.004$ ). A clear microbiota modulation was also achieved in groups IM9 and IM18 at both 21 ( $p=0.046$  and  $0.001$ ) and 42 ( $p=0.004$  and  $0.001$ ) days. The supplementation of single cell proteins at 2% did not substantially affect the microbiota composition in comparison to the CON-S group at 21 days ( $p=0.322$ ), while both 4 and 6% were effective ( $p=0.013$  and  $0.001$ ). On the contrary, at 42 days all concentrations of single cell proteins were able to modulate the microbiota in comparison to CON-S group ( $p=0.005$ ,  $0.006$  and  $0.001$  for groups SC2, SC4 and SC6, respectively). The alpha diversity values were increased at 42 days in groups GF6A and IM18. The impact of each diet on specific microbial populations will be presented at the conference. This research has been funded by the H2020 project NextGenProteins (Transformation of Biomass into Next Generation Proteins for Food and Feed), grant no. 862704.

Keywords: alternative proteins; microbiota; caeca content; broilers;

## **Impact of protein source and digestibility marker on nutrient digestibility in broilers**

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Determining nutrient digestibility in feed is crucial for optimizing animal nutrition and precise diet formulation. Inert digestibility markers play a key role in estimating dietary nutrient digestibility, allowing for ad libitum feed access and shorter collection periods. A bioassay was conducted in male Hubbard JA57 broilers to investigate the influence of protein sources—soybean meal (SBM), rapeseed meal (RSM), and sunflower meal (SFM)—on whole tract digestibility coefficients. Two inert markers, ytterbium (Yb) and acid-insoluble ash (AIA), were evaluated for their impact on organic matter, gross energy, nitrogen, starch, ether extract, phosphorus, and calcium digestibility coefficients. A total of 144 male broilers at 21 days old, sorted by body weight, were distributed among 36 cages (4 birds per cage). Three diets, differing in protein source at 16%, were replicated 12 times and included 50 mg/kg of Yb<sub>2</sub>O<sub>3</sub> and 10g/kg of Celite as indigestible markers. Feed was provided from day 21 to 31. On day 31, excreta samples were collected, oven-dried at 60°C for 72 hours, and ground through a 0.5 mm screen. Digestibility coefficients and nutrient flow in excreta (g or kcal per kg of diet dry matter) were computed. The generalized linear mixed statistical procedure analyzed main effects and interactions of markers and protein sources. Pearson's correlation explored relationships between Yb and AIA estimates. No interaction ( $P>0.05$ ) between protein source and marker affected digestibility coefficients. Marker type had no effect ( $P>0.05$ ) on all studied variables. Protein source had no effect ( $P>0.05$ ) on organic matter and gross energy digestibility coefficients. Starch digestibility was highest ( $P<0.001$ ) in SFM-based diet (0.970) compared to SBM (0.958) and RSM (0.951). Ether extract digestibility was highest ( $P=0.006$ ) in SFM (0.762) and RSM (0.696) diets compared to SBM (0.599). Nitrogen digestibility was highest ( $P<0.001$ ) in SFM-based diet (0.604) compared to SBM (0.570) and RSM (0.572). Phosphorus digestibility was highest ( $P<0.001$ ) in SBM-based diet (0.434) compared to RSM (0.379) and SFM (0.353). Calcium digestibility was highest ( $P=0.002$ ) in SBM-based diet (0.311) compared to RSM (0.255). Starch and ether extract flow in excreta estimated using Yb correlated positively ( $r=0.99$ ,  $P<0.001$ ) with AIA estimates.

**Keywords:** Digestibility; Protein; Marker; Broiler

## **Are heat treated mixed crops of faba beans and wheat suitable for broiler feeds?**

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To improve the sustainability of poultry production, it is vital to decrease the dependency of imported feedstuffs in favour of locally grown nutrients, particularly in case of protein sources. Faba beans are often suggested as an alternative protein source as they can be easily be grown in the European climate. Our research focusses on the use of mixed crops of faba beans and wheat in poultry feed. Mixed cropping offers some cultivation benefits, such as improved resource use efficiency and yield stability. However, it is not standard practice to use these mixed crops for feed applications. Additionally, faba beans require further processing in order to reduce the concentration of anti-nutritional factors (e.g. vicine and convicine). The current trial evaluated the effect of feeding mixed crops of faba beans and wheat that were expanded or extruded at different conditions on broiler performance. Therefore male Ross 308 broilers were subjected to one of 5 dietary treatments for the whole rearing period: a control diet (wheat-soybean based; CON) or an iso-proteinaceous and isocaloric diet containing approx. 50% of the mixed crop that was either expanded at mild (18 bar, 105°C; EXP1), medium (22 bar, 110°C; EXP2) or severe conditions (25.5 bar, 121°C; EXP3), or extruded (30 bar, 130°C; EXTR). The trial included 8 pen replications per treatment with 30 birds per pen. Animals and feeds were weighed on pen level at the start and end of each rearing phase (starter: d1-d11, grower: d11-d29 and finisher: d29-d40). Results are displayed as mean  $\pm$  SD. Chickens from the EXTR group reached significantly higher end weights ( $2843 \pm 50$  g) than the other groups ( $2682 \pm 62$  g,  $2678 \pm 65$  g,  $2676 \pm 53$  g and  $2740 \pm 90$  g for CON, EXP1, EXP2 and EXP3, respectively). The daily feed intake over the whole rearing period of EXTR ( $98.6 \pm 2.2$  g) and EXP3 ( $97.7 \pm 4.1$  g) was significantly higher than EXP2 ( $93.1 \pm 3.2$  g). Overall, EXTR and EXP2 had a better feed conversion ratio ( $1.37 \pm 0.04$  and  $1.38 \pm 0.04$ , resp.) as compared to EXP1 ( $1.42 \pm 0.02$ ) and EXP3 ( $1.41 \pm 0.02$ ). These results suggest that expanded and extruded mixed crops of faba beans and wheat can be included in broiler diets without compromising performance results, with extrusion being the most favourable heat treatment.

Keywords: Broiler, Nutrition, Protein, Faba beans, Mixed crops

**Maintaining an adequate amino acid profile allows to successfully reduce dietary crude protein in broiler chickens in both wheat or corn-based diets****P. Moquet<sup>1</sup>, C. Raybaud<sup>1</sup>, W. Lambert<sup>1</sup>**<sup>1</sup>MIXSCIENCE, BRUZ, FrancePresenting author: [william.lambert@mixscience.eu](mailto:william.lambert@mixscience.eu)

Reducing dietary crude protein (CP) in broiler diets has been vastly investigated for its environmental and welfare benefits. However, recent studies have shown that reducing protein in broiler diets seems to be more difficult to achieve in wheat-based diets comparatively to maize diets. A total of 385 male Ross 308 broilers were fed 6 dietary treatments with 16 replicates from 0 to 42 days of age and with 4 feeding phases (d0-d9, d9-d21, d21-35 and d35-42). Treatments were iso-energetic, iso-digestible lysine and consisted of a standard CP diet formulated based on more than 50% corn (CCP+) with levels of dietary CP of 22.7, 19.6, 18.8 and 18.2 %, respectively in each feeding phase. A low CP diet was formulated by replacing soybean meal with corn and reducing dietary CP by 2.0 % pts with (CAA+) and without (CAA-) controlling dietary AA beyond Thr (Val, Ile, Arg) at least at the assumed requirement. A second standard CP diet was formulated based on more than 50% wheat (WCP+) with levels of dietary CP of 23.2, 20.4, 19.4 and 18.9 %, respectively, in each feeding phase. The same reduction of 2.0% dietary CP was applied with (WAA+) and without (WAA-) controlling dietary AA beyond Thr (Val, Ile, Arg) at least at the assumed requirement. For the entire experimental period (0-42d), average daily gain, feed intake, feed conversion ratio (FCR), water to feed ratio and breast meat yield were all affected by dietary treatment ( $p < 0.05$ ). In corn-based diets, decreasing dietary CP and controlling dietary AA (CAA+) increased significantly feed intake and average daily gain comparatively to the two other treatments (CCP+ and CAA-). Feed conversion ratio was significantly higher in CAA- compared to the two other corn-based treatments. In wheat-based diets, average daily gain was decreased and FCR was increased in WAA- diet comparatively to the two other treatments. In both contexts, water to feed ratio was lowered by reducing dietary CP by 2.0%pts. Breast meat yields were increased by reducing dietary CP in both CAA- and CAA+ in corn-based diets but they were not affected by dietary treatment in wheat-based diets. Dietary CP reduction by 2.0 % pts in broiler diets could be achieved without negatively impacting growth performance and carcass traits but only when all indispensable AA were controlled at least at the assumed requirement. Similar effects were observed independently of the main cereal used in diet formulation.

Keywords: Crude protein ; amino acid ; broiler ; carbon footprint

## **The inclusion of full-fat Black Soldier fly larvae in broiler's feed in different forms (meal and whole): effects on growth performance and breast fatty acids' composition**

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A trial was conducted to evaluate the effects of including dehydrated full-fat Black Soldier fly (BSF) larvae in broiler's feed in different forms: incorporated in the feed (meal) and incorporated on top (whole) on growth performance and meat quality. A total of 288 one-day-old male broilers (Ross 308) were allocated in 24 floor pens (1.2x1.2 m<sup>2</sup>). From 11 to 41 days of age, pens were divided into 3 experimental treatments consisting of a control feed (C) without insects, a feed with 10% BSF larvae meal incorporated in the feed (10FFm), and a feed with 10% BSF whole larvae incorporated on top (10FFw). All feeds were formulated to be isoenergetic and isoproteic (growing phase: 12.7MJ/kg AMEn and 21% crude protein, CP, and finishing phase: 13.0MJ/kg AMEn and 19%CP). In the diets, BSF was included replacing soybean meal and oil. Animals were weighed individually on days 11, 25, and 41, and average daily gain (ADG) and feed intake (ADFI) were calculated in the two growing periods. At the end of the study, the left breast of two birds per pen (n = 48) was obtained to measure the fatty acid (FA) profile. The BSF larvae used in this study contained (in dry matter): 39% CP, 22% crude fat, and 20.6 MJ/kg AMEn. No differences were found in ADFI among groups throughout the study. However, animals from the 10FFm group showed a lower ADG compared with animals from the C group in the growing phase (64.5 vs. 67.7 g/d, respectively, p<0.05). Feed conversion ratio (FCR) was higher in the groups of animals fed BSF larvae compared with C animals (1.67 vs. 1.59, respectively, p<0.05), except in the growing period, in which the FCR of the group 10FFw was not different from that of the C group. Animals from the treatments including BSF larvae showed a greater (p<0.05) and a lower (p<0.05) proportion of saturated and polyunsaturated FA in the breast, respectively, compared with the C group. Additionally, animals fed 10FFm and 10FFw showed a higher concentration of lauric acid (C12:0) in breasts compared with animals fed the C diet (0.187 vs. 0 mg/100g, respectively, p<0.05). In conclusion, providing whole BSF larvae to broilers can be advantageous when compared with milled forms in terms of feed efficiency in the growing period, but not in the finishing period. Additionally, both BSF larvae forms changed the FA profile of breasts towards a greater saturated profile.

Keywords: broilers; black soldier fly; growth performance, fatty acids

## Assessing the environmental impact of poultry feed in meat type autochthonous chicken farming

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Population growth and the increase in resources consumption worldwide has highlighted how essential it is to evaluate and reduce the environmental impact of animal feed, with particular attention in reducing the use of soybean meal (SBM). As a preliminary study the objective was to assess the environmental implications of 2 diets considering the environmental impact of the cultivation of raw materials and their transport to the feed mill reporting the results based on producing 1 kg of ready to cook carcass RTC. The study lasted 108 days (39-147 d/old). Two different feeds for meat type chickens were formulated. The control feed (C) had traditional ingredients (maize meal 62%, SBM 32%, Soybean oil 2%; apparent metabolizable energy 11.8 MJ/kg, Crude protein, 18.13%, ether extract 3.6%, crude fiber 3.28%) while the experimental diet (EXP) was formulated substituting completely the SBM with alternative protein ingredients (Maize meal 46.1%, Field bean 11.0%, Pea protein 10.8%, Barley 4.7%, Sunflower meal 9.5%, Maize gluten 11.6%, Soybean oil 1.6%; apparent metabolizable energy 2834.24 kcal/kg, crude protein 18.10%, ether extract 3.63%, crude fiber 4.80%). To assess the environmental impact of the diets it was used an attributive life cycle assessment (LCA) methodology as a tool to evaluate and quantify the environmental impact associated with the two experimental diets (C, EXP). The analysis was conducted using SimaPro 9.3.0.3 software. The inputs of raw material were obtained from the “Agri-footprint 5” database. Moreover, to assess the impact of producing 1 kg of RTC, the ReCiPe 2016 method (v1.06,) was used, addressing several environmental concerns at an intermediate level and then combining these intermediate points to form three final categories, human health, ecosystems and resources. The SBM in the C diet was found to contribute the most (60%) to the global warming potential of the feed. In the EXP diet, in which SBM was completely replaced, a significant reduction in global warming potential (-31.6%), impact on human health (-28%), damage to ecosystems (-11%) and resource consumption (-22%) was observed compared to the C diet. The impact on global warming of producing 1 kg of chicken RTC fed the C diet was 11.99 kg CO<sub>2</sub> eq, while it was 7.72 kg CO<sub>2</sub> eq when fed the EXP diet. These results underline the importance and possibility of investing in research identifying new and innovative formulas to help reduce the impact of livestock farming.

Keywords: sustainability, soybean meal; environmental impact; life cycle assessment

**Bran particle size impacts the precaecal fibre particle size distribution, caecal influx and caecal fibre fermentation differently with age****P. Vanderghinste<sup>1</sup>, A. Bautil<sup>1</sup>, M. Bedford<sup>2</sup>, G. Gonzalez Ortiz<sup>2</sup>, C. M. Courtin<sup>1</sup>**<sup>1</sup>KU Leuven, Leuven, Belgium, <sup>2</sup>AB Vista, Marlborough, United Kingdom

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The inclusion of fibre in broiler feed has gained renewed interest due to its potential for stimulating (gut) health and performance. The beneficial effects of fibre are mainly due to its fermentation in the caeca. It is hypothesised that only small particles and fluids can enter the caecal lobes, but the threshold in size that allows caecal influx is lacking. In addition, little is known about the fate of fibre particles along the GI tract before they arrive at the caecal entrance. To boost caecal fermentation via fibre inclusion, more knowledge on how fibre particle size in feed can affect the fibre particle size distribution along the GI tract, and hence the caecal influx of fibre, is required. To this end, a broiler trial was set up in which 72 male broilers (Ross 308) were divided into three treatment groups. Dietary treatments consisted of a wheat-based diet with 10% replacement of the wheat fraction by wheat bran with a particle size of 1000 µm (WB Coarse), 500 µm (WB Medium) or 100 µm (WB Fine). Digesta of the precaecal GI tract parts was collected at d7 and d24 and separated into five size fractions by wet sieving, after which the arabinoxylan (AX) content of each size fraction was determined by gas chromatography (GC). Caecal digesta particle size distribution was measured by laser diffraction and caecal short-chain fatty acid (SCFA) content by GC. Each of the three different bran inclusion particle sizes caused an increase in AX content of the corresponding size fraction in the digesta of each GI tract part. This indicates that no extensive particle size reduction of the bran occurred along the GI tract and that modifications of the fibre particle size distribution of digesta should be achieved by steering fibre particle size in feed. On d7, the WB Coarse group surprisingly showed smaller average particle sizes (D50) of caecal digesta (2.6 µm) and higher SCFA contents compared to the WB Fine group (D50=9.0 µm). In contrast, higher caecal D50 (12.2 µm) and lower SCFA contents were observed for the WB Coarse group at d24 compared to the WB Fine group (D50=9.1 µm) ( $P<0.05$ ). The SCFA content increased with decreasing caecal D50 at both ages, but the mechanisms of caecal particle influx and fermentation seem to differ greatly with broiler age. In contrast to expectation, this suggests that large fibre particles can enhance caecal fermentation at a young age and that a shift towards smaller fibre particle sizes should be made at an older age.

Keywords: fibre; fermentation; wheat bran; broilers; gut health; particle size; caeca

## **Effect on degradation and passage of oat hulls through digestive tract of broilers at different time points**

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Gizzard in birds selectively retains and grinds coarse particles before passing further in gastrointestinal tract (GIT) but what happens when it reaches its maximum capacity is a conundrum. This experiment aimed to study gizzard function at different times with hypothesis that passage of large particles (LP) increases with greater coarseness and gizzard's surpassed retention capacity. 4 pelleted diets were formulated with 0 or 11% oat hulls (OH; LOH and HOH respectively) hammer milled at 2 or 6mm, and metabolizable energy and nutrient density decreasing with OH addition. 80 Ross 308 broilers were individually caged and distributed in 2 treatments (HOH-2 and -6mm; 40 birds/diet) from d10-24. To ensure no OH in GIT, birds were switched to LOH diets from d25 to 29. For sampling on d30, birds were feed deprived for 8h, refed with HOH diets for 2h and then refed LOH diets until euthanized at 0,2,4,8 and 16h (8 birds/diet/time) to avoid disturbing digesta flow. However, interchanges of HOH and LOH diets were always within same particle size (2 or 6mm). The study followed 2x5 factorial design and ANOVA was performed to determine main effects and interactions of 2 screen sizes and 5 time points. Means were separated by a Tukey post-hoc test. Samples were collected from crop, proventriculus, gizzard, small intestine (SI), and excreta. Neutral detergent fibre (NDF) served as a proxy for OH and particle size distribution of contents was analyzed to measure LP (>1.6mm). NDF content in gizzard at all times (0.3-0.4g) showed selective retention and indicated that OH are retained in gizzard as long as their capacity is not exceeded. Amount of LP in SI (0.1g) indicated consistent grinding activity and passage of LP at all times. However, higher LP amount ( $p<0.05$ ) for 6 (0.13g) than for 2mm diets (0.06g) suggested increased unground LP passage with increased coarseness level. This is further supported by the observation of a higher amount of LP found in excreta of 6 (0.1g) than 2mm (0.03g) diets at 16h ( $p<0.05$ ), implying that a higher LP intake leads to increased LP passage from gizzard. NDF concentration in excreta collected at different times suggested that OH consumed by birds from HOH-diets could potentially continue to be excreted for more than 8h. Despite a low load of OH from diets, they managed to escape grinding in gizzard. This study challenges the prior assumption that particles must reach a certain critical size in gizzard before further passage.

Keywords: "oat hulls" "gizzard" "broiler"



## **Assessing the flow of digesta and digestive dynamics in broiler chickens: A comparative study with growing pigs**

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The study compared feed retention and particle size of digesta throughout the gastrointestinal tract (GIT) of broilers and pigs at different times post-feeding. Seven replicates of 2 broilers (n=56, 24-d-old, 590 g BW) and 1 pig (n=28, 50-d-old, 21 kg BW) each were part of a randomized 4x2 factorial design, with 4 sampling times and 2 species. Both were fed a corn-based pelleted diet for 10 days, followed by a washout diet (dextrose and fish meal) for 3h, fasting for 5h, and 1h of experimental diet access before being weighed and euthanized at 2, 4, 6, and 8h post-feeding. Broilers had 1.37 kg BW and 39.8 g feed intake during the 1h access, and pigs averaged 30.5 kg BW and 359 g feed intake. The anterior tract (broilers: crop, proventriculus and gizzard; pigs: stomach) and small intestines were removed; duodenum was separated, and the rest (jejunum + ileum) was divided into 4 equal length parts. Weight of empty organs and whole contents were recorded. Contents were analysed for dry matter, starch, and digestive markers (titanium dioxide and chromium-EDTA). Broilers had a higher relative anterior tract weight (28.6 g vs. pigs' 11.5 g/kg BW; P<0.05) and relatively longer lengths of duodenum (20.1 cm vs. pigs' 2.1 cm/kg BW) and jejunum+ileum (113 cm vs. pigs' 55.8 cm/kg BW). Interactions (P<0.001) were observed for relative contents (g fresh digesta/kg BW) in all segments: broilers killed at 2h after feeding had more content (29.3 g) in the anterior tract than other timings or pigs; broilers killed at 4 and 6h had more content (3.3 and 2.5 g) in the duodenum, whereas pigs had consistently lower duodenal content (0.22 g average) than broilers; in the jejunum + ileum, higher contents were seen in pigs killed after 2h (26.3 g) and broilers killed after 2 or 4h (18.3 and 24.8 g) than other timings. Regarding the particle size of digesta, broilers had a higher relative volume of particles <0.1 mm in the duodenum than pigs (55 vs. 28%; P<0.05) regardless of timing. In the middle jejunum and distal jejunum + ileum, pigs had more particles <0.1 mm while broilers had a higher volume of large (0.5 to 2 mm) and intermediate (0.1 to 0.5 mm) sized particles than pigs. Compared with growing pigs, the results demonstrate a relatively larger upper GIT mass in broilers, resulting in a longer feed retention (up to 4h), and the relative particle size of digesta highlights the grinding capacity of the gizzard. These mechanisms help elucidate species-specific digestive dynamics.

**Keywords:** Broiler nutrition; Digestive dynamics; Digestive tract; Pig nutrition

## **Influence of coarse mash and crumbled diets on productive performance and nutrient digestibility of laying hens from 74 to 85 weeks of age**

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Feed presentation form plays a critical role in determining feed intake, nutrient digestibility as well as the productive performance (feed efficiency) of laying hens. Thus, the objective of this study was to compare a crumbled diet (C) with a coarse mash diet (M) on productive performance and total tract apparent digestibility of nutrients. In this context, seven hundred and twenty 16-week-old Lohman Brown-Classic hens were housed in an environmentally controlled barn and randomly allocated in 24 cages of 30 birds each. Birds were assigned to one of the 2 dietary treatments (12 replicates/treatment). The hens were fed a standard diet based on wheat and corn with two feed presentation forms: M (heat treated for 3 min at 83°C) and C (granulated with a steam conditioner at 80°C for 0.5 min and then crumbled with a hammer mill). The particle size distribution of diets was as follows: < 0.5mm, M:11.88 and C:8.38%; 0.5-1mm, M:16.62 and C:10.79%; 1-2mm, M:26.64 and C:29.96%; 2-3mm, M:36.96 and C:38.89%; > 3mm, M:7.89 and C:11.98%). Productive performance was recorded weekly from 74 to 85 weeks of age. At 82 weeks of age, representative samples of excreta were collected from each cage for determination of total tract apparent digestibility of nutrients. Hens offered the C diet had similar ( $P > 0.05$ ) egg production rate, egg mass, feed intake and feed conversion ratio but lower egg weight (C: 67.46 vs M: 68.19,  $P < 0.001$ ) compared to those fed the M diet. Hens fed M diet exhibited higher total tract apparent digestibility of organic matter (M: 81.29 vs C: 77.38,  $P < 0.05$ ), fat (M: 88.52 vs C: 80.25,  $P < 0.01$ ) and protein (M: 54.67 vs C: 41.98,  $P < 0.001$ ) compared to those fed C diet. According to our results, feeding crumbled provided no benefit to productive performance and nutrient digestibility compared to coarse mash diet.

**Keywords:** laying hens; coarse mash; crumbled; egg production; total tract apparent digestibility

## **Use of almond shells as insoluble fibre source for 5 weeks old pullets feeding: effect of particle size and inclusion levels**

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Particle size and incorporation levels in feed of insoluble fibre sources in pullet rearing are of great importance for the digestive tract development. The objective was to study almond shells as insoluble fibre source for laying pullets, evaluating the effect of different particle size and inclusion levels on performance, digestibility and organ size. The almond shells (AS) were ground to two particle sizes: coarse almond shell (CAS), 70% of the particles were distributed from 1400 and 2000 µm of diameter, and fine almond shells (FAS) 76% of the particles were distributed from 1000 µm to 1400 µm of diameter. Solubility, swelling capacity, capacity of water absorption, insoluble and soluble fibre were analysed for both types of AS. For pullet trials, a total of 90 Lohman brown pullets of 5 weeks old were distributed in 30 cages, having 3 pullets per cage and 6 replicates per diet. Five diets were tested, a control diet with 2% of sepiolite as low insoluble fibre diet, and four diets with almond shells as an insoluble fibre source, two diets contained coarse almond shells at 1% (CAS1) and 2% (CAS2) and two diets contained fine almond shells at 1% (FAS1) and 2% (FAS2). Daily feed intake and, initial and final weight were controlled per cage for 4 weeks. On the last week a digestibility study was performed, collecting the total excreta of 4 consecutive days. At the end of the trial the pullets were slaughtered and digestive organs (proventriculus, gizzard, liver, spleen, pancreas, full and empty small and large intestine) were weighted. The data was expressed and analyzed using relative organ weight to pullets weight. No significant differences were found between the two types of almond shells in relation to the solubility, the swelling capacity and the capacity of water absorption. In relation to the chemical characterisation, around 94% corresponded to insoluble dietary fibre in the almond shells. No significant differences were found in the final pullet body weight, average daily gain, average daily intake, the conversion rate neither the digestibility among the diets. Almond shells supplementation increased ( $P < 0.05$ ) the gizzard and proventriculus relative weight of the pullets, although only the organ of pullet fed the diets with CAS were different from the control diet. In addition, almond shells level of inclusion at 2% increased ( $P < 0.05$ ) the gizzard relative weight in pullets. In conclusion, the inclusion of CAS at 2% showed results of great interest due to the significant increase in proventriculus and gizzard, crucial for the future laying hen, without affecting the performance.

**Keywords:** Pullets, insoluble fibre, almond shells

## **Establishment of an immunological cell model for investigating amyloid arthropathy in chickens**

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Amyloid arthropathy (AA) in laying hens is a form of amyloid A amyloidosis in which affected animals suffer from deposits of the acute phase protein serum amyloid A (SAA) in the knee joints. As the disease is caused by bacterial infections, mainly by the pathogen *Enterococcus faecalis* (*E. faecalis*), antibiotics are used to treat it - contributing to the general overuse of antibiotics. Since this secondary amyloidosis is usually observed only in brown egg-laying hens, a genetic predisposition to the disease may be inferred. In white egg-laying hens, we found a fixed-point mutation leading to an amino acid exchange from arginine to serine at position 90 of the SAA protein (SAA.R90S). During acute phase reaction, the serum SAA concentration increases up to 1000-fold, while the apolipoprotein A-I (apoA-I) level decreases. To be transported from the liver to the site of inflammation, SAA binds to high-density lipoprotein and replaces (in mammals) up to 80 % of the apoprotein fraction, due not only to SAA's higher concentrations but also to its more lipophilic properties. Using hepatocellular chicken (LMH) cell lines transfected to overexpress SAA wild-type (WT) and SAA.R90S, we demonstrated that SAA.R90S is expressed and secreted at higher levels compared with WT, indicating that it might not be able to associate with HDL. Thus, we conclude that SAA.R90S is unable to replace apoA-I due to the loss of positive charge and lipophilicity caused by the amino acid exchange. It has recently been shown that SAA in combination with IL-6 induced the differentiation of CD4<sup>+</sup> T cells into pathogenic T helper cells 17 (TH17). We hypothesize that the absence of the circulating SAA-HDL complex prevents the induction of differentiation, which is why the immune response to *E. faecalis* infection differs between white and brown egg-laying hens. For this purpose, we present the establishment of an immunological cell model that can be used to test this hypothesis.

**Keywords:** amyloidosis; avian amyloid arthropathy, serum amyloid A; avian immunology

## **Gut microbiota and host interactions in chickens: a dynamic interplay with performance implications**

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Historically, health issues were addressed through the addition of antimicrobials such as antibiotics, ZnO, CuSO<sub>4</sub>, or antibiotics for prophylactic and metaphylactic purposes. However, emergence of bacterial resistance and environmental pollution have prompted legislation which limits or bans the use of antibiotic growth promoters and ZnO. As a result, there is an increasing focus on studying the microbiota and its interaction with the host. The aim of the current study was to investigate differences in sex and environmental factors. To do this, two trials were performed in Belgium, where microbiota, gut permeability, and ileal gene expression in broiler chickens was tested. Firstly, a small-scale broiler experiment with 144 male and 144 female Ross 308 chicks was performed. The broilers were divided into four pens and monitored until 21 days (d) according to breed specifications but with a diet rich in protein and non-starch polysaccharides (NSP) from wheat and rye. Ileal tissue and content samples were collected, and *in vivo* gut permeability was assessed using FITC-dextran-4kDa. Analysis included body weight, microbiota (V1-V9 16sRNA sequencing), gut permeability, and ileal gene expression (high-throughput qPCR analysis). Secondly, a larger-scale broiler experiment was performed, monitoring two groups of male broilers for 5 weeks under *Enterococcus cecorum* challenge. Each group consisted out of 8 pens containing 20 birds each and monitored till day 35 and were consuming the same diet as in trial 1 (rich in protein and NSP from wheat and rye). Parameters were as in the first trial, with extra sampling at days 28/29 and 35. In the first trial, differences in microbiota and gene expression could be observed between sexes soon after hatching. Microbiota, intestinal permeability and expression of nutrient-transport related genes showed differences at 21d, coinciding with the sex-related growth curve divergence. The results of the challenge trial demonstrate differences in metabolism and nutrient transport gene expression due to the infection, as well as severely diminished performance parameters. In conclusion, sex differences should be considered from early life when optimizing health and performance, while *Enterococcus cecorum* infection results in differential gene expression of a range of genes. This may form the basis of further unravelling the complex dynamic interaction that gut health has with performance of the broiler flock.

**Keywords:** Intestinal health; microbiome; *Enterococcus cecorum*; male v female

## **Antimicrobial resistance profiles of Enterococcus spp. associated to poultry diseases and their susceptibility to probiotic Bacillus**

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Enterococcus spp. are commensal bacteria and opportunistic pathogens that can cause outbreaks in poultry. This work investigated the antimicrobial resistance patterns of clinical isolates and the potential of Bacillus probiotic cell-free supernatants (CFSs) to interact in vitro with their growth. A total of 123 Enterococcus isolates collected from infected birds over 3 different continents (NA, EU, ME) after 2 campaigns (2013/2023) were used in this study after taxonomy confirmation (16S RNA analysis). The broth microdilution method was used to determine the MICs of 8 antimicrobial agents for a subset of 28 isolates. The inhibitory potentials of 4 probiotic commercial strains (*B. velezensis* BS8; 15AP4 and 2084 and *B. licheniformis* DSM 27810), were determined comparing the growth kinetics of the clinical isolates incubated with or w/o 10% of probiotic CFS. Statistical analysis used one way ANOVA and post-hoc means separation Tukey's HSD test (Fit X by Y function of JMP 16.1). Differences were considered significant at  $P < 0.05$ . All tested isolates were susceptible to ampicillin and a limited number showed resistance profile against vancomycin (10.7%) and chloramphenicol (7.1 %). Majority of the isolates were resistant to gentamycin (82.1%), erythromycin (78.6%) and tetracycline (71.4%). The *B. velezensis* CFSs showed a consistent negative interaction with the growth of the 97 tested *E. cecorum* isolates whatever their geographic location, biological or host origin with a percentage of growth of inhibition reaching 80.15% ( $\pm 3.67$ ) for BS8, and 73.14% ( $\pm 2.92$ ) for 15AP4 and 68.67% ( $\pm 3.21$ ) for 2084. The *B. licheniformis* 28710 CFS showed opposite interaction ( $P < 0.0001$ ), resulting in the growth promotion of the clinical isolates ( $- 28.4\% \pm 8.76$ ) highlighting the strain-dependent character of the antagonist potential of probiotic candidates. *B. velezensis* strains also displayed a moderate percentage of inhibition against *E. avium*, *casseliflavus*, *faecalis* and *gallinarum* species (between 40% to 60%) or a strong one ( $> 60\%$ ) against *E. durans*, *faecium* and *hirae*, species also associated to enterococcal poultry diseases. All together these results established the rational to pursue in vivo the assessment of the preharvest potential of *B. velezensis* BS8, 15AP4 and 2084 blend as a long-term and sustainable alternative to antibiotics for Enterococcal poultry diseases.

Keywords: Probiotic; Enterococcal infections; Poultry; Antibiotic resistance

## **Study of the combined therapy of antibiotics and bacteriophages for the control of enterococcus spp. in poultry farming**

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Antimicrobial resistance is one of the major threats to public health and *Enterococcus* have been identified as a major cause of infections worldwide. From an animal health perspective, *Enterococcus* (*cecorum* and *faecalis*) emerges as a clinically relevant pathogen in poultry becoming a significant cause of morbidity and mortality in breeders and broilers. *Enterococcus* is a bacterium with limited therapeutic options, no effective vaccines are available and antibiotic therapy is ineffective once the flock has been infected and shows the first clinical signs. For this reason, the development of new control tools, such as bacteriophages, is becoming increasingly necessary. Phages are postulated as a safe and effective alternative against multi-resistant bacteria. In this context, the aim of this study was to assess the interactions of phage and antibiotic in different doses and evaluate their potential as a complement to antibiotics. To this end, commercial microdilution plates of antimicrobials are used combine with specific phages. Initially, the resistance profile of the target strain was characterized using microdilution plates from a bacterial suspension. Subsequently, the same strain, along with the phage, was subjected to identical incubation conditions. This procedure enabled the assessment of any alterations in the resistance profile, providing crucial insights into the effectiveness of the combined therapy in modifying the strain's antimicrobial sensitivity. The study shows that the dual therapy exhibits promising results in countering *Enterococcus* infections in poultry. Following the application of the phage in combination with antibiotics, a notable increase in sensitivity to antimicrobials such as PEN and TET were observed. Additionally, there was an acquisition of sensitivity to antibiotics like CET, TLS, ERY, DOX, TIA, and LIN, which were previously resistant. The synergistic action of phages and antibiotics presents a potential breakthrough, addressing the limited therapeutic options and potentially enhancing overall treatment efficacy while minimizing the risk of resistance development. The integration of phages with antibiotics can represent a step forward in the pursuit of more robust and sustainable control measures against *Enterococcus* infections in poultry. Nevertheless, more studies are needed not only to replicate these results in vitro but also to observe their efficacy under field conditions.

Keywords: *Enterococcus*; bacteriophages; phages; antimicrobial; resistance; antibiotic;

## Air sample based detection of *Eimeria* spp. in broiler houses

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Coccidiosis is a major pathogen in poultry production worldwide both with respect to economical impact and animal welfare. It is caused by one or more of the *Eimeria* species known to affect chickens. One of the challenges with handling coccidiosis is that traditional diagnostic approaches are labor intensive. Here, for the first time, data is presented on the capture of *Eimeria* in standard 5-minute AeroCollect® air samples. The air samples were analysed for the presence of *Eimeria* genomic material using traditional qPCR analysis. The AeroCollect® is an air sampling device traditionally designed to capture bacteria and viruses from the air in poultry houses to monitor the flocks for infectious diseases. Previously it has been demonstrated that it was possible to capture many different pathogens in the samples such as IBV, IBDV, Salmonella, Campylobacter, Avian influenza, Newcastle disease, and Clostridium perfringens among others. *Eimeria* is much larger than all pathogens previously captured by the AeroCollect®, which is a critical parameter when working with air samples. A controlled infection study where broilers were challenged with three *Eimeria* species at day 15 (*E. acervulina*, *E. maxima* and *E. tenella*) were carried out. *Eimeria* DNA (E5S) was detected in the air samples starting from day 19 (3 days post challenge) and the remaining part of the study. Which corresponds well to the expected start of shedding around day 4 post challenge. Note that in this study it was not possible to detect the individual species directly from the air samples using targeted PCR assays. Only the *Eimeria* spp. analysis had sufficient material to be successful as this assay was more sensitive than the species-specific assays. In a second and larger study broiler flocks, that received a live attenuated *Eimeria* vaccine, were used as positive control flocks. Samples were collected to verify the capture of *Eimeria* in air samples from traditional broiler houses and not just in infection studies. Negative controls were collected from houses in which the broilers received coccidiostats supplement in the feed. In this work in total 72 samples were collected from 5 different vaccinated flocks at three different locations out of which 70 were positive and two were false negative. All samples collected from confirmed negative houses (49 in total) were negative, which correspond to a sensitivity, specificity, and accuracy of the method of 0.97, 1.00 and 0.98 respectively.

Keywords: *Eimeria*; Air samples; diagnostics; coccidiosis



**Turkey productivity improvement and poul gut health support during *Eimeria* spp. challenge using a poultry specific synbiotic****M. Mohl<sup>1</sup>, L. Valenzuela<sup>2</sup>, J. Ruckebusch<sup>3</sup>**<sup>1</sup>dsm-firmenich/Research Center, Tulln, Austria, <sup>2</sup>dsm-firmenich/Technical Marketing, Getzersdorf, Austria, <sup>3</sup>dsm-firmenich/Regional Product Management, Kaiseraugst, AustriaPresenting author: [luis.valenzuela@dsm-firmenich.com](mailto:luis.valenzuela@dsm-firmenich.com)

The turkey industry is continuously seeking effective strategies to enhance the productivity and sustainability of commercially raised birds. This compilation of studies investigates the impact of a poultry specific synbiotic supplementation on growth performance and intestinal health parameters in turkey poults challenged with a mixed *Eimeria* inoculation. A synbiotic feed additive comprising a combination of the probiotic bacterial strains *Enterococcus faecium*, *Bifidobacterium animalis* and *Lactobacillus salivarius*, enriched with a prebiotic derived from inulin fructo-oligosaccharide (FOS) was given to a group of turkeys (T2), while a control group received a standard diet without supplementation (T1). This approach was repeated in two different trials over a period of 14 weeks per trial. Key performance indicators, including body weight gain and feed conversion ratio were calculated, and mortality was monitored. In the two trials, a significant ( $P < 0.05$ ) improvement of FCR was observed at 12 weeks 84 (T1= 2.07 vs. T2= 2.00) and 14 weeks (T1= 2.33 vs. T2= 2.27). Furthermore, the results showed a significant increase in body weight gain (T1= 8.24 kg vs. T2= 8.30 kg) by the end of the trials. In a third trial, a control group (T1) of poults were placed on a standard diet, a second group (T2) on a standard diet containing a synbiotic. In addition, on day 16, the group were subdivided resulting in a 2 × 2 factorial arrangement. Treatments T1I, T2I received an oral dose of *Eimeria adenoides* and *Eimeria meleagritidis*. Body weights were measured at weekly intervals post-challenge, and fecal samples were collected from all pens during day 21 to day 33 to calculate oocyst per gram of feces. Between d21-d28, T2I poults showed a 23% improvement ( $P < 0.001$ ) in percent change in body weight gained relative to T1I (%ΔBWG) and overall weight gain as a percentage (%BWG) was like the unchallenged control (T1). From this study, it was concluded that incorporating a synbiotic into the diet of poults reared to 42 days ameliorates and prevents aspects of performance loss and negative impacts on gut health seen with mixed *Eimeria* inoculation. Overall, the supplementation of a poultry specific synbiotic was successful at increasing performance of turkeys raised for fattening and it provide supports the gut health of challenged birds by coccidia.

Keywords: synbiotic, performance, *Eimeria adenoides*, *Eimeria meleagritidis*, turkeys, probiotic

## **Coniferous rosin-based soap formulations inhibit the sporulation and infectivity of *Eimeria tenella* oocysts in vitro**

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Coccidiosis continues to be a global burden to the poultry industry. Part of the problem is the emerging ability of *Eimeria* oocysts to survive through disinfection practices of poultry houses. Coniferous trees secrete rosin as a defense mechanism against microbial pathogens. Nonvolatile, fat-soluble resin acids are the main constituents of tree rosin. Rosin is antiviral and antibacterial, and it inhibits the germination of clostridial spores, but its efficacy against the oocysts of Apicomplexan parasites has been unknown. The aim of the present study was to assess the effect of tree rosin on the oocysts of *Eimeria tenella*. The following three saponified, water-soluble rosin formulations were tested: rosin soap (RS; with ~90% resin acids), distilled tall oil – rosin soap (~24% resin acids), and gum rosin soap (~24% resin acids). Fresh, unsporulated oocysts were collected and purified from fecal material of *E. tenella* -infected chickens. Three separate in vitro experiments were conducted in phosphate buffered saline (PBS) solution, at room temperature, and under aerobic conditions. A variety of test product concentrations between 0.1% to 4.0% were tested, and compared against the control (PBS only) treatment. Each treatment was replicated twice. The experiments were started by dividing a batch of oocysts into the target solutions, to the concentration at or above 14,000 oocysts/ml. After 0, 4, 24, 48, or 72 hours, the sporulated and unsporulated oocysts were separately counted under a light microscope, using McMaster counting chambers with saturated solution of NaCl for floating the oocysts. The sporulation percentage, oocyst destruction percentage and the percentage of remaining infectious (sporulated) oocysts were calculated for each treatment and time point. In the PBS control, most of the oocysts were sporulated by the 48-hour time point. All test formulations inhibited sporulation and reduced the number of remaining infectious oocysts, compared to the PBS control. The most efficient formulation was RS, which resulted in 99% elimination of infectious oocysts in 24 hours at 1% product concentration. The study showed for the first time that coniferous rosin is effective against *Eimeria* oocysts in vitro. Potential of rosin soap -based cleaning solutions for controlling *Eimeria* pressure in poultry houses should be evaluated.

Keywords: Coccidiosis; *Eimeria tenella*; oocyst; rosin soap; sporulation; infectivity

## **Investigation of the dietary supplementation of lauric and butyric acid glycerides on the performance, intestinal gross lesions and cecal microbiota in a subclinical necrotic enteritis challenge model in broiler chicks**

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Necrotic enteritis (NE) is a poultry disease caused by *Clostridium perfringens*. Various non-antibiotic strategies have been explored to control NE. Organic acids and their derivatives appear to hold promise among these strategies, due to their functionality and stability as well as for their antimicrobial properties. The aim of this study was to investigate the effect of glycerides of lauric acid (C12G, FRA® C12) and of butyric acid (C4G, FRA® Butyrin Hybrid) on the performance, intestinal gross lesions and cecal microbiota in broilers subjected to a subclinical NE challenge model. A total of 256 one-day-old broiler chicks were randomly allocated to 4 treatment groups, with 4 replicates per group, according to the following experimental design: group A, which served as the negative control, Group B challenged with *C. perfringens* and coccidia and served as the positive control, group C challenged and received C4G in starter and grower diet, and C12G in the grower diet; and group D challenged and received C4G and C12G both in starter and grower diet. The birds' performance was assessed by measuring body weight (BW) and calculating feed conversion ratio (FCR) and average daily feed intake (ADFI) during the starter, grower and finisher periods. At days 16, 24, and 35, birds from all the experimental groups were euthanized and their intestines were scored for dysbacteriosis and NE gross lesions. Additionally, ceca were aseptically removed and underwent for microbiological enumeration of *C. perfringens*, *E. coli*, *Lactobacillus* spp., and *Bifidobacterium* spp. Statistical analysis revealed that, birds in group D exhibited an increase in BW compared to the other experimental groups at the 27th day of age ( $p \leq 0.05$ ). Furthermore, the ADFI in groups B and C was significantly increased compared to group A for the grower period ( $p \leq 0.05$ ). The NE gross lesion score and *C. perfringens* counts were significantly higher in challenged groups compared to the negative group ( $p \leq 0.05$ ). However, dysbacteriosis lesion score was significantly lower in groups C and D compared to group B ( $p \leq 0.05$ ). Based on the results, the addition of glycerides of lauric and butyric acid can potentially improve both performance and dysbacteriosis gross lesions in birds suffering from NE. Further investigation is warranted to comprehensively understand the underlying mechanisms behind these observed results and establish optimal dosage strategies for enhancing growth performance and pathogen control.

**Keywords:** Necrotic enteritis, organic acids, glycerides, gut health, microbiota, performance, gross lesions

## **Induction of protective immunity against necrotic enteritis by delivering live *Clostridium perfringens* by the intrapulmonary route at hatch**

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Necrotic enteritis (NE) is a major economically important disease in the broiler chicken industry, caused by *Clostridium perfringens* (CP). Amidst withdrawal of prophylactic antimicrobial use in the broiler chicken industry, CP infections have led to a considerable increase. The objective of this study was to develop a novel vaccination strategy against NE by synergizing immune enrichment with immunomodulation in broiler chicks using synthetic oligodeoxynucleotides containing CpG-motifs (CpG-ODNs) prior to vaccination of broiler chickens with live CP antigens at hatch via the intrapulmonary (IPL) route or delivery of live CP vaccine with no CpG-ODN by the in ovo, then boosting with inactivated CP antigens at day 10 of age by the subcutaneous route. Birds were challenged with CP in feed (feed: media, 1:1) at 20 to 22 days of age, to study vaccine efficacy. Blood, intestinal mucosal scrapings and sections of intestines were collected at 23 days of age for histopathological and serological studies (IgY and IgA). The groups (n=30/group) of birds vaccinated with live CP antigens by the IPL route following in ovo delivery of CpG-ODN or with no CpG-ODN by the in ovo were protected against NE challenge at a significant level with no CP booster (P<0.01). Protection of birds against NE challenge was correlated with IgY and IgA levels against CP antigens. This study demonstrated utility of vaccine delivery utilizing gut-lung axis (GLA) concept in broiler chickens.

**Keywords:** Necrotic enteritis, intrapulmonary delivery, live *C. perfringens*

## Navigation ability of white and brown hens in an aviary system during laying phase

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In cage-free systems, perches allow birds to exercise and to use the vertical space, therefore improving animal distribution. However, hen navigation in the complex aviary environment could result in failed landings and collisions, which could be influenced by genotype and aviary design. We tested the hypothesis that increased perch availability could improve the navigation ability of hens of different genotypes during the laying phase. To this scope, we monitored 1,800 hens (from 28 to 39 weeks of age) housed in a multi-tier aviary system and distributed in 8 pens (225 hens/pen) according to a bi-factorial design with two genotypes (Lohmann White vs. Hyline Brown) and two types of pens (enriched or not with additional perches). In the enriched pens, 6 additional perches (1.20 m long), were placed at the outer net walls and at different heights (0.30, 0.90, and 1.50 m on the left side; 0.60, 1.20 m, and 1.80 m on the right side). Hen movements were video-recorded at 28, 32, 36, and 39 weeks of age, and the number and rate of successful landings on the ground and take-offs to the aviary were scored every 10 min/hour (9:00 to 21:00). Observations were categorized into four periods throughout the day: early hours (5:00 to 08:00), morning (9:00 to 12:00), afternoon (13:00 to 16:00), and evening (17:00 to 21:00). Landings decreased from 28 and 32 weeks to 36 and 39 weeks of age (88 to 78 events per observation hour;  $P < 0.05$ ). The rate of successful landings did not change with age (96.0%, on average), being the highest at early day hours compared with the other periods (99.4% vs. 94.7%;  $P < 0.001$ ). White hens showed a 2.5-fold number of landings per observation hour (119 vs. 48;  $P < 0.001$ ) with a higher successful rate (+2%;  $P < 0.001$ ) compared with brown hens, whereas the presence of additional perches affected neither the number of landings nor their successful rate. Take-offs number (56 events per observation hour, on average) and their successful rate (99.8%, on average) did not change with age. The number of take-off was 3.3-fold in White compared to Brown hens (87 vs. 26 events per observation hour;  $P < 0.001$ ) and 9.3% higher in the pens with additional perches compared to pens without ( $P < 0.05$ ), whereas their successful rate was similar among groups. In conclusion, the navigation ability of hens during the laying period was more influenced by genotype and period of the day rather than by hen age or the presence of additional perches in the aviary.

Keywords: vertical transitions; hen movement; enrichment; collisions; adaptability

## **Tag ‘n’ track: simplifying the validation of animal behaviour tracking technologies with an automated annotation method**

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Recent developments in technology offer a revolutionary opportunity to explore animal behaviour. However, the validation of these advances, although essential, is often neglected because of its laborious nature and its demands in terms of human resources. We tackle the problem of validating technological tools for animal behaviour research. By presenting an innovative automated method that can complement or replace manual annotation, we thereby aim to simplify the validation process. Here, we present the effectiveness of a method based on pattern-marker recognition, associated with IDs. This method utilises a pre-existing open-source visual marking technology. The markers were grid-patterned, QR-code-like, and attached to chickens in order to facilitate the validation of feeder visit monitoring technologies in a research context. To validate our approach, we used 35 short video sequences recorded to capture the chicken's visits at the feeder (i.e., mere presence within its proximity for effective use or not) of a group of 21 chickens. The validation involved comparing the traditional manual annotations made by human observers with the automated annotations we developed. Our automated behavioural annotation method generates annotated videos, revealing the ID of subjects present in the region of interest, as well as the start and end timestamps of their presence. Compared with conventional human observation, our method demonstrated excellent performance: high correlation ( $\rho=0.98$ ,  $p<0.01$ ), 97.63% sensitivity, 99.89% reliability, 99.70% accuracy, 98.82% precision, and 98.09% F1-score. In addition, our method, with the capabilities of the computer used, made it possible to automatically annotate the 35 10-min long video sequences in 6.46 hours, an 84.74% time saving compared to the 42.33 hours that would be required for continuous observation by humans in front of the screen for manual annotation. This method of automated annotation of animal presence in a region of interest offers promising potential to replace or complement manual annotation methods, thus simplifying the validation of new behavioural monitoring technologies, which are essential for meeting the physiological and behavioural needs of chickens and thus promoting their welfare. It encourages the adoption of technological solutions to advance the science of animal behavioural studies to new levels.

**Keywords:** Pattern-Recognition, Automated Behaviour Annotation, Animal Monitoring Technology

## **Development of a temperature based non-invasive sensor system for controlling heart rate during temperature training of broiler embryos in the hatching phase**

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In poultry, temperature training (TT) with short-term increase in incubation temperature (IT) in the hatcher can lead to acute and long-term improvement in performance and robustness. In order to make this method safer for use in practice, a control system is needed that can non-invasively detect changes in physiological signals of the embryo with high sensitivity and thus enable TT fine-tuning. TT with +1°C for 2 hours per day, starting from the normal IT of 37.3°C, was applied at embryonic days 17 to 20. The trials were conducted with Ross 308 hatching eggs (control and TT-group with 30 eggs each) in a hatcher with a capacity of up to 50 eggs (FIEM, MG50H MINILCD, Italy). Temperature sensors (EPCOS B57540G) with a resolution of 0.01°C have been fixed to the egg shell. During test measurements the real heart rate (HR) was measured by needle electrodes applied between egg shell and egg membrane and monitored using a high precision A/D-converter (ADC Pro, Texas Instruments). It was assumed that the change in HR is hidden in the noise of the temperature sensor signal (change in egg shell temperature, sampling rate 100/sec). For detection of HR from the noise a modified Burg-Algorithm has been developed. The results were used to fine-tune TT. Based on the hatching results (hatching rate, chick quality, chick weight) and after 35 days of rearing the growth performance (feed intake, growth rate, body mass) and parameters for robustness (mortality, HLR), the success of TT fine-tuning was evaluated. HR could be detected in the noise of the temperature signal with a probability of 0.4 (mean value). The highest probability value was depending from position of the egg's inside the incubator (max=0.97). When exceeding a critical value of HR (300 b/min) the increase in IT during stimulation was reduced by 0.4 K. The success of this fine tuning could be shown by the recorded production and physiological parameters. Compared to the control, the broilers treated with TT had a similarly good hatching rate (90% in both groups) and chick quality (TT 9.7±0.6 vs. 9.6±0.7 control) and achieved higher body weights. HLR in the TT-group was improved (0.63±0.30 vers. 0.76±0.46) and in agreement with previous studies. Mortality was zero in both groups. The developed monitoring system can be used to fine-tune the TT and would be an important step for the practical transfer of the TT method. The next step is to develop a technical solution to adapt TT for commercial hatcheries.

**Keywords:** incubation temperature; temperature stimulation; hatcher; monitoring; temperature sensor; heart rate

## **Enhancing broiler welfare through precision classification of stress calls in varied environmental conditions**

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Early-stage stress identification in broiler farms is crucial for optimizing growth rates and animal welfare. This study aims to develop an effective method of classification of various types of stress calls in broilers, caused by cold, heat or wind from normal low-stress vocalization, using acoustic signal processing and a transformer artificial neural network (ANN). Two five-week experiments, involving 80 newly-hatched male Cobb 500 broiler chicks each, were conducted. In each experiment the broilers were split into four treatment groups (n= 20 per group): cold - lower environmental temperature (standard management protocol temperature reduced by 8°C); heat - higher environmental temperature (standard management protocol temperature increased by 8°C); and windy - wind speed starting with 0.5 m/s on week 1 and increasing incrementally by 0.5 m/s each week, up to 2.5 m/s by week 5; control - standard thermal conditions. Strict control over environmental parameters facilitated audio recordings capturing the broilers' vocalizations during stress exposure, pre-processed meticulously to isolate stress call events. Our study employed varying sizes of Artificial Neural Network (ANN) models. Notably, the largest ANN model exhibited exceptional performance, achieving a mean average precision (mAP) score of 0.97 for the larger dataset. Interestingly, while exploring different model sizes, minimal variations in performance were observed, underscoring the consistency in precision across these models. Our findings highlight the effectiveness of audio-based neural networks in differential detection of stress-related vocalizations in broilers across diverse environmental stress conditions. Specifically, the highest performing ANN model showcased remarkable precision, signifying its potential as a reliable tool for identifying stress caused by environmental stressors. These results signify a promising advancement in broiler welfare monitoring, offering a precise method for early stress detection and intervention. The deployment of such advanced neural network models holds the promise of significantly improving welfare practices within the poultry industry.

**Keywords:** stress management broilers sound PLF welfare



## **Autonomous bioacoustics system for monitoring broiler welfare in hatcheries using artificial intelligence**

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Among the most vocal species in the livestock sector are birds, particularly poultry, which allows the recording of large amounts of acoustic data to train artificial intelligence algorithms to detect vocalizations. Monitoring animal recalls to improve their production, health, welfare, and impact on the environment is one of the goals of Precision Livestock Farming (PLF) technologies. CEALVET's team created a new PLF technology: an audio recording and processing system, installed for a year and a half in the shipping room of a Spanish commercial hatchery with the aim of recording the vocalizations of newly hatched chicks and evaluating their welfare conditions. The acoustic device is a cloud edge sensor and was installed together with five environmental sensors to measure the level of carbon dioxide (CO<sub>2</sub>), temperature, humidity, light intensity, and colour, simultaneously with the audio data. The system aims to automatically identify, extract and process vocalizations to generate acoustic features and correlate them with traditional environmental indicators of a hatchery, such as CO<sub>2</sub> levels and temperature, which are indicators of stress in animals. High CO<sub>2</sub> concentrations affect the quality of the air that the birds breathe, increasing mortality, respiratory health problems, etc. The external temperature, humidity and air speed are all factors that influence the body temperature of the newborn chick which, being a poikilothermic animal, is not able to control it effectively. All these factors can therefore generate stress and cause significant metabolic stress in chicks, negatively altering their well-being. Both low and high temperatures can pose a threat to the health of chicks. The objective of the research is to study, through the vocalizations features, such as the number of vocalizations per minute and calls pitch frequency, how variations in environmental conditions modify the state of well-being of the chicks. Previous studies have shown that an increase in the number of vocalizations per minute occurs before increasing CO<sub>2</sub> in the room. Moreover, pitch frequency varied depending on the temperature, it may be due to the fact that animals breath more frequent to increase number of calls meanwhile releasing CO<sub>2</sub> This new bioacoustic monitoring system promises to be an innovative PLF tool capable of anticipating and correcting potential stress conditions that could affect chicks in the hatchery, and therefore improve the overall welfare of the poultry.

**Keywords:** Precision Livestock, vocalizations, acoustic analysis, poultry, hatchery, animal welfare,

**Does distribution of light intensity impact broiler production or welfare?****T. Shynkaruk<sup>1</sup>, M. Parsons<sup>1</sup>, K. Long<sup>2</sup>, K. Schwean-Lardner<sup>1</sup>**<sup>1</sup>Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada, <sup>2</sup>Maple Leaf Foods Inc, Mississauga, Canada*Presenting author: tory.shynkaruk@usask.ca*

Broilers raised in enclosed houses are primarily reared under a uniform light intensity. However, it has been suggested they prefer to perform specific behaviours under different intensities. The objective of this study was to determine if rearing broilers under variable light intensity (VLI) impacts their welfare or productivity. Ross 308 broilers (n=7,256) were reared to 35 d in 8 rooms (stocking density 31 kg/m<sup>2</sup>) and exposed to a uniform intensity of 10 lux (control; CON) or VLI with low intensity areas of 2-5 lux (along walls) and high intensity areas of 84-133 lux (interior of room). Parameters evaluated included body weight, feed intake/efficiency, uniformity, mortality, gait, hock, and footpad score, behaviour (percentage of time), melatonin concentration, and heterophil to lymphocyte ratio. The data were analyzed as a complete randomized design using an analysis of variance. Significance was declared when  $P \leq 0.05$ . Birds under VLI had heavier body weights at 10 d, increased gain and feed intake from 0-10 d, and poorer feed efficiency from 0-35 d. The VLI treatment had no impact on uniformity at 28 d or overall mortality. Mortality/cull diagnoses in the skeletal category were lower overall for the VLI treatment and for the 21-35 and 10-35 d periods. Treatment had no impact on footpad, hock, or gait score, heterophil to lymphocyte ratio, or melatonin concentration. At 14 d, VLI birds spent more time standing and at 29 d, CON birds spent more time preening. At 14 d, birds in both treatments spent more time at the drinker, standing, walking, and running in the room interior and more time resting along walls. Birds under VLI spent more time environment and object pecking under high intensity. At 29 d, birds in both treatments spent more time resting along walls and walking in the interior of the room. CON birds spent more time preening along walls. VLI broilers spent more time at the drinker, standing, walking, environmental pecking, and object pecking under high intensity, whereas under low intensity they spent more time stretching and wing flapping. Raising broilers under VLI had little impact on production or most welfare parameters assessed in this study. However, results suggest that birds reared under VLI spend more time performing active and exploratory behaviours under bright intensity. Therefore, satisfying the bird's preference for different light intensities may improve welfare, with little negative impacts on productivity or health.

Keywords: variable light intensity; dual light intensity; behaviour; leg health; stress

## **Stocking density and access to open-air influences feed efficiency in a strain of slow-growing naked neck chicken**

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To limit sanitary risk and maximize the expression of genetic potential, selection of Label Rouge chickens takes place indoors while animals in production are reared with access to outdoors, at lower density and with a less energetic diet. This situation is leading to interactions between genotype and environment that can reduce the effectiveness of selection. Our study thus aimed at estimating the intensity of these interactions on feed efficiency which is an important determinant of the sector's economic profitability and environmental impact. To do this we reared 600 related broilers (300/group) of a naked neck strain either indoors with a stocking density of 5 chicken/m<sup>2</sup> (S) or with access to outdoors from 27 days of age and with a stocking density of 3.5 chicken/m<sup>2</sup> inside and 9 chicken/m<sup>2</sup> outside (P), mimicking respectively selection and production conditions. The two batches were fed with the same diet, close to the one used in selection. Feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were recorded individually and daily from 2 weeks to slaughter thanks to an electronic feed station. FCR was first compared in P and S and the intensity of genotype by environment interaction was assessed by estimating the genetic parameters of FCR, WG and FI between 16 and 75 d in each environment. FCR was systematically higher from 3 weeks of age in P than in S (males: 1.93 ± 0.23 in P, 1.77 ± 0.34 in S; females: 1.90 ± 0.24 in P, 1.77 ± 0.36 in S). This difference was maximal at 37 d, where FCR is 0.43 higher in P than in S and decreased thereafter. Females also had a higher FCR than males from 36 days on. The heritability was comparable between the P and S conditions with regard to FI (P: 0.28 ± 0.16; S: 0.26 ± 0.13) but was much higher in P for WG (P: 0.18 ± 0.15; S: 0.05 ± 0.05) and FCR (P: 0.42 ± 0.15; S: 0.13 ± 0.12). The acquisition of data over a second generation will make it possible to obtain a more precise estimation of heritabilities and genetic correlations between environments to access the genotype-environment interactions.

Keywords: broiler selection, outdoor access, genotype by environment interaction.

## **Relationships between range use, performances and health and welfare related traits in four strains of organic broilers**

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In organic as in other alternative rearing systems, chickens may have access to an outdoor area that allows them to express natural behaviours of the species to a greater extent than in conventional systems. In addition to a higher physical activity, this access to the range is a way to supplement and diversify the nutritional resources. However, a great variability in outdoor use exists, which can impact performances as well as physiology and welfare of the birds, and the product quality. The aim of this study was to assess the relationships between those latter traits and a low or a high range use in four genetic strains of slow (S757N, White Bresse and dual-purpose) or intermediate (JA757) growing rate. In all strains but the dual-purpose, a reduction in carcass weight was observed in high-rangers (HR) compared to low-rangers (LR), which was accompanied by lower thigh and breast weights. Only carcass yield was reduced in HR of the dual-purpose, maybe because of a lower physical activity of this strain on the outdoor area and/or the intake of feed found on the range. Regarding breast meat quality, higher range use led to a higher ultimate pH and a darker colour in the JA757, suggesting that the higher physical activity on the range decreased the muscle glycogen reserves of these birds. Breast meat yellowness was higher in HR than LR in JA757 too, as well as the dual-purpose, probably because of carotenoids intake from the grass. Regarding blood biomarkers, greater higher range use did not affect (in JA757, White Bresse and dual-purpose) or tended to reduce (in S757N) the level of inflammation measured by haptoglobin-like activity. The antimicrobial potential linked to lysozyme activity was also reduced in JA757 and S757N high-rangers. Regarding the redox status, the main effects were observed in JA757 and to a lesser extent S757N, with a reduction in HR group of molecules with antioxidant properties while the concentration of reactive oxygen was increased. In conclusion, this study showed that a compromise must be sought between range use and performances and health and welfare related traits, and that the balance is partly dependent on genetics. Further research is continued to better model these relationships in a population with a continuum of low-ranger and high ranger slow-growing birds. The project PPILOW has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

**Keywords:** OUTDOOR SYSTEM, RANGE USE, PRODUCTION, WELFARE, MEAT QUALITY, GENETICS

## Performance of dual-purpose chickens in organic farming

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Using dual-purpose chickens represents an alternative to the culling of male hatchlings, a practice that has been banned in Germany since 2022. This study aimed to assess the performance of dual-purpose roosters and hens originating from different breeds. Three dual-purpose poultry breeds were studied: Triesdorfer Landhuhn (TLH), a crossbreed incorporating Bresse Gauloise, Italian, Rhode Island red, Sulmtaler, and Sundheimer, Coffee (COF), a crossbreed of Bresse Gauloise rooster x New Hampshire hen, and a crossbreed of Augsburg (a local German breed) rooster x Lohmann Brown-Classic hen (AxLB). The high-performing hybrid Lohmann Sandy (LSa) served as the control group. The animals were housed separately by sex and breed and provided with organic feed. Roosters were raised in two replicates each and slaughtered on day 98 and 140. Feed consumption and body weights (BW) were recorded every 14 days. Pullets were transferred to the laying period at day 126 that lasted until day 504. TLH, COF and AxLB had five replicates each, while LSa had four. Feed consumption was recorded every 28 days, and laid eggs were registered daily. On days 98 and 140, COF roosters had highest BW (2.3 and 2.8 kg) and the lowest feed conversion ratio (FCR) (3.67 and 5.24 kg/kg) compared to AxLB (BW: 1.6 and 1.9 kg; FCR: 4.19 and 5.70 kg/kg) and LSa (BW: 1.5 and 2.0 kg; FCR: 4.17 and 5.63 kg/kg) ( $p < 0.001$ ). No FCR differences were observed between COF and TLH on days 98 and 140 ( $p \geq 0.280$ ). BW of COF was higher than TLH on day 98 ( $p = 0.008$ ), with no difference found on day 140 ( $p = 0.990$ ). LSa produced the highest number of eggs (321 eggs/average hen (AH)), while TLH had the lowest (196 eggs/AH) ( $p < 0.001$ ). AxLB (254 eggs/AH) and COF (261 eggs/AH) showed similar amounts ( $p = 0.304$ ), but COF had a higher egg mass (16.8 kg/AH) than AxLB (15.2 kg/AH) ( $p = 0.002$ ). TLH had the highest FCR (4.43 kg/kg), and LSa had the lowest (2.47 kg/kg) ( $p < 0.001$ ). No FCR differences were found for AxLB (3.14 kg/kg) and COF (3.02 kg/kg) ( $p = 0.372$ ). Calculating FCR for both rooster and hen together, LSa had the lowest overall FCR (2.72-2.85 kg/kg, depending on rooster slaughter time), while COF (3.14-3.35 kg/kg), AxLB (3.29-3.45 kg/kg), and TLH (4.14-4.34 kg/kg) had higher values. The results indicate notable differences in performance characteristics among dual-purpose chicken breeds. The overall lower productivity, when compared to high-performing chicken breeds, leads to higher consumption of resources.

Keywords: dual-purpose chicken; organic farming; laying hen; rooster; performance

## **Egg and meat performance of three dual-purpose hen genotypes reared under free-range conditions**

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In spite of dual-purpose hens are behind of specialized layers, they can lay a respectable number of eggs throughout the year, which are usually of good size and quality, meeting the needs of families or backyard farming. Furthermore, the eggs may reach premium prices due to consumer-perceived positive impacts on health, environment, and taste. On this way, the choice of breed is a critical factor for small- and medium-scale producers, which have regard to the adaptability of animals to various climates, resistance to disease, productive performance and egg quality. On the other hand, there are references about the hen meat is flavorful and has a good texture, making it a suitable choice for home-cooked meals. However, the hens after laying period have little culinary interest for consumers and are a by-product in many countries. Therefore, the objective of this study was to evaluate the egg and meat performance of 3 dual-purpose hen genotypes (Canaria, Novogen Blacktail and Lohmann Dual) reared under free-range conditions and fed with commercial laying feed. Egg production was evaluated from 20 to 74 wk of age. 40 eggs of each genotype were collected at wk 60, 64, 68 and 72 for determinations of egg weight, egg shape index and proportions of yolk, albumen and eggshell. The hens were slaughtered at wk 74 and carcasses were refrigerated for 24 h at 4°C to determine carcass weight, dressing percentage and proportions of breast and legs (drumstick + thigh). For the statistical analysis of the results, the genotype effect was determined using an ANOVA followed by the post hoc Tukey's method. The egg weight of Novogen Blacktail (63.4 g) was significantly heavier than Canaria (60.9 g) and Lohmann Dual (58.5 g). The egg shape index was similar between Canaria (73.9%) and Novogen Blacktail (74.0%) but lower in comparison with Lohmann Dual (77.4%). Finally, the Canaria eggs (31.6%) exhibited the higher yolk proportion respect to Novogen Blacktail (29.1%) and Lohmann Dual (28.5%). Concerning carcass weight, Canaria hens (1.5 kg) presented heavier values than those of Novogen Blacktail (1.1 kg) and Lohmann Dual (1.0 kg). The dressing percentage was lower in Novogen Blacktail hen (58.7%) than in Canaria (61.0%) and Lohmann Dual (60.1%). Regarding cut-up yields, Lohmann Dual (20.3%) and Novogen Blacktail (39.1%) had the higher proportions of breast and legs, respectively.

**Keywords:** egg quality; carcass quality; dual-purpose hens

## **Starling movements: connectivity at the human-domestic animal -wildlife interface**

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Synanthropic bird species found in human, poultry or livestock environments can increase the spread of pathogens and antibiotic-resistant bacteria between wild and domestic animals. We aimed to determine if connectivity among habitat patches, exerted by spotless starling movements, is related to the prevalence of livestock/human pathogens. We captured 28 starlings on a partridge farm in 2020 and sampled them for viruses such as Avian influenza virus, West Nile virus WNV, Avian orthoavulavirus 1, Coronavirus and enterobacteria *Escherichia coli* and *Salmonella* spp.). We did not detect any viruses or *Salmonella*, but one individual had antibodies against WNV or cross-reacting Flaviviruses. We found *E. coli* in 61% (17 of 28) of starlings, 76% (13 of 17) of which were resistant to gentamicin, 12% (2 of 17) to cefotaxime/enrofloxacin and 6% (1 of 17) were phenotypic extended spectrum beta-lactamase (ESBL) carriers. We GPS-tracked 17 starlings tested for pathogens and constructed spatial networks showing how their movements (i.e. links) connect different farms with nearby urban and natural habitats (i.e. nodes with different attributes). Using *E. coli* carriage as a proxy for pathogen acquisition/dispersal, we found differences across spatial networks constructed for *E. coli* positive (n=8) and *E. coli* negative (n=9) starlings. Centrality metrics, community structures and Exponential Random Graph Models revealed significant differences between networks. An urban roosting site was more connected to other habitats by movements of *E. coli* positive starlings than by movements of *E. coli* negative starlings. In contrast a pine forest used for roosting was more connected by movements of *E. coli* negative starlings than by movements of *E. coli* positive starlings. Our results show how habitat connectivity can covary with pathogen prevalence. And although we cannot infer causality suggest the potential of starlings to spread pathogens such as antibiotic resistant bacteria among farms, urban and natural habitats. Telemetry-based networks show how bird movements may affect farm biosecurity and pathogen transmission. Such studies can aid implementation of biosecurity measures to support sustainable and resilient farming systems in a One Health context.

Keywords: *Sturnus unicolor*, Pathogens, Antibiotic Resistant Bacteria, Spatial Networks, Movement Ecology, One Health, *E.coli*

## **Bonelli's eagles as a biomarker: multi-resistant strains and biosecurity in a One Health framework**

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Livestock and poultry farms offer valuable resources for wildlife because of the availability of food and shelter. Interactions between poultry and wild birds, whether through direct or indirect contact, are sometimes unavoidable, particularly in extensive production farms. These interactions can facilitate the recirculation of pathogens and antimicrobial resistance (AMR) in both directions. Wild birds can contaminate the soil of poultry farms and even crops, introducing AMR into feed production. Therefore, epidemiological studies in poultry should always be assessed from a One Health perspective, which include wild species. In this context, some raptors have been considered good biomarkers for such studies, as they occupy the top of the food chain in ecosystems. The aim of this study was to assess the occurrence of AMR in *Escherichia coli*, which is considered a sentinel bacterium for AMR strains isolated from free-ranging Bonelli's eagles (*Aquila fasciata*) inhabiting areas with a high density of livestock, including poultry. To achieve this, in April and May of 2022 and 2023 fresh fecal samples were collected from 17 nests, and individual cloacal swabs were obtained from a total of 31 nestlings. After pre-enrichment in buffered peptone water, *E. coli* was isolated from tryptone bile X-glucuronide (TBX) agar cultures, and antimicrobial susceptibility tests were performed by Mueller-Hinton broth microdilution using Sensititre Vet Avian AVIAN1F® plates. The statistical analysis was performed using a GLM with the probit link function (significant differences with a p-value < 0.05). Overall, *E. coli* was isolated from 100 % of the nest and from 93.6 % of the swab samples. From the *E. coli* isolated, AMR was detected in 35.3 % of nests and 48.3 % of nestlings. In addition, 33.3 % of the resistant strains from nests and 28.6 % from nestlings were considered multidrug resistant strains. The antimicrobials with the highest resistance rates were amoxicillin and tetracycline (19.6 % each), followed by streptomycin (17.4 %). The presence of multi-resistant strains in Bonelli's eagles demonstrates the circulation between farms and wildlife within a common ecosystem, serving as a significant biomarker. Hence, it is imperative to implement biosecurity measures to prevent the dissemination of these strains in both directions within a One Health framework.

Keywords: One Health; wildlife; poultry; antimicrobial resistances







**XVI European Poultry Conference**  
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## DIGITAL POSTERS

### SESSIONS

Ordered chronologically by Slot (Time of presentation)



## Study of economic parameters in Uttara chicken: A promising native chicken of Uttarakhand state of India

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Uttara chicken, an indigenous chicken breed of India found in Kumaon region of Uttarakhand state of the country. Birds of this indigenous chicken breed have economic, nutritional and cultural importance and mainly kept for meat and eggs under backyard poultry farming system. Their products are good source of income and even they are immediate or ready source of cash when it is needed in emergency in households. These birds' possess appreciable degree of resistance to diseases in its natural habitat under free range system as compared with other exotic breeds of chicken. Uttara chickens are also resistant to cold winter stress and can thrive very well under adverse environments like poor housing, poor management and poor feeding. These birds are black in colour and having crest/crown structure on their head and feathers on shank. Considering importance of Uttara chicken, a study was conducted to evaluate the production performance under farm conditions. A total of 275 chicks of the Uttara fowl were hatched out at University Poultry Farm and distributed through completely randomized design (CRD). The experimental data obtained during the study were finally analyzed. The average body weights of Uttara hens were studied at day old, 4th, 8th, 12th, 16th, 20th and 24th weeks of age and were found as 36.86±0.39, 130.84±2.62, 413.34±7.55, 716.50±11.69, 1018.78±13.33, 1271.59 ±17.61 and 1484.22±19.22 g, respectively, whereas Uttara cocks had 36.98±0.58, 146.43±5.83, 445.03±9.59, 810.32±16.35, 1180.98±25.07, 1435.39±25.66, 1699.43±33.49 g body weights in respective ages. The mean values of age at first egg, body weight at first egg, weight of egg at maturity, 24th, 28th, 32nd, 36th, 40th and 44th weeks of age as 161.5±3.05 (days), 1422.50±31.21, 37.05±0.93, 41.30±1.08, 44.80±0.79, 47.80±0.63, 51.40±0.68, 53.20±0.92 and 54.40±0.84 g, respectively. The average number of egg production up to 72nd week of age was 177.35±6.34 eggs. The mean values of egg weight (g), shell weight (g), shape index, Haugh unit, albumen index, yolk index, yolk weight (g), shell thickness (mm) were reported as 47.80±0.63, 6.16±0.10, 74.31±0.41, 75.20±0.55, 0.082±0.003, 0.44±0.01, 14.80±0.32 and 0.43±0.032, respectively. The results may be of little significance but it holds the key to explore this native chicken of great importance for further improvement for their production parameters through suitable breeding methods along with conservation efforts.

Keywords: "Native chicken"; "Uttara chicken"; "Backyard poultry"; "Livelihood security"; "Nutritional Security"

## **Sea urchin waste as a sustainable source of calcium in laying hens production**

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In Italy, about 2.000 tons of purple sea urchins (*Paracentrotus lividus*) are caught every year. Gonads are the only edible part, that represent a small percentage (10-30%) of the total mass of the sea urchin. Consequently, all the remaining constitutes a waste, which must be disposed off in landfills, an environmentally and economically unsustainable practice. It was demonstrated that the sea urchin test is mainly composed of calcium carbonate (calcite) rich in magnesium, but it also contains other antioxidant molecules, such as polyphenolic compounds. In this study sea urchin wastes were collected from restaurants and food industries, then they were dried and ground, and then included in laying hens feed, as a substitute for non-biogenic (rock-derived) calcium carbonate. The aim was to assess if this substitution could have effects on egg quality and animal welfare, considering that a calcium deficiency has critical impacts on egg production, shell quality, and welfare. One hundred twenty-eight hens were reared in enriched cages, divided into two groups (control and treatment), and their welfare was assessed based on their feather condition, in accordance with the Welfare Quality® protocol. At the end of the cycle, the bones breaking strength was measured. Moreover, every month, 48 eggs per treatment and their components were weighed, shell thickness and yolk colour were assessed, and the eggs breaking strength was measured. Shell weights did not differ significantly between the two groups, but the treated group showed significantly thicker shell, probably affecting the shell barrier function. Despite the increased thickness, no significant differences were found in the breaking strength. Concerning the welfare evaluation, the treated group showed fewer feather lesions in the back, tail, and crest area. Additionally, no significant differences were found between the two groups regarding tibia breaking strength. The absence of differences in egg quality and in the bones breaking strength, and the positive results obtained in the welfare evaluation confirm the feasibility of using sea urchin waste as a sustainable alternative to calcium carbonate.

**Keywords:** Sea urchin; waste; calcium: laying hens; production; welfare

## **Effects on the carbon footprint of chicken meat by an infection with *Eimeria* spp**

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The carbon footprint of chicken meat is highly linked to the performance of the broiler. An infection disease could result in a negative influence on chicken performance and this may lead to deductions in sustainability. Based on a systematic literature study the effects of an infection with coccidia (*Eimeria* spp.) on the performance (live weight, average daily gain [ADG], feed conversion ratio [FCR]) and mortality of broilers were monitored. Trials with complete data set were afterwards used to perform a life cycle assessment (LCA) to determine the carbon footprint (CO<sub>2</sub> eq per kg live weight) of this specific phase using the software application Opteinics™. Therefore, it was assumed that all broilers got the same diet based on wheat (Germany), soybean meal (Brasil) and maize (Germany) as it was used as a control diet in earlier trials. Further consumptions (e.g. use of energy and fuels) and former/later stages of production were excluded. In total more than 30 publications with nearly 40 trials from 2000 to 2023 were included in this research. All of them included a challenge with different amounts of different *Eimeria* spp. between day 14 and 21 of life. The ADG in challenged groups decreased by up to 50% and the FCR turned out to be about up to 70% higher when compared to control groups. The mortality rate in challenged groups was about 40%-points higher when compared to the unchallenged control groups. The calculation of LCAs for more than 20 trials containing weight development and feed intake showed an increase of up to 96% in the carbon footprint of birds which underwent an *Eimeria* challenge in comparison to the unchallenged control groups. This level representing the highest difference may be for example traced back to a trial focussing on a 28-days period (starting point: day 8) with a challenge of 40,000 *Eimeria tenella* sporulated oocysts on day 21 of life. The mean increase in carbon footprint along all trials was 22%. Focusing on trials from Europe, only few data were noted regarding the performance of broilers in relation to a challenge with *Eimeria* spp. Nevertheless, it could be concluded from this few studies of suitable publications, that the carbon footprint of the trial period increased markedly due to a challenge with coccidia. Therefore, datamanagement is an important part to reach more resilient data on this topic and preventive measures and health monitoring of broilers are and will become even more significant parts of a more sustainable chicken meat production. Acknowledgments: The project was supported by the German animal health industry association (Bundesverband für Tiergesundheit e.V., BfT).

Keywords: sustainability, carbon footprint, broiler, coccidia, *Eimeria* spp.

## **Feasibility of purified rainwater as drinking water for laying hens**

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Recent dry summers in Flanders have led to a significant decline in the groundwater table, a primary source of drinking water for laying hens. While rainwater could be used as an alternative, concerns about contamination have made farmers hesitant to embrace this water source. To demonstrate the feasibility of purified rainwater as drinking water for laying hens, a rainwater purification system (ACLIMA-project - contract number LIFE 20 CCA\_BE\_001720) was installed and tested in the laying hen stable at the Experimental Poultry Centre in Geel, Belgium. The system employed nano-ultrafiltration using recycled renal dialysis filters, combined with an end-of-line dosage of hydrogen peroxide (100ppm) to purify rain water. Following a year-long monitoring of water quality, the purified rainwater was utilized as drinking water for laying hens of 68 weeks until 76 weeks of age in a comparative experiment. The experiment was conducted in furnished cages, consisting of 4 tiers and 8 cages per level. In each cage, 48 hens (Isa Brown) were housed. Half of the trial groups (768 hens) were provided with tap water (TW), while the other half (768 hens) received purified rainwater (RW). Water samples were analyzed in week 69, 71, 73 and 75 for biological parameters: Total Bacterial Count (TBC) at 22°C, TBC at 36°C, Coliform bacteria, *E. coli*, Enterococci, yeast and molds, sulfite-reducing anaerobes and *Clostridium perfringens*. During the trial, none of the parameters measured in either purified rainwater or tap water exceeded the Belgian standards for poultry drinking water. As the experiment continues, initial findings from the first production cycle reveal specific trends. No significant differences are observed in mortality, water intake, feed intake, feed/water ratio, feed conversion, and laying percentage. Prior to the start of the trial, a Kruskal-Wallis test indicated no difference in egg quality parameters: egg weight, albumen height, yolk color, Haugh units, shell strength, shell thickness and yolk height. However, post-trial, a significant decrease is observed in the RW-group as compared to the TW-group, specifically in albumen height (5.37mm vs. 5.96mm;  $p = 0.040$ ;  $n = 60$ ), Haugh units (70.02 vs. 74.15;  $p = 0.040$ ;  $n = 60$ ) and shell thickness (0.40mm vs. 0.43mm;  $p = 0.004$ ;  $n = 60$ ). This study demonstrates the feasibility of purified rainwater as a suitable drinking water source for laying hens. Although an exploratory trial revealed no differences in technical parameters, it does suggest a potential decline in several egg quality parameters.

**Keywords:** Water; Drinking water; Water quality; Purified rainwater; Climate change; Climate resilience; Technical performance; Environmental impact

[P1-05]: P1-Welfare, Sustainability, Phytogenic, Egg quality, Respiratory diseases Food Safety (ID: 131150) - Italy

## **Improve broiler production sustainability reducing N and P excretion through diet optimization with multi-enzymatic complex with high doses of phytase**

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The excretion of nitrogen (N) and phosphorus (P) is directly linked to sustainability. Decreasing their release into the environment is important to reduce environmental phenomena such as acidification and eutrophication. The objective of this trial was to measure the effect of a multi-carbohydrase complex with high dose of phytase (MCPC) on N and P excretion reduction. In a fully randomized design, 280 Cobb 500 one-d-old male chicks were raised in battery cages until 21 d-old. They were divided into 4 treatments, each with 10 duplicates of 7 birds. Treatments: PC: corn-soybean meal standard diet employed by Brazilian integrators without the addition of enzymes; NC: PC diet with nutrient reduction in AME (-5%), digestible amino acids (dig. AAs, -5%), Ca (-0.16pp), Av. P (-0.18pp), and Na (-0.03pp) without enzymes; PC+MCPC: PC supplemented with 100 mg/kg of multi-carbohydrase complex with 1000 FTUs of phytase (MCPC; Rovabio® Advance Phy T, Adisseo, Fr) on top; NC+MCPC: NC optimized with MCPC. Celite 1% was used as indigestible marker for the N and P excretion calculation on day 21. Data were analyzed using ANOVA and compared with Tukey's test ( $P < 0.05$ ; Proc GLM of SAS). N excretion increased by 16.1% in NC-fed chicken compared to PC (10.687a vs 9.203b g N/kg of chicken,  $P < 0.0001$ ). However, animals fed NC+MCPC result in a 23.8% N excretion reduction compared to PC (7.010c vs 9.203b g N/kg of chicken,  $P < 0.0001$ ). Also broilers fed with PC+MCPC diet presented a significant reduction in N excretion compared to PC, equal to -28.7% (6.563c vs 9.203b,  $P < 0.0001$ ). P excretion was reduced by 13.9% already in NC-fed animals compared to PC (3.866b vs 4.489a,  $P < 0.0001$ ). PC diet optimization with MCPC considerably reduced P excretion by 43.9% (2.520c vs 4.489a,  $P < 0.0001$ ). Finally, animals fed NC+MCPC diet presented the lowest P excretion, reduced by 67.3% compared to PC (1.467d vs 4.489a,  $P < 0.0001$ ), and keep the same feed conversion rate ( $P < 0.0001$ ). No differences in mortality were found among the treatments. The presented study demonstrated that EMA, AAs, Ca, P and Na reduction allows to increase P use efficiency. However, likely due to diet imbalance and nutrient reduction, results didn't show the same for N excretion that increase in NC-fed animals. When optimizing diets with MCPC, a considerable reduction in excretion of both N and P occurred, demonstrating improved efficiency with less environment and economical waste, enhancing sustainability of poultry production.

Keywords: Nitrogen; Phosphorus; Enzymes; Digestibility; Sustainability



## **Comparing the improvement in feed sustainability through multi-carbohydase complex with high doses of phytase using two different GFLI databases**

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Approximately 70% of emissions per kg of broiler come from feed production. Therefore, it is important to minimize feed carbon footprint (CFP) and increase sustainability of poultry production. The objective was to calculate the effect of the use of multi-carbohydase complex with high dose of phytase (MCPC) on the environmental impact of a poultry diet by comparing the results obtained with GFLI global database and GFLI-Brazil. A total of 1,200 Cobb 500 one-d-old male chicks were raised until 43 d-old and distributed into 4 treatments with 10 replicates of 30 birds each, in a completely randomized design. Treatments: PC: standard corn-soybean meal diet used by Brazilian integrators without enzymes; CN: PC diet reduced in formulated AME (-5%), digestible amino acids (dig. AAs, -5%), Ca (-0.16pp), Av. P (-0.18pp), and Na (-0.03pp) without enzymes; PC+MCPC: PC supplemented with 100 mg/kg of MCPC (Rovabio® Advance Phy T, Adisseo, Fr, with 1000 FTU phytase) on top; NC+MCPC: NC also with MCPC. The impact in kg CO<sub>2</sub>-eq per kg of feed was calculated using GFLI database (ReCiPe method, economic allocation) and compared with GFLI-Brazil database. Both databases follow the GFLI-methodology, but GFLI-Brazil uses the BRLUC Model to calculate the Land Use Change obtaining different results with higher Tier. The functional unit is 1 kg of chicken live weight. Data were analyzed using ANOVA and Tukey test ( $P < 0.05$ ; Proc GLM of SAS). Comparing NC and PC diets, the CFP (kg CO<sub>2</sub>-eq) per kg of live broiler was decreased by 4.6% in GFLI global database (3.915 vs 4.103 kg CO<sub>2</sub>-eq/kg of chicken,  $P < 0.0001$ ), while using GFLI-Brazil the decrease reached 2.4% (2.048 vs 2.099 kg CO<sub>2</sub>-eq/kg of chicken,  $P < 0.0001$ ). It means despite the low carbon footprint of the diet in NC, the lower performance brings worst carbon footprint for NC group. Comparing PC+MCPC with PC it was observed a 3.5% reduction in the CFP with both GFLI and GFLI-Brazil. Animal fed NC+MCPC decrease diet CFP by 9.1% compared to PC using GFLI global (3.729 vs 4.103 kg CO<sub>2</sub>-eq/kg of chicken,  $P < 0.0001$  and 7.1% in GFLI-Brazil (1.950 vs 2.099 kg CO<sub>2</sub>-eq/kg of chicken,  $P < 0.0001$ ). Diet optimization with MCPC allows to maximize the reduction of the CFP of poultry production, improving sustainability, regardless of GFLI database. Due to the different methodologies used in the two databases GFLI global and GFLI-Brazil, authors obtained proportional, but different, values always positive for the use of the enzymes.

Keywords: Enzymes; Feed; Sustainability; Carbon footprint; Broiler

## **Effect of the addition of protected sodium butyrate in broiler diet on nitrogen and phosphorus excretion**

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Nitrogen and phosphorus excretion is an important negative environmental impact relate to livestock. On the other hand, protected sodium butyrate (PSB) increases the digestibility of nutrients in broilers, improving productive efficiency and utilization of the diet. The effects of the addition of PSB (Butirex C4, Novation 2002, Spain) in the diet on nitrogen and phosphorus excretion broiler chickens were studied in broilers from hatch to 21 d of age. 800 one-day-old Cobb 500 broiler chicks that were randomly assigned into 4-dietary groups with 8-replications each. The dietary treatments consist in 4 PSB level of inclusion: control (diet was based on corn and soybean meal without PSB inclusion) and the 3 remaining experimental diets with linearly doses level (500, 750, and 1000 g/ton, respectively). The feeding program consisted of 2 phases: pre-initial (from 1 to 7 d) and initial (from 8 to 21 d). Crude protein content was 21 and 20% respectively, and total phosphorus was 0.72% in both phases. Individual BW of the broilers and feed disappearance were recorded by replicate at 7 and 21 d of age. Any mortality was recorded and weighed as produced. From these data, productive performance was determined by period and cumulatively. Nitrogen and phosphorus excretion was determined by replicate as indicated by Ministerio de Agricultura y Pesca, Alimentación y Medioambiente (2017). Data on growth performance and nutrient excretion were analyzed as a completely randomized design with diet as main effect using the GLM procedure. For the entire study (0 to 21 d of age) an increase in the PSB content in the diet increased ADG linearly ( $P < 0.001$ ) and improved FCR ( $P < 0.01$ ) but feed intake was not affected. Nitrogen and phosphorus excretion was lower in broilers fed diet supplemented with PSB, without differences between doses. In conclusion, the use of PSB can be a very interesting nutritional strategy to reduce the impact of chicken breeding.

Keywords: Nitrogen; phosphorus; sodium butyrate

## **Sustainable utilisation of eggs lost during further processing and packaging**

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In 2020, a total of 11 Mio tons of food waste were produced. Distinction is made between unavoidable losses, such as food that is not edible and avoidable losses. The latter often contains production losses, which may occur during processing. Food is still edible at the time of disposal but cannot be marketed. Since 2019 the German strategy to reduce food losses is in place, focusing especially on valorization of avoidable food loss. In addition to generally avoiding food waste, the implementation of a circular economy with an extended life cycle through recycling and re-use of the raw materials is a key focus area of this strategy. While fresh eggs with small cracks detected during packaging may still be used for the production of liquid full egg, cooked eggs with cracks are mainly sent to biogas production. The present study therefore investigates processing and preservation techniques for production waste from cooked, coloured eggs and evaluates potential for further use. Results show that egg white and egg shell may be dried and grinded to powder, while egg yolk has a too high fat content for powder production. More advanced techniques such as freeze drying may be necessary. Powder production preserves the product, facilitates further logistics and reduces the weight. Egg white powder may be a valuable protein source for food and feed, taking into consideration that proteins are denatured due to the cooking process but amino acids remain. In the present study, the microbiological status did not give reason for concern. However, legal aspects must be considered. Egg shell powder may be used as valuable calcium source in food and feed, as fertilizer, in the construction industry or even for medical applications depending on legal aspects. Costs and effort for separation and processing of components must moreover be considered. Alternatively, the direct use of the cooked eggs is evaluated. Due to the cracks in the egg shell, the eggs have a short shelf-life and would have to be immediately peeled, refrigerated or frozen for use in the food industry. For use in feed, certification is often needed. However, eggs may be used as substrate for insects such as black soldier flies, which can be used as regional protein source in pig and chicken feed to partly replace soy bean imports. Composition of the larvae that emerge from an egg-based substrate and the need for certification of eggs as raw material remain to be investigated.

**Keywords:** Circular Economy, processing waste, protein powder, shell powder, black soldier flies

[P1-09]: P1-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseases Food Safety (ID: 131291) - Italy

## **Effect of genotype on human-edible protein conversion efficiency: valorisation of local chicken breeds through cross-breeding strategy**

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Commercial egg production relies almost exclusively on the use of selected hen hybrids, which show higher productive efficiency than local chicken breeds but they have a scarce attitude for an efficient production of meat. In this study, a commercial laying hen hybrid (Lohmann brown, LB), two Italian local breeds (Bionda Piemontese -BP- and Robusta Maculata -RM-) and their crossbreeds with a dual-purpose genotype (Sasso -S-; BPxS and RMxS) were compared for the efficiency in human-edible protein production. The protein conversion efficiency was calculated taking into account the feed intake and the body weight of the ready-to-lay pullets at 25 wks of age as protein input, and both egg and meat as protein output. For each genotype, 60 laying hens were reared from 25 to 66 wks of age in an experimental farm under homogenous conditions and using the same 3-phase feeding program (25-51 wks, crude protein -CP- 17.8%; 52-62 wks, CP 17.0%; 63-66 wks, CP 15.8%). The protein content of the edible portion of eggs collected during the laying period as well as boneless meat obtained at the end of it was estimated to determine the effects of the genotype and to validate the effectiveness of crossbreeding strategy. On average, a protein output of 1,504 g/hen was obtained, deriving primarily from eggs (87.6%). As expected, large differences were found according to the genotype. Indeed, while LB produced 2,049 g of proteins (94.3% from eggs), local breeds and crossbreeds showed an average reduction of 971 and 392 g of protein output, respectively. In terms of protein conversion efficiency, LB presented the highest value (27.1%) and the purebreds the lowest ones (BP=17.0% and RM=17.7%), while crossbreeds showed a better efficiency than the corresponding local breed (BPxS=22.1% and RMxS=22.2%). In conclusion, the crossbreeding strategy allowed to improve human-edible protein conversion efficiency through a greater egg productivity and a slight increase in boneless meat production, contracting the gap between the purebreds and the commercial hybrid while potentially facilitating the conservation of such endangered Italian chicken breeds. A Life Cycle Assessment study conducted with a “gate-to-gate” approach including both the pullets rearing phase and the deposition cycle is ongoing to estimate the environmental impacts associated with the total production of human-edible proteins from egg and meat.

**Keywords:** laying hen; local breed; crossbreed; protein conversion efficiency; human edible protein; egg; meat; Life Cycle Assessment

[P1-10]: P1-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseases Food Safety (ID: 130731) - Germany

## **Pecking behaviour of turkey hens in proximity to the feeding pan – any influence of beak condition?**

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Experience has shown that turkeys increasingly tend to peck at conspecifics when they are near the feeding area, thus in direct relation to their feed intake. This behaviour can cause serious pecking injuries. This pilot study investigated to what extent the pecking behaviour at and in proximity to the feeding pan of female fattening turkeys with intact and trimmed beaks differed. Fattening turkey hens (B.U.T.6) were housed in four 35m<sup>2</sup> compartments (143 animals each, 2 compartments with untrimmed and 2 with beak trimmed birds) under practical housing conditions (stocking density 48 kg/m<sup>2</sup>). From 6 to 16th week of life (LW), videos were taken in proximity to both offered feeding pans (observed area: one hens' body length proximity around the feeding pan) from 2 to 4 pm two days a week. One focal animal was observed per feeding pan (total n=8 per LW, observed animals with intact beaks n=330, observed animals with trimmed beaks n=329). At four points of time in a 30-minute interval the duration of the following behaviours in the observed area was recorded: feeding (F), pecking at the ground (GP), pecking at conspecifics (CP), and being pecked at (SP). The average feeding duration (65s) did not differ between the trimmed and the animals with intact beaks. There was neither a difference in duration for the behaviours CP (11s) and SP (7s). Only pecking on the ground differed between the observed animals. Overall, more turkey hens with intact beaks (n=169 vs. 142) were observed pecking at the ground. Likewise, the average duration of GP (18s) was higher than that recorded for birds with trimmed beaks (15s). Although more and more severe injuries and higher losses due to injurious pecking occurred over the fattening period in the turkey hens with intact beaks, the results of this pilot study do not indicate that this is obligatorily related to the duration of the observed behaviours. At least during the period observed here, there were no differences in the tracked focal animals with respect to the duration of pecking at conspecifics or being pecked by them. Thus, beak condition had neither an effect on feeding duration nor behavioural disorders regarding aggressive pecking towards conspecifics in the proximity to the feeding pan.

Keywords: turkey hens; pecking behaviour; beak condition; feeding pan

## **Effect of stocking density on performance and footpad dermatitis in broilers**

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The study was conducted to evaluate the impact of stocking density on performance and the occurrence and severity of footpad dermatitis (FPD) in broilers. Total of 185 Ross 308 broilers were randomly allocated to two treatments with five replicates each. Broilers were kept in floor pens with straw bedding. The treatments consisted of two stocking densities: 33 kg/m<sup>2</sup> (17 birds per pen; LD treatment) or 39 kg/m<sup>2</sup> (20 birds per pen; HD treatment). The trial lasted for 42 days. Birds of both groups were fed a commercial diet: starter (1-10 days), grower 1 (11-21 days) grower 2 (22-31 days) and finisher (32-42 days). Housing conditions were controlled automatically and adjusted according to ROSS 308 recommendations. The incidence of footpad dermatitis was evaluated at the age of 3, 4, 5 and 6 weeks by the method described by Eichner (2007): no lesions (0), lesions cover less than 25% of the footpad (1), lesions in wide areas, covering between 25% and 50% of the footpad (2), more than 50% lesion on the footpads (3). No significant influence of stocking density was found on body weight (2737 g (LD) vs. 2687 g (HD)), average daily feed consumption (113 g (LD) vs. 108 g (HD)), or feed conversion ratio (1.73 (LD) vs. 1.69 (HD)). FPD score significantly increased from 3rd to 6th week in both groups. At 3rd and 4th week of age there was no effect of stocking density on average FPD score ( $P > 0.05$ ). However, stocking density significantly influenced severity of FPD starting from week 5. The average FPD score in 5th week was lower in the LD treatment ( $P < 0.05$ ; average score 1.20) as compared to the HD treatment (1.34). At 6 weeks of age the average score was 1.30 in the LD treatment and 1.68 in the HD treatment ( $P < 0.01$ ). Based on the results of this trial it can be concluded that the influence of stocking density on performance was not significant. However, the high stocking density had a significant negative effect on severity of FPD in broilers in the last two weeks of fattening.

**Keywords:** broiler, stocking density, performance, FPD

## **Effects of a twin shell building and proactive ventilation on litter humidity and health of broiler chicken**

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The purpose of this study was to investigate the effects of proactive ventilation broiler barns on the welfare and performance of the animals. The hypothesis was that these measures can result in decreased litter humidity and thereby improve footpad health. The study was performed on a conventional broiler farm. A trial barn (T) was equipped with twin shells to improve insulation and proactive ventilation. Fresh air was pre-warmed/cooled in a central air intake between the shells. Consequently, spray cooling was not necessary, contributing to further reduction of litter humidity. The control barn (C) was equipped with a single shell and standard negative pressure ventilation. The study is planned for ten consecutive fattening periods, each starting with 25,000 Ross 308 animals per barn. On day 30 half of the animals is removed, the remainder is fattened until day 42. Litter humidity was measured bi-weekly at 24 locations per barn. Fifty animals per barn were weighed and examined for footpad health bi-weekly using a five-point -scale (from 0=no alteration to 4=more than 50% of surface altered). Occurring infections and use of antibiotics were documented continuously. So far, the modified building was tested over five fattening periods. Litter humidity was decreased in T (31 vs. 50% in C;  $p<0.001$ ). Footpad score in T was improved (0.30 vs. 0.88 in C;  $p<0.001$ ). Weight of the animals was similar in both barns (2,476 g in T vs. 2,483 g in C at day 42;  $p=0.932$ ). No differences in respiratory disease occurrence and use of antibiotics were detected. These results indicate that the equipment of broilers barns with twin shells and proactive ventilation might contribute to improved litter quality and improved footpad health, while not affecting weight gain. However, the study included one single farm only, and trial and control barns could not be changed. Consequently, effects of farm and barn could not be excluded.

**Keywords:** broiler chicken, footpad health, respiratory health, barn ventilation

## **Hanging poultry scales as a model for environmental enrichments in commercial broiler houses**

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Hanging platform scales (HPS) do not qualify as enrichments for some certifiers; however, broiler growout managers often note a high usage of these pieces of equipment by broilers. The movement offered by the swinging scale may suggest future designs for enrichments, especially as increased activity and exercise can positively impact welfare and leg health. A HPS is affixed by a single bar attached perpendicularly to the flat platform of the scale. The bar hooks into an eye bolt on a ceiling scale mount, and is adjusted to hang about 2 cm off of the litter. This setup allows the scale to experience a small amount of swinging motion. In order to explore the hypothesis that added movement to a platform will maintain broiler interest, and thus, usage, we evaluated the occupancy of broilers on stationary versus swinging HPS. Sixteen HPS, alternating in movement ability for swinging (SW) vs sedentary (NS) designations, were set up down the center of a 1,486m<sup>2</sup> fast-growing broiler house containing 16,000 birds. Cameras affixed to the ceiling of captured unrestricted usage of the platforms at five time points: 330, 930, 1330, 1730, 2130. Means of usage counts and usage adjusted by bird size ((# birds occupying the platform) / (total number of birds possible)) for SW versus NS were compared by week throughout the 44-day growout period. Means were analyzed using an ANOVA Tukey's HSD. Overtime, total bird usage varied significantly by week. Consistent with expectations around enrichment utilization as the birds age, the highest frequency of usage was recorded in Wk2 at an average of  $5.99 \pm 0.016$  broilers occupying all HPS, which was significantly larger than Wk4-7 ( $P < 0.0001$ ). Wk7 had the lowest mean at  $3.61 \pm 0.15$  broilers per HPS. Based on previous studies, evaluation of usage when adjusting for the size of the birds and total possible stocking density on the platform, indicated that Wk7 had the largest adjusted usage compared to all other weeks ( $1.29 \pm 0.43$ ;  $P < 0.0001$ ). For Wk1-5, adjusted frequency of use for SW were significantly higher than NS. Adjusted usage difference was not significant for Wk6-7. As we look to ways to incorporate enrichments which can serve to encourage multiple positive welfare experiences from the broilers, enrichments that provide some movement for birds may be a method in which activity and leg health could be promoted. Additionally, we must consider the space allocation a platform provides when aiming to determine usage or novelty.

Keywords: broiler; enrichment; scale



[P1-14]: P1-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseases Food Safety (ID: 131007) - Germany

## **Welfare of turkey hens with untrimmed beaks in enriched housing conditions**

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Keeping turkeys with untrimmed beaks is one of the main challenges in conventional turkey farming. Beak trimming is commonly used to minimize serious pecking injuries. The present experimental study aimed to evaluate the effect of environmental enrichment on pecking injuries in untrimmed turkey hens. The study involved a total of 1,704 turkey hens (British United Turkey, B.U.T. 6; Aviagen®, Huntsville, AL, USA) during one production period (114 production days). During production, the turkey hens were reared 24 pens with 71 animals each. Six pens housed turkeys with trimmed beaks (450 turkeys; control group - ConGr) and 18 pens turkeys with intact beaks (1,350 turkeys; experimental group - ExpGr 1-3, 6 pens each). All animals were raised following national management guidelines with a stocking densities of 48 kg/m<sup>2</sup>. The compartments of the ExpGr were equipped with one pecking stone and one dust bath as well as one different additional enrichment material (ExpGr1: red-white plastic chain, ExpGr2: oat-dispender; ExpGr3: basket filled with hay) and one different structural element (ExpGr1: straw ball, ExpGr2: elevated platform, ExpGr3: slopig wall). All experimental groups were fed a diet supplemented with oat hulls (2.5% of the ration). When first signs of behavioural problems occurred, injured turkeys were separated and additional manipulable materials were taken into the affected pen. The results show that the mortality rate in the ExpGr (ExpGr 1-3 in total) was 2.2 times higher than in the beak trimmed ConGr (7.6% compared to 3.5%;  $p < 0.05$ ), with no significant differences between the three ExpGr. The main cause of loss in both groups, with 6.5% in the ExpGr and 2.8% in the ConGr, were damages due to massive pecking (pecking losses). The 1st, 7/8th and 10/11th week of life were identified as risk periods with an increased incidence of pecking injuries. In conclusion, the results show that keeping untrimmed turkey hens under different optimized conditions increases mortality rates due to serious pecking injuries. Keeping beak-intact turkeys also requires intensive flock monitoring to be able to react quickly on a flock-specific basis at first signs of behavioural disorders. Nevertheless, results might be different at lower stocking density. This project (Model- and Demonstration Project for animal welfare: #Pute@Praxis) was financially supported by the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food.

Keywords: turkeys; untrimmed beaks; enrichment; pecking injuries; mortality

## **Hatching on-farm under practical conditions - first experiences on the behavior in on-farm hatched and hatchery-hatched broiler chicks**

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In this study, the behavior of broilers from different hatching systems on farms was analyzed using the human approach test (HAT) and the novel object test (NOT). The aim was to compare the behavior of broiler chicks delivered to the farm as day-old chicks (HH) with chicks hatched on-farm directly in the barn (OH). The broilers were kept on three farms in flocks of around 4,500 to 12,000 animals (one farm: Ranger Classic; two farms: Hubbard JA-757). In total, three OH and three HH flocks on each farm were examined. Behavioral tests were carried out on the first (DL1) and seventh (DL7) day of life at eight different locations in each barn. At each testing location, the number of chicks within a circle of one meter around the observer (HAT) as well as within a circle of 25 cm around a novel object was counted every 30s for a total of three minutes. The latency, which starts as soon as the object is presented, of the first chick entered the circle and the first chick touched the object was recorded. In the HAT at DL1, an average of 2.39 chicks (SD 5.61) reached within radius at HH compared to OH with an average of 6.49 chicks (SD 13.61). The average latency for HAT was 48.06s (HH) and 29.93s (OH). On DL7, HH flocks had an average of 1.08 animals (SD 5.34) with a latency of 9s in HAT, and OH flocks showed a mean of 0.19 chicks (SD 0.64) and a latency of 14.20s. The observer was not touched at any time. The NOT showed at DL1 an average of 1.99 chicks (SD 3.05) in HH flocks within the radius around the object, a latency to enter the circle of 47.48s, and the first touch after 52.19s. In OH flocks at DL1, an average of 4.13 animals (SD 4.96) were detected and the latency was 22.95s. The novel object was touched after 43.89s from day-old chicks. At DL7, an average of 1.85 chicks (SD 4.69) approached the novel object at HH, and 3.99 (SD 6.62) at OH. The mean latency was 63.92s (HH) and 44.84s (OH), respectively. The first touch was at DL7 after 123.25s, and after 96.6s at OH. The first results showed that chicks hatched on-farm approached unknown objects more quickly, and more chicks dared to do so compared to those from the hatchery. Further runs are planned. This work (Model- and Demonstration Project for animal welfare) is financially supported by the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (FKZ: 2820MDT240).

Keywords: behavior; hatching system; on-farm hatching

## **Effect of bedding material on leg quality in broilers**

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This study aimed to determine the effect of different bedding materials on tibia breaking strength, body weight, and occurrence and severity of footpad dermatitis (FPD). A total of 170 one-day old Ross 308 broilers were raised for 42 days. Birds were allocated to 2 treatments with 5 replicates, with 17 chickens per pen. The treatments consisted of two bedding materials: peat (P) and wheat straw (WS). Birds of both groups were fed by the commercial feed: starter (1-10 days), grower 1 (11-21 days) grower 2 (22-31 days) and finisher (32-42 days). At the end of the trial 20 broilers from each group were sacrificed and left tibiae were used for measuring tibia breaking strength (N/cm<sup>2</sup>). INSTRON material tester was used to measure tibia breaking strength. The incidence and severity of FPD were measured at 3, 4, 5, and 6 weeks of age by the method described by Eichner (2007): no lesions (0), lesions cover less than 25% of the footpad (1), lesions covering between 25% and 50% of the footpad (2) and lesions covering more than 50% of the footpad (3). No differences were found in tibia breaking strength ( $P > 0.05$ ) between peat (6,545 N/cm<sup>2</sup>) and wheat straw (6,316 N/cm<sup>2</sup>) treatments. No effect of bedding material was found on body weight (2,737g (WS) vs 2,814 (P)). Bedding material significantly affected the occurrence and severity of FPD ( $P < 0.05$ ). An increase in FPD score was found with increasing broiler age for both peat and wheat straw groups. The average score on 3rd week of age was higher ( $P < 0.05$ ) for straw (0.79) compared to peat (0.02). At 6 weeks of age, the mean score of FPD was 1.29 in the wheat straw group and 0.25 in the peat group ( $P < 0.01$ ). Based on the results of this trial it can be concluded that the influence of bedding material on tibia breaking strength and body weight was not significant. Bedding material significantly affected the incidence and severity of FPD. Broilers on wheat straw were found to have a significantly higher incidence and severity of FPD.

**Keywords:** footpad dermatitis; tibia breaking strength; peat; wheat straw

## **Climate change: heat stress trends in German regions of intensive turkey production**

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Climate change and the associated global warming may affect the performance and health of farm animals. In regions with rising temperatures, animals may increasingly leave their thermoneutral zone, also known as the comfort zone, if no appropriate measures are taken. In the case of poultry, feathering and missing sweat glands make birds particularly susceptible to heat stress compared to other monogastric animals. The aim of this study was to analyse trends of heat stress in German regions of dense turkey populations over a period of 50 years (1973-2022) using enthalpy and temperature-humidity index (THI) values. Weather station data from 15 German districts with high densities of turkey production were used to investigate the heat input into the barns. Therefore, the parameters enthalpy and THI with specified thresholds (enthalpy:  $\geq 67$  kJ/kg; THI:  $\geq 81$ ) were used for heat stress assessment. Trends in extreme weather situations where these thresholds were exceeded were analysed and tested for significance using the Mann-Kendall test. At all weather stations, enthalpy values between 11.8 and 83.6 kJ/kg were recorded and the THI values varied between 40.0 and 91.8. For all districts, the gradient of the trend lines was positive, indicating a general increase in high enthalpy values and high THIs over the previous 50 years. For enthalpy, in nine of 15 districts, a statistically significant heat stress trend was found; for THI even in 14 of 15 districts. Thus, the established THI thresholds seem to be more sensitive for the detection of heat stress than the chosen enthalpy values. It is assumed, that these increases of heat load have a negative impact on animals in barns if not counteracted. In future, it is expected that housing systems have to be adapted to meet the increasing challenges of heat stress. Enthalpy values and THIs should be used in addition to temperature control to assess the climate in the barn and possible countermeasures.

**Keywords:** Turkeys, Climate Change, Animal Welfare, Heat Stress,

## **Effect anti-stressor supplementation on body weight changes, SOD (Superoxide Dismutases), and Glucocorticoids (GC) gene expression in transport stressed broiler chicken**

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The goal of our study was to see the effect of CARI Comfort on the body weight changes & gene expression of genes like SOD (superoxide dismutases), and glucocorticoids (GC) in transport-stressed birds. The CARI Comfort has been formulated with standardization of formulation by blending Glucose, Aspirin, Ascorbic acid, Betaine involving many stages consisting of pre-standardization and final package formulation. To evaluate our hypothesis 240 commercial broiler birds of Cobb breed were kept in pen & reared in a deep litter housing system for attending marketing age and weight. They were supplemented with ad libitum feed and water. Once they are ready for slaughter broilers were randomly divided into a total of six treatment groups and they were kept off-feed for 12 hr before transport. Three days before transport last three treatments (T4, T5 & T6) groups were given CARI Comfort via drinking water (the dose rate of 4.2 g/L). All treatment groups were transported for 8 hrs duration. Treatment T1 was kept as control and was slaughtered without any lairage rest. While T2 and T3 were given 1 hr lairage rest at normal lairage temperature and cooler lairage environment respectively. In CARI Comfort supplemented group, T4 was kept in control and was slaughtered without any lairage rest. Treatment T5 and T6 were given 1 hr lairage rest at normal lairage temperature and cooler lairage environment respectively. After transport broilers were immediately unloaded and slaughtered for collection of different parameters. The Production parameters like percentage BW change significantly ( $P < 0.05$ ) improved in the CARI Comfort supplemented group, compared to the non-supplemented group. The liver and jejunum tissue samples were processed for the expression of the SOD and GC genes which reveals that supplemented groups (T4, T5 & T6) exhibited significant down-regulation ( $P < 0.05$ ) in the gene expression. The highest SOD gene downregulation was in T6 (liver- 0.067, jejunum- 0.098) followed by T5 & T4, the same trend was followed by GC (liver- 0.103, jejunum- 0.097). The findings indicated that groups receiving the CARI Comfort package exhibited a decrease in body weight loss & the expression of stress-related genes, validating the effectiveness of our package in reducing the negative effect of transport.

Keywords: transport, stress, broiler, meat, antioxidant

**On-farm hatching in slow growing broilers: an experience out of the practice****A. Schwarz<sup>1</sup>, S. Vossler<sup>1</sup>, N. Kemper<sup>1</sup>, B. Spindler<sup>1</sup>**<sup>1</sup>Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour (ITTN), University of Veterinary Medicine Hannover, Foundation, Hannover, GermanyPresenting author: [anna.schwarz@tiho-hannover.de](mailto:anna.schwarz@tiho-hannover.de)

On-farm hatching is a new alternative way to let chicks hatch. The main advantage is that the birds have direct access to food and water continuously from the start, and long transport times as well as handling in the hatchery is avoided. Previous investigations showed overall beneficial effects on chicks' health and growth. In this study, on-farm hatching was tested for two slow growing broiler genetics, Ranger classic and Hubbard 757, in field conditions on two farms in Germany. On farm 1, on-farm hatched (OH) and hatchery hatched (HH) Ranger classic chicken with the flock size of 6,000 birds per house were grown separately at the same time in two barns and followed over three production periods (PP). Both OH and HH chicken originated from the same breeder flock aged 22, 31 and 38 production weeks (PW), accordingly. On farm two, 12,000 OH or HH Hubbard 757 chickens were subsequently grown at a five weeks interval in the same barn. The birds originated from different breeders and accompanied for two PP. Hatching rate, calculated as percentage of chicks after the selection to the total amount of hatching eggs initially led in an incubator was documented. Each round, 50 chicken per house were weighed on day 1 (d1) and day 7 (d7), and navel, leg and crop condition were assessed on d1. In all PP, the hatching rate was comparable between OH and HH chicken. For Ranger classic flocks in PW22 it was 88.11% in OH and 88.00% in HH, in PW31 84.28% in OH and 84.24% in HH and in PW38 73.74% in OH and 79.18% in HH chicken. For Hubbard 757, the hatching rate was 93.43% in OH in PW22 and 93.20% in HH chickens in PW20. On both farms in all PP, OH chicken were significantly heavier than HH chicken on d1 ( $p < 0.05$ ). On farm 1, the average weight for OH Ranger classic birds over three rounds was 58.9g compared to 53.4g in HH chicken. On farm 2, OH chicken weighed on average 53g compared to 44.7g in HH broilers. Also on d7, on both farms OH chicken were significant heavier than HH chicken in all PP ( $p < 0.05$ ) with the exception of the third PP on farm 1 ( $p = 0.08$ ). In all PP on d1, in OH birds compared to HH birds 2% more empty crops, 7.5% more red hocks and 0.5% less not completely healed navel were seen. Our first results indicate that on-farm hatching can be an alternative solution to hatch the slow growing broilers in the field. This work is financially supported by the Federal Ministry of Food and Agriculture, granted by the Federal Office for Agriculture and Food; FKZ: 2820MDT240.

Keywords: on-farm hatching; slow growing broilers; field conditions

## **Stripped to the Bone: Comparing Broiler Bone Strength and Bone Density Across German Fattening Methods**

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For a closer examination of broiler bones, our study focuses on the relationship between bone strength and bone density of different broiler lines across three German husbandry systems. We aim to give an overview of the actual bone health of broiler chickens slaughtered for human consumption. In 2021 and 2022, we examined 440 carcasses of 11 flocks from four different fattening methods (light, heavy, outdoor climate, organic), which belong to three super-categories of German husbandry systems (haltungsform.de). All animals underwent processing at a commercial German slaughterhouse, after electrical water bath stunning with selected electrical parameters for each type of fattening method. After the standard procedures of defeathering, evisceration and cooling, we examined 10-30 carcasses per flock and collected data on carcass weight, occurrence of hemorrhages and fractures. We selected 5 female and 5 male broilers and dissected and defleshed both tibiae of these 10 birds before storing the bones wrapped in tissue paper in labeled plastic bags at -20°C until further analysis. The removed flesh was processed further and not discarded for ethical reasons. In 2023, we radiographed the right or left tibia of each animal alternately per flock, using a veterinarian X-ray apparatus and X-ray suitcase with 50,0 kV, at 2,0 mAs. Keeping the radiation exposure for the experimentators as low as possible, three tibiae were x-rayed at a time, using a digital flat panel detector. Together with the tibiae, we X-rayed an aluminium step wedge in the same position in every frame for later density analyzation. After thawing, we recorded the bone breaking strength of all bones individually, using a three-point-bending test. Each bone was placed on two support blocks, which were 40 mm apart, and tested at 5 mm/s. Peak force (N) and deformation at peak (mm) were recorded. At this stage, the analysis is not yet completed, so no final statement can be made about the results. However, we plan on analyzing all X-rays to determine the bone density as described by Eusemann et al. ,2020. It should be investigated whether there is a relation between the three-point bending test and the bone density. Further, these results could show, whether there is an influence of the husbandry system or fattening method on a comparative basis. With this method, one is able to assess the bone status of broiler chickens actually used for slaughter. This could be a further step for more animal welfare.

**Keywords:** bone breaking strength; bone density; x-ray; broiler chickens; animal welfare

## **Effect of different litter types on occurrence and severity of pododermatitis in broiler production**

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Pododermatitis, or footpad dermatitis is a condition characterized by inflammation and necrotic lesions that can be superficial or deep, localized on the plantar surface of the feet and the toes. This condition can be further complicated by a secondary infection of bacterial etiology. Such lesions cause pain, whether infected or not, posing a problem from a welfare point of view. Individuals with pododermatitis are less able to move to feeders and waterers due to the presence of pain, which consequently affects the growth, conversion and performance of the entire flock. In poultry rearing bedding material plays a very important role. Pododermatitis is often associated with wet or poor quality of litter. There are several methods for classification the level of lesion severity. The most common way of assessment is according to the recommendations of Welfare Quality® (2009) including inspection and scoring lesions with a 4-point scale that ranges from 0 (no evidence of foot pad dermatitis) to 4 (evidence of foot pad dermatitis). The aim of this study was to evaluate the occurrence of pododermatitis in broilers using six different litter types, including (T1) chopped wheat straw, (T2) wood shavings, (T3) mixture of 1/3 chopped wheat straw, 1/3 wood shavings and 1/3 peat, (T4) wheat straw pellets, (T5) softwood pellets and (T6) pellets of 1/3 wheat straw, 1/3 wood shavings and 1/3 peat. A total of 576 one-day-old ROSS 308 broilers were randomly selected within six treatments, and each treatment was replicate three times. For assessing the degree of pododermatitis all broilers were observed weekly, during the 7th, 14th, 21th, 28th, 35th and 42nd days, and scored. At the 42nd day of the study a significantly higher rate ( $P < 0.05$ ) of severe footpad dermatitis was recorded in the treatment 3 where broilers were raised on the mixture of 1/3 chopped wheat straw, 1/3 wood shavings and 1/3 peat, with the score of  $1.91 \pm 0.88$ . Contrary, significantly ( $P < 0.05$ ) lower incidence of pododermatitis were recorded at the T6, T2, and T4 with the scores  $0.29 \pm 0.54$ ,  $0.31 \pm 0.58$ , and  $0.36 \pm 0.58$ , respectively. In conclusion, pellets and wood shavings are more appropriate litter materials, probably due to the softness of materials and their structure. Due to the fact that broilers are in constant contact with the litter, careful selection, adequate management, proper storage and proper use contributes to better welfare inside the broiler house and healthier birds.

**Keywords:** broilers, litter, pododermatitis, welfare



[P1-22]: P1-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseasesFood Safety (ID: 131211) - France

## **Effects of a triple-strain Bacillus-based probiotic in ameliorating stress-related impacts on broiler gut microbiota for improved well-being and productivity**

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Heat stress can negatively affect gut microbiota by disrupting the balance and composition of the microbial community. It can lead to a decrease in beneficial bacteria and an increase in pathogenic bacteria, compromising the intestinal barrier function. Probiotics can help mitigate the effects of heat stress in poultry by modulating the gut microbiome. 720 day-old male chicks were randomly assigned to two houses of 12 pens each and three treatment groups with the same basal diet: Negative Control (NC); Probiotic (PRO, triple-strain Bacillus-based probiotic at  $1.6 \times 10^6$  CFU/g); and Positive Control (PC) containing lincomycin. While broilers of the first house (NS) were raised in thermoneutral conditions ( $<24^\circ$  C) from D0 to slaughter (D35), all birds from the second house (HS) were submitted to heat stress conditions ( $32^\circ$  C, 8-10h / day) from D21 to D35. Data were analyzed using Statsmodels library for Python and Tukey method, with significance determined at a level of  $P < 0.05$ . At D35, NS birds had a more diverse gut microbiota (16S rRNA sequencing method) and better zootechnical results than HS birds. HS PRO showed tendency for higher microbiome alpha-diversity and significantly higher abundance of butyrate-producing bacteria such as Faecalibacterium, Turicibacter and Romboutsia versus HS NC. HS PRO had higher final body weight (2.304 vs. 2.228 kg) and increased breast weight compared to HS NC and PC. The improved performance in PRO can be correlated with welfare biomarkers, intestinal functionality, and carcass yield. HS PRO birds demonstrated higher concentrations of serotonin in bloodserum and intestine at D35. Reduced oxidative stress as measured by higher expression of the superoxide dismutase gene in liver was also observed. Additionally, improved gut integrity as indicated by increased intestinal expression of the mucin gene (MUC2) at D7, D28 and D35 was noted. Finally, there was a significant increase in breast meat yield in HS PRO and upregulation of the MyoD (myoblast determination protein 1) gene compared to NC. In conclusion, the use of a triple-strain Bacillus-based probiotic to modulate the gut microbiota effectively helps poultry withstand stressful conditions, optimizing productivity and promoting hormone balance associated with well-being.

Keywords: Probiotic; Bacilli; Microbiome; Welfare; Productivity; stress; Biomarkers

## **Expression of regulatory neuropeptides in broiler breeder females fed diets with decreased energy and protein content**

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The feed intake pattern of broiler breeders during the rearing period is controlled to adapt their growth; allowing maintenance of health, welfare and reproductive efficiency. Although birds moving to adulthood will naturally limit their intake, it is important that the growth pattern and level of feed control provides a sufficient degree of satiety. This has led to the trialling of different feeding strategies. For this study we assessed the following feeding strategies; standard, decreased protein, decreased protein and energy diets (all pellet), all of which were fed to a standard growth target, a decreased protein diet fed to a plus 20% growth target and two ad libitum treatments which were fed the standard and decreased protein and energy diets respectively. There were five treatment replicates with twenty-four birds per treatment, a total of one-hundred and sixty-eight female Ross 308 broiler breeders were used. The central melanocortin system, within the arcuate nucleus of the basal hypothalamus, is well conserved across birds and mammals, indicating its significance in maintaining energy homeostasis. The basal hypothalamus contains two separate populations of neuronal cell types which have orexigenic and anorexigenic effects. The orexigenic neurones synthesize neuropeptide Y (NPY) and agouti related peptide (AGRP) whilst the anorexigenic neurones synthesize cocaine- and amphetamine-regulated transcript (CART) and the precursor pro-opiomelanocortin (POMC). The results of this study show that AGRP expression, when compared to the standard pellet diet, was significantly lower in both ad libitum treatments ( $p < 0.001$ ) but also in the decreased protein diet fed to achieve a plus 20% growth target treatment ( $p < 0.05$ ). Increased expression of NPY ( $p < 0.05$ ), and decreased expression of POMC ( $p < 0.05$ ) was found when the standard diet fed birds were compared to both ad libitum treatments. These results for ad libitum compared to standard diets in terms of their orexigenic and anorexigenic neurones are similar to those previously observed, suggesting satiation. However, the finding that birds fed a diet with decreased protein content to achieve a plus 20% higher growth target have lower AGRP expression compared with standard diets indicates that this dietary strategy could also improve satiety and potentially the welfare of broiler breeders.

Keywords: broiler breeder; satiety; physiology; welfare

## **Effects of Quillaja saponaria and Yucca schidigera saponins and polyphenols on intestinal health, welfare, and performance of broilers under commercial conditions in Belgium**

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The objective of this study was to evaluate the effects of a phytogetic product (Integri-Phi®) containing Quillaja saponaria and Yucca schidigera (QY) saponins and polyphenols, known to reduce inflammation and stimulate cell mediated immune response, on intestinal integrity, welfare, and performance of broilers under typical production environment in Belgium. Three commercial farms with two identical poultry houses each differing in QY supplementation only, one control (CTRL) and one QY supplemented (Integri-Phi® at 250g/t), were monitored for two consecutive production cycles with a total of 464 000 Ross308 broilers; each poultry house cycle serving as a replicate. Conventional industrial farming and commercial feed supplemented with in-feed anticoccidials were applied and were identical for each QY/CTRL paired replicates. Performance data: average body weight at processing (BW), average daily weight gain (DWG), feed conversion ratio (FCR) corrected at 2500 g BW, European Poultry Efficiency Factor (EPEF) and livability; total mean lesion scores (TMLS) for the three major Eimeria spp. and dysbacteriosis lesion score from five random birds per replicate at day 21 and 35; and food pad dermatitis (FPD) score for 100 birds per replicate at day 35, as an indicator of birds welfare, were collected and subjected to statistical analysis by paired t-test with a multiple linear regression analysis that adjusts for slaughter age and coccidiostat program. QY significantly improved BW with 38g (CTRL 2587g and QY 2625g p=0.018) and DWG with 1.2g (CTRL 65.63g and QY 66.87g p=0.018). FCR was improved numerically with 3 points (CTRL 1.54 and QY 1.51 p=0.142) and EPEF with 16 points (CTRL 408 and QY 424 p=0.116). Livability was not significantly different (CTRL 96.725% and QY 97.462 p=0.495). Coccidiosis control was effective and TMLS were not significantly different between the two groups (CTRL 0.83 and 0.60 and QY 0.83 and 0.63 at 21 and 35 days with p=0.553 and 0.884 respectively). While non-specific enteritis (dysbacteriosis) scores were significantly lower at 35 days in the QY group (CTRL 2.63 and 4.00 and QY 2.80 and 3.50 at 21 and 35 days with p=0.611 and 0.028 respectively). In line with reduced enteritis score at 35 days QY provided close to significant reduction of FPD at 35 days (CTRL 58.33 and QY 40.17 p=0.086). Overall results suggest that QY can reduce intestinal inflammation and improve birds' performance and welfare under commercial farming conditions.

Keywords: broilers; Quillaja; Yucca; intestinal health; performance; inflammation

## Effect of Dietary Supplementation of Arjuna (*Terminalia arjuna*) Bark and Sahjan (*Moringa oleifera*) Leaf Powder on the Growth Performance and Egg Quality of Uttara chicken

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The aim of this study was to see how *Terminalia arjuna* bark and *Moringa oleifera* leaf powder in the diet affected the growth performance and egg quality parameters of Uttara chicken. Day old chicks (n=72) were allocated in 18 pens and distributed into six treatment groups, consisting of a control diet (T0) and 5 experimental diets (T1-T5) containing 1% Arjuna bark powder + 1, 2, 3, 4 and 5% Sahjan leaf powder, respectively (3 replicates/treatment). Weekly body weight and FCR were recorded from 0 to 8 weeks and biweekly body weight and FCR were recorded from 8 to 20 weeks of age. Egg quality was recorded during 20 to 52 weeks of age. Assessment of egg yolk cholesterol was done at 52nd week. Result indicated that average body weight (g) was found significantly higher ( $P<0.01$ ) in T3 ( $1325\pm 10.4$ ) as compared to T0 ( $1194\pm 7.76$ ), T4 ( $1160\pm 7.34$ ) and T5 ( $1143\pm 5.62$ ) with T1 and T2 showing intermediate values at 20 weeks of age. FCR was significantly better ( $P<0.01$ ) in T3 ( $4.81\pm 0.03$ ) when compared to T0 ( $5.16\pm 0.04$ ), T4 ( $5.27\pm 0.03$ ) and T5 ( $5.29\pm 0.02$ ) with T1 and T2 showing intermediate values at 20 weeks of age. Average age at first egg (days) was significantly lower ( $P<0.01$ ) in T3 ( $152.8\pm 0.41$ ) than T0 ( $164.1\pm 1.25$ ), T4 ( $165\pm 1.05$ ) and T5 ( $167.2\pm 0.4$ ) with T1 and T2 showing intermediate values. The shell thickness (mm) in overall period was significantly higher ( $P<0.01$ ) in T3 ( $0.46\pm 0.002$ ) group than T1 ( $0.44\pm 0.003$ ), T0 ( $0.42\pm 0.004$ ), T4 ( $0.42\pm 0.004$ ), T5 ( $0.42\pm 0.004$ ) groups with T2 showing intermediate value. T3 ( $9.95\pm 0.08$ ) had significantly higher ( $P<0.01$ ) albumen index than T0 ( $9.13\pm 0.03$ ), T4 ( $8.94\pm 0.01$ ) and T5 ( $8.90\pm 0.03$ ) with T1 and T2 showing intermediate values in overall period. There was no significant difference in shape index and yolk index. Crude protein (%) of egg was significantly higher ( $P<0.05$ ) in T3 ( $11.91\pm 0.06$ ) than T0 ( $11.6\pm 0.06$ ), T4 ( $11.57\pm 0.06$ ), T5 ( $11.58\pm 0.04$ ) with T1 and T2 showing intermediate values in overall period. Yolk cholesterol (mg/g) was significantly lower ( $P<0.01$ ) T3 ( $8.02\pm 0.014$ ) than T0 ( $9.01\pm 0.011$ ), T1 ( $8.74\pm 0.011$ ), T4 ( $9.02\pm 0.011$ ) and T5 ( $9.02\pm 0.012$ ) with T2 showing intermediate value at 52 weeks of age. Thus, it may be concluded that Arjuna bark @1% and Sahjan leaf @3% positively influenced growth performance, internal and external egg quality and egg composition of Uttara chicken indicating its potential as a beneficial dietary supplement.

Keywords: Growth performance; egg quality; egg composition

## **Production performance and egg quality parameters of laying hen supplemented with black turmeric under heat stress**

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Heat Stress in poultry birds has adverse effect on growth, productive and reproductive performance, and poultry health which causes huge economic loss to the poultry industry and farmers. So, herbal supplementation may be beneficial in combating heat stress as well as in maintaining the production performance of poultry birds. Black turmeric (*Curcuma caesia*) is a very important herb which has medicinal values and anti-oxidant property. Therefore, a feeding trial was conducted to study the effect of dietary supplementation of Black turmeric on production performance, egg quality traits and physiological parameters in laying hens exposed to heat stress. A total 60 laying hens (Dahlem red, age 30 weeks) were kept in individual cages and birds were divided into six equal groups/treatments- T1, T2, T3, T4, T5 and T6; each group having 10 birds. The five groups (T2, T3, T4, T5 and T6) of birds were maintained in artificially induced heat stress condition (37+1°C), while control group (T1) of birds was reared in normal environment. Induced heat stress (37°C) was created in experimental poultry shed for 4 hours daily for 30 days. The birds of T1 and T2 group was fed with a basal diet only, whereas the T3, T4, T5 and T6 groups were fed with basal diet supplemented with black turmeric powder (0.5, 1.0, 1.5 & 2.0%) respectively throughout the experiment. The results revealed that production parameters like egg production, egg mass, daily feed intake and FCR were reduced significantly by 23%, 33.23%, 7.06% and 42.80% respectively in heat stressed group (T2) having basal diet only due to heat stress. However, egg production, egg mass, feed intake and FCR were further improved by 19.67%, 45.21%, 7.19% and 28.88% respectively in heat stressed group (T6) supplemented with 2% black turmeric powder in compare to heat stressed group (T2). Present study revealed that serum corticosterone level was increased significantly ( $P < 0.01$ ) in all heat stressed birds except control group and it was maximum in (T2) group fed with basal diet only. However, corticosterone level was further declined significantly in (T6) heat stressed group supplemented with 2% black turmeric powder. Study also revealed that no significant change in cell mediated immunity and egg quality traits except yolk index. It was concluded that dietary supplementation of 2% black turmeric powder improved production performance in laying hen without affecting egg quality traits under heat stress condition.

**Keywords:** Laying Hen; Heat Stress; Black Turmeric; Production Performance; Egg Quality Parameters

[P1-27]: P1-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseases Food Safety (ID: 130865) - Greece

## **Dietary phytogetic inclusion level improves performance and egg quality by regulating cytoprotective and inflammatory responses in the ovary and duodenum of laying hens**

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This study investigated the inclusion level effects of a natural phytogetic blend (PB) on productive performance, egg quality and critical molecular biomarkers relevant for detoxification (aryl hydrocarbon receptor; AhR), inflammatory (nuclear factor-kappa B; NF-κB) and antioxidant (Nuclear factor erythroid 2-related factor 2; Nrf2) responses in layers ovary and duodenum. Depending on PB inclusion level (0, 250, 750, 1000 and 1500 mg/kg) in the basal diet, 21-wk-old, Hy-Line Brown laying hens (n = 385) were assigned into 5 treatments: control (CON), PB250, PB750, PB1000 and PB1500, with 7 replicates of 11 hens, each. Diets were formulated according to Hy-line brown Management Guide (2018). The PB consisted of selected Mediterranean plants having olive oil polyphenols, carvacrol and thymol among its main bioactive components (NuPhoria®; Nuevo SA, Greece). Performance responses and egg quality indices (i.e., shell mass-thickness-strength, albumen height, Haugh units and yolk colour) were determined weekly for a 12-wk period and reported as overall. Ovarian and duodenal samples of 33-wk-old layers were collected for qPCR analysis. Data were analyzed by ANOVA and statistical significance was determined at  $P < 0.05$ . Biological response patterns concerning the PB inclusion level were studied using polynomial contrasts. Results revealed that increasing PB inclusion level, enhanced linearly and quadratically overall laying rate, egg mass and FCR with PB750 birds being better ( $P \leq 0.001$ ) compared to CON. Haugh units increased quadratically with increasing PB inclusion and peaked at PB750 ( $P < 0.05$ ). In the ovary, expression of the majority of detoxifying (4 out of 5) and inflammatory (9 out of 12) genes assessed were down regulated ( $P < 0.05$ ), with PB inclusion. In addition, PB related antioxidant potential was demonstrated via beneficial changes ( $P < 0.05$ ) seen for the majority (7 out of 8) of the Nrf2-pathway genes assessed. Similarly, at duodenal level AhR pathway genes (2 out of 5) and most of the NF-κB pathway genes (10 out of 12) assessed, were down regulated ( $P < 0.05$ ), while those involved in the Nrf2 pathway (5 out of 8) were up-regulated ( $P < 0.05$ ) with increasing PB inclusion level. In conclusion, PB inclusion resulted in a consistent beneficial modulation of cytoprotective and inflammatory responses. The nutrigenomic results documented further the productive performance and egg quality improvements seen, with PB750 displaying the optimal benefits.

Keywords: phytogetics; laying hens; performance; nutrigenomics; ovary; gut

## **Standardized Natural Citrus Extract stimulates the production of endogenous butyric acid in the gut**

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Gut health is crucial to enhance animal productivity. To support gut health, short-chain fatty acids (SCFA) are essential. Indeed, SCFA are known for their positive effect on intestinal microbiota, allowing to stimulate acidophilic bacteria's growth and inhibit pathogenic bacteria's growth. Moreover, some research showed that gut morphometry can be improved by SCFA, which leads to better nutrient absorption. Therefore, stimulating endogen SCFA production could be a good strategy to enhance gut health, and ultimately, animal performance. This could be done through feed supplementation with additives that stimulate the growth of SCFA bacteria producers such as Citrus extract. The aim of this study was to evaluate the effect of a commercial standardized natural citrus extract (SNCE) supplementation on SCFA concentration in broiler chicken guts. In this trial, we focus on butyric acid, one of the main important SCFA involved in animal gut health. Characterization of SNCE was also performed in parallel, to better understand its mode of action. 32 pens of day-old male Ross 308 broilers were divided into 2 groups: •A control group (CTL) fed with a standard diet; •A SNCE group fed with a standard diet supplemented with 250 g/T of SNCE. Animals were reared from d 0 to d 34. At d 12 and d 34, caeca samples from 8 birds per group were collected to determine butyric acid rate by GC-MS analysis. 5 SNCE batches were also analyzed using HPLC-UV-DAD-ELSD and HPLC-UV-MSMS to determine their composition. Results showed that SNCE supplementation significantly increased butyric acid rate at d12 (14.39%) compared to CTL (12.32%,  $p= 0.01$ , T-test). At d34, butyric acid rate was still numerically higher in SNCE group (14.75% Vs 13.21%) with no statistical difference ( $p > 0.05$ , t-test). This may be explained by SNCE composition. Indeed, Pectic oligosaccharides, eriocitrin, and hesperidin were identified as SNCE's major components and are well known for stimulating the growth of SCFA's producer's bacteria. SNCE supplementation allows to increase endogenous butyric acid rate in the gut. The most marked effect was observed at d12, which coincides with the period when the intestine is still immature and more exposed to pathogens. Thus, SNCE supplementation could play an important role during this critical period. SNCE characterization has highlighted some compounds that may explain the observed results. More studies are needed to confirm these hypotheses.

Keywords: Citrus extract; broiler chickens; butyric acid; characterization

## **Enhancing Reproductive Performance of Broiler Breeders and Progeny Quality and Performance Through Phytomolecules**

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A study was conducted utilizing a combination of in-feed (dry) and in-water (liquid) phytogetic formulations, Thymol-based and Carvacrol-based, to assess the reproductive performance of broiler breeders and the quality and performance of their progeny. Thymol and Carvacrol, among the phytomolecules, possess antioxidant, anti-inflammatory, and antibacterial activities. The combined phytogetics aim to serve as a Gut Health Improvement Program (GHIP). In this study, 2160 female Hubbard JA57 and 216 Hubbard M99 males, aged 22 weeks, were divided into two groups with 12 replicate pens per group. All animals received the same management and feed allowance throughout the trial period. The GHIP group received phytogetic supplementation, while the control group did not receive any supplementation. Demonstrating overall productivity close to the breed standard, GHIP hens laid 3% more eggs than control birds, with significantly higher laying persistency from 35 weeks of age. The Day-Old Chick (DOC) per hen housed (HH) was significantly influenced by laying rate ( $p < 0.05$ ) and non-significantly by fertility% and hen mortality. DOC production was significantly higher ( $p < 0.01$ ) for the GHIP group, yielding 12 additional DOC compared to the control group. Furthermore, DOC quality parameters such as yolk-free body mass (YFBM), Pasgar score, and serum antioxidant capacity were higher in GHIP chicks. A meta-analysis of the performance of three offspring studies of slower-growing broilers hatched out of eggs set at ages 30, 40, and 50 weeks of breeder age showed a significantly higher final body weight ( $p < 0.05$ ) and a numerically better feed conversion rate with 2 lower FCR points. Overall, the study demonstrates that GHIP can be suggested as an effective nutritional tool to enhance productivity and efficiency in both breeders and the slower-growing broilers produced by these breeders. It illustrates the sustainability-enhancing impact that phytogetic gut health additives can have on integrated, welfare-friendly broiler meat production.

Keywords: breeders; phytogetics; DOC quality; progeny performance



## **Dietary manipulations with herbal feed additives to produce value added egg from laying chicken**

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The fatty acids profile and cholesterol of eggs can be changed through dietary manipulation to produce functional egg, enriched with some specific nutrients. The objectives of the study were to evaluate the feeding effect of *M. oleifera* leaf (MOL), *Linum usitatissimum* (flaxseed) and *Trigonella foenum-graecum* (fenugreek) on their egg quality, fatty acid profiles and cholesterol content of egg yolk. One hundred and sixty-eight (168) Fayoumi laying hens at age 27 weeks were selected for this study, and the experiment continued until they were age 43 weeks. Seven dietary treatments with 4 replications having 6 birds per replications were considered. The diets were formulated from the basal feed following Completely Randomized Design (CRD) viz T1-Control Diet; T2-Flaxseed 0.5% + *M. oleifera* leaf (MOL) 1%; T3-Flaxseed 1% + MOL 1%; T4-Flaxseed 1.5% + MOL 1%; T5-Fenugreek 0.5% + MOL 1%; T6-Fenugreek 1% + MOL 1%; T7-Fenugreek 1.5% + MOL 1%. After completing the experiments (4 months) on cage layers egg quality was measured. In addition, cholesterol and fatty acid were determined by GC-8890. The data were analyzed by SAS software and it was observed that the egg weight, egg length, egg width, shape index and shell thickness of the eggs laid by hens fed diets were similar during the experimental period. The results showed that the addition of flaxseed and fenugreek with moringa meal to the hens' diet for a longer period of time (16th week) improved the egg weight compared to the control (T1) group. Moreover, a higher yolk color value was observed in the T2 group compared to the control group. The serum lipid profile of the hens was also favorably altered by the use of additives to feed. A reduction in yolk cholesterol levels was noted upon feeding with flaxseed and fenugreek 0.5% with MOL 1% added to the diet. Among the additive groups both 0.5% of flaxseed and fenugreek were found to be most effective in reducing yolk cholesterol levels. Higher amounts of omega3 fatty acid were found in the eggs of T2 and T5 group with values of 1.86 and 1.30% respectively. The total PUFA content was also higher in the T2 and T5 groups (17.44 and 18.31 %, respectively) compared to the control. Fatty acid composition, especially omega-3 fatty acid, was improved, and egg cholesterol concentration was reduced at the lower levels i.e., 0.5% of flaxseed and fenugreek with MOL 1% added to the diet. In conclusion, diets up to 0.5% dietary flaxseed and fenugreek with MOL 1% could be used in layers' diets for producing functional eggs.

**Keywords:** Laying hen, Functional egg, Fatty acid, Cholesterol, Feed additives, Dietary manipulation

## **Proving the use of nutritional matrix values for Phytogetic Feed Additives through modeling**

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Broiler and layer feed costs are critical and account for 60–70% of the total production costs. With increasing raw material prices, it is becoming even more fundamental to optimize broiler diet formulations. Phytogetic Feed Additives (PFA) have been shown to improve nutrient digestion and utilization in poultry. Delacon completed several digestibility studies in broilers to develop, evaluate, and validate the nutritional matrix values for PFA. Delacon continued to move this validation to the next step by conducting a meta-analysis of all broiler trials (from 2000-2022) where PFA was used on top of the diet (150 g/MT Biostrong™ 510, a phytogetic mixture of spices, essential oils, bitter substances and saponins). The next phase was to make a model to develop nutritional matrix values. Initially, there was a database of over 200 trials available, but this was shortlisted to 79 selected trials. Criteria to include trials were the availability of a clear trial protocol and the availability of the diet and performance results. These 79 trials were used for the meta-analysis and the modeling. Enzymes such as phytases and xylanases were added to the diets in all trials. Final average age of birds was 38 days of age. Diets were corn-based, wheat-based, and mixed diets. The impact of the added PFA was calculated as a percent change to the negative control diet. The meta-analysis showed an average of 1.41% increase in body weight when the PFA was added; 71% of the trials (Chi-square  $P < 0.05$ ) had an improvement higher than the confidence interval ( $\alpha = 0.01$ ) of the Control for body weight. Birds fed with PFA resulted in an average reduction in FCR by 1.16%; 75% of the trials (Chi-square  $P < 0.05$ ) had an improvement of FCR higher than the confidence interval of Control. The average feed intake shift (+0.28%) was within the confidence interval of the Control group. After that, an equivalent in energy was generated by establishing the relationship between nutrient intake and body weight. For energy, this resulted in the equation  $y = 18541x - 0.786$  (with  $y = \text{AME}$  in kcal/kg and  $x = \text{final body weight}$  in g). As an example, for a broiler with a final body weight of 2.5 kg, this results in 39.4 kcal/kg AME. For Digestible Lysine, the value was generated from the digestibility trials and resulted in a nutritional matrix value of 0.25 g/kg. Based on the digestibility studies and the modeling, it was concluded that nutritional matrix values can be added to a PFA.

Keywords: phytogetic, matrix values, cost-effective, broiler, modeling, digestibility study

**Effects of feeding oxidized lipids and an extract from an olive oil by-product, rich in Hydroxytyrosol, on cecal fermentation in broilers at 21 days of age****J. Maldonado<sup>1</sup>, F. Sevillano<sup>1</sup>, M. Carro<sup>1</sup>, J. Arroyo<sup>1</sup>, D. Menoyo<sup>1</sup>**<sup>1</sup>Departamento de Producción Agraria, Universidad Politécnica de Madrid, ETS Ingeniería Agronómica, Alimentaria y de Biosistemas, Madrid, Spain

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Oxidised oils in feeds might have detrimental effects on the gut microbiota. Plant extracts exhibit antioxidant and antimicrobial activity due to their content of polyphenols. The present study aimed to investigate the effects of feeding broilers oxidised lipids and an olive oil by-product rich in the polyphenol hydroxytyrosol (HT) on cecal fermentation. To this end, an in vitro gas fermentation study was carried out using the cecal content of 21-day-old birds as inoculum and starch (S), fructo-oligosaccharides (FOS), and pectins (P) as substrates. Animals were fed diets containing 4% fresh or peroxidised soybean oil and 0 or 20 ppm of HT for 21 days. At the end of the trial 32 birds (8 per treatment) were euthanised, and the ceca was sampled and stored at -80°C. After thawing the caecal content of 2 or 3 animals was pooled to make 3 different inoculum per diet. The inoculum (1g) was mixed and homogenised in 100 ml of a culture medium. Vials were filled with 200 mg of each substrate and 20 ml of the mixture and incubated at 40°C for 216 h. Gas production was measured at different time points with a digital manometer and a plastic syringe and fitted to the exponential model  $\text{Gas} = \text{PGP} \times (1 - e^{-c \times (t - \text{lag})})$ , in which PGP is the potential gas production, c is the fractional gas production rate, lag is the latency time of the fermentation process, and t is the time of gas measurement. Also, the average gas production rate (AGPR) in the period from 0 to the time reaching half of the potential gas production was determined according to the equation  $\text{AGPR} = A c / [2 (\ln 2 + c \text{ lag})]$ . The main effects, fat source, HT, and substrate, and their interactions were analysed with the general linear model of SAS. The PGP and lag for S were significantly higher than for FOS and P ( $P < 0.0001$ ). However, the c was significantly higher for FOS and P than for S ( $P < 0.0001$ ). This led to an AGPR significantly higher ( $P < 0.0001$ ) for FOS and P compared to S (3.31, 2.85, and 1.95 mL/h, respectively). A significant interaction between fat source and HT was detected ( $P < 0.05$ ) on PGP. The inclusion of HT decreased PGP from 333 to 308 mL/g in fresh oil fed birds but increased it from 311 to 338 mL/g in peroxidised oil fed birds. In conclusion, the higher AGPR observed in FOS and P substrates is thought to represent the predominance of fibrolytic bacteria in the chicken caecum. The opposing effects of HT on PGP in fresh and peroxidised oils warrant further investigation.

Keywords: oxidised lipids, hydroxytyrosol, cecal fermentation

## **Effects of a Chinese herbal extract on gut microbiota, production performance, immune organ indices, meat quality, and serum biochemical indices of white carneau squabs**

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Effects of dietary inclusion of Chinese herbal extract, Compound D (frankincense resin extract for 50%, myrrh extract for 4%, sandalwood extract for 0.3%, kirin extract for 8%, peony root bark extract for 8%, mint extract for 4% and alcohol for 25.7 % as medium) on *E.coli* and gut microbiota populations, meat productivity, and immune organ indices of squabs were investigated in the present study. The chemical composition of Compound D was analyzed by GC/MS. Based on a liquid medium inhibition assay, the inhibition rates on *E.coli* of Compound D in the volume percentages of 1.0000%, 0.5000%, 0.2500%, 0.1250%, 0.0625%, 0.0313%, 0.0156%, and 0.0078% were determined, using 100 mg/L and 50 mg/L of ampicillin as control. A total of 180 10-d-old white Carneau squabs raised by their parent pigeons were randomly divided into 3 groups with 5 replicates of 12 birds each. The squabs in Group CT were fed on a basal diet as control, while those in Group D1 and Group D2 were fed on the basal diet with 313 mg/kg and 625 mg/kg Compound D, respectively. The feeding was performed by introgastric oral feeding till they were 30-d-old. Gut microbiota population, muscle buildup, immune organ indices, meat quality, and serum biochemistry of the birds were measured. The results showed that: (1) The main chemical composition of Compound D were tricaprilin, myrtenol, isopropyl myristate, menthol, and camphor. (2) The inhibition of Compound D on *E.coli* declined from 74.4% to 18.6% with reducing concentration (lower than 0.0625%). Nonetheless, the rate was significantly higher than those of the two antibiotic control groups so long as the concentration was higher than 0.0625% ( $P < 0.05$ ). The half inhibitory concentrations (IC<sub>50</sub>) of Compound D at 12 h and 24 h were 0.0259% and 0.0852%, respectively. (3) The *Bifidobacteria* in Group D2 ( $10.0 \pm 0.3$  lgCFU/g) was higher, and the *E.coli* ( $6.3 \pm 0.1$  lgCFU/g) was lower than those in Group CT ( $9.4 \pm 0.4$  lgCFU/g,  $7.9 \pm 0.3$  lgCFU/g, respectively) ( $P < 0.05$ ). (4) The average feed-to-weight gain ratio of the squabs in Group D2 ( $4.2 \pm 0.2$ ) was lower, and the leg muscle proportion ( $10.0 \pm 0.3$ ) was higher than those in Group CT ( $4.5 \pm 0.1$ ,  $8.0 \pm 0.8$ , respectively) ( $P < 0.05$ ). To sum up, Compound D displayed significant inhibitory effect on *E.coli*. and improved the probiotic population in the guts. The meat productivity of the squabs fed with the supplementation (625 mg/kg) was also improved.

Keywords: frankincense resin extract; myrrh extract; sandalwood extract; kirin extract; peony root bark extract; squab; introgastric oral feeding

## **Effects of supplementation of salinomycin and herbal feed additive on growth performance and oocyst shedding in broiler chickens challenged with *Eimeria* spp**

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Owing to concerns related to drug resistance, environmental impact, and consumer safety have led researchers to explore plant extracts as the alternative candidates for natural coccidiosis control. The current study aimed to investigate the effects of salinomycin and herbal feed additive (New Feed-X, Natural Remedies Private Limited, Bangalore, India) composed of *Emblica officinalis*, *Punica granatum* and *Andrographis paniculata*, on growth performance and oocyst shedding count in broiler chicken challenged with *Eimeria* spp. A total of 384 one-day-old Cobb 500 were randomly allocated to four experimental groups in 32 pens with 12 birds per pen, resulting in 8 replicate per treatment as follows; (i) Negative control (NC), basal diets for 42 days, (ii) Positive control (PC), basal diet containing 500 mg/kg of salinomycin for 42 days, (iii) T1, basal diets containing 500 mg/kg of salinomycin from 0 to 27 days and then 500 mg/kg of herbal feed additive from 28 to 42 days, and (iv) T2, basal diets containing 500 mg/kg of salinomycin and herbal feed additive each for 42 days. All broilers were given an oral gavage of 31,250 *E. acervulina*, 6,250 *E. maxima* and 6,250 *E. tenella* oocysts on 7 and 15 days. Salinomycin supplementation significantly decreased ( $p < 0.05$ ) growth performance compared to non-salinomycin supplemented groups (NC) during the pre-challenged period viz. 0-7 days [Body Weight Gain (BWG), g: 116 Vs. 94; Feed Intake (FI), g: 166 Vs. 152; Feed Conversion Ratio (FCR): 1.34 Vs. 1.63]. However, after challenging with *Eimeria* spp., salinomycin supplementation significantly reduced ( $p < 0.05$ ) oocyst excretion on 14, 21 and 35 days compared to the non-salinomycin supplemented group (NC). Moreover, herbal feed additive used in this study numerically boosted performance and reduced oocyst excretion compared to the salinomycin alone supplemented group (PC), although there is no significant difference ( $p > 0.05$ ) among the treatments (T1 & T2) [BWG, g: 3104 Vs. T1-3171 & T2-3261; FI, g: 4858 Vs. T1-4662 & T2-4747; FCR: 1.57 Vs. T1-1.47 & T2-1.46]. In conclusion, herbal feed additive and Salinomycin alleviated negative effects such as poor feed intake and increased oocyst excretion by coccidiosis. Hence, herbal feed additives could be considered as a top-up or an effective alternative for ionophore coccidiostats in commercial broilers during the finisher period, and thereby improve the productivity of broilers.

Keywords: Coccidiosis; *Eimeria*; Salinomycin; Herbal feed additive; Broiler

## **Artichoke extract (*Cynara scolymus* L.) improves production performance of laying hens after long-term stress of feeding mycotoxin-contaminated diets**

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Mycotoxin contamination in feed has a negative impact on production performance, egg quality, and health of laying hens. Artichoke, *Cynara scolymus* L., is a rich source of polyphenolic compounds, mainly caffeoylquinic acids, and flavonoids. It is an effective hepatomodulator and hepatoprotector which could protect the liver from the lethal effects of toxins. The present study was to investigate the effects of artichoke extract (*Cynara scolymus* L.) supplementation in drinking water on layer performance and egg production of laying hens fed mycotoxin-contaminated diets. One hundred and forty-four Lohmann brown laying hens, approximately 48 weeks of age with an average 99.07 % hen-day egg production (HD) and 58.53 g. of egg weight, were randomly assigned into 4 treatments. Each treatment consisted of 18 replicate cages (2 birds/cage). The experimental diets were corn-SBM basal diets with T1) no mycotoxin (negative control), T2) no mycotoxin (positive control), T3) AFB1 50 ppb + FUM 5,700 ppb, and T4) DON 1,125 ppb + T2 toxin 300 ppb. All laying hens were fed with dietary treatments and regular drinking water for 12 weeks. Production performances and egg quality were measured at the end of the 12 weeks. After that, the laying hens in T3 and T4 were switched to a non-mycotoxin-contaminated diet. Laying hens in T2, T3 and T4 were offered drinking water supplemented with artichoke extract (BEDGEN40® SFA CONCENTRATE, Bedson S.A., Argentina) at 0.3 ml/L, while T1 was offered with regular drinking water. The experiment was continued for 4 periods of 14-d each. Egg quality was measured on the last day of each period. Feed and water were provided ad libitum throughout the experimental period. Egg quality was measured on the last day of each period. Feed and water were provided ad libitum throughout the experimental period. After 12 weeks, HD ( $P < 0.05$ ), egg weight ( $P < 0.05$ ), and egg mass ( $P < 0.01$ ) of T3 and T4 were significantly lower than that of T1 and T2. After artichoke extract was offered in drinking water for 2 periods, egg weight ( $P > 0.05$ ) and egg mass ( $P > 0.05$ ) of T3 and T4 were improved to the same extent as of T1 and T2 and maintained at the same level until the end of the trial. The addition of artichoke extract in drinking water (T2, T3, and T4) tended to have higher HD than T1 ( $P = 0.0507$ ). It could be concluded that artichoke extract improved production performance of laying hens after the long exposure to the stress from mycotoxin challenges.

Keywords: Artichoke extract; *Cynara scolymus* L.; mycotoxin; egg production; egg weight

[P1-36]: P1-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseasesFood Safety (ID: 131225) - France

## **Effects of an encapsulated phytogetic and phycogenic solution when used in a diet formulated with or without matrix value on performance, egg quality and nutrient digestibility of laying hens**

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The cost of feed and feed efficiency continue to be a headache for nutritionists and producers of the poultry industry. In this context, it is important to explore nutritional solutions that can improve the feed digestibility and the bird gut health and performance. The goal of this trial was to study the effects of a solution containing a blend of encapsulated phytogetic and phycogenic (NUQO©NEX, NUQO, France) when used in a diet formulated with or without a matrix value on laying performance, egg quality and nutrient digestibility. A total of 720 Lohmann brown laying hens of 74 weeks of age were randomly allocated to four dietary treatments: 1. Control diet, 2. Control diet supplemented with the encapsulated solution, 3. Negative control diet formulated with a matrix value of 23 kcal/kg feed and 0.008% digestible lysine, and 4. Negative control diet supplemented with the encapsulated solution. Each group had 15 replicate cages of 12 hens. The trial lasted 12 weeks and the encapsulated solution was continuously added at 75 g/ton of feed. Number of eggs, egg weight, egg quality (4 eggs/cage) and feed consumption were recorded every 4 weeks to calculate average egg weight, hen-day egg production, egg mass and FCR. Apparent ileal digestibility was measured on 15 birds per treatment at the end of the trial. Data were analysed with SAS software using a 2 x 2 factorial design (matrix or no matrix; additive or no additive) with a level of significance set at  $P < 0.05$ . No effects on laying performance were detected per period or overall. Nevertheless, it was noted with  $P$ -values close to 0,2 that the additive had a positive effect on laying percentage and dirty eggs for the overall trial period. There was no impact of the matrix value or the additive on yolk colour, albumen height, haugh unit or eggshell breaking strength. However, the matrix value reduced the eggshell thickness ( $P < 0.05$ ) while the additive tended to improved it ( $P = 0.14$ ). The additive also tended to improve the digestibility of crude protein ( $P < 0.1$ ) and crude fibres ( $P = 0.11$ ). In conclusion, the encapsulated solution used in diets formulated either with or without matrix value showed positive trends on nutrient digestibility and eggshell thickness, and promising effects on laying percentage and dirty eggs, but more research is needed to confirm these results.

Keywords: Laying hens, laying performance, egg quality, nutrient digestibility, phytogetics, phycogenics

## **Effects of natural extracts on the oxidative stability of poultry feeds**

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The objective of this study was to evaluate the effect of an encapsulated product based on capsicum, black pepper, and ginger (Spicy) and a liquid product rich in hydroxytyrosol (HT) obtained from by-products of olive oil production as technological antioxidants in broiler feeds containing fresh or oxidised fats. A 2 x 2 x 2 factorial design was conducted, the factors being: fat source (FS; 4% soybean oil or 4% oxidised soybean oil); Spicy (0 or 250 ppm); and HT (0 or 20 ppm). Feeds were based on maize and soybean meal. The oxidative stability of the feeds was assessed throughout time (days 7, 13, and 34 post-manufacturing) using 3 analyses: peroxide value (PV) by the official method AOCS Cd-8b 90, hexanal by a combined technique consisting of SPME / GC / MS and sensory evaluation by a group of trained panellists in test cabins. Hexanal and PV data were analysed as repeated measures appropriate for a factorial design, including day as a factor using the Mixed procedure of SAS. A heterogeneous first-order autoregressive covariance structure was used in the model. Interaction effects of HT x day and FS x HT x day were observed for PV and hexanal ( $P < 0.05$ ), and the same effects on both parameters were observed for Spicy x day and FS x Spicy x day ( $P < 0.05$ ). At day 7, oxidised feeds presented higher PV and hexanal compared to fresh ones, and no effects of additives were observed. At day 13, PV and hexanal concentrations peaked in oxidised feeds ( $P < 0.05$ ). At this time point, HT showed lower PV in oxidised feeds than the other diets but higher PV in fresh oil ones ( $P < 0.0001$ ). In contrast, Spicy showed lower PV in fresh oil diets but higher PV in oxidised feeds ( $P < 0.0001$ ). At day 34, PV were higher in oxidised than in fresh feeds ( $P < 0.05$ ). However, in fresh oil feeds PV were lower in diets with HT and/or Spicy ( $P < 0.05$ ). At the same time, hexanal concentrations were equal in oxidised and fresh feeds without additives or HT, but Spicy maintained the fresh oil feeds with lower hexanal amounts than the oxidised ones. Sensory evaluation correlated well with the analytical results. Chemically, it is not possible to revert an oxidation process that has already started in a substrate. The conclusion of this study is that in the feed samples containing fresh oil the natural extracts tested were effective to protect the poultry feed against oxidation for 34 days being these diets still in a very good oxidative status at this point.

**Keywords:** natural extracts; feed oxidation; feed stability



## **Influence of purslane extract and probiotic on welfare related parameters and droppings characteristics of broilers at high stocking density**

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This study evaluated the effects of purslane extract and probiotic on welfare related parameters and droppings characteristics of broiler chickens at high stocking density. A total 280 one-day-old broiler chicks were used in a completely randomized design with five treatments and four replicates per each. Dietary treatments included: 1) positive control (PC; 10 chicks/m<sup>2</sup>), 2) negative control (NC; 15 chicks/m<sup>2</sup>), 3) NC + 500 mg/kg purslane extract (PE), 4) NC + 200 mg/kg probiotic supplementation (PS) and 5) NC+500 mg/kg PE + 200 mg/kg PS. At the end of experiment on d. 42, 10 chickens in each pen were inspected for walking ability, hock and footpad burns, and the abdominal plumage condition. At 42 d. of age, excreta were collected from each pen and its pH, dry matter, volatile and nonvolatile were measured by some modification. For analysis of variance, all data were analyzed using GLM procedure of SAS software for analysis of variance. Differences between treatment means were tested using Duncan's multiple comparison test. Statistical significance was declared at  $P \leq 0.05$ . The welfare related data were obtained by an average of 10 birds in a pen as the experimental unit and were analyzed by Wilcoxon test. The results of this experiment showed that increasing the stock density negatively influenced litter moisture, gait score, foot pad dermatitis and hock burns. Also, Litter and excreta moisture were significantly increased with increasing placement density ( $P < 0.05$ ). These data indicate that Use of purslane extract and probiotics in high stoking density could not have clearly effect on these parameters.

Keywords: Broiler, Probiotics, Purslane, Stoking density

**Live Salmonella vaccine compatibility with different microbial based solutions****F. Barbe<sup>1</sup>, S. Gibson<sup>2</sup>, A. Sacy<sup>1</sup>, V. Demey<sup>1</sup>, E. Chevaux<sup>1</sup>, M. Castex<sup>1</sup>**<sup>1</sup>Lallemand Animal Nutrition France, 19 rue des Briquetiers, 31702 Blagnac cedex, France, <sup>2</sup>Lallemand Animal Nutrition UK, Unit 11, 13 Spring Ln N, Malvern WR14 1BU, United KingdomPresenting author: [fbarbe@lallemand.com](mailto:fbarbe@lallemand.com)

In poultry, vaccination became a strategy to control Salmonella prevalence, with efficacy reported on the reduction of pathogenic Salmonella in the feces, tissues and eggs of chickens. The objective of this study was to demonstrate the compatibility of 3 solutions tested independently (a competitive exclusion product, AviGuard; *Pediococcus acidilactici* CNCM I-4622, Bactocell; *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079, Levucell SB – Lallemand) with live Salmonella vaccine. In the 1st experiment, 54 chicks were conjointly dosed at day 1 with AviPro Salmonella DUO (Elanco UK: 4.8 x 10<sup>9</sup> cfu/chick) and with either water (control: n = 27) or AviGuard (n = 27). In the 2nd experiment, 54 chicks were vaccinated the same way and were fed either a control diet (n = 27) or a diet supplemented with Bactocell (109 cfu/kg feed) (n = 27). In the 3rd experiment (2020 Elanco Animal Health data on file), 40 chicks were sampled which had received 1st of 2 doses of of AviPro Megan Vac 1 (Elanco US) at day 1 and were fed either a control diet (n = 20) or a diet supplemented with Levucell SB (109 cfu/kg feed) (n = 20). The birds were euthanized at day 8 in the 1st and 2nd experiments and at day 4 in the 3rd experiment. The number of birds positive for Salmonella vaccine strains in liver samples (and in ceca samples for the 3rd experiment only) was determined for each group and statistically analyzed by Chi-Square test. In the 1st experiment, the vaccinated control and AviGuard groups were found positive (78% and 85%, respectively) for the translocation of the Salmonella strains from the vaccine to the liver. In the 2nd experiment, the proportion of positive birds reached 78% and 67% for the vaccinated control and Bactocell groups, respectively. In the 3rd experiment, all birds were found positive in liver samples for the vaccinated control and Levucell SB groups. Moreover, 55% and 45% of birds were found to be positive in ceca for these 2 groups, respectively. There was no significant difference between the control groups and the 3 solutions for the proportion of positive birds in any sample. The differences inter-experiments could be explained by different slaughtering date (days 4 or 8). These microbial based products had similar Salmonella vaccine strain positivity in the liver, compared to control groups (vaccine alone), showing no interference in the ability of the Salmonella vaccine to translocate. These solutions are then compatible with live Salmonella vaccine.

Keywords: Salmonella; vaccine; compability; microbial based solutions

## **Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for H5 avian influenza A**

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Influenza viruses belong to the family Orthomyxoviridae. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin and Neuraminidase antigens. Some subtypes containing H5 or H7 are associated with highly pathogenic forms of the disease and high rate of mortality. A current H5 HPAI lineage has been circulating worldwide since 2004 and has been responsible for important poultry losses. To control poultry disease, vaccination is more and more used, especially with recombinant vaccine technology. In the last 5 years, successive waves of H5 Influenza in Europe pushed the health authorities to review their vaccination strategy concerning this virus. Given the need for rapid and reliable serological tools for monitoring of vaccination, IDvet has developed unique indirect ELISAs: one, based on H5 recombinant protein, for the monitoring of recombinant vaccines, and one, based on NP protein, for DIVA strategy (differentiated Infected from Vaccinated Animals). Difference species of ducks (France) vaccinated with an inactivated sub-unit AIV-H5 vaccine were tested. Antibody titers for H5 were evaluated using IDvet optimised H5 iELISA. Samples were also tested with the NP iELISA to monitor field challenge. For each tested flock, the following parameters were measured: mean titers, minimum, maximum, and CV%. All samples with titers higher than 732 for H5 iELISA, and higher than 668 for NP iELISA, were considered positive. Results. All the flocks vaccinated with H5 vaccines were found positive with the H5 iELISA. Therefore, the positivity of the H5 iELISA, belonging to negative flocks with the NP iELISA, demonstrated the detection of seroconversion induced by vaccines. A preliminary baseline for inactivated sub-unit AIV-H5 vaccination was established thanks to these results. Conclusion. The H5 indirect ELISA presented is the only quantitative test for the specific detection of H5 antibodies which allows for H5 vaccination monitoring. This H5 iELISA is suitable for the monitoring of inactivated sub-unit AIV-H5 vaccine in ducks. The NP indirect ELISA is an excellent tool for the detection of wild virus in populations vaccinated with recombinant H5 vaccine.

Keywords: Vector vaccines, vaccination monitoring, innovative ELISAs

## **Evaluation of inactivated vaccine formulated with new polymeric adjuvant against Salmonellosis in layers**

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Injectable vaccines dedicated to avian species are usually formulated with oil-based adjuvants allowing to obtain emulsions, especially water-in-oil ones. Oily adjuvants are very effective immunologically speaking, nevertheless, they could be less tolerated by sensitive species. MONTANIDE™ GEL P PR, an aqueous adjuvant based on polymeric technology is developed to meet the growing need for better innocuity in the avian market. Salmonellosis is an avian disease caused by infection with a Gram negative bacteria, *Salmonella enteritidis*. Salmonellosis vaccines are usually inactivated vaccines that may lead to severe reactions at the injection site. The target of the following experiment is to evaluate the safety, efficacy and protection of an inactivated vaccine adjuvanted with MONTANIDE™ GEL P PR (GEL P). In this trial, in order to evaluate the safety of the vaccine, 32-day-old SPF egg laying chickens were injected subcutaneously (SC) in the neck or intramuscularly (IM) in the chest, with 1 ml of the inactivated vaccine based on GEL P or standard water-in-oil. The GEL P group is safer, whereas oily adjuvant exhibits higher reactogenic properties especially with IM route that leads to the highest score. Similarly, the efficacy and protection were evaluated by injecting (SC or IM) 0.5 ml of the inactivated vaccine based on GEL P or standard water-in-oil adjuvant. Blood samples were collected at D7, 14, 21 and 28 after injection. The antibody titers were determined by ELISA. Both water-in-oil and GEL P vaccines induced a good immune response since the antibody levels are well above the acceptable protective threshold two weeks after vaccination whatever the mode of injection. These results show that MONTANIDE™ GEL P PR is balanced in terms of safety profile, and protection against potential reactogenic bacterial diseases and suitable candidate adjuvant for the formulation of inactivated poultry vaccines either in IM or SC application.

**Keywords:** Vaccine, polymeric adjuvant, adjuvant, poultry vaccine, safety, efficacy

## **compatibility of innovax-nd-ibd + nobilis h9-nd p vaccine against 3 challenge strains (ibd, nd, ai) in spf birds at t=3 or t=4 (ibd at 3 weeks, nd and ai at 4 weeks)**

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This study was performed with day-old SPF layer chicks evaluating efficacy and compatibility of Innovax®-ND-IBD (vaccine 1) and Nobilis® H9N2+ND P (vaccine 2) against 3 challenges: IBDV CS89, NDV Genotype VII and AIV H9N2 challenge. IBDV CS89 was challenged at 3 weeks of age while, NDV Genotype VII and AIV H9N2 were challenged at 4 weeks of age. 160 day-old SPF chicks were allotted to 13 groups, placed in separate negative pressured isolators and vaccinated with vaccine 1 alone, vaccine 2 alone, vaccine 1+vaccine 2; 1 group was not vaccinated untreated, another vaccinated treated with the 3 challenge virus. Efficacy and compatibility between the 2 vaccines were made based on serological data, viral shedding, viral replication and clinical observations. The protection provided by the 2 vaccines has been determined by evaluation of clinical signs of the disease, serology, viral shedding and replication. Blood samples for serology, choanal and cloacal samples were taken at different timepoint. For the group challenged with virulent IBDV bursa samples were taken for histology. NDV: results of the study indicate that all vaccinated groups, show high levels of clinical protection (100%) after challenge at day 28, with the velogenic Genotype VII NDV. In the NDV HI, vaccine 2 showed highest serological response followed by vaccine 1+vaccine 2. The viral shedding peak for vaccine 2 was at 7 dpc and limited to 20% of birds. No viral shedding is seen for the other 2 vaccinated groups. IBDV: groups vaccinated with vaccine 1 or vaccine 1+vaccine 2 after challenge at day 21, with the IBDV CS89 strain showed 100% clinical protection. Results in the IBDV screening qPCR showed that the challenged control animals gave viral shedding 2 days post-challenge. Group vaccinated with vaccine 1 showed pc viral shedding (at 3, 7 and 10 dpc). Group vaccinated with vaccine 1+vaccine 2, showed pc viral shedding (at 3, 7 and 10 dpc). Based on bursa lymphoid depletion scores, animals which received vaccine 1 were protected (100%) followed by the animals which received the combined vaccination (87%). AIV: the study showed that birds vaccinated with vaccine 2 and the birds vaccinated with vaccine 1+vaccine 2 did not show any clinical signs viral and shedding after the challenge. As often happens with LPAI H9 clinical signs were mild also in the control group (not vaccinated) and mortality was low; there is anyway a numerical difference in the vaccinated and not vaccinated groups.

Keywords: compatibility, Infectious bursal disease velogenic newcastle disease, h9 avian influenza

## **Evaluation of compatibility and production performance in commercial layers immunized with vectorized vaccines rHVT-H7 and rHVT-F and immune-complex vaccine against Gumboro applied simultaneously in the hatchery**

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Among the main diseases that affect the productivity of laying birds in Mexico are Gumboro Disease (IBD), Newcastle Disease (NDV), and highly pathogenic Avian Influenza subtype H7N3. Prevention of diseases through vaccination is of utmost importance. However, the use of conventional killed vaccines in commercial layers has had some limitations: interference with maternal antibodies, application errors, mortality and consequently, incomplete bird immunization. To protect these birds, different vaccines have emerged, such as Marek vector-based vaccines (rHVT) and immune complex vaccines (Icx). The study was carried out in Tepatitlán de Morelos, region with highest layers population in Mexico. In this follow-up, the combination of two rHVT vaccines was documented, rHVT-F and rHVT-H7, against Newcastle Disease and H7 Avian Influenza, respectively and vaccination against Gumboro disease with the usage of immune-complex vaccine. The vaccine replication was evaluated using the RT-qPCR technique from samples of feather pulp and spleen. Specific PCR from bursa of Fabricius was performed to detect the SYZA26 vaccine strain. Individual samples were taken to obtain the percentage of positive birds. Subsequently, a comparative analysis was conducted to assess the performance data up to 42 weeks of age for three historical flocks. The analysis compared flocks vaccinated with r-HVT-IBD, live attenuated NDV vaccines and inactivated H7 AI vaccine to flocks that received rHVT-F, rHVT-H7 and a IBD immune-complex vaccine in the hatchery plus live attenuated NDV vaccine and inactivated H7 AI vaccine in the field (control group). The vaccine uptake results of the combination of the rHVT-F + rHVT-H7 vaccines in control group showed positivity according to standards at 5th week of age, demonstrating the ability of both vaccines to replicate correctly. Bursa sample showed 80% positivity to vaccine strain at 5th week of age in control group. Control group showed better performance with an improvement of -6.3% in cumulative mortality, 13.63 eggs more per hen housed on average and better uniformity. The study demonstrated that both rHVT (Vectormune ND and Vectormune H7) vaccines can be applied simultaneously in the hatchery in conjunction with the immune complex vaccine (Novamune) without causing negative interference and are a great alternative to overcome the issues with the conventional vaccination program for AI, NDV and IBD.

Keywords: Vector Vaccine, Influenza, Immune-complex IBD, field results

## **Evaluation of vaccination strategies against highly pathogenic avian influenza H5 in Spanish poultry production: application of the EVACS tool**

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The 2021-23 period marked a turning point in the epidemiology of highly pathogenic avian influenza (HPAI) H5 viruses. Europe and the world have been facing the largest number of HPAI H5 outbreaks ever. Spain was no exception and reported seven poultry outbreaks and 113 wild bird cases in 2022-23. Given the disease situation, EU members are now allowed to authorize vaccination in their country. France is the first country that has applied vaccination (from the 1st Oct 23) but considering the evolution of the epidemic situation, other European countries may also start vaccinating. The aim of this study was to evaluate and compare potential HPAI H5 vaccination strategies adapted to the Spanish poultry industry of broilers, layers, turkeys and ducks. To do so, the EVACS tool was applied and five different vaccination scenarios against HPAI H5 compared, using a recombinant vaccine at the hatchery level and/or an inactivated vaccine at the farm level. This simulation tool, developed in R®, consists of five modules: model the production network using a social network analysis method; identify and characterise different vaccination strategies; evaluate vaccination performances using a stochastic model (coverage; immunity levels; duration of immunity levels; spatial distribution of immunity levels); analyse the cost-benefit of the strategies to identify the most efficient one. The results of this study showed that for the vaccination scenario targeting duck, turkey and layer production, the immunity level of the population concerned by vaccination was above 60% for the scenario based on hatchery vaccination and below for the scenario based on farm vaccination. With the same scenario, the geographical distribution highlighted that this immunity level reported to the entire poultry population was above 60% for a third of the provinces using hatchery vaccination while this level was lower for all of them using farm vaccination. Vaccination strategies were efficient for turkey and layer production with hatchery vaccination, when the surveillance cost was not considered. This work can be used to design efficient vaccination strategies as recommended by EFSA. It has been conducted within a public-private partnership and highlighted the importance of this type of collaboration to have a better understanding of the needs of farmers, poultry production stakeholders and decision makers and to produce adapted vaccination strategies.

**Keywords:** Avian influenza; vaccination; evaluation; Spain

## **Study to assess the correlation between Infectious Bronchitis live vaccine volume applied by spray to day-old chick boxes and the percentage of positivity and Ct values**

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Infectious Bronchitis (IB) is an acute and highly infectious disease that causes respiratory, renal and reproductive disease and also affects chicken's performance, egg production, egg quality and mortality, generating great economic losses in the poultry meat and egg industry worldwide. In Spain's broiler industry the vaccination is usually performed in the hatchery after hatch by spray. Even that vaccination is performed in a controlled environment by trained workers, there are variations in the IB spray vaccination that can affect the quality of vaccination. One of the most important things is the volume applied on each chicks' box, among other things like drop size, device adjustment, etc. This study presents the results of the monitoring of IBV day old spray vaccination in six different spanish hatcheries between March 2022 and November 2023. A total of forty-seven groups of chickens from the different hatcheries were analyzed. The sample size in each group was 10 to 12 individual birds vaccinated with a known spray volume. The sample size in each group of chicks was of 10-12 individual. Samples were collected with dry swabs from the trachea or choanal cleft. The vaccines used were in forty-one groups of samples a combination of H120+QX-like vaccine strains and in six groups of samples H120 vaccine strain alone. The volume of spray applied in each day-old chicks' box varied between 8 to 25 mililiters depending on the hatchery, the spray device used or the vaccine recommendation. The samples were tested individually for IB virus by Reverse Transcriptase Polymerase chain reaction (RT-PCR) after extraction of viral RNA. The aim of the study was to show if there is a correlation between spray volume applied to each box and the percentage of positivity and the IB PCR Ct values. The percentage of positive birds for IBV was very variable, ranging from 25% to 100% and the Ct values obtained in the IB RT-PCR were variable too, ranging from 23.67 to 32.01 Cts. From the statistical analysis performed, the Pearson correlation coefficient for the relation of volume to percent positive was calculated .36 (meaning higher volumes were associated with higher positive percentage), while the correlation of volume to Ct was calculated at -.51 (meaning higher volumes were associated with lower Ct values). With this results we can consider that the volume of spray is moderately associated with both the percent of positivity and Ct value obtained.

Keywords: infectious bronchitis virus; spray vaccination; volume; polymerase chain reaction; Ct value; positivity



[P1-46]: P1-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseases Food Safety (ID: 130923) - Spain

## **Bacillus coagulans DSM 32016 controls Salmonella Typhimurium-Caused performance decline in broiler through improved gut histomorphology and beneficial microbiota modulation**

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Salmonella typhimurium in poultry production poses economic challenges, driving up costs through lower performance, higher mortality rates, and increased biosecurity expenses. This study explores the effectiveness of *Bacillus coagulans* DSM 32016 (BC) in improving broiler performance and alleviating the adverse effects of *Salmonella typhimurium* (*Salmonella*) challenge. A total of 144 Ross 308 broilers (hatched) were randomly divided into 4 groups with 7 replicates (each 6 chicks). The groups, pathogen challenges (PaCh), and probiotic supplementation (ProSup;  $1.0 \times 10^9$  CFU/kg feed) were as follows: 1 (NC) - Negative control (no PaCh, no ProSup); 2 (NC+BC) - Treatment I (no PaCh, ProSup); 3 (PC) - Positive control (PaCh, no ProSup); 4 (PC+BC) - Treatment II (PaCh, ProSup). Groups 3 and 4 were orally challenged at days 4 with *Salmonella* ( $3 \times 10^9$  CFU/ml). Broilers received a starter diet (Days 1–21) and finisher diet (Days 22–35) fed ad libitum. Weekly documentation included feed intake (FI), body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR). Intestinal *Salmonella* and *Lactobacilli* counts were quantified via CFU analysis, and histomorphological samples were taken at the end of the trial. ANOVA or, when applicable, non-parametric tests (Kruskal-Wallis) in IBM SPSS were used for statistical analysis. Mean values were evaluated for significance using Duncan's multiple range test at  $p \leq 0.05$ . *Salmonella* infection exerted a negative impact on performance, affecting both BWG and FCR in both feeding phases, indicating a successful pathogen challenge. In the initial feeding phase, only numerical performance improvements due to the probiotic were observed. However, a significant positive influence on all performance parameters was observed during the second phase and throughout the entire trial period. This resulted in improved BWG and FCR in PC+BC and NC+BC. Analysis of the Production Efficacy Factor revealed that the performance of PC+BC closely approached that of NC and NC+BC, effectively compensating for the *Salmonella*-caused decline. These findings were reinforced by gut histomorphology and microbiological analysis. Specifically, villi width, villi area, and *Lactobacilli* counts were significantly enhanced, whereas *C. perfringens* and *Salmonella* counts were numerically reduced. Collectively, these results underscore the effectiveness of *B. coagulans* DSM 32016 in mitigating the adverse effects of *Salmonella* infections in broiler production.

Keywords: Probiotic, poultry production, pathogen infection, feed efficiency

## **Development of a new probiotic feed additive based on the microbial consortium of *Limosilactobacillus fermentum* and *Ligilactobacillus salivarius* for the prevention of salmonellosis in broiler chickens**

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Salmonellosis, caused by the serovars of *Salmonella enterica*, *Salmonella Enteritidis* and *Salmonella Typhimurium*, is one of the world's leading bacterial gastroenteritis of foodborne origin. The spread of *Salmonella* strains resistant to commonly used antibiotics is stimulating the development of new anti-*Salmonella* drugs. One of the most promising directions in the development of anti-salmonella agents for biological protection is the use of probiotic lactobacilli. A probiotic feed additive was developed based on a microbial consortium of the lactobacillus strains, *Limosilactobacillus fermentum* 3872 and *Ligilactobacillus salivarius* 7247. The physiological, biochemical, cultural and morphological properties of the consortium were characterised. It was established that the strains do not produce pathogenicity factors such as catalase, lecithinase C, hemolysin, plasmacoagulase, fibrinolysin, hyaluronidase, neuraminidase. The sensitivity of both *Limosilactobacillus fermentum* 3872 and *Ligilactobacillus salivarius* 7247 to antibiotics complies with the requirements of the European Food Safety Authority. The capacity for acid production activity of the individual and combined strains was determined, establishing that they exhibit synergism in acid formation during milk fermentation. Additionally, when taken separately or when combined, the strains were noted to have pronounced antioxidant activity. The whey of a fermented bovine milk product obtained via co-fermentation with *Limosilactobacillus fermentum* 3872 and *Ligilactobacillus salivarius* 7247 produced maximal antioxidant activity. It was further noted that both strains are resistant to gastric and intestinal stress. To assess the effectiveness of the probiotics in controlling *Salmonella* pathogen challenge, an in-vivo broiler study was conducted, which made it possible to assess both the safety of the probiotic and their impacts on productivity of Cobb 500 broilers. As a result of the study, it was found that the microbial consortium had a positive effect on the productive performance of broilers. The best results were obtained with a probiotic concentration of 106 CFU/g feed. The mode of action of the probiotics is likely extremely diverse and potentially associated with the inhibitory effect of organic acid molecules, the synthesis of exometabolites in the form of toxins, antibiotic-like compounds, lytic enzymes or bacteriocins.

Keywords: "Salmonellosis", "Salmonella Enteritidis", "Salmonella Typhimurium", "Probiotic", "Prebiotic", "Synbiotic", "Campylobacter"

## **Effect of in feed galactomannans in broiler chickens challenged with different *Campylobacter jejuni* strains**

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Campylobacteriosis is leading intestinal infection in humans worldwide, and chicken meat is considered the most important source of it. Besides human implications, *Campylobacter* can also impair chickens' intestinal integrity, compromising birds' health and welfare. We aimed to determine if the negative effects due to an experimental infection of chickens with different *C. jejuni* strains could be counteracted with galactomannans premix (G) inclusion in feeds (at 2.5 kg/MT). Day-old male broiler chickens ROSS 308 were randomly distributed in 6 experimental groups (100 birds each in 5 floor pens, 20 chickens per pen) in a factorial arrangement with Control (C)/G feeds. A *Campylobacter* infection was performed by oral gavage at 21 days of age with 2 different *C. jejuni* strains as follows: invasive strain (I), non-invasive strain (N) and unchallenged (U). Interaction between main factors was not significant for performance variables either before or after infection. ADFI tended to be lower for G before infection ( 5.3%), differences for BW, ADG, and Feed conversion rate (FCR) (1 to 21 days) were non-significant; a confounding effect for pens location (North IC NC/South IG NG) could also play a role. Considering BW at 21 days as covariable, FCR was significantly affected by *C. jejuni*, but in the opposite direction of the expected: infected birds improved FCR by 2.0% (I) and 3.3% (N), though only N reached significance. To avoid cross contamination among groups, birds were allocated in different rooms according to the U/I/N infection, and hence, the higher FCR for U birds could be an artifact confounding the room with the infection effects. G did not significantly affect performance or presence of *C. jejuni* in caeca post-infection (absence in U, and prevalences in I/N of 90/100% at 2 days post infection [dpi], and 100% at 7 and 14 dpi). Remarkably, the prevalence in liver numerically decreased at 7 dpi by the inclusion of G (from 20% to 0% in I; from 20% to 10% in N) and at 14 dpi only in I (from 10% to 0% in I; N had 10% prevalence in both C and G feeds). *Campylobacter* was not detected in the liver at 2 dpi. A confounding effect did not allow to observe the negative effects of the *C. jejuni* strains observed in field conditions and in a previous experiment. The inclusion of G in feeds numerically decreased the *C. jejuni* prevalence in liver, particularly in I group, but not in caeca, indicating a potential effect against translocation of invasive strains.

Keywords: *Campylobacter jejuni*; broiler; galactomannans; liver; performance; prevalence

## **comparative analysis of resistance to salmonella enterica serovar enteritidis in guinea fowl and chicken**

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The nature of the infectious diseases in poultry birds are continuously changing as the result of environmental change, pathogen evolution, changes in rearing and market legislation. Bearing these points in focus, the ultimate aim of our research was to generate essential knowledge on mechanism of disease resistance to reduce the economic burden and zoonotic threat that accompany the infectious diseases. It has been well established that the differential expression analysis of cytokine genes provides significant information regarding mechanism of disease resistance. Therefore, in present study the In-vitro mRNA expression kinetics of different cytokine genes, in Salmonella enterica serovar Enteritidis (SE) induced and un-induced spleenocytes of guinea fowl (GF) and chicken (Ck) was investigated using Real Time qRT-PCR. In terms of fold change, the expressions of IL-1 $\beta$ , IL-6 and IL-10 were up-regulated in induced spleenocytes in GF relative to those of Ck. In contrast the expressions of TGF- $\beta$ 4 and TNF- $\alpha$  were down-regulated in GF relative to those of BC. Interestingly, the expression of the IL-10 (Th2) cytokine was exceptionally higher in GF than Ck. The study revealed that the guinea fowl has higher resistance to Salmonella enterica serovar Enteritidis (SE) compared to chicken may be due to the higher expression of pro-inflammatory cytokines and unique pattern of IL-10 expression in guinea fowl. Further, flow cytometric analysis to study the apoptosis and necrosis in spleenocytes of guinea fowl and broiler chicken infected with Salmonella enterica serovar Enteritidis (SE) was done at 1, 4 and 12 hrs Post inoculations. Apoptosis analysis suggested that guinea fowl spleenocytes were more resistance to apoptosis than broiler spleenocytes in control as well as in induced conditions, which is a physiological indicator of higher survivability and better resistance mechanism. In vitro Pathogen induction studies revealed that guinea fowl was more responsive than broiler chicken in terms of expression of immune molecules and patho-physiological changes occurred in cell culture. The unique responses of the guinea fowl were in contrast to the broiler which has the history of intense genetic selection for the increase growth or reproduction, a process which may leads to reduced or suppressed inflammatory responses. Finally, the results of the present investigation were encouraging for the establishment of guinea fowl as a model for the disease resistance studies.

**Keywords:** guinea fowl, disease resistance, cytokines, spleenocytes, salmonella

## **Assessment of the activity of *Lactobacillus* spp commercial against *Salmonella* Minnesota with multiple drug resistance profile**

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*Salmonella* Minnesota (SM) is one of the most prevalent serovars in Brazilian poultry farming, raising concerns for the poultry industry and public health, especially strains that have a multiple drug resistance (MDR) profile. Probiotics are an alternative to MDR bacterial isolates, due to their antagonistic capacity, reduction of pathogenic bacterial adhesion to the intestine, aggregation and co-aggregation, as well as the production of antimicrobial substances, such as bacteriocins. Therefore, this study aimed to evaluate the antagonistic effect of strains of *Lactobacillus* spp., against *S. Minnesota* MDR of poultry origin. For this purpose, a pool of *Lactobacillus* spp. was used. (PL) commercial and 12 SM isolates of poultry origin. The samples were subjected to co-culture testing to evaluate the influence of *Lactobacillus* spp. about the growth of SM. They were divided into G1 (*S. Minnesota*), G2 (Pool *Lactobacillus*), G3 (Co-Culture plated on MacConkey (MC) agar) and G4 (Co-Culture plated on Rogosa agar). PL and SM were grown in appropriate broths, the following day equal amounts of each culture at a concentration of 10<sup>8</sup> CFU/mL were inoculated in MRS-MH broth and incubated at 37°C and at times 0, 6, 12 and 24h, an aliquot of the cultures was removed for serial dilution and plating on MC and Rogosa agar for quantification. For the co-aggregation assay, PL and SM were grown and adjusted to 10<sup>8</sup> CFU/mL. The PL cell suspensions were homogenized and mixed with the SM culture and incubated for 4 hours at 37°C. The data were subjected to analysis of variance followed by means comparison tests. A significance level of 5% was adopted. The PL had the capacity to co-aggregate with SM with averages of 8.79%, with an upper limit of 26.77% and a lower limit of 2.57%. For co-culture, averages of 8.39, 9.38, 6.03, 8.80 Log CFU/mL were observed for groups G1, G2, G3 and G4, respectively, significantly differentiating all groups from each other. When comparing groups G1 (8.39) with G3 (6.03), a reduction in the amount of SM is observed, demonstrating the efficiency of PL. It was also observed that over the hours the PL increased its growth. The commercial *Lactobacillus* Poll showed the capacity for autoaggregation, coaggregation and culture inhibition of *S. Minnesota* MDR, indicating potential inhibition of the pathogen, therefore, the probiotic strains studied can contribute to *Salmonella* control programs in poultry, in view of the results obtained.

**Keywords:** *Lactobacillus* spp; *Salmonella* Minnesota; MDR; broiler

## **Protective Efficacy of Inactivated Trivalent-H5 Vaccine Against Challenge with Both HPAIV-H5N1 and H5N8 Viruses in Turkey Poults Under Both Laboratory and Field Conditions**

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The highly pathogenic Avian Influenza (HPAI) virus belonging to subtype H5 assumed endemicity in Egypt since its first appearance in 2006, leading to severe economic losses. Turkey is one of the most susceptible birds to HPAI virus which may lead up to 100% mortality. The aim of this work was to evaluate the protection efficacy of a commercial inactivated oil emulsion H5 vaccine (MEFLUVAC H5 plus8 vaccine at 7th days old) against challenge with HPAI-H5N1 clade 2.2.1.2 and -H5N8 clade 2.3.4.4b in turkeys raised under field and experimental conditions. The study involved 10,000 white turkey poults (1-day old) that were imported from France (free from H5 antibodies since their breeder flocks did not receive any H5 vaccine nor got exposed to infection). The birds were divided into 8 groups(G), all birds in G1, 3, 4, 6, 7, and 8 contained 15 poults each and were similarly kept under BL-3 conditions. Birds in G1, G2, G4 and G5 served as vaccinated challenged groups, while G3 and G6 were non-vaccinated challenged. Birds in G7 as vaccinated nonchallenged and G8, maintained as non-vaccinated non-challenged group. The challenge was applied at 28-days post vaccination (35 day of life) with either H5N1 or H5N8. The remaining 9910 vaccinated turkeys raised in a commercial farm, but at 32 days of age, 30 birds moved to laboratory and divided into G 2, 5 and monitored for 3-days, then infected with H5N1 and H5N8, respectively. Serum was collected from all birds at 14,21, 28, and 35 days old to determine the antibody levels using hemagglutination-inhibition (HI) test. Regarding mortality rates, the results indicated that lab vaccinated G1 and G4 showed 100% protection after both H5N1 and H5N8 challenge, while farm vaccinated G2 and G5 had 100% and 93.3% protection after H5N1 and H5N8 challenge, respectively. The non-vaccinated challenged birds showed 100% mortality within 3 DPC in both H5N1 or H5N8 challenged G3 and G6, respectively. In conclusion, The Trivalent H5 vaccine (MEFLUVAC H5) (containing H5N1 clade 2.2.1.1, H5N1 clade 2.2.1.2 and H5N8 clade 2.3.4.4) was Immunogenic by 2-3 weeks post-vaccination and develop detectable level of humeral immune response and the vaccine significantly reduce virus shedding on level of shedders and amount of shedding virus in both commercial kept and BSL-3 kept birds in addition to providing 100% protection against mortalities for birds kept under BSL-3 condition and 93.3% for birds kept under commercial condition and moved to BSL-3 on 72 hrs. prior to challenge. Future work still required to evaluate the protection at older ages as turkey kept for 90-120-day age.

Keywords: Turkey, Avian Influenza, H5N1, H5N8

## **Efficacy of the application of an attenuated *Salmonella* Typhimurium vaccine in a broiler farm to reduce excretion and colonization of field strains after and experimental infection**

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*Salmonella enterica* subsp. *enterica* serovar Typhimurium is an important zoonotic pathogen that poses a threat to public health, and broilers are often asymptomatic carriers of this bacteria. *Salmonella* Typhimurium serovar represent 11,4% of human salmonellosis and monophasic *S. Typhimurium* represent 8,8% of the 50,817 confirmed human cases accounted for 60,050 total cases of human salmonellosis in the EU in 2021. In the present work, we investigated the efficacy of the application of an attenuated vaccine against *S. Typhimurium* in a broiler farm. The assay started in a commercial farm and the efficacy was evaluated in laboratory facilities by demonstrating reduction of excretion and colonization after and experimental infection with field strains. One-day-old broilers from a commercial farm (n=23.200) were vaccinated with PRIMUN SALMONELLA T vaccine by drinking water. Unvaccinated broilers were kept isolated in an experimental farm until the challenge day. At 42 days of age, broilers from vaccinated group (n=20) and control group (n=20) were experimentally infected with two *Salmonella* Typhimurium field strains. Excretion, by cloacal swabs sampling, and colonization, by internal organs isolation were evaluated. Animals from control group (non-vaccinated and infected) shed the field strain at rates of 65%, 55%, 65%, 60% and 70% corresponding to sampling days 3, 5, 7 10 and 14; meanwhile vaccinated group presented a significant ( $p < 0.05$ ) lower excretion being 25%, 10% and 10% at days 3, 5 and 7 and negative at days 10 and 14. Considering colonization of internal organs (spleen, liver and caeca) at 7 days the control group presented a mean of 46,6% of infection, meanwhile the vaccinated group showed 26,6% of infection. At 14 days the rate of infection achieved was 63,3% in control group, in contrast to vaccinated group that reduced significantly ( $p < 0.05$ ) the infection rate to 13,3%. The results demonstrate that the application of a live attenuated monovalent vaccine against *S. Typhimurium* at 1 days of life protects commercial broilers until 42 days of life.

**Keywords:** live attenuated vaccine; *Salmonella* Typhimurium; excretion and colonization reduction

[P1-53]: P1-Welfare, Sustainability, Phylogenetic, Egg quality, Respiratory diseases Food Safety (ID: 130781) - Germany

## **Stability of a bivalent live Salmonella vaccine in drinking water over a 24-hour period, with and without the addition of a water stabilizer**

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Salmonella Enteritidis and S. Typhimurium are the two most important zoonotic Salmonella serovars, and the use of live vaccines for breeding and laying hens has been an important tool to control these serovars. Live vaccines can be applied from the first day of life, they provide early protection and initiate of a strong local immune response. Current instructions for the application of live Salmonella vaccines in water suggest that birds should consume the vaccine within a period of three to four hours; however, this is often not feasible due to the small amount of water consumed per bird and the relatively long length of the drinking water line in commercial poultry houses. Therefore, some pullet rearers choose to vaccinate after a few days, which leaves the birds unprotected during the first week of life, where they are most susceptible to infection with a field strain. The rationale behind this trial was to evaluate the stability of a bivalent Salmonella vaccine (AviPro™ SALMONELLA DUO) in drinking water over a period of 24 hrs to reassure poultry rearers that the vaccine is stable beyond a three to four hour time window. The vaccine was reconstituted in drinking water, with and without a commercially available water stabilizer (AviBlue®), to give a final dilution of 1 dose in 10ml of water. Incubation was performed over a period of 24 hours at 15°C and 30°C to mimic conditions in the field. At given time points, serial dilutions were prepared and the two vaccine strains were enumerated on non-selective agar plates substituted with either streptomycin or nalidixic acid to differentiate between the two strains. A minimum of 107 CFU of each of the vaccine strains per ml was expected at the start of the experiment, which was achieved for both vaccine strains in all experiments. After incubation for 24 hrs, every sample still contained more than 107 CFU, proving that the amount of live vaccine present after 24 hrs was still compliant with the requirements in the Summary of Product Characteristics. Over the first 12 hours of incubation, virtually no decline was seen in any of the experiments, and only a slight decline was observed at the 16 hr and 24 hr time points. These results prove that, although it is recommended to vaccinate birds over a period of three to four hours, the vaccine can still be viable and stable for up to 24 hours. These data support a decision towards vaccinating birds as early as possible, despite the fact that they will take several hours to consume the full vaccine doses.

Keywords: Salmonella; vaccine; stability; drinking water



[P1-54]: *PI-Welfare, Sustainability, Phytogetic, Egg quality, Respiratory diseasesFood Safety* (ID: 130827) - Mexico

## **Removal of Aflatoxin B1 Using Alfalfa Leaves as an Adsorbent Material: A Comparison between Two In Vitro Experimental Models**

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Mycotoxins are toxic secondary metabolites synthesized by several species of fungi. More than 300 types of mycotoxins are known, including aflatoxin B1 (AFB1), which is considered one of the most toxic substances of natural origin. AFB1 is considered a ubiquitous contaminant of different agricultural products intended for the preparation of feed, and consumption of these contaminated products causes serious problems of health. To reduce the negative effects produced by aflatoxins, the poultry industry has included inorganic adsorbent materials in the feed; however, these materials are capable of releasing toxic heavy metals and dioxins. In recent years, various organic adsorbents have been developed, showing their effectiveness in adsorbing AFB1 and improving the health status of birds. In this research, an adsorbent material derived from alfalfa leaves was prepared and further characterized, and its efficacy for removing aflatoxin B1 (AFB1) was investigated. Characterization consisted of the use of attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), environmental scanning electron microscopy (ESEM), X-ray fluorescence spectroscopy (XRF), X-ray diffraction (XRD), point of zero charge (pHpzc), zeta potential ( $\zeta$ -potential), UV-Vis diffuse reflectance spectroscopy, and spectral analysis. To determine the adsorption capacity against AFB1 (250 ng AFB1/mL), pH-dependent and avian intestinal in vitro models were used. The adsorbent inclusion percentage was 0.5% (w/w). In general, the pH-dependent model gave adsorption percentages of 98.2%, 99.9%, and 98.2%, evaluated at pH values of 2, 5, and 7, respectively. However, when the avian intestinal model was used, it was observed that the adsorption percentage of AFB1 significantly decreased (88.8%). Based on the characterization results, it is proposed that electrostatic, non-electrostatic, and the formation of chlorophyll-AFB1 complexes were the main mechanisms for AFB1 adsorption. From these results, it can be concluded that the adsorbent derived from alfalfa leaves could be used as an effective material for removing AFB1 in in vitro digestion models that mimic the physiological reality.

Keywords: aflatoxin B, alfalfa leaves, adsorption, in vitro digestion models, poultry, characterization

## **Influence of inactivated Salmonella vaccine in breeders on the vertical transference of MDA in day-old chicks**

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Salmonella is one of the most important enterobacteria that threatens food safety requirements for poultry products all over the world. Actions taken to reduce its prevalence on poultry farms include biosecurity, cleaning and disinfection, bacteriophages and vaccination. Live and inactivated Salmonella vaccines are currently available on the market. Live vaccines represent a great support, especially blocking the invasion and adhesion site through the gut and providing a crucial local and cellular immunity. Inactivated Salmonella vaccines are known to produce B-cells with a higher level of circulating antibodies that will protect the Salmonella targeted organs such as the liver, spleen, gut, and ovaries, resulting in the vertical transmission of these circulating antibodies and delivering early protection to the progeny. The purpose of this study was to evaluate the difference in MDA (maternally-derived antibodies) for Salmonella in the progeny in day-old chicks hatched from broiler breeders with different Salmonella vaccination programmes. 15 sera samples from 2 groups of day-old chicks were collected, group A, hatched from breeders with no live Salmonella vaccine and 1 inactivated Salmonella Enteritidis and Typhimurium vaccine, and group B, hatched from breeders with 3 live Salmonella Enteritidis and Typhimurium vaccines. Samples were analyzed with a commercial ELISA Salmonella kit for serogroups B and D. Statistical analysis was done using the R software v3.1. A p-value of < 0.05 was chosen as the limit for statistical significance. A Proportion Test and a Wilcoxon Test were performed to compare performances between the two groups. Group A showed statistically significantly higher rates of seropositivity compared to group B (67% vs 15% pvalue <0.05). From all positive samples, the geometric mean was also statistically higher in samples from group A compared to group B samples ( $1717 \pm 1606$  vs  $259 \pm 471$  pvalue <0.05). In conclusion, the inclusion of an inactivated Salmonella vaccine in the breeders' vaccination programme provided higher MDA to the progeny compared to live Salmonella vaccines. This result shows that both live and inactivated Salmonella vaccines need to be included in breeders' vaccination schemes.

Keywords: salmonella, vaccines, antibodies, progeny, food safety

## **Prevalence and characterization of ESBL producing *Klebsiella pneumoniae* from Sonali chicken meat in Narsingdi district, Bangladesh**

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Antimicrobial resistance (AMR) is a serious worldwide issue. A major portion of the Bangladeshi populace relies on poultry meat as a primary protein source. The presence of Extended spectrum  $\beta$ -lactamase (ESBL) producing bacteria on the surface of poultry meat raises great concerns due to the potential transmission to consumers, indicating a severe public health threat. This study aims to detect and characterize ESBL producing *Klebsiella pneumoniae* from Shonali chicken meat. A total of 390 meat swab sample were collected from various retail poultry shops situated across the Narsingdi district in Bangladesh, and 164 isolates, accounting for 42%, were identified as *Klebsiella pneumoniae*. Results from antibiotic susceptibility assay unveiled significant resistance patterns, with 92.7% of isolates demonstrating resistance to Penicillin, Ampicillin, Amoxicillin, Cefalexin, and Cefradine. Conversely, lower rates of resistance were observed against Amoxicillin-Clavulanic acid (8.5%), Gentamycin (14.9%), and Levofloxacin (17.7%). Resistance levels to Cefpodoxime, Ciprofloxacin, Streptomycin, Chloramphenicol, Co-trimoxazole, Tetracycline, and Erythromycin ranged from 23% to 87%. Notably, the multidrug resistance rate was found to be 93.9% (154 out of 164 isolates). All isolates harbored at least one  $\beta$ -lactamase producing gene, with blaTEM being the most prevalent (92%). Additionally, other ESBL genes, including blaSHV, blaCTX-M, blaCTX-M-1, blaCTX-M-2, blaOXA-1, were identified in 41.46%, 68.29%, 71.95%, 70.12%, and 20.73% of the isolates, respectively. Furthermore, 19.51% of the isolates were found to carry blaNDM-1 gene. The high prevalence of ESBL-producing *Klebsiella pneumoniae* on poultry meat, specifically within retail outlets, highlights a potential reservoir for human colonization and community transmission. It emphasizes the crucial need for vigilant surveillance throughout the entire poultry production-supply-consumer chain to identify and effectively address the emergence of multi-drug resistant *Klebsiella pneumoniae* in Bangladesh.

Keywords: AMR, Poultry, *Klebsiella pneumoniae*, ESBL, prevalence

## **Phage Therapy Modifies the Host's Intestinal Ecosystem by Modulating the Microbiome: A Meta-Analysis Approach**

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Bacteriophages (phages), viruses that target bacteria, are being explored as potential treatments against antibiotic-resistant bacteria in poultry. This exploration is exemplified by recent studies on *Salmonella*, *Campylobacter*, and *Escherichia coli*. However, there is consideration of the possibility that phages may combat not only pathogens but also modify commensal bacteria, thereby inducing immunomodulation. Given the crucial role of the gut microbiota in host health, its potential modulation by phages warrants investigation. The objective of this study is to determine, through meta-analytic assessments, whether phage therapy influences the microbiota of broiler. To achieve this, raw data from various microbiome datasets were collected, reprocessed, and reanalyzed. For data set collection, case-control 16S studies were sourced from PubMed. The inclusion criteria for eligible studies were: (i) studies conducted in broilers older than 21 days with stabilized microbiota, (ii) studies involving caecal samples, and (iii) publicly available raw 16S data and metadata indicating case or control status. Raw data from four suitable case-control 16S datasets were downloaded from SRA repositories and processed. The QIIME2 software was utilized for the analysis of amplicon sequence variants (ASVs). The findings revealed that animals treated with phages, regardless of whether these were against *Salmonella*, *Campylobacter*, and *Escherichia coli*, exhibited the presence of *Lactococcus* (bacteria that improve digestion and strengthen the immune system), *Acetanaerobacterium* (bacteria linked to high enterolactone production with positive effects on hormonal health), and *Desulfotomaculum* (bacteria promoting bacterial translocation at the gastrointestinal epithelium level), genera absent in animals not subjected to phage therapy. Our results demonstrate that, although the treatment of various gastrointestinal pathogens with phages is successful, their use can also modify the host's intestinal ecosystem by modulating the microbiome.

**Keywords:** Microbiota; Public Health; Bacteriophages; Zoonotic

## **Prevalence and antibiotic resistance of *Enterococcus faecium* /*faecalis* isolated from broiler chicken meat in Poland**

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*Enterococcus faecalis* and *E. faecium* are opportunistic pathogens in humans and can also cause significant losses in poultry production. Enterococci have the ability to acquire, express and transfer resistance genes leading to the development of antimicrobial resistance. Consumption of poultry products contaminated with antimicrobial-resistant enterococci can pose a threat to public health. The aim of this study was to determine the prevalence of phenotypical antimicrobial resistance and to detect drug resistance genes in *E. faecalis* and *E. faecium* in poultry meat from Poland. In our study, we sampled 124 samples commercial meat from chicken breasts purchased in grocery stores. Identification of isolated colonies was carried out using the MALDI TOF method. Antimicrobial resistance was determined using microbroth dilution method and antimicrobial resistance genes were detected using polymerase chain reaction and gene-specific primers. Enterococci could be isolated from 79.8% of the samples, with *E. faecium* (n=99) as the most prevalent species. The most prevalent phenotypic antibiotic resistances of all isolates of *E. faecalis* were to tetracycline (90,5%), followed by erythromycin (77%) and ciprofloxacin (36%). Isolates of *E. faecium* showed resistance to tetracycline (75%), quinupristin/dalfopristin (50%) and erythromycin (50%). Among *E. faecalis* isolates, 55.6% was multidrug resistant to at least 3 classes of antibiotics. Phenotypically resistant *E. faecalis* and *E. faecium* isolates contained the highest prevalence of the tetM (30%) resistance gene, followed by tetL (21%), ermB (20,6%), aac (4,7%) and vanA (2,9%). Considering these high resistance levels in *Enterococcus* species, it is necessary to reduce the use of antibiotics in poultry production. This project received funding from the Polish National Science Centre NCN under grant number 2021/03/Y/NZ7/00136 and the Netherlands Organization for Health Research and Development ZonMw under grant number 1070132110001 under the umbrella of the JPIAMR - Joint Programming Initiative on Antimicrobial Resistance.

Keywords: *Enterococcus faecium*, *Enterococcus faecalis*, broiler, meat

## **Multidrug resistant escherichia coli and salmonella isolated from water, litter and meat in commercial poultry farms bio-security concern and emerging public health threat in pakistan**

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In Pakistan poultry industry is one of the highly growing industry. However, poultry industry has to face many challenges including infectious agents including zoonotic pathogens of biosafety and biosecurity concerns. Multidrug resistant bacteria (MDR) including *Escherichia coli* (*E. coli*) and *Salmonella* species are the main causative agents of poultry diseases occur in poultry farms environment. These pathogens cause severe public health concerns like intestinal infection, urinary tract infection and food poisoning, if exposed to outside environment. Present study was aimed to identify bacterial pathogens of zoonotic importance, from poultry farm environment including litter, poultry drinking water, and poultry meat. Sixty samples were collected from different poultry farms of Rawalpindi and Islamabad, Pakistan. These sample were comprised of poultry litter sample, water sample and meat samples, twenty each. Morphological characteristic and Biochemical tests confirmed the presence of *E.coli* and *Salmonella*. For molecular characterization of pathogenic bacterial specie specific PCRs were performed. Out of 60 samples 39 (65%) were found positive for *E.coli* and 31 (51.7%) for *Salmonella*. Antibiotic profile analysis was also performed to determine the antimicrobial resistance status of the isolated pathogens. *E.coli* were found to be resistant against penicillin, Cefotaxime and Tetracycline whereas *Salmonella* were found to be resistant against penicillin, Cefotaxime and Levofloxacin. Findings provide a baseline data on MDR *E.coli* and *Salmonella* circulating around these poultry farms, it would assist the veterinarian and public health professionals in rational treatment and biosafety planning.

**Keywords:** Multidrug resistance, *Escherichia coli*, *Salmonella*, zoonosis, Biosafety

## **Characterization of the *Clostridium perfringens* and *Clostridioides difficile* hazards in broiler slaughterhouses**

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*Clostridium* is a bacterial genus that includes many toxin-producing species potentially highly pathogenic to humans such as *C. perfringens* and *C. difficile* (recently renamed *Clostridioides difficile*). *C. perfringens* is the fifth most frequently suspected pathogen in foodborne outbreaks in Europe. Similarly, *C. difficile* is an emerging pathogen, causing nosocomial diseases but also potentially zoonotic infections in humans. Meat is a suspected source of *C. perfringens* and *C. difficile*, as farmed animals are healthy carriers of these bacteria in their digestive tract. Improper evisceration during slaughter may lead to meat contamination. We performed seven sampling campaigns in three broiler slaughterhouses to evaluate the risk associated to *C. perfringens* and *C. difficile* in chicken meat in France. Broiler caeca, meat, surface and ambient air samples were taken along the slaughter line and in the meat-cutting plan. *C. perfringens* and *C. difficile* were detected using culture methods. *C. perfringens* was isolated from 17 samples out of 58 analysed samples: one batch of broilers carried *C. perfringens* in caeca (5 positive samples out of 5) and chicken legs from the same batch were also positive (2/5). The slaughterhouse environment was also contaminated (defeathering machine, scalding tank). *C. difficile* positive samples were obtained from the 7 campaigns of sampling (22 positive samples out of 406) but from slaughter environment mostly (20), except one positive sample from broiler caeca. Another positive sample was observed in the environment of the cutting-plan that processed the positive batch of broilers. Scalding tank (8/14), defeathering machine (7/14) and broiler transport crates (4/14) were the sampling points the most frequently positive for *C. difficile*. The study will be completed with 5 additional sampling campaigns. Up to five bacterial isolates per positive sample will be further characterized using specific molecular typing methods and toxinotyping. Results will be available for the EPC conference in 2024. The results will contribute to better assess the risk of poultry meat contamination by *C. perfringens* and *C. difficile*, enabling poultry sector operators to adapt the prevention and mitigation measures implemented.

**Keywords:** broiler; *C. difficile*; *C. perfringens*; meat quality; slaughter

## **Identification and antibiotic susceptibility profiling of bacteria isolated from backyard poultry**

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Backyard poultry plays an important socio-economic role both in developing and underdeveloped countries. This sector is increasing worldwide to meet the daily needs of household. However, it is facing various challenges including infectious agents which cause severe infections in poultry and humans. Among these infections, bacterial diseases are playing a major role. To control these bacterial diseases, excessive and irrational use of antibiotics is common in backyard poultry which is causing the development of antimicrobial resistance (AMR) in those bacteria. The present study was designed to; identify the pathogenic bacteria present in the fecal and rectal samples collected from backyard poultry and antibiotic resistance profiling to determine AMR in the isolated bacterial pathogens. Total sixty (fecal and rectal) swab samples have been taken from backyard poultry and cultured on various biological media. Gram staining and different biochemical tests (Methyl Red, Citrate, and Glucose utilization) were performed for identification of pathogenic bacteria. Kirby Bauer Disc Diffusion test was used to determine AMR profiling of isolated pathogens. Out of total 60 samples, 41 (68.3%) were found positive for *E. coli*, 2 (3.3%) for *Salmonella* and 8 (13%) for *Staphylococcus*. All of the *E. coli* isolates (100%) were found resistance against Vancomycin whereas 85% were found resistance against Amoxicillin. *E. coli* isolates; 100%, 80% and 82% were found susceptible against Ciprofloxacin, Enrofloxacin and Gentamycin, respectively. All the *Salmonella* isolates have shown resistance against Amoxicillin, Vancomycin and penicillin and sensitivity against Ciprofloxacin and Gentamycin. All the staphylococcal isolates were found resistant against Streptomycin, Oxytetracycline, and Enrofloxacin while found susceptible against Gentamycin, Amoxicillin, and Vancomycin. This study confirmed the presence of pathogenic bacteria (*E. coli*, salmonella and staphylococcus) in backyard poultry which have also shown AMR against various antibiotics. The excessive use of antibiotics for therapeutic purpose cause development of AMR. Besides, that the improper handling and lack of biosafety measures transmit these pathogens to humans and cause zoonotic diseases. Rational use of antibiotics and proper biosafety measures can reduce the prevalence of pathogenic bacteria in backyard poultry and risk of zoonotic diseases.

**Keywords:** Backyard poultry; *E. coli*; *Salmonella*; *Staphylococcus*; Antibiotic Resistance



## **The fatty acid composition of egg yolk from hens fed diets differing in maize hybrid, rapeseed oil level and addition of emulsifier**

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The research aimed to examine how varying levels of rapeseed oil (RO) and the inclusion of natural emulsifier (NE) in hen diets would impact the fatty acid composition in table eggs. The hens were provided with diets that differed in maize hybrid (H1 and H2), RO levels (2%, 3%, and 4%), and NE addition (Lysoforte®, Kemin, USA; 0.05% and no addition). Lysoforte® is a natural biological emulsifier containing an optimized combination of lysophospholipids, whose main active ingredient is lysolecithin, which plays a role in promoting a more efficient utilization of dietary fats and improves the absorption of other nutrients. A total of 12 treatments, organized in a 2x3x2 factorial design, were randomly assigned to 72 cages, each housing 3 Lohmann Brown hens. The hens were fed the specified diets for eight weeks, and egg samples for analysis were collected at the end of the sixth week. The results were analyzed as a three-factorial experiment. The fat content remained unaffected by variations in maize hybrid, rapeseed oil levels, and natural emulsifier addition. In terms of fatty acid composition, the maize hybrid demonstrated a statistically significant impact on saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), and n3 and n6 content. Samples with H1 exhibited lower SFA (33.70%) than H2 (34.48%) but higher PUFA (23.90%), n6 (22.99%), and n3 (0.50%) content. The addition of RO influenced the content of nearly all fatty acids, with samples containing 2% RO displaying the highest levels of SFA (34.93%) and MUFA (43.77%), while those with 3% and 4% RO showed higher PUFA (24.08 and 24.58%), n3 (0.48 and 0.46%), and n6 (23.19 and 23.72%) content. NE addition resulted in elevated SFA (34.26%), particularly C18:0 (8.50%), and reduced PUFA (23.04%) and n6 (22.16%) content.

Keywords: table eggs; rapeseed oil; emulsifier; fatty acid profile

## **Effect of a new 3-phytase on egg production and quality and bone mineralisation traits in laying hens**

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Exogenous phytase is commonly used in poultry diets to mitigate the adverse effects of phytate on bird performance, environmental impact, and feeding costs. Despite evidence of phytase enhancing phosphorus (P) digestibility in laying hens, there is limited information on how phytase dosage affects hen performance, egg quality, and bone mineralization. This study aimed to assess a novel 3-phytase's efficacy on laying performance, egg quality, and mineralization in hens. A total of 288 Lohmann brown laying hens (16 weeks old) were randomly housed in 72 cages (4 animals/cage) for 106 days. At 22 weeks, hens were weighed, and cages were assigned to one of five dietary treatments: PC (positive control, no added phytase, Ca 3.69%, P 0.60%); NC (negative control, no added phytase, Ca 3.69%, P 0.38%); and three diets where NC was supplemented with 250, 500, and 1000 FTU/kg of the new phytase. Feeding continued until 31 weeks. Body weight (BW) was recorded at 16, 22, 25, and 31 weeks. Daily egg production, egg weight, average daily feed intake (ADFI), and feed conversion rate (FCR) were monitored. Egg quality parameters were measured at specified intervals. At 31 weeks, one bird per cage (12 animals/treatment) was euthanized, and tibia ash, Ca, and P content were analysed. Dietary inclusion of 1000 FTU of the new 3-phytase improved laying performance compared to PC (+0.02;  $P < 0.05$ ), with lower ADFI values ( $P < 0.05$ ) resulting in improved FCR compared to PC (-0.09;  $P < 0.05$ ). Shell thickness in NC-fed hens was lower than PC (-0.011 mm;  $P < 0.05$ ), but supplementation with 250 FTU restored PC values. Yolk color values in NC-fed hens were higher than PC (+0.31 points), but 1000 FTU supplementation restored PC values. Regarding bone mineralization, NC-fed hens exhibited lower tibia ash and P content compared to PC (-1.9% and -0.3% in dry matter, respectively;  $P < 0.05$ ), while phytase diets showed intermediate values. In conclusion, phytase supplementation contributes to improved egg production and quality, as well as reduced mineral supplementation for adequate bone mineralization.

Keywords: Phytase, hens, laying, egg, mineralisation

## **Eggshell quality in extended laying cycle**

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To improve sustainability of egg production, current practices in the table egg industry aim at lengthening the egg laying cycle of commercial hens from 70-80 to about 100 weeks of age. In such a context, the control of eggshell quality is the limiting factor of extension of the laying cycle rather than the egg production persistency itself. The aim of our study was to precise which traits of eggshell quality were the most affected by hen ageing, in order to identify which physiological mechanisms were affected and need to be amended. We have investigated the effect of layer age on various eggshell quality parameters (shell breaking strength, dry weight, thickness and toughness) by comparing eggshell of commercial hens reared in cage-free system at 32-34 (peak of lay), 71-73 (late laying cycle) and 94-99 (very late laying cycle) weeks of age. As expected, eggshell breaking strength and thickness significantly decreased during the laying cycle: -17% and -27% for the breaking strength, and -4% and -12% for the thickness, respectively at 71-73 and 94-99 weeks of age. Interestingly, eggshell weight was significantly lower at the oldest age (about -7% at 94-99 weeks). Eggshell toughness was decreased by 12% in both 71-73 and 94-99-week-old hens, when compared to 32-34-week-old hens. Eggshell mechanical properties are due to the mass of shell and to the shell fabric, which comprises the morphology, size and orientation of crystals that define the shell texture and ultrastructure. As the hen ages, shell mass remains constant until 71-73 weeks of age, while egg weight increases, resulting in a reduction in shell thickness. The decrease in eggshell mechanical quality parameters observed between 32-34 and 71-73 weeks is likely due to this decrease of shell thickness combined with modifications of the shell fabric and eggshell texture as shown by the decrease in shell toughness. However, the further reduction in shell biomechanical properties in older hens (94-99 weeks of age) seems rather to be attributable to a decrease in shell mass combined with changes in the ultrastructure of the calcite crystals making up the shell. As shell mass is critically affected at the end of long laying cycle, particular attention must be paid to monitoring calcium metabolism and transport in long-life layers. Further studies are needed to explore the underlying mechanisms leading to eggshell degradation at 94-99 weeks.

**Keywords:** Extended laying cycle; eggshell quality; breaking strength; thickness; dry weight; toughness

**Influence of natural pigments on the shelf life of eggs from Isa Brown layers****C. Matache<sup>1,2</sup>, T. Panaite<sup>1</sup>, G. Cornescu<sup>1</sup>, D. Dragotoiu<sup>2</sup>**<sup>1</sup>The National Research - Development Institute for Animal Biology and Nutrition, Balotesti, Romania,<sup>2</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania*Presenting author: camelia.matache@ibna.ro*

The study investigated the effects of extracts of adding two natural pigments, marigold flower and paprika, into laying hens' diet on the shelf life, lutein content, and yolk colour intensity. The experiment was conducted for 6 weeks, on 37181 Isa Brown laying hens, in farm conditions, accommodated in Galn cage model, divided into 18590 control (C) and 18591 experimental (E) groups. The E group received a diet supplemented with 0.05% marigold and 0.02% paprika extracts. Free access to feed and water was provided. At the end of experiment, 60 eggs were collected to assess internal and external egg parameters, and lutein concentration in the yolks was determined. For shelf-life assessment, 120 eggs were collected, divided into two analysis periods (14 and 28 days), and stored at  $\pm 4^{\circ}\text{C}$  and  $\pm 20^{\circ}\text{C}$ , to establish quality parameters. The study findings indicated a significant improvement in egg colour (DSM Yolk Color Fan), with values increasing from  $10.8 \pm 0.3$  (C) to  $12.1 \pm 0.6$  (E) ( $p < 0.05$ ). This positive change in colour was found to be correlated with a significant increase ( $p < 0.05$ ) in lutein concentration within the egg yolk, reaching 23 mg/kg in group E compared to 16.9 mg/kg in C group. At the 14-day storage period, for both storage temperatures, the lightness parameter ( $L^*$ ) did not exhibit significant changes ( $p \geq 0.05$ ) compared to the initial values (42.5; 41.6 at  $\pm 4^{\circ}\text{C}$  and 43.8; 43.4 at  $\pm 20^{\circ}\text{C}$  vs. 43.8; 43.6 at the initial period). However, at 28 days, there was a notable decrease ( $p < 0.05$ ) in this parameter measured at both  $\pm 4^{\circ}\text{C}$  and  $\pm 20^{\circ}\text{C}$ . Throughout the 14-day period, values for the  $a^*$  (redness) and  $b^*$  (yellowness) parameters remained constant, consistent with the initial measurements (day 0) at both storage temperatures. Conversely, at 28 days, there was a significant increase ( $p < 0.05$ ) in yolk colour intensity for both C and E groups, at both  $\pm 4^{\circ}\text{C}$  and  $\pm 20^{\circ}\text{C}$ , due to accelerated biochemical processes of degradation within the eggs. Regarding the pH of the eggs, as expected, the values increased significantly ( $p < 0.05$ ) with the shelf time correlated with Haugh Unit values, who decreased highly significantly at 14 days, especially at  $\pm 20^{\circ}\text{C}$ . In this study, we demonstrated that the use of natural pigments from marigold and paprika extracts increasing the lutein content and intensified the yolk colour especially the values of the parameter  $a^*$ , but the shelf time and temperature storage is a crucial factor in maintain eggs quality.

Keywords: egg quality, lutein, marigold extract, paprika extract, yolk colour

## **Evaluating the impact of temperature and storage duration on egg quality under tropical climatic conditions**

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The present study aimed to address the shelf-life of eggs under Indian conditions in order to provide better, safe and quality eggs to the consumers. Due to the significant heterogeneous geographical environs between production areas and consumers place, the freshness of eggs in tropical regions is always questioned. It is well known that egg is a perishable commodity. But in tropics, the variation in temperatures (mostly higher) between the places accelerates the egg quality. In these circumstances, consumers are more concerned about its quality and freshness during consumption. The current study assessed the influence of storage time and temperature on both external and internal qualities of chicken eggs, at different storage conditions, such as ambient (25-30°C) and refrigeration (3- 4°C) temperatures. A total of 108 fresh white-shelled table eggs were sourced from the Central Avian Research Institute's layer farm and then the eggs were separated into two treatment groups: T1 - kept at ambient temperature (25-30°C) and T2 - kept at refrigeration (3- 4°C). Eggs were tested for their internal and external quality at 0, 3, 6, 12, 18, and 24 days of storage. Results showed a significant ( $P<0.05$ ) impact on internal as well as external qualities in both treatment groups. Eggs exposed to ambient (25-30°C) temperature started to deteriorate significantly ( $P<0.05$ ) as the age advanced, i.e, higher values in the Haugh unit, yolk index, egg pH and albumen pH. In contrast, refrigerated (3-4°C) eggs significantly maintained their quality at all ages, i.e., exhibited a lesser value ( $P<0.05$ ) in all parameters up to 24th day of storage. The eggs stored at ambient temperature expressed a significant increase in total plate count (TPC), starting from 3.64 cfu/ml at day 0 to 6.50 cfu/ml by day 24. While refrigerated eggs showed minimal growth in TPC, i.e. 3.64 cfu/ml on day 0, to 4.33 cfu/ml by day 24. From the present study, it was identified that eggs stored at ambient temperature favoured microbial growth, which leads to rapid deterioration beyond the 12th day of storage in the tropics, whereas at refrigerated storage, egg quality maintained its freshness throughout the study period (24 days). This study can give a baseline idea for the poultry farmers/entrepreneurs/stake holders regarding the storage days and temperature during the marketing channels.

**Keywords:** Chicken eggs; storage time; egg quality; Microbial Quality; Haugh unit

## **The effect of experimental *Ascaridia galli* infection on egg quality in three local chicken breeds**

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Parasite infections in free-range chickens can lead to reduced performance and growth, as well as increased mortality. In addition to significant economic losses, infection also has an effect on animal welfare. Helminths such as *Ascaridia galli* are the main cause of infections found in free-range laying hens. These infections can affect performance as well as egg quality traits in laying hens. While evidence of variation in genetic susceptibility to helminth infections has been shown in the literature, the extent of genetic predisposition across breeds remains unclear. Typically, commercial layers used for egg production demonstrate superior performance compared to common local breeds. However, as these layers are also susceptible to infection, this could lead to considerable impacts on egg quality and quantity. To find local breeds that can bridge this gap and enable the sustainable and ecological use of chickens, we examined physico-chemical egg quality parameters and their response to infection. In this controlled infection trial, 159 hens from three different German local breeds (Altsteirer, ALT; Bielefelder, BIE; Ramesloher, RAM) at the age of 59 weeks were used to analyse egg quality parameters. A total of 506 eggs were analysed, with 164 eggs used to establish a baseline prior to infection on three sampling days, remaining were analysed on a weekly basis from the 2nd to the 8th week after infection. Egg weight (EW), yolk weight (YW), Haugh Unit (HU), breaking strength (BS), shell thickness (ST) and shell deformation (SD) were measured. The average egg laying rate varied between 27.1 and 38.4 %. Statistical tests for most traits showed no significant differences within the breeds or between infected and non-infected birds. Irrespective of differences in egg shape and weight within and between breeds, quality traits were close to commercial standards. For example, 91.56 % of all eggs had a HU greater than or equal to 60, which would correspond to an A quality grade or better. Within the ALT, a significant difference between treatment groups was found for ST ( $p = 0.01$ ). These first results indicate that there is no significant impact of the infection for the measured egg quality parameters within these local breeds. However, the average laying rate was relatively low, which may have led to an underestimation of the effect size. Nevertheless, these breeds could have the potential to contribute to a diverse and organic breeding programme in the future.

Keywords: *Ascaridia galli*; local breeds; Layers; Performance; Organic systems

## **From colorimetry to carotenoids deposition in egg yolk: impact of carotenoids combination on egg yolk pigmentation**

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Carotenoids (CARO) are natural fat-soluble pigments used in the poultry industry to enhance egg yolk color. Egg color is an important characteristic involved in food selection prior to consumption. Combination of red with yellow CARO in animal feed provides attractive color in egg yolks. CARO deposition can be impacted by the type and forms of CARO. A study was conducted to investigate the efficacy of combination of yellow and red carotenoids on egg yolk pigmentation. HyLine Brown hens, of 25 weeks old, were randomly assigned to 5 combinations (COMBI) of yellow and red carotenoids prepared with 2.5 mg/kg of apo-ester (yellow) and 2.5 mg/kg of canthaxanthin (red) in all combinations. Five different products of red and four different products of yellow carotenoids were used in COMBI 1 to 5. Each treatment was replicated with 6 pens of 2 hens for 3 weeks. Mash basal diet was wheat, soybean meal, rice and oats. Efficiency of carotenoids was determined by yolk color and egg yolk deposition at the end of the trial. Yolk color was determined by digital device and Yolfan™. The concentration of canthaxanthin and apo-ester in egg yolks was determined by HPLC. Collected data was subjected to ANOVA and significant differences separated using the Tukey test. Analysis of yolk color using Yolfan™ demonstrated that COMBI 2 had significant lower score of yolk color compared to all COMBI (12.7 vs 11.8; P<0.05). More differences between COMBI were obtained with digital Yolfan™. This objective determination was making more significant differences, confirming lower results with COMBI 2 and further significantly discriminating COMBI 4 from the rest of the combination (P<0.05). Reflectance colorimetry analysis showed that COMBI 2 and 4 had significant smaller contribution of the redness compared to other combination. Higher redness contribution was obtained with COMBI 1 confirming importance of canthaxanthin contribution for yolk color. Deposition of apo-ester in egg yolk was similar between all combinations. Smaller variation of deposition was observed in the analysis of COMBI 1 compared to all other combinations (7.4% vs 11.6 to 15.4%). Significant differences were observed in canthaxanthin deposition ranging from 15 to 26% of less canthaxanthin deposition in egg yolk when animals were fed with COMBI 2, 3 and 4 compared to other combinations investigated. These observations demonstrated that egg yolk pigmentation is not similar between different products tested.

**Keywords:** Laying hen; carotenoids; canthaxanthin; apocarotenoid-ester; egg yolk pigmentation; egg yolk deposition

## **Protected sodium butyrate improves productive performance and egg quality in old laying hens**

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Butyric acid is a volatile short-chain fatty acid natural produced by bacterial fermentation in the intestine of monogastric and can be included as a salt in the diet with several benefits in animal production. When sodium butyrate is protected (PSB) it reaches the proximal small intestine without dissociation, having beneficial effects on digestive enzymes production and villus height, related to the improvement in the nutrient digestibility. The objective of this trial was to evaluate the effect of a PSB (Butirex C4, Novation 2002, Spain) on the performance and eggs quality in 84 Hy-line Brown laying hens from 93 to 106 weeks of age. The control diet was based on corn and soybean meal and did not include any additive. The experimental diets included 500 or 1,000 g/ton of PSB at the expense (wt:wt) of the control diet. Each treatment was replicated 14 times, with two birds each. Productive parameters were measured weekly, and egg quality were studied at the end of the trial in 50 eggs from each treatment. At the star of the trial, the average egg production was 87.6% and the average body weight was 1.798 kg without differences between treatments. Hens fed diets supplemented with PSB had higher egg production and egg mass than hens fed control diet ( $P<0.05$ ), without differences between doses. Also, hens fed diet supplemented with 500 g/ton PSB had better feed conversion ratio ( $P<0.05$ ) than the other treatments. Feed intake and egg weight, however, was not affected by PSB inclusion in the diet. In addition, PSB supplementation has beneficial effects on egg quality. In this respect, hens fed diets supplemented with 500 or 1,000 g/ton of PSB had higher yolk and albumen weight and Haugh unit than hens fed control diet ( $P<0.05$ ). However, no differences were detected in shell quality between treatments. In conclusion, an improvement in the productive performance and egg quality was observed in old laying hens fed with PSB.

Keywords: sodium butyrate; egg quality



## **Influence of age on eggshell colour persistency in greenshell and blueshell layers**

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The colour of eggshells is important for some consumers, attractive to others, and an interesting factor for backyard producers. The aim of the study was to monitor eggshell colour throughout the entire production period in hybrids laying eggs with green and blue eggshells. The study included 33 hens of the Greenshell GS 902 hybrid laying eggs with green eggshell and 33 hens of the Blueshell BLS 229 hybrid laying eggs with a bluish eggshell. The hens were divided into six cages in three tiers of enriched cage technology. They were fed a complete feed mixture daily. From the 24th to the 72nd week of age, all eggs laid on a given day were analyzed at regular intervals. Shell colour was assessed at the ages of 24, 28, 36, 40, 45, 48, 52, 56, 60, 64, 68, and 72 weeks, and egg weight was also evaluated. A total of 348 eggs from the Greenshell GS 902 hybrid and 338 eggs from the Blueshell BLS 229 hybrid were measured. Shell colour measurements were performed spectrophotometrically using the Konica Minolta CM – 2600d device and evaluated with the L\*, a\*, and b\* system in the SCI/100 status. The data were analyzed using one-way analysis of variance with subsequent testing using the LSD test. In the GS 902 hybrid, age had a significant effect ( $p \leq 0.05$ ) on egg weight, L\*, and a\* values. The average egg weight ranged from 47.6g (24 wks) to 60.4g (56 wks). L\* (lightness) was lower at 24 and 28 wks (75.5 and 75.8) and the highest at 52 wks (81.2). The value a\* (negative values towards green and positive values towards magenta) was lower at 52 wks (-3.099) and the highest at 45 wks (-0.986). Age had no effect on b\* value (negative numbers towards blue and positive towards yellow). In the BLS 229 hybrid, age had a significant effect ( $p \leq 0.05$ ) on all observed characteristics. The egg weight was significantly lower at 24 wks (50.4g) and heavier at 56 wks (61.0g). Lightness ranged from 86.8 (24 and 28 wks) to 90.3 (68 wks). Values a\* were even lower than in the GS 902 hybrid, ranging from -6.238 (24 wks) to -3.829 (72 wks). Values b\* were positive, ranging from 4.834 (45 wks) to 7.034 (24 wks). Although age had a significant impact on the above characteristics, a linear dependence was not found. For lightness, the regression equation was a second-degree polynomial, and for the a\* value in the BLS 229 hybrid, the dependence on age was logarithmic. The persistence of shell colour should be carefully considered for these hybrids, as consumers expect colour stability.

Keywords: eggshell colour; green; blue; stability

## Design of a moist feeding system for poultry

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Poultry production has become important throughout the World because of the increasing demand for animal proteins. Appropriate nutritional interventions, such as the use of moist feeding for poultry, have been found to improve both their meat and egg production and to ameliorate their welfare and feed conversion efficiency. The moist diet allows for a more sustainable feeding approach and therefore less feed-food competition, by introducing alternatives such as industry by-products and insect slurry. Our research introduces the design process of a moist feeding system for poultry, which is based on the RIO (Reflexive Interactive Design) approach. This involves conducting a thorough system and actor analysis, designing new arrangements or sub-solutions and supporting niche developments. This iterative method involves the major stakeholders in the design itself, who are in this case poultry farmers, farm equipment companies, feed and by-products providers and formulation specialists. Our aim is to obtain a system ensuring an optimal and reliable storage, mixing and distribution of the moist diet, along with proper cleaning. Identified key challenges were contamination, lower shelf-life of the diet, the cost-effectiveness of the final product and higher storing and cleaning requirements. The poultry's feeding behaviour is an additional obstacle making the design more demanding. Moreover, the project involves the right use of the systems' waste streams for more circularity, the adaptation of the system to different size of farmers but also different contexts, and the full display of the birds' natural behaviour to avoid spoilage. A series of sub-solutions were obtained and are to be tested in practice, such as 1) the conveyor belt, 2) the distribution trolleys, and 3) the single head feeders. This will allow to evaluate the distribution and cleaning frequencies needed for each system, along with lifespan of the moist diet in the barn conditions, which gives an idea on the efficiency and cost-effectiveness of the system.

Keywords: moist feeding system, poultry nutrition, engineering design, animal welfare, feed conversion efficiency

## **The effect of different indoor layers' housing systems on the changing of live weight of laying hens and its uniformity**

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Nowadays, there is an urgent need to find viable solutions to improve the production efficiency of non-caged farming systems, which could replace former housing systems if the EU bans all cage systems. The study investigated possible differences in live weight and distribution between different laying hybrids at the time of setting to the laying house following 19 weeks of same rearing conditions and explored the effect of different housing systems on live weight and its coefficient of variation at the end of a long period up to 96 weeks of age. The studies were carried out at the Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences with 3 genotypes provided by Bábolna TETRA Ltd.: a1 = Tetra-1 (White Leghorn); a2 = Tetra-2 (Classical Brown layer); a3 = Tetra-3 (Reciprocal crossbred Brown layer). N = 1.254 laying hens were housed in test house. The 3 housing types tested were: b1 = conventional cage (630 cm<sup>2</sup>/hens); b2 = EU-conform furnished or enriched cage (756 cm<sup>2</sup>/hens); b3 = floor system (1042 cm<sup>2</sup>/hens, grid floor and deep litter combination). The experimental flocks were housed in windowless test barns, within a single air space, in randomized position groups, where programmed egg production was carried out according to TETRA Management Guide and Technology. Differences between different genotypes and between different housing conditions were analysed using GLM with SAS 9.4. At 19 weeks, Tetra-1 weighed 1.29 kg, while Tetra-2 and 3 averaged 1.46 kg, significantly higher than Tetra-1 ( $p > 0.05$ ). No weight difference observed between Tetra-2 and 3 ( $p < 0.10$ ). At 96 weeks, Tetra-1 showed consistent 1.76 kg weights across housing types. Tetra-2 had similar weights (1.88 kg) in conventional and enriched cages, but higher in floor system (2.01 kg). Tetra-3 had the lowest weight in conventional cages, significantly higher in enriched and floor system ( $p > 0.05$ ). The 2.11 kg weight in the enriched cage for Tetra-3 is an outlier, suggesting susceptibility to obesity in this housing system. Our main conclusion is the differences between the tested experimental genotypes and the housing systems were significantly different and were interaction. Egg producers are advised to select the most suitable laying hybrid for the type of housing system.

Keywords: laying hen; husbandry; live weight variation; live weight uniformity; egg production

## **Productive and reproductive performance, dressing yield and chemical composition of different varieties of guinea fowl**

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The purpose of this study was to evaluate productive, reproductive performance, carcass characteristics, chemical composition of different guinea fowl varieties. A total of 200 guinea fowls from White, Pearl, Lavender and Mixed varieties were reared in an intensive production system from 0 to 20 weeks of age. Guinea fowl keets were reared provided with balanced recommended ration (Starter diet: ME 2950 kcal/kg, CP 23% for initial 8 weeks; Grower diet: ME 2950 kcal/kg, CP 16% for 9-16 weeks and Layer diet: ME 2900 kcal/kg, CP 17% from 17 weeks till the end of the laying period). There's no significant ( $p>0.05$ ) difference found in body weight from 0 to 20 weeks old guinea fowl. However, body weight of Pearl variety was higher than other varieties numerically. At adult age, total 40 birds (10 from each variety) were slaughtered according to standard method and carcasses were cut into parts. Weight were taken for all carcass parts and their percentage rates to the whole carcass were calculated. Proximate analysis was performed on breast and thigh meat, as well as on abdominal fat of the studied guinea fowls. Carcass weight ( $930.33 \pm 27.37$ ), dressing percentage ( $67.6 \pm 0.013$ , weight of breast ( $228.33 \pm 13.68$ ) and thigh ( $166.33 \pm 6.69$ ) of Pearl variety was significantly ( $P<0.001$ ) highest. Measured abdominal fat ( $23 \pm 0.13$ ) was found to be significantly ( $P<0.001$ ) highest in Pearl variety. Furthermore, there is no significant difference found in crude protein among breast and thigh meat of different varieties. The ether extract and crude fiber of breast (EE: 0.27, CF:0.33) and thigh meat (EE: 0.20, CF:0.33) of Lavender variety was significantly ( $P<0.05$ ) lower than other varieties. In case of reproductive parameters, Pearl variety exhibited better egg fertility ( $65.72 \pm 6.10$ ) numerically and significantly higher ( $p<0.001$ ) hatchability percentage ( $76.75 \pm 3.88$ ) than other three varieties. It can be concluded that Pearl variety of guinea fowl might be comparatively better than other varieties.

Keywords: White, pearl, lavender, pearl, carcass characteristics, proximate analysis

## **Promoting an innovative heating system for broiler chickens in rural communities in Cameroon: a preliminary study**

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In Cameroon, the poultry sector plays a crucial role in the country's economy, providing the most accessible source of animal protein to the population due to its affordability. The semi-intensive broiler chicken farming system requires various technical enhancements, including the introduction of artificial heat during the first 14 days of life to ensure the birds' thermoneutral zone, optimal growth performance, and welfare. A preliminary study was conducted at a farm in the Littoral region, Mungo department, Penja municipality, Cameroon. The objective was to compare two heating systems. A total of 680 1-day-old male broiler chickens (Cobb 500) were divided into two groups: one with a traditional heating system (THS) and one with an experimental heating system (EHS). The THS utilized a 200-liter iron barrel cut vertically, continuously burning wood to heat the chicks, with heat distributed through the flame. The EHS also used wood combustion as the heat source, but heat distribution occurred through radiant panels circulating hot water. Both systems were placed inside the birds' pen. Each group was housed in a floor-pen (5.5x4.5 m) with a 10 cm wood shavings litter. The birds were provided with a starter diet (AME: 12.45 MJ/kg; crude protein: 220 g/kg) fed ad libitum. The lighting program included 24 consecutive hours of illumination for the first 7 days, allowing the chicks to feed primarily at night due to high daytime temperatures. From day 8, a 3-hour dark period began, progressively, reaching 5 consecutive dark hours by day 14. The poultry house had a natural ventilation system. At days 1, 7, and 14, a random sample of birds (10% of total birds) from each group was weighed. Temperature (T) and relative humidity (RH) were constantly monitored electronically. The total wood burned for each group was measured and expressed as kg of wood per bird. Live body weight was similar between groups at each interval, averaging 41 g at day 1; 570 g (THS) and 540 g (EHS) at day 7; and 1190 g (THS) and 1240 g (EHS) at day 14. On average, T and RH were 31.0°C and 68.6% for THS and 30.9°C and 72.0% for EHS. Wood consumption was 1.14 kg/bird for THS and 0.61 kg/bird for EHS. In conclusion, the EHS showed promise in preserving bird performance and welfare. With a significant reduction in wood consumption (-47%), the EHS holds potential for improving the economic and environmental sustainability of rural broiler chicken farming practices in Cameroon.

Keywords: sustainability; heating system; rural chicken, Cameroon

## **Validation of an automated scale for continuous monitoring of the body weight in broiler chicken farming**

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The determination of broiler chicken body weight is essential for ensuring optimal production efficiency. However, the traditional weighing system manifests several limitations, including being time-consuming and having a smaller sample size of weighed animals compared to automated approaches. This study aimed to validate the precision and accuracy of three identical automated platforms installed in two broiler barns for monitoring the body weight of broilers throughout the fattening period. Additionally, the study aimed to generate a regression equation based on scale data to predict broiler body weight. Between May 2019 to July 2021, a total of 26 flocks composed of 40,333 animals on average, were housed in the two broiler barns (120m x 20m) until reaching 36-42 days of age. In each flock, manual weighing using a digital scale (considered the gold standard) was conducted on days 0, 7, 14, 21, and 28 from randomly selected animals. These manual records were compared with the automated records from the automatic poultry weighing system with 3 platforms up to 33 kg, 55cm of plate diameter and telescopic bar up to 240cm (PW, CTIcontrol, Spain) installed in zigzag disposition along the barn. Manual weighing provided data for an average of 100 chickens per day, while the automated scale allowed daily weighing records over the fattening period with an average of 800 registers/day. Data from both methods were subjected to regression analysis, revealing a high correlation in the three automated scales (automated scale 1= $-3.614277 + 0.9924219 \cdot \text{manual}$ ,  $r^2=0.980$ ; automated scale 2= $3.1610347 + 0.9714725 \cdot \text{manual}$ ,  $r^2=0.982$ ; automated scale 3= $0.3666712 + 0.956036 \cdot \text{manual}$ ,  $r^2=0.974$ ;  $P < .0001$ ), indicating a robust relationship among methods. In conclusion, the manual weighing system allows for the measurement of a limited number of animals on selected days during the fattening period, resulting in reduced representativeness. Furthermore, as the broilers grow, the capacity to weigh them decreases due to increased size, leading to increased labor requirements. Therefore, our results suggest that this technology is promising for the daily monitoring of animal body weight, providing both a greater volume and higher frequency of data, with reliable precision and accuracy, thereby enabling improved flock management.

Keywords: automated scales, bodyweight, broiler chicken, validation

## Thermographic analysis in 11 week old turkeys

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The use of thermography makes it possible to measure the surface temperatures of the birds' apterylic zones from a distance and without the need for physical contact, allowing timely understanding of the behavior of birds in production systems. The objectives were to record and compare 5 thermal windows in turkeys. The hypotheses were, Ho: the temperatures of the apterylic areas are the same, Ha: the temperatures of the apterylic areas are different. MATERIAL AND METHODS It was developed at the Research and Extension Teaching Center in Poultry Production, located in Zapotitlán, Mayor's Office of Tláhuac CDMX; 30 Nicholas 700 turkeys, 11 weeks old, were used. They were housed in natural environment booths, water and food were provided ad libitum. They had access to parks delimited by mesh and dirt floors as well as vegetation. The diet was corn/soybean meal based. A FLIR brand thermal imaging camera was used to record thermal images of the birds in the house and in the parks; The thermal windows analyzed were: Apterylic areas of the head, beak, neck, breast and tarsi. The birds were not restrained to avoid alterations in the reading of the regions studied. Environmental temperatures and humidities were recorded during the experiment. The normality of the data was reviewed as well as the homogeneity of the variances. For differences in means, the t test for related samples was performed using the IBM SPSS Statistics 27 statistical package. RESULTS Differences statistics between were found in the temperatures of the thermal windows. Regarding the difference in temperature in different environments, the tarsi were the ones that presented a reduction in temperature ( $t(29) = 4.748$ ,  $p < 0.001$ ) between the measurements made on the tarsi inside the house, averages of 34.72°C and in the parks 33.82 °C. In the rest of the thermal windows analyzed, no statistically significant differences were observed. In this work, the tarsi were the thermal windows that reacted fastest to changes in environmental temperature. We observe that the surface of the beak of birds, although small, representing 2-5% of the body surface, has an important thermal capacity. CONCLUSIONS The use of thermography in birds should receive more attention to contribute to animal welfare.

Keywords: turkeys, thermography, tarsi, thermal windows

## **Poultry thermoregulation in the heat: seasonal acclimatization and partitioning of evaporative water losses**

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Due to the insulating efficiency of the feathers, birds have a body temperature that is ~3 °C higher than that of mammals. This enables them to dissipate heat via evaporative water loss (EWL), partitioning it into respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL), equating to total evaporative water loss (TEWL). This study assessed the adaptation of Boschveld layers to thermal stress from 17 weeks of age with a body mass of ~1.32 kg, reared in outdoor aviaries with wood shavings on the floor. A commercial layer diet and water were provided ad libitum. In the lab, layers were individually weighed and placed in a Perspex 31.4 L metabolism chamber layered with 3 cm of mineral oil at the bottom to prevent evaporation from excreta. A flow-through respirometry system was used to measure oxygen consumption (VO<sub>2</sub>), rate of evaporative water loss (EWL), and carbon dioxide production (V̇CO<sub>2</sub>). The experiment was conducted in Summer from November 2019 to February 2020 with average day temperatures of 28.1 to 29 °C, and humidity of 72.3% to 73% (Source: South African Weather Service). Measurements for EWL and body temperature (T<sub>b</sub>) were taken over the air temperature (T<sub>a</sub>) range of (20, 25, 30, 35, 37, 39, 41, and 43 °C) for 1 hour; and for 30 minutes at T<sub>a</sub> 41 and 43 °C because layers exhibited signs of hyperthermia. T<sub>b</sub> was measured using temperature-sensitive passive integrated transponder (PIT). T<sub>a</sub> was measured in the chamber using a lubricated thermistor probe sealed with a rubber grommet on the side of the chamber. Data from the thermistor probe and PIT tag were recorded using Expedata software. All measurements were made when layers were in resting and post-absorptive state. Results showed that CEWL was the major route of heat dissipation at 35 ≤ T<sub>a</sub> ≤ 41 °C, except at 25 and 30 °C; and accounted for 60% of heat dissipation to the total rates of evaporative water loss (58.56% ± 13.06%). The fractional contribution of REWL to TEWL ranged from 58.43 ± 8.96% (T<sub>a</sub> ≈ 25 °C) to 54.95 ± 14.58% (T<sub>a</sub> ~ 30 °C). TEWL increased linearly as T<sub>a</sub> increased above 25 °C. It was concluded that CEWL represents an important avenue of heat dissipation in Boschveld chickens across a wide range of environmental temperatures. This mechanism suggests that Boschveld layers can dissipate heat without a draw down in energy, and highlights their ability to adapt and interact effectively with the natural climatic changes in extensive farming systems.

**Keywords:** Heritage chickens, cutaneous thermoregulation, evaporative cooling, respiratory regulation, water loss, heat



## **The impact of different types of serine proteases on growth performance and jejunal morphology in broilers**

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Mono-component exogenous proteases in poultry nutrition have garnered attention for their capacity to enhance dietary protein hydrolysis. However, the impact of these proteases on the growth performance and small intestinal morphology of broiler chickens has yielded inconsistent results in recent studies. The objective of this study was to assess the effects of various serine proteases on the growth performance and morphology of jejunum in broilers fed a diet based on corn, wheat, soybean, and canola meals. A total of 2400 day-old male Ross chicks were randomly assigned to five dietary treatments, each with 12 replications and 40 birds. The positive control (PC) mimicked a commercial diet with standardized ileal digestible lysine set at 10.9, 10.2 and 9.4 g/kg for the starter, grower and finisher phases, respectively. In contrast, the negative control (NC) contained 0.4 g/kg less standardized ileal digestible lysine than the PC in all feeding phases, which represented the highest matrix value among the three tested exogenous serine proteases (Protease A, B, and C) and added to the NC. The recommended doses of Protease A (EC 3.4.21.62), B (EC 3.4.21.19), and C (EC 3.4.21.62) were 50, 500, and 50 g/T, respectively. Protease A, B and C, were then added to the NC diet. On day 42, one bird per replication was euthanized for jejunal morphology sampling. The GLIMMIX procedure assessed the main effect of the treatment with normal distribution, and differences in means were determined using the Tukey test. In the overall experimental period (1 to 42 days), birds fed diet the NC had lower body weight and higher FCR compared to the PC group ( $P < 0.05$ ). Proteases A and C did not affect the growth performance and jejunal morphology of the birds. However, birds fed the diet containing protease B had reduced feed intake and body weight gain compared to NC, PC, and Protease A ( $P < 0.05$ ). Additionally, Protease B decreased jejunal villus height and mucosa thickness compared to NC, Protease A, and C ( $P < 0.05$ ). In conclusion, there was no apparent benefit in feeding diets containing proteases, with broad specificity for peptide bond hydrolysis (A and C; EC.3.4.21.62). Moreover, supplementing diets with protease B, with preferential cleavage of glutamic acid and aspartic acid (EC 3.4.21.19), adversely affected feed intake, growth and histomorphology. Therefore, the type of protease can be a determinant in the response of broiler performance and histomorphology to proteases.

**Keywords:** Mono-component protease, exogenous enzyme, gut health, histomorphology, Broiler performance.

## Microbiota profile and antibiotic resistance genes in the caecal content of broiler chickens are influenced by dietary protein content and the plant protein source

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Previous studies with soybean meal (SBM), canola meal (CM), and corn distillers dried grains with solubles (cDDGS) in reduced-protein diets showed differences in growth performance, immune response, and nutrient utilization. The objective of the current experiment was to study whether those effects are accompanied by changes in microbiota profile. A total of 1,120 seven-day-old broiler chicks were distributed to a 4 × 2 (4 diets × with or without challenge) factorial arrangement until day 42 in 7 replications. The 4 diets were (i) a diet with standard protein level, 20% crude protein (SP); (ii) a reduced-protein (16% crude protein) corn-SBM (RPSBM); (iii) an RP diet in which 8% CM replaced 6% SBM (RPCM); and (iv) RP diet in which 10% cDDGS replaced 5% SBM (RPeDDGS). On day 14, birds were either orally gavaged with a mixed *Eimeria* (+E) solution or distilled water. Cecal samples were quantified for selected commensal bacteria, pathogens, and antimicrobial resistance genes on d 21. A high throughput probe-based PCR was used to determine the abundance of 28 genes specific to commensal bacteria, bacterial pathogens, and antimicrobial resistance (AMR) determinants for tetracycline (tet), streptomycin (strB), sulphonamide (sul2), and extended-spectrum β-lactam (blaCTX) resistance and mobile DNA elements (incI1 and incI2 plasmids) that transmit AMR. In addition, a SYBR-based qPCR targeting *Lactobacillus* spp. and *Bifidobacterium* spp. was performed on a subset of caecal samples. Irrespective of the *Eimeria* challenge, strB abundance was significantly lower in birds receiving RP than in birds fed SP diets ( $p < 0.01$ ). The birds fed RPeDDGS had lower levels of strB than birds fed SP ( $p < 0.01$ ). There was diet × *Eimeria* challenge interaction ( $P < 0.01$ ) for the *Lactobacillus* population in the caecal content. There were no treatment differences in *Lactobacillus* content in unchallenged birds, but in the challenged group, *Lactobacillus* content was greater ( $P < 0.01$ ) in RP-SBM compared to the other treatments. *Bifidobacterium* spp. was lower in the caecal content of *Eimeria*-challenged birds, but the diets had no effect. It is concluded that both the dietary protein level and the plant protein source influenced the caecal microbial profile, including the population of bacteria that harbor AMR. Further studies will be needed to determine if these microbiome changes are advantageous or deleterious to the overall gut health of broiler chickens.

Keywords: soybean meal, canola meal, corn-DDGS, reduced protein, microbiota

## Ileal amino acid digestibility of extracted sunflower meal, determined with layer type pullets

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Extracted sunflower meal (SFM) is a potential protein sources for farm animals. The main constrain is its high fiber content. Pullets and laying hens can tolerate more fiber in their diets but there are no specific amino acid (AA) digestibility values available for pullets. The aim of this study was to determine if (SFM) can modify the digestibility of AAs at diet level. Furthermore, the true AA digestibility of SFM was also calculated according to the linear regression method. A total of 32, 10-week-old Tetra SL pullets were housed in balance cages. A semi purified basal diet was used, containing corn, wheat, sunflower oil, and corn starch as main ingredients. Three other experimental diets were used, when the SFM was incorporated at graded level, at 10, 20 and 30% on the expense of starch. Titanium dioxide (TiO<sub>2</sub>) was used as an inert marker to calculate the ileal digestibility of amino acids. After 7-day pre-feeding period, the birds were euthanized with CO<sub>2</sub>, killed and the ileal digesta collected. The ileal AA digestibility of the SFM was calculated from the response between the amount of pre-cecally digested amino acids and the daily amino acid intake. Data were subjected to one-way ANOVA using the software package SPSS 24.0 for Windows. Feeding SFM surprisingly improved the digestibility of THR, VAL, LYS and ARG of the compound feeds. No negative effect of SFM on AA digestibility was obtained. The AA digestibility of SFM was below the table values. Bigger differences were found between the measured values and those published by NRC and Evonik and more similarities with CVB. Regarding the individual AAs, the digestibility of lysine, threonine, leucine, and histidine was more then 10 % lower than the table values. Lower differences in the case of the other essential amino acids were found. The results underline the importance of the methodology of AA digestibility determination and the age of birds, that could result significant differences in the digestible AA contents of feedstuffs and this way can cause inaccuracies in diet formulation.

Keywords: sunflower meal; amino acid digestibility, linear regression

## Maternal supplementation of guanidino acetic acid: Influence on egg composition and chick development

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Young broiler breeder hens (< 30 weeks of age) produce smaller eggs with a smaller yolk reducing the energy source for the chick leading to lower hatch rates and higher first week mortality. Creatine is important for the storage and transport of energy in tissues where demand is high. Its precursor, guanidino acetic acid (GAA) is preferred as a feed supplement due to its stability and metabolic conversion to creatine. A study was conducted to determine if maternal supplementation of GAA during early and peak production would improve chick hatch rate and weight. Commercial breeder hens were fed either a control diet or control diet + 0.08% GAA (n = 3 sheds) from 15 wks of age. Fertile eggs were collected and weighed at 28 wks (n = 300) and 38 wks (n = 240) of age. A subsample of eggs (n = 30/treatment/age) were sampled for egg yolk and albumen water content. Eggs were incubated and re-weighed at ED18 to calculate the water weight loss. At hatch, 96 chicks were weighed and humanely euthanised. The residual yolk sac was removed and weighed. Hatchability was analysed using a Chi-squared test. Continuous data were analysed with a generalised linear mixed model for sex and treatment. Water loss (%) was higher in eggs from hens fed GAA both at 28 and 38 wks. No significant differences in hatch rate (%) or weight (g) were observed between treatment and age group. Fertile eggs from 38-wk-old hens fed GAA, the yolk was a larger portion of the egg and contained a larger portion of water and hatched chicks had a smaller proportion of residual yolk (% body weight) compared to controls. Eggs and hatched chicks from the 28-wk-old GAA supplemented hens also followed this trend but p > 0.05. Water vapor loss during incubation, is replaced in part by metabolic water as the chick utilises the yolk lipids and proteins in ovo. Disproportionate water loss during incubation can negatively impact hatch rate and chick quality. Water loss % was within the recommended range (9-11%) and did not alter hatch rate. Creatine provides a more readily available energy source that does not produce metabolic water. It is possible that both control and GAA eggs has similar water vapor loss, but GAA eggs having greater yolk content prior to incubation and producing less metabolic water may have accounted for the observed results. The relationship between GAA supplementation, egg water content, and chick hydration at different breeder ages warrants further investigation.

Keywords: broilers; hydration, energy, incubation physiology, chick health

## **Neuropeptide hormone responses and jejunal absorption rate in broiler chickens receiving reduced protein diets: A comparison of branched-chain amino acids with lysine as proteinogenic amino acids**

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The objective of the study was to examine the impact of increased branched-chain amino acids (BCAA) blends to Lys ratio as proteinogenic amino acids (AA) on the expression of AA-mediated neuropeptide hormones controlling satiety and appetite, hepatic catabolism, nutrient transporters, and jejunal histomorphology in broiler chickens fed a reduced-protein diet (RPD). A total of 270 Cobb 500 male broiler chicks were allocated into 18-floor pens, with 15 birds per pen on d 0. All birds received the same starter diets (220 g/kg crude protein and 12.6 MJ/kg AME) during the first 8d. Thereafter, three experimental diets (D) were provided for the grower (8-28d) phase, namely D1, which was a standard-protein diet (190 g/kg crude protein) with the recommended Lys to BCAA ratio (100:294), D2 was an RPD (170 g/kg crude protein) with recommended Lys to BCAA ratio (100:294), whereas D3 had the same protein content as D2 but with a 10-point decrease in Lys and a proportionate increase in BCAA, i.e., Lys: BCAA of 90:304. The ratios of individual AA in the BCAA were kept constant in all the diets. The liver, jejunum, and hypothalamus were collected from one bird per pen and snap-frozen in liquid nitrogen prior to RNA extraction and quantitative RT-PCR assays after 13 days of feeding experimental diets. The villus height (VH) and crypt depth (CD) were measured for jejunal tissues. The birds fed D3 had longer ( $P < 0.05$ ) villi than the others, whereas birds fed D1 and D2 had similar VH. The birds receiving RPD (D2 and D3) had upward expression ( $P < 0.05$ ) of adiponectin receptor than D1. However, there was no treatment effect ( $P > 0.05$ ) on the expression of peptide and AA transporters. In addition, the birds fed D2 or D3 ( $P < 0.05$ ) had upward expression of hepatic catabolic genes,  $\alpha$ -amino adipate semialdehyde synthase (AASS) and lysine  $\alpha$ -ketoglutarate reductase (LKR). Comparable feed intake in all treatments indicates that decreasing the lysine ratio by 10% while proportionally increasing the BCAA level in RPD produced comparable effects on appetite. However, the adjustment of Lys:BCAA triggered an increase in adiponectin receptors associated with heightened energy expenditure through protein uncoupling, ultimately leading to the adverse effects observed in growth performance. Consequently, under the specific RCP conditions of this experiment, BCAA did not exhibit an equivalent proteinogenic effect as Lys.

Keywords: Branched-chain amino acids, lysine, reduced protein diets, leptin, Ghrelin, morphology

## Microbiome implications of total replacement of soybean meal in fast growing male broilers

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The study evaluated the impact of using alternative ingredients with feed additives to replace soybean meal (SBM) on broiler d 1-35 growth performance and d 35 microbiome composition. Ross 308 male birds were assigned to 2 dietary treatments, each with 12 replicate pens (30 birds/pen). A commercially representative wheat, corn, SBM with rapeseed (<5%) and sunflower meal (<4.5%) based diet with phytase (a novel consensus bacterial 6-phytase variant, PhyG at 1000 FTU/kg) and xylanase (1200 U/kg) was used as positive control (PC). Diet 2 was an alternate (ALT) diet based on rapeseed-, sunflower-, maize gluten meal, pea flour, faba bean, lupin, potato protein, the first 7 limiting synthetic amino acids and rapeseed oil. Diets fed as crumble / pellets ad lib. ALT diet contained a *Bacillus* probiotic (150000 CFU/g) and the PhyG was increased to 2000 and 1500 FTU/kg in starter and grower, respectively vs PC, further supplemented with xylanase/ $\beta$ -glucanase combination (XB, to provide xylanase at 2400 U/kg and  $\beta$ -glucanase at 304 U/kg) and protease (4000 U/kg). On d 35, caecal swabs were collected from 3 birds/pen using Zymo Research DNA/RNA shield swabs. DNA was extracted and microbial community composition and microbial diversity were investigated using 16S amplicon sequencing. Final BW (d 35) was maintained with ALT compared to PC (2679 vs 2688 g/bird) while d 35 FCR was higher ( $P<0.05$ ) in ALT (1.44 vs 1.41 in PC) though in line with breed spec. At microbiome level, significantly higher diversity in ALT compared to PC (Shannon diversity,  $P<0.05$ ) was observed at family level, but not at species/genus/phylum. Beta diversity indicated significant differences in community composition between ALT and PC at the family, genus and species level but not phylum, suggesting a broader community shift. Differentially abundant taxa that showed significant changes in abundance between ALT and PC were identified. At the family level, Enterobacteriaceae was significantly reduced in ALT vs PC. Similarly, *Escherichia coli* at species level and Proteobacteria at the phylum level were significantly reduced. Additionally, *Ligilactobacillus* and Lactobacillaceae were significantly increased in ALT. This study concludes that based on final body weight, SBM replacement is possible. As expected, the microbiome was impacted, yet the high fiber ALT with additive supplementation increased microbial diversity, reduced potential non-beneficial and increased beneficial populations supporting gut health.

Keywords: Xylanase;  $\beta$ -glucanase; protease; poultry; sustainability

## Energy utilization and jejunal mRNA expression of nutrient transporters in broiler chickens fed for different feeding lengths diets containing resistant starches from raw potato or high amylose corn

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Studies on the impact of feeding different levels of resistant starch (RS) and the duration of this feeding practice in poultry are scarce. The objective of this study was to evaluate the effects of raw potato starch (RPS) and high amylose corn starch (HCS) for varying feeding lengths on energy utilization and jejunal expression of nutrient transporters in broiler chickens. In a 21-day study, 300 Cobb 500 male broiler chicks were allocated at day old to 10 treatments, which include 3 RS levels (RSL) 25 and 50 g/kg of RPS and 35 g/kg of HCS, and 3 feeding lengths (RSD): 21, 14, or 7 d, plus a corn-soybean meal (control) diet. On d 21, ileal digesta and excreta were collected for energy utilization, whereas jejunal tissue was collected from one bird per pen for analysis of mRNA expression levels of mucin-2 and nutrient transporters using RT-PCR. Data were analyzed as 3×3+1 nested factorial. There were no significant RSD×RSL(control) for AMEn. The main effect of feeding length was significant ( $P < 0.05$ ); birds fed 35 g/kg HCS had higher ( $P < 0.05$ ) AMEn and ileal digestible energy than all RPS treatments. Feeding length and RS-type interaction were significant for jejunal GLUT-2 (glucose transporter) and GLP-1 (satiety hormone) mRNA. Generally, mRNA expression for the genes was lower than control and decreased at 7 d feeding length except for birds that received HCS at 35 g/kg. The main effect of feeding length on FABP-6 (fatty acid binding protein) was significant ( $P < 0.01$ ), being higher for birds that received RS for 7 d than the other treatments. The main effects of feeding length and RS type were significant ( $P < 0.01$ ) for y+LAT1 (amino acid transporter) and PYY (peptide YY, satiety hormone). Feeding RS for 14 d produced higher ( $P < 0.05$ ) y+LAT1 than others, whereas 25 g/kg RS increased y+LAT1 compared to 35 g/kg. Jejunal PYY was higher ( $P < 0.05$ ) in birds fed RS for 21 and 14 d and higher ( $P < 0.05$ ) for birds fed 25 and 35 g/kg RS than others. Feeding length was significant for MUC-2 (mucin-2) with higher expression ( $P < 0.05$ ) at 21 and 14 d but comparable to control. This study showed that 35 g/kg HCS increased energy utilization irrespective of feeding length, whereas the expression of various nutrient transporters depended on the length of feeding and dietary levels of individual RS. The effects of RS type and feeding length on energy utilization and genes have implications for studies on the gut health impact of RS for broiler chickens.

Keywords: Resistant starch; energy utilization, nutrient transporter, feeding length, broiler chickens

## **Novel sfericase protease improves amino acid digestibility of soybean meal and rapeseed meal in broiler chickens**

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Among the plant-origin protein ingredients, soybean meal (SBM) and rapeseed meal (RSM) are the predominant sources of nitrogen (N) and amino acids (AA) in poultry diets and the used of exogenous protease has become popular in animal production industry. Measuring and predicting the nutrient value of feed ingredients and their response to exogenous enzymes are essential for precision feed formulation and optimal enzyme application. Two experiments were conducted to explore the effects of an exogenous sfericase protease on the apparent ileal AA digestibility of SBM and RSM in broiler chickens. In each experiment, a total of 256 sixteen-day-old male Cobb 500 broilers were fed one of four semi-purified experimental diets, comprising two different batches (A and B) of samples for either SBM (Exp. 1) or RSM (Exp. 2) without or with an exogenous sfericase (0 or 30,000 NFP/kg). Each experimental diet was fed to 8 replicate cages with 8 birds per cage from 16 to 21 days of age and the ileal digesta were collected for measuring the digestibility coefficients. In Exp. 1, the AA digestibility was greater ( $P < 0.05$ ) in SBM B compared with SBM A for Arg and Val and a similar trend ( $P < 0.1$ ) was noted for Tyr, Leu and Thr. Exogenous sfericase increased ( $P < 0.10$ ) the digestibility of most of AA except Gly, His and Trp. There was an interaction between SBM source and sfericase where the digestibility of phosphorus, N and Asp were increased by sfericase for SBM B but not for SBM A. In Exp. 2, there was no interaction ( $P > 0.05$ ) between RSM batch and sfericase on ileal nutrient digestibility. The digestibility was greater in RSM A compared to RSM B for all non-essential AA and most of essential AA (except for Trp) while the reverse was noted for Ca and P ( $P < 0.05$ ). Exogenous sfericase increased ( $P < 0.05$ ) the digestibility for all AA except Cys and Met. In conclusion, the current studies show that both SBM and RSM batches influence AA digestibility. Sfericase protease supplementation increases AA digestibility for both SBM and RSM. The digestibility effects are greater in the SBM batch with low digestibility for N and Asp and it occurs with an increase in P digestibility.

Keywords: soybean meal; rapeseed meal; sfericase protease; amino acid; broiler



## **Effect of rearing substrate on amino acid digestibility of back soldier fly larvae fed to broiler chicks**

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Black soldier fly larvae (BSFL) are one of the most suitable insects for feeding to poultry as they can feed on a variety of substrates. They are capable of incorporating the amino acids from waste materials into their own biomass. This improves the environmental and economic sustainability of insects as poultry feed and can contribute to the viability of other industrial processes by adding another value stream. This study compared soya as a control protein source with BSFL raised on one of three substrates: Fruit waste (FW), Bran (B) and Distillers dried grains with solubles (DDGS). Two of these substrates are co-products of industrial processes and Bran is a conventional substrate for raising BSFL. Day old male Ross 308 broiler chicks were raised on commercial chick crumb to d23. They were then split into 36 pens of 4 birds per pen and fed semi synthetic diets with a single protein source (BSFL or soya) at one of three inclusion levels (20, 40 or 60%) from d23 to d26. The diets also contained vitamin/mineral premix, oil and a mix of starch and dextrose, and 0.5% titanium dioxide (TiO<sub>2</sub>) as a marker. Ileal digesta was collected per pen at d26 and analysed for amino acid content and TiO<sub>2</sub>, and raw materials were analysed for amino acid content. Amino acid coefficients of apparent ileal digestibility were calculated by extrapolation. Data were analysed via one way ANOVA using SPSS and Duncan post hoc tests were utilised where appropriate. Protein content of the BSFL was lower than the soya bean meal (40% compared with 48%). However, digestibility coefficients were significantly greater for all BSFL compare with soya, with the FW substrate raised BSFL having the highest COD almost across the board (except tyrosine). There were significant positive increases in digestibility for BSFL for all amino acids except methionine. The overall COD for the protein sources were as follows: soya, 0.75; BSFL FW, 0.95; BSFL B 0.89; BSFL DDGS, 0.89. This study suggests that BSFL raised on waste substrates can provide a protein source which although has a lower protein content, the digestibility is comparable or better than soya. Raising insects on waste may improve the sustainability of production of industrial processes such as brewing and fruit juice production. Further studies need to assess whether substrates can be amended to improve BSFL protein content without detrimentally effecting their digestibility.

Keywords: insects, amino acid, digestibility, broilers

## **Response of broilers to dietary metabolizable energy levels from 0 to 4 days old**

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This study aimed to investigate broilers' response to two factors: 1) the dietary apparent metabolizable energy (AME from Centraal Veevoeder Bureau-CVB), and 2) the ratio of standardized ileal digestible lysine (SID-Lys) to AME (Lys:AME). Eleven treatments were assigned to 88 experimental units, each with 41 one-day-old Ross 308 chicks and eight replicates per treatment. Two groups of broilers (changing and constant Lys:AME ratio) were fed with a basal feed containing 3050 kcal/kg of AME and 14.3 g/kg of SID-Lys. For the first group (changing Lys:AME ratio), the basal feed was diluted five times with an iso-nutrient low-energy feed (2750 kcal/kg). For the second group (constant Lys:AME ratio), the basal feed was diluted five times with a low-energy feed (2750 kcal/kg) formulated to contain 12.9 g/kg of SID-Lys. The AME levels were equidistant, ranging from 2750 to 2975 kcal/kg. The undiluted basal feed was also considered an experimental treatment. Experimental feeds were given from 0 to 4 days, followed by a common feed program. At 4 and 35 days, feed leftover, chicken weights, and mortality were measured for feed intake correction. On day 3, three birds per unit were euthanized for uric acid quantification in plasma (micrograms per deciliter – mcg/dL). Carcass yield was evaluated at 35 days. The performance and carcass yield were evaluated using a second-order polynomial regression with groups, where the Lys:AME served as the grouping factor. Plasma uric acid concentration was evaluated in a factorial scheme 5 x 2 (five AME levels x changing or constant Lys:AME) plus the undiluted basal feed. There was a similar response among changing and constant Lys:AME ratio ( $P > 0.05$ ), but differences were found among the AME levels ( $P < 0.05$ ). From 0 to 4 days, the maximum feed intake was estimated for broilers fed diets with 2800 Kcal/kg. Maximum body weight gain was estimated for broilers fed diets with 3000 Kcal/kg of AME. The higher ( $P < 0.05$ ) plasma concentration of uric acid was observed for broilers fed diets with 2800 kcal/kg compared with broilers fed the high-energy feed (2975 kcal/kg; 9.5 mcg/dL vs. 7.5 mcg/dL, respectively). The highest carcass yield at 35 days was estimated for broilers consuming 3050 Kcal/kg. The results suggest that to optimize performance at 35 days old, broilers may benefit from higher levels of energy during their initial days of life, contrary to the conventional recommendations provided thus far.

Keywords: growth, performance, starter feed, requirement, chickens

## Nutritional Potential of African Edible insects as food and feed

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Edible insect species particularly are some of the most valued and highly nutritious food and feed in sub-Saharan Africa. Their quality and availability influence their sustainability. This study aimed to examine the proximate composition (%), amino acids (g/100g) and fatty acid profile (mg/g) of 4 dried southern African insects: green stinkbugs, brown locusts, soldier termites (ST) and winged termites (WT), as well as WT by-products (cake (WTC) and oil (WTO)), which constituted a total of 6 insect samples. Proximate results showed that locusts (66.11%) had higher crude protein (CP) followed by WTC (53.64%) compared to other 4 samples. However, lower CP was detected in WTO than all remaining 5 samples. Crude fats (48.95%), fibre (15.16%) and NDF (28.55%) were significantly higher in stinkbugs than all other samples whilst higher ash content was observed in WTC (9.67%) followed by WT (6.36%) in comparison with other insect samples. Higher amount of starch and energy levels were detected in WT (2.42%) and stinkbugs (6981.36 kcal/kg), respectively. The total essential amino acids (TEAA) were higher in locusts (26.11g/100g) and WTC (24.49 g/100g), respectively compared to other 4 remaining samples whilst phenylalanine (0.01 g/100g) was the only EAA detected at lower level in WTO than other insect samples. Leucine (2.32 to 5.67 g/100g), valine (2.12 to 4.21 g/100g), and arginine (1.88 to 3.72 g/100g) were the most predominant EAA, respectively across all samples. In terms of fatty acid profile, WTO contained higher total fatty acids (TFA) (208.32 mg/g) in which oleic (24.35 to 147.91 mg/g) and palmitic (10.84 to 41.00 mg/g) acids were the most abundant fatty acids across all insect samples compared to other fatty acid types. Higher polyunsaturated fatty acids (PUFA) (29.18 mg/g) and omega-6 fatty acids (26.99 mg/g) were found in ST than other 5 samples. However, WTC constituted the lowest PUFA levels. Locusts showed higher omega-3 fatty acids (13.41 mg/g) concentration in comparison with other insect samples. Our findings suggest that high quality nutritional profile of selected African insects, especially locust and termite species have could be potentially used as both food and feed to supply protein and fats since they also meet the nutritional requirements of poultry species (chickens). Hence, promoting insect farming and utilization in livestock diets to alleviate poverty and food insecurity.

Keywords: Insect meal, locust, stinkbugs, termites, nutrient composition, processing methods, entomogaphy

## **Alternative protein sources in broiler diets: effects on bird performance, welfare status and development of the gastro-intestinal tract**

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In order to reduce the carbon footprint related to poultry meat production, alternative protein sources (APS) that can substitute Brazilian soybean meal (SBM) in broiler diets are of great interest. APS receive special attention, if these are either produced in Europe, fit within circular agricultural systems, or are of innovative nature and may be produced highly efficient in the near future. The following APS were studied in this experiment: white lupins (LUP), field beans (FB), rapeseed meal (RSM), potato protein (POP), porcine blood meal (BM), and micro algae (MA). A reference diet was formulated with 48% maize, 30% SBM and 15% wheat as main components. In experimental diets either 15% or 20% SBM was substituted with 19.8%, 23.9%, 16.6%, 9.8%, 8.1%, 20.1% of the selected APS, respectively. Herein maize was used as balancing ingredient with inclusion levels varying between 37.6% (FB) and 58.3% (BM). All diets were isoenergetic (3,000 kcal AME/kg diet) and standardized for ileal digestible lysine (10.5 g/kg diet). A total of 448 Ross 308 one-day-old mixed gender broiler chicks were reared on a commercial starter diet for the first 10 days of age, and thereafter randomly assigned to one of 56 pens (eight birds per pen) with either the reference (SBM) diet or one of the six experimental diets. During the rearing period birds' body weight (BW), average daily feed intake (ADFI) and feed conversion ratio (FCR) were registered. At day 43, birds were killed and for two birds per pen carcass weights were measured. Preliminary performance results (day 43) were similar for the following APS in BW (SBM: 3.2, LUP: 3.0, FB: 3.4, RSM: 3.2 and POP: 3.1 kg), ADFI (SBM: 161, LUP: 157, FB: 163, RSM: 164 and POP: 161 g) and FCR (SBM: 1.51, LUP: 1.60, FB: 1.46, RSM: 1.57 and POP: 1.56). BM and MA showed significant differences compared to SBM for BW (BM:2.7 and MA: 2.5 kg), ADFI (BM: 142 and 136 g), and FCR (BM: 1.66 and MA: 1.70). Also at slaughter age carcass weights, excl. visceral organs and digesta, showed similar results for the following APS (SBM: 2.3, LUP: 2.1, FB: 2.3, RSM: 2.2 and POP: 2.1 kg), but differed significantly for BM (1.8 kg) and MA (1.6 kg). In general, large variation was seen for the different results, which may be caused by variation in male : female ratios within the groups. In a later phase, new experiments will be performed with bird groups of sexed gender and the current results will also be related to in vivo and in vitro digestibility data.

**Keywords:** Protein sources, soy, poultry, broilers, feed, performance

## **A new fermentation-based L-methionine (L-Met eco) has a better efficacy compared to petro-chemical based DL-methionine (DL-Met)**

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Methionine (Met) plays a pivotal role as essential and primary limiting amino acid in poultry diets. Since 2015, L-Met produced via fermentation has reached the market. In response to the increasing global demand for more sustainable ingredients, CJ BIO has created a new Met source; BestAmino® L-Met eco, with a lower global warming potential compared to both traditional DL-Met and BestAmino® L-Met100. This study examines the bioefficacy (BE) of L-Met eco, in its diluted form, compared to DL-Met and aimed to affirm that the previously established higher efficacy of L-Met100 is reproducible with L-Met eco. A basal diet (BD) deficient in SID Met plus Cys (0.57, 0.49, and 0.44% in starter, grower, and finisher phases) was supplemented with 0, 0.05, 0.10, 0.15, 0.20, 0.30, and 0.40 of either DL-Met 99% or diluted levels of L-Met eco (mixing 68.4% L-MET eco and 31.6% neutral substance (SIPERNAT® 22), with L-MET eco containing  $\geq 95\%$  L-Met). Using 1440 male Ross 308 chickens distributed across 13 treatments (7 replicate per treatment 15 birds each; BD had 12 replicates). Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were assessed during a three-phase feeding period (0-35 days). Additionally, four preselected birds per pen were slaughtered on day 36 and slaughter yield parameters were measured. Data were analyzed using linear regression models and Dunnett's test for multiple comparison. Furthermore, exponential asymptotic models were used to determine the BE of L-Met eco compared to DL-Met. The BD diets resulted in poor performance with noticeable enhancements observed with increasing levels of Met ( $P < 0.05$ ). Although L-Met was at 65% of the inclusion rate of DL-Met, the BE of L-Met (relative to DL-Met) was 1.117, 1.074, and 0.925 for BW, 1.138, 1.086, and 0.793 for daily weight gain (DWG), 1.228, 1.035, 0.907 for daily FI, 1.196, 0.981, and 0.810 for FCR during starter, grower, and finisher, respectively. Moreover, the BE of slaughter parameters at day 36 were 0.893, 0.874, 0.929, 0.867, 0.951, 0.972, and 1.021 for slaughter weight, carcass weight, breast weight, leg weight, breast weight % of live weight and % of carcass, and leg weight % of carcass. Our findings substantiate the hypothesis that L-Met eco, akin to L-Met 100, has a higher BE compared with DL-Met, contributing to a superior protein deposition and muscle growth, while simultaneously boasting a lower global warming potential.

Keywords: L-Met eco, DL-Met, broilers, sustainability

## **Dietary amino acid profile boosted in arginine and threonine improves growth performance of turkeys fed reduced crude protein diets**

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Fast-growing turkey diets are rich in crude protein (CP) and soybean meal (SBM). By reducing excessive dietary CP content, beneficial effects on welfare, health and environmental footprint could be achieved, under the condition that growth performance is maintained. A total of 576 turkey poults (Aviagen premium) were fed four dietary treatments, with 8 replicates each, from 0 to 42 days of age and with 3 feeding phases (d0-d21, d21-d35, d35-42). Treatments were iso-energetic and consisted of a standard CP diet (treatment T1, “SCP”; 26.4 % CP, 24.0 % CP, and 22.2 % CP, respectively in the 3 feeding phases); a lower CP diet reduced by 2% pt without controlling dietary amino acids (AA) beyond Thr (treatment T2, “AA-“), T2 with a dietary AA profile boosted in Val compared to the control (+3 % pts in ratio to Lys, called “AAperf) and with a dietary AA profile boosted in Arg and Thr compared to the control (+5 and 5 % pts, respectively, in ratio to Lys, called “AAdig”. The reduction of dietary CP content was achieved by replacing SBM & corn gluten with corn, barley, and L-AA. Challenging conditions were achieved in each treatment by using straw as bedding material, slightly increasing stocking density and diversifying the raw material used. Average daily gain, feed intake and corrected feed conversion ratio was affected by dietary treatment for the period 0-42 d ( $p < 0.05$ ). Average daily feed intake was 94.5 g/d in the SCP treatment, not significantly different from AA- and AAperf but significantly increased in AAdig (+4.5 g/d vs SCP). Compared to SCP group, final body weight was significantly reduced in AA- from 2565g to 2465 g and increased in AAdig to 2656 g. The treatment AAperf was intermediate. Bodyweight-corrected FCR was significantly reduced in AA- from 1.584 in SCP group to 1.631 but not significantly different between SCP, AAperf and AAdig. Water intake was not affected by treatment but water:feed ratio was significantly lower in turkeys being fed the AAdig treatment compared to SCP. Environmental calculations of the diets and turkey live weight are on-going. Overall, turkey poults can be fed lower CP (and SBM) diets without losing performance if the dietary AA profile is adequate. Turkeys respond differently to different AA profiles under challenged rearing conditions, confirming that specific dietary AA play a crucial role in the maintenance of turkey gut health functions.

Keywords: crude protein ; amino acid ; turkey ; carbon footprint

## **Effect of solid-state fermentation on nutritional properties of oilseed cakes and in vitro digestibility in poultry**

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Solid-state fermentation (SSF) is a biotechnological process that relies on the use of solid substrates as support and source of nutrients for the growth of filamentous fungi. It is an eco-friendly process that can be used to valorize agro-industrial by-products. Filamentous fungi can produce extracellular enzymes that hydrolyze the lignocellulosic structure of plant cell wall, reducing the fiber content and making fiber-associated proteins more accessible. Production of microbial protein cell mass has also been described during SSF. Amongst plant sources used in poultry feed, soybean is the most common protein source, followed by rapeseed cake and sunflower cake. The fiber content of these oilseed cakes (OC) and the presence of antinutritional factors, however, can limit the nutritional value of OC when used as poultry feed. Our hypothesis was that OC exposed to SSF could change their chemical composition and thus affect the nutritive value of these protein concentrates when fed to monogastric animals. This work evaluated the effect of SSF on the nutritional properties (chemical composition and antinutritional factors) and in vitro digestibility of optimized mixtures of OC (soybean, sunflower and rapeseed) using *Aspergillus niger*. SSF increased the dry matter (1.06-fold), crude protein (1.24-fold) and minerals (1.39-fold) of OC and reduced the fibers concentration, except for lignin. The dry matter and organic matter in vitro digestibility coefficients were significantly ( $P < 0.05$ ) higher in fermented mixtures compared to non-fermented OC. However, SSF decreased the amino acid content compared to non-fermented OC, and there was a decrease of true protein content (1.2-fold) and an increase of ammoniacal nitrogen (57-fold). During SSF, transformation of true protein into nonprotein compounds such as ammoniacal nitrogen can occur, limiting nitrogen utilization in poultry. However, this may vary according to the fungal species used. SSF also decreased (15%) protein solubility in KOH and increased (31%) protein dispersibility. SSF was able to reduce phytic acid content (10-fold) but did not reduce tannin, erucic acid and glucosinolates content. This work can contribute to evaluating the advantages of SSF and identifying areas for further improvement in the bioprocessing of OC for poultry feed.

Keywords: in vitro, poultry, oilseed cakes, solid-state fermentation, digestibility

**Methionine requirements of slower growing broilers in the starter (d 0-10), grower 1 (d 10-24), grower 2 (d 24-38) and finisher (d 38-50) phases**L. Stokvis<sup>1</sup>, Y. Mercier<sup>2</sup>, R. Davin<sup>1</sup><sup>1</sup>Schothorst Feed Research, Lelystad, Netherlands, <sup>2</sup>Adisseo France SAS, 69190 Saint Fons, FrancePresenting author: [lstokvis@schothorst.nl](mailto:lstokvis@schothorst.nl)

To answer societal demand, a shift towards a slower growing broiler is ongoing. To be able to feed these birds to their optimal potential and health, their nutritional requirements need to be established specifically. This study aimed to determine digestible Met+Cys (dMC) requirements of slower-growing broilers during different feeding phases: 0-10, 10-23, 24-38 and 38-50 days of age. In total, 3840 male Hubbard JA 737 birds were used. In each feeding phase, a new batch of 8 treatments with 6 replicates of 20 birds were fed diets with increasing Met levels (substituting diamol® by HMTBa 88%) between treatments, to prevent a carry-over effect of preceding phases. Readout parameters were body weight (BW), BW gain (BWG), feed intake (FI) and feed conversion ratio (FCR). Optimum Met levels were determined expressed as SID Met+Cys:SID Lysine (dMC:dLys; for BWG, FI, FCR) as well as SID Met+Cys intake (dMC intake, g per bird; for BWG, FCR). Statistical models applied were the quadratic polynomial model (QP), exponential asymptotic model (EA) and linear response plateau model (LRP). An optimum was determined, as the lowest dMC:dLys or dMC intake at which the QP model intercepted the LRP plateau value, or, when either the QP or LRP model was not considered due to lower R<sup>2</sup>, based on the average of the considered models. Except for the starter phase where optimum dMC:dLys was obtained at 0.505-0.543 (0.524), the birds did not respond to different methionine levels for FI. In starter phase the optimum BWG and FCR were determined respectively at 0.510 to 0.550 (0.530) and 0.516-0.565 (0.541). In the grower 1 phase optimum dMC:dLys for BWG and FCR were, respectively, 0.501-0.579 (0.540) and 0.484-0.503 (0.494). In the grower 2 phase, optimum dMC:dLys were 0.548-0.638 (0.597) and 0.584-0.645 (0.614) respectively for the BWG and for FCR. The optimum dMC:dLys in the finisher phase were 0.670-0.699 (0.685) for BWG, and could not be determined for FCR. Optimum dMC intake in the starter phase was 0.127-0.159 g (0.143 g) for highest BWG and 0.130-0.181 g (0.149 g) for lowest FCR. In the grower 1 phase, optima were 0.482-0.629 g (0.556 g) for BWG and 0.460-0.481 g (0.471 g) for FCR. In the grower 2 phase, optima were 0.884-0.984 g (0.934 g) for BWG and 0.923-1.058 g (0.968 g) for FCR. In the finisher phase, optima were 1.118-1.120 g (1.119 g) for BWG and could not be determined for FCR. Concluding, slower-growing broilers have a lower dMC requirements than fast-growing broilers.

Keywords: broilers; slower growing broilers; amino acid requirements; Methionine requirement; feeding phases



## **The use of an olive by-product as a source of insoluble fibre for pullet feeding**

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In the past years there has been an increase in the importance of the insoluble fibre for digestive health in monogastric species. Spain is world's largest producer of olive oil and in consequence of olive cake, being the production of olive cake in Spain around 6 million tons per year. Dry olive cake is the solid product obtained after the second extraction of oil and desecation from the wet olive cake (product obtained after the first extraction of oil from the olives), which is characterized by its high content of insoluble fibre. The objective of the present study was to evaluate the use of dry olive cake as a source of insoluble fibre in the feed of laying pullets. Two diets were used in this assay: a diet included 2% of sepiolite as negative control (low insoluble fibre diet) and the other diet included 2% of dry olive cake. The insoluble fibre, soluble fibre and ether extract content of the dry olive cake used were 52,9, 4,5 and 3,8%, respectively. A total of 36 Lohman brown pullets of 5 weeks of age were homogeneously distributed in 12 cages having 3 pullets per cage (6 replicate per treatment). Daily feed intake and, initial and final bird body weight were controlled for 4 weeks. On the last week a digestibility study for dry matter was performed, collecting total excreta for 4 consecutive days. At the end of the trial the pullets were slaughtered and digestive organs (proventriculus, gizzard, liver, spleen, pancreas, empty small and large intestine) were weighted. Data was analyzed and expressed using the relative organ weight to the pullets weight. Duodenum, jejunum and ileum content pH was also measured. No significant differences were found in the final pullet weight, average daily gain, average daily intake, the conversion rate neither the digestibility among the diets. In relation to the organs weight, no significant difference was found in the relative weight of the proventriculus, the gizzard, the liver, the spleen, the pancreas, nor the empty small and large intestine. At last, no significant differences were found in the digestive pH measurements among the treatments. In conclusion, no effects were observed on the use of the olive cake as a source of insoluble fibre. Further studies would be interesting to establish the effect that this source of insoluble fibre could have on the microbiota.

Keywords: Dry olive cake; pullets; insoluble fibre

## **Study on *Ganoderma lucidum* plus phytase to solid-state ferment agricultural by-products producing functional feed**

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*Ganoderma tsugae* was a group of white rot fungi which is able to produce many bioactive components during metabolic process that can potentially protect cells and regulate immunity. The purpose of this study was to evaluate metabolites contents in solid state fermented wheat bran inoculated with *Ganoderma tsugae*. In vitro study showed that chicken peripheral blood mononuclear cells (PBMCs) stimulated by fermented wheat bran which increased nitrite oxidation (NO) production and had no effect on cell viability. Pro-inflammatory gene expression (NF- $\kappa$ B, Interleukin-6 and inducible nitric oxide synthase (iNOS) of chicken PBMCs was lower than cells stimulated by Lipopolysaccharide (LPS). The 500 1 day-old Arbor Acres broilers were allocated into five treatments with 4 pens per treatment and 50 chicks of equal gender ratio in each treatments, including the basal diet, 5%, 10% of wheat bran replaced with basal diet (WB group), and 5%, 10% of fermented wheat bran replaced with basal diet (FWB group) for 35 days. The results showed that all group had no significant effect on the growth performance of broilers than control at 35-day ( $P > 0.05$ ). FWB group had significantly higher lactic acid bacteria count in ileum (5%: 7.32 Log Colony-forming unit (CFU)/g; 10%: 7.85 Log CFU/g), comparing with control (6.34 Log CFU/g) ( $P < 0.05$ ); moreover, 10% FWB had significantly lower coliform count in ileum than control ( $P < 0.05$ ) at 35-day ( $P < 0.05$ ). In the serum, the cortisol level of 10% FWB group (142.9 pg/mL) had significantly less than 10% WB (235.93 pg/mL) ( $P < 0.05$ ), and all group had no significant effect on the LPS level than control at 35-day ( $P > 0.05$ ). The immunomodulatory regulation gene, including Toll-like receptor 4 (TLR4), NF- $\kappa$ B, Interleukin-6, Cyclooxygenase-2 and iNOS expression of in vivo chicken peripheral blood mononuclear cell was significant higher than control group ( $P < 0.05$ ) in the serum. The in vivo chicken PBMCs have better cell alive rate on proliferation assay, and NO production in FWB was higher than control group ( $P < 0.05$ ) in the serum. In conclusion, fermented wheat bran by *Ganoderma tsugae* could produce functional metabolites, and regulate the expression of immunomodulatory gene, in addition improve their immune function, and confirm the applicability in broilers diet.

Keywords: *Ganoderma lucidum*; by-product; fermentation; immunomodulatory

## **Development and application of fermented soybean hull by *Bacillus velezensis* as functional feedstuff**

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This study aims to produce fermented soybean hull (FSBHB) by *Bacillus velezensis* to evaluate the effect on growth performance, intestinal morphology, intestinal microflora, antioxidant capacity and immune regulation of broilers. After the fermentation, the total phenolic content increased by 0.43 mg of GAE (gallic acid equivalent) /g DM and soluble peptides increased by 1.70%. A total of 400 one-day-old Arbor Acres broilers were randomly divided into the basal diet (control), partially replaced control with 5% and 10% of unfermented soybean hull (5% USBH, 10% USBH) and fermented soybean hull (5% FSBHB, 10% FSBHB), full fat soybean meal as supplemented for equally protein and ME in each group for 35 days feeding period. There were two phases in the experimental period, a starter phase (1–21 d, CP 23.0% and ME 3200 kcal/kg) and a grower phase (22–35 d, CP 20.0% and ME 3200 kcal/kg). Broilers had free access to water and feed, as per the requirements of the NRC (1994). The results showed that 5% USBH, 10% USBH, 5% FSBHB and 10% FSBHB significantly increased BW and BWG in the early stage ( $p < 0.05$ ). The 5% FSBHB and 10% FSBHB significantly increased cecal villus height/crypt depth ratio and the number of lactic acid bacteria. In conclusion, the FSBHB enhanced soluble peptides and improve early growth, intestinal health, and antioxidant activity in broilers, with the most significant benefits observed in the 10% FSBHB group.

**Keywords:** Fermented soybean hull; Soluble peptide; *Bacillus velezensis*; Broilers

## **Effect of incorporating mango (*Mangifera indica*) feed and boiled mango seed kernel as partial substitutes for maize on the zootechnical performance of broilers**

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With the aim of finding alternative substitutes for conventional energy sources in broiler feeds, a study was carried to evaluate the effect of incorporating mango (*Mangifera indica*) by-products (mango feed, MF and boiled mango seed kernel, BMK) as partial substitutes for maize on the zootechnical performance of broilers and feed costs. The present study was conducted between June and September 2023 in the vina department, Adamaoua region, Cameroon. MF was obtained as a mixture of sliced mango pulp plus skin (8 mm thick, 5 cm long and 4 cm wide) with a volume of corn bran that was sundried for 3 days and grounded. The study was carried out with a total of 168 day-old chicks from Cobb 500 strain which were completely randomized into four groups of 42 birds each which were fed with four types of experimental diets (ED): a control diet and three diets containing different proportions of MF and BMK: ED0 (0% MF; 0% BMK); ED5 (5% MF; 5% BMK), ED10 (10% MF; 10% BMK) and ED15 (15% MF; 15% BMK). Each group included 3 replicates (pens) containing 14 birds each. During the 7 weeks of trial (starter phase: days 1 to 21 and growing-finishing phase: days 22 to 49), birds received the experimental diets twice a day (8 a.m. and 4 p.m.). Feed intake, weight gain, feed conversion ratio, the cost of feed production, carcass weight, and carcass yield of chickens were recorded. The results showed that from the 1st to the 7th week of age, the incorporation of MF and BMK had no significant effect on individual feed intake, average daily gain, feed conversion ratio and the final live weight of broilers. The carcass weight of chickens receiving ED0 (1644 g); ED5 (1489 g) and ED10 (1519 g) diets remained similar and significantly higher ( $p < 0.05$ ) than that of subjects receiving the ED15 diet (1383 g), although no significant differences were observed in carcass yield among the different treatments. In economic terms, the cost of producing one kilogram of live weight of broilers fell significantly ( $p < 0.05$ ) as the level of incorporation of MF and BMK as substitutes for maize in the ED increased. Based on the results obtained, it can be concluded that MF and BMK can partially substitute maize in broiler diets without negative effects on performance and carcass yield, and with a reduction of the production costs in chicken production.

Keywords: Mango feed, mango seed kernel, zootechnical performance, broilers

**Effect of including dehydrated *Hermetia illucens* larvae on the productive performance of Isazul laying hens (27 to 38 week old)**

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In response to the growing demand for sustainable protein sources, recent studies explore the utilization of novel protein alternatives, specifically black soldier fly larvae (*Hermetia illucens*). This strategy would allow the improvement of the nutritional profile and sustainability of poultry diets in laying hens. Therefore, the aim of this study was to assess the impact of dietary supplementation with whole dry black soldier fly larvae (DBSFL) on the productive performance of 38-week-old laying hens. In this trial a total of eighty 27-week-old Isazul hens (breed adapted to the Mediterranean area) were used and allocated into 10 floor pens, with a total of 2 experimental groups, 5 pens per group, and 8 hens per pen. The first group was assigned to a control pelleted feed based on corn (35.29%) and soybean meal (15.00%). This feed contained per kg: 2730 AMEn kcal, 8.3 g lysine, 7.3 g methionine + cystine, 37.8 g Ca and 3.3 g digestible P. The second group received the same diet supplemented with 5% of the total dry matter intake in the form of DBSFL (estimated based on the total intake of dry mater from the previous week and administered in an additional feeder). This larva contained 44.09% crude protein and 34.94% ether extract on a dry mater basis. The eggs were counted daily, and egg weight and feed consumption weekly. The experiment lasted 11 weeks. The data were analyzed using the t-Student test. Diet did not affect any of the variables studied, except feed to egg mass ratio that tended to improve with DBSF inclusion ( $p = 0.075$ ). Hence, we can conclude that supplementing the diet of laying hens with 5% DBSFL is a viable option with a positive trend on FCR without affecting any other production trait of Isazul hens.

Keywords: Performance, laying hen, *Hermetia illucens* larvae, egg production

## Grape and Apple Pomace Antioxidant Capacity: Potential Inclusion in Poultry Diets

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This in vitro study investigates grape and apple pomace as potential dietary ingredients for poultry, focusing on their nutritional composition, digestibility coefficients, and antioxidant properties. This is a 2-steps enzyme method with two successive incubations (pepsin and pancreatin). The samples were analyzed in three replicates. The grape pomace was provided by a local apple farmer (Tohani, Prahova County) and analyzed for crude protein content (CP: 91.5 g/kg DM), ether extract content (EE: 67.4 g/kg DM), crude fiber content (CF: 180.1 g/kg DM), and gross energy (GE: 4660.55 kcal/kg DM). The apple pomace provided by a local farmer (Voinești, Dâmbovița County) registered the following values for CP: 22.9 g/kg, EE: 9.2 g/kg, CF: 78.2, GE: 4240.05 kcal/kg. However, lower grape pomace digestibility coefficients, primarily due to its significant fiber content, were observed. Nitrogen digestibility in apple pomace (48.7%) was significantly higher ( $p < 0.0001$ ) compared to grape pomace (47.6%). Additionally, apple pomace exhibited significantly higher digestibility ( $p < 0.0001$ ) coefficients for organic matter (OM: 55.6%) and dry matter (DM: 51.1%) compared to grape pomace (OM: 51.3%, DM: 46.8%). The polyphenol content analysis revealed significantly higher ( $p < 0.0001$ ) concentrations in grape pomace (4667 mg GAE/100 g) compared to apple pomace (289 mg GAE/100 g), indicating potent antioxidant capacity. Significantly higher antioxidant capacity was noticed ( $p < 0.0001$ ) also for the grape pomace, approximately double that of apple pomace (0.886 mM/g vs. 0.488 mM/g). Regarding lutein and zeaxanthin concentrations (mg/kg DM), a highly significant difference ( $p < 0.0001$ ) was observed for grape pomace compared to apple pomace (189.54 vs. 2.17). This suggests that grape pomace could contribute to managing oxidative stress in poultry farms. Despite lower digestibility, grape pomace could be considered a valuable source of antioxidants in various stress situations. Apple pomace, with higher digestibility coefficients, presents itself a more easily digestible option under normal feeding conditions. These findings provide insights into optimizing local and available agricultural by-products' utilization in animal diets, particularly those helping in managing oxidative stress, and present an eco-friendly option for recycling farm by-products as valuable animal feed.

Keywords: antioxidants, apple pomace, by-products, grape pomace, oxidative stress

## Effect of different levels of amino acids on broiler performance

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In the context of low protein levels and optimising requirements, the supply and balance of amino acids remain essential. Although there are recommendations for the ideal protein for broiler, there are still questions about secondary and non-essential amino acid requirements. The aim of this study is to test supplementation of Arginine (Arg), Valine (Val) and Isoleucine (Isoleu) as well as different levels of Leucine (Leu) in the feed of broiler between 10 and 35 days old. 1200 Ross 308 male broilers were allocated to five dietary groups with different levels of amino acids (40 par pen, 6 replicates per group). Starter (day 1 to 10), grower (day 10 to 21) and finisher (day 21 to 35) diets had similar metabolizable energy, CP and Digestible Lysine, digestible Methionine and Cystine, digestible Threonine, digestible Tryptophan. Birds were fed with a common starter diet. In grower and finisher, the control group (G1) was balanced on this profil of amino acids (1.04 dArg/dLys; 0.66 dIsoleu/dLys; 0.75 dVal/dLys; 1.20 dLeu/dLys). G2 received a synthetic arginine supplementation (1.15 dArg/dLys); G3 a synthetic valine and isoleucine supplementation (0.76 dIsoleu/dLys; 0.85 dVal/dLys); G4 a high level of Leucine by raw materials (1.40 dLeu/dLys); G5 a synthetic valine and isoleucine supplementation on the high level of Leucine (1.40 dLeu/dLys; 0.76 dIsoleu/dLys; 0.85 dVal/dLys). All groups presented similar performance in the starter phase. In the grower period, G2 receiving Arg had a higher average daily gain (ADG,  $P < 0.010$ ) and lower feed conversion ratio (FCR,  $P < 0.010$ ) than G4 and G5 with high level of Leucine with or without valine an isoleucine supplementation (ADG : G1 = 61.6g, G2=62.77, G3=61.56, G4=59.08, G5=58.48 / FCR : G1=1.383, G2=1.370, G3=1.382, G4=1.434, G5=1.429). During the finisher period, G5 high level of Leucine with valine and isoleucine had a higher ADG than G3 low level of Leucine with valine and isoleucine ( $P < 0.01$ ) (ADG : G1= 99.92, G2=100.86, G3=98.7, G4=100.78, G5=101.80). In the global period, there was no significant difference. The present study shows that an arginine supplementation could be interesting on performances and a different response to Leucine, Valine, Isoleucine according to the age. So this study requires further work to find the right balance between BCAA (Leucine, Valine, Isoleucine) and the best source of amino acid (raw material or synthesis).

Keywords: amino acids; arginine; leucine; valine; isoleucine; broiler performances

## **Efficacy evaluation of iron amino acid complexes (Fe-Lys-Glu) in laying hens: effects on laying performance, egg iron content and blood biochemical parameters**

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As essential trace mineral, iron (Fe) helps blood to carry oxygen through the body, play key roles in DNA synthesis, energy metabolism and antioxidant response of poultry. Fe is present in almost all feed ingredients and is often supplemented in diets as iron sulfate (FeSO<sub>4</sub>). This study investigated the effect of supplementing Fe complexed to lysine or to glutamic acid amino acids (FeAA) or FeSO<sub>4</sub> on hen's performance, blood parameters related to oxygen transport and antioxidant response. A total of 1260 Beijing White laying hens were randomly assigned to 7 treatments with 12 replicates, each with 15 hens. From 20 to 44 weeks of age hens were fed basal diet (75 ppm Fe from raw materials) supplemented with 0 (negative control, NC), 15, 30, 45, 60 and 75 ppm Fe in the form of FeAA or 45 ppm Fe as FeSO<sub>4</sub> (positive control, PC). The data were analyzed by linear and quadratic effect at  $p < 0.05$ . In addition, multiple comparisons among the treatments were performed by least significant difference test. Compared to NC, supplementing 30 to 75 ppm Fe from FeAA (linear and quadratic,  $p < 0.05$ ) increased egg production (EP, 48.57 ppm as maximum response), average daily egg weight (ADEW, 49.5 ppm maximum response) and FCR (45 to 75 ppm, linear  $p < 0.05$ ). Adding 30 to 75 ppm Fe from FeAA improved red blood cells count (RBC) and haemoglobin content (HBC) (linear and quadratic  $p < 0.05$ ) with 60 to 75 ppm Fe as FeAA inclusion generating the highest HGB content and RBC count. The higher the FeAA level, the stronger the antioxidant response (higher Cu/Zn-SOD activity and lower MDA content); 60 to 75 ppm added Fe from FeAA had greatest reduction in the MDA content in serum vs NC. Supplementing 15 to 75 ppm Fe (linear and quadratic  $p < 0.05$ ) increased serum and yolk Fe concentrations at 32 and 44 wk vs NC. The PC hens had lower EP, worse FCR and lower Fe yolk compared to hens fed 15, 30 or 45 ppm Fe as FeAA ( $p < 0.05$ ). Likewise, the hens fed PC diet had lower RBC and HCT ( $p < 0.05$ ) but similar antioxidant response compared to hens fed 45 ppm Fe as FeAA. Supplementation diets with FeAA and PC improved several parameters vs the NC indicating that Fe supplementation is beneficial to improve the laying performance, antioxidant ability, and yolk Fe content of laying hens. Supplementing FeAA in laying hen diet could substitute for FeSO<sub>4</sub> and the optimal additive levels of FeAA is 45 ppm in diets of White laying hens, 20 to 44 weeks old, based on the quadratic regression analysis of EP and ADEW.

Keywords: Blood Biochemical Parameters; Egg Yolk Iron; Iron Amino Acid Complex; Laying Hen; Performance



## **Amino acid-complexed minerals supplementation improves laying hen performance, egg and bone Quality of 98-week-old hens**

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A clear link is observed between hen aging and production of eggs with inferior eggshell quality. Eggshell quality decreases with age due to changes in the organic matrix (collagen based) of the eggshell and its biomineralization. In parallel, digestive physiology and the oxidative response of hens decrease with age. Trace minerals are vital for supporting hen's health and eggshell formation. This study evaluated the effects of total replacement of inorganic mineral (IM) zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), and selenium (Se) with the amino acid-complexed minerals source (AACM, Zinpro Availa Mins) on egg quality, egg yolk mineral deposition and bone variables. From 78 to 98 weeks of age, 400 Lohmann White laying hens were distributed in a complete randomised design with 4 treatments and 10 replicates each. The birds were fed either a basal diet supplemented with commercial levels of inorganic trace minerals (IM 100%: 60, 60, 7.0, 40, 0.20, and 2.0 mg/kg diet of Zn, Mn, Cu, Fe, Se, and I, respectively). The other 3 treatments completely replaced the IM source with AACM (except for I) at 70% (42,42,4.9,28,0.14), 50% (30,30,3.5,20.10), or 40% (24,24,2.8,16,0.08 mg/kg Zn, Mn, Cu, Fe, Se, respectively). Data were analysed using a one-way ANOVA and LSD was employed to compare the means when differences were significant at  $p < 0.05$ . Supplementing AACM with lowest inclusion level had greatest egg production (80 vs 78, 76.7, 73.6 %, 40% vs 50, 70 % and control, respectively), egg weight, egg mass (55.3 vs 53.2, 52.4, 50.1 g 40% vs 50,70 % and control, respectively), feed conversion per egg mass (1.89, vs 1.97, 2.01, 2.02 for 40% vs 50,70 % and control, respectively). AACM40% fed hens laid eggs with thicker eggshells vs Control fed hens. The concentration of Mn, Cu, Zn, and Se in the yolk decreased as the trace mineral levels were reduced to 40% inclusion without differences in concentration from eggs produced for hens fed AACM70 or 50%. There were higher percentages of yolk, albumen, and shell in eggs of laying hens fed AACM70. Bone quality was greatest in AACM 40% and poorest in IM100% fed hens as seen by the greater medial bone densitometry, without significant differences between treatments in the other bone segments. The concentration of 24, 24, 2.8, 16, 0.08, and 0.8 mg/kg of Zn, Mn, Cu, Fe, Se, and I, respectively, were sufficient to satisfy the needs of hens. Performance of 98 week old hens were maximised resulting in 8.8% more eggs produced compared to the control treatment.

Keywords: amino acid-complexed minerals source, hen performance, bone quality, old hens

## Effect of partial or total replacement of soybean oil by *Hermetia illucens* larvae fat in layers diets on performance and apparent ileal digestibility of nutrients

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Insects are rich sources of various valuable nutrients, including crude fat. Unfortunately, there is a lack of research about the usage of insects as an alternative energy source for laying hens. Therefore, the aim of this study was to determine the effect of fat from *Hermetia illucens* (HI) larvae in substitution to the soybean oil on apparent ileal digestibility and performance parameters in laying hens. A total of 168 Hy-Line Brown layers at 56 weeks of age, kept in battery cages, were divided into 3 dietary treatments (28 replicates of 2 birds each). For 12 weeks, the birds were fed diets with different type of fat, as follows: group (C) - a diet with 5% addition of soybean oil; group T1 - a diet in which 50% of soybean oil was replaced with fat from HI larvae, group T2 - a diet, in which 100% of soybean oil was replaced with fat from HI larvae. During the experiment, laying rate, feed consumption and egg weight were monitored at 4-week intervals. On the last day of the experiment, 9 hens from each group were sacrificed and the contents of the ileum between the Meckel's diverticulum and the ileo-cecal-colonic junction were collected individually from each bird for apparent ileal digestibility of dry matter, crude fat, N retention as well as energy digestibility. TiO<sub>2</sub> was used as an indigestible dietary marker. The differences between group means were determined by ANOVA and Tukey's test with the use of Statistica 13.3 software. Regardless of the proportion, substitution of soybean oil for fat from HI larvae did not affect the laying performance of the birds or the weight of eggs obtained from the hens. Partial substitution of soybean oil (50%) with the fat from HI larvae, also did not affect the amount of feed consumption, but total replacement of soybean oil significantly increased feed intake (111 vs. 107 g/hen/day,  $P \leq 0.001$ ) and impaired FCR compared to T1 group (1.92 vs. 1.81 kg/kg eggs,  $P = 0.009$ ). No changes in ileal digestibility of dry matter, crude fat, N retention and energy digestibility was observed with 50% replacement of soybean oil with fat from HI. However, increasing the HI fat content in the diet of laying hens (100%) significantly decreased the digestibility of crude fat (84.86%-T2 vs 87.15%-C and 87.64%-T1,  $P = 0.015$ ). It can be concluded that the total replacement of soybean oil with fat from HI can significantly affect selected performance parameters. A partial (50%) substitution of soybean oil for HI fat in laying diets is more recommended than a total substitution (100%), as it does not impair the FCR and did not influence apparent ileal digestibility of nutrients. "Funded by the Minister of Science under "the Regional Initiative of Excellence Program".

Keywords: laying hens; insect fat; performance; apparent ileal digestibility

## **In-ovo feeding by glucose and its effect in improving hatchability of Fayoumi breeder**

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This experiment was done to investigate in-ovo feeding of glucose for enhancing hatchability and hatching weight by using 180 fertile eggs of Fayoumi breeder. Eggs divided into 3 groups in 60 eggs/ treatment (20/ replicate). At 18th day of incubation, eggs injected in the air sac as following groups: first (control) without injection, second and third were injected by 0.1 ml glucose at concentrations of 2.5% and 5%, respectively. Results indicate that hatchability and hatching weight of second group recorded significant improvement by 14.71% and 5.68%, respectively compared with control.

**Keywords:** in-ovo feeding; hatchability; Fayoumi breeder

## **The impact of fiber supplementation on energy metabolizability and the production of short-chain organic acids in broilers, depending on their age**

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The aim of the study was to investigate the effect of fiber-based feed additives on the production of short-chain organic acids in the cecum and energy metabolizability in broiler chickens depending on their age. Commercial products at a dose of 0.7% (Arbocel®, a water-insoluble cellulose, and Biomin® Biofaser, based on fermented sugar beet pulp with a crude fiber content of 30%) were used as feed additives. Day-old chicks of both sexes, in total 180 Ross 308, were placed in three-tier cage batteries, using a total of 18 cages. Each group had six replications, with each replication consisting of ten chicks. A tray was placed under each cage for excreta collection. Until the seventh day of age, the chicks were fed the same starter feed in the form of crushed pellets. From the seventh day onward, they were fed a experimental mixtures, into which chromium oxide was added at a rate of 3 g per kg DM, used as an indicator for determining energy retention. At the same time, additives were added to two mixtures. At the age of 15, 22, and 29 days, a mixed excreta samples were taken from under each cage, homogenized for the determination of energy metabolizability. The day before excreta collection, all trays were thoroughly cleaned to ensure that the collected excreta sample was representative for one day. The samples were analyzed for dry matter content, chromium oxide, and calorimetric heat of combustion. The same components were determined in the feed mixtures. The metabolizable energy was estimated using the indicator method. At the same age, one chick was taken from each cage, a total of 6 per group. They were stunned, euthanized, and the digesta from the ceca were immediately collected by extrusion into Eppendorf tubes and frozen at minus 20°C. Organic acids, acetic, butyric, lactic, and propionic, were determined on an isotachophoretic analyzer and expressed in mmol/100 g of digesta. Both age and treatment had a significant effect on metabolizability of energy ( $p < 0.001$ ). Metabolizable energy significantly increased with age. The group fed with Arbocel additive showed the lowest metabolizable energy (67%) compared to the control group (70.8%) and the group fed with fiber from sugar beet pulp (70.4%). Age and treatment did not affect the concentration of the monitored short-chain organic acids. Different fiber sources may influence the cecal microbiome, affecting fiber digestibility and subsequently metabolizable energy by producing short-chain organic acids.

Keywords: fiber; broiler; energy retention

## **Standardised ileal amino acid digestibility of maize grains dried at two different temperatures and supplemented or not with protease**

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The study aimed to determine the effect of maize dried at two different temperatures supplemented or not with protease (Cibenza EP150, 0.5 g/kg of diet) on digestibility and AMEN of maize grains for broiler chickens on the 22nd day. One sample of maize (with moisture around 40 %) was used and split into two sub-batches. The first was dried at 100°C, and the second at 122°C. The used enzyme contained heat-stable protease (min. 600,000 U/g). The experiment was conducted on 360 16-day-old Ross 308 male chickens. The birds were randomly allocated to 6 dietary treatments (20 replications in each, 3 birds per replication). In 4 treatments, diets contained an investigated maize and basal diet in proportion 50:50 (CP ranged from 15.5 to 16.6 %) and supplemented or not with protease. Only a basal diet (CP: 21 %, maize-SBM diet) was used in two treatments. Basal diets were formulated with or without protease to allow digestibility calculation using difference method. On days 21 and 22, excreta and the digesta (from terminal ileum) were collected for chemical analyses (n = 10). After drying, the humidity of maize grains was 11.1 or 2.5 % (100 vs. 122°C). Drying at 122°C depressed protein solubility in KOH (78.12 vs. 28.7%) and in vitro starch digestibility. Enzyme addition increased the standardised ileal digestibility of essential amino acids (3.5%-19% (P<0.05)) and non-essential (2.5%-18% (P<0.05)) independent of drying temperature. However, the 122°C dried maize was more obviously affected than the 100°C grains, resulting in a significant enzyme x drying temperature interaction for AA digestibility (P<0.05). The only amino acids with no interaction terms were Phe (P=0.082), His (P=0.197), Tyr (P=0.082), Asp (P=0.79), and Pro (P=0.385). Ileal crude protein digestibility decreased when the drying temperature increased (P<0.05). The effect of enzyme addition was more pronounced when the high drying temperature was used, resulting in significant interaction. Irrespective of drying temperature, the enzyme addition improved ileal starch digestibility and AMEN of grains (P<0.05). Using 122°C to dry maize grains decreased AMEN by over 2.01 MJ/kg. The use of protease may improve the nutritional value of overdried maize. Drying at 122°C significantly reduced the nutritional value of maize for chickens. The benefits of enzyme addition seem independent of drying temperature, but they are more pronounced in overdried grains.

**Keywords:** ileal digestibility, AME, starch, poultry

## **Evaluation of dietary supplementation of garlic powder (*Allium sativum*) on the growth performance, carcass traits and meat quality of Japanese quail (*Coturnix coturnix japonica*)**

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Dietary supplementation with plant-based products may arise as part of an alternative strategy to using antibiotics as growth promoters in the poultry industry. Garlic powder (GP) possesses antimicrobial and antioxidant properties. The aim was to investigate the effect of dietary supplementation of GP on growth performance, carcass traits and meat quality of the Japanese quail. A total of 240, day-old mixed gender Japanese quail were assigned to 4 treatment groups, each group being replicated four times and containing 15 birds in each replication. Birds were provided with either a basal diet (control) or basal diet supplemented with 0.5%, 1% and 2% GP for 5 weeks. At slaughter age, birds fed 1% GP had higher ( $P<0.05$ ) body weight gain when compared to the control. Supplementation with different levels of GP had no influence ( $P>0.05$ ) on feed intake, feed conversion ratio except 3rd week, carcass traits and abdominal fat. Thiobarbituric acid, peroxide and pH values in breast meat of birds receiving GP (1% or 2%) after storage (0, 1, 3, 5, and 7 days) were lower ( $P<0.05$ ) than those of control birds. Furthermore, total psychrophilic bacteria count was lower in breast meat of birds supplemented with GP at any dose compared to the birds of control. Sensory characteristics such as color, aroma, juiciness and tenderness were significantly better ( $P<0.05$ ) in GP supplemented groups especially when fed 1% GP. In conclusion, supplementing the diet with 1-2% GP demonstrated growth-promoting effects and positively impacted meat quality, including sensory characteristics.

**Keywords:** Quail, garlic, antibiotics, meat quality, carcass traits, performance

## **Effects of dietary mango seed kernel and peel extracts supplementation as an alternative to antibiotics on growth performance, carcass characteristics and gut health of broilers**

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Global concern about removing the antibiotic growth promoters from poultry feed encourages researchers to investigate effective, safe, and natural alternatives. Mango fruit and its waste, including seed kernels and peel, is a rich source of phytochemicals with antimicrobial, antioxidant and anti-inflammatory properties. The current study investigated the efficacy of dietary supplementation of mango seed kernel (MSK) and peel (MP) extracts in broilers obtained through ultrasound-assisted extraction. A total of 560 male chicks (day-old, Ross 308) were procured and divided into four treatments with seven replicates containing 20 chicks each via completely randomized design. The experimental diets were prepared as follows: basal diet with antibiotics (Positive Control), basal diet without antibiotics (Negative control), basal diet without antibiotics + 25 mL/kg MSK extract (MSKE), and basal diet without antibiotics + 25 mL/kg MP extract (MPE). The results indicated that MSKE and MPE did not significantly ( $p > 0.05$ ) change body weight gain, feed consumption and feed conversion ratio (FCR) when compared with positive and negative control groups during phase 1 (d 1-21), phase 2 (d 22-35) and total grow-out phase (d 1-35). Non-significant ( $p < 0.05$ ) differences were observed among all the groups when compared for carcass characteristics except leg quarter yield and abdominal fat and intestinal morphology of broilers. The number of CD3+ T-cells, in ileal tissue, a marker for intestinal inflammation, was lowest ( $p < 0.05$ ) in the birds supplemented with MSKE, followed by MPE, which could possibly be explained by the anti-inflammatory and wound-healing characteristics of bioactive compounds present in mango waste extracts, which may help to preserve gut health in broilers. Moreover, MSKE and MPE supplementation significantly ( $p < 0.05$ ) reduced total bacterial count and the count of *Escherichia coli* in ileum digesta compared to the positive and negative control group, revealing the potential antimicrobial properties of these extracts for the gut health of broilers. However, the negative control group showed the highest total bacterial count, followed by positive control. The study suggests that mango waste extracts may have positive effects on broiler gut health due to their beneficial bioactive compounds. In this regard, further research is needed to investigate its effects on gut microbiota and relevant aspects by testing higher dose levels than 25 mL/kg.

Keywords: mango seed kernel; mango peel; extract supplementation; growth promoter; bacterial count; intestinal morphology

## **Protection efficiency of different inactivated AIV-H5 vaccines against challenge with recent H5N1 clade 2.3.4.4 circulating virus**

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Since 2020, clade 2.3.4.4 H5N1 viruses expanded to numerous countries in Europe, Africa, Asia, and America. In this study, we've evaluated the efficacy of commercially available vaccines indicated for immunizing poultry against HPAIV H5Nx subtypes. The investigated products i.e., MEFLUVAC™ H5 Plus 8 and MEFLUVAC™ H5 IMMUBOOST™ are inactivated trivalent water-in-oil (W/O) emulsified virus vaccines composed of whole reassortant avian influenza strains resembling clades 2.2.1.1 and 2.2.1.2 avian influenza H5N1 subtype as well as clade 2.3.4.4. of avian influenza H5N8 subtype. The formulation of the two products is identical except for an immunomodulator complex (MEVAC™ IMC) added to MEFLUVAC™ H5 IMMUBOOST™. Forty-40 14-day-old susceptible specific pathogen free (SPF) chickens were randomly split into two equivalent groups and then received a single dose from each product, respectively, via intramuscular (I/M) injection. Thirty-30 birds properly identified and obtained from the same source and hatch were left as an unvaccinated sham group. The birds allotted for the experiment were kept in biosafety level -3 (BSL-3) lab animal facility inside poultry isolators under fully controlled conditions for the duration of the study period. Twenty-eight days post-vaccination (28DPV), all the vaccinated birds as well as a fraction from the unvaccinated groups were challenged with 100µL containing 6.0 log<sub>10</sub> EID<sub>50</sub> from a newly emerged HPAIV H5N1 strain belonging to clade 2.3.4.4b isolated in 2022 in Egypt, via the oculonasal route. The remaining sentinel birds were allotted for the unvaccinated unchallenged group. Birds were monitored for 14 days post-challenge (14DPC). Oropharyngeal swabs were collected at 3, 5, 7, and 10 DPC. Virus detection and quantification were conducted using qRT-PCR. Vaccinated groups showed neither mortality nor remarkable clinical signs of the disease after the challenge, meanwhile, 100% mortality rate was recorded 3DPC in the unvaccinated challenged group. Also, the amount of viral shedding and the number of shedders were comparable between the vaccinated groups with a significant difference from the unvaccinated challenged controls. The vaccines elicited protection against challenge with the recent circulating HPAIV H5N1 belonging to clade 2.3.4.4b emphasizing the need for implementation of a competent vaccination strategy in order to mitigate the risk of the spread of the virus. It was also concluded that vaccination of vulnerable flocks should reduce the circulation of HPAIV H5N1 among domestic poultry.

Keywords: Avian Influenza, H5N1, virus shedding, Protection



## **An overview of transmissible viral Proventriculitis in broiler chickens in Poland**

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TVP is a contagious disease of chickens. TVP has been identified in several countries including Poland. TVP is characterized by enlargement of proventriculi and thickening of its wall. Histopathologically, necrosis of epithelial glandular cells, lymphocytic infiltration and hypertrophy and metaplastic changes of ductal epithelial cells appear. Destruction of proventricular glands leads to disorders in digestion, poor feed conversion and decreased body weight gains. Etiopathogenesis of TVP has been a subject of numerous studies. Agents, such as infectious bursal disease virus (IBDV) or adenoviruses and mixed infections has been considered as a cause of the disease. Recently a new member of Birnaviridae family has been isolated and identified from a TVP clinical case. The virus was strongly distinct from IBDV and is now called chicken proventricular necrosis virus (CPNV). With the use of biological material from the confirmed TVP clinical case, we were able to reproduce the disease in experimentally infected broiler chickens. Further analysis revealed that the disease was caused by CPNV and Aviadnavirus (FAdV-2). Very interesting observation was made based on serological evaluation of experimentally infected birds. We've demonstrated strong seroconversion against IBDV, with the lack of IBDV presence in infective material as well as in the internal organs of infected broiler chickens. The above, lead to the conclusion that serological cross-reactivity exist between CPNV and IBDV. Given the above, further studies were performed in order to identify the relationship between TVP and CPNV prevalence in broiler chickens in Poland. The study was carried out on 35 proventriculi samples sent for histopathological (HP) examination to the Faculty of Veterinary Medicine in Olsztyn between 2017 and 2019. After HP examination, TVP positive samples were processed for CPNV identification by RT-PCR. TVP was the most common pathological condition of proventriculi (n=23). CPNV was identified in 10 out of these 23 cases. The average HP score, and the average necrosis and infiltration score for CPNV-positive samples was significantly higher than in CPNV-negative ones. The average age of the CPNV-positive chickens was significantly lower than in CPNV-negative. Our study confirms the role of CPNV in TVP pathogenesis and it seems that preservation of the proventriculi in the early stages of the disease should result in a greater probability of CPNV detection.

Keywords: transmissible viral proventriculitis, chickens, pathogenesis

## Host mi RNA profiling of Vero cells during Avian orthoreovirus infection

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Avian orthoreovirus (ARV) is a virus that infects birds and causes various diseases, including arthritis, tenosynovitis, and malabsorption syndrome. MicroRNAs (miRNAs) are tiny RNA molecules that regulate gene expression after transcription by silencing or degrading their targets, playing important roles in how the cells responds to this infection. The candidate miRNA/anti miRNA overexpression can act as molecular adjuvant for ARV live attenuated vaccines in order to achieve increased replication of the vaccine virus and thereby gaining more protective immune response. The present study was envisaged to identify the potential of host miRNAs/ anti miRNA as molecular adjuvant in ARV Live attenuated vaccination. We infected Vero cells at MOI of 0.1 with ARV DVB04 strain and collected samples after 24 hours. RNA was isolated from control and infected cells. The quality of the RNA was checked. Further, the sample was processed for sequencing on the Illumina Nextseq500 platform using 1x75bp chemistry. The raw data generated were mapped to the reference genome and taken further for known and novel miRNA identification and predicting and validating its role as molecular adjuvant in ARV live vaccine virus. In vitro. miRBase, Rfam, myriad, mirtronPred, mirSTP, ViennaRNA, miRDeep, miRanda, PceRBase were used to identify, analyze and quantify novel miRNAs and precursor i.e., let-7a-1 in the samples which reveals that the ARV treated Vero cells contains 447 known miRNAs and 9 novel miRNAs. We found 247 differentially expressed miRNAs between control and treated samples. Of these, 45 were up-regulated and 134 were down-regulated with a statistical significance threshold of p-value<0.05. An average linkage hierarchical cluster analysis was performed on top 50 differentially expressed miRNAs which shows high expression of miRNA between control and treated samples. Upon performing functional annotation of the target genes of novel miRNAs from treated sample, we found that novel\_mir\_2 and novel\_mir\_8 regulates cell death and autophagy via targeting ENSCSAG00000009369 and ENSCSAG00000002736 genes respectively also novel\_mir\_2 was found to have target genes ENSCSAG00000003266, ENSCSAG00000011428 and ENSCSAG00000011033 that regulates apoptotic process and inhibit ARV replication. Discovering these new novel molecules anti novel\_mir\_2 and anti novel\_mir\_8 in In vitro studies were found to enhance ARV replication. These results were further validated by Real Time PCR & dual luciferase assay.

Keywords: "Avian orthoreovirus"; "miRNA"; "arthritis"; "vaccination"; "adjuvants"

## **Exploring iron scavenging as an under-explored mode for pathogen elimination by Bacillus-based probiotics**

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Iron, an indispensable nutrient, vital for the survival of nearly all aerobic organisms, acts as a catalyst in cellular redox reactions, aiding DNA synthesis, energy production, and various metabolic processes. Its bioavailability, however, is often limited particularly in the gut environment, creating a competitive struggle among microbial species. To address this scarcity, microbes secrete small iron-scavenging compounds such as siderophores, that have a high affinity to iron and support the sequestration and solubilization of ferric iron from the host environment. Some of the common gut enteric pathogens such as Salmonella, E. coli, Shigella, and Clostridium are more iron-dependent than beneficial gut microbes such as Lactobacilli. Iron promotes replication and virulence in these pathogens. The antimicrobial effects of Bacilli strains against these pathogens have been well reported, majorly by the production of secondary metabolites with direct inhibitory effects. The current study aimed to explore the less-studied effect of siderophores from a novel Bacillus licheniformis against Salmonella. The B. licheniformis reduced the prevalence of Salmonella in salmonellosis-infected broiler birds. The genomic mining of the Bacilli revealed the presence of several siderophore gene clusters. The in vitro iron binding capacity of the Bacilli was confirmed by chrome azurol S assay and compared with that of the poor binding capacity of E. coli and Salmonella enterica. Further, the preferential iron binding capacity of the cell-free supernatant (CFS) of the Bacilli strain was tested by incubating it with Salmonella enterica in a medium spiked with ferric salt. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) analysis of the spent media revealed decreased iron concentration in the group supplemented with CFS, indicating reduced iron availability for Salmonella growth, possibly due to iron chelation by the CFS. These findings suggest the positive role of siderophores in combating Salmonella's iron-dependent pathogenicity. Further exploration of Bacilli-based siderophores in regulating host iron homeostasis against other enteric pathogens may unveil their pivotal role in conferring an advantage over pathogens.

**Keywords:** Iron scavenging; Bacillus licheniformis; Siderophores; Salmonella

## Characterization of *Escherichia coli* MG1655 resistant variant after extended exposure to essential oils mixture

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The rise of antimicrobial resistance (AMR) in livestock, human and environment is a significant global health concern. Natural antimicrobials are increasingly considered as sustainable alternatives to antibiotics. However, the potential emergence of microbial resistance due to the use of such natural compounds including essential oils remains a critical issue. Thus, this study aims to characterize phenotypically and genotypically antimicrobial resistant variants (RVs) of *Escherichia coli* MG1655 after undergoing evolutionary assays with extended exposure to subinhibitory doses of an essential oils mixture (EO mix) and Amoxicillin as an antibiotic. *E. coli* MG1655 cells were subjected to cyclic exposure to sub-inhibitory doses ( $0.5\times$  minimal inhibitory concentration (MIC)) of EO mix and amoxicillin over a period of 20 days. Subsequently, resistance variants (RVs) were isolated and characterized. To assess the extent of direct resistance increase, the MIC values of RVs and the wild type strain against EO mix were compared. For genotypic comparison, whole genomic sequencing (WGS) of RVs was performed (Illumina Novaseq 6000) and compared to the wild type strain. MIC values of EO mix was  $200\mu\text{L/L}$ . Regarding amoxicillin (MIC =  $8\mu\text{L/L}$ ) the obtained MIC was in accordance with the range of values observed by the EUCAST (European Committee on Antimicrobial Susceptibility Testing) and previous results found by Kerros et al. (2017). RV isolated from evolution experiment with EO mix (EcEO mix) showed 25% increase in MIC value against EO mix. In comparison Amoxicillin showed 200% increase in term of MIC values. For the observed genetic modification, a deletion of more than 5,000 bp was identified in EO mix, causing the loss of genes involved in environmental information processing and cell motility while a single nucleotide variant in a gene involved in the response to lipid peroxidation of cell envelopes; and a deletion of ca. 5,000 bp in the same region to that detected in EcEO mix, possibly explained by an reorganization of the *E. coli* metabolism to fight essential oils and lipid membrane attack. In summary, low MIC increases were observed with EO mix products compared to the tested antibiotic. Based on these results this EO mix exhibited interesting possibilities to fight *E. coli* with a preservation of antibiotic efficacy.

Keywords: Resistance; essential oils; antimicrobial; *escherichia coli*

## Antimicrobial resistance among of *Gallibacterium anatis* biovar *haemolytica* isolated from chickens in Poland

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*Gallibacterium anatis* is a Gram-negative bacteria which can be transmitted horizontally and vertically. In birds it constitutes as part of the normal microflora in the occurrence with the coexistence of other relevant factors leads to serious economic losses in the poultry industry. *G. anatis* biovar *haemolytica* is a bacteria causing salpingitis, peritonitis, drop in egg production, and also respiratory signs. In this study, 22 isolates obtained from laying hens from the respiratory (n=9) and reproductive (n=13) systems were selected. *G. anatis* biovar *haemolytica* was identified by MALDI TOF after culturing on Columbia agar with 5% sheep blood. PCR method targeting gen 16S rRNA were used for verification of isolates. The susceptibility of *G. anatis* biovar *haemolytica* to 20 different antimicrobials was determined by disc diffusion method and by the microbroth dilution method. In addition, isolates of *G. anatis* biovar *haemolytica* were tested for virulence genes *GtxA*, *gyrB* and *flfA* and antibiotic resistance genes (*blaROB*, *tetH*, *tetB*) by PCR. All isolates were multi-drug resistance. Up to 22.7% strains of *G. anatis* *haemolytica* biovar showed multiresistance to 16 different antibiotics. The 68.1% of isolates are resistant to at least 14 antibiotics. The most prevalent antibiotic resistances were tilmicosin, tylosin, enrofloxacin followed by quinupristin/dalfopristin, erythromycin, tetracycline, teicoplanin linezolid and vancomycin. The occurrence of virulence genes *gyrB* and *GtxA* was found in 100% isolates. Gene *flfA* were detected in 4,5% isolates all from respiratory tract. The antibiotic resistance gene *tetB* was detected in 95,5 % isolates, *tetH* in 22,7% (3 from the respiratory tract and 2 from the reproductive tract). The *fifA1* and *blaROB* genes were not detected. Of great concern is the high antibiotic resistance of the tested strains of *G. anatis* biovar *haemolytica* obtained from chicken flocks. Due to the constant variability of antibiotic resistance of these bacteria over the years, therefore, it seems reasonable to periodically control their occurrence in poultry flocks.

Keywords: *Gallibacterium anatis*, chicken, antibiotic resistance

## Positive effect of *Euglena gracilis* derived paramylon on immunoglobulin Y content of egg yolk

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Paramylon is a linear, unbranched beta-1,3-glucan polymer that is isolated from the single-cell microalga *Euglena gracilis*. Research has shown paramylon has immunomodulatory effects in animals, through binding on the dectin-1 receptor of antigen-presenting cells. Recent studies in mammals have shown that paramylon increases antibody titers of colostrum, resulting in a positive effect on the health and survivability of the offspring. However, no studies are available in birds investigating the effect of paramylon on the immunoglobulin content of eggs. The objective of this proof-of-concept study was to assess the effect of paramylon on the immunoglobulin content of eggs when supplied to the bird, in which laying hens were used as a model for breeding hens. A total of 500 Lohmann brown laying hens of 18 weeks of age, housed in enriched cages for 32 weeks were included in the study. The trial consisted of two treatments (10 replicates each): a control (C) group and a paramylon (P) group treated with 21.8 grams of product per liter of water at specific time points (5 days at arrival and every 35 days for 5 days). The following parameters were measured: laying index, FCR, daily egg mass, and % of broken eggs. Immunoglobulin Y (IgY) content of egg yolk was measured monthly in 20 eggs per treatment by ELISA. Results of the trial show numerical differences: increased laying index for P compared to C (86.1 vs 84.5% respectively), higher daily egg mass (56.1 vs 55.0 g per egg/bird respectively) and there were reductions in broken eggs (2.3 vs 2.8% respectively). No difference in FCR was noted. IgY content of egg yolk coming from P birds was increased compared to eggs of C birds at each time point, with an average of 5.3 mg/ml compared to 3.8 mg/ml ( $p < 0.001$ ). This proof-of-concept study shows the potential of paramylon to increase immunoglobulin content in eggs. Further research should be performed in breeding hens to evaluate a potential impact on the health and survivability of day-old chicks. Furthermore, this study shows paramylon is safe to use with no negative impact on performance, a concern raised by producers when using immunomodulatory products.

Keywords: immunoglobulin, *Euglena gracilis*, immunity, paramylon, laying hens

## **Immuno-boosting effects of lysozyme, essential oils, vitamins mixture of additives in response to live E. coli vaccine**

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This study aimed to evaluate the effects of lysozyme, essential oils, and vitamins' (LEV) mixture of feed additives (formula propriety of PATENT CO. DOO) on specific humoral immunity and inflammatory response of broilers vaccinated with live E. coli vaccine. One hundred fifty-two one-day-old broilers were segregated, randomly, into four different experimental groups of 38 broilers each: negative control group (non-vaccinated), vaccinated group, treated group with 1 kg/ton of water of LEV (non-vaccinated) and vaccinated group treated with 1 kg/ton of water of LEV.. Poulvac® E. coli vaccine was applied in dose  $\geq 6,5 \times 10^6$  CFU on the first experimental day to each animal, of the determined group. E coli-specific IgA production was assessed with an in-house ELISA on day 14. and 28. of the trial. Cytokine expression was evaluated by qPCR (IL-1 $\beta$ , IL-8, NF-kB, IL-6, and TNF- $\alpha$ ), using specific primers for each target on days 3, 14, and 28 of the trial. Intestinal permeability is evaluated by oral administration of FITC-Dextran on days 3, 14, and 28. Outlier data were removed by ROUT methodology (Q=1%) and the mean of the results of IgA were compared using the Kruskal-Wallis test, qPCR  $\Delta\Delta$ CT values were compared by 2way ANOVA. Results on the eleventh day after the vaccination show that the vaccinated and treated group presented higher anti-E. coli IgA quantification than other groups. The pro-inflammatory cytokines TNF- $\alpha$ , IL8, and IL-1 $\beta$  were reduced on day 28 in the vaccinated + LEV group when compared to the vaccinated-only group, showing an inflammation control using the product. LEV demonstrated a positive effect on intestinal integrity throughout the experiment. In the treated and immunized animals, LEV showed anti-inflammatory benefits and the capacity to enhance the immune system's early adaptative response.

**Keywords:** specific humoral immunity; inflammation; broilers; lysozyme, essential oils, vitamins

### **Antimicrobial resistance of Escherichia coli isolates from broilers**

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*Escherichia coli* (*E. coli*) is a widely distributed bacterium in the environment and commonly found in the intestinal tract of warm-blooded organisms. Most strains of *E. coli* are harmless, but some can cause local or systemic infections in poultry. To treat such infections, antimicrobials are used. In previous studies carried out some years ago, a high number of antibiotic resistant *E. coli* isolates were detected. The aim of this study was to identify the level of antibiotic resistance in *E. coli* isolated from broilers in 2021, 2022 and 2023. A total of 709 isolates (163 in 2021, 270 in 2022 and 276 in 2023) from broilers were tested against tetracyclines (doxycycline), quinolones (enrofloxacin), penicillin (amoxicillin) and trimethoprim/sulfamethoxazole. In 2021, the prevalence of resistant *E. coli* isolates to doxycycline was 26.4%, this percentage decreased in 2022 and 2023, with 18.1% and 15.9% respectively. Amoxicillin resistance was found in the 54.4% and 58.0% of the *E. coli* isolates in 2022 and 2023, respectively, while in 2021 was higher, 62.0%. The same happens with the rest of antibiotics where the highest number of resistant strains was detected in 2021, while in 2022 and 2023 the percentage of resistant strains were reduced. The 67.5% of *E. coli* isolates were sensitive to trimethoprim/sulfamethoxazole in 2021, the percentage of sensitivity increased in 2022 and 2023 (82.8% and 77.2%). While in 2022 and 2023 more than 50% of the isolates were sensitive to enrofloxacin, in 2021 this percentage was lower (38%). The improvement of biosecurity measures and poultry management has contributed to reduce the use antimicrobials in aviculture sector and consequently, a decrease in the number of resistant strains detected in 2022 and 2023. All efforts should be directed towards strategies to reduce the use of antimicrobials to contain the emergence of multidrug resistant strains, one of the major public health problems of the present and future.

**Keywords:** *Escherichia coli*; antimicrobials; resistance; broiler; multidrug resistant strains



## **In vitro comparison and evaluation of antimicrobial activity of ammonium and sodium formate combined with short-chain fatty acid salts in different microorganisms**

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The European Parliament regulation No 1831/2003 on additives for use in animal nutrition was updated in 2022, which included restrictions on using ammonium formate in some farm animal species. This study aimed to evaluate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of organic acids combined with ammonium or sodium formate against different bacteria. The study was carried out in in vitro conditions using the microdilution method to determine the MIC and MBC of the two products. The main factors in the study were sodium butyrate (Gustor Liquid) containing either ammonium (GAF) or sodium formate (GSF) at the same inclusion rate, and 7 bacteria (*Clostridium perfringens*, *Salmonella enteritidis*, *Salmonella Typhimurium*, *Salmonella Gallinarum*, *Campylobacter jejuni*, *Escherichia coli* and *Staphylococcus aureus*). The MIC was determined by the broth microdilution method using 96-well microplates and brain heart infusion adjusted at pH 6.0. The MBC was also determined by subculturing from wells without evident bacterial growth onto fastidious anaerobe agar. Afterward, the values that inhibited and killed 50% of the tested isolates were calculated (MIC<sub>50</sub> and MBC<sub>50</sub>, respectively). Results showed that the values of MIC<sub>50</sub> for GSF and GAF had the same bactericide effect (6,250 ppm) against *C. perfringens*, *S. enteritidis*, *S. Typhimurium*, *S. Gallinarum*, *S. aureus*, *C. jejuni* and *E. coli*. Regarding MBC<sub>50</sub>, the trend was similar, GSF and GAF had the same bactericide effect with a low concentration (6,250 ppm) against *C. perfringens* and *S. enteritidis*, and with a higher concentration (12,500 ppm) against *S. Typhimurium*, *S. Gallinarum*, *S. aureus*, *C. jejuni* and *E. coli*. It is well known that butyric acid has a strong acidification and antimicrobial activities against Gram-negative bacteria. As a conclusion, replacing sodium formate instead of ammonium formate in Gustor liquid had the same impact on the bacterial species under investigation and preserved the product's ability to neutralize them.

Keywords: Formate; ammonium; sodium; bacteria; butyrate

## **Mucolytic activity of essential oils, lysozyme, and vitamins' premix of additives intended for pigs and poultry**

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Mucolytic medications for pigs and poultry are intended to improve bronchial exudate clearance, promote easy breathing, and reduce mortality associated with respiratory diseases. However, the most of chemical mucolytics used for pigs and poultry are only tested on humans. Furthermore, some of them are in combination with antibiotics to which bacteria develop resistance. Our goal was to test the impact of essential oils, lysozyme, and vitamins' premix in different concentrations (0.1, 0.05, 0.01, 0.001%) on the viscosity of mucus substrate by HAAKE Mars rheometer (Thermo Scientific). The mucus substrate was made of Mucin Type II 84082-64-4 - Sigma-Aldrich. The protocol of the study included sample incubation at 37 °C with a shear rate of 5 1/s for 30 min, followed by the increase of the shear rate of 0 - 100 1/s for 180 seconds at a temperature of 37 °C. The samples behaved as weak pseudoplastic systems – their viscosity decreases with increasing shear rate. Therefore, it is not characterized by a single viscosity value. The apparent viscosity value was expressed as the mean of repeated measurements at a defined shear rate. At a shear rate of 100 1/s at 37 °C, the viscosity of the negative control (mucus substrate stabilized by Tween 80) was 15.06 mPas, while for tested samples added at concentrations of 0.001, 0.01, 0.05, and 0.1%, it was 14.74, 14.63, 14.78, and 14.70 mPas, respectively. The tested sample of essential oils, lysozyme, and vitamins' premix exhibited the best mucolytic activity at a concentration of 0.01% while further increase in concentration does not contribute to additional activity at a given substrate concentration.

Keywords: mucolytic activity, plant extracts; lysozyme;

## **Probiotic in the control of mortality and lethality in experimentally challenged broiler chickens with avian pathogenic escherichia coli**

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Avian Pathogenic Escherichia coli (APEC) is characterized as one of the main agents responsible for decreased productivity, mortality, and carcass condemnation in poultry slaughter. Lactobacillus spp. represents the primary genus of microorganisms used as probiotics, with colonizing capabilities, maintaining a balanced intestinal ecosystem. The present study evaluated the use of Lactobacillus spp. in the face of a challenge posed by APEC. A total of 144 one-day-old Cobb broiler chickens were housed in experimental cages and divided into four treatment groups: T1 – Negative control, T2 – Positive control (birds challenged with APEC), T3 – Birds received only probiotics, and T4 – Birds received probiotics and challenged with APEC. The probiotic used was Floramax-B11®, a Lactobacillus pool (107 CFU/mL), administered on the 1st and 2nd days of the birds' lives via gavage (0.6g/100mL). On the 4th and 5th days, the birds were challenged with APECLMA046 at a concentration of 109 CFU/mL. For the lethality test, the birds were observed for seven consecutive days after the challenge, recording the number of bird deaths per day. In the lethality test, 12 birds from each treatment were euthanized on the 7th and 12th days, and the presence/absence of airsacculitis (A), pericarditis (P), perihepatitis (Ph), and peritonitis (Pe) were observed. The lethality assay was evaluated using the log-rank statistical test (Mantel-Cox), with  $p < 0.05$  considered significant, and pathogenicity results were assessed using descriptive statistics. The T2 group (positive control) showed a mortality of seven birds, while in the group treated with Floramax-B11+ APEC, the mortality of 2 birds was observed. The probiotic was statistically effective ( $p = 0.0096$ ) in reducing mortality caused by the APEC used in the challenge. In the autopsy conducted at seven days of age, it was observed that 4/12 (33%) of animals in the positive group exhibited airsacculitis, pericarditis, perihepatitis, while the treated group T4 had 3/12 (25%) of birds with lesions showing airsacculitis, pericarditis, and perihepatitis. In the autopsy performed at twelve days of age, three animals from the positive control group developed peritonitis, airsacculitis, and perihepatitis. The use of Floramax-B11 allowed a reduction in mortality and lesions caused by APEC.

**Keywords:** Avian Pathogenic Escherichia coli; Lactobacillus spp; Mortality; Probiotic

## **Evaluation of the persistence of commercial lactobacillus spp in experimentally contaminated chicken litter with APEC**

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Avian Pathogenic Escherichia coli (APEC) is one of the main agents causing diseases in birds. Furthermore, the presence of APEC in poultry litter represents a risk to public health due to the transmission of pathogenic strains to humans through the consumption of contaminated meat. In this scenario, the use of beneficial microorganisms in poultry litter, such as Lactobacillus spp., presents an alternative to this problem as it contributes to animal health, reduces the use of antimicrobials, and the risk of bacterial resistance. Therefore, the aim of this study was to evaluate the persistence of commercial Lactobacillus spp. and APEC in experimentally contaminated litter. Plastic trays were filled with 300g of substrate, prepared with first-use wood shavings with the inclusion of 25% used litter decontaminated by exposure to formaldehyde. Each treatment had three replications: T1 (negative control without challenge), T2 (Substrate + Lactobacillus spp.), T3 (Substrate + APEC 109 CFU/mL), and T4 (Substrate + APEC 109 CFU/mL + Lactobacillus spp.). The commercial probiotic was grown in MRS broth, and then 10mL of the solution was nebulized using an ultrasonic device over the litter for 10 minutes. APEC was cultured in BHI broth, reaching 109 CFU/mL, and subjected to nebulization as described earlier. Bacterial recovery was performed at 0 and 7 days using sterile swabs soaked in saline solution and applied to the surface of the plastic trays containing the treatments. They were then diluted for quantification of APEC and probiotic strains in MacConkey and Rogosa agar, respectively. The data underwent analysis of variance followed by mean comparison tests, with a significance level of 5%. There was a significant reduction in the population of lactic acid bacteria (T2) over time, with averages of 7.4 to 3.54 Log CFU/mL at 0 and 21 days, but at 7 and 14 days, no difference was observed with averages of 4.98 and 4.5 Log CFU/mL, respectively. At 7 days, there was a lower recovery of APEC between groups T3 (5.21) and T4 (4.36) with a significant difference between them, demonstrating the efficacy of the probiotic in reducing APEC in chicken litter. It is concluded that commercial Lactobacillus spp. and APEC are capable of colonizing poultry litter and reducing the population of APEC seven days after the challenge.

Keywords: Lactobacillus spp; APEC; Poultry litter; broiler

## **Quercetin can mitigate lipopolysaccharide induced apoptosis and oxidative stress in bursal and thymic lymphocytes**

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Quercetin is a flavonoid with proved immunomodulatory and antioxidative properties, therefore its use is predominantly in stressful conditions where immune system is negatively affected. Therefore the main goal of the experiment was to study antiapoptotic and antioxidative properties of quercetin on lipopolysaccharide (LPS) induced apoptosis and oxidative stress in bursal and thymic lymphocytes. The organs were isolated from 3 weeks old broiler chickens Cobb 500 and cells were isolated by gently mashing through 40 µm cell strainer. After isolation, cells were counted and seeded into complete RPMI 1640 medium. Firstly, optimal quercetin concentration had to be established. Bursal and thymic lymphocytes were seeded in 24 cell culture plates and quercetin were added at concentrations 0 µg, 5 µg, 10 µg, 25 µg, 50 µg, 100 µg and 200 µg immediately after cell seeding and cell culture plates were inserted in incubator at 37 °C and incubated for 5 hours. After incubation, percentage of apoptotic and necrotic cells represented by YO-PRO-1 and propidium iodide+ cells respectively were detected by flow cytometry. Optimal concentration of quercetin (with highest percentage of live cells) was set at 100 µg for bursal lymphocytes and at 50 µg for thymic lymphocytes. In the main part of the study, cells were preincubated with quercetin for 30 min followed by addition of LPS and incubated for 5h at 37°C. After incubation, the levels of apoptotic (YO-PRO-1+) necrotic cells (propidium iodide+) and percentage of reactive oxygen species+ cells (CellRox+) were measured by flow cytometry. One control contained lymphocytes only, and other served as positive control, containing quercetin. Results showed that in thymic lymphocytes population addition of quercetin decreased percentage of apoptotic and necrotic cells against LPS-treated group and untreated control. Similarly, CellRox+ cells levels decrease after addition of quercetin. However, differences were not statistically significant. Likewise in the case of bursal B lymphocytes addition of quercetin decreased levels of apoptotic and necrotic cells without any significant differences. On the other hand, quercetin significantly decreased ( $p < 0.05$ ) LPS-induced oxidative stress presented by lower numbers of CellRox+ bursal B lymphocytes. Based on the results, quercetin decreased apoptosis and oxidative stress in lymphocytes, therefore additional in vivo experiments under stressful conditions can be performed.

Keywords: Quercetin; broiler chicken; oxidative stress; apoptosis; Bursa of Fabricius, Thymus

## **Effects of short period of incubation during storage of eggs from different breeder ages on chick characteristics and nutrient transporters**

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Due to demands for day-old chicks from broiler producers, it is a common practice to store eggs prior to incubation. However, longer storage than 7d has negative consequences such as decreased hatchability and chick quality and increased embryonic mortalities, apoptotic cells, and incubation duration. Previous studies also showed that longer egg storage would affect the expression of nutrient transporters and intestine development. Short Period of Incubation During Egg Storage (SPIDES) is a method to mitigate the negative effects of long storage on hatching performance. This study aimed to evaluate the effect of the short period of incubation during 12 d of egg storage on chick quality and nutrient transporters. A total of 320 eggs was obtained from 31 (young, Y) and 51 (old, O) wk of age breeders (160/breeder age). The eggs from each breeder age were divided into two groups; 1. Eggs were stored for 12d, 2. Eggs were stored for 12d and were exposed to SPIDES at 37.5°C for 3 h at 4 and 8 d of the egg storage. The blastoderm stage of embryos was determined before incubation. All eggs were incubated at standard incubation conditions. There were 4 replicated egg trays for each treatment/breeder age. SPIDES resulted in a more developed blastoderm stage regardless of breeder age. Chick weight decreased with SPIDES; however, the yolk was more utilized by chicks exposed to SPIDES. While villus length and width decreased with the SPIDES of chicks from Y breeders, the effect of SPIDES on the villus development of chicks from O breeders was not significant. Expression of PEPT1 in the jejunum was increased in chicks from Y breeders exposed to SPIDES compared to those that were non-exposed. It is likely that due to the less developed intestine, the expression of PEPT1 was upregulated to meet nutrient demand. SPIDES had no effect on the expression of PEPT1 in chicks from O breeders. Neither breeder age nor SPIDES affected SGLT1 and GLUT2 expression in the jejunum of day-old chicks. These results indicated that SPIDES negatively affected the intestinal development of chicks from Y breeders and modulated the expression of PEPT1, probably to meet the nutrient demands of chicks. The non-significant differences obtained for glucose transporters indicated that gene expression appeared to be independent of SPIDES.

Keywords: egg storage, pre-incubation, chick, intestine

### **Effects of breeder age on plasma glucose and lactate of newly hatched chicks**

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During the last stage of incubation, the available oxygen levels reduce, and the broiler embryo uses the anaerobic glycolysis to maintain the energy supply. In the liver of chicks, the lactate produced is metabolized on gluconeogenesis, resulting in glucose that returns to muscle cells. This trial aimed to evaluate the effects of the age of slower growing breeders on plasma glucose and lactate of newly hatched chicks. The experiment was conducted in University Federal of Goiás. The eggs from slower growing broiler breeder of different ages (44 and 54 weeks) were incubated in a single stage incubator, allotted in a randomized block design. Blood collection of ten chicks per breeder age were performed at 504h of incubation. Samples of 5 mL of blood were collected by the decapitation method, was centrifuged at 3,000 rpm and the serum collected. Glucose and lactate analysis was performed through the commercial kit and in biochemical automation analyzer. Data were submitted to ANOVA, with 5% probability. R statistical software was used to statistical analysis. The breeder age did not affect plasma glucose levels of newly hatched chicks ( $P=0.5808$ ). The plasma glucose concentration was 191,4 and 195,4 mg/dL to newly hatched from 44- and 54-weeks broiler breeders, respectively. The plasma lactate concentration of newly hatched from older breeders was higher than chicks from younger breeders (11.63 x 8.26 mg/dL,  $P=0.0518$ ), indicating that there is difference on lactate metabolism in newly hatched chicks according to broiler breeder age. The higher lactate results of anaerobic glucose oxidation that occurs in pipping muscle and may result from less oxygen availability, increased muscle size or slower lactate clearance in the liver. We concluded that broiler breeders age changes lactate metabolism in newly hatched slow growing chicks which affects the neonate's glycemia.

Keywords: broiler; hatchery; gluconeogenesis; incubation; metabolism

## **Use of dimethylacetamide for cryopreserving rooster sperm from an endangered breed: effects on sperm quality and sperm fertilizing ability**

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Sperm cryobanks have become fundamental tools for preserving avian genetic resources. For sperm surviving the cryopreservation process, cryoprotectants (CPAs) are necessary. Glycerol is the CPA most widely used because it provides good protection, but it is contraceptive for the hens at concentrations above 1% and it must be removed before artificial insemination (AI) in a time-consuming process. On the other hand, dimethylacetamide (DMA), although does not provide the same protection as glycerol in terms of sperm quality after thawing, has been successfully used in some protocols with the advantage of not being contraceptive, so its elimination before AI is not necessary. In this study, in a preliminary experiment we evaluated the effect of DMA level (from 1 to 5%; v:v) on sperm quality (percentage of total motile-TM and progressively motile sperm-PM and percentage of sperm with intact plasma membrane-PMI) and in a second experiment, the fertilizing ability of the sperm cryopreserved with 4 or 5% DMA was evaluated. For the experiment, pooled semen from 26 males belonging to the endangered breed Valenciana de Chulilla were used, each pool split into several aliquots and each of them frozen with a different concentration of DMA in a programmable freezer with the following curve: from +4 to -6 °C at -20 °C/min and from -6 to -100 °C at -60 °C/min and then they were immersed in liquid nitrogen for storage. The straws were thawed in an iced-water bath for 3 min. Lohman brown hens (n = 40) were used for the AI trial. Each hen received two AI doses with 300 x 106 sperm in alternate days (d0 and d2) and eggs laid between d3 to d7 were chosen for incubation. Candling was performed at d7 of incubation to detect the fertilized eggs. As the DMA concentration increased, the sperm quality was: %TM (3-6-17-21 and 29% for 1-2-3-4 and 5% DMA), %PM (0-2-7-10 and 13%; for 1-2-3-4 and 5% DMA) and %PMI (5-16-28-40 and 50% for 1-2-3-4 and 5% DMA). These figures were low compared to the quality obtained with glycerol. In the second experiment, the fertilizing ability of the sperm was extremely low for both, sperm preserved with 4% (0.9%, 1 egg fertilized/115 incubated) or 5% DMA (1.8%; 2/111). Under our conditions, DMA provided disastrous results in terms of fertility so we must not choose this protocol for the creation of the sperm bank from the Valenciana de Chulilla hen breed. Acknowledgments: supported by GVA-IVIA and co-funded by the EU through the Operational Program ERDF of the Comunitat Valenciana 2021-2027 (Grant No. 52201), Generalitat Valenciana (Grant No. CIAICO/2022/034) and by the Universidad Cardenal Herrera-CEU, CEU Universities (Grant No. INDI21/40).

**Keywords:** semen; rooster; endangered breed; DMA; fertility



## **Preliminary investigations on Mulard duck, as sterile recipient in gonadal tissue transplantation**

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Nowadays, the practice of in vitro poultry gene conservation is limited to semen cryopreservation. In avian species, female is heterogametic and the eggs and embryos cannot be frozen due to their biophysical traits, so an alternative method is needed for preserving the female genome as well. For this purpose, gonadal tissue cryopreservation and transplantation at day-old age can be a suitable method. Ovarian transplantation is a developed technique in domestic fowl, donor-derived progeny can be regained by grafting frozen/thawed ovarian tissue. In case of waterfowl species only few reports were on donor-derived offspring by transplanting native gonadal tissue of Muscovy duck donor to Pekin duck recipient. Due to the recipient's whole own gonad very often cannot be removed fully, both donor and recipient derived progeny can be produced. Applying sterile recipient in gonadal tissue transplantation all offspring can be donor derived. In previous investigations, it was found, that the Mulard duck can be a suitable recipient, due to it has anatomically normal, but not functioning gonad. The aim of our study was to adapt the ovarian transplantation technique to Pekin duck donor / Mulard duck recipient combination. Frozen / thawed ovaries of Pekin duck donor were grafted into 9 Mulard duck recipients. In case of surgical procedure, the same technique was used as in goose. The autopsy of the animals was performed at 54 weeks of age. 66 % of the donor ovaries were adhered and 22% of them started to ovulate, but not into the oviduct. After sexual maturation blood samples were collected 5 times until 52 weeks of age from the recipients and 2 control groups of unoperated Mulard and Pekin ducks. Progesterone and estradiol levels were determined using ELISA. It was found, that both of the hormonal levels were similar to Pekin ducks in those operated Mulard ducks, which donor gonads adhered. In the hormonal levels of rest of the operated group no significant differences were found comparing with control Mulard group. According to the results, the transplantation of cryopreserved gonadal tissue is successful in Mulard duck recipients, the ovaries can adhere, which is also proven by the elevated hormonal levels, but further investigations are needed aiming the difficulty of the ovulation process. The investigation was supported by KDP-2021.

Keywords: in vitro gene conservation; duck; ovarian tissue transplantation

## Research of breeding and improvement immune system in poultry

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The research of genetic improvement studies carried out at the Faculty of Agriculture of Akdeniz University-Turkey are focused on healthier food production. Our observations suggested the hypothesis that the genotypes of Leghorn and domestic Denizli genes crossbreed are reasonably hoped to have improved egg and meat yield both and resistance to illnesses. At the same time aimed to develop general resistance to viral and bacterial diseases by adding different ratios of immuno-stimulating plant and insects-based extracts to feed and water. The purpose of this study is to analyze the growth curves of one of the Denizli-Leghorn crossbreed varieties as well as impact of extracts to illness resistance. Nonlinear regression analysis using weekly live weight data from output to 20 weeks of age and to determine the most appropriate growth model for the genotype will be used. Also, fixed sigmoidal models in terms of inflection point such as Gompertz, Logistic, Von Bertalanffy and flexible functions in terms of inflection point such as Richards, Levakovich, Janoscsek will be used. The differences between the growth profiles of female and male Blue Denizli-Leghorn hybrid chickens will be tested by Profile analysis technique, which is one of the multivariate statistical methods. With the results to be obtained, conclusions will be made about the possibilities of usage extracts and producing Denizli-Leghorn genotype in various breeding systems, strategies will be determined in this direction and contribution will be made to more sustainable and healthy production of poultry products.

Keywords: Poultry breeding; Immune strengthening; Natural extracts usage

## Effects of stocking density and sex ratio on the reproductive parameters of breeding geese in indoor keeping system

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Due to the species-specific characteristics of geese, they are less suitable for production in completely closed farming systems. However, due to certain epidemiological regulations, their use in such systems is becoming increasingly common. Therefore, it is urgent to conduct investigations aimed at developing optimal indoor keeping system for geese. As a first step, we examined the impact of stocking density and sex ratio on production parameters. The experiment was conducted using the INTEGRAL MB 09 breed in the first production cycle. The experimental groups were produced at densities of 0.8, 1, and 1.2 geese/m<sup>2</sup>, with three different sex ratios (1:2.5, 1:3, and 1:4) applied at each stocking density. In total, nine groups were formed. Egg production and candling data were continuously collected during production. Every two weeks, the success of sperm transport was assessed in 10 eggs per group (a total of 630 eggs) by determining OPVL sperm counts. Additionally, using ELISA, we determined the average corticosterone values from fecal samples of each group. During the 14-week production period, egg production in all groups fell below the breed standard (19.1-29.6 vs. 40 eggs/layer), but no significant differences were found among the groups. Through candling, the average fertility ranged from 67% to 91%, with the lowest observed at 0.8 geese/m<sup>2</sup> with a 1:3 sex ratio, and the highest at 1.2 geese/m<sup>2</sup> with a 1:2.5 sex ratio. The quantity of OPVL sperm in eggs was lowest in the 0.8 geese/m<sup>2</sup> with a 1:3 sex ratio group. The number of sperm in eggs decreased only in three groups as production progressed (1 geese/m<sup>2</sup>, 1:4 and 1:2.5; 0.8 geese/m<sup>2</sup>, 1:3), while it remained at the same level in others. According to fecal corticosterone values, even the highest stocking density (1.2 geese/m<sup>2</sup>) did not increase stress in geese, and no correlation was found between corticosterone values and production parameters. Overall, egg production in groups kept in closed systems with different stocking densities and sex ratios fell below the expected values for the breed. However, fertility compensated for lower egg production in several cases. No significant differences were found in production parameters among the groups, but the expected number of day-old goslings per layer was highest at 1.2 geese/m<sup>2</sup> with a 1:2.5 sex ratio. Our results provide a good basis for further investigation of other elements in indoor keeping systems.

Keywords: geese, stocking density, sex ratio

## **GWAS for FCR and breast meat yield traits in Pekin ducks**

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Genome wide association studies are only recently used in ducks (Liu et al, 2021). As an example, 1116 Orvia Pekin ducks (males and females) have been genotyped using 60k SNP Orvia array (60281) with the help of gentyane INRAE platform. Those ducks have been grown under commercial conditions. Individual feed intake, growth and slaughter performances have been measured. AxAS, Plink and R software have been used to perform the entire process of GWAS. The following filtering criteria have been applied: individual with more than 10% missing data, SNP with more than 10% of missing data and with MAF below 0.05 have been removed. Then the missing SNP have been imputed and the GWAS analyse using a mixed linear model based on 2 traits: FCR and meat yield have been performed. Based on the Bonferroni threshold 9 significant SNP have a major effect on breast meat yield. They are located on chromosome 1, 2, 4, 8 and 10 with respectively 3, 3, 1, 1 and 1 SNPs. Similarly, 6 significant SNP have major effect on FCR. They are on chromosomes 1, 2 and 10 with respectively 3, 2 and 1 SNPs. All genes that have significant impact on FCR had also impact on breast meat yield. FCR is a complex trait. It is influenced by feed intake behaviour, digestion and absorption capacity and meat production. Breast meat yield is also complex. Indeed, it is linked to FCR and involves the metabolic pathways that promotes breast meat production instead of fat deposition for example. From NCBI (<https://www.ncbi.nlm.nih.gov>), the duck genome (ZJU1.0) data base was searched for potential candidate genes, FGF13 on chr 10 and PHACTR1 on chromosome 2 were identified as having the potential to be responsible for the GWAS result observed. FGF13 is part of the fibroblast growth factor family and is involved in a variety of biological processes like cell growth and tissue repair. PHACTR1 is known as playing a role in cells motility and vascular genesis, which are logical regarding the traits studied. This study identifies genomic regions that affect traits under selection. Using such information could improve duck broiler breeding programs.

Keywords: Duck; GWAS; FCR; breast meat yield

## **Decoding selective sweeps associated with tropical adaptability in Guinea fowl through whole-genome sequencing**

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The helmeted Guinea fowl is a domesticated poultry species, plays a crucial role in agriculture due to its low-input requirements, remarkable adaptability and strong resistance to diseases. In Guinea fowl, the investigation into the genetic basis of adaptive evolution remains largely unexplored, thereby presenting a significant opportunity for advancing the identification and exploration of genes linked to adaptability. This is the first work in Indian Guinea fowl population which is expected to have been selected differently from guinea fowl populations in other parts of world (Vignal et al., 2019). The aim was to identify potential genomic selective sweeps linked with thermo-tolerance in the course of this study. Six individuals of the Pearl variety of Guinea fowl were selected and blood was aseptically collected. Isolation of Genomic DNA was carried out and the integrity and quantity were assessed. Subsequently, samples underwent whole-genome sequencing (WGS), and next-generation sequencing (NGS) libraries were constructed for sequencing on the Illumina platform. Quality checks for sequence reads, data pre-processing, mapping, variant calling and annotation were performed using appropriate bioinformatics tools. Fixation index test (FST) statistics was employed to explore the selective sweep regions. Several genes viz. HSP90AB1, HSPA12A, CACNA1B, KIT, RAD, RAF1, MAP2K1, PACRG, MITF, BOK, ARPP21, ARHGAP1, MLH1, and MTF were found to be potential thermo-tolerant candidate selective sweeps. These putatively selected genes were significantly enriched for diverse biological pathways and molecular functions. In addition, these functional candidate genes are significantly involved in different signaling pathways which are potential candidates underlying physiological adaptation to heat stress in Guinea fowls to radiation. The genetic mechanism facilitating adaptation to hot environmental conditions may be evolved through the influence of these candidate genes, serving as functional targets within the genomic framework. Our strategic findings provide crucial insights into distinctive candidate genes that could serve as the foundation for future molecular breeding focused on achieving rapid and sustainable improvements in thermo-tolerance and disease resistance of bird.

**Keywords:** DNA; WGS; NGS; Illumina; SNP; Gene

## **Molecular characterization of Chicken B cell marker (ChB6) in native chicken of Poonch region from international borders of India and Pakistan**

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Present study was undertaken with the main objectives of molecular characterization of ChB6 gene in local native chicken of Poonch region located at international borders between India and Pakistan. The chicken B-cell marker (ChB6) gene has been proposed as a candidate gene in regulating B-cell development. Material and Method: RNA was isolated by Blood RNA Purification Kit (HiPura) and Trizol method from whole blood samples. Positive PCR products with size 1110 bp were selected for further purification, sequencing and analysis. The amplified PCR product was sequenced by Sangers dideoxy chain termination method. The obtained sequence of ChB6 gene of Poonchi chicken were compared by MEGAX software. BioEdit software was used to construct phylogenetic tree and Neighbor Joining method was used to infer evolutionary history. In order to compute evolutionary distance Maximum Composite Likelihood method was used. Results: The positively amplified samples of ChB6 genes were then subjected to Sanger sequencing with “Primer Walking. The sequences were then analyzed using MEGA X and BioEdit software. The sequence results were compared with other reported sequence from different breed of chicken and with other species obtained from the NCBI (National Center for Biotechnology Information). ClustalW method using MEGA X software was used for multiple sequence alignment. Sequencing results showed variations between different species. It was observed that AT content were higher then GC content for ChB6 gene. The lower AT content suggests less thermostable. It was observed that there was no sequence difference within the Poonchi population for ChB6 gene. The high homology within chicken population indicates the conservation of ChB6 gene. Conclusion: The results of immune related gene Chb6 shows between population genetic variability. Therefore, further association studies of this gene with some prevalent diseases in large population would be helpful to identify disease resistant/ susceptible genotypes in the indigenous chicken population.

Keywords: : ChB6, Sequencing, ClustalW, Genetic distance, Poonchi chicken, SNP

## Novel MHC-B haplotypes in Chantecler chickens

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Major histocompatibility complex (MHC) is a highly polymorphic cluster of genes which contribute to immune response. Located on chromosome 16, the chicken MHC has great influence over disease resistance and susceptibility. Through the use of a high-density SNP panel which encompasses the MHC-B region, haplotypes can be easily identified. This study aims to use an MHC-B SNP panel to evaluate the MHC-B variability in the Chantecler breed. This breed is native to Québec, Canada, and is a dual-purpose breed known for its strong resistance to extreme cold temperatures. The Chantecler breed faced a near extinction event in the 1970s, which most likely resulted in a genetic bottleneck and loss of diversity. Despite this, SNP haplotype diversity was observed among 4 Chantecler populations. A total of 8 haplotypes were observed. Of these haplotypes, 6 were previously defined in other breeds, and the other 2 were unique to the Chantecler. Within the populations, the number of haplotypes ranged from 4 to 7, with 3 haplotypes, including the novel BSNP-Chant01, being present in all the groups. This study shows existence of reasonable diversity in the MHC-B region of the Chantecler breed and our results further contribute to understanding the variability of this region in chickens.

Keywords: Chantecler; major histocompatibility complex (MHC); SNP genotype; haplotype

## **Inclusion of eggshell thickness in a genetic improvement program in laying hens**

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Shell quality is one of the most important traits in the production of both table and hatching eggs. This is related to the need to take this trait into account in the genetic improvement programs of laying hen populations. The aim of the study was to estimate the genetic variability of eggshell thickness as well as genetic and environmental correlations with other production traits including to routine performance evaluation. The research covered eggs of 807 laying hens (currently the study is extended to subsequent lines and generations) from a breeding farm. This trait has not been selected for so far on this pedigree farm. Egg shell thickness was measured using the ultrasonic method (ESTG-2), with the simultaneous use of a desktop application enabling the registration of measurement results in the SQL Server 2019 database. Genetic and environmental (co)variances were estimated by the use of the REML method under a six-trait animal model. Estimated heritability of average eggshell thickness [AET] is 0.294. Positive genetic correlations were estimated between AET and body weight [BW], and average egg weight [AEW] and age at first egg [AFE] whereas negative correlations were estimated for initial egg production [IEP] and rate of initial egg production [RIEP]. Similar values of environmental correlations were estimated only of BW and AEW. In the case of IEP, RIEP and AFE, opposite (to genetic) environmental relationships were observed. These results are currently being verified for larger number of individuals. The study is financed by the GENDROB research project of „DZIAŁANIE 16 WSPÓŁPRACA” contract 00038.DDD.6509.00101.2019.06

Keywords: Chicken, genetics, egg production.



## **Application of impedance spectroscopy for the determination of body condition and pelvic fat content in breeder broiler hen**

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Genetic selection aims to enhance productive performance in both broilers and breeders, with a focus on increased meat breast yield and reduced abdominal fat. Pullets must attain optimal body condition, including weight, fleshing, and fat deposition, by the age of photostimulation to initiate egg production. Traditionally, subjective palpation has been employed to assess abdominal fat around the pelvis bone. The objective of this study was searching a more precise measure of breast fleshing and pelvic fattening in breeders at various ages using impedance spectroscopy. In a first trial, 2160 1-day-old chicks were used, which were housed at the Trial Farm of Cobb Española, following Cobb 2020 weight and feeding standards. At 147 days, birds were photostimulated, and the BIOBEE miniaturized bioimpedance device collected electrical data for spectroscopy analysis. Measurements were taken at 21 and 24 weeks via clamp electrodes on the birds' legs. Subsequently, 72 breeders were sacrificed for live weight, breast weight, and abdominal fat recording, under animal welfare regulations (RD 1099/2099). The second trial focused on assessing abdominal fat in the pelvic area using a new BIOBEE device with improved manageability. For this trial, 7920 chicks were used and managed under identical conditions to the previous trial, and 216 breeders were sacrificed at 21 weeks, under animal welfare regulations (RD 1099/2099). The device BIOBEE directly measured pelvic fat surface, and the ratio between pelvic fat and total weight was crucial (above 1.5% or below 1%). Results from the first trial indicated variations between real and estimated data by bioimpedance. At 21 weeks, variations were 2.3%, 6.5%, and 7.5% in breast weight, pelvic fat weight, and live weight, respectively, and at 24 weeks, the variations were 6.8%, 0.04%, and 3.4%, respectively. In the second trial, 87.5% of samples coincided in range when comparing in situ fat measurement and bioimpedance. About 73.15% of the total sample showed a  $\Delta$  of  $\pm 0.75\%$  and coincided in range. Results indicate that miniaturized bioimpedance provides objective and valid data for measuring bird fat levels.

Keywords: breeder hens; bioimpedance; fattening, body condition

## **Semen quality parameters of Italian chicken breeds: characterization at the molecular level by proteomics**

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Innovative proteomic studies have revealed an exceptional diversity in the protein composition of sperm, and new molecular markers have been suggested to assess sperm fertilizing ability in domestic mammals, whereas very few studies are available in birds. The aim of the present study was to assess sperm proteomic profile and semen quality parameters to characterize between breed variability in chickens. Semen quality parameters and proteomic profile were assessed in the following Italian chicken breeds: Mericanel della Brianza (n=4), Bionda Piemontese (n=9), Bianca di Saluzzo (n=9), Pepoi (n=10) and Robusta Maculata (n=5). Semen was routinely collected twice weekly and the following quality parameters recorded: volume (mL), concentration (bill/mL), viability (%), total motility (TM, %), progressive motility (PM, %), VCL ( $\mu\text{m/s}$ ), VSL ( $\mu\text{m/s}$ ), VAP ( $\mu\text{m/s}$ ), LIN (VSL/VCL), STR (VSL/VAP), ALH ( $\mu\text{m}$ ), BCF (Hz) and WOB (VAP/VCL). Sperm pellets were separated by centrifugation and frozen at  $-20^{\circ}\text{C}$  until further analyses. The proteomic profile of sperm was evaluated by a shotgun label-free proteomic approach. The entire protein content was extracted from each sample, reduced, alkylated and digested with trypsin. The obtained tryptic digest mixture was subjected to nano LC-MS/MS analysis using a high-resolution mass spectrometer (Orbitrap Fusion Tribrid, Thermo Fisher Scientific). Acquired raw files were analysed by MaxQuant software (version 1.6.1.0) for protein identification and relative quantification in each dataset. The good reproducibility of the protein extraction method, the efficacy of the whole proteomics workflow, as well as the low sample variability within breed, were assessed evaluating (I) the number of identified proteins, (II) the percentage of MS/MS analyses performed, (III) the sum of the LFQ (Label Free Quantification) signal intensity values and (IV) the Pearson's coefficient values between the different datasets. This analysis allowed to choose the datasets (n=4 for each breed) to be subjected to the differential analysis, for the identification of proteins commonly and exclusively present in the different breeds. The bioinformatic analysis performed by GSEA (Gene Set Enrichment Analysis), highlights the high correlation between those proteins and sperm quality parameters above mentioned, in particular for those concerning sperm mobility.

Keywords: proteomics; nano LC-MS/MS; GSEA; sperm quality; chicken breeds

## Single Nucleotide Polymorphism related to maternal ability trait of Thai and Laos native chickens

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Thailand and the Lao People's Democratic Republic (Lao PDR) are countries known for their high genetic diversity of native chickens. Maternal ability is considered one of the crucial traits contributing to the sustainability of this genetic diversity. Therefore, this study aimed to identify the single nucleotide polymorphism (SNP) markers associated with maternal ability traits in Thai and Lao native chickens using genotyping-by-sequencing (GBS) technique. Genotyping was performed on DNA samples from 50 Thai native, Leung Hang Khao, and 5 Lao native chickens, comprising 32 Ou, 10 Black bone, 9 Horn Chou, 121 Yolk, and 41 Chae chickens were cleaved with the EcoRI and MseI enzymes, and sequenced on Illumina platform. Raw SNPs 647,463 were revealed, after quality filtering, a total of 1,484 common SNPs were retained and these data were used to carry out bioinformatic analysis. Annotation analysis revealed that 11 genes related to maternal traits, broody and incubation, —PGR, PRL, PRLH, PRLHR, PRLR, VIP, VIPR1, VIPR2, GHR, GnRH-I, and LHCGR—were significantly enriched in biological processes such as positive regulation of multicellular organism growth and negative regulation of apoptotic process. Also, a significant enrichment was evident in the KEGG pathway related to neuroactive ligand-receptor interaction. The findings of the present work provide critical insights into functional molecular markers and pathways associated with maternal ability traits for poultry breeding programs to enhance efficient productivity.

Keywords: Lao native chickens; Thai native chickens; Genetic diversity; Genomic DNA; Single nucleotide polymorphism

## Comparison of the nesting behaviour of white and brown pure line laying hens

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Due to the shift in egg production from cages to cage-free housing systems for laying hens, data on nest utilisation, nest duration time and the oviposition time throughout the entire laying period is becoming increasingly important. Layer breeding programmes should take nesting behaviour into account to select birds that adapt well to cage-free environments and increase egg production in the nest. The electronic nest system (Funnel Nest Box) enables the automatic daily recording of this data from laying hens in floor housing systems. This technology provides animal-specific performance and behaviour data. The data is recorded using RFID transponders on the legs of the hens and an antenna inside the nest box. In this system, Rhode Island Red and White Leghorn pure line hens were housed in several pens with a group size of 190 hens per pen and a nest-to-hen ratio of 1:4.8. Daily data recording started at 24 weeks of age. The first 4 weeks were used for acclimatisation of the hens to the system. The aim of this study was to compare the nesting behaviour of white and brown hens regarding oviposition time and the time spent in the nest before and after the egg is laid. The peak laying activity of the White Leghorn hens was 3.5 hours after the onset of light, whereas the brown hens showed laying activity immediately after the lights were switched on. The peak laying activity of brown hens was already reached 2 hours after start of light. The results show that brown hens stay in the nest for a shorter time, with an average nest duration of 34 minutes compared to the white layers, with an average nest duration of 47 minutes. White layers remain relatively long in the nest after oviposition, around 50 % of the time spent in the nest, whereas for brown layers the nest duration time after oviposition only account for 30 % of the total time spent in the nest.

Keywords: nesting behaviour; oviposition time; nest duration time; alternative housing systems

## **Key miRNA of chicken seminal plasma extracellular vesicles related with sperm motility regulation**

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Extracellular vesicles are small vesicles containing small RNAs, proteins, and lipids, etc., with a diameter of 30-150 nm. To explore the important role of SPEVs miRNA profile in seminal plasma extracellular vesicles (SPEVs), we used computer-aided semen analysis to detect semen quality in Beijing-You chicken, and identified high sperm motility (HSM, n=8) and low sperm motility (LSM, n=8) groups. SPEVs was extracted from two groups by ultracentrifugation at 100000 g for 90 min, and confirmed by nanoparticle tracking analysis, transmission electron microscope, and western blot for EVs-specific proteins. The small RNA sequencing identified 706 known miRNAs and 300 novel miRNAs in HSM group, and 692 known miRNAs and 392 novel miRNAs in LSM. A total of 34 miRNAs were identified as differentially expressed (DE) miRNAs, of which 15 were up-regulated and 19 were down-regulated in the HSM group. GO and KEGG pathway analyses showed that the targeted genes were enriched for functions such as SNARE interactions in vesicular transport, Metabolic pathways, Apelin signaling pathway, Hippo signaling pathway, FoxO signaling pathway. This indicated that SPEVs may play a key role in spermatogenesis and maturation via these miRNAs. The DE SPEVs miRNAs were found to target 38 and 48 functionally important mRNAs in the potential recipient cells including sperm, and sperm storage tubules (SST) cells, respectively. The pathways that enriched by target mRNAs revealed that the SPEVs-coupled miRNAs may rule the fertility by affecting the sperm maturation and amino acid metabolism. When SPEVs were used as exogenous additives in fresh or frozen sperm of chickens, it improved the fertility. The knockout of gga-miR-24-3p, one of the DE miRNAs, significantly reduced the fertility. In summary, this study introduces a unique library of SPEVs-conjugated miRNAs associated with sperm motility in chickens, expanding our understanding of their potential role in sperm maturation, capacitation, storage, and fertility. It also indicated that SPEVs could be used as exogenous additives to improve fertility.

Keywords: chicken, seminal plasma, extracellular vesicles, miRNA

## Genetics of daily feed efficiency in broiler lines divergent for digestive efficiency

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Poultry diets are more diversified and less optimized compared to the past. While digestive efficiency was not a significant concern for feed conversion ratio (FCR) in the past as optimized diets were easy to digest, it has thus gained greater importance in the current. Therefore, selection for a better FCR and digestive efficiency is one of the main challenges to decrease poultry production costs and reduce its environmental impact. Recent developments of electronic feed stations now enable to collect feed intake of animals reared on floor and in group, i.e. in conditions similar to production conditions. Feed intake and efficiency data collected by this way are thus more reliable than data collected in individual cages and more precise as they provide daily data. The present study aimed to estimate the genetic parameters of traits related to feed efficiency in two lines divergently selected for their digestive efficiency in order to find the best strategies of selection of feed efficiency fed with suboptimal diets in chicken. We used a total of 316 birds from two lines divergently selected for high (D+) or low (D-) digestive efficiency at 3 weeks. Birds were identified at hatch with a RFID chip. Each time a chicken ate, its chip was read by antennae placed on electronic feed stations, enabling 24 animals to feed at the same time. The machine gives body weight and feed intake at each visit from day 3 to day 46. Using these raw data, cumulative daily feed intake (DFI), body weight gain (BWG), and FCR were calculated for each individual broiler. Line and sex effects were estimated by an analysis of variance with the R software. We will then calculate the genetic parameters of feed and digestive efficiency traits with VCE software and an animal model. BWG differed between the two lines on most days between 8 and 33 d, but the difference was very low, D- birds showing a 2% higher weight gain than D+ birds. At the opposite, DFI and FCR differed during the whole production cycle, showing that the difference in efficiency between the 2 lines was mostly driven by DFI. Differences between lines were the highest at 5 d and between 20 and 30 d, i.e. at age at selection (21-24 d). At 5 d, D- ate 27% and had a 0.7 higher FCR than D+ birds. Between 20 and 30 d, D- ate 19% more and had a 0.26 higher FCR than D+ birds. We are currently calculating genetic parameters of feed efficiency and its components in these two lines and will present the results at the conference.

Keywords: selection; feed efficiency; digestibility

## **A crossbreeding as a proposal in a conservation program of Spanish ‘Murciana’ chicken breed**

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Spanish “Murciana” Chicken is a local endangered breed that has suffered a sharp reduction in population size due to the replacement by fast-growing hybrids. “Biodiversity” and “From Farm to Fork” strategies have become a priority for the European Green Deal. Murciana chickens are highly resistant to diseases despite environmental challenges and are well adapted to low input rearing conditions. Thus, chickens are usually reared in organic, extensive or grazing systems, ensuring high animal welfare standards. This breed is also characterized by low productivity ratios and its products are perceived as healthier and tastier. National initiatives have been undertaken to promote these local products that are certified with quality labels such as the “100% Autochthonous Breed”. Since Murciana Chicken is a slow growing breed with tasty meat, a crossbreeding with a faster growing commercial line to combine the meat characteristics of the Murciana Chicken (MC) with improved productivity traits from fast growing lines is proposed in this work. Thus, a crossbreeding between Hubbard JA757 strain cockerel with MC hen was conducted. In this context, growth, feed conversion efficiency and carcass and meat quality of parental genetic lines 100% Murciana Chicken (MC100) and 100% Hubbard JA757 (MC0), and of the crossed breed result 50% MC x 50% Hubbard JA757 (MC50) were analyzed. In order to analyze their growth performance, animals were weighted regularly by calculating the average daily gain (ADG) from hatching to 49 days old. At this final age, MC100, MC50 and MC0 were weighed, averaging 762±50, 1267±43 and 3401±41 g, respectively, and showing ADG<sub>0-49</sub> values of 14.6±1.02, 25.0±0.89 and 68.6±0.83 g/day, respectively. Concerning carcass quality, MC50 was more similar to MC100 than to MC0 performance, showing non-significant differences for carcass yield percentages between them, albeit exhibiting higher breast than MC100 (13.9±0.51%, 17.8±0.3% and 28.4±0.38% for MC100, MC50 and MC0, respectively) but with lower ‘thigh + drumstick’ values (31.3±0.41%, 30.3±0.25% and 26.4±0.32% for MC100, MC50 and MC0, respectively). The findings of this study identify crossbred animals as a commercially viable option that enhances the productive indices of the Murciana Chicken, while concurrently supporting the breed conservation. This research is being extended to include an analysis of meat quality traits and the assessment of animal welfare.

Keywords: local breeds; biodiversity; sustainability; poultry farming; productivity traits

## Detection of male gametes from successful primordial germ cell implantation in a sterile interspecies hybrid

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In the case of avian species, it is difficult to preserve the female genome by cryopreservation, as the egg, and therefore the embryo, cannot be deep-frozen. Therefore, our studies have focused on primordial germ cells, which can be used to solve this problem. In our experiments, we performed several tests on crossbred guinea fowl and domestic fowl hybrids to assess their suitability to receive primordial germ cells (PGCs). Our preliminary results were encouraging and we subsequently injected GFP-expressing PG cells into the bloodstream of 3-day-old hybrid embryos to monitor the implantations. The GFP-expressing PGC lines were isolated from transgenic White Leghorn chicken embryos. The injected eggs were incubated at 37.8 °C with 70% relative humidity. Of the 156 hybrid embryos injected, twelve chickens died immediately before hatching (perinatal death) and five hatched. Two hybrids reached sexual maturity. Comparing the speed of testicular development of hybrids with that of domestic fowl, it can be seen that the maturation of hybrids is much slower. This could be due to the deficiency of hormonal regulation. Histopathological examination of the gonads confirmed that both injected hybrids were chimeric, i.e. the injected PG cells were incorporated into the gonads. The testes of the two mature hybrids were heavily colonised by the implanted GFP-labelled PGC cells. In one of the specimens, spermatogonia, spermatocytes and mature spermatozoa at different stages of maturation were observed in the testes. The other individual was 4 weeks younger, so mature spermatozoa were not yet observed, but testicular tissue was demonstrably colonised by the implanted cells. The control individuals were found to be sterile. No gametes or progenitor cells were observed in these. Based on our studies, it is likely that hybrid combinations of some species might be suitable hosts for the capture of stem cells and thus the production of mature gametes. The research was supported by the European Union Horizon 2020 project 677353, VEKOP-16-2017-00008 and the European Social Fund.

Keywords: sterile hybrid; Guinea fowl; domestic fowl; sperm cells; PGCs; gonad development



## **Growth performance of male layer-type chickens reared at different stocking density**

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Two experiments with male layer-type chickens of the Lohmann Brown Classic breed was carried out at the Institute of Animal Science-Kostinbrod, Bulgaria, aiming to investigate the growth performance of the birds when reared at different stocking densities. The experiments were carried out in 2022 and 2023. In both trials the birds were reared in a controlled microclimate, with an initial stocking density of 22 birds/m<sup>2</sup> for the first trial and 9 birds/m<sup>2</sup> for the second one. At five weeks of age, fragmentation of the stocking density was applied, decreasing the number to seven birds/m<sup>2</sup> and 3 birds/m<sup>2</sup> for the first and second experiment respectively. Chickens were slaughtered at five and nine weeks of age. The dynamics of changes in the growth performance traits of the birds showed that the live weight of the male layer type chickens remained higher in the birds reared at lower density. On the other hand, the weight gain was higher in the chickens reared at lower density until the end of the 5th week and from 6th week the values of this trait decreased in these birds. The feed intake was higher in the chickens reared at lower density for the first two weeks, however on the third week of age its values increased ( $P < 0.0001$ ) in the birds from the first trial and remained higher until 6 weeks. Significantly decreased feed intake was also noted on the 8th weeks old chickens reared at lower density. The chickens reared at lower density displayed significantly lower values of the feed conversion ratio ( $P < 0.0001$ ) until 5 weeks of age, however after 7 weeks the FCR values increased in these birds. When assessing the effect of the stocking density and age on the performance traits of the male layer type chickens we observed significant interaction of the two factors in regard to live weight ( $P < 0.0001$ ), body weight gain ( $P = 0.0023$ ) and FCR ( $P < 0.0001$ ). Feed intake was affected by both factors being higher in the older birds ( $P < 0.0001$ ) and in those reared at higher density ( $P = 0.0011$ ). Acknowledgement: The study was supported by the Bulgarian National Science Fund, Ministry of Education and Science in Bulgaria (Project INOVAMESPRO, Contract No KP-06-N56/10, 12 November 2021).

Keywords: male layer-type chickens; stocking density; growth performance

## **What emergency killing methods should be used for poultry weighing less than or more than 5kg?**

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On any poultry farm, the presence of sick or injured animals is inevitable, despite the quality of care provided by the farmer. When recovery or pain management is not possible, on-farm killing may be necessary, while respecting animal welfare. The aim of this study is to evaluate the different methods of individual killing available, in terms of animal protection. In order to cover the range of weights found on commercial and breeder farms, tests were carried out on male and female broilers (1.6 kg), female turkeys (> 7 kg) and male fattened ducks (3.5 kg). For each species, we evaluated, on a small number of animals (around ten), the use of a non-penetrative captive bolt (TED gun), mechanical cervical dislocation (dislocation tweezers) alone (on poultry weighing less than 5 kg so only on broiler chicken and ducks), manual cervical dislocation (on chickens only) and, finally, only head electrical stunning followed by mechanical cervical dislocation. Evaluation of the presence of corneo palpebral reflexes (CPR) indicated the birds' state of consciousness (a positive CPR means that the animal is conscious and that stunning has failed). With the non-penetrative Captive bolt (TED gun), it was not possible to assess the presence and persistence of CPR in chickens. In other species, the gun appears to be effective, with a failure rate (CPR present) of 1/9 and 3/19 for turkeys and ducks respectively, probably due to poor positioning of the gun on the skull. Even when unconscious, the animals could take more than 2 minutes to die. However, all the animals died following the application of this method. The use of mechanical cervical dislocation alone is not an option, as it does not provide good stunning in the birds tested (all birds had a persistent CPR lasting several minutes -no loss of consciousness - and died after more than 2 minutes following application of the method). For birds weighing less than 3 kg, manual cervical elongation remains an effective method but requires skill in the technique. In fact, 3 out of eight chickens lost their CPR 30 seconds after application of the manual cervical elongation method, and the others showed no reflex after this application, then all the chickens died without regaining consciousness. The combination of only head electrical stunning followed by mechanical cervical dislocation is a satisfactory approach, provided that the current delivered is sufficiently high and the time of exposure to the electric current is sufficiently long. Indeed, in our study, the reappearance of CPR was observed in 2/8 of chickens, 10/10 of turkeys and 8/10 of ducks, which suggests that the intensity delivered was not sufficient.

Keywords: Emergency killing; Animal welfare; Duck, Turkey; Chicken

## **Weight lifting hens? The impact of sensor weight on the movements of aviary housed hens**

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The validity of behavioral data acquired using hen-mounted sensors relies on the assumption that the birds' behaviors are not altered by presence of the sensor. It is often presumed that sensors weighing less than 5% of the animal's body weight do not significantly impact its behavior. This assumption has not been examined in poultry. Therefore, we tested whether the presence and weight of a hen-mounted sensor affected the frequency of vertical movements made by aviary-housed hens. Dekalb White hens were housed in previously established groups across six multi-tiered aviary pens. One to two hens per pen were assigned to each of five treatment groups (10 hens/pen total): four sensor weight categories ranging from 3.3 to 7 % of the hen's body weight, and a no-sensor control. None of the hens had previously been fitted with sensors. All sensors were sized matched. The frequency of each hen's movements up and down the aviary tiers was evaluated using continuous sampling (3hrs/day) at 1, 6, and 12 days after sensors were fitted, and the day after the sensors were removed (day 14). Data analysis was conducted in R using a generalized linear mixed effects model, with hen ID within pen as a random effect. With alpha set to 0.05, the frequency of movements across aviary tiers was not significantly impacted by treatment ( $p=0.08$ ), day ( $p=0.22$ ), or the interaction of treatment and day ( $p=0.33$ ). The treatment trend reflects that, on average, control hens made numerically more transitions than hens wearing backpacks, regardless of backpack weight. Although sensor weight, including sensors weighing over 5% of the hen's body weight, did not impact transitions in this study, more research is needed to further explore the observed trend. Future work should explore the impact of hen-mounted sensor on a larger range of hen behaviors in light of hen strain, age, size and experience, hen housing structure, and sensor attachment method.

Keywords: PLR, sensor, laying hen, aviary, movement

## **Development of an automatic device for the administration of mother hen uropygial secretion analogue in hatching eggs and its effect on vocalization at hatch**

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Day-old chicks are exposed to multiple stress factors from pre-hatch to housing on the farm. These stress factors can lead to welfare issues, immunodepression, and decreased productivity. The mother hen uropygial secretion analogue (MHUSA) is a synthetic analog (SecureChick®) of a maternal semiochemical secretion produced by the uropygial gland. The application of MHUSA has demonstrated its potential to reduce stress-related reactions in chicks as well as to influence their behaviour. The objectives of this study were to develop an application system to administer the pheromone in the hatchers and to study the effects of the application on the day-old chicks. The study was conducted under commercial conditions and was carried out in a hatchery located in Catalonia, Spain. To carry out the first objective of the study, three tests were performed. The first two tests focused on testing the application concept through a prototype. The third test was aimed at validating the administration of the pheromone through this application system. For the second objective the hatchery sourced fertile eggs from two distinct broiler breeder flocks of the Ross 308 variety. The trial was made in four production days from consecutive weeks, the first two days were used to apply the pheromone. In each day it was used 190,080 embryonated eggs with an embryonic development age of 19 days - 95,040 as treatment and 95,040 as control. The two days eggs were placed across Chickmaster hatchers with ID F1 (treatment) and F2 (control). To assess the effect of MHUSA during the following two days corresponding to the hatching of the chicks 66 vocalizations records were made from 6,600-day-old chicks. The statistical analysis revealed two different patterns of vocalizations between the chicks treated with the pheromone and the control chicks. The effect of the pheromone was reflected by the more frequent identification of comfort vocalizations, higher probability (0.65-0.80) in the treated group versus the control group. In conclusion, the results of the sound analysis confirmed that there is 'pheromone intake' after spraying the hatching eggs in the hatcher with effect on the day of hatching. And the effect of the pheromone is reflected by the more frequent identification of comfort vocalizations in the treated group versus control group.

**Keywords:** hatching eggs; MHUSA; pheromone; stress; broiler

## **Differential effect of two different doses of organic selenium on production performance in White Leghorn layers**

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Laying period in the hen causes stress which in turn affects biological parameters. It is known that selenium (Se) a trace mineral acts like an antioxidant and further organic form is more beneficial than the inorganic form for improvement of production performance. However reports are contradictory. Reports are not available as to how the lower or higher dose affects the production performance. Hence the present experiment has been taken up to observe the differential effect of doses on different parameters in White Leghorn layers. A hundred fifty number of White Leghorn (WLH) layers were selected with average body weight of 1300g at 22 weeks of age . The study is being conducted for the laying period from 24-36 weeks of age. This tenure was again divided in to two groups i e 24-28 weeks (Early laying period) and 32-36 weeks (Mid laying period). The hens were divided in to three groups with equal number of 50 birds in each. Ten replicates with five birds in each replicate. Birds were reared from 8 weeks of age till they attained 22 weeks of age. WLH birds were reared on deep litter system till 16 weeks of age . They were provided with feed and water. Later on they were transferred to individual cages in the farm. From 24 weeks onwards basal feed based on maize and soybean @ 110g/bird/d was offered to control group (C). The basal feed contained inorganic selenium @ 0.3ppm which is routinely added at our institute and 0ppm of organic selenium. Treatment groups were offered basal feed along with 0.3ppm and 0.6 ppm of organic selenium (selenium enriched yeast) to T1 and T2 groups respectively throughout the course of the experiment. The two different doses had different effects. The lower concentration of the dose increased egg production significantly ( $P < 0.05$ ) over that of control and T2 group. The higher concentration of the dose increased body weight and transport of selenium significantly to muscles and egg. Both the concentrations had beneficial effect on the histomorphology of the jejunum (portion of digestive tract). When the birds were in the mid phase of the laying period, the higher dose increased egg production more effectively than the lower dose.

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Keywords: Egg production, Body weight, Organic selenium, White Leghorn

## **Assessment of welfare and meat quality parameters of backyard native birds reared under deep litter versus cage rearing system**

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The poultry industry has experienced rapid growth in the livestock sector globally with 621% increase in slaughter of poultry between 1961 and 2001. Chickens experience emotions such as pain and frustration that's why ethical considerations must be applied to poultry farming, to ensure the well-being of birds. Looking at poultry welfare, it entails meeting the fundamental needs of birds daily, which encompass accessible food and water, the freedom to move, stand, turn, stretch, sit, and lie down, as well as visual contact. At global level lot of work & discussion regarding cage vs deep litter rearing affecting bird's welfare is done. The welfare concept in India is new and very limited data is available with respect to cages vs deep litter rearing in Indian farming conditions. With this context the present is designed to study the welfare, stress and meat quality parameters of the native birds reared under deep litter versus cage system. To study this hypothesis total of 400 birds are divided equally and kept in deep litter as well as cage rearing for period of 12 weeks with 4 replicate each treatment. The standard management practices were followed and welfare as well as meat quality parameters were done at end of the experiment. The welfare parameters such as fecal score, feather score, hock burn score, and footpad dermatitis score revealed significantly ( $P < 0.05$ ) positive impact in birds under deep litter system. However, parameters such as Gait score, Runaway test, and tonic immunity did not exhibit any statistically significant differences between the treatments. Furthermore, the meat quality parameters like pH, WHC, shear force and drip loss were comparable among the different treatments. Interestingly the stress parameters (H/L ratio) also not display significant variations in both rearing system types. From our results we can confirm that in Indian rearing conditions, the cage rearing does not create stress or compromise meat quality in early stage of bird's life i.e. upto 12 weeks. Further study especially in adult birds (up to 40 week) is needed for final conclusion about cage vs deep litter in Indian farming conditions.

**Keywords:** Cage, Deep litter, desi birds, welfare

## Stakeholders' attitudes toward painfulness of keel bone damage in laying hens: an empirical study from Serbia

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Recent research revealed relatively low egg sector stakeholders' awareness of some aspects of laying hen welfare in Serbia. Particularly low is the level of knowledge about keel bone damage (KBD), which is one of the important animal-based indicators of laying hen welfare. For the unambiguously necessary improvement in the welfare of laying hens, it is important to increase the awareness and interest of key stakeholders in this issue. A precondition for this is to understand the factors that influence their attitudes. This could help the development of appropriate awareness-raising measures. There is still no scientific consensus about the extent to which KBD is painful for birds. However, it is known that an individual's sensitivity to animal welfare increases if/when animals suffer. That is why this paper aims to understand attitudes toward the painfulness of KBD and to examine whether and to what extent they are influenced by the basic demographic characteristics of respondents (gender, age, and educational level). To that end, a survey was conducted, involving 560 respondents, coming from key egg sector stakeholder groups (citizens, poultry farmers, and poultry experts/professionals). To process the data IBM SPSS Statistics 21 software was used. The chi-square test was selected to test differences in attitudes toward painfulness of KBD. The majority of respondents (74.2%) think that KBD is painful for birds. The chi-square test of independence confirmed the statistical significance of the difference in opinion of male and female respondents regarding the painfulness of KBD ( $\chi^2(1, n=535) = 12.398, p = 0.000, \text{fi} = 0.157$ ), i.e. females are more likely to think that KBD is a painful condition. No significant effect of age ( $\chi^2(4, n=537) = 7.722, p = 0.102, \text{Cramer's } V = 0.120$ ) and education level ( $\chi^2(3, n=536) = 3.977, p = 0.262, \text{Cramer's } V = 0.087$ ) on respondents' opinion about KBD painfulness was found. To provide broader support for laying hen welfare improvement in Serbia, gender-sensitive awareness-raising activities about KBD should be designed and targeted to the key stakeholders.

Keywords: laying hen; keel bone damage; painfulness; stakeholders; attitudes;

## **Efficacy of alliaceous extracts in modulating cytokine response under heat stress in poultry: insights from in vitro macrophage studies**

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The effects of global warming, characterized by rising temperatures, have adverse impacts on both animal welfare and the productivity of broiler chickens. Increased ambient temperatures cause thermal stress, leading to reduced feed intake, heightened metabolic demands, and compromised growth rates in poultry. To address these issues, the use of phytochemicals in feed emerges as a promising solution. This study examined the effectiveness of different concentrations of alliaceous extracts, rich in organosulfur compounds primarily derived from onions, like propyl propanethiosulfonate (PTSO), in mitigating heat stress-induced cytokine production in poultry. Using the RAW 264.7 murine macrophage cell line, the cells were subjected to a 42°C environment to mimic heat stress conditions, with LPS induction as a model for inflammatory response. Three concentrations of alliaceous extracts—1 µg/mL, 5 µg/mL, and 10 µg/mL—were tested. Cytokine production levels were measured by enzyme-linked immunosorbent assay (ELISA) utilizing specific kits. A negative control (without LPS induction) and a positive control (induced with LPS) were also included. Results showed that while the lowest concentration (1 µg/mL) didn't significantly affect cytokine levels, higher concentrations (5 µg/mL and 10 µg/mL) significantly reduced pro-inflammatory cytokines. At 5 µg/mL, TNF-α and IL-6 levels decreased by 20% and 25%, respectively, and at 10 µg/mL, these reductions were 35% and 45%. Notably, IL-1β exhibited a significant decrease only at the highest concentration, showing a 30% reduction. These findings suggest a potential mechanism involving membrane receptor activation and subsequent signaling pathways influencing cytokine transcription. In summary, pending further in vivo trials, employing alliaceous extracts rich in organosulfur compounds appears as a potential strategy to alleviate the negative impacts of heat stress in the poultry production sector.

Keywords: Allium; heat stress; inflammatory; phytochemicals; cell culture



## **Early characterization of behavioural reactivity in two Italian chicken breeds: a preliminary study**

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Behavioural plasticity plays a pivotal role defining chicken breeds' coping ability. The present study focuses on fear responses and their antipredator significance in two chicken breeds particularly adapted to extensive en plein air rearing conditions. A total of 42 7 days old chicks of the following breeds were tested: Milanino (n=21, MIL), Mericanel della Brianza (n=21, MRB). Chicks were simultaneously hatched, reared in a warm controlled room (3 pens/breed; 1 sqm/pen) and fed ad libitum a standard starter diet and fresh water. Tonic Immobility (TI) and Emergence Test (ET) were applied. Bird's live weight (LW), number of induction (N, max 3; TINI), TI duration (s, max 180 s, TIDU), number of vocalization (n, TIVO), vocalization rate (N/s, vocalization/TI duration, TIVR) in TI, head emergence out of the box latency (s, ETHE), first step out of the box latency (s, ETFS), bird's complete emergence out of the box latency (s, ETCE), number of vocalization (n, ETVO), defecation (n, ETDE) in ET (max latency time 180s). Data have been analysed using GLM procedure of SAS® 9.4 software to assess the breed effect; Student's t-test was used to compare LSMeans. Significant differences were described in LW: MIL=63.95 (SE 1.25); MRB=39.70 (SE 1.25), in TIVO: MIL=5.20 (SE 1.04); MRB=39.70 (SE 1.04) and TIVR: MIL=0.30 (SE 0.05); MRB=0.00 (SE 0.05). No significant differences were recorded in ET parameters. However, ETVO was higher in MIL (38.80 vs. 17.95). High intensity vocalizations are considered an antipredator response in domestic chickens. The breed specific differences found at early age in intensity vocalization, considered an antipredator response, suggest the uniqueness of the behavioural repertoire of heritage chicken breeds at every life stage. A standardized comprehensive description of behavioural breed/population reactivity is suggested to complete the assessments of distinctive traits used for phenotypic characterization in biodiversity conservation program and promotion of local breeds for productive purposes.

Keywords: chickens; breeds; behaviour; vocalization

## **Measuring the chronic metabolic demands of keel bone fractures in brown-feathered end-of-lay hens**

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Keel bone fractures (KBF) are an epidemic among the laying hen population and affect up to 90% of hens by the time they reach end-of-lay. Much of the current body of research on KBF focuses on its implications on younger, peak-lay hens rather than end-of-lay hens. However, the landscape of egg farming is shifting, and many countries keep their laying hens for longer than 70 weeks of age, making research into KBF in the end-of-lay greatly important. These fractures heal by the time hens reach the end-of-lay. Still, the formation of a hard callus during the healing process and behaviour modifications stemming from the fracture's presence may be impactful on production and welfare. To investigate how KBF impacts brown-feathered end-of-lay hens, we used doubly-labelled water to measure the differences in daily energy expenditure (DEE) in hens with (n = 8, 2076.5 ± 83.75 g) and without KBF (n = 8, 2006.3 ± 57.06 g) aged 92 weeks of age. Additionally, we used open-flow mask respirometry to determine differences in the metabolic rate of brown-feathered hens at the end-of-lay with and without KBF when performing running and jumping activities (KBF n = 19, 2144.63 ± 172.17 g; no KBF n = 20, 2117.74 ± 172.84 g) aged 71 weeks of age. The DEE of hens with and without KBF did not differ significantly between groups; however, the allometric slopes of hens with KBF (0.99) and without KBF (-1.14) did differ significantly (p = 0.0309). The metabolic rate (open-flow respirometry) of hens with and without KBF did not differ when running or jumping. The duration for a hen with and without KBF to complete the running and jumping activities was not different; however, there was a 43% difference between the jumping means of the two groups, and the metabolic rates in hens with KBF visually showed greater variance than in hens without KBF. This research lays the groundwork to investigate the potentially chronic effects of KBF and is the first to determine some associated factors for differing metabolism in end-of-lay hens with and without KBF.

Keywords: bone fracture; metabolism; doubly-labelled water; DLW; respirometry; chicken

## **Occurrence of feather damage, skin injuries, and keel bone deviations in dual-purpose hens in organic farming**

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In Germany, the killing of male day-old chicks from laying hen lines has been prohibited since 2022. As alternatives, options such as in-ovo sex determination, the rearing of male laying hybrids, and the utilization of dual-purpose chickens are being considered. The aim of the study was to identify potential differences in the integumentary condition among dual-purpose poultry breeds during the laying period (weeks 21-72). The investigation encompasses three dual-purpose breeds: Triesdorfer Landhuhn (TLH), a hybrid incorporating Bresse Gauloise, Italian, Rohde Island red, Sulmtaler, and Sundheimer; Coffee (COF), a crossbreed involving a Bresse Gauloise rooster and a New Hampshire hen, and a crossbreed of Augsburg (an ancient local German breed) rooster with a Lohmann Brown-Classic hen (AxLB). The high-performing hybrid Lohmann Sandy (LSa) serves as the fourth origin, acting as the control group. The hens were kept in a fixed stable with an outdoor climate area and were provided with organic feed. For AxLB, COF, and TLH, five compartments each (142 hens/origin), and for LSa, four compartments (104 hens) were investigated. All present animals were examined from the 20th week of age at 10-week intervals up to the 70th week of age for the following integumentary features using a standardized schema: feather loss (back, belly, dorsal neck), skin injuries, toe injuries, footpad swellings, and palpable deviations from the midline of the keel bone. A multivariate analysis was performed using binary logistic regression with the effects of age and origin. Toe injuries were not observed in any of the origins at any examination point. The regression models indicated an influence of origin on feather condition, skin injuries, and footpad swellings (each  $p < 0.001$ ) with consistently present changes associated with age ( $p < 0.001$ ). LSa, in comparison with AxLB, exhibited the least feather loss ( $p \leq 0.012$ ) and, in comparison with all other origins, the significantly lowest prevalence of skin injuries ( $p \leq 0.029$ ). Footpad swellings were higher in TLH and LSa than in COF, which, in turn, exhibited more changes than AxLB ( $p \leq 0.043$ ). Origin had no effect on keel bone deviations ( $p = 0.397$ ). The results indicate that there are no general advantages of the examined dual-purpose chickens over the evaluated high-performance hybrid in the animal welfare-associated features of feathers, skin, footpads, and keel bone.

Keywords: dual-purpose hen; organic farming; feather damage; skin injuries; keel bone

## **The effect of crossbreeding of local chicken breeds with commercial parental lines on animal welfare**

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With the ban on killing day-old male layer-type chicks in Germany, there is a high demand for finding an alternative approach that is both animal-friendly and economically viable, especially in the context of organic poultry farming. Using crosses of commercial parental lines and local chicken breeds seems to be a promising approach by combining performance parameters of commercial lines with positive welfare parameters of local chicken breeds, and at the same time contributing to the conservation of genetic diversity through their use. Here, we examined the effect of crossbreeding of males of two local chicken breeds (Bielefelder BIE and Malines M) with females of layer- (White Rock WR, Lohmann Breeders) and meat-type commercial parental lines (Ranger RG, Aviagen) on several parameters related to animal welfare in the crossbreds (BIE<sub>x</sub>WR, BIE<sub>x</sub>RG, M<sub>x</sub>WR, M<sub>x</sub>RG) and their corresponding purebred parental breeds (BIE, M). All animals (BIE: n = 128, M: n = 118, BIE<sub>x</sub>WR: n = 66, BIE<sub>x</sub>RG: n = 50, M<sub>x</sub>WR: n = 26, M<sub>x</sub>RG: n = 61) were kept in a floor housing system under conditions in compliance with organic farming. We assessed welfare parameters in a bi-weekly rhythm on 15 randomly chosen hens per genetic and per assessment from 2 to 36 weeks of life as well as at the end of the production period in week 72. Specifically, feather scores (head/neck, back, cloacal region), beak length, comb injuries, foot pad health and keel bone health were investigated according to the Welfare Quality® Assessment Protocol for Poultry. Both BIE-crossbreds (BIE<sub>x</sub>WR: 2 % score 1 and 2; BIE<sub>x</sub>RG: 5 % score 1 and 2) showed significantly better feather scores for the cloacal region than the purebred BIE (8 % score 1 and 2;  $p < 0.001$ ). Both meat-type crossbreds (BIE<sub>x</sub>RG: 36.5 % score 1 and 2; M<sub>x</sub>RG: 19 % score 1 and 2) had a higher prevalence for foot pad lesions than the layer-type crossbreds (BIE<sub>x</sub>WR: 12 %; M<sub>x</sub>WR: 5 %) or the purebred breeds (BIE: 7 %; M: 3 %;  $p < 0.001$ ). Further, keel bone condition was significantly better in BIE<sub>x</sub>WR (6 % keel bone damages) than in BIE<sub>x</sub>RG (16 %) and BIE (10 %,  $p < 0.001$ ). These results indicate that the combination of local chicken breeds with commercial parental lines can improve some of the welfare parameters in the crossbreds compared to the local chicken breeds, but other parameters need to be given a special attention, e.g., foot pad health. Overall, crossbreeding seems to be a promising approach for organic poultry production.

**Keywords:** chicken; welfare; crossbred; local chicken breed

## **Judgement bias test in dual-purpose chickens exposed to different environments**

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There is an increasing interest in dual-purpose chickens in poultry production. However, how environmental enrichment affects different affective states of dual-purpose chickens is still unknown. This study investigates the impact of enrichment material interchanged on a regular base on the affective state of chickens in a modified judgement bias test. This test queries optimistic or pessimistic responses to ambiguous stimuli. In total, 24 layer-type crossbreds (local breeds Altsteirer, Bielefelder and Ramelsloher x commercial parental layer line White Rock, Lohmann Breeders) were observed simultaneously in pairs. These social pairs — trained on visual and local stimuli — were tested with near-positive, neutral, and near-negative stimuli cues of gradual opposite colour and location to elicit optimistic or pessimistic responses. Preliminary results suggest that the chickens were able to successfully discriminate between cues ( $p = 0.001$ ). The results also show that judgement bias between layer-type crossbreds raised in enriched and non-enriched environments tend to differ ( $p = 0.078$ ). For example, chickens from the enriched environments showed a longer latency to approach the near-negative cue than those from non-enriched environments, indicating a more pessimistic or at least a different affective state. The outcome of other standardized testing procedures with these animals such as novel object test, tonic immobility, and attention bias test will be set in context and discussed. Our study provides valuable insights into the welfare of layer-type crossbreds, highlighting the role of environmental conditions in judgement bias and other welfare assessments. Understanding how chickens perceive and respond to their environment is crucial for optimising their husbandry and production system.

Keywords: poultry welfare; environmental enrichment; husbandry system

## **Field study on the behaviour, feed consumption and performance of laying hens in an aviary system**

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Following the banning of conventional cages, aviary systems have developed in Europe to a significant extent. Today, there is limited data available to understand the behavioural adaptation of laying hens to these three-dimensional systems. The aim of the study is to analyse the behaviour of 40,000 24-week-old Babcock hens in the Volito® Vivo aviary over a 24-hour period. This includes their social interactions and feed consumption. Six cameras were installed to record behavioural data and the physical condition of 120 birds was assessed. Eight feed samples were collected from each of the two feed chains over a 24-hour period and analysed for particle size, starch, protein and calcium content. Animal data were recorded. Performance is in line with the standards of the Babcock strain. The body weight (BW) of the hens is significantly lower by 141 g ( $p<0.05$ ) on the top floor compared to the first floor. Keel bone condition differs between the floors ( $p=0.07$ ), particularly with a 36.7% increase in deviations for hens on the top floor compared to the first floor. Total feed consumption is higher in the lower feed chain. In addition, a particle size selection is observed, with a preference for particles larger than 2 mm, resulting in different nutrient intakes for different chains and at different times of the day. The results indicate that (i) starch is consumed predominantly throughout the day, (ii) calcium is consumed more from 3 pm to 9 am and (iii) protein is consumed more from 10 am to 5 pm, with differences between floors. There are significantly more eggs laid outside the nest on the upper floors ( $p<0.01$ ). Hypotheses about dominance relationships and their effects on the spatial distribution of animals can be proposed based on BW differences, damage to the keel bone, distribution of eggs laid outside the nest and competition in the feed chain. This observational study therefore allows to hypothesize about the feeding trends of laying hens in an aviary system and the effect on the health and social interactions of the animals. To confirm these hypotheses, replications would have to be carried out and the study would have to be conducted in different systems.

**Keywords:** Aviary system ; Laying hens ; Behaviour ; Social interactions ; Feed consumption

## **Investigation of the use of different environmental enrichment elements in the case of different hens genotypes (preliminary results)**

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In our preliminary study, we aimed to investigate the use of environmental enrichments of different hen genotypes, thereby helping practitioners with our experience in selecting the ideal environmental enrichment used in alternative housing. The study was carried out at the Kaposvár Campus of the Hungarian University of Agricultural and Life Sciences with a total of 13 hen genotypes (17-21 wks. old): (N=131). Layer genotypes: TETRA SUPER HARCO parent stock (p.s.); TETRA-L SUPERB p.s.; TETRA-SL LL p.s.; TETRA-SL LL commercial hybrid (c.h.), Meat type genotypes: TETRA-HB COLOR p.s.; ROSS 308 p.s.; ROSS 308 (c.h.). Ancestral Hungarian dual-purpose 6 genotypes. The flock was observed after a 5 day adaptation period, the study lasted for one week. The animals were housed in 4 m<sup>2</sup> (2x2 m) volieres. Five different environmental enrichments were suspended up to the animals' back height: Pumpkin (PK); Red 'Jonathan' apple (RA); Corn cob (CC); Green 'Mutsu' apple (GA); Mixed meadow hay (H). Our results show that laying hens frequently consumed the environmental enrichments except for the corn cob: Bábolna HARCO p. s.: (PK: 6.3 g/day/hen; RA: 3.0 g/day/hen; CC: 0.1 g/day/hen; GA: 3.3 g/day/hen; H: 9.0 g/day/hen); TETRA-L SUPERB p.s.: (PK: 3.0 g/day/hen; RA: 7.7 g/day/hen; CC: 0.2 g/day/hen; GA: 0.7 g/day/hen; H: 3.2 g/day/hen); TETRA-SL LL p.s.: (PK: 2.4 g/day/hen; RA: 1.9 g/day/hen; CC: 0.1 g/day/hen; GA: 1.7 g/day/hen; H: 3.7 g/day/hen); TETRA-SL LL c.h.: (PK: 2.0 g/day/hen; RA: 7.4 g/day/hen; CC: 0.1 g/day/hen; GA: 3.2 g/day/hen; H: 4.9 g/day/hen). The meat-type parent pairs preferred hay, followed by green and red apples in similar proportions. TETRA-HB COLOR p.s.: (PK: 3.1 g/day/hen; RA: 3.6 g/day/hen; CC: 0.1 g/day/hen; GA: 3.5 g/day/hen; H: 10.0 g/day/hen); ROSS 308 p.s.: (PK: 2.2 g/day/hen; RA: 4.4 g/day/hen; CC: 0.1 g/day/hen; GA: 3.7 g/day/hen; H: 7.6 g/day/hen). Overall, ROSS 308 c.h. used relatively low rates of each of these environmental enrichments: (PK: 2.8 g/day/hen; RA: 0.7 g/day/hen; CC: 0.1 g/day/hen; GA: 0.7 g/day/hen; H: 2.2 g/day/hen). On average, native Hungarian dual-purpose genotypes consumed high overall levels of all environmental enrichment elements: (PK: 8.4 g/day/hen; RA: 4.6 g/day/hen; CC: 0.3 g/day/hen; GA: 3.2 g/day/hen; H: 5.6 g/day/hen). Acknowledgements: This research was „SUPPORTED BY THE ÚNKP-23-4 AND THE ÚNKP-23-3 NEW NATIONAL EXCELLENCE PROGRAM OF THE MINISTRY FOR CULTURE AND INNOVATION FROM THE SOURCE OF THE NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION FUND.” and supported by the Hungarian Government and by the "Scientific Replacement Programme" (TUEP) project, funded by the Hungarian University of Agriculture and Life Sciences.

Keywords: hen; behaviour; preference; environmental enrichment; alternative housing

## **Effect of including whole *Hermetia illucens* larvae in the diet on the stress levels in laying hens**

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Currently, there is a growing interest in reducing stress levels in production animals, including laying hens. This is potentially beneficial not only for the welfare of the animals but also for increasing their productivity. In addition to this, there is a pursuit of more sustainable production practices, and the reduction of soybean meal and imported ingredients could be a strategy to reduce the environmental footprint of the feeds. Thus, insects can be an alternative due to their high protein content. The aim of this study was to assess the influence of the effect of an experimental diet including whole dry black soldier fly larvae on the stress levels of 38-week-old laying hens. For this trial, eighty 23-week-old hens were distributed into 10 floor pens. These groups were divided into two distinct dietary treatments: a control diet comprising a standardized diet for laying hens based on corn and soybean meal, and an alternative diet supplemented with black soldier fly larvae, constituting 5% of the total feed intake. After 105 days, 2 hens per pen were randomly selected, and blood samples were collected from the brachial vein of the wing for determination of the heterophil/lymphocyte ratio. From the same animals, mature feathers were collected for corticosterone analysis in the inter-scapular area (4-6) and of the wings (2 and 8 primary feathers). The heterophil/lymphocyte ratio was determined through the examination of blood smears (Wright-Giemsa stain). The extraction of corticosterone from feathers was performed using a methanol-based method, and subsequently, the determination using an ELISA Kit. The U-Mann Whitney test was employed to assess differences between treatments. No differences were observed in the corticosterone content between hens fed with the two different diets in either of the two locations (inter-scapular ( $p=0.838$ ) or primary wing feathers ( $p=0.143$ )). Additionally, there was no difference in the heterophil-to-lymphocyte ratio of animals fed with the two different diets ( $p=0.539$ ). Therefore, it can be concluded that the supplementation of black soldier fly larvae does not negatively affect the stress levels of 38-week-old laying hens.

Keywords: Laying hen, *Hermetia illucens*, stress levels, sustainability



[P3-17]: P3- Fats (Nutrition), Welfare, Physiology, Meat Quality, Pathology and Immunology (ID: 131226) - Hungary

## **Investigation of the use of different environmental enrichment elements for various waterfowl genotypes (a preliminary study)**

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In our preliminary study, we aimed to investigate the use of environmental enrichment elements for different waterfowl genotypes. Our goal is to assist practitioners by sharing our experience in selecting the ideal environmental enrichment elements for duck and geese husbandry technologies. The study was conducted at the Kaposvár Campus of the Hungarian University of Agricultural and Life Sciences involving a total of 8 waterfowl genotypes (at different ages): (N=32, 8 genotypes, 1 male and 3 female birds/genotypes). Domesticated duck genotypes 3: WHITE HUNGARIAN DUCK parent stock (p.s.); MALLARD COLOURED HUNGARIAN DUCK p.s.; ORVIA ST5 LOURD p.s.. Geese 5 genotypes: WHITE HUNGARIAN GEESE p.s.; INTEGRÁL-MB 09 p.s.; ORVIA SI4 p.s.; ORVIA SI14 p.s.; DUNAI LÚD MAGYAR EGYES commercial. The flocks were observed after a 5 day adaptation period, and the study lasted for 7 days. On the first day, different environmental enrichment elements were introduced. The animals were housed in a 4 m<sup>2</sup> (2x2 m) voliere with wood chip litter. Throughout the experiment the birds were provided ad libitum access to feed and they had continuous access to water. Five different environmental enrichment items were suspended up to the animals' back height: Pumpkin (PK); Red apple (Jonathan type) (RA); Corncob (CC); Green apple (Mutsu) (GA) and Mixed meadow hay (H). Each morning, we measured the weight loss of the different items, from which we inferred the preference. Our results show that ducks exhibited limited interest in the environmental enrichments, except for hay and pumpkin. The consumption of the elements: WHITE HUNGARIAN DUCK (p.s.) 13.6 g/day/duck; MALLARD COLOURED HUNGARIAN DUCK p.s. 53.0 g/day/duck; ORVIA ST5 LOURD p.s. 8.5 g/day/duck hay, and WHITE HUNGARIAN DUCK (p.s.) 15.0 g/day/duck; MALLARD COLOURED HUNGARIAN DUCK p.s. 8.1 g/day/duck; ORVIA ST5 LOURD p.s. 7.0 g/day/duck pumpkin. The consumption of the other elements ranged from 0.3 to 2.0 g/day/duck. The most popular item for geese was pumpkin, followed by hay. The consumption of the elements: WHITE HUNGARIAN GEESE p.s. pumpkin: 73.6, g/day/geese, hay: 74.8 g/day/geese; INTEGRÁL-MB 09 p.s. pumpkin: 47.2, g/day/geese, hay: 12.2 g/day/geese; ORVIA SI4 p.s. pumpkin: 13.6, g/day/geese, hay: 63.8 g/day/geese; ORVIA SI14 p.s. pumpkin: 75.3, g/day/geese, hay: 61.8 g/day/geese; DUNAI LÚD MAGYAR EGYES comm. pumpkin: 10.1, g/day/geese, hay: 13.6 g/day/geese. SUPPORTED BY THE ÚNKP-23-4 AND THE ÚNKP-23-3 NEW NATIONAL EXCELLENCE PROGRAM and by the "Scientific Replacement Programme" (TUEP) project, funded by MATE.

Keywords: waterfowl; duck; geese; preference; environmental enrichment; behaviour

## **Welfare of spent hens in Flemish slaughterhouses: current situation**

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Despite the rising concern about welfare of laying hens, comprehensive animal-based monitoring systems to evaluate their welfare are rarely taken up by the industry. In this context, a pilot version of a ready-to-use welfare monitoring protocol for spent hens at the level of the slaughterhouse was developed. Data was gathered by means of questionnaires in a custom made mobile application according to the following protocol. Firstflock behavior and climate were inspected during lairage. Next, a range of animal-based indicators were scored on a random subset of 100 animals per flock after stunning. Plumage (6 body parts), skin injuries (3 zones) and pododermatitis was done on a 1 (severe damage) to 4 (perfect condition) scale. Comb injuries were scored on a 0 (no injuries)-2 point score. Keel bone fractures and deviations were detected by palpation. After defeathering, fresh fractures and bruises on wings, legs and breast were counted during 2 times 5 min inspection at the slaughter line. Lastly, death on arrivals, rejects and information on rearing conditions (e.g. housing system, egg color, flock age) were copied from the slaughterhouse documents. Between November 2021 and February 2023, 49 Flemish flocks were monitored using this protocol. For each indicator the mean score or the prevalence per flock (experimental unit) was calculated. Statistical analysis was performed using the GLM procedure in RStudio (version 2022.07.2). Values are displayed as means  $\pm$  standard error. Mean plumage scores were  $2.2 \pm 0.05$ ,  $1.9 \pm 0.08$ ,  $2.0 \pm 0.07$ ,  $2.4 \pm 0.06$ ,  $1.4 \pm 0.05$  and  $1.6 \pm 0.08$  for the neck, back, tail, wings, breast and cloaca zone, respectively. Injuries were rather rare, with mean scores ranging from  $3.8 \pm 0.02$  for the breast region to  $4.0 \pm 0.01$  for the backside. The mean prevalence of keel bone fractures ( $53.5 \pm 2.84\%$ ) and deviations ( $54.5 \pm 2.92\%$ ) was high, with a significantly lower prevalence of keel bone fractures in hens from enriched cages ( $36.7 \pm 4.45\%$ ) as compared to floor housing ( $54.5 \pm 3.74\%$ ) and aviaries ( $65.4 \pm 3.98\%$ ). Remarkable was the high prevalence of bruises at the wing tips ( $11.9 \pm 1.94\%$ ) as compared to other body parts ( $<3\%$ ). Based on these data, the final set of indicators which will be assessed at the slaughter line, has been chosen. The tool allows the egg industry or local authorities to set thresholds for each welfare indicator in order to strive for evidence-based improvements in laying hen welfare.

Keywords: laying hens; welfare; slaughterhouse

**Gene expression of two egg proteins, VMO1 and AvBD11, in the laying hen****M. Chessé<sup>1</sup>, O. Hervé<sup>1</sup>, N. Bernardet<sup>1</sup>, S. Réhault-Godbert<sup>1</sup>, J. Gautron<sup>1</sup>, T. Moreau<sup>1</sup>, N. Guyot<sup>1</sup>**<sup>1</sup>INRAE, Université de Tours, BOA, Nouzilly, FrancePresenting author: [magali.chesse@inrae.fr](mailto:magali.chesse@inrae.fr)

VMO1 (Vitelline Membrane Outer layer protein 1) and AvBD11 (Avian Beta-Defensin 11, formerly known as VMO2) are two egg proteins, particularly abundant in the proteinaceous structure surrounding the egg yolk, namely the outer vitelline membrane. Both proteins are expressed in the oviduct and secreted into the forming egg. AvBD11 is likely involved in egg immunity, while the biological function of VMO1 still remains unknown. It is thought that these proteins might also play structural roles in contributing to the integrity of the vitelline membrane. Indeed, as the vitelline membrane weakens during egg storage at 20°C, protein levels of VMO1 and AvBD11 are known to decrease in the vitelline membrane, suggesting that they are potential biomarkers of quality of the vitelline membrane. In this work, our aim was to investigate (i) the expression pattern of VMO1 and AvBD11 genes in different hen tissues and (ii) the effects of hen age on the expression profile of these genes in the oviduct. In a first experiment, gene expression was analyzed by RT-qPCR in reproductive tissues including the oviduct (infundibulum, magnum, white isthmus, uterus, vagina) and the ovary (theca, granulosa), and in non-reproductive tissues (bone marrow, skin, lung, duodenum, kidney, liver) from laying hens. Our results demonstrated that VMO1 and AvBD11 genes are mainly expressed in the oviduct, while no or very low expression is detected in the other tissues analyzed. The magnum (involved in the secretion of albumen) is the oviductal region showing the strongest expression for both genes. These genes are also expressed but at lower levels, in other parts of oviduct, namely the infundibulum (secretion of outer vitelline membrane) for VMO1 and AvBD11, and the white isthmus (eggshell membranes) for AvBD11. Our data thus suggest that VMO1 and AvBD11 are presumably two egg-specific proteins. These expression sites are consistent with the presence of these proteins in egg albumen and vitelline membrane. Considering that the quality of these two latter egg structures is altered by hen age, especially in late laying cycle, a second experiment was conducted in which tissues sampled from the upper oviduct (responsible for the secretion of albumen and vitelline membrane) were collected from laying hens at three periods of egg production (32-34, 73-75 and 99-100 weeks of age). RT-qPCR analysis of VMO1 and AvBD11 genes in these tissue samples is in progress.

Keywords: Laying hens; gene expression; RT-qPCR; VMO1; AvBD11; oviduct

## **Herbal adaptogen feed-additive modulates the hypothalamic expression of feeding-related neuropeptides in heat-stressed broilers**

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Triggered by global warming and climate change, heat stress (HS) constitutes a disquieting economic, welfare, and vital burden to the global poultry production sustainability. In the current NAE era and fueled by consumer preferences for natural products, plant extracts became a hot research spot. Here we sought to determine the effect of herbal adaptogen (stress response modifier) on growth performance in heat-stressed broilers. Day-old male Cobb 500 chicks (n=720) were randomly assigned, in environmental chambers (n=12, 24 pens), to 3 diet-treatments: a 3-phase corn-soybean based diet fed as such (Control, C), or supplemented with the herbal adaptogen at 500g/1000kg control diet (NR-PHY-500) or at 1kg/1000 kg control diet (NR-PHY-1000). From d29 to d42, birds from 9 chambers were exposed to cyclic heat stress (HS, 35°C from 9:30 am-5:30 pm), however the rest of the chambers were maintained at thermoneutral conditions (24°C, TN), which created 4 experimental groups: C-TN, C-HS, NR-PHY-500HS, and NR-PHY-1000HS (6 pens/group, 168 birds/group). Two birds per pen were randomly selected and equipped with a ThermoChron temperature logger for continuous monitoring of core body temperature. The surface temperature was measured by an Extech Flir I5 thermal imaging infrared camera on d41. Feed and water intake were measured daily, and body weights were recorded weekly. Data were analyzed by one-way ANOVA and the means were compared by Tukey HSD multiple range test using Graph Pad Prism software (version 9.5.1 for Windows, Graph Pad Software, La Jolla, CA, United States). HS altered growth performance via depression of feed intake and body weight. Adaptogen supplementation stimulated feed intake and averaged 65.95g and 83.25g better body weight and 5 and 10 points better FCR at low and high dose, respectively, compared to heat-stressed birds. This increase in body weight was mirrored in enhanced weights of body parts (breast, tender, wings, and legs). To delineate the mode of action of adaptogen on feed intake, we first measured the expression profile of hypothalamic feeding-related neuropeptides by qPCR and 2- $\Delta\Delta$ Ct method. The expression of orexigenic peptides, Orexin, AgRP, and neuropeptide Y4 receptor, was significantly upregulated by adaptogen compared to HS control. In summary, herbal adaptogen supplementation ameliorated growth performance in heat-stressed broilers by modulating the hypothalamic expression of orexigenic neuropeptides (orexin, Y4, AgRP) and stimulating feed intake. Further mechanistic studies related to the effect of herbal adaptogen on broiler muscle growth are warranted.

**Keywords:** Broilers, adaptogen, growth performances, gene expression, hypothalamus

## **Bone quality and incidence of urolithiasis in male broiler breeders fed a female or male ration**

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Separate sex feeding is practiced in breeder farms, but sometimes the males are given the female ration, rather than a specifically formulated male ration, for practical reasons, such as the requirement for another feed silo, less quantities required and the risk of mixing up the feed and feeding females the male ration. The drawback is that the female ration contains high crude protein (CP), calcium (Ca), and phosphorous (P), at levels excess to recommendations for male requirements. A total of 40 male broiler breeders fed a male-specific ration (MM) and 40 male broiler breeders fed a female ration (MF) at depletion were acquired from various KwaZulu-Natal farms. The birds were euthanised at the farms and transported to the Animal Science laboratory at Pietermaritzburg campus (University of KwaZulu-Natal) for examination. The kidneys and tibia bones were examined to assess the effects of excess protein, Ca and P in female rations given to male broiler breeders. Kidney weight and lesion scores were measured to determine urolithiasis. Bone mechanical properties (weight, thickness, and breaking strength) and bone mineral analysis (bone ash and organic matter) were quantified on some samples. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to quantify the Ca and P percentages of charred tibia bones. To compare the two variables, a two-sample t-test at 95% confidence interval was used. Pale and enlarged kidneys were observed in some MF birds but the lesion score had no significant difference ( $P > 0.05$ ) to the MM birds. Although there were no significant differences in kidney lesion scores, the kidney weight of MF birds was higher than MM ( $P < 0.05$ ). No visible uroliths were observed. Relative tibia weight was significantly lower in MF compared to MM ( $P < 0.05$ ). Bone thickness was not significantly different. The tibia bones of MF had a lower breaking strength ( $P < 0.05$ ). Tibia bone ash and organic matter showed no significant difference. Calcium and P content of tibia bone was not different. The results suggested that aspects of bone quality were poorer in male breeders fed a female ration, but that there was little evidence to show a negative effect on kidney function. For those producers still feeding a female ration, there are potential detrimental effects on kidney function and bone strength but these may not be severe enough for birds to experience problems. Therefore feeding a ration formulated to male requirements is recommended, however, there may be no detrimental affects observed if a female ration is still fed.

Keywords: kidney; calcium; feed; breeder

## **Role of novel natural nutritional emulsifier in optimizing fat utilization and energy saving in poultry diets**

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The integration of nutritional emulsifiers in diets can indeed play a pivotal role in improving the energy efficiency of fats and oils, leading to reduced feed costs and a more sustainable approach to poultry production. Considering the significant role of fats and oils as high-energy sources in feed formulation for high-performing broilers, it is essential to ensure their optimal utilization for economic benefits. By using nutritional emulsifiers to enhance fat digestibility, the overall energy efficiency of these raw materials can be improved. This improvement in energy efficiency subsequently leads to reduced feed costs, benefiting the economic aspect of animal production. An experiment was undertaken in VenCobb 430Y broiler model (0-6 weeks) to evaluate physiological effect of supplementing Emulsifier on broiler performance. Birds of control and treatment are fed with low energy ration (-2.5% ME) (pre-starter, ME: 2929 Kcal, CP: 22.52%, Oil: 1.72%), [starter, ME: 3049 Kcal, CP: 21%, Oil: 3.26%) and (finisher, ME: 3175 Kcal, CP: 19.50 %, Oil: 4.75%). Treatment group birds were supplemented with BioEmulsin DS@250g/ton supplemented to treatment group 1 (T1) throughout lifecycle of 6 weeks. A significant ( $P < 0.01$ ) improvement in zootechnical performance was recorded in BioEmulsin DS supplemented group. Mean BW at 6th week was higher by 111g, FCR was optimized and lowered by 1.9%, mortality was lowered by 1.04% Supplementing BioEmulsin DS significantly ( $P < 0.01$ ) increased serum levels of bile acids and pancreatic lipase activity at 6th week and allowed energy saving upto 60Kcal and reformulation of ration and cost saving. The use of nutritional emulsifier serves as an effective strategy for optimizing energy efficiency in poultry diets. This, in turn, not only reduces feed costs but also contributes to a more economical and sustainable model of poultry production, addressing the key concerns of the agricultural industry.

**Keywords:** nutritional emulsifier, bile secretion, fat utilization

## **Broiler chickens fed a low digestible diet improved intestinal health and growth performance when supplemented with a multi-component solution alone or combined with a protected blend of organic acids and essential oils**

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Today's scenario of high-cost raw materials has encouraged the poultry industry to use diets with low-cost ingredients, which has led to poor intestinal health and growth performance. This study aimed to investigate the dietary supplementation of a multi-component solution alone or in combination with a blend of protected organic acids and essential oils on the intestinal health and growth performance of broiler chickens fed a low digestible diet. A total of 1400 one-day-old Ross 308 male broiler chicks were assigned to receive 1 of 4 dietary treatments in a completely randomized design study for 35 d. Each treatment consisted of 14 replicates of 25 birds/replicate. Treatments: 1) Standard diet (ST: corn-soybean meal), 2) Low digestible diet (LD: corn-wheat-soybean meal-corn DDGS), 3) LD + Multi-component Solution (Bacterial origin enhancer of protein assimilation, Jefo Inc.) at 125 g/t (LD+MS), 4) LD + MS at 125 g/t + protected blend of Organic Acids + Essential Oils [P(OA+EO), Jefo Inc.] at 300 g/t (LD+MS+OA&EO). Feed and water were provided ad libitum. At the end of the study, gene expression of jejunal tight junction proteins, cecal concentration of biogenic amines and overall growth performance were determined. Data was analyzed by ANOVA using the GLM procedure of SAS and comparisons of treatment means were done using Duncan's test. Body weight gain was similar among treatments, but all groups fed LD diets had higher feed intake ( $P<0.05$ ) compared to ST. Feed conversion ratio (FCR) was higher in LD as compared with ST, but broilers fed LD+MS or LD+MS+OA&EO had a FCR similar to ST ( $P<0.05$ ). Gene expression of tight junction occludin-1 and claudin were upregulated ( $P<0.05$ ) in broilers fed LD+MS and LD+MS+OA&EO, respectively, as compared to ST and LD. High expression of tight junctions may be interpreted as better intestinal integrity. Cecal ammonia was higher in all groups fed LD diet ( $P<0.05$ ), but biogenic amines putrescin and cadaverin were lower ( $P<0.05$ ) in ST, LD+P and LD+MS+OA&EO compared to LD. Biogenic amines are toxic metabolites of protein fermentation, thus their lower concentration indicates better intestinal health. In conclusion, feeding broiler with low digestible diets negatively affect intestinal health and growth performance, but the supplementation of a multi-component solution alone or in combination with a blend of protected organic acids and essential oils can be used as a strategy to alleviate these negative effects.

Keywords: biogenic amines; essential oils; intestinal integrity; organic acids; poultry

## **Butyric and valeric glycerides alleviate sub-clinical necrotic enteritis effect on performance in broiler**

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Organic acids are readily absorbed in the upper gastrointestinal tract, which hinders their optimal delivery to segments where gut health issues mostly occur. Glycerol esters of butyric and valeric acid (BVg) are known for their capacity to release acids within the small intestine. This study aimed to elucidate the impact of these esters on broiler performance under sub-clinical Necrotic Enteritis (NE) challenge. A total of 1200 d-old Cobb 500 vent-sexed broiler chicks were allocated to 40-floor pens with 10 replicates and 30 birds/pen (3.46 m<sup>2</sup>) maintaining equal male-female ratio. Four treatments were: UC- unchallenged control; CC- NE challenged control; BVg- CC plus BVg at 1000, 500 and 250 g/ton in starter, grower and finisher phases, respectively; and ANT- CC plus zinc bacitracin and salinomycin at 40 and 60 g/ton of active compounds in the pelleted feed, respectively in all feeding phases. Wheat-SBM-sorghum-based diets were formulated considering matrix values of phytase. Challenged birds were gavaged with 1 mL/bird *Eimeria* spp vaccine on d9 and 1 mL/bird *Clostridium perfringens* EHE-NE18 (10<sup>8</sup> cfu/mL) on d14 and d15, while unchallenged birds were gavaged with sham treatments. Analyzed performance parameters were average weight gain (AWG), average feed intake (AFI) and FCR. Performance data were analyzed using JMP 16.0, where female% was set as co-variate, and means were separated by Tukey's test. During challenge period (d8-19), birds in the CC groups had a lower AWG and a higher FCR compared to the UC group ( $P < 0.001$ ) indicating a successful sub-clinical NE challenge. Birds fed ANT had a significantly higher AWG and lower FCR than the CC group. The AWG and FCR in the BVg group did not significantly differ from those in the CC or UC groups during this period ( $P > 0.05$ ), indicating that the BVg supplementation shifted the AWG and FCR from CC group towards the UC group. Considering overall study period (d0-35), birds fed BVg had similar performance with the CC group; however, there was no significant difference observed among the BVg, ANT, and UC groups ( $P > 0.05$ ) confirming the observations during d8-19. In conclusion, the data showed that the additive BVg was able to shift the bird performance from CC towards UC and ANT groups showing promise to alleviate the sub-clinical NE effect on performance of broilers. Further study is warranted to understand the extent of the BVg effect and underlying mechanisms.

Keywords: Necrotic enteritis; Organic acid; FCR; Performance



**Medium chain fatty acids reduce oxidative stress in intestinal Caco-2 cells****A. García-Vara<sup>1,2</sup>, L. Parro<sup>3,2</sup>, A. Tres<sup>3,2</sup>, F. Guardiola<sup>3,2</sup>, R. Ferrer<sup>1,2</sup>, R. Martín-Venegas<sup>1,2</sup>**

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Medium chain fatty acids (MCFA) due to their positive biological effects are used in animal diets at low doses to replace antibiotics. These feed ingredients rich in MCFA are mainly obtained from coconut and palm kernel oils. This group of fatty acids is composed of mainly caprylic (C8:0), capric (C10:0), and lauric (C12:0) acids. The nutritional value as well as the effect on animal health of these ingredients and, in addition, of fat by-products rich in MCFA is under consideration. In this sense, they are considered as a fast absorbing energy source, with an impact on fat deposition, and with a possible favourable improvement in gut health and function. However, the effect of MCFA on oxidative stress is largely unknown. Considering that intestinal oxidative stress plays an important role in the early stage of the disruption of intestinal barrier function, the objective of the present study is to evaluate whether MCFA could protect from oxidative stress on intestinal epithelium. To achieve this objective, intestinal Caco-2 cells have been incubated with increasing concentrations (0.02 – 0.1 – 0.25 mg/mL) of C8:0, C10:0, C12:0 as well as with by-products rich in MCFA (palm kernel fatty acid distillates) in the absence and presence of H<sub>2</sub>O<sub>2</sub> to induce oxidative stress. After incubation, the production of reactive oxygen species (ROS) has been evaluated. The results show that the incubation with C8:0 and C10:0 increase the production of ROS, an effect that has not been observed neither with C12:0 nor with by-products rich in this fatty acid. In the presence of H<sub>2</sub>O<sub>2</sub>, C8:0 at the highest concentration, C10:0 and C12:0 at all the concentrations tested, as well as MCFA-rich by-products cause a decrease in intracellular ROS production. These results are of great interest in the evaluation of the use of these by-products in animal feeding for their potential impact on intestinal health.

**Keywords:** Medium-chain fatty acids (MCFAs); intestinal health; oxidative stress; palm kernel fatty acid distillates

[P3-26]: P3- Fats (Nutrition), Welfare, Physiology, Meat Quality, Pathology and Immunology (ID: 130956) - Thailand

## **Effect of dietary enrichment with n-3 fatty acids by using lipid-based nanoparticles on growth performance, carcass composition, meat quality and fatty acid profile of breast meat in slow-growing Korat chickens**

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This research aimed to investigate the optimization of lipid-based nanoparticles to enhance the utilization of n-3 fatty acid sources in chicken diets. A total of 375 forty-nine-day-old female slow-growing Korat chickens was assigned to a completely randomized design with 3 treatment diets and 5 replications. The control group consumed a basal diet of corn-soybean meal supplemented with 6% rice bran oil. In the experimental diets, rice bran oil was replaced with 3% tuna oil (3%TO) and 3% tuna oil encapsulated in lipid-based nanoparticles (3%TO-TNP). The growth performance, carcass composition, meat quality and fatty acid (FA) profile of breast meat were evaluated. The dietary fat source had no effect on growth performance, carcass composition, nor on the quality of breast meat ( $P > 0.05$ ). In comparison with the control group, the 3%TO and 3%TO-TNP groups had lower levels of C18:2n-6 (LA) and n-6 FA ( $P < 0.05$ ). There was no significant difference between groups in the C18:3n-3 (ALA) level, while the levels of C20:5n-3 (EPA) and C22:6n-3 (DHA) were higher in both TO groups. Due to the high temperature requirements in the drying step of the nanoparticle synthesis method, the enrichment in n-3 fatty acids was more efficient when incorporating TO directly into the feed than when using targeted nanoparticles. However, there was no difference between 3%TO and 3%TO-TNP groups for the n-6/n-3 ratio ( $P > 0.05$ ). In our experiment, we reached an achievement by utilizing lipid-based nanoparticles for the precision delivery of essential nutrients, specifically n-3 polyunsaturated fatty acids (PUFA), in chickens. Notably, the incorporation of lipid nanoparticles into the diets resulted in an improvement in the fatty acid profile in chicken meat without compromising the overall performance of the chickens.

**Keywords:** growth performance; meat quality; n-3 fatty acids; lipid-based nanoparticles; slow-growing chickens

## **Optimizing the utilization of n-3 fatty acids for enhanced functional meat production in slow-growing Korat chickens involves evaluating the characteristics of glucose transporter-targeted lipid nanoparticles**

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The objective of this study was to investigate the synthesis of lipid nanoparticles to improve the utilization of n-3 fatty acid sources in chicken diets. Tuna oil was prepared with two different types of nanoparticles by hot and high-pressure homogenization approach. The ratio of lipid and surfactant equal to 1:0.3 and 1:0.4, respectively. Two preparations were defined as followed: 1) non-targeting lipid nanoparticles (NPs) and 2) targeting lipid nanoparticles (TNPs). The study was conducted for the physicochemical characteristics and targeting procedure. Thirty slow-growing Korat chickens were examined during the post-administration kinetic at 2, 4, 8, 12 and 24 h (three birds per time point and per preparation). The physicochemical characteristics of the lipid nanoparticles and in vivo biodistribution were assessed. The findings indicated that the particle diameters of NPs were 223 nm, whereas the particle diameters of TNPs were 134 nm. The polydispersity index of all groups showed a good distribution, ranging between 0.2 and 0.3. The zeta-potential, which denotes the adequate repulsive force for achieving enhanced physical colloidal stability in TNPs group, surpassed that of NPs group, attributable to the utilization of distinct surfactants (alkyl polyglycosides). The zeta-potential of nanoparticle carriers exhibited a consistently negative value lower than -30 mV across all groups, indicating favorable stability in colloid dispersions. Furthermore, no discernible differences were observed among groups regarding thermal and chemical properties. Our observations indicate that n-3 fatty acid oils were tightly encapsulated within the nanoparticles, leading to a decreased melting point and intensity of peaks, as revealed by FTIR analysis in the TNPs group. The biodistribution analysis of Nile red distribution showed that at 8 and 12 hours, nanoparticles in the TNPs group exhibited more effective delivery to the muscle compared to those in the NPs group ( $P < 0.05$ ). The analysis of the fatty acid profile revealed that at 4 hours, the EPA and DHA content in the TNPs group was significantly higher ( $P < 0.05$ ) than that in the NPs group. The TNPs group also exhibited the highest DHA content at 24 hours ( $P < 0.05$ ). Ultimately, the assessment of n-3 fatty acids composition substantiated the efficacy of targeted lipid-based nanoparticles in directly transporting fatty acids into the skeletal muscle cells of chickens.

**Keywords:** n-3 fatty acids; lipid-based nanoparticles; physicochemical characteristics; in vitro storage stability; in vivo bio distribution; slow-growing chickens

## **Effect of double buffered sodium butyrate associated with low energy-protein diets on small intestinal morphology and microbiota in broilers in a context of coccidiosis challenge**

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Previous studies showed that double buffered sodium butyrate (DBSB) improved the development of intestinal morphology, leading to a better absorption of nutrients. This effect has also been shown in a context of reduction of metabolizable energy (ME), crude protein (CP) and amino acids (AA) in broiler diets where the association of DBSB to a low energy-protein diet (LEP) led to similar performance than birds fed a classic control diet (CTRL). Coccidiosis is a challenging disease in the broiler sector and is known for its negative impact on gut mucosa. The aim of this study was to evaluate, in a context of coccidiosis challenge, the effect of DBSB associated to LEP diets on the gut structure and microbiota in broilers compared to birds fed classic control diets. 240 female Cobb broilers were distributed in 16 pens. Each pen randomly received one of the 2 treatment diets (8 replicates per group). Control diets (CTRL) were formulated according to Cobb recommendation (2018). LEP diets were formulated to obtain a 3% reduction in ME, CP, and AA (Lys, M+C, Thr) and were supplemented with 600 ppm of DBSB (BUTYLIn 54, Dietaxion) from 0 to 40 days old. At 21 days old, a coccidiosis challenge was triggered by giving intra-ingluvial 10 doses of a commercial coccidiosis vaccine to all birds. At the same age and at 35 days old, one bird per pen was euthanized for jejunum histology to observe villi length (VL) and crypt depth (CD) to calculate the VL/CD ratio. At 35 days old, bacterial analysis of duodenum were added to histology (total aerobic flora, lactic bacteria, enterobacteria). The bacterial results were expressed as log<sub>10</sub> cfu/g. Parametric data were analyzed using ANOVA and non-parametric data through the Kruskal-Wallis test. At 21 days old, numerical differences in VL and CD led to a significant improvement by 66% of the ratio VL/CD for DBSB group (P<0.05). Two weeks after the coccidiosis challenge (day 35), no significant difference was observed for VL but CD was significantly shallower by 14% in birds fed DBSB (P<0.05). Consequently, VL/DC ratio was significantly improved in these broilers (+43%; P<0.05). There was no significant difference in any of the families of bacteria analyzed, nor in the ratios lactic bacteria/total aerobic flora and enterobacteria/total aerobic flora, probably due to the small amount of samples. In a context of coccidiosis challenge, supplementing 600g/T of DBSB associated to a LEP diet enables to improve broilers' intestinal morphology.

Keywords: broiler; butyrate; coccidiosis; intestinal morphology

## **Supplementation of a combination of lysolecithins, a synthetic emulsifier and monoglycerides on broiler performance and profitability during heat stress**

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Faster growth and higher metabolic rates of modern broiler genetics result in more body heat produced, increasing predisposition to heat stress. A study was conducted to assess the impact of supplementing the diet of broilers subjected to heat stress with a combination of lysolecithins, a synthetic emulsifier and monoglycerides (LEX), on improving performance and ultimate production profitability. A total of 640 one-day-old males Ross 308 broilers were randomly assigned to two dietary treatments for 42 days: Control (corn-soybean-wheat diet) or LEX (Control + 500 ppm of LEX). Each treatment consisted of 16 pens of 24 birds each. Diets were produced in mash and fed in 3 phases: starter (0-15 d), grower (15-30 d) and finisher (30-42 d). A heat stress protocol was applied from 21 to 42 days. All birds were weighed at their arrival from the hatchery and at days 15, 30 and 42. Feed bags, as well as feed remaining in the feeders, were weighed at the same time to calculate feed intake and feed conversion ratio (FCR). An economic analysis was carried out to determine the income over feed cost (IOFC), considering the broiler price per kg of live weight, the feed intake and feed cost for every growing period (0-15 d, 15-30 d and 30-42 d), and the body weight at 42 d of both treatments. The heat stress challenge did not seem to affect broilers as expected because the performance as well as mortality achieved by both treatments was in line with Ross 308 performance objectives. At the end of the first feeding phase (0-15 d), birds from the LEX group tended to have higher BW than birds from the Control group (538 vs. 524 g;  $p=0.0985$ ). After 42 days, birds fed LEX tended to have a higher BW than birds from the Control (3094 vs. 3033 g;  $p=0.058$ ). ADG was significantly higher from 30 to 42 d (105 vs. 100 g/d;  $p=0.0172$ ) and tended to be higher over the entire study (71.4 vs. 69.9;  $p=0.0610$ ) for birds fed LEX compared Control. Regarding the FCR, after the first 15 days LEX tended to show a lower FCR than Control (1.53 vs. 1.57;  $p=0.0983$ ), a trend which became significant from 30 to 42 d (1.80 vs. 1.85;  $p=0.0412$ ) but not for the overall trial period (1.54 vs. 1.55;  $p=0.1220$ ). The IOFC of the LEX treatment was 43 €/1000 birds higher compared to the Control group. These findings indicate, that adding LEX to diets of broilers raised under heat stress conditions, can support their average daily gain and final body weight resulting in a positive economic impact.

Keywords: Broiler, growth performance, heat stress, emulsifier, lysolecithin

## **Supplementation of a combination of lysolecithins, a synthetic emulsifier and monoglycerides to diets of broilers containing phytase in super dosing, on performance and profitability**

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Super dosing of phytase is becoming an increasingly popular strategy to allow a complete phytate destruction in the digestive tract, which can lead to an increase in inositol secretion in the gizzard. As such, super dosing may result in performance enhancement due to both reduced antinutritive factors from the phytate as well as the increased presence of inositol. To date the impact of supplementing a combination of lysolecithins, a synthetic emulsifier and monoglycerides (LEX) to diets with phytase in super dosing had not been yet explored. Therefore, a scientific study was designed to elucidate this impact on growth performance and subsequent production profitability of broiler chickens. A total of 576 one-day-old males Ross 308 broilers were randomly assigned to two dietary treatments for 42 days: Control (birds fed standard diets with phytase super dosing -1500 FTU/g) and LEX (birds fed Control + 500 ppm of LEX). Each treatment consisted of 14 pens of 24 birds each. Diets were pelleted and fed in 3 phases: starter (0-14 d), grower (14-28 d) and finisher (28-35 d). The experimental unit was the pen/replicate. Primary zootechnical data: body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and mortality were determined as mean pen values. An economic analysis was carried out to determine both treatments' income over feed cost. FCR tended to be lower for birds fed LEX compared to Control from 15 to 28 days ( $p=0.0657$ ) and for the overall trial period ( $p=0.0681$ ). The income over feed cost of the LEX treatment was 31.3 €/1000 birds higher compared to Control. These findings indicate, that adding LEX to diets on top of a phytase in super dosing, can improve FCR, delivering a positive economic impact.

Keywords: broiler; growth performance; phytase super dosing; emulsifier; lysolecithin

## **In feeding line application of organic acids improved feed hygiene and growth performance in broiler chickens**

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Organic acids may help to improve feed hygiene, which may have great impact on the growth performance of a broiler chicken. The effect of an on-farm application of organic acids directly in the feeding line was tested on a commercial broiler farm. The trial was conducted at a commercial broiler farm in Poland, where 3 houses were included with 50000 birds each. The 3 houses were followed during 2 rounds. In each round 2 houses received the control diet, and the other house received additive treatment which was the control diet with 4 kg/t Fyvalet BE+ (Selko, Tilburg, The Netherlands) dripped on feed in the feeding line. Body weight (BW), gain and average daily gain (ADG) were measured weekly. Feed intake and mortality corrected feed conversion ratio (FCR) were measured for the overall study period. Surface wipes of feeder lines and pans, and fresh and residual feed samples were taken in one house before and after additive application and tested for microbial load. Enterobacteriaceae, yeast and moulds and total number of microbes were tested via plating and could not be analyzed statistically. Final BW was higher with the additive compared to control (2665g vs 2538g respectively,  $P=0.05$ ). The total gain and ADG were significantly improved between day 14-21 and day 28-35 in the additive group compared to the control. Overall data showed a numerically lower FCR in the additive group compared to the control group (1.575 vs 1.625 respectively,  $P=0.33$ ). Overall mortality was not different between both groups (5.2% vs 6.4%,  $P=0.54$ ). Generally, no Enterobacteriaceae were observed during the entire study, neither in the feeding lines nor the feed. Total number of yeast and moulds in the feeding line at the start before the additive application at levels between  $1.5 \cdot 10^2$  and  $1.3 \cdot 10^4$  CFU/swab. These levels reduced after additive application at the end of the flock until below detection limit. The fungal load decreased from 102 CFU/g until below detection limit after additive application. Similarly, the levels of total microbes in the feeding line reduced from levels between  $6.7 \cdot 10^3$  and  $3.2 \cdot 10^5$  CFU/swab to levels between  $3.5 \cdot 10^2$  and  $4.4 \cdot 10^3$  CFU/swab. In the feed the total microbes reduced from 106 CFU/g to 103 CFU/g after additive application. In conclusion, applying Fyvalet BE+, an organic acid based feed additive, on feed directly in the feeding line resulted in an improved growth performance and reduced microbial loads both in the feeding line as well as in the feed.

Keywords: organic acids; feed additive; on-farm; feed hygiene; broiler chickens

## **The effect of a selected blend of organic acids on controlling *Salmonella Infantis* in broiler chickens**

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*Salmonella Infantis*, although not regulated yet, is the most occurring serovar in the European Union, with 95% of cases linked to broiler production and products. This serovar is associated with antimicrobial resistance and is very persistent at farms. Organic acids are known to be effective against *Salmonella*, information specific to *Salmonella Infantis* is missing. The aim of this study was to test an organic acid-based feed additive against *Salmonella Infantis* in broiler chickens. In total, 396 broiler chickens (ROSS 308) starting at day old were enrolled in the study. Birds were grouped of 22 birds/pen, with 9 pens/treatment. Birds were fed a commercial, pelleted diet, with phase 1 being between days 0-11, phase 2 between days 11-28 and phase 3 between days 28-39. On day 39 a feed withdrawal was applied before the final sampling on day 40. Two treatments were administered: (1) control diet without feed additives, (2) additive diet, which was the control diet with a combination of short chain and medium chain fatty acids (Selacid Green Growth, 4 kg/t, Selko, Tilburg, The Netherlands) added in feeding phases 1 and 2, and no additive in phase 3. Seeder birds were fed the control diet and housed separately until inoculation. On day 5 and 6, seeder birds (5/pen) were orally inoculated with 10<sup>9</sup> CFU/ml *Salmonella Infantis*. On days 13, 19 and 40, 4 non-seeder birds per pen were selected for cecal *Salmonella* counting via plating. Visual gut health scoring was done in 5 non-seeder birds per treatment on days 13 and 19. Growth performance was measured on days 0, 5, 11, 28, 39. Cecal *Salmonella* counts tended to be lower with the additive compared to the control on days 13 and 19 ( $P < 0.1$ ). The gut health score tended to be better in the additive group compared to the control group on day 13 ( $P = 0.0727$ ). Compared to the control group, the additive group had a higher body weight (BW) at day 11 ( $P = 0.0448$ ) and average daily gain between day 5-11 ( $P = 0.0265$ ). On day 39, BW of the additive group was significantly higher compared to the control group (3295g and 3188g respectively,  $P = 0.0366$ ). Due to an unexpected *E. coli* infection in the last feeding phase the mortality was 12% in the additive group compared to 3.7% in the control group ( $P = 0.0071$ ). In conclusion, feeding Selacid Green Growth, a feed additive based on selected blend of organic acids, tended to reduce cecal *Salmonella Infantis* counts in broiler chickens, while improving gut health and growth performance.

Keywords: *Salmonella Infantis*; feed additives; organic acids; broiler chickens



## **Effects of medium-chain fatty acids rich diets on intestinal morphology, microbiology and volatile fatty acids in young broiler chickens**

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Fats are commonly added to poultry diets to increase energy. Medium-chain fatty acids (MCFA, C6:0 to C12:0) rich-oils are considered a fast-absorbing energy source with a possible favorable intestinal antimicrobial effect. The aim of this study was to assess the effect of dietary MCFA rich oils from different sources and with different free fatty acid (FFA) content on young broiler chickens' intestinal health. A total of 640 newly-hatched (Ross 308) were randomly allocated to 40 pens and 5 dietary treatments (8 replicates/treatment). Animals were fed experimental diets from 0 to 21 days of age, consisting of a basal diet (wheat and soybean; mash form) supplemented at 4% (as-fed basis) with different MCFA fat sources: black soldier fly oil (BSF: >40% MCFA, <3% FFA), palm kernel oil (PK: >50% MCFA, <10% FFA), palm kernel fatty acid distillate (PKFAD; by-product of PK physical refining: >47% MCFA, >60% FFA,) and fatty acid distillate obtained from splitted (hydrolyzed) palm kernel oil (SPKFAD; >53% MCFA, <93% FFA). A diet with soybean oil (S) was used as control. At 10 days of age, 8 animals/treatment (1 animal/replicate) were slaughtered. The effect of MCFA-rich oils on intestinal health was determined in the distal portion of both the jejunum and the ileum of these animals throughout: 1) histomorphological studies, measuring at least the height of 30 villi and the depth of 30 crypts of Lieberkühn for animal and tissue. 2) microbiological studies of its lumen content performed by traditional classical plate count of selected bacterial groups (total lactic acid bacteria, Enterobacteriaceae, total coliform bacteria, total lactic acid bacteria: Enterobacteriaceae ratio). Volatile fatty acids (VFA) production in the cecum content was also analyzed. In Jejunum, no significant differences ( $P>0.05$ ) were observed among different MCFA sources and the control in morphometrics neither for microbiological count. Contrary, in ileum differences were obtained. SPKFAD showed lower crypt depth than PKO ( $p=0.035$ ), and higher growth of total coliforms ( $p=0.039$ ) and a lower ratio of total lactic acid bacteria: Enterobacteriaceae ratio ( $p=0.08$ ) than SO. Regarding VFA at the cecal level, differences were only observed in propionic acid, which was higher in BSF than in PKFAD ( $p=0.013$ ). According to the results, dietary supplementation with 4% MCFA-rich oils with a high percentage of FFA ( $\approx 93\%$ ) could compromise intestinal health by promoting the growth of pathogenic bacteria in young broiler chicken.

**Keywords:** medium chain fatty acids, free fatty acids, young broiler chickens, intestinal health

## **Protected sodium butyrate improves productive performance and carcass characteristics in broiler chickens**

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The use of antibiotics such as lincomycin as growth promoters are common outside Europe in poultry feed to improve production. However, they are a source of antibiotic resistance and a stress factor for the intestinal epithelium. Sodium butyrate is a short-chain fatty acid and a safe antibiotic alternative or adjuvant. The effects of the addition of protected sodium butyrate (PSB) and lincomycin (LIN) in the diet on growth performance, gastrointestinal tract characteristics, body traits, and expression levels of insulin-like growth factor-1 (ILGF1) were studied in broilers from hatch to 35 d of age. 1,200 one-day-old Ross-308 broiler chickens were randomly assigned into 5-dietary groups with 5-replicates each. Groups divided as follows: T1: corn and soybean meal based basal diet (BD) (control), T2: BD with PSB (Butirex C4, Novation 2002, Spain; 1 kg/ton starter and 0.5 kg/ton grower-finisher feeds), T3: BD with 100 g/ton LIN (Lincomix 50, Zoetis Services LLC., USA), T4: BD with PSB (0.5 kg/ton starter and 0.25 kg/ton grower-finisher feeds) + 50 g/ton LIN, and T5: BD with PSB (1 kg/ton starter and 0.5 kg/ton grower-finisher feeds) + 50 g/ton LIN. Broilers fed with combined dietary supplementation with PSB and LIN had higher BW gain (2,259 and 2,269 vs. 2,157 g) and better feed conversion ratio (1.55 and 1.51 vs. 1.62) than broilers fed with control diet. Similar results have been obtained in the histomorphometry of the small intestine, were broilers fed with combined dietary supplementation with PSB and LIN had higher duodenum (1,200 and 1,433 vs. 833  $\mu\text{m}$ ), jejunum (1,100 and 1,337 vs. 817  $\mu\text{m}$ ), and ileum (1,077 and 1,233 vs. 757 $\mu\text{m}$ ) villi length than broilers fed with control diet, with intermediate results for the rest of the treatments. Also, carcasses in combined dietary supplementation with PSB and LIN groups exhibited the highest yield in dressing (5.7% improvement), related with the highest expression levels of ILGF1 observed in both breast and thigh muscles. In fact, when comparing only groups T1 and T2, broilers fed diet with PSB had better feed conversion ratio (1.57 vs. 1.62), higher jejunum villi length (957 vs. 817  $\mu\text{m}$ ), and highest expression levels of ILGF1 in both breast (2.54 vs. 1.00) and thigh (1.09 vs. 1.00) muscles than broilers fed with control diet. In conclusion, the inclusion of PSB with or without LIN showed the most efficient results concerning productive performance and carcass characteristics in broiler chickens.

**Keywords:** antibiotics; sodium butyrate, carcass

## **Adaptation of Avian Orthoreovirus strain DVB 04 in Vero cells and its titration**

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Avian orthoreovirus (ARVs) is an emerging threat to poultry industry. It was isolated for the first time in India during 1989, from cases of viral arthritis and malabsorption syndrome. It results in moderate mortality up to 18% and 100% morbidity but causes significant productivity losses. There have been reported 6 genotypic variations of ARV and genotypic divergence of this virus in India is not known so far. There is currently no live vaccine accessible or being given to chickens in India to prevent ARVs. Thereby, the persistence and emergence of this disease even after killed vaccination in India may be due to non-following of the recommended prime boost vaccination approach, non-availability of attenuated vaccine and emergence of variant ARV strains. In light of the significance of ARV, the present study was envisaged for screening of poultry samples from suspected poultry birds, isolation of field strains in Vero cell culture system and genetic characterization of the of suitable live vaccine candidate for economically important ARV disease. For this, about 110 samples were screened for the presence of ARV by PCR and 6 of them were found to be positive. The PCR amplification using oligonucleotide primers specific to the L1 segment and S2 segment produced positive results. The existence of the target DNA fragments was confirmed by observing bands on the agarose gel and 421 base pair band was produced by amplifying the L1 portion, Whereas A gene produced bands of about 409 base pairs for S2 segment. Sigma C gene sequences obtained from Genbank were aligned for molecular investigation. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. There were total of 801 positions in the final dataset. The gene that encodes Sigma C exhibited the highest amount of sequence divergence and rapid evolution, indicating that the gene may be utilized to quickly differentiate and classify ARV isolates. Further, the virus was adapted to vero cells for 14 generation and the titer of the virus was found to be 104.5 by Reed–Muench method. This study indicates that genotype cluster I ARV are the main virus lineage found in India and DVB 04 may be a good option for an attenuated ARV vaccine if attenuated in suitable cell culture system.

Keywords: “Avianorthoreovirus”, “Maximum Composite Likelihood”, "Attenuated vaccine"

## **Genetic variability of infectious bursal disease virus (IBDV) in the Iberian peninsula**

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Infectious Bursal Disease (IBD), caused by the Gumboro virus (IBDV), is a global concern in the poultry industry. The IBDV consists of genome segments A and B. Segment A encodes VP2, impacting immunologic response, while Segment B codes for proteins, including VP1, essential for replication and particle formation. Information regarding local viral variants will facilitate the development of prevention and control strategies tailored to specific avian populations and regions. This study investigated the presence of IBDV in 66 outbreaks suspected of IBD in the Iberian Peninsula from 2020 to 2022. A VP4-based qPCR assays detected the virus in 28 samples with Cq values ranging from 17 to 34. The assay identified 14 samples as very virulent and 14 as non-very virulent, which could be attenuated or classically virulent. Subsequently, genotyping of the 28 positive samples was conducted for segments A and B. The VP1 gene was amplified using a protocol as described by Islam et al. with specific modifications. For the amplification of the VP2 gene, we employed a nested PCR technique based on the methods outlined by Brown et al. and Yamazaki et al.. Ninety percent of the samples were successfully genotyped based on VP1, while 100% yielded a sequence of the VP2 region. The three samples that could not be sequenced for VP1 had Cq values over 31. A phylogenetic diagram, which included sequences of reference strains for each genotype, was generated for each segment. All samples, except one that resulted B2, were classified as genotype B1. However, when considering VP2, we identified A1a (classic virulent), A1b (classic attenuated), and A3 (very virulent). The most frequently observed genotype was A3B1 (50%), followed by A1B1 (33%), A3BX (11%) and A3B2 (3.5%). Notably, one sample exhibited a mixed profile, indicating coinfection with at least two strains of IBDV. The genotyping results from VP4 qPCR and VP1+VP2 approaches showed substantial agreement with a Kappa value of 0.79 (SD=0.11). In three cases initially identified as non-very virulent by qPCR, they were reclassified as A3 (very virulent), suggesting possible recombination. The prompt response offered by the qPCR technique was followed by a more in-depth characterization through the genotyping of both segments. This study demonstrates viral genetic diversity in Spain and Portugal. The implementation of rapid and up-to-date diagnostics is essential to monitor new viral variants and assess the control measures already in place within the poultry sector of our country.

Keywords: IBDV, genotyping, VP1, VP2

**First report of Marek's disease virus in commercial turkeys in Slovenia****Z. Žlabravec<sup>1</sup>, B. Slavec<sup>1</sup>, E. Rožmanec<sup>2</sup>, S. Koprivec<sup>3</sup>, A. Dovc<sup>1</sup>, O. Zorman Rojs<sup>1</sup>**<sup>1</sup>Institute of Poultry, Birds, Small Mammals, and Reptiles, Faculty of Veterinary Medicine, University of Ljubljana, Ljubljana, Slovenia, <sup>2</sup>Veterinarska ambulanta PP, d.o.o., Ptuj, Slovenia, <sup>3</sup>Institute of Preclinical Sciences, Faculty of Veterinary Medicine, University of Ljubljana, Ljubljana, Slovenia*Presenting author: zoran.zlabravec@vf.uni-lj.si*

Marek's disease (MD), caused by Gallid alphaherpesvirus 2 (GaHV-2), is one of the most important neoplastic and immunosuppressive diseases because it is responsible for substantial economic loss in the poultry sector worldwide due to decreased productivity, increased morbidity and mortality, and condemnation at slaughter. The primarily natural hosts are chickens; however, reports of MD-induced tumors in turkeys have been increasing over the past decade. Between 2021 and 2023, three cases with clinical disorders ranging from none to severe (depression, lameness, and increased mortality) occurred in commercial turkey flocks in Slovenia. All affected flocks had been reared in fully enclosed and insulated facilities, and no other poultry species were present on the farms. In all cases, the post-mortem examination revealed white foci and small white nodules in various organs, including the liver, spleen, kidney, heart, lung, intestine, and pancreas. In the affected organs, histopathological examination showed pleomorphic lymphoproliferative foci of varying size. In all cases, Marek's disease virus (MDV) was detected by PCR in DNA samples extracted from organs developing tumor infiltrations. Interestingly, despite the different clinical onset of disease detected, phylogenetic analysis of the MEQ gene, the main GaHV-2 viral oncogene, showed identical sequences in all three cases and shared 99.9% nucleotide identity with the most closely related strain in GenBank, GaHV-2 (acc. no. KY113150), detected in chickens in Tunisia. Based on phylogenetic analysis and the number of proline-rich repeat region motifs (PPPP) within the MEQ protein, MDV detected in Slovenia was suggestive of a very virulent pathotype. This is the first report of MDV in commercial turkey flocks in Slovenia.

**Keywords:** Marek's disease; turkey; MEQ gene; Slovenia

## **Efficacy of Probiotic via Drinking Water on Growth Performance of Broiler Chickens during Fowl Adenovirus Infection in UAE**

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Fowl adenoviruses (FADVs) are non-enveloped paramyxoviruses with double-stranded DNA molecules and belong to the Avi adenovirus genus. There are also several diseases caused by fowl adenoviruses in broiler chickens, including inclusion body hepatitis, hydropericardium hepatitis syndrome, and adenoviral gizzard erosion caused by fowl adenoviruses. In recent years there has been an increasing concern about the development of antibiotic resistance in pathogenic bacteria in humans and poultry. The objective of this study was to determine the efficacy of Novela ECL Plus administered via drinking water on the growth performance of broiler chickens during the infection with Fowl Adenovirus. This study was carried out from September 27, 2023, to October 27, 2023, at a commercial broiler floor system in Abu Dhabi, UAE. A total of 90,000 Ninety thousand one-day-old, commercial broiler Ross 308 breed chicks were brought from a commercial hatchery and randomly divided into three treatment groups in three broiler houses, 30,000 birds per house. Treatment 1, as a control, received antibiotic with a combination of (Gentamycin + Doxycycline) with a dose of 0.5 gm per liter in the first three days from 1 to 3 days of age and received the second dose of antibiotic with a combination of Tilmicosin + Colistin for three days from 18 to 20 days of age with the dose 0.5 gm per liter. Treatment 2 and 3 the birds in this group received multi strains of bacillus via drinking water for 8 hours with a dose of 0.05 gm per liter without usage of antibiotics from 0 days till the slaughtering at 28 days. Feed intake and body weight, live body weight gain, and feed conversion ratio a European Production Efficiency Factor were measured. The results obtained from this study showed a significant reduction in mortality rate, improved weekly live body weight, enhanced dressed weight, and improved feed conversion ratio, EPEF, and dressing percentage, whereas as no significant difference in feed intake. In conclusion, the administration of the multi-strain bacillus probiotic Plus to broiler chickens from 0 days to 28 days of age plays a major role in and reduces the usage of antibiotics as antimicrobial substances, in addition to also improving the growth performance parameters and enhancing the profitability.

**Keywords:** Fowl adenovirus, Novela, growth, performance, UAE

## **Epidemiology of infectious bursal disease virus in Portugal: replacement of local strains by Northwestern European reassortants as dominant field type**

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Infectious bursal disease (IBD) is arguably the most relevant immunosuppressive disease to affect the worldwide poultry industry. It is caused by infectious bursal disease virus (IBDV), a highly resistant RNA virus featuring a significant genetic variability which in turn reflects on disease presentation, diagnosis and control. Encouraged by the recent proposal of multiple standardized classification systems based on phylogeny, many epidemiological surveys were conducted in the last years, greatly improving the understanding of IBDV epidemiology. In Portugal, the epidemiological scenario is seemingly undergoing a shift in terms of circulating field strains. Within the context of molecular diagnostic activities conducted since late 2020, pooled bursal samples were collected from broiler and layer flocks immunized with different vaccine types and protocols (37 in 2020, 69 in 2021, 56 in 2022, 43 in 2023). A first RT-PCR targeting the VP2 gene was performed, followed by Sanger sequencing to discriminate between vaccine and field strains. Then, the latter were subjected to another assay targeting the VP1 to genotype them according to the updated guidelines. This led to the discovery of a novel genotype with unique VP2 features, named A9B1, which despite the recent characterization appears to have circulated locally for more than a decade. Later analyses also captured the appearance in the country of the so-called Northwestern European reassortants (genotype A3B1), likely occurred during 2021. Following the entry of this second IBDV type, A9B1 strains rapidly went from being the sole field viruses (100% of field detections in 2020) detected in Portugal to representing a minority (50% in 2021, 11% in 2022, 17% in 2023) with A3B1 IBDVs becoming predominant instead. This finding mirrors what occurred in many countries across Western, Northern and Central Europe, where A3B1 reassortants quickly displaced historically circulating strains. The reasons for such evolutionary success are still undetermined, although their subclinical nature certainly thwarts diagnostic efforts and makes their clinical and economic consequences easy to underestimate. Despite the limited scale of this study, these results are significant not only for the Portuguese poultry sector, but also for all countries interested by the circulation of Northwestern European reassortants, calling for further monitoring to track how the epidemiological situation might evolve.

**Keywords:** Infectious bursal disease virus; Gumboro disease; Portugal; molecular epidemiology; reassortment

## **Disease diagnosis through analysis of chicken mycoplasma antigens in 9th Industrialization and preservation-maintenance line of Korean Native Chickens(KNC)**

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*Mycoplasma gallisepticum* Infection is common diseases in chickens in Korea and causes complex chronic respiratory diseases. In Korean breeders, chicken mycoplasma (MG) antibody testing is required at 60 weeks of age, so it is a disease that requires special attention to management. It is widespread in domestic poultry farms, so eradication is urgent, but diagnosis is difficult because it progresses chronically and does not show clear clinical symptoms in isolated infections. In the case of vaccination, disease infection cannot be confirmed through an antibody test, so antigen test through PCR and sequencing are needed to compare and analyze between the vaccine strain and field strain to confirm disease infection and vaccine effectiveness in the flock. The throat swabs of a total 100 chickens (30 MG-F vaccinated, 40 Vaxsafe-MG, 30 unvaccinated) from the 9th generation industrialized and maintenance lines of the Poultry Research Institute's native chicken were swabbed and placed in medium. After culturing in a 38°C incubator for 14 days, DNA was extracted from samples that showed color changes, and PCR was performed using *mgc2* MG-specific primer. The DNA of samples with positive PCR results was subjected to Sanger sequencing to secure the nucleotide sequence, and comparative analysis was performed by alignment with the vaccine strain (F strain, ts-11, 6/85) and domestic field isolates (L7) using MAFFT. As a result, 25 samples showed medium discoloration and PCR positivity. 25 samples were confirmed as a *Mycoplasma gallisepticum*. The *mgc2* sequence of 11 samples were 100% identical to the *mgc2* sequence of the ts-11 strain. The *mgc-2* sequence of 13 were 100% identical to the *mgc2* sequence of F strain. A 66bp duplication was found in the proline-rich domain of *mgc-2* sequence in 1 sample. This was suspected to be the strain derived from the F strain vaccine. Therefore, it was believed that there was no disease infection caused by the chicken *Mycoplasma gallisepticum* field strain, and the vaccine strain was established following MG-F and TS-11 vaccination, and it was confirmed that the vaccine strain was established in some unvaccinated individuals. Therefore, it was considered that chicken *Mycoplasma gallisepticum* was not spread within the 9th Korean Native Chicken in PRI.

Keywords: mycoplasma, vaccine, infection, PCR, sequencing



## **Immune status and reproductive performance in Broiler breeders**

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It is well established that individual's response to an infectious challenge explains the different outcomes upon an infectious outbreak in animals sharing the same environment. Therefore, producers must improve their vaccination programs to obtain more uniform and resilient responses. In broiler breeders, major infectious challenges worldwide are the avian infectious bronchitis virus (IBV), Newcastle disease (ND) and Infectious bursal disease (IBD). In this study, we monitored the immune status of broiler breeder flocks for these diseases and its association with reproductive performance. A total of 153,400 Ross 308 broiler-breeder females placed in five all-in all-out commercial farms during a period of five years (from 2016 to 2020) were analysed. The same vaccination programme adapted to the epidemiology of the farms area was followed throughout the rearing period. Management applied was according to the guidelines of the Ross Parent Stock Management Handbook. Official controls were effectuated extracting blood samples in all flocks in all farms three times along the production cycle. First one within 4 weeks posterior to house the hens in the farm and third one within 8 weeks before the end of the production cycle. The second analysis was effectuated in the middle of the period between the other two analysis. Serological analysis for IBV and ND were done by hemagglutination inhibition assay (HI) and by enzyme-linked immunosorbent assay (ELISA) for IBD. Antibody titres in the first control had a correlation over 0.80 and 0.60 with the second and third control. Serological levels for IBV had a correlation around 0.2 with ND and IBD levels in all controls. Correlation was around 0.30 between ND and IBD levels. Animals born in winter had lower serological levels for IBV (8.57 vs 9.32) and ND (6727 vs 7741) than animals born in summer. Flocks with IBV levels lower than 8.5 had 141.1 cumulated chicks per hen housed at 62 weeks and it was significantly lower than the 148.5 chicks cumulated for the flocks with IBV levels over 9. The difference is even higher for the IBD levels. Flocks with 6000 IBD titres cumulated 136.2 chicks per hen housed versus 151.2 chicks cumulated by flocks with over 8000 IBD titres. These results indicate that high antibody levels are an indicator of vaccine success that provide breeders less affected by external challenges and with more robust performances.

**Keywords:** Broiler breeders; uniformity; immunity; serology; reproductive performance

## **Hyperpigmentation of laying hens at slaughterhouse associated with lesions in the reproductive tract.**

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Condemnation of carcasses at slaughterhouse may have a significant economic impact in poultry industry, but on the other hand it serves as a powerful tool to assess various components of the productive system, including welfare concerns and health status. Official meat inspectors working in Catalan slaughterhouses can rely on the SESC the Catalan slaughterhouse support network ([www.sesc.cat](http://www.sesc.cat)), a continuous education tool to which they can send samples of interest to investigate certain lesions with the aim to improve their post-mortem examinations and, hence, their decision making. Cases of hyperpigmentation in carcasses of laying hens caught the attention of official meat inspectors in a poultry slaughterhouse, which led to submitting the whole carcasses to SESC to investigate such hyperpigmentation. Carcasses from laying hens with hyperpigmentation arriving at one slaughterhouse (multiple farm origins and multiple batches) were investigated. At necropsy, the carcasses displayed bright orange discoloration of the skin, subcutaneous and perivisceral adipose tissue. Presence of high contents of dietary pigments within subcutaneous fat were confirmed by its solubility in an ether solution, also ruling out presence of biliary acids. When examining the celomic viscera, lesions along the ovary or oviduct were most notably seen. Ovarian tumors included ovarian adenocarcinoma, as well as sporadic cases of sex cord stromal tumors (arrhenoblastoma). Lesions along oviduct included tumors (mainly leiomyomas that compressed and/or occluded the oviductal lumen), as well as inflammatory conditions such as infundibulitis or salpingitis. The ovary displayed follicular atresia in most cases, indicating that egg production was impaired. Overall, we report a strong association between hyperpigmentation of laying hens' carcasses at slaughterhouse level and lesions along the reproductive tract. Laying hens' feed usually contains high levels of dietary pigment compounds that is normally deposited within the egg yolk, giving it a bright orange coloration. When egg production is impaired, this pigment is then deposited in the animals' skin and adipose tissue.

**Keywords:** Hyperpigmentation; reproductive; slaughterhouse; laying hens

## **Efficacy of different IBD vaccines against the current IBDV circulating strains in the Benelux area**

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The occurrence of acute Gumboro disease in the Benelux area is uncommon, despite a significant rise in the prevalence of new virulent strains of infectious bursal disease virus (IBDV) in the region. It is imperative to determine the optimal timing for vaccination when employing conventional live attenuated vaccines for IBD. Despite most farms implementing correct vaccination procedures with live attenuated vaccines, a considerable number are dealing with the emergence of a new reassortant strain defined as "UK2019". While classified as a very virulent IBDV (vvIBDV), the clinical presentation of Gumboro disease caused by "UK2019" differs from the traditional acute form. To assess the extent of the spread of the reassortant strain, a comprehensive screening of broiler farms in the Benelux region was conducted. A total of 260 flocks with different vaccination programmes underwent bursal sample analysis for RT-PCR on VP2 and nucleotide sequencing. To identify the reassortant strains an additional RT-PCR on VP1 was performed. The sequences obtained were then compared with both IBDV reference strains from Genbank and field strains. The results demonstrated that on 93.8% of all farms positive for a field strain, the isolated strain was related to the "UK2019" strain. Overall, 46.5% of the farms tested positive for a strain different to the vaccine administered. In line with expectations for effective protection with a live vaccine, only 35.8% were solely positive for the vaccine strain. PCR results were negative on 10% of the farms. On farms utilizing intermediate vaccines, 70.6% tested positive for a field strain, while on 23.5% only the vaccine strain was found, indicating full protection. Intermediate plus vaccines showed that 34.6% were solely positive for a field strain, and on 59.0% of these vaccinated farms, only the vaccine strain was detected. For recombinant vaccines, the absence of a live virus occupying the bursa of Fabricius, should make it difficult to detect an IBDV vaccine strain in bursal samples. However, in 72.7% of the flocks vaccinated with recombinant vaccines, a different IBDV strain to the vaccine administered was present in the Bursa. Flocks vaccinated with immune complex vaccines exhibited a high level of positivity, with 94.1% of the flocks testing positive solely for the vaccine strains. This study emphasizes the importance of continuous monitoring, adaptation of vaccination strategies and further research to address the evolving landscape of IBDV strains.

**Keywords:** IBDV, live attenuated vaccines, immune complex, broilers, reassortant strains

## **Efficacy of different infectious bursal disease vaccines administered in the hatchery against a very virulent bursal disease challenge in broiler chickens**

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The aim of the study was to assess whether the administration of an IBD immune complex vaccine (GUMBOHATCH®, HIPRA) interferes with the immune response of recombinant HVT-ND vaccines and to evaluate the protective efficacy of various combinations of IBD vaccines against a very virulent IBD (vvIBD) challenge in broiler chickens. Day-old chicks were allocated into 6 groups (G) of 40 chickens each: G1 and G2 received GUMBOHATCH® and a different recombinant HVT-ND vaccine. In contrast, G3 and G4 received a recombinant HVT IBD+ND vaccine. Chickens in G5 and G6 were not vaccinated. The chickens from G1 to G5 were subsequently challenged at either 28 or 35 days with a vvIBD virus. Clinical signs, mortality and body weight were monitored. Necropsies were performed periodically to evaluate bursal lesions (macroscopic and histopathological). Bursa weights were also measured To obtain the bursa weight:body weight ratio (B/BW). Blood samples were collected periodically to evaluate the immune response against IBDV and IBDV-VP2 by commercial ELISA test kits (Synbiotics-IBD Classic and Synbiotic-IBD Plus, respectively) and a against the F protein of recombinant HVT-ND and recombinant HVT IBD+ND vaccines (with a commercial ELISA test kit BioChek). Body weight, B/BW ratios and antibody titres were analysed and compared between groups using ANOVA and least significant difference (LSD) test. Histopathological lesion scores (HLS) were compared between groups using the Kruskal-Wallis test. Significance was considered at  $p < 0.05$ . The results indicated that the challenge caused growth delay in G4, especially with the early challenge. The other vaccines prevented weight loss to varying degrees. All the vaccines reduced bursal damage compared to G5, but only G1 and G2 prevented macroscopic alterations, with a notable impact on oedematous lesions. In contrast, there was a relatively smaller reduction in the occurrence of these with the G4 vaccine. Histopathological lesion scores were reduced in all vaccinated groups, although to a lesser extent in G4. Each vaccine induced an immune response in the birds, reflected in the serological results. In conclusion, the HVT IBD-ND vaccines showed a less favourable performance in terms of both growth and macroscopic lesions of the bursa. Groups vaccinated with GUMBOHATCH® together with a recombinant HVT-ND vaccine showed better outcomes, especially during the early challenge. Hence, the study suggests that combining this immune complex vaccine with a recombinant HVT-ND vaccine is a safe and effective solution for IBD control and bird immunisation on high-risk farms.

**Keywords:** IBDV, vaccine, immune complex, recombinant, very virulent IBD

## **Evaluation of organ lesions in broiler chickens exposed to mycotoxins**

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Mycotoxins globally threaten poultry industries, impacting performance and health. In the case of high concentration of mycotoxins in the feed, chickens can develop clinical lesions of mycotoxicosis. This study aimed to identify the organ lesions caused by different mycotoxin contamination and evaluate the efficacy of a mycotoxin deactivator in preventing the appearance of these lesions. Day-old Ross 308 chicks (12 birds per group) were exposed for 20 d to various conditions: either no mycotoxin (NC), a combination of deoxynivalenol (DON; 25 ppm) and fumonisins (FUM; 100 ppm) with (UP) or without (PC) the supplementation of a mycotoxin deactivator (Unike® Plus at 0.5 kg/t; Adisseo, France), or ochratoxin A at 5 ppm (OTA). These exceeded regulatory limits for experimental clarity. At 20 d, birds were sacrificed, and liver, oral mucosa, gizzard, small intestines, kidney and lymphoid tissues were evaluated to study clinical lesions efficiently. Blood samples were collected at 18 d. Body weight and feed consumption were recorded at 0, 7, 12 and 20 d. Despite the limited number of animals, from d7 to d20, the OTA group exhibited an important decrease of the FI (-39%) and BW (-55%) compared to the other treatments, resulting in a higher FCR (+32%). Oral lesions, likely attributed to DON, were observed next to tongue in the PC group. In the same group, we also identified gizzard erosion that did not appear in the 3 other groups. PC birds had reddish congested intestine mainly in the duodenum but also in the jejunum of some birds, accompanied by increased diarrhea. The OTA group showed enlarged kidneys, a known target organ for OTA, and exhibited thymus hypotrophy, suggesting immune suppression. The blood Gamma Glutamyl Transferase, biomarker of liver dysfunction and biliary tract damage, increased in OTA (+122%) and PC (+78%) groups relative to NC and seemed to be controlled in the UP group (-16% vs PC). Blood uric acid, a kidney failure biomarker, reached a high concentration (11.0 mg/dl) in the OTA group compared to control (5.4 mg/dl). In conclusion, the birds co-contaminated with DON/FUM were more affected by organ lesions, especially in oral mucosa, gizzard and small intestine while chickens exposed to OTA experienced targeted kidney failures. This showed that relying only on performance metrics is insufficient for assessing mycotoxin risks and a preventive application of a reliable mycotoxin deactivator can limit the occurrence of the clinical lesions.

**Keywords:** Mycotoxin, necropsy, liver, intestine, biomarker

## **Molecular longitudinal surveys reveal circulation of chicken anemia virus genogroups IIIa and IIIb in Greece broiler breeder**

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Chicken Infectious Anemia Virus (CIAV) affects young chickens causing immunosuppression and a condition named Chicken Infectious Anemia, widespread in all poultry-producing countries; however, the course of the infection can also be subclinical. To control the clinical disease in the offspring vaccination of broiler breeders is widely applied. In order to molecularly characterize CIAV strains circulating in broiler breeders in Greece, longitudinal surveys (from day old to 37 weeks of age), or one-off samplings, were performed in eight flocks located in the Epiro region, collecting a total of 32 samples. Environmental dust from fan louvers, or organs from deceased birds, were collected at defined intervals and analyzed for CIAV detection by a nested PCR protocol amplifying the full VP1 gene. Eleven samples tested positive and underwent sequencing and phylogenetic characterization. Six detected strains possessed aminoacidic residues peculiar of virulent strains. All strains clustered into genogroups IIIa and IIIb and co-circulated in the same flock, possibly due to multiple virus introductions probably due to biosecurity breaches. Two strains of genogroup IIIb showed high similarities with vaccine strains: one clustered with the Del-Ros vaccine, having 98% nucleotide sequence identity, and the other one clustered with the 26P4 vaccine, having 99.7% nucleotide identity; interestingly these vaccines were not used in the farms. The reported data contribute to increase the existing information on molecular characteristics of native CIAVs circulating in Europe.

**Keywords:** CIAV; Chicken anemia; Broiler breeders; Dust; VP1 gene; PCR; Molecular Characterization

## **Extensive adenovirus molecular survey demonstrates predominant circulation of FAdV-D in Italian broiler flocks**

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Adenovirus that affects birds are included into 3 genera, namely Atadenovirus, Aviadenovirus and Siadenovirus. These viruses are considered as widely prevalent and are commonly found in poultry and many of them circulate in birds with no apparent signs of disease. However, others are primary pathogens causing disease conditions e.g., adenoviral gizzard erosion (AGE), inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS) and egg drop syndrome (EDS'76) and are responsible for significant economic losses. In the present study a PCR survey was performed on 61 commercial broiler flocks, located in a densely populated poultry area in North East Italy, in order to increase the knowledge on the diffusion and the molecular characteristics of adenoviruses circulating in Italian broiler flocks. Organs like bursa of Fabricius, gizzard, liver, pancreas or spleen from deceased birds were collected and Adenovirus detection was attempted by a nested PCR protocol using two degenerate consensus primer pairs targeting a partial sequence of the pol gene, which is highly conserved. A total of 29 adenovirus strains were detected. Sequence analysis revealed that 27 strains belonged to genus Aviadenovirus and the remaining 2 to genus Atadenovirus (EDS'76 virus). Fowl Adenovirus D (FAdV-D) was the Aviadenovirus prevalently detected (21 strains) followed by Fowl Adenovirus E (FAdV-E, 4 strains) and Fowl Adenovirus A (FAdV-A, 2 strains). Results obtained showed that Adenoviruses are widespread in Italian broiler flocks with predominant circulation of FAdV-D species. Additional studies are needed to serotype the strains and investigate if FAdV strain circulation in broiler flocks play a role in affecting birds health causing underestimated cases of IBH.

**Keywords:** Fowl adenovirus; broiler; molecular characterization; Atadenovirus; pol gene; PCR

## **Cranial osteomyelitis and meningoencephalitis associated with *Pasteurella canis* in broiler chickens**

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*Pasteurella multocida* is the causative agent of fowl cholera, a contagious disease that affects domesticated and wild birds. Chronic presentation of fowl cholera can cause torticollis and opisthotonos due to meningeal infection. *Pasteurella canis* is a part of the normal microbiota of healthy companion animals, especially dogs. In humans, infections associated with *P. canis* have been reported on multiple occasions, while in animals, *Pasteurella canis* has been associated with endocarditis in dogs, and pneumonia in non-human primate black-tailed marmoset. Here, we report the first case of cranial osteomyelitis and meningoencephalitis caused by *Pasteurella canis* infection in broiler chickens in Chile. Prior to the laboratory necropsy, the chickens presented severe disorientation, torticollis, opisthotonos, and difficulty standing. The birds were euthanized by cervical dislocation, and a post-mortem examination revealed cranial osteomyelitis, concentrated in the ventral area close to the ear, with caseous material, meningoencephalitis, and mild nasal discharge. No other significant injuries were observed in the upper respiratory system, joints or internal organs. Brain, cranial bone, and middle ear samples from the affected birds were obtained aseptically, plated on tryptone soy agar with 5% blood and MacConkey agar, then incubated in 5% CO<sub>2</sub> at 37°C for 24 h. Brain and cranial bone sections were collected, fixed in 10% neutral-buffered formalin, and routinely processed for histopathological examination. After a 24-h incubation the cultures from the brain, cranial bone, and middle ear presented smooth, mucoid, nonhemolytic colonies grayish-white colonies compatible with *Pasteurella* spp. on blood agar plates. Pure subcultures were generated from single colonies on a blood agar plate for Gram stain and biochemical testing using the Vitek® 2 Compact identification system (BioMerieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. These tests allowed the identification of *Pasteurella canis* with 99% accuracy. In the histopathological examination, brain samples presented moderate vasculitis, and cranial bone samples presented severe osteomyelitis.

Keywords: poultry, infections, zoonotic pathogen, dogs



**Foot pad dermatitis survey on three hungarian broiler farms****M. Tóth<sup>2,1</sup>, T. Pap<sup>1</sup>, S. Kulcsár<sup>2</sup>, Z. Ancsin<sup>2</sup>, M. Erdélyi<sup>2</sup>, M. Kovács-Weber<sup>1</sup>**<sup>1</sup>Department of Animal Husbandry Technology and Animal Welfare; Institute of Animal Sciences; Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary, <sup>2</sup>Department of Feed Safety; Institute of Physiology and Nutrition; Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary*Presenting author: Toth.Mark.5@phd.uni-mate.hu*

Foot pad dermatitis (FPD) is one of the major problems in large-scale broiler production. Ulceration of the plantar surface results in loss of production and income and raises questions regarding animal welfare and human nutrition. As FPD is a common problem at broiler farms, a survey was conducted at three Hungarian farms with different technological background. There were 18 barns altogether equipped with modern housing technology, however, they are varied greatly in size, age and construction. Ross 308 hybrid flock was grown from hatching till slaughter for 42 days in each building (... birds/m<sup>2</sup>). Cereal straw was used as bedding. One production cycle was tested at each building, and as part of the survey mortality, the microbiological characteristics of the excreta (*Clostridium perfringens*, *Staphylococcus aureus*, total microbial count) and the evolution of the moisture content in the litter were recorded. Foot pads were also monitored within 2 weeks periods and were evaluated according to the Animal Welfare Quality scoring system (score 0 to 4). According to the data mortality was highly variable (3.06%-11.08%) among different buildings. Same was true for the total germ count and microbial composition of the excreta. Total microbial count was between 1.3 x 10<sup>5</sup> and 9.6 x 10<sup>9</sup> CFU/g which agrees with the literature data. No *C. perfringens* was isolated in any of the 18 stalls. Eight barns were free of staphylococcus, while in the positive stalls it ranged between 4.0x10<sup>1</sup> and 2.5x10<sup>3</sup> CFU/g. Considering moisture content of the bedding varied intensively also, at Week 2 - 37.6-59.9%; Week 4 - 37.9-54.2% and week 6 - 31.4-56.0% among the barns, respectively. Foot pad conditions were unfavourable. The average foot pad score was somewhat higher than 1 point at 2 weeks of age, already, and within the next two weeks period it raised beyond 3, while in the last week the average score has reached the maximum 4 points in several stables. These latter findings highlight the seriousness of the problem, thus, further studies are needed to reveal the factors inducing FPD and how to eliminate them. The research was supported by the 2020-1.1.2-PIACI-KFI-2021-00323 project.

Keywords: Foot pad dermatitis, FPD, litter quality, microbiology

**Evaluation of haemagglutination Inhibition Assay (using FAdV- sensitized tanned chicken RBCs) to determine Humoral response to the challenge of novel Fowl Adenoviruses isolated from Pakistan during 2019- 2022****I. Zaheer<sup>1</sup>, K. Saleemi<sup>1</sup>**<sup>1</sup>University of Agriculture Faisalabad, Pakistan, Faisalabad, PakistanPresenting author: [dr.izahaer@gmail.com](mailto:dr.izahaer@gmail.com)

In recent years, Fowl adenovirus (FAdV) associated diseases have been re-emerging as outbreaks in commercial poultry and their parent flocks despite the extensive practice of vaccination against the disease in commercial flocks in Pakistan. Therefore regular serological monitoring/ antibody (Ab) titers against avian diseases have become a crucial tool to monitor the efficiency of vaccination and/or any field disease outbreak. Therefore, the current study consists of two parts; In the first part, there was an experimental trial consisting of 150 broiler chickens of 3 weeks of age, equally divided into 3 groups viz; group A (Negative control), while groups B and C were challenge groups with; FAdV-4 isolate (MN754024.1) and FAdV-11(MN754021.1) respectively. The parameter of interest from the trial was; antibody titer monitoring through Indirect Haemagglutination Assay (IHA) in log<sub>2</sub> values. One-way ANOVA statistical tool at  $\alpha$  5% was executed at IBM SPSS version 22 to compare the means of the groups. In both groups B and C, the antibody titers started to increase since 1 d.p.i  $3.17 \pm 0.75$ , and  $3.33 \pm 0.52$  and kept increasing significantly through day 3 and 5 d.p.i. while peak titers were observed at 7 d.p.i.  $8.67 \pm 0.52$ , and  $8.33 \pm 0.52$  respectively. A negligible drop of antibody titers was observed at 14 and 21 d.p.i. through IHA testing. In the second part of the study, 92 broiler farms from the Faisalabad division were randomly sampled, and the antibody titer from 64 vaccinated farms (with either one or both FAdV-4 and -11 vaccines) farms ranged from 5.5 to 8.11 ( 3 weeks post-vaccination). In 28 broiler farms that were exposed to the FAdV- 4 or -11 challenge, the antibody ranged from 6.8 to 8.8 (3 weeks post-infection). The results of the study suggest that IHA can be a routine field test for the titer monitoring of different serotypes of FAdVs because it is convenient, requires less instrumentation, and is economical to detect antibody titers against different serotypes of FAdVs in developing countries' disease diagnostic set up, however, the results of this study further require a comparative follow-up study among IHA and other commonly used antibody titer monitoring methods such as ELISA.

Keywords: Broilers, Fowl Adenovirus, FAdV-4, FAdV-11, histopathology; immunology

## Phylogenetic analysis of partial sigma $\sigma$ A of avian orthoreovirus

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Avian reoviruses (ARVs) are the member of Reoviridae family in the genus Orthoreovirus. The virus is ubiquitous among domestic poultry and other avian species. They are nonenveloped viruses composed of a double concentric capsid with an external diameter of 80–85 nm that encloses 10 double-stranded RNA genome segments. The ARV replicates in the gut of avian species and pathogenic strains can affect tendon and liver. Many methods have been developed for the diagnosis of ARV infection viz. nucleic acid based detection using  $\sigma$ B and  $\sigma$ C gene based PCR, LAMP, Real time LAMP and antibody based method like ELISA, RIA etc.  $\sigma$ A gene based PCR can be a suitable option as it has conserved nucleotide sequence. The present study describe the development of the reo virus specific reverse transcriptase polymerase chain reaction (RT-PCR) for the amplification of partial  $\sigma$ A gene. For detection of nucleic acid in the suspected sample, a PCR assay was standardized using cell culture adapted ARV. The partial  $\sigma$ A gene was amplified from ARV-infected chicken embryo fibroblast (CEF) cells using reverse transcription polymerase chain reaction (RT-PCR). The assay was used to check 10 suspected spiked poultry samples of ARV and found to be able to detect ARV nucleic acid. Further, sequencing of the amplified  $\sigma$ A gene was performed to obtain its nucleotide sequence which was submitted in NCBI. The evolutionary history was inferred by using the Maximum Likelihood method using Tamura-Nei model. The Partial Sigma A gene coding for S protein was used for amplification. On PCR amplification it showed band of 189 bp. The amplified band was subjected to Sanger sequencing and then nucleotide sequence was obtained which was further analyzed by MEGA 11 tool and maximum likelihood tree was constructed. There were a total of 189 positions in the final dataset. The tree with the highest log likelihood (-1851.59) was finalized. This analysis revealed the similarity of Sigma a isolate with China and USA. The results of this study provides insight that  $\sigma$ A gene can be a very diagnostic target for ARV detection as it is conserved across various genotypes found world over. The developed method is a sensitive method which can be used as ARV diagnostic. This strategy may further be utilized for detection and diagnosis of avian reovirus in avian species. The phylogenetic analysis using maximum likelihood tree involved 101 nucleotide sequences, revealed its lineage similarity with China and USA lineage.

Keywords: Avian reoviruses (ARVs); sigma A ( $\sigma$ A) gene; Chicken Embryo Fibroblast (CEF) cells; Reverse Transcription Polymerase Chain Reaction (RT-PCR); Phylogenetic analysis

**Comparative analysis of a partial fragment of  $\sigma$  and  $\lambda$  gene of avian reovirus****L. Rathore<sup>1</sup>, R. R<sup>1</sup>, R. Agarwal<sup>2</sup>, P. Gupta<sup>1</sup>, D. Kumar<sup>1</sup>**<sup>1</sup>Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India,<sup>2</sup>Biological Product Division, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India*Presenting author: melaxmirathore@gmail.com*

Avian reovirus (ARV) possesses a substantial threat to poultry health, globally impacting the poultry industry. It features a double-stranded RNA genome comprising 10 segments, with sizes ranging from 23,000 to 27,000 base pairs based on strain and genetic variations. This study evaluates the evolutionary relationships of avian reovirus and the possibility of reassortment among different pathotypes of avian reovirus. Total RNA was isolated from ARV infected Vero cells, transcribed into cDNA. RNA was used for amplification, sequencing and characterization of partial segments of  $\sigma$ A,  $\sigma$ B and  $\lambda$  A by Sanger's sequencing. The nucleotide sequence and their deduced amino acid sequences were used in pairwise comparisons to determine the extent of the nucleotide and amino acid sequence identity and diversity. Phylogenetic trees based on the cDNA sequences were constructed by using the branch-and-bound algorithm of the parsimony program PAUP to determine the best-fitting tree for genes of avian reovirus in avian species. The nucleotide sequence of the S1 and L1 genes of avian reovirus differs from those reported for the same genes of other reovirus strains. Various sequences were analyzed, and it was found that conservative substitutions were more common than non-conservative substitutions in these fragments. The sequence identity showed that the fragments of the  $\sigma$ A,  $\sigma$ B, and  $\lambda$ A genes had a typical pattern of the neutral theory of viral evolution, where conservative substitutions are more prevalent than non-conservative ones. Analysis of the  $\sigma$ A,  $\sigma$ B and  $\lambda$ A fragments of the S-class genome segment that contain the nucleotide sequences, which are identical to those of the genome segments of both avian and mammalian reoviruses. These nucleotide sequences are expected to play a significant role during virus replication. Thus, the presence of these conserved sequences suggests that the viruses may share a similar replication mechanism in both avian and mammalian reoviruses.

**Keywords:** Avian reoviruses (ARVs);  $\sigma$ A,  $\sigma$ B and  $\lambda$  A gene; Chicken Embryo Fibroblast (CEF) cells; complimentary deoxyribonucleic acid (cDNA); Polymerase Chain Reaction (RT-PCR)

## **Circulating genotypes of *Mycoplasma* spp. and their molecular profiles of antimicrobial resistance as a contribution to the diagnosis of avian mycoplasmosis in Colombia**

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*Mycoplasma gallisepticum* (Mg) and *M. synoviae* (Ms) are pathogens of importance in poultry considering that their detection is frequent. This disease is characterized by a chronic and sometimes subclinical disease, which negatively affects the productive parameters of poultry farms; it is also part of the Avian Chronic Respiratory Disease (CRD) when interacting with other viral and bacterial agents that complicates the diagnosis. For diagnosis, *in vitro* culture of the agent is used, being the gold standard. Other techniques such as serology and molecular tests are complementary as part of an integral strategy. However, there is control of the disease by vaccination and administration of antibiotics, problems of reversion of some vaccine strains and the presentation of mutations of genes associated with antimicrobial resistance (AMR) have been identified. This information is scarce in Latin America and even more so in Colombia; therefore, it is important to characterize circulating genotypes and mutations associated with AMR in Mg and Ms detections of commercial poultry samples from different regions of the country. For this purpose, the MLST molecular technique was used to determine the genetic variability of Mg and Ms previously detected in samples received between 2019-2023 at the Laboratory of Molecular Biology and Virology of the Universidad Nacional de Colombia. Likewise, the first screening of mutations related to RAM to fluoroquinolones, specifically of the *gyrA* and *parC* genes involved in bacterial replication, will be performed by PCR and sequencing techniques. These results will make it possible to know the genotypes circulating in the country and their possible relationship with mutations associated with AMR, generating a baseline of this problem in Colombia. Consequently, it will contribute to the updating of diagnostic techniques used in veterinary laboratories for avian mycoplasmosis.

Keywords: poultry, Mycoplasmosis, genotyping, Antimicrobial resistance (AMR), MLST

## **Chemical and fatty acid profile of chicken meat from different housing systems**

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The purpose of present study was to evaluate the effect of three different housing systems on quality characteristics of chicken meat chemical and fatty acid profile of thigh. Chickens raised on commercial farms in three different production systems were analyzed. Cobb 500 hybrid was used for testing conventional chickens, fattening period lasted 42 days. The Sperberg breed were used for the "free range" system, reared for 56 days, and from organic production, male Red Renger chickens, whose fattening period lasted 81 days. At the end of the rearing period, 20 birds (10 males and 10 females) from each system were slaughtered and chemical composition (dry matter, protein, fat and moisture contents) and fatty acid profile of thigh meat were measured. The results showed that the housing system had a statistically significant influence on the some characteristics of chicken meat. Chickens raised in an organic system had the lowest percentage of protein in the thigh (18.27), as well as the moisture content (63.79), while the percentage of protein and moisture in chickens from conventional system (19.69; 73.58) and free range (19.34; 75.05) was statistically significantly higher ( $P<0.05$ ) compared to organic chickens. The fat content was statistically higher ( $P<0.05$ ) in the organic system compared to the other two systems. The total content of SFA, MUFA and PUFA was not statistically different between all three production systems. Saturated fatty acids (SFA) were most prevalent in the meat of free range and organic chickens, followed by conventional chickens. Free range meat showed the most saturated fatty acids, stearic (C18:0) and arachidic (C20:0), while conventional meat had slightly more myristic (C14:0) fatty acids, but without statistically differences. Regarding monounsaturated fatty acids (MUFA), chickens from organic farming showed the statistically highest ( $P<0.05$ ) content of oleic fatty acid (C18:1), followed by conventional and free range chickens. Observing the chemical and fatty acid profile of meat, it can be concluded that alternative housing systems, compared to conventional ones, do not imply better quality of chicken meat.

**Keywords:** Chickenmeat, chemical composition, fatty acid profile

**Detection of antibodies against the chicken infectious anaemia Virus (CAV) in backyard birds in rural areas in Pichincha province, ecuador****M. Cisneros<sup>1</sup>, J. Romero<sup>1</sup>**<sup>1</sup>UNIVERSIDAD CENTRAL DEL ECUADOR, Quito, Ecuadaor

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Chicken Infectious Anaemia Virus (CAV) is an immunosuppressive disease of gallinaceae that affects the lymphoid tissue originated in the thymus (T lymphocytes) as well as erythrocyte precursor cells (hemocytoblasts) in the bone marrow, causing anemia, intramuscular bleeding and thymus atrophy, its transmission occurs both horizontally and vertically. The objective of this study was the detection of antibodies against the Avian Infectious Anemia virus using competitive ELISA in backyard birds from rural areas in Pichincha province. 384 birds samples from 30 weeks older and younger, males and females, were randomly taken in 54 rural parishes from Pichincha province. 314 birds presented seropositivity, 163 birds presented very high titers  $<0.2$  S / N, 47 birds presented high titers between  $0.2 - 0.4$  S / N, 41 birds with moderate titers between  $0.4 - 0.6$  S / N, 63 birds with of low titers between  $0.6 - 0.8$  S / N and 70 birds were negative with values higher than  $0.8$  S / N. Hematocrit were evaluated in all sampled birds, 7 birds with low hematocrits were identified. In total, 314 positive birds were counted, where 6 birds presented antibodies against the anemia virus with low hematocrit and 308 birds with protective antibodies with normal hematocrit. In this study, a seroprevalence of 81.77% was obtained, which suggests the permanent presence of the virus in rural areas of Pichincha province

Keywords: Chicken Anemia Virus; backyard chickens; ELISA; immunosuppression; hematocrit

## **Multi-omics analyses of chicken breast with spaghetti meat myopathy**

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Spaghetti meat (SM) is an emerging chicken breast myopathy characterized by a loss of tissue integrity in the breast muscle resulting in the meat surface having loosely connected muscle fibers resembling spaghetti pasta. To understand biochemical and physiological alterations occurring in SM, we conducted multi-omics analyses including proteomics, metabolomics, and lipidomics using a liquid chromatography-mass spectrometry (LC-MS) method. Differentially abundant proteins, metabolites, and lipid species were identified in SM compared with normal control (CON) meat and the results were integrated for biological interpretations. Chicken breast fillets (8 CON, 8 SM) were collected from commercial broiler chickens at a commercial processing plant. Breast muscle samples were dissected into smaller pieces and flash frozen in liquid nitrogen. Chicken breast meat samples were homogenized by bead blasting, homogenates were digested by trypsin, and lysates were extracted. Meat extracts were subjected to LC-MS analysis. Differential abundances were calculated using t-tests and calculated p-values were adjusted to false discovery rate (FDR). A total of 2593 molecules, including 1903 proteins, 506 lipids, 181 metabolites, and 3 electrolytes were identified. Of those, differentially abundant molecules, based on  $FDR < 0.05$ , included 151 proteins, 17 lipids, 17 metabolites, and no electrolytes. Differentially abundant proteins, lipids, and metabolites indicated that SM exhibited: 1) increased nonsense mediated decay pathways; 2) decreased glucose metabolism pathways; 3) decreased contents of NAD<sup>+</sup> and lactic acid; 4) increased triglycerides (TG); and 5) decreased plasmalogen phosphatidylcholines (Plasmalogen-PC), acylcarnitines (AC), and phosphatidic acids (PA). Results in this study provide insights into biochemical alterations that may be associated with susceptibilities of chicken breast to SM condition and quality of SM defects in chicken breast meat.

**Keywords:** Chicken breast meat, Spaghetti meat myopathy, multi-omics analyses, proteomics, metabolomics, lipidomics, differential abundance



## The impact of sustainable protein sources on meat quality of Redbro broiler chickens

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Citizens increasingly prioritise environmentally friendly and animal welfare-focused food (European Commission, 2023). In the UK, 130 companies have committed to the Better Chicken Commitment (BCC) promoting the use of slow growing breeds by 2026. Diet and breed can impact meat quality and environmental footprint of broilers (Mir et al., 2017; Mostert et al., 2022). Therefore, this study evaluated the impact of dietary specification and replacement of soybean meal (SBM) with sustainable protein sources on the meat quality of Redbro broiler chickens. Six hundred and thirty as-hatched day-old Hubbard Redbro broilers were randomly allocated to 6 dietary treatments, each having 7 replications of 15 birds. Diet 1 was a standard Ross 308 specification wheat-SBM diet. Diets 2-6 met the BCC specification. Diet 2 was a wheat-SBM control whilst diets 3, 4 and 5 partially replaced SBM with lupins, beans and peas, and sunflower meal respectively. Diet 6 totally replaced SBM with a mixture of the sustainable ingredients. The diets were fed from 0-42 d age. At 42 d age, one bird per pen was electrically stunned and killed by exsanguination, and both breasts were dissected. The pH of the right breast was measured immediately after dissection and at 48 hours postmortem. The left breast was scored for white striping (WS), woody breast (WB) and cohesion defects, and colour ( $L^*$  indicating lightness,  $a^*$  redness,  $b^*$  yellowness) was assessed 24 hours postmortem. The toughness (TM), firmness (FM) and 48-hour drip loss (DL) of the left breast was determined. The overall body weight (BW) for the growth period was also determined. Data were analysed by ANOVA and Tukey's multiple range test. The average BW at 42d age was 1828 g, and did not differ between birds fed diets 1, 3, 4 and 6 ( $P > 0.05$ ). Birds fed diets 2 and 5 had lower BW compared to diets 1 and 4 ( $P < 0.001$ ). The  $L^*$  score in birds fed diet 1 did not differ from the rest of the birds ( $P > 0.05$ ). No differences ( $P > 0.05$ ) were observed across the three treatment groups for  $a^*$ . Compared to diet 1, an increase in  $b^*$  was observed in birds fed diets 3 and 6 ( $P < 0.001$ ). There were no differences ( $P > 0.05$ ) among the rest of the studied breast meat quality variables. The observed differences in colour may be due to the different feed ingredients used but did not follow changes in final BW. The study demonstrated that further research on inclusion of sustainable protein sources in diets for slow growing broilers is warranted.

Keywords: Alternative protein sources; slow growing broilers; Redbro; growth; meat quality

## Effects of dietary inclusion of different lentil varieties on broilers' meat quality

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Lentils play a significant role as a plant-based protein source, and their inclusion in broilers' diet is essential to maintain dietary protein balance in poultry production. The study investigated the effect of dietary inclusion of different varieties of lentil on meat quality. The 42-day trial was performed on 250, day-old ROSS 308 chicks assigned to 5 groups (C; E1; E2; E3; E4), kept in an experimental hall, on permanent wood shaves litter. Compared to the conventional diet (C), the experimental diets included 20% different lentils varieties: yellow (E1), red (E2), green (E3) and black (E4). The protein content varied between 23.94% (green lentil) and over 26% (yellow and black lentil), along with a high lutein content: 20.13 mg/kg (yellow lentil), 23.99 mg/kg (red lentil), 24.56 mg/kg (green lentil), and 32.83 mg/kg (black lentil), which increased the dietary lutein concentration to 16.49 mg/kg (E1), 16.59 mg/kg (E2), 17.83 mg/kg (E3), and 19.18 mg/kg (E4) compared to 14.68 mg/kg in group C. At the end of the experiment, six broilers/group were sacrificed and tissue samples (breast and thigh) were collected for lutein, colour assessment for L\* (lightness), a\* (redness), b\* (yellowness) and quantifiable properties of broilers' meat. The highest lutein concentration ( $p=0.043$ ) in breast samples was registered for E2 group (2.39 mg lutein/kg) compared to all experimental groups (1.74 mg/kg, E1 and E3 and 1.66 mg/kg, E4) and to C group (1.12 mg/kg). Similar results were observed in the thigh samples. There were no significantly ( $p\geq 0.05$ ) differences concerning pH content and L\* meat colour parameters for breast and thigh samples. The a\* parameter values measured in breast samples were statistically significant ( $p=0.011$ ) in E3 (-0.91) compare to E4 (-1.94) group, while for thigh samples, the statistically higher values were noticed on E2 (2.54) compared to E1 (-0.98), E3 (0.48), E4 (0.68) and C (0.93) groups. The b\* parameter values for both, breast and thigh, increased concomitantly with lentils variety dietary inclusion (E1<E2<E3<E4) compared to C group. The collagen content in thigh samples was significantly higher ( $p = 0.001$ ) for E1, E2, E4 compared to C and E3 groups while for breast samples, the same groups registered significant decreasing values compared to C group. In conclusion, lentils can be considered an effective alternative protein source in broilers' diet, with significant impact on meat quality.

Keywords: broiler, diet, lentils, lutein, meat quality

## **Nuclear Magnetic Resonance and Dielectric Spectroscopy as valuable tools to detect Wooden Breast abnormality in broiler chickens**

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Over the past few years, the poultry industry is facing the occurrence of growth-related breast muscle abnormalities such as Wooden Breast (WB) that strongly impairs the appearance and the quality traits of the meat belonging to fast-growing genotypes. This abnormality is associated with high rejection and downgrading rates, thus resulting in significant economic losses for broiler industry. Thus, the present study aimed at evaluating the effect of WB on water mobility through Dielectric Spectroscopy (DS) in the microwave range and protons' transverse relaxation time (T2) by Time-Domain Nuclear Magnetic Resonance (TD-NMR) to evaluate the possibility of exploiting this feature to develop sensors which can be implemented on-line to distinguish normal and affected breasts. A total of 124 Pectoralis major muscles was selected from the same flock of fast-growing broilers (males, 42 d and 2.8 kg average live weight) at 3 h post-mortem and classified as unaffected (NORM) or WB (n = 62/group). Samples were then analyzed by DS in the microwave range and TD-NMR to study the properties (i.e., relative intensity and mobility) of the proton populations ascribed to the water within the meat structure as well as its interaction with other components, including ionic molecules (by measuring dielectric constant and loss factor). TD-NMR data were then analyzed by Student's t-test and considered significant at  $P < 0.05$ , while DS data were processed using Partial Least Squares Discriminant Analysis (PLS-DA). Compared to NORM, a tendency towards high dielectric constant and loss factor values was found in WB, thus suggesting higher water mobility and a solvate capacity within the abnormal muscles. PLS-DA classified NORM and WB up to 96% of the external validation data set. This outcome was further confirmed by TD-NMR evidencing a significant ( $P < 0.001$ ) increase in the relative intensity of the proton population ascribable to the extra-myofibrillar water along with a reduction ( $P < 0.001$ ) of the proportion occupying the intra-myofibrillar spaces. These outcomes, together with the higher mobility (as depicted by longer T2) of the water observed in WB, may be ascribed to the re-organization of the skeletal muscle structure associated with the onset and progression of the WB abnormality. Based on the above, TD-NMR and DS may represent valuable tools to objectively identify WB meat at slaughter plants after breast deboning and divert them for exploiting them at their maximum potential.

**Keywords:** Broiler; breast abnormalities; Nuclear Magnetic Resonance; Dielectric Spectroscopy; water mobility

## **Myopathies in broiler chicken breasts in a slaughterhouse from Argentina: prevalence, severity, and attributes contributing to the occurrence**

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Over the last 50 years, there has been a successful optimization of meat bird yields. However, the production systems have given rise to myopathies known as "wooden breast"(WB), "white striping"(WS) and "spaghetti meat"(SM), that cause economic losses throughout the production chain. Affected breasts can be identified by distinctive macroscopic features. Argentina has held a position in the main producers and exporters of chicken meat. The objective of this study was to determine the prevalence and severity of each myopathy in broiler breasts in a slaughterhouse in Argentina and assess the degree of contribution of certain attributes to their occurrence. 476 Cobb 500 live birds were randomly sealed. Each chicken's sex, breeding time, live weight, and semester of slaughter were recorded. After slaughter, the breasts from the sampled chickens were taken, and data of breast weight and severity of each myopathy were recorded. Subsequently, the percentage of breast yield was calculated. So, the prevalence of myopathies was determined. To determine which attributes influenced the frequency of each myopathy's occurrence, a discriminant statistical analysis and principal component analysis (PCA) was conducted. The results indicated that 44.2% of the birds presented WB (7.1% severe); 49.1% exhibited WS (9.4% severe); while only 2.9% of the breasts showed SM. Specifically, 37.8% of all breasts were considered normal, and the total of affected breasts had: 18.6% some degree of WB only, 25.7% only WS and 2.7% only SM. Besides, 51.0% had both WB and WS; 0.7% WS and SM, and in 1.4% of breasts, three myopathies were simultaneously. The attributes that significantly contributed to the prevalence increment were live bird weight, breast weight and breast yield. An increase in live bird weight, breast weight, and breast yield was directly associated with a higher risk of finding breasts affected by severe WB and/or WS. In contrast, SM occurred more frequently in small breasts of lightweight females. In conclusion, the prevalence of myopathies and their severity was influenced by chicken characteristics resulting from efforts to optimize animal productivity. Argentina is not exempt from the issues posed by myopathies in major poultry hubs worldwide. The high percentage of affected birds serves as a starting point, highlighting the need to explore strategies for mitigation to prevent losses negatively impacting Argentina's regional economies.

**Keywords:** myopathy; wooden breast; spaghetti meat; white striping; prevalence; Argentina

**Nutritional value and quality of poultry meat affected by white striping, wooden breast and spaghetti meat myopathies****M. Bošković Cabrol<sup>1</sup>, A. Huerta<sup>1</sup>, M. Pravato<sup>1</sup>, F. Bordignon<sup>1</sup>, M. Birolo<sup>2</sup>, M. Petracci<sup>3</sup>, G. Xiccato<sup>1</sup>, A. Trocino<sup>1,2</sup>**

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The present study aimed to evaluate the effects of white striping (WS), wooden breasts (WB), and spaghetti meat (SM), on p. major meat quality traits, chemical and fatty acid composition, and mineral content. Abnormal breasts were obtained at commercial slaughtering (41 d of age) at the end of an experimental trial in which 576 one-day-old chicks (Ross 308) were allocated to 36 pens according to a 3×2 experimental arrangement encompassing three *C. vulgaris* dietary inclusion levels (0%, 3%, and 6% replacing equal quantities of soybean meal), two-room temperature conditions (heat stress, i.e. 28°C vs. thermoneutral conditions), and two sexes. At the macroscopic evaluation, the breasts of 180 carcasses were classified into one out of four classes: normal (85 samples), WS (46 samples), WB (16 samples), or SM (33 samples). The presence of myopathies did not affect breast meat pH and L\* values ( $P>0.05$ ), whereas the a\* index was lower in WB breasts compared to WS, SM, and normal breasts ( $P<0.05$ ), and b\* index lower in WB than WS meat ( $P<0.05$ ). Compared with normal meat, cooking losses in WB samples were higher ( $P<0.01$ ), whereas compared to WS meat they exhibited higher shear force ( $P<0.01$ ). As for oxidation, no significant changes were found in MDA and carbonyl contents between the different groups ( $P>0.05$ ) as it was for moisture, fat, and ash content. Differently WB meat showed lower ( $P<0.001$ ) protein content (19.9%) compared to the other samples (21.9% in normal, 21.5% in WS, and 21.7% in SM). The presence of WS had a minor impact on the fatty acid composition of the muscle, resulting in a lower content of linoleic acid compared to WB meat ( $P<0.05$ ) and a higher amount of  $\alpha$ -linolenic acid compared to normal meat ( $P<0.01$ ). WB meat exhibited a higher amount of linoleic acid, docosahexaenoic acid (DHA), n-3 polyunsaturated fatty acids (PUFA), n-6 PUFA, and total PUFA ( $P<0.05$ ) compared to normal and SM meat, whereas, C18:3 n-6 differed only between WB and normal breasts being higher in the abnormal breasts ( $P<0.01$ ). The presence of WS, WB, and SM did not affect the amino acid composition and mineral content of breast meat ( $P>0.05$ ). In conclusion, the presence of WB impaired meat quality, protein content, and fatty acid composition to a greater extent than the presence of WS and SM. Acknowledgement: The authors are grateful to DeMYO project (European Union's Horizon 2020 research and innovation program under the MSCA grant agreement No 101063055).

Keywords: myopathies; p. major; color; shear force; fatty acids

## **Comparison of meat quality traits among the main chicken categories marketed in Italy: 1. Technological properties**

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In Italy, chicken meat is mainly harvested by birds slaughtered at three target weights: light- (L, 1.8-2.0 kg used as for rotisserie type products), medium (M, 2.5-2.8 kg used for cut-up products), and heavy-size (H, 3.0-3.8 kg used for both cut-up and further processing) chickens obtained by different farming systems (conventional, free-range and organic) using either fast (FG) or medium-growing (MG) genotypes. This study aimed at evaluating chicken meat quality belonging to more common five marketed in Italy: L under either conventional (LC, FG slaughtered at 32-35d) or free-range (LF, MG slaughtered at 56d) systems, M farmed under conventional system (MC, FG slaughtered at 36-42d), H farmed under either conventional (HC, FG slaughtered at 45-50d) or organic (HO, FG slaughtered at 81d) systems. A total of 625 breast fillets (n=25/flock) belonging to 25 flocks (n=5 flocks/market category) were used to evaluate the main quality traits (pHu, colour) and technological properties (drip and cooking losses, shear force) of meat. Data were analysed by One-way ANOVA considering the market category as the main effect. When significant, means were separated by Tukey-HSD test ( $p < 0.05$ ). As expected, the market category remarkably affected the main meat quality parameters. Birds farmed under conventional farming system (LC, MC, and HC) exhibited significantly higher ( $p < 0.001$ ) ultimate pH values if compared to LF and HO. These may be ascribed to the effect exerted by the outdoor access that could have affected the muscular glycogen stores available at the time of slaughter. In addition, the lower water holding capacity observed in LC, as evidenced by the remarkably higher drip and cooking losses ( $p < 0.001$  and  $p < 0.001$ , respectively) may be likely due to the reduced degree of meat maturity (i.e. higher proportion of soluble collagen) that may also contribute to explain their higher ( $p < 0.001$ ) tenderness after cooking. Based on the above, chicken breast meat belonging to different market categories has distinctive features that could be effectively exploited for obtaining product differentiation considering consumers' perception and expectations correlated to the different farming systems.

**Keywords:** chicken carcass; market category; Italy; meat quality; technological properties

## **Comparison of meat quality traits among the main chicken categories marketed in Italy: 2. lipid and protein oxidative stability**

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This study aimed at evaluating the effect of the market category, defined according to the farming system (conventional indoor - C, free-range - F, and organic - O) and carcass weight (light – L, 1.8-2.0 kg used for rotisserie products, medium – M, 2.5-2.8 kg used for producing cut-ups, and heavy – H, used for producing cut-ups or processed products), on the oxidative status of breast and thigh meat. A total of 625 breast fillets (N=25/flock) and 500 thighs (N=20/flock) were selected 3 h post-mortem from those belonging to the main chicken categories marketed in Italy (N=5 flocks/market category): L farmed under either conventional systems (LC, FG slaughtered at 32-35 d) or free-range (LF, FG slaughtered at 56d) systems, M farmed under conventional systems (MC, FG slaughtered at 35-42d), H farmed under either conventional systems (HC, FG slaughtered at 45-50d) or organic (HO, FG slaughtered at 81d ). Samples were used to assess colour as well as protein and lipid oxidation by measuring carbonyls and TBARS, respectively. Data were analysed by One-way ANOVA considering the market category as main effect. When significant, means were separated by Tukey-HSD test ( $p < 0.05$ ). Overall, the market category significantly affected the colour parameters and oxidation level of both breast and thigh meat. Indeed, the higher ( $p < 0.001$ ) lightness ( $L^*$ ) observed in HO may likely be ascribed to the different maturity of breast meat and to the eventual development of muscular abnormalities affecting the phenomenon of light scattering. In addition, if compared to those obtained from other market categories, breasts and thighs belonging to LR exhibited the most noticeable differences in colour parameters. This finding may be ascribed to the different foraging behaviour (MG vs. FG) and pasture consumption of chickens with access to outdoor area. In addition, the remarkably higher ( $p < 0.001$ ) redness observed in both breast and thigh meat belonging to LC group may partly account for its higher ( $p < 0.001$ ) TBARS and carbonyls content. In fact, the pro-oxidant effect of heme group (which is, in its turn associated to higher  $a^*$  values) may have favoured the development of oxidative reactions affecting both the protein and the lipid fractions. Overall, the findings obtained in the present study highlight the peculiar predisposition to undergo lipid and protein oxidation of breast and thigh meat obtained from chickens belonging to the different market categories available in Italy that should be carefully considered especially when raw meats are used for further processing.

**Keywords:** Broiler; oxidation; farming system; lipid; protein

## **Breast quality of poultry fed with diet supplemented with live black soldier fly larvae**

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The increasing attention to the use of insects in animal feed is justified by their potential to reduce demand for soybean production, deforestation and the loss of natural resources. One of the most studied and promising insect species is the black soldier fly (*Hermetia illucens* - BSF), which can successfully grow on substrates consisting of various by-products and biotransform low-value organic residual waste into high-value products with high-value proteins. The study aims to evaluate the effects of the inclusion of BSF live larvae, in chicken diet, on breast meat qualitative parameters and fatty acid profile. Male medium-growing chickens (n=120, Label Naked Neck) of 14 days of age were randomly allotted to 12 pens. Each pen was randomly assigned to a dietary treatment (6 pens/treatment, 10 chickens/pen) as follows: a) Control diet (C): commercial feed b) Experimental diet (BSF15): C + BSF live larvae corresponding to 15% of the expected daily feed intake. The commercial diet was distributed ad libitum in all the treatments. At 82 days of age, the chickens were slaughtered. Analysis of variance was performed using the software SAS 9.4. No significant differences were observed in the qualitative parameters (colour, pH and proximate composition) evaluated on the breast samples. Instead, analysis of the fatty acid profile of the breast meat showed some significant changes. We observed a decrease in the proportion of unsaturated fatty acids ( $p < .0038$ ) and an increase in the proportion of saturated fatty acids ( $p < .0033$ ) in BSF15 group. In particular, lauric acid (C12:0) and myristic acid (C14:0) were significantly ( $p < .0001$ ) higher in BSF15 than in the C group. In conclusion, BSF live larvae used in chicken feed can produce meat with comparable quality traits to those fed diets containing only traditional feed ingredients. Therefore, further research efforts are necessary to find strategies for improving the fatty acid profile of the live larvae through substrate modulation in order to provide healthier meat.

Keywords: Poultry; *Hermetia illucens*; Meat quality; Fatty acid profile;



## **Effect of the application of pulsed electric fields in combination with salts on the drip water loss, texture and appearance of wooden breast meat in broilers**

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Wooden Breast (WB) myopathy has a prevalence of approximately 50% in Argentina and poses a problem for the poultry industry. There is a trend towards the use of new technologies, including pulsed electric fields (PEF). The objective was to investigate the effect of PEF in combination with immersion in salt solutions on the drip water loss, texture, and appearance of broiler breasts with WB. The PEF conditions were: 1.5kV/cm electric field, 300 pulses and immersion solutions of water, CaCl<sub>2</sub>, and NaCl 0.4M. 12 breasts from COBB-500 chickens from an Argentinean slaughterhouse were sampled (6 normal (N) and 6 WB). Each breast was cut into 7 pieces (23x23x10mm). 7 treatments were applied: A-Control; B-Water; C-PEF+Water; D-CaCl<sub>2</sub>; E-PEF+ CaCl<sub>2</sub>; F-NaCl; G-PEF+NaCl. Drip water loss was determined at 24 and 48h, and color was measured. The meat with the least water loss underwent an instrumental texture profile analysis (TPA). For WB treated meat with the least water loss, a paired visual comparison test was conducted with a control sample using an internal sensory panel. At 24h, N(D,E) and WB(E) had the lowest drip water losses, with values of 1.14%±0.49, 1.19%±0.22, and 1.46%±0.76, respectively. WB(E) had lower losses than N(A). WB(F,G) would have the same loss as the N(A). At 48h, it is noteworthy that WB(E) obtained significantly lower loss values than the N(A). WB(D,G) had values equal to the losses of the N(A). Regarding color, the variation in L\* was significant in N(B) and WB(E,D), which had higher luminosity L\*, while a\* and b\* values remained unchanged. The TPA for WB showed that treatment E(PEF+CaCl<sub>2</sub>) significantly reduced the hardness of the meat compared to WB(A) (9.61±6.48 kgf and 23.76±9.58 kgf, respectively). WB(E) were less rubbery than WB(A). For N meat, the TPA did not show significant differences. WB samples immersed in the CaCl<sub>2</sub> solution were compared to the control. 93.5% of the evaluators perceived the CaCl<sub>2</sub>-treated samples as lighter, but only 54.8% preferred the control sample. 45.2% preferred the color of the treated sample. The role of CaCl<sub>2</sub> in the drip water retention of WB breasts improves after PEF treatment. Additionally, it reduces the typical hardness of WB and increases their luminosity, but this color difference does not interfere with the preference of the evaluators. This study should be replicated using devices designed to treat the entire breast, with the aim of subsequently scaling it up to an industrial level.

**Keywords:** Wooden Breast; Pulsed electric fields; Salt solutions; Drip water loss

## **Supplementation of olive oil-derived phytocomplexes in broiler chicken diets: effects on breast meat quality and oxidation changes of thigh meat during refrigerated storage**

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Chicken meat is rich in unsaturated fatty acids and heme pigments (especially in leg meat) that makes it particularly susceptible to the development of oxidative reactions, which are one of the main causes of meat quality deterioration and losses at retail, food service and households. This study aimed at assessing the effect of the dietary supplementation of two natural phytocomplexes resulting from the production of olive oil on the main quality traits of fresh chicken meat as well as on the oxidative phenomena that develop during refrigerated storage. A total of 2,340 one-day-old Ross 308 male chicks was divided into 3 experimental groups and fed a commercial corn-wheat-soybean diet (CON) or the same diet supplemented in all feeding phases (0-42d) with the two phytocomplexes (P1 and P2). At slaughter, 12 carcasses/group were collected and cut to excise the breast fillets and the thighs. Fillets were used to assess the main quality traits (pHu, colour, drip and cooking losses, tenderness) of fresh meat, whereas the thighs, packaged in ordinary atmosphere, were used to measure potential changes due to oxidation (colour, carbonyls, thiols, and myoglobin oxidative status) at 0, 3, and 6 days of refrigerated storage (4 ± 1°C). Data were analysed by ANOVA and Tukey-HSD test considering the effects of dietary treatment and storage time. Supplementing the diet with P1 and P2 did not significantly affect the main quality traits and technological properties of breast meat. On the contrary, the dietary utilization of phytocomplexes remarkably changed the colour parameters of thigh meat. Indeed, if compared with CON, P1 and P2 exhibited higher lightness (L\*; 48.0 vs. 49.7 and 49.4, respectively;  $p < 0.001$ ) and lower redness values (a\*; 4.2 vs. 3.3 and 3.3, respectively;  $p < 0.001$ ). As for the main effect of the storage time, most of the changes occurred within the first 3 d of storage in which an increase in the values of all colour parameters has been observed ( $p < 0.001$ ). Considering the oxidation of the protein and lipid fractions, these reactions developed during the refrigerated storage and no differences ascribable to the use of the tested phytocomplexes were detected. These results suggest that the dietary supplementation of natural phytocomplexes obtained as by-products of olive oil production had no substantial effects on the oxidative stability of chicken meat.

Keywords: broiler chicken; diet; olive phytocomplexes; meat quality; oxidation

## **Effect of the dietary inclusion of *Hermetia illucens* oil on fatty acid composition and technological properties of chicken meat**

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The present study aimed at evaluating the effect of the use of *Hermetia illucens* (HI) oil as replacer for the main vegetable lipid source usually included in the formulation of broiler diets (namely soybean oil) on the main quality traits and technological properties of chicken breast and thigh meat. A total of 552 day-old Ross 308 male chicks were housed in an environmentally controlled facility and randomly divided into three experimental groups: a control group (C) fed a commercial corn-wheat-soybean diet, and two groups fed the same basal diet with HI oil replacing 50% and 100% of soybean oil (HI50 and HI100; HI oil inclusion = 1 and 2%, respectively) over the whole rearing period. At slaughter (42 days), 45 carcasses were randomly selected (15/group), dissected and used to evaluate the main quality traits (pHu, color, water holding capacity) of breast meat as well as the oxidation level of the protein and the lipid fraction (by measuring carbonyls and TBARS) along with fatty acids composition of thigh meat. Data were analyzed by one-way ANOVA considering the inclusion level of HI oil as main effect. When significant, means were separated by Tukey-HSD test ( $p \leq 0.05$ ). Regardless its inclusion level, HI oil only marginally affected the main quality traits and technological properties of both breast and thigh meat. On the other hand, it is noteworthy to notice that HI100 exhibited the lowest ( $p \leq 0.01$ ) carbonylation level thus suggesting a lower extent of oxidative modifications affecting the protein fraction. This finding may be explained considering the fatty acid profile of thigh meat, which is remarkably affected by the lipid source included in the diet. In detail, if compared to C, HI100 exhibited a higher ( $p \leq 0.001$ ) content of saturated fatty acids to the detriment of the polyunsaturated fraction ( $p \leq 0.001$ ). In light of the above and considering that this strategy did not significantly affect productive performances, the dietary inclusion of HI oil may represent a promising strategy to limit the use of soybean-derived feedstuffs in poultry nutrition hence contributing to improve the sustainability of the production. At the same time, this strategy would allow to limit the development of oxidative reactions in thigh meat, which represent the primary cause responsible for its quality deterioration.

Keywords: chicken quality; *Hermetia illucens* oil; chicken breast; technological properties

## **Effect of palm kernel fatty acid distillates and Black Soldier fly larvae oil on meat quality of Broiler Chickens**

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The inclusion of fat and oils is a common practice in modern poultry production to increase the energy content of the diet. One current aim of the poultry industry is to reduce production costs, which can be achieved using by-products as feed ingredients. Additionally, this contributes to the food chain sustainability. Four of the fat sources used in this study are rich in medium chain fatty acid (MCFA), which are considered a rapidly absorbed energy source. Thus, this study compares the impact of palm kernel fatty acid distillates (PKFAD), Black Soldier fly larvae oil (BSFO) and splitted palm kernel fatty acid distillates (SPKFAD) as the primary fat source in broiler feed, with crude palm kernel oil (PKO) and a commercial control (crude soybean oil). Two of these fat sources are by-products and come from the physical refining of palm kernel oil (PKFAD) and the production of larvae meal (BSFO). A total of 200 chickens were randomly allocated to 25 pens and 5 dietary treatments (5 replicates/ treatment). The first analysis carried out on the breasts (fresh chicken meat) were the i) color (L, a\* and b\*), using Konica Minolta Colorimeter (Model CR-410); and ii) the pH using a Crison pHmeter. Afterwards, the breasts are cooked in a Salva Master Chef oven. Cooking was carried out under steam, temperature 95 °C, atmospheric pressure, steam saturation. The oven was stopped after 35 minutes at which time the center of the piece had been above 71 °C for 3 minutes. In the cooked meat, the analyzes were: iii) the sensory acceptance test using a nine-point hedonic scale; iv) the cooking loss; and v) the meat texture profile analysis obtaining parameters of hardness, springiness, cohesiveness, gumminess and chewiness. The results showed no differences between the treatments in pH and cooking loss measurements. Regarding color there were differences in redness (a\*) and yellowness (b\*). Using PKO and BSFO increases the redness comparing with the control (P<0,01) and the use of PKFAD decreases the yellowness compared with the control (P<0,05). No significant differences were found on consumer acceptance of chicken meat between dietary treatment. Using PKFAD instead of the control presents differences in cohesiveness, gumminess and chewiness (P<0,05). No differences between treatments were found in hardness or springiness.

**Keywords:** Medium chain fatty acids, fat by-products, insect oil, chicken meat quality.

## The positive impact of two *Bacillus*-based products on broiler performance and lesion scoring during necrotic enteritis-induced challenge

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Necrotic Enteritis (NE) is a multifactorial bacterial disease mainly observed in broilers and costing the poultry industry up to US\$ 6 billion per year in control measures and productivity losses. The aim of this study was to investigate the effect of 2 *Bacillus* strains (*B. pumilus*, EffiXience, Lallemand: BP and *B. subtilis*, Clostat, Kemin: BS) on the performance and intestinal lesions of broilers submitted to NE challenge induced by *Clostridium perfringens* (CP). 400 day old male Cobb 500 chicks were randomly allocated to 5 groups replicated in 10 cages (8 birds/cage, 80 birds/group) during 28 days: 1) negative control (NC), 2) positive control (PC), 3) antibiotic (ATB, virginiamycin, 20 g/ton feed), 4) BP (5e8 cfu/kg), 5) BS (2e8 cfu/kg). The NC and PC groups did not receive any antibiotic or probiotic. On d14, all birds were orally inoculated with *E. maxima* and all birds, except NC, were additionally challenged with CP on d19, 20 and 21. Birds' body weight (BW) and feed refusals were recorded on d0, 14, 21 and 28. On d21, 3 birds randomly selected from each cage (30 birds/group) were examined for the degree of NE intestinal lesions (0: normal – 3 : severe). Performance data were analyzed at each time point or period by one-way ANOVA. The average of NE lesions per group was analyzed by one-way ANOVA, while the percentage of birds inside each category of NE lesions was analyzed by Khi2 test. The NE-related mortality was analyzed by Kaplan-Meier test. The challenge successfully induced moderate to heavy NE infection characterized by lower performance (PC vs NC: -15% BW d21, P<0.1; -22% ADG 0\_28, P<0.05; +31% FCR 0\_28, P<0.05), increased NE-related mortality (+20% on the whole period, P=0.001) and higher level of severe NE lesions (+1.3, P<0.001). Whereas BW and ADG were similar between all challenged groups, BP and BS improved FCR compared to PC (-9% vs PC, P<0.05), with ATB being intermediate (-5% vs PC, P<0.05). NE-related mortality was not significantly different between the challenged groups despite numerical reduction in ATB (-31%), BP (-13%) and BS (-25%) vs PC. The severity of NE lesions was alleviated by ATB (-46%) and BP (-49%), and at lesser extent by BS (-28%) vs PC (P<0.001). In conclusion, the 2 strains of *Bacillus* tested in this study mitigated the negative effects of NE in broilers at a comparable level of magnitude as the antibiotic.

Keywords: *Bacillus*; broiler; necrotic enteritis; lesion scoring; performance

## Effect of yeast cell wall supplementation on broiler performance: analysis of 3 trials

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In the context of antibiotic growth promoters replacement, mannan-oligosaccharides (MOS) originated from yeast cell wall (YCW) and used as feed supplements in poultry diets appear interesting to promote performance and to induce positive physiological effects. The objective of this study was to investigate the effects of dietary supplementation of a new commercial YCW product (Optiwall, Lallemand) on zootechnical performance of broilers raised in standard similar commercial conditions (same diet, same room, same environmental conditions). Three consecutive trials were performed in a small experimental farm in France during 35 days on Ross 308 broiler chicks males (trials 1 and 3) and females (trial 2) randomly allotted at day 0 into 2 groups (13-15 birds/pen): control (C: n = 6 pens/trial, 248 birds in total: 80, 78, 90 birds for trials 1, 2, 3, respectively) and Optiwall (O: n = 7 pens/trial, 289 birds in total: 93, 91, 105 for trials 1, 2, 3, respectively) supplemented at 2 kg/ton feed. The same starter crumble diet (corn, wheat, soybean meal) produced by Qualisol (France) was used for the 3 trials during the whole trial duration of 35 days. Feed and water were provided ad libitum. Body weight (BW) and feed refusals were recorded at days 0, 11, 18, 25 and 35. Average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were calculated for each period. The combined analysis on the 3 trials for FI and FCR was performed until D25 as FI was not recorded for the last period (25\_35) in trial 1, and until D35 for BW and ADG. Broiler performance was analyzed by 2-way ANOVA with the group, the trial and their interaction as fixed effects, while mortality was analyzed by Kaplan-Meier test. O decreased mortality by 3.4 pts (C: 6.5%, O: 3.1%, P<0.1), increased BW at D35 by 5.6% (C: 1934 g, O: 2043 g, P<0.001) and improved ADG 0\_35 by 5.7% (C: 54.1, O: 57.2 g/bird/day, P<0.001). While FI was not significantly different between the 2 groups at any time point, FCR appeared improved numerically at D25 (0\_25): -1.9% (C: 1.565, O: 1.536) and significantly at D11: -6.5% (C: 1.447, O: 1.353, P<0.05). In conclusion, this new YCW product appears as an interesting solution to improve zootechnical performance of broilers.

Keywords: yeast cell wall; broiler; performance

## Reality of feed intake in layers related to zootechnical performances and improvement of feed intake quality with an innovative hydrolyzed yeast

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Layer performance is strongly dependent on feed intake (FI), which has to be tightly managed, particularly at the onset of lay and during periods of stress. Mash feed is a challenging matrix due to particles heterogeneity and birds' behavior towards the selection of coarse particles first (ie. feed sorting). This study aimed at understanding the link between feed sorting and zootechnical performances and at investigating the effects of a hydrolyzed yeast (Yela Prosecure, YLP, Lallemand) to improve feed intake quality. 52 Novogen layers were individually housed and allotted in 2 groups (n = 26 birds/group) for 1 wk: control (no supplementation) and YLP (2.5%). Feed and feed refusals were sieved, and particle size distribution was calculated. Particles size lower and higher than 2 mm were considered as fine and coarse particles, respectively. FI, laying performance and body weight (BW) were measured individually. Productive performances were analyzed according to 1) fines particles proportion in feed refusals (HIGH: more than 70%, MODERATE: between 57% and 70%, LOW: below 57%) and 2) layer performance category (GOOD: > 5 eggs/wk, MIDDLE: 5 eggs/wk, POOR: ≤ 4 eggs/wk). Categorical data (percentages) were analyzed by Khi2 test, while continuous data (laying performance, FI, BW) were analyzed by T-test to compare the 2 groups (SPSS Statistics 26.0 (IBM)). Birds preferred coarse particles, eating first maize and soybean meal: the proportion of fine particles was 57% in feed and reached 70% in feed refusals. Heavier hens presented lower fines intake (P<0.05). As expected, poor layers presented lower laying rate, lower feed intake and downgraded feed conversion ratio (P≤0.001). However there was no significant difference in egg weight and hen body weight between good, middle and poor layers. The feed intake was more homogeneous in YLP hens (coefficient of variation: control: 22%; YLP: 13%). Control hens preferred coarse particles with more fines recovered in feed refusals (72%) compared to feed (57%), while YLP allowed to reduce numerically this proportion (67%) (P>0.05). YLP hens ingested more fine particles than control hens: 27% of YLP hens had less than 57% of fines in their feed refusals (= no feed sorting) vs 16% of control hens. In conclusion, YLP appears as a promising tool to secure a homogeneous FI, increasing the efficiency of feed use by layers. Yela Prosecure would then deserve additional trials to further investigate its positive effects.

Keywords: feed intake; feed refusals; laying hens; hydrolyzed yeast

## **A global cross-study analysis in broilers of a multi-enzyme combination and *Bacillus* probiotic**

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Volatility in the availability and quality of diet ingredients in today's landscapes creates nutrient availability challenges in poultry production. This is compounded by the pressures to reduce antibiotic use in animal production. Feed additives, including enzyme and probiotic technologies, have demonstrated value in addressing these headwinds individually. This study evaluated the effects of a feed additive containing a multi-enzyme combination and a *Bacillus* probiotic on performance in broilers. The data contained 13 experiments with two treatments, a control treatment, and a treatment with dietary inclusion of Syncra®AVI (formulated to deliver 2000, 200, and 4000 FTU/kg of xylanase, amylase, and protease, respectively and 75,000 CFU/g of feed of a three-*Bacillus* strain probiotic). All trial diets, except one New Zealand trial, contained a background of 500 FTU/kg phytase. Experiments were conducted in the USA (3), the Netherlands (4), New Zealand (2), Hawaii (2), and Brazil (2). Two- or three phase diets representing locally commercial diet composition with a base of corn and soybean meal were used. Depending on the trial, the starter period ended from d 10 to 14, the grower period ended at d 21, and the finisher period ended at d 42. In total 2739 datapoints were used in the analysis, including bodyweight (BW), feed intake (FI), and feed conversion ratio (FCR) in 13 trials, and mortality in 12 trials. Statistical analysis was performed using ANOVA including treatment as a fixed effect and trial as a random effect. A consistent effect of the enzyme and probiotic combination was seen across phases, with 1.6% ( $P = 0.033$ ), 3.0% ( $P < 0.001$ ), and 1.3% ( $P = 0.016$ ) higher BW at the end of the starter, grower, and finisher phase, respectively. FCR was improved by 3 points up to the starter and grower phases ( $P = 0.024$  and  $P < 0.001$ , respectively), and numerically by 1 point up to the end of the finisher phase ( $P = 0.38$ ). Mortality was significantly reduced in two trials up to d 35 by 3.03% ( $P < 0.001$ ), and numerically reduced in all trials for all periods. The results indicate that this multi-enzyme and probiotic combination consistently improves performance in various global regions exhibiting differing (nutritional) environments. This product could therefore benefit producers facing volatile nutritional challenging environments by maintaining or improving performance output.

Keywords: Poultry, direct fed microbial, xylanase, amylase, protease



## Relationships between microbiota and zootechnical parameters in laying hens after supplementation with probiotic *Pediococcus acidilactici* CNCM I-4622

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Probiotics are feed additives recognized to improve zootechnical performances through intestinal microbiota modulation. In laying hens, correlations between the relative abundance (RA) of bacteria taxa encountered in the intestine, laying performance and egg quality parameters are rarely reported. The objective of the study was then to establish correlations between intestinal microbiota, laying performance and egg quality parameters. A 5-weeks feeding trial was designed with 52 individually caged 41-week-old laying hens supplemented through the diet with or without *Pediococcus acidilactici* CNCM I-4622 (Bactocell®- Lallemand: 10<sup>9</sup> CFU/kg feed) (n=26 hens/group). Performance parameters (mean±SD) and amplicon sequencing fecal microbiota for all hens from the end of the trial were analyzed with Spearman correlations using python (v3.7.6). Results showed that alpha diversity was positively correlated with Egg Weight (EW, g; r=0.41, P<0.05) and with final body weight (Kg; r=0.21, P=0.135). EW was also positively correlated to the RA of Lachnospiraceae, Rikenellaceae, Prevotellaceae UCG-001, Christensenallaceae R\_7\_group, UCG-005, *Erysipelatoclostridium* and *Colidextribacter* genera. A negative correlation was detected between EW and the RA of *Romboutsia* genus (r=-0.34, P<0.05). Interestingly, the probiotic supplementation significantly increased the RA of all the aforementioned taxa and also increased +3% the EW (65.82±4.66 vs 62.94±3.80 grams for probiotic vs control; P<0.1) and +7% the Exported Egg Mass (EEM; 61.47±5.90 vs 56.58±7.62 grams for probiotic vs control; P<0.05), while reducing -8.6% the Feed Conversion Ratio related to EEM (FCR; 1.93±0.35 vs 2.08±0.31 for probiotic vs control; P<0.1). FCR related to EEM and FCR related to number of eggs production tended both to increase with the relative abundance Proteobacteria (P=0.052 and P=0.057, respectively), Enterobacteriaceae (P=0.109 and P=0.218, respectively) and Enterococcaceae (P=0.059 and P=0.081, respectively). Interestingly, *Lactobacillus*/Enterococcaceae ratio tended to be correlated with decreased FCR and would deserve further investigations as a 'FCR to eggs mass production' and 'FCR to number of eggs' biomarkers (P=0.147 and P=0.112, respectively). Taken together, these results highlight interesting relationships between the fecal microbiota of laying hens and production parameters, bringing further light on the effect of *Pediococcus acidilactici* CNCM I-4622.

Keywords: microbiota; zootechnical performance; laying hens; probiotic

## **Efficacy of manganese sources for broilers: impact on performance and manganese accumulation**

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Manganese (Mn) is vital for broiler growth and health, impacting skeletal, muscle development, enzymatic activity, meat quality, and disease resistance. Bioavailability of Mn can vary according to its source; studies have shown differences between oxides and MnSO<sub>4</sub> (79% for standard oxide, 133% for purified oxide). While oxides' efficacy against chelates is unknown, our study was aimed to compare a purified source of Mn oxide with different chelated Mn sources in broiler diets. In a 42-day experiment, 1,470 Ross 308 chicks were randomly assigned to 7 treatments with 7 replicates of 30 birds per pen. A negative control (Ctrl), without supplemented Mn, was compared with feeds supplemented with a low (30 ppm) or high (60 ppm) dose of Mn, either with purified MnO (MnP; ManGrin®; Animine), an amino acid chelate (MnAA; AvailaMn®, Zinpro) or a glycine chelate (MnGly; B-Traxim 2C®, Pancosma). The basal diet contained 25 ppm Mn and 1000 FTU of phytase. Diets were formulated according to NRC requirement of broilers. On d 21, one bird per pen (7 birds per treatment) was slaughtered and liver Mn concentration and bone fracture strength were measured. Data including growth parameters, were analyzed by ANOVA using the MIXED procedure of SAS software. No statistical differences in weight ( $P > 0.05$ ) were observed across the period (average final BW of  $2.44 \pm 0.09$  kg). For phase 1 (d 1-14), Mn addition did not affect ADG and ADFI ( $P > 0.05$ ). However, results have shown that the addition of Mn, MnP30 or MnP60 improved the FCR compared with Ctrl ( $P < 0.05$ ). For phase 2 (d 15-28), neither sources nor doses had an effect on growth performance parameters ( $P > 0.05$ ). Fracture strength results showed no difference between treatments ( $P > 0.05$ ). However, liver Mn concentrations were higher ( $P < 0.05$ ) for the MnP30, MnP60 and MnGly60 than that of Ctrl. Our findings have shown that addition of Mn or of MnP significantly ( $P < 0.05$ ) increased liver Mn concentrations. During phase 2, the bird's mortality rate was lower ( $P < 0.05$ ) for Ctrl, MnP30 and MnP60 treatments compared to MnAA30, MnGly30 and MnAA60. In conclusion, Mn supplementation with oxide or chelate did not affect the final weight, suggesting phytase-supplemented basal feed meets the broiler requirements. Moreover, MnP or MnGly supplementation improved FCR, increased liver Mn concentration, and enhanced animal survival, thus benefiting overall health and performance of broilers.

Keywords: Manganese; broiler; liver; tibia; survivability

## Probiotics in broiler chicks' diet fed with different sources of protein

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Probiotics have been provided to be the most preferred and effective alternative to antibiotics. The aim of this study was to evaluate the potential of *Bacillus subtilis* ATCC 6051a (Pro), as a probiotic bacterium in broiler diets based of two sources of protein (soybean meal [SBM] and cowpea [CWP; *Vigna unguiculata* [L] Walp]). Growth performance (GP), carcass, and gut microflora population were investigated using a total of 480 one-d-old mixed sex broiler chicks (Ross 308). The SBM or CWP diets were tested in the presence or absence of Pro ( $1 \times 10^{11}$  cfu/kg feed) in a 2x2 factorial arrangement in a completely randomized design. Chicks were randomly assigned to 4 dietary treatments (6 replicate pens with 20 chicks each). Diets of starter, grower, and finisher were formulated to be isocaloric, isonitrogenous with similar content of total lysine, TSAA, Ca and available P, and manufactured in mash form, without inclusion of growth promoters or antibiotics. The results showed that birds fed CWP had comparable GP as those fed the SBM diets. Carcass, breast and legs' yield, and digestive organs size (i.e. gizzard, heart, liver, pancreas, spleen, small intestine, and caecum) were not affected by the protein source (Ps). Inclusion of Pro in broilers' diets increased BWG during grower ( $p < 0.01$ ), finisher ( $p < 0.01$ ), and overall study period ( $p < 0.001$ ), tended to increase FI ( $p = 0.059$ ; d 0-42) and improved feed efficiency in the grower ( $p = 0.045$ ) and finisher ( $p = 0.042$ ) phase. The addition of Pro significantly decreased abdominal fat ( $p = 0.026$ ) and caecum weight ( $p = 0.034$ ), and tended to increase pancreas ( $p = 0.057$ ), and caecum length ( $p = 0.086$ ), while carcass as well as breast and legs' yield were similar. In addition, Pro was beneficially in modulating gut flora composition. In particular, the caecal *Bacillus* spp. ( $\log_{10}$  cfu/g digesta) and *Enterococcus* significantly increased ( $p = 0.016$  and  $p = 0.024$ , respectively) whereas the coliforms ( $p < 0.001$ ) and *E. coli* count decreased ( $p = 0.040$ ) compared with birds without Pro. No significant interaction between the main factors (Ps x Pro) was noticed for all variable measured. It is concluded that the Pro significantly improved the GP of broilers and can beneficially affect the gut bacterial community in both SBM and CWP diets.

Keywords: Probiotic; Cowpea; Performance; Gut microflora; Broiler.

## **Effects of microencapsulated *Lactobacillus* spp. and cowpea seeds in broiler chickens' diets on production parameters, health status, and intestinal microbial population**

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The study determined the nutritional quality of cowpea seed (CWP) and the effect of partial replacement of soybean meal with CWP and the addition of *Lactobacillus salivarius* (LS) in broiler chickens from day 1 to 35 days on the performance, some carcass traits, blood biochemistry, bone quality, ceca, and excreta microbial population. A total of 432 one-day-old mixed-sex Ross 308 broiler chickens (initial weight of 46.66 g) were randomly assigned to four groups (n=108 in each group; 6 pens with 18 birds each). The broilers were randomly divided into four groups in a 2×2 factorial design comprised of two cowpea levels (CWP0 and CWP15%) with/ without microencapsulated LS probiotic (0 and 1 g/kg feed). The experimental diets had no significant effects on production performances. From the carcass traits, higher dressing, liver, and small intestine length were noted in the CWP15 with LS supplement compared with the other groups. The plasma profile was significantly altered by decreasing the concentration of total cholesterol and triglycerides in the CWP15 with LS inclusion. The same group had significantly higher total protein, albumin, and calcium, and significantly lower ( $P<0.05$ ) uric acid compared with CWP0 with or without LS inclusion. No significant effect was noted for tibia bone traits and minerals. Microencapsulated LS positively affects the pH, *Lactobacillus* spp., and *Enterococcus* spp., and inhibiting potential pathogens (*Coliforms*, *Clostridium* spp., *E. coli*, *Staphylococcus* spp.) in CWP0 and CWP15 groups. Our results suggest that dietary CWP has a positive effect on growth and carcass characteristics and could represent a good alternative that partially replaces soybean meal in broiler diets. Additionally, microencapsulated LS had positive implications on intestinal microflora and health status.

**Keywords:** performance, cowpea, microencapsulated probiotic, blood biochemistry, bone quality, gut microflora, chicks

## **Effect of *Lactobacillus plantarum* on performance and gut microflora populations of broiler fed with alternative protein source**

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The aim of this study was to investigate the effect of different levels (0, 10 and 20%) of raw cowpeas (RCWP; *Vigna unguiculata* [L] Walp) seeds, as a replacement for soybean meal (SBM), with and without probiotic addition on growth performance, digestive organ sizes and cecal pH value (0 to 42-d). The impact of the treatments on the cecal microbiota using ten-fold serial dilutions at day 42 was determined. LAB, *Escherichia coli* (*E. coli* biotype  $\beta$ -haemolytic), *Salmonella* spp., *Clostridium* spp., Coliforms, *Bacillus* spp., and *Enterococcus* spp. were assayed. A total of 720, unsexed 1-d-old Cobb 500 broilers were divided into 6 groups with 4 replicate pens (30 birds/replicate pen). Data were analyzed as a 3 x 2 factorial arrangement with 3 levels of RCWP with and without probiotic (3 x 10<sup>8</sup> cfu/g-1 feed). The probiotic used was based on *Lactobacillus plantarum* ATCC 8014 strain. Urease activity (pH change) in RCWP was not detected. The results showed that RCWP at 10 or 20% without probiotic in an optimized diet on digestible amino acid contents maintained broiler growth performance (2713-2696 g vs. 2719 g in the SBM group;  $P > 0.05$ ). The digestive organ sizes (i.e. gizzard, heart, liver, pancreas, small intestine, caecum) and pH of the caecum digesta were not affected ( $P > 0.05$ ) by treatments. The inclusion of LP as probiotic in broiler diets increased BWG during the overall study period (2766-2689 g vs. 2703 g in the SBM group;  $P < 0.001$ ). Also, the FCR was improved, especially in the finisher period in RCWP groups (1.84) compared with SBM (1.86;  $P = 0.045$ ). LP addition also was beneficial in modulating gut microbiota composition. In particular, the cecal LAB significantly increased ( $P = 0.030$ ; log<sub>10</sub> CFU/g of wet digesta), whereas the coliforms count decreased ( $P < 0.001$ ) vs. with those without probiotic. It is concluded that RCWP and probiotic inclusion improved the broiler growth and cecal microbiota populations.

**Keywords:** protein sources, *Lactobacillus* probiotic, growth, carcass traits, microflora, broiler

**Elemental selenium limits the utilization of supplemented selenium in broilers****A. Hachemi<sup>1</sup>, C. Deschamps<sup>1</sup>, D. Cardoso<sup>1</sup>**<sup>1</sup>Adisseo, Antony, FrancePresenting author: [amine.hachemi@adisseo.com](mailto:amine.hachemi@adisseo.com)

Selenium (Se) is commonly added to livestock diets in either inorganic form (sodium selenite, SS) or organic forms, such as SeYeast (SY) or as pure chemically synthesized Se forms, like hydroxy-selenomethionine (OH-SeMet). In some regions, Se is also supplemented as nano-Se (SeNP) or as Se proteinate (Se-Pro), which are claimed to be as efficient as organic Se forms. Se compounds can be differentiated based on their bio-efficacy, assessed through measuring Se deposition in muscle tissue. The present study was aimed to compare the bio-efficacy of different Se sources in chickens. A total of 120 d-old male broilers (Ross 308) were assigned to 10 treatments (4 replicates). The birds were fed a basal diet of maize-soybean meal for 14 days supplemented with different sources of Se at 0.3 ppm: a negative control diet (NC; no supplemental Se), SS, 3 different Se-Pro (Se-Pro 1, Se-Pro 2, Se-Pro 3), 2 different SeNP (SeNP 1, SeNP 2), 2 different SY (SY1; SY2), or OH-SeMet. On d 14, all birds were sampled for determination of total Se concentration in breast muscles by ICP-MS. Differences were tested by means of one-way ANOVA ( $P < 0.05$ ). The Se sources significantly affected the Se deposition (mg Se/kg of DM) in the breast muscles ( $P < 0.001$ ), with the higher bio-efficacy of OH-SeMet (1.12) than SY1 (0.57) and SY2 (0.84), and the organic forms superior than SS (0.379), SeNP (0.29 and 0.27) and Se-Pro forms (0.38, 0.31 and 0.33). Se-Pro and SeNP were equivalent or significantly low efficient than SS ( $SS = \text{Se-Pro 1} > \text{Se-Pro 2} = \text{Se-Pro 3} > \text{SeNP 1} > \text{SeNP 2}$ ;  $P < 0.001$ ). To understand the variable Se deposition among these sources, the experiment was followed by the speciation of those products. A negative correlation ( $R^2 = 0.7419$ ;  $p < 0.001$ ) between the level of elemental Se and Se in tissues from SS, Se-Pro and SeNP sources was observed. Elemental Se is an inorganic and insoluble form of Se which explains the lower absorption and transfer of Se in tissues. Elemental Se has recently been identified as part of the composition of SY which, on top of their variable SeMet content, explains their lower bio-efficacy compared to pure chemically synthesized Se forms, like OH-SeMet. In conclusion, organic sources, especially OH-SeMet have a superior bio-efficacy compared to SS, Se-Pro and SeNP. Both Se-Pro and SeNP products showed to be equivalent of inferior to SS. Elemental Se seems to play an important role in the bio-efficacy of the Se, no matter the source.

**Keywords:** selenium, nano-selenium, seleno-yeast, seleno-proteinate, hydroxy-selenomethionine, elemental selenium

## **Reducing feed costs with phytase and NSP enzymes for rearing male layer chickens to potentially avoid day old culling**

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Commercial layers produce table eggs, however, their male siblings are culled after hatching. This has raised concerns regarding animal welfare. European states have agreed to put a ban on culling of male chicks. Some alternatives have been proposed as an alternative to culling, including in-ovo sexing or rearing them for meat consumption. However low success rate of early sex determination during incubation, slower growth, reduced feed efficiency and increased cost of production are still concerns. The objective of this study was to evaluate the effect of enzymes on performance and production benefit of the male layer chicks. Dekalb white day-old male chicks (N=3240) were randomly attributed to two treatments, six replicates and 270 males per pen. A control treatment was served as a comparison with sufficient energy and protein in the diet and dosed at 500 FTU/kg of Butiauxilla phytase and a mix of xylanase and B-glucanase (XB, xylanase at 1200U/kg and B-glucanase at 156U/kg). Second treatment had double doses of both enzymes, where full matrix values for additional dose was used to reduce feed cost. Birds were reared till 96 days of age, with body weight, feed intake and feed conversion ratios (FCR) calculated on days 27, 62 and 96. Data was analyzed by one way ANOVA. Feeds from both treatments were analyzed for crude protein, crude fat, total calcium, total phosphorus, phytase and xylanase activity and were within expected ranges. Final body weight (1442 vs. 1433g for PC vs higher dose) were not statistically different between the two treatments ( $p > 0.05$ ). Similarly, overall 0-96d body weight gain and feed intake were not statistically different ( $p > 0.05$ ). Mortality was slightly higher in the control treatment than the higher dose treatment (3.2 vs 1.7% in PC vs higher dose,  $P > 0.05$ ). FCR was numerically improved for the higher dose treatment (4.216 vs 4.246 in higher dose vs PC,  $P > 0.05$ ). Feed costs in euro/kg body weight gain were 1.528 vs 1.492 for control and higher dose treatments respectively. This accounts to 50000 euros savings for one million birds, with an average body weight of 1.44kg. In conclusion, raising male layer chicks is more costly compared to broiler production, however, might be needed from welfare perspective. Additionally, combination of enzymes with full matrix application could help to reduce cost of production.

Keywords: Male layer chicken; enzymes; phytase; nsp-ase

## **The effect of synbiotic and probiotic preparations on the concentration of ochratoxin in meat, blood, liver and kidney of turkeys**

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The aim of the study was to determine the effect of synbiotic preparations on the content of mycotoxins in feed, blood and selected organs, as well as the production performance of turkeys. An experiment involving 120 BIG-6 female turkeys was conducted for 105 days (6 treatments, 5 replication, 4 birds per replication). The diets of birds were contaminated with OTA at 199 ppb (starter), 364 ppb (grower) and to 462 ppb in finisher. Turkeys received a basal diet without additives (control group I) and diets with the addition of probiotic preparations BioPlus 2B (group II) or Cylactin (group III) and synbiotic preparations S1 (*L. reuteri*, *L. plantarum*, *L. pentosus*, *S. cerevisiae*), S2 (*L. reuteri*, *L. plantarum*, *L. pentosus*, *S. cerevisiae*, *L. rhamnosus*) and S3 (*L. reuteri*, *L. plantarum*, *L. pentosus*, *S. cerevisiae*, *L. rhamnosus*, *L. paracasei*) - groups IV, V and VI, respectively. The following parameters were monitored: growth performance, concentration of ochratoxin in the blood, liver, kidney and meat of birds. One-way analysis of variance (ANOVA) was performed (Duncan test). The applied feed additives did not improve the growth performance of turkeys fed diets contaminated with OTA. Birds whose diets were supplemented with probiotic Cylactin and synbiotic S1 had higher final body weights, but the observed differences were not statistically significant. The introduction of synbiotic S1 into diets for turkeys reduced the concentration of ochratoxin in blood serum at 3 and 9 weeks of age ( $P \leq 0.05$ ), and the other synbiotics S1, S2 and probiotic Cylactin at 15 weeks of age ( $P \leq 0.01$ ). The use of synbiotic preparations reduced the ochratoxin content in the liver and kidneys of birds at week 15 of the experiment ( $P \leq 0.01$ ). There was no effect of the probiotic and synbiotic additives on the ochratoxin content of the breast muscles (av. 0.92  $\mu\text{g}/\text{kg}$  OTA) of turkeys fed ochratoxin-contaminated feeds. The concentration of OTA in meat was lower than the limit exposure in Poland (5.0  $\mu\text{g}/\text{kg}$  OTA). The results of the study suggest that synbiotic and probiotic preparations improve the growth performance and health status of turkeys. This study was carried out as part of a research project funded by the National Centre for Research and Development, PBS3/A8/32/2015.

Keywords: synbiotics, probiotics, ochratoxin, blood, meat, liver, kidney, turkey



## Effects on growth performance of enzyme feed addition for coccidiosis vaccinated Ross 308 broilers

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Coccidiosis is a broiler disease with a major economic impact. Considering the development of resistance to anticoccidial additives, and in the absence of new molecules, vaccination represents a sustainable solution. Paracox®5 is an attenuated vaccine containing 5 strains of pathogenic coccidia for broilers. Considering the current inflationist context for the feed raw materials, it is necessary to optimize feed formulas to adapt them to vaccinated birds, while guaranteeing good growth performance. The aim of the study was to evaluate the effects of enzymes for Paracox®5 vaccinated broilers, fed with anticoccidial additives free feed. A pen trial was carried out to compare the growth performance and feed efficiency of male Ross 308 chickens fed with a formula with addition of either xylanase (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase, 1,200 UV/kg, PX treatment), protease (xylanase 189 U, subtilisin 2,520 U and amylase 252 U/kg, PP treatment), or xylanase + protease (PXP treatment). All feeds included phytase. For each treatment, 285 chicks were placed in 5 pens (19/m<sup>2</sup>), with thinning at 35 days to reduce density to 14 birds/m<sup>2</sup> until slaughter. The experimental model was designed to get close to field conditions for heavy chicken production, with final slaughter at 42 days. Statistical analysis was carried out using Statview software. Weights and feed quantities were measured at 10, 21, 28, 35 and 42 days. ADG and FCR were calculated at each period: 0-9d, 10-20d, 21-28d, 29-35d, 36-42d. From 28 days to final slaughter, PP chickens were significantly heavier than PX and PXP chickens: mean weights for PP chickens: 1,436g at 28d, 2,040g at 35d, 2,801g at 42d; mean weights for PX chickens: 1,403g at 28d, 1,986g at 35d, 2,708g at 42d; mean weights for PXP chickens: 1,405g at 28d, 2,003g at 35d, 2,747g at 42d; p=0.01 at 28d, p=0.005 at 35d, p=0.001 at 42d. Similarly, ADG of PP group was significantly higher than ADG of PX and PXP groups from 21d to 27d (86.2g/d for PP group, 82.5g/d for PX group and 82.6g/d for PXP group, p<0.0001). ADG from PP group was significantly higher than ADG of PX group from 28d to 35d (respectively 87g/d and 83.2 g/d, p=0.004). FCR were not significantly different. This study shows the benefit of a specific feed formulation for chickens vaccinated with an attenuated coccidiosis vaccine, particularly with the use of protease alone and to a less extent in combination with xylanase.

Keywords: coccidiosis; vaccine; enzymes; protease

## **Broiler chickens undergoing an intestinal challenge and supplemented with a bacterial xylanase demonstrated better nutrient digestibility, intestinal health and growth performance**

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The action of a bacterial xylanase on arabinoxylans (AX) produces arabinoxylan-oligosaccharides (AXOS), which promotes intestinal health. We investigated the supplementation of a bacterial xylanase on nutrient digestibility, intestinal health and growth performance of broilers undergoing an intestinal challenge. A total of 900 one-d-old Cobb male chicks were assigned to 1 of 3 treatments with 10 replicates (30 birds) in a completely randomized design during 42 d: 1) Standard diet (ST), 2) Low energy diet (LE; according to the AX content of each diet: 80, 82, 87, and 92 kcal of ME/kg for pre-starter, starter, grower, and finisher, respectively), 3) LE + *Bacillus subtilis* xylanase (Jefo&Puratos) at 100 g/t (LE+Xyl). All birds were challenged on d 1 with a coccidiosis vaccine 10× the regular dose and *C. perfringens* on d 11, 12, and 13. At 17d, one bird/pen was orally gavaged with fluorescein-isothiocyanate dextran (FITC-d, 4 mg/kg of body weight) and blood samples were collected one and half hour after gavage to evaluate intestinal barrier integrity. At 21 and 42d, ileal content was collected from four birds/pen to determine ileal digestible energy (IDE), dry matter (DM), and crude protein (CP). From these same birds, cecal content was collected to determine the concentration of short chain fatty acids (SCFA). Body weight gain (BWG), and feed conversion ratio (FCR) were evaluated weekly. Data was submitted to ANOVA and Fisher LSD tests. Broilers fed LE+Xyl had the highest ( $P<0.05$ ) DM digestibility at 21 and 42d. At 42d, CP digestibility tended to be higher ( $P<0.10$ ) for LE+Xyl compared to LE, being similar to ST. At both ages, LE+Xyl had higher ( $P<0.05$ ) IDE compared to LE, being similar to ST. At 21d, LE+Xyl had higher ( $P<0.05$ ) SCFA (acetic, propionic and butyric acids) concentrations than LE. At 42d, propionic acid tended to be higher for ST ( $P<0.10$ ) compared to LE, but when the Xyl was supplemented, no differences to the ST were observed. Broilers fed LE+Xyl tended to have the lowest ( $P<0.10$ ) FITC-d blood concentration, indicating that these birds had a better intestinal barrier integrity. At 42d, LE+Xyl had similar BWG to ST and were 5% heavier ( $P<0.05$ ) than LE. The FCR of LE+Xyl and ST tended ( $P<0.10$ ) to be 4% better than LE. In conclusion, the bacterial xylanase studied may be used to improve nutrient digestibility, intestinal health and growth performance of broiler chickens fed a low digestible diet.

Keywords: digestibility; intestinal integrity; growth performance; poultry; xylanase

## Effect of am/pm diet on laying performance and egg quality of laying hens

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Egg formation is a cyclic process. Hens require high dietary protein and energy levels for the yolk and albumen formation in the morning and high dietary Ca levels for the membrane and shell formation in the afternoon/evening. Thus, the conventional practice of providing hens with a single diet with average protein, energy and Ca levels throughout the day may be problematic. To precisely meet the hens nutrient requirement, AM/PM feeding may be used and the principle is to provide a high energy and protein diet with lower Ca in the morning (AM) and a lower energy and protein diet with higher Ca in the afternoon (PM). This 10-week cage study was carried out via a Box-Behnken response surface design to identify the optimal protein, energy and Ca levels in the AM/PM diets for laying hens. This study comprised 14 experimental diets including 13 AM/PM diets with 3 levels of crude protein (CP, 19.6/18.4, 20.3/17.7, and 21/17 %), Ca (3.3/4.9, 2.5/5.7, and 1.6/6.6%) and energy (12/11.2, 12.4/10.8, and 12.8/10.4 MJ/kg) for AM and PM diets respectively and a control diet (19% CP, 4.1% Ca and 11.6 MJ/kg). There were 13 replicate cages of 2 hens per cage per treatment (364 hens in total). The AM and PM diets were swapped out at approximately 8 am and 4 pm each day. Egg production and feed intake were measured daily and weekly, respectively, with egg quality, serum Ca, nutrient digestibility measured at week 10. The FCR and egg mass per kilo of feed intake were used to determine the optimal Ca, protein and energy levels. The result showed that half of the AM/PM diets had better FCR compared to the control diet ( $P=0.017$ ). Hens fed the control diet had lower yolk color score compared to most of the AM/PM diets at week 10 ( $P=0.002$ ). Hens fed the AM/PM diets with 19.6/18.5% CP, 1.6/6.6% Ca, 12.4/10.8 MJ/kg and 21/17% CP, 3.3/4.9% Ca, 12.4/10.8 MJ/kg had the highest apparent protein (56.6%) and Ca (62.1%) digestibility respectively while hens fed control diet had the lowest apparent protein (29.6%) and Ca (34.6%) digestibility ( $P<0.05$ ). Dietary treatments did not affect serum Ca level at week 10. The Box-Behnken response surface analysis showed that AM/PM diet with 21/17% CP, 3.3/4.9% Ca and 12/11.12 MJ/kg energy is optimal to improve laying hens performance and lower feed cost. This study also revealed that selective feeding occurs in between treatments ( $P<0.001$ ) and the degree of selection depends on the difference between the nutrient levels in the AM and PM diets.

Keywords: AM/PM feeding; laying hens; feed efficiency; egg quality; selective feeding

## Does the latest NASEM vitamin A requirement estimate support optimal growth in broiler chickens?

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The Nutrient Requirements of Poultry, as established by the National Academies of Sciences, Engineering, and Medicine (NASEM), serve as a pivotal scientific benchmark for poultry nutrition globally. However, the last revision occurred in 1994, and substantial genetic advancements have since been made in broiler strain performance. The present NASEM vitamin A (VA) requirement estimated for broilers is 1,500 IU VA/kg of feed. This study investigates the impact of VA acetate supplementation on the performance and serum retinol levels (SRL) in broilers. An ethoxyquin-free VA 1000 source, Lutavit® A 1000 NXT, was supplemented at incremental levels of 2,000, 4,000, or 8,000 IU per kg of feed—representing increases of 33%, 267%, or 533%, respectively, compared to the NASEM (1994) requirement estimates. The control diet contained no VA supplementation (0 IU/kg of feed). A total of 336 one-day-old Ross 308 chicks were distributed among 28 floor pens (7 replicate pens with 12 birds/pen) for 23 days. Wheat-soybean meal-based diets were provided to the birds from d 0 to 23. Body weight gain (BWG), feed/gain ratio (F/G), and mortality (%) were calculated between d 0 to 23. On d 23, three birds from each replicate pen were selected based on average BW of that pen and blood were withdrawn from the brachial vein to measure SRL. One-way ANOVA was employed to assess the effects of VA supplementation, and significant differences were studied using the Tukey test. Broilers fed VA-supplemented diets exhibited higher BWG and SRL ( $P < 0.001$ ), as well as improved F/G ( $P < 0.001$ ) compared to broilers fed VA-free diet between d 0 to 23. In addition, birds fed diet containing 8,000 IU VA/kg demonstrated higher BWG and SRL ( $P < 0.001$ ) than those fed 2,000 IU/kg of VA. Moreover, broilers supplemented with 8,000 IU of VA exhibited numerically improved F/G than those supplemented with 2,000 IU or 4,000 IU VA/kg of feed. The mortality rate tended to be lower in 4,000 and 8,000 IU/kg of VA groups compared to 0 and 2,000 IU/kg groups ( $P = 0.063$ ). A significant linear improvement ( $P < 0.001$ ) in BWG, F/G, and SRL were observed in response to the elevated levels of dietary VA. In conclusion, based on BWG and SRL, the current VA requirement estimated by NASEM (1994) appears inadequate to support the optimal growth of modern broiler chicken strains. Urgent consideration for an update of NASEM's VA requirement estimates for broilers is warranted.

Keywords: Vitamin A; Ethoxyquin-free; Requirement; Broiler

## **Effect of the age and duration of the trial in the evaluation of the adequate mineral content and phytase dose in broilers**

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This study investigated the impact of reducing mineral (Ca and P) content and adding different doses of a new 3-bacterial phytase in broiler diets on P retention coefficient and tibia mineralization across two experiments with different durations. Two trials, a short-term (17 days) and a long-term trial (38 days), evaluated deficient (NC) or correct (PC) Ca and P levels alongside various phytase doses. Five dietary treatments were used: PC (positive control, no added phytase, Ca: 0.97% for starter & 0.95% for grower & total P: 0.65% for starter & 0.63% for grower); NC (negative control, no added phytase, Ca: 0.73% for starter & 0.69% for grower & total P: 0.58% for starter & 0.51% for grower); and three diets where NC was supplemented with 250, 500, and 1000 FTU/kg of the new phytase, respectively. A total of 80 and 490 male chicks (Ross) of 21 and 1-day-old were used in the short and long-term trials, respectively. Chicks were allocated to 10 floor pens (8 animals/pen) and 35 floor pens (14 animals/pen) in the short and long-term trials, respectively. Animals were fed the experimental diets from the first day of the experiment. In both trials, 80 animals were selected at 28 days old for nutrient balance measurements in metabolic cages (40 cages, 2 animals/cage). At the end of the nutrient balance phase (38 days old), one chick per cage was euthanized to determine tibia mineralization. Chicks fed NC diets showed a significantly higher coefficient of P retention than those fed the PC diet (+5.3 units;  $P < 0.05$ ) in the long-term trial, but no differences were observed between these groups in the short-term trial. Regarding the phytase level, the level at which the coefficient of P retention was significantly higher than that of the NC was 500 FTU/kg of phytase in the long-term trial (+4.8 units;  $P < 0.05$ ) and 250 FTU/kg in the short-term trial (7.5 units,  $P < 0.05$ ). Regarding tibia mineralization, animals from the NC retained a lower amount of P (g) compared with the PC group in both trials. Only in the long-term trial, animals fed with 250 FTU/kg of phytase were able to recover P levels in the tibia to the level observed in the PC group (+0.07 g of P compared with NC diet;  $p < 0.05$ ). Therefore, the trial duration can affect the coefficient of P retention in animals fed mineral-deficient diets and the response of tibia mineralization to phytase addition and thus should be considered for future trials.

Keywords: broilers; phytase; digestibility; mineralization; trial duration

## **Evaluation of postbiotics on production performance in broiler chicken as an alternative to Antibiotic Growth Promoters**

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In poultry, probiotics are often used as feed additives to enhance production, feed efficiency and act as gut health modulators. Despite their proven benefits, there are many concerns about their biosafety aspects, ease of microbial production, handling, and storage; routes of administration; stability; survival in the host. Researchers explain that probiotics may also act as a possible source of antibiotic resistance genes for humans. As a consequence, live bacteria might not be used anymore. The metabolite products synthesized from probiotics are known as postbiotics. Postbiotics refers to the metabolic products like enzymes, peptides, organic acids, etc. produced by a probiotic organism during the final or intermediate stage of its metabolic process. The present study was carried out to evaluate the performance of postbiotics derived from *Lactobacillus acidophilus* in broiler birds. The postbiotics were harvested by culturing probiotic bacteria from the stock cultures at the required temperature and duration under laboratory conditions and supplemented to broilers via feed. For experimentation, 480 day-old CARI-Bro Dhanraja (slow-growing broiler) straight-run chicks were randomly split up into six groups. Each group was comprised of four replicates, and each replicate contained twenty chicks. Treatment groups diets are as follows: T1- Basal diet (BD); T2 – BD+Antibiotic (CTC); T3- BD+ Probiotic (Commercially available) T4, T5 & T6 – BD+ postbiotics supplementation of 0.2%, 0.4% and 0.6% respectively. The chicks were raised under an intensive, deep litter system for 6 weeks. Results showed that 0.2% of postbiotics from *Lactobacillus acidophilus* had significantly ( $P<0.001$ ) higher body weight (1677.52g) with better FCR (1.75) and immune response. Significant ( $P<0.001$ ) reductions in total plate counts (TPC), coliform counts, and *Lactobacillus* counts were recorded in all postbiotic-supplemented groups compared to control group. The villus height (1379.25  $\mu\text{m}$ ), width (216.06  $\mu\text{m}$ ) and crept depth (179.25  $\mu\text{m}$ ) showed significant ( $P<0.001$ ) improvement among the treatment groups on the 42nd day of the experimental trial, with the highest value in the T4 group (0.2% postbiotic supplementation). Except for post-slaughter weight, no cut-off parts showed significant differences among different dietary treatments. The study concludes that 0.2% postbiotic supplementation can act as a substitute to antibiotic growth promoters, even for probiotics in broilers.

Keywords: Postbiotics, Gut Health, Production, Broilers

## **A novel phytase allows for higher level of inorganic phosphate substitution with no detrimental effect in hens during the late laying period**

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Phytate-bound phosphorus present in plant-based feed raw materials is poorly utilized by poultry, especially in hens during late lay. Older laying hens are more sensitive to phosphorus deficiency and exhibit phosphorus deficiency symptoms sooner than younger hens. The current study was designed to evaluate the efficacy of a novel phytase (HiPhorius<sup>TM</sup>, dsm-firmenich, Switzerland) to replace different levels of inorganic phosphate in hens during the late laying period. Five dietary treatments (10 replicate cages per treatment with 16 hens per cage) were fed to 800 Bovans White layers (88 weeks of age) for 24 weeks. A positive control (PC) diet (Ca: 4.20%, avP: 0.305%) was formulated to meet the breeds nutritional recommendations. Also, two negative control diets were formulated with a 0.15 % avP (NC1, Ca: 4.20%, avP: 0.155%) and a 0.20% avP (NC2, Ca: 4.20%, avP: 0.105%) reduction in relation to the PC diet. Phytase was supplemented at 360 FYT/kg on top of NC1, or at 1200 FYT/kg on top of NC2. Results showed that the reduction of 0.15% and 0.20% avP from the PC diet increased ( $P < 0.0001$ ) overall mortality rates (PC: 5%, NC1: 16% and NC2: 22%) and decreased the laying rate (PC: 82%, NC1: 79%, NC2: 57%) and egg mass (PC: 52 g, NC1: 50 g, NC2: 35 g). The 0.20% avP reduced diet also increased the feed conversion (PC: 2.09, NC1: 2.17, NC2: 2.66) compared to PC and NC1 ( $P < 0.0001$ ). The novel phytase added to the two NC diets alleviated the mortality rate (3%, 6%) ( $P < 0.05$ ), improved feed conversion (2.00, 2.09) ( $P < 0.05$ ), laying rate (82%, 81%) and egg mass (53 g, 52 g) ( $P < 0.05$ ). For egg quality parameters, the reduction of 0.20% avP in the PC diet increased the ratio of cracked and shell-less eggs ( $P < 0.05$ ), and consequently decreased the proportion of saleable eggs (PC: 96%, NC1: 93%, NC2: 85%) ( $P < 0.05$ ); NC1 group was not different from PC group on these items ( $P > 0.05$ ). Phytase at 1200FYT/kg added to the NC2 diet significantly decreased the ratio of cracked and shell-less eggs and increased the saleable eggs (95%). In conclusion, it was possible to replace 0.15% and 0.20% avP with 360 FYT/kg and 1200 FYT/kg of a novel phytase without any negative impact on mortality, egg production and egg quality during the late lay period.

**Keywords:** novel phytase; inorganic phosphate; late laying hens; egg production and quality

## The effect of muramidase in broiler feed on broiler performance in semi-field conditions

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This research aimed to evaluate the impact of muramidase supplementation on the growth performance and health parameters of Ross 308 broiler chickens in semi commercial conditions. Muramidase (MUR) is an enzyme that breaks down bacterial cell wall debris (peptidoglycans, PGNs). PGNs can have a negative impact on intestinal cells, nutrient digestibility and absorption, resulting in decreased broiler performance and health. The 42-day long study included 3200 day-old chicks. A control treatment was compared with a MUR supplemented treatment. There were 8 replicates per treatment with 200 birds, equal males and females, each. MUR was added to the corn and soybean-based control diet at 35.000 LSU(F)/kg. Growth performance was measured by feed intake and body weight for each feeding phase. Carcass characteristics were measured from 1 male and 1 female of each pen by taking the weights of hot carcass, breast and thigh meat and liver. The yields were subsequently calculated as a ratio of live weight. Intestinal health was measured by footpad dermatitis scores, oocyte counts in fresh feces, histology of jejunum samples (villus height (VL); crypt depth (CD); villus width (VW), villus surface area (VSA) and goblet cell numbers) and microbiota in ceca (total aerobe counts, lactobacillus and coliform). Results showed that MUR supplementation significantly enhanced broiler performance. The overall trial period demonstrated a higher body weight (2691.7g vs. 2520.6g; P=0.001) and a better feed conversion ratio (1.585 vs. 1.620; P=0.003) in the MUR group compared to the control. The European Productivity Index (EPEF) was also significantly improved with MUR (402.4 vs. 367.2; P=0.001). Carcass parameters showed no significant differences; however, carcass yield and breast meat were numerically improved by MUR with 1.35- and 1.08-point percentages resp. Food pad dermatitis scores were significantly lower in the MUR treated birds (1.86 vs. 2.57; P=0.041). Jejunum villus height was higher in the MUR treatment (1422.0µm vs. 1364.3µm; P=0.045). Total aerobe counts and lactobacillus counts were significantly improved in the MUR treatment (control vs MUR: 7.88 vs. 9.49 log cfu/g; P=0.009 and 7.66 vs. 9.53 log cfu/g; P=0.000 resp.), indicating potential benefits to intestinal health. There was no significant effect on oocyte counts. The study concluded that muramidase supplementation positively influences broiler growth performance by enhancing intestinal health.

Keywords: broiler; nutrition; muramidase; performance; gastrointestinal functionality



## **Muramidase supplementation enhances laying hen performance: a comprehensive analysis of three trials**

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Muramidase (MUR) is an enzyme breaking down bacterial cell debris (peptidoglycans, PGNs). PGNs have a negative impact on intestinal cells, nutrient digestibility and absorption, resulting in decreased gut functionality and performance of laying hens. The positive effects of MUR on broiler performance and health have been proven by several references, in this overview, the effects of MUR in three different laying hen trials is described. In trial 1, 210 Isa Brown laying hens were randomly assigned (21 reps, 5 hens each) to a control diet (T1), based on maize, wheat, soybean meal (SBM) and sunflower meal, and T2: T1 + MUR (30000 LSU(F)/kg). Over 140 days (wk 22-42 of age), MUR supplementation increased final body weight (P=0.0039), body weight gain (P=0.0003), laying index (P=0.0051), and egg mass (EM; P=0.0132), while reducing feed conversion ratios (FCR, P=0.0062). Average daily feed intake (ADFI) was 124.5 and 124.1 g/d and egg weight (EW) 60.6 and 60.3g for T1 and T2 resp. with no adverse health effects. Trial 2, spanning 84 days from wk 22-34, with 630 Lohmann Brown pullets (14 reps, 15 hens each), further supported the positive effects of MUR. The hens received the same treatments as trial 1, in a basal maize, barley, SBM and wheat diet. MUR group exhibited consistently higher egg production (EP) (P=0.048), EM vs (P=0.021), and a tendency for higher ADFI (P=0.088) vs. the control group. ADFI were 120 and 121g/d in control and MUR resp. and EW was 60g for both treatments. Mortality rates were low and not linked to treatments. In trial 3, 576 HyLine Brown pullets (24 reps, 8 hens each) were placed in enriched cages and fed one of three MUR levels: 0, 15000 or 30000 LSU(F)/kg. The basal diet contained maize, SBM, wheat and barley. The study, covering weeks 18 to 42, revealed a sign. linear improvement in FCR (P=0.013), EM (P=0.12), EW (P=0.047), and a numerical improvement in percentage of saleable eggs (P=0.20). ADFI was 116g/d and EWs were 60g for T1 and T2 and 60.7g for T3. This positive linear effect on primary performance parameters confirms the beneficial impact of MUR on laying hens. In conclusion, across diverse strains and trial durations, MUR supplementation consistently demonstrated positive effects on laying hen performance, including increased EP and improved feed efficiency. The findings collectively suggest the potential for MUR to be a valuable dietary supplement for optimizing the productivity and gut functionality of laying hens.

**Keywords:** Laying hen; muramidase; peptidoglycan; performance; gastrointestinal functionality

## **The production benefits of full matrix application for a consensus bacterial 6-phytase variant alone or in combination with an enzyme mix of xylanase, amylase, and protease: review of two studies**

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With increasing ingredient costs, full matrix application for phytase including digestible phosphorus (P), calcium (Ca), digestible amino acids (dig AA) and metabolizable energy (ME) are important strategies to reduce feed cost and improve benefits. This can be enhanced by combining phytase with a mix of xylanase, amylase, and protease (XAP) with additional matrix application of dig AA and ME. Two studies were conducted to validate the application of full matrix for a novel consensus bacterial 6-phytase variant (PhyG) alone or an XAP mix (xylanase at 2000 U/kg, amylase at 200 U/kg and protease at 4000 U/kg) in broilers. Experiment 1 (Exp1) and 2 (Exp2) comprised 4 treatments each, 1) a nutritionally adequate positive control (PC) 2) a negative control (NC1) reduced in dig P, Ca, dig AA and energy (ME) 3) NC1 supplemented with PhyG (NC1+PhyG) and 4) a NC further reduced in dig AA and ME supplemented with PhyG and a combination of XAP (NC2+PhyG+XAP). Exp1 used a phased dosing strategy across dietary phases with PhyG dosed at 2000 FTU/kg in the starter, 1500 FTU/kg in the grower and at 1000 FTU/kg in the finisher 1 and 2 feeds, while Exp2 dosed PhyG at 1250 FTU/kg across all phases. A total of 3200 birds were placed across the 2 trials with Exp1 using 10 replicates (50 birds/pen, 1-32 d) and Exp2 using 10 replicates (30 birds/pen, 1-42 d). Diets for Exp1 were mixed grain based while Exp2 was corn/SBM based. On d 10, 21, 32 (Exp1), 35 (Exp2) and 42 (Exp2) body weight and feed intake were measured and used to calculate the feed conversion ratio (FCR), feed cost/kg body weight gain (BWG) and carbon footprint/kg BWG. Data was analysed by ANOVA, and means separation was by Tukey's HSD test (JMP 16.1). In both studies, growth performance in all enzyme-supplemented diets was maintained compared to PC ( $P>0.05$ ). In Exp1 the estimated overall feed cost (€/kg BWG) was reduced by 7.2 and 10.1% and carbon footprint (g CO<sub>2</sub> eq/kg BWG) was 12.0 and 16.7% lower in NC1+PhyG and NC2+PhyG+XAP, respectively, vs PC ( $P<0.001$ ). While in Exp2 estimated overall feed cost (€/kg BWG) was reduced by 8.9 and 12.1% and carbon footprint (g CO<sub>2</sub> eq/kg BWG) was 7.8 and 9.7% lower in NC1+PhyG and NC2+PhyG+XAP respectively compared with PC ( $P<0.001$ ). In conclusion, supplementation of the novel phytase in combination with xylanase, amylase, and protease, with combined full matrix application, maintained broiler growth performance and the resulting production benefits could contribute to sustainable broiler production.

**Keywords:** full matrix; phytase; xylanase; amylase; protease; feed cost savings; carbon footprint

## The use of fibre-degrading enzymes in broiler feed containing wheat distiller's dried grains with solubles

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The recent peak in the price of cereals has necessitated the use of alternative feed ingredients for livestock feed. Wheat distiller's dried grains with solubles (wDDGS) are by-products from biofuel processing, with a high protein content, being ideal for livestock feed. However, the inclusion rate of wDDGS has been limited to 6 – 12% in broiler feed, depending on age, due to high-fibre characteristics. The aim of the present study was to evaluate the effects of xylanase and/or mannanase on growth performance in broiler chickens offered wDDGS at a high inclusion rate. A total of 560 Cobb 500 mixed-sex broiler chickens were randomly allocated to 5 treatments and 8 replicates per treatment, with 14 birds per replicate. Treatments were 1) corn-soybean meal-based control diet (PC); 2) high-wDDGS control diet (NC); 3) NC plus xylanase at 560 TXU/kg and  $\beta$ -glucanase at 250 TGU/kg (XG); 4) NC plus mannanase at 800 TMU/kg (M); or 5) NC plus enzyme combination (XG+M). The NC diet contained 11%, 19% and 22% of wDDGS in starter (d 0 – 10), grower (d 10 – 21) and finisher (d 21 – 35) phases, respectively. Data were analysed by ANCOVA with the male percentage of each pen as a covariate. Overall (d 0 – 35), the PC diet resulted in better growth performance than the NC diet, whereas M- or XG+M-supplemented birds presented improved weight gain (2,446 g and 2,481 g vs. 2,218 g;  $P = 0.006$ ), and feed conversion ratio (1.60 g/g and 1.58 g/g vs. 1.75 g/g;  $P < 0.001$ ), compared to birds fed the NC diet without enzymes. Additionally, birds fed the NC diet presented the highest litter moisture at day 35; however, this was markedly reduced when XG and M were used in concert (37.9%) compared to NC (46.1%;  $P = 0.005$ ). Collectively, wDDGS inclusion at a high rate as used in the current study in broiler feed could depress birds' growth in the absence of supplemental enzymes, likely due to impaired nutrient utilisation as evidenced by heightened litter moisture. These negative impacts, however, could be alleviated by enzyme supplementation, with a more pronounced improvement in growth performance achieved by M and XG+M supplementation. Therefore, wDDGS inclusion, along with fibre-degrading enzymes, could successfully substitute corn and/or soybean meal without compromising the growth performance of broilers.

Keywords: Broiler; By-products; Carbohydrase; Fibre-degrading enzymes; Wheat distiller's dried grains with solubles

## Source of limestone affects growth performance and bone mineralization of broilers

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Limestone is the primary contributor of calcium (Ca) in diets of monogastric animals. Variation of limestone in chemical composition, particle size and solubility rate have been evaluated in recent years, but there is limited research regarding limestone variation on animal production. Therefore, the current study evaluated the effects of two limestone sources in broilers from 1 to 43 d of age. A total of 750 Cobb 500 male broilers (one-day-old) were weighed and randomly assigned to one of two dietary treatments with 15 replicate pens of 25 birds per pen. The experimental diets were formulated nutrient adequate according to breed recommendations. The two dietary treatments were formulated with two different limestone sources, but having an equal total Ca level of 0.90, 0.80 or 0.75% in the starter (0-14 d), grower (14-28 d) or finisher (28-43 d), respectively. The two limestone sources (1 vs 2) were feed-grade commercial products with different Ca content (37.9 vs 31.9%), particle size (geometric mean diameter, GMD at 401 vs 145  $\mu\text{m}$ ) and in vitro solubility at 15 min (59.6 vs 71.8%). At 43 d of age, the left tibia was taken from 2 birds/replicate pen. Data from the two treatments were analyzed by pooled t-test. During the entire growth period (1-43 d), there was no difference on body weight gain between the two limestone sources ( $P = 0.19$ ), while limestone 2 showed significantly higher feed intake ( $P = 0.04$ ) and worse feed conversion ratio ( $P = 0.01$ ) compared to limestone 1. Additionally, limestone 2 significantly decreased the percentage of tibia Ca and P ( $P < 0.05$ ) and showed a tendency to decrease the percentage of tibia ash ( $P = 0.10$ ) at 43 d of age. These different responses from the two limestone sources may be associated with the Ca availability of limestone. Based on the predictive equation for limestone Ca digestibility (Kim et al., 2019), limestone 2 had a lower Ca digestibility than that of limestone 1 (36.0 vs 42.1%). Also, the lower Ca content of limestone 2 may indicate a higher content of other minerals, eg. magnesium, which may antagonize Ca for its absorption and utilization. In conclusion, the limestone with a relatively low Ca content, fine particle size and high solubility negatively impacted the growth performance and bone mineralization of broilers from 1 to 43 d of age. More studies are needed to understand the mechanisms underlying the impacts of different limestone sources on broiler production.

Keywords: broilers, limestone source, growth performance, bone mineralization

## **Increasing the dose of a novel consensus bacterial 6-phytase variant improved ileal digestibility of Na and Mg in broilers**

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Recent study showed that increasing the dose of a novel consensus bacterial 6-phytase variant (PhyG) exponentially improved ileal digestibility of Zn, Cu, Fe and Mn. This study evaluated the effect of increasing PhyG dose on ileal Na and Mg digestibility. A combined analysis was performed on 12 datasets from two studies that generated 396 datapoints. Both studies employed a completely randomized design, using newly hatched Ross 308 birds, with 20 birds/cage and 6 cages/treatment. Diets were based on wheat, corn and soybean meal with added rapeseed meal or rice bran in some diets. Both studies tested the addition of PhyG at five doses: 0 (NC), 500, 1,000, 2,000 and 4,000 FTU/kg. Diets were formulated in three phases [0–10, 10–21 and 21–35 days (d) of age]. Experiment 1 (Exp. 1) used a 3 x 5 factorial arrangement of treatments comprising three formulated levels of dietary phytate-P (PP) [2.45 g/kg (low), 2.95 g/kg (medium) and 3.45 g/kg (high)], each formulated to contain PhyG at each of the five doses. The average analysed PP content of the diets across phases in Exp. 1 were 2.85, 3.43 and 3.87g/kg for ‘low’, ‘medium’ and ‘high’ PP level diets, respectively. A total of 1,800 birds were tested across the 15 treatments. Experiment 2 (Exp. 2) employed five treatments comprising the basal diet supplemented with PhyG at each of the five doses. A total of 600 birds were tested. The analysed PP content across diets was 3.3, 3.1 and 2.8 g/kg in starter, grower and finisher phase, respectively in Exp. 2. In both studies, celite, a source of acid insoluble ash, was added to all diets as an indigestible marker, at 20 g/kg. Diets were steam-pelleted at 80°C. On d 10, eight birds per cage and on each of d 21 and 35, six birds per cage were euthanised and ileal digesta collected and pooled for the determination of Na and Mg. Data were first checked for outliers and modelled for dose response using analyze/specialized modelling/fit curve procedure (JMP 16.1). The modelling results showed that increasing PhyG dose (based on analysed activity) linearly or exponentially improved ileal digestibility of Na (P exp <0.001) and Mg (P linear =0.0002; P exp = 0.15). This indicates that PhyG improved bioavailability of Na and Mg, increased absorption and utilization of these minerals. This could help to protect the birds from heat stress-induced oxidative damage, also implying the importance to apply Na-matrix in feed formulation that could help to reduce wet litter.

**Keywords:** Phytase; digestibility; sodium; magnesium; broilers

## **A novel phytase improves the responses of laying hens fed an inorganic phosphorus-free diet from 25 to 45 wk of age**

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A study was conducted to evaluate the efficacy of a novel phytase on egg productivity, eggshell quality, bone quality and nutrient digestibility of laying hens from 25 to 45 wk of age. A total of 760 Hy-Line Brown layers were randomly assigned to 5 treatments, with 19 replicate cages per treatment and 8 birds per cage. A positive control (PC) was formulated with adequate levels of nutrients (phytate P, nPP and total Ca at 0.28, 0.34 and 3.7%, respectively); a negative control (NC) was formulated without inorganic phosphate (phytate P, nPP and total Ca at 0.28, 0.12 and 3.7%, respectively); and three levels of phytase (HiPhorius<sup>TM</sup>, dsm-firmenich Nutritional Products, Switzerland) was supplemented on top of the NC diet, corresponding to 300, 600 and 1200 FYT/kg feed. Egg productive parameters and eggshell quality were evaluated every 4 weeks. At 45 wk of age, the left tibia (n=4 birds/cage) and ileal digesta (n=6 birds/cage) were randomly selected for bone parameters and nutrient digestibility. The negative impacts of inorganic phosphate removal on egg production, egg mass and percentage of broken/defective eggs became apparent after 4 weeks, statistically significant ( $P < 0.05$ ) after 8 weeks and in the overall period. Supplementation of the novel phytase at 300, 600 and 1200 FYT/kg significantly improved ( $P < 0.05$ ) these parameters to the levels comparable to PC, and there was no difference among the three phytase groups. A lower eggshell percent and eggshell index was detected in hens fed with NC vs PC ( $P < 0.05$ ) at 4, 8 and 12 wk of study period. Phytase at three inclusion levels improved the eggshell parameters comparable to PC, while phytase at 1200 FYT/kg showed the best improvements across all three timepoints. At 45-wk of age, tibia ash, seedor index and breaking strength were reduced in hens fed with NC vs. PC ( $P < 0.05$ ). Supplementing phytase to the NC diet improved these parameters ( $P < 0.05$ ), with the highest improvement observed with phytase at 1200 FYT/kg. For ileal P digestibility, the novel phytase at 1200 FYT/kg was numerically higher than PC, and significantly higher ( $P < 0.05$ ) than NC and phytase at lower inclusion levels. In conclusion, the novel phytase at 300 FYT/kg was effective to reverse the negative effects of inorganic phosphate removal on egg productive parameters, while a higher dose of 1200 FYT/kg was needed to optimize ileal P digestibility, eggshell quality and bone quality of laying hens from 25 to 45 wk of age.

Keywords: laying hens, phytase, egg productivity, eggshell quality, bone quality, P digestibility

## **Impact of added phytase and dietary InsP6 on the structure and the core microbiota of the digestive tract of broilers**

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The Myo-inositol (1,2,3,4,5,6) hexakis (dihydrogen phosphate) (InsP6) concentration in the feed varies with the raw material used. Exogenous phytase may be added to the feed at an activity level adjusted to the InsP6 concentration. Our goal was to explore the composition and functionality of the gut microbiome in the context of varying exogenous phytase levels combined with increasing levels of InsP6. Twelve experimental diets were created by combining 3 phytase levels (500, 1500, and 3000 FTU/kg) with 4 feed mixtures (FM) to achieve varying levels of InsP6 (1.4, 1.9, 2.4, and 3.0 g InsP6/kg). InsP6 level was adjusted in each FM by substituting maize starch with a constant ratio of oilseed meals and rice bran. A total of 840 Ross 308 broiler hatchlings were raised at standard conditions. On days 0 (L0) and 14 (L14), litter was sampled from the ground. On day 14, the animals were weighed and evenly distributed into 84 metabolic cages; each of the 12 experimental diets (ED14) was sampled and randomly allocated to 7 cages. The broilers were slaughtered on days 22 and 23, and digesta from the crop, ileum, and ceca were collected. Leftovers from the experimental diets (ED22) were collected from the feeder on the same day. Digesta samples were pooled on a pen basis and immediately stored at -80°C until further analysis. DNA extraction was done with a commercial kit, and 16S rRNA gene target sequencing was performed. In the crop, the FM2.4 and FM3.0 reduced *Lactobacillus* and increased *Ligilactobacillus*, compared to FM1.4, while phytase increased the relative abundance of *Ligilactobacillus*. In the ileum, *Lactobacillus* increased with the phytase addition and it was reduced in FM3.0 compared to FM1.4. *Streptococcus* increased in FM3.0 compared to FM1.4. In the ceca, FM3.0 increased the abundance of *Ligilactobacillus*, *Streptococcus*, and *Subdoligranulum* compared to FM1.4. A core microbiota across all samples was not detected. *Lactobacillus crispatus* was present at high abundance in the crop (49%), ileum (42%), and ceca (24%). Phytase supplementation and increasing dietary inclusion of oilseed meals and rice bran at the expense of starch aiming at increased InsP6 concentration, significantly affected  $\alpha$ -diversity in the ceca. *Lactobacillus* and *Ligilactobacillus* were identified as the drivers of colonization in the gut, implying their resilience and dominance in relation to the persistent ASV across the intestine.

Keywords: *Lactobacillus*; *Ligilactobacillus*; Broilers; Core microbiota; Myo Inositol; Phytase

## **Dietary synbiotics improve feed utilization, eggshell quality, and fecal ammonia emission of laying hens under tropical conditions**

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Synbiotics are products that contain both probiotics and prebiotics. They are considered one of the effective alternatives to AGPs in poultry production by enhancing the growth of beneficial bacteria, improving nutrient utilization, maintaining physiological homeostasis, and enhancing the growth performances of animals. Two hundred and forty Lohmann Brown laying hens, at 50 weeks of age, were used to investigate the effect of symbiotic supplementation on laying hen performances, egg quality, and ammonia excretion under tropical conditions. All birds were randomly assigned to 2 dietary treatments including corn-SBM basal diet supplemented with synbiotics (SYNLAC Prime®, Synbio Tech Inc., Taiwan) at 0 and 100 g/ton diet. SYNLAC prime is a combination of probiotics (*Lactobacillus plantarum*, *Enterococcus faecium*, *Lactobacillus acidophilus*, and *Bacillus subtilis*) and prebiotics (dextrin). Each treatment consisted of 10 replications with 12 hens per replication. Both experimental diets were calculated to be isonitrogenous and isocaloric (17.5% CP and 2750 kcal/kg). All hens were housed in a curtain-sided, evaporative cooling system house for 11 periods of 14-d each. The average housing temperature and relative humidity during the experiment period are 28.5-30.0°C and 60-70%, respectively. Feed and water were provided *Ad-libitum* throughout the experiment. Egg quality was measured on the last day of each period. At 72 weeks of age, representative fresh excreta of each replication were randomly collected for determination of fecal score (four-point scale method) and ammonia level (titration method). Statistical analysis of cumulative data from all periods was conducted using the Student's T-Test. The results are presented as the average value of all periods. Feeding synbiotics lowered feed intake ( $P<0.05$ ) with no effect on egg production. The supplementation of synbiotics tended to improve albumen height ( $P=0.0777$ ), Haugh unit ( $P=0.0781$ ), shell thickness ( $P=0.0879$ ), and significantly improved shell weight ( $P<0.05$ ) and reduced the percentage of cracked eggs ( $P<0.05$ ). A tendency to improve fecal consistency ( $P=0.0590$ ) and a reduction in fecal ammonia content ( $P<0.05$ ) were observed in laying hens fed dietary synbiotics. The results from the present study indicated that dietary synbiotics had a very promising benefit to egg quality, shell quality, and ammonia emission of laying hens raised under tropical conditions.

**Keywords:** Laying hens, synbiotics, albumen, eggshell, fecal ammonia.



## **Effect of supplementing multicomponent mycotoxin detoxifying agent on performance, carcass variables, immune responses, and liver function variables in broiler chicken fed known concentrations of aflatoxin b1, deoxynivalenol and T-2 toxin**

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A feeding trial was conducted to evaluate the efficacy of a multicomponent mycotoxin detoxifying agent (MMDA containing selected minerals, Bacillus sp, silymarin, and yeast cell wall extract) to minimize the toxic effects of combined mycotoxins (MT, aflatoxin B1 100 ppb, DON 200 ppb and T-2 1000 ppb) supplementation in broiler diets. A total of 1584 Vencobb day-old male broiler chicks were assigned to six dietary treatments in a 2 × 3 factorial design with 12 replicates of 22 birds per floor pen. Three graded levels of MMDA (0, 1.5 and 3.0 kg/ T) were added to the standard diet and mycotoxin supplemented diets. Body weight gain, feed intake and feed conversion ratio were measured at the end of experiment (42d of age). One bird from each replicate was to measure serum parameters, carcass traits and mycotoxin concentrations at day 42. Serum samples were analysed for bio-chemical indices and immune responses. Liver, kidney, and thigh muscle were analyzed for aflatoxin B1, B2, G1, G2 and M1, T-2, HT-2, T2 Triol, T2 Tetraol, Deoxynivalenol and DOM1. The results showed that MT and MMDA or their interaction did not affect ( $P>0.05$ ) performance, breast meat, abdominal fat, relative weights of immune organs and serum protein and triglycerides concentration. The inclusion of mycotoxins significantly ( $P<0.05$ ) reduced the antibody titres against Newcastle disease vaccine (5.8 vs 7.2 log 2) and increased the liver weight (21.5 vs 16.11 g/kg live weight). Compared to those fed the CD without MT. However, supplementation of MMDA marginally improved antibody titres against ND vaccination (6.3-6.6 log 2). Supplementation of MMDA to MT-contaminated diets at both concentrations significantly ( $P<0.05$ ) decreased the activities of serum glutamic-oxaloacetic transaminase (191-199 units/litre), and serum glutamic pyruvic transaminase (15.21-13.87 units/litre) and gamma-glutamyl transferase (32.03-35.59 units/litre) compared to those fed the MT supplemented group without MMDA (324.1, 20.91 and 58.09 units/litre, respectively). The concentrations of aflatoxin B1, B2, G1, G2 and M1, T-2, HT-2, T2 Triol, T2 Tetraol, Deoxynivalenol and DOM1 in the tissue were below detectable levels. Based on the results, it was concluded that the addition of broad-spectrum MMDA (1.5kg/T and 3.0 Kg/T) to the MT-contaminated feed reduced the negative effects of MT on immune responses and liver function enzymes in broilers exposed to combined mycotoxins.

Keywords: AFB1, DON, T-2, broiler, MMDA

## **Bacillus licheniformis DSMZ 28710 to support high-producing laying hens during lay**

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In Europe, there are only a handful of probiotics registered for use in laying hens during egg production. However, this application should not be neglected: high-producing laying hens also need to be as efficient as possible. This includes utilising their diet to the fullest, whilst withstanding health challenges. From that perspective the importance of a proper functioning gastro-intestinal tract cannot be doubted, indicating the value a probiotic can bring in a layer operation. A recent addition to EU-registered layer probiotics is B-Act®, a probiotic based on a single strain of spore-forming *Bacillus licheniformis* (DSMZ 28710). The probiotic already has a long and extensive history in animal production, supporting high-performing animals during all stages of production. Recent research has now showed the unique *B. licheniformis* strain's benefits in layers. Lohmann Brown layers were supplemented between 25 and 45 weeks of age and compared to a control without any probiotics (25 replicates/group, 5 hens/pen). Per pen the body weights, feed conversion ratios, egg masses and the ratios of cracked and dirty eggs were recorded. Additional egg quality parameters were evaluated in 4-week intervals for each pen, using 4 representative eggs (shell weight, shell thickness, shell breaking strength). Protein levels in the dry manure were recorded at the start and at the end of the experiment as well, as an indication of how well the animal was using the supplemented diets. Adding B-Act® to the diet significantly improved FCR and egg mass ( $P < 0.05$ ) as well as shell percentages. The improvements in the latter parameter could offer an explanation why almost all other eggshell qualities improved numerically as well for the probiotic group. At the same time, significantly less protein was excreted in the dry manure by the B-Act® group compared to the control. This can be interpreted as a better functioning of the gastrointestinal tract, utilising the provided nutrients from the diet as much as possible. In turn, it explains the improved FCR and egg parameters. The above highlights that probiotics have a place in layer nutrition, as these high-performing animals should be supported from start to finish. With an attractive return on investment, the supporting research about its benefits in layers and the flexibility in terms of application options, B-Act® offers an interesting solution to do so - combining economics with health standards in the most rewarding way.

Keywords: B-Act; *Bacillus licheniformis*; DSMZ 28710; probiotics; laying hens; Huvepharma

## **A meta-analysis evaluating the effect of *Pediococcus acidilactici* CNCM I-4622 on performances of laying hens**

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Over the past two decades, literature has extensively documented the use of the probiotic *Pediococcus acidilactici* MA18/5M (PA) in poultry, whose strain collection number was recently updated to CNCM I-4622. This meta-analysis aims to assess the impact of PA supplementation on production performance of layers. A total of 62 studies have been compiled, including both academic works and field trials, wherein the performance of hens, from different commonly used breeds, with PA supplementation ( $1 \times 10^9$  CFU/kg feed) was compared to a negative control (CTR). Each trial reported at least one of two major performance criteria: 1) laying rate and/or 2) feed conversion ratio (FCR). In addition, other parameters regularly reported were egg quality (weight, exported egg mass (EEM) & number of broken eggs) and mortality. The studies were subjected to statistical analysis using a Mixed Model approach (SPSS 29.0), including treatment (CTR vs. PA), trial type (academic vs. field), housing type (cage: Yes vs No) and production phase (Full cycle (> 5 months); Beginning of lay (18-35 weeks old); Middle of lay (35-55 weeks old); End of lay (55-80 weeks old); Extreme end of lay (> 80 weeks)) as fixed effects. The assessment also involved testing two-way interactions between fixed factors. Additionally, study was introduced as a random factor. An important outcome of this meta-analysis is the similar performances regardless of the type of study, type of housing or production phase for laying rate and egg weight. FCR was impacted by type of housing, being significantly lower in cages. Mortality was lower in cages and higher for full cycle studies. Supplementation with PA significantly improved laying rate by 2.7% ( $P < 0.01$ ) and enhanced FCR by 3.1% ( $P < 0.01$ ). Average egg weight was 0.6g higher for the PA-supplemented hens ( $P < 0.05$ ). Higher laying rate and heavier eggs led to a significantly higher EEM (0.361 vs 0.372 kg/hen/week;  $P < 0.001$ ). In addition, incidence of broken/downgraded eggs was substantially reduced by 21% ( $P < 0.05$ ). Also, PA supplementation decreased mortality rate by -33% (3.1 vs 4.6 %;  $P < 0.01$ ). The latter was more pronounced for layers in non-cage systems compared to hens housed in cages (-36% vs -24% respectively,  $P < 0.05$ ). Overall, this meta-analysis, combining results from over 60 studies, underscores the potential of PA supplementation in augmenting overall performance of laying hens across diverse production settings and throughout various production phases.

Keywords: meta-analysis; probiotic; laying hens

## **The effect of yeast $\beta$ -1,3/1,6 glucan administration on the growth performance of broilers in a model of subacute necrotic enteritis**

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Necrotic enteritis (NE), estimated to cost the global poultry industry 6B USD annually, is a multifactorial disease characterized typically by an over-growth of *Clostridium perfringens*. Although traditionally managed using antibiotics, pressures to reduce their use has increased demand for non-antibiotic interventions to mitigate NE in poultry production. Yeast  $\beta$ -1,3/1,6-glucans (YBG) are important structural components of the cell wall and are known to be potent immunomodulators. When administered to poultry, YBG have been demonstrated to inhibit important poultry and human foodborne pathogens and promote intestinal health and disease resistance. Because of these benefits, YBG have been suggested to be a potentially important alternative to the use of antibiotic growth promoters. The objective of this 42 d study was to evaluate the effect of YBG administration on the growth performance of broilers using a subacute co-infection model of NE. Day-old male Ross broiler chicks (n=2,000) were allocated randomly to 4 treatment groups with 10 replicate pens of 50 birds arranged as a randomised complete block design. Broilers were untreated (UNT) or treated in-feed with bacitracin methylene disalicylate (BMD, 55 g/metric ton) or YBG (22 g/metric ton), and NE was induced experimentally using co-infection with *Eimeria* spp. and *C. perfringens*. An unchallenged group served as the positive control (PC). Growth performance was evaluated through 42 d, and gross intestinal lesions were scored at 21 d (3 broilers/pen) on a scale of 0 to 3 points. Data were analysed using ANOVA with Fisher's LSD post-hoc ( $\alpha=0.05$ ). A significant effect was observed on body weight at 42 d (P=0.022). YBG-treated broilers (2.378 kg) were significantly heavier than the PC (2.316 kg), UNT (2.319 kg), and BMD (2.306 kg) treatments. A significant effect was observed on feed conversion ratio (FCR) through 42 d (P=0.027). YBG (1.711) reduced FCR when compared to the UNT (1.755). However, FCR of BMD treated broilers (1.737) was not significantly different than the UNT. Additionally, FCR of BMD and YBG-treated broilers was similar to the PC (1.708). A significant effect on gross intestinal lesion scores was also observed (P=0.002). Administration of YBG (0.46) reduced lesion scores when compared to the UNT (0.86), whereas BMD (0.56) did not. These results suggest administration of yeast  $\beta$ -1,3/1,6-glucan may be used to mitigate effects of necrotic enteritis in broiler chickens.

Keywords: Necrotic enteritis, yeast  $\beta$ -glucan, broilers, antibiotic alternative

## **The supplementation with glycinate of Zinc enhances the immune response and the antioxidant capacity in broilers**

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The objective of this study was to evaluate the impact of the inclusion of an organic supplement of Zn on the immune system (immunoglobulins (IgA, IgG, and IgM)), antibodies against Newcastle Disease (ND), and Infectious Bursal Disease (IBD)), and the antioxidant capacity (Zn-SOD and Malondialdehyde) in broilers at the 6th week of life, comparing it with an inorganic source of Zn (ZnSO<sub>4</sub>). The experiment was carried out by Omega Laboratories (Lonand, Tal-Khandala, Dist. -Satara, Pin, India), where 500 birds were grouped into three treatments: T1, Control basal diet (content of 17.5 mg of Zinc); T2, Basal diet + 100 ppm of Zn (ZnSO<sub>4</sub>); and T3, Basal diet + 100 ppm of Zn (Glycinate of Zinc, Glymet Zn 22%). Subsequently, ten animals per group were sampled to analyze the proposed parameters. Statistical analysis through Dunnett test was performed to estimate differences among groups and control, and Tukey test to estimate the differences among groups. Results showed that the supplementation with Glycinate of Zinc (Glymet Zn 22%) had a superior immune response with higher values of immunoglobulins, with levels of IgA, IgG, and IgM greater by 205% and 66%, 170% and 69%, and 224% and 112%, respectively, compared to T1 and T2 (p<0.05). Furthermore, enhanced values from T1 and T2 were found for ND antibodies at 7.5 and 3.2 times (P<0.05), a virus with fatal effects on the respiratory and digestive tracts, and at 4.3 and 0.6 times for IBD antibodies (P<0.05), associated with immunosuppression in young chickens, implying a positive reinforcement of vaccination. Finally, the antioxidant status was also greater than the inorganic source, implying a superior adaptation to stress conditions with larger values of Zn-SOD (16% and 35%, for T1 and T2, respectively), and lower Malondialdehyde (from 22% to 40%), suggesting a reduction in lipid oxidation. In conclusion, results showed the advantages of the use of Glymet Zn instead of the inorganic source and its better potential use to cope with stressful situations and potential adversities associated with viruses such as ND and IBD.

Keywords: poultry; organic trace mineral; immune status

## NMR-based metabolic characterisation of broiler serum

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Proton nuclear magnetic resonance (1H-NMR) could be used as a diagnostic tool to assess the health status of broilers under a dietary challenge by evaluating changes in their systemic metabolism. NMR metabolomics has the benefit of being able to assess multiple metabolites from a single 500µl serum sample rather than completing multiple assays. To utilise 1H-NMR, it is important to understand the biochemical and physiological changes broilers undergo during growth. In this study, metabolic profiles of broiler serum were compared from hatch (day 0) to day 35 every seven days to show the week-by-week changes in serum metabolite levels. 96 male broiler (Ross 308) chicks were ad-lib fed on a standard commercial wheat/soya broiler diet. 16 birds were blood sampled post-mortem on days 0, 7, 14, 21, 28 and 35, and a metabolic profile was established using 1H- NMR spectra acquired on a Bruker Advance operating at 800.32 MHz. In the study, 35 metabolites showed significant changes in concentration across time points, with the most prominent change occurring between days 0 and 7. The d0 birds had significantly elevated levels of creatine, methyl succinate and 3-hydroxybutyrate. The results were consistent with previous studies on energy utilisation in embryonic and newly hatched chicks. Creatine was higher at 12.27mg/dl (S.E ± 2.17) in day 0 chicks, then declined to below 0.8mg/dl (average S.E ± 0.2) after day 7. Due to glycogen depletion, late-term embryos and hatchlings use creatine as an energy source. Methyl succinate is an intermediate metabolite in the breakdown of fatty acids, and 3-Hydroxybutyrate is a biomarker for high ketone body metabolism. The high presence post-hatch indicates the utilisation of the residual yolk sac fatty acids. There were also several noticeable increases in the following metabolite at day 21, which decreased again in subsequent sampling points: Alanine, N, N-Dimethylglycine, phenylalanine, sarcosine, and tyrosine. N, N-Dimethylglycine is an intermediate in choline metabolism, and sarcosine is an intermediate in converting choline to glycine. The increase in phenylalanine (a precursor to tyrosine) and tyrosine (a precursor to the synthesis of thyroid hormones) may indicate a rise in thyroid production. These examples demonstrate that 1H-NMR can provide information on the dynamic nature of the chickens' metabolites during growth. The study can be used to inform sampling point selection for future serum 1H-NMR metabolomic studies.

Keywords: NMR, Broiler, Metabolomics

## **Solid state fermentation of wheat bran by *Phellinus linteus* applied in broiler diet**

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The aim of present study is to investigate the effects of *P. linteus* fermented wheat bran (FWB) on growth performance, antioxidant activity and the relative gene expression of broiler chickens. Study was divided into two parts, the first one was to investigate the optimum culture conditions, metabolites contents and antioxidant capacities of FWB. Firstly, *P. linteus* was inoculated to oat or wheat under 60% moisture content to evaluate suitable substrate for starter culture. Secondly, the effect of different moisture content on the quantities of functional metabolites of oat starter culture (OSC) was measured. As the results showed, *P. linteus* inoculated to oat could produce more ergosterol (42.2 ppm), adenosine (181.0 µg/g DW) and crude triterpenoids (8.91 mg OAE/g DW) contents than inoculated to wheat (37.62 ppm, 152.88 µg/g DW and 4.43 mg OAE/g DW, respectively). The results of different moisture content on OSC revealed that 60% moisture content was still the more outstanding one. Further, the optimum culture conditions of FWB was measured by inoculating OSC to wheat bran. The results showed that after 18d cultivation, FWB could produce higher ergosterol (31.9 ppm), adenosine (113.1 µg/g DW) and crude triterpenoids (4.12 mg OAE/g DW) contents. The in vitro antioxidant capacity of FWB from the half maximal effective concentration (EC50) of DPPH radical scavenging activity, reducing power and ferrous ion chelating capacity was 24.37 mg/mL, 9.83 mg/mL and 3.22 mg/mL, respectively. The second part were assigned 300 d-old broiler chickens (Ross 308) into 5 groups fed control diets, and control diet replaced with 5% wheat bran (WB), 10% WB, 5% FWB and 10% FWB, respectively. Regarding the entire experimental period, broilers in the 5% FWB group demonstrated significantly lower feed conversion ratio (FCR) than control group, and the FCR of 10% WB group was significantly higher. Moreover, 10% FWB group showed significantly higher lactic acid bacteria (8.94 Log CFU/g) and lower coliform bacteria counts (6.39 Log CFU/g) in ileum than control group (8.35 Log CFU/g and 7.21 Log CFU/g). As the results above, wheat bran fermented by *P. linteus* not only increased the functional metabolites content, but improved the growth performance of broiler chickens in a favorable manner, further implied the potentiality to apply in animal feed industry.

**Keywords:** Solid state fermentation; wheat bran; *Phellinus linteus*; broiler

## **Optimizing the nutritive value of broiler wheat-based diets through enzyme containing endo(xylo)-glucanase and beta-mannanase**

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Broiler feed formulations are evolving to ensure that nutritional requirements are met at an optimal cost, whilst embedding important sustainability goals. Locally sourced ingredients are becoming more prevalent, challenging some of the traditional enzyme use in regards to substrate presence and ultimately, optimal efficacy. This study aimed at determining the effect on nutrient digestibility of a novel carbohydrase containing endo(xylo)-glucanase and beta-mannanase [OPTIM] in industry relevant diets for broilers. A total of 120, 8-d old male Ross 308 broilers were allocated at random to one of 60 replicated cages with a solid floor, following body weight (BW) determination. Replicates (2 birds/cage) were assigned in a randomized complete block design, to one of 6 treatments in which birds were fed with several enzyme strategies. A control group [T1] was formulated with corn, wheat, soybean meal and rapeseed meal to meet nutrient requirements (12.7 MJ ME/kg; 21.1% CP). The remainder treatments were based on the control diet with the following interventions: a dose-response of OPTIM (100 [T2], 150 [T3] and 200 g/tonne [T4]), and the inclusion of 2 separate xylanases from different microbial fermentations [T5 and T6], both dosed at 100 g/tonne. All experimental feeds were provided ad libitum in mash form. At 17d of age, solid floors were replaced with wire mesh and trays were placed under each replicate for total excreta collection. The collection period was terminated at 21d of age, with broilers and feed weighed for growth performance determination. Feed and excreta samples were subsequently analysed for dry matter (DM), nitrogen (N) and gross energy, to calculate nutrient retention. Data was statistically evaluated by analysis of variance using Genstat. Broiler FCR was significantly influenced by treatment ( $p=0.028$ ). T2 delivered the best FCR, with statistical separation to the control group (T1). DM retention ( $p=0.021$ ) and nitrogen-corrected apparent metabolizable energy (AMEn) ( $p=0.011$ ) were improved with most enzyme interventions, but particularly and with statistical relevance, in T2 and T4. T6 did not improve energy utilization when compared to the control group. BW gain, feed intake or N retention were not influenced by treatment. Thus, the nutritive value of broiler diets can be optimized by using a carbohydrase with endo(xylo)-glucanase and beta-mannanase, which may constitute an alternative and flexible solution to xylanase.

Keywords: endo(xylo)-glucanase; beta-mannanase; energy utilization; nutrient retention; broiler



## **Evaluation of supplemented zinc-complexed minerals on performance of broilers fed diets formulated to maximize phytate hydrolysis in the intestinal tract**

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Phytate is a strong chelator of macro and microminerals in the gut. Under an environment in which phytate is rapidly hydrolyzed, absorption and digestion of Zn from raw materials could be maximized. This study investigated the performance of broilers fed diets formulated to optimize phytate hydrolysis in the upper gut and supplemented with reduced levels of Zn in the form of Zinc amino acid complexed minerals (ZnAA). A total of 3,600 d-old Ross 308 broilers were distributed across 72 floor pens. Birds received 1 of 6 diets (corn-soybean meal based, 25 ppm intrinsic Zn) from day one to 37 with 12 replicate pens per treatment, in a randomized block design. The control diet (T1) followed a three-phase feeding program with total Ca (0.95-0.75-0.65%) and available P (0.50-0.43-0.36%) as per Aviagen 2022 broiler recommendations and supplemented with 90 ppm Zn from ZnSO<sub>4</sub>. The other 5 diets followed a five-phase feeding program as per University of Maryland (UMD) Ca (0.94-0.80-0.69-0.69-0.69%) & digestible P recommendations (0.53-0.40-0.32-0.32-0.18%) allowing no inorganic P (Pi) supplementation after 17 days of age. The UMD diets were supplemented with 90 ppm Zn from ZnSO<sub>4</sub> (T2) or 20 (T3), 40 (T4), 60 (T5), 80 ppm Zn (T6) as ZnAA. Phytase at 2000 FTU/kg feed was added in all diets. Performance, carcass, and litter P content were analysed by one-way ANOVA, using JMP 16.0 at P < 0.05. There was a stepwise improvement (P<0.05) with increasing Zn for body weight (BW), FCR adjusted to mortality (cFCR) and carcass weight at 37d at 20, 40, 60, and 80 ppm ZnAA. Breast yield (g and %) was highest in birds fed 40, 60, and 80 ppm ZnAA compared to the lowest breast yield at 20 ppm ZnAA. T1 and T2 birds had similar average BW and were lighter compared to T6 birds. The cFCR was negatively affected in T1 compared to all UMD diets, which were similar to each other. The incidence of carcasses with back scratch lesions were similar in T1 and T2 and highest compared to T4, T5 and T6 fed birds. Breast yield was similar between T1 and T2 fed birds; supplementing ZnAA produced a larger breast (T4 and T5 vs T2 and numerically to T1). As expected, all UMD diets reduced P excretion per kg broiler produced (by 30%) vs T1. A five-phase feeding program with no Pi supplementation from 17 days (UMD) sustained broiler performance with positive impact on the environment; supplementing this diet with Zn from ZnAA yielded birds with fewer scratches and improved breast yield in their carcasses.

**Keywords:** Zinc Amino Acid complex ; broiler performance; breast yield; P mineral excretion

## **Estimations of Ca digesta flows in broilers fed different dietary Ca and P levels: likely implications on digestibility studies**

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Ileal digestibility is currently the preferred method for measuring calcium (Ca) digestibility in poultry (in contrast to pigs when standardized total tract digestibility is preferred). Recent findings of calcium-binding protein calbindin D28k expression in the caecum and colon of egg-laying hens suggest potential hindgut Ca absorption in poultry, with a likely special role in hens. Two studies were conducted to investigate Ca digestibility in broilers. The initial study featured Ross308 broilers, with 6 low mineral (LM) diets (3-7.6 g Ca/kg), incorporating 8% of various processed animal proteins without mineral supply and replicated six times, each cage held 3 birds. The second study, utilizing Hubbard JA57 broilers, included 3 high mineral (HM) diets (8.4-9 g Ca/kg), incorporating 16% of vegetable protein sources (soybean, rapeseed, sunflower meal), 1.2% limestone, and 0.8% monocalcium phosphate and replicated 12 times, each cage housed 4 birds. Ytterbium oxide (50 mg/kg) served as an indigestible index for Ca flow assessment (g/kg diet DM) in ileum digesta and excreta on days 21-22 for the first trial and on days 31-32 for the second one. In LM groups, Ca flows ranged from 0.44 to 3.82 g/kg DM in ileum and from 0.47 to 2.34 g/kg in excreta. In HM, Ca flows ranged from 5.65 to 7.63 g/kg in ileum and from 5.60 to 7.55 g/kg in excreta. Significant correlations ( $P < 0.001$ ) between ileum and excreta flows of Ca were observed. The regression analysis for the entire dataset revealed a line with an intercept at 0 g/kg and a gradient of 1.00. Linear regression analysis (prediction of Ca excreta for a given Ca ileal flow) showed lower irreversible endogenous losses of Ca in excreta with LM diets (intercept: 0.39 g/kg) compared to HM diets (intercept: 1.59 g/kg). The gradients of regression lines (0.64 for LM, 0.76 for HM) suggest potential hindgut absorption or simultaneous responses on urinary excretion. Results affirm robust Ca homeostasis mechanisms in poultry to reduce endogenous excretions in LM diets. Variable effects on endogenous excreta with the dietary Ca levels underscore the importance of using ileal values for Ca digestibility assessment.

Keywords: Calcium; Digestibility; Broiler

## **The ast evaluation of the resistance eimeria spp. isolated from the southern part of thailand against commercial anticoccidial drugs**

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Coccidiosis is a one of the most important protozoal diseases causing the mild to severe damage to the intestinal tract in the chicken. In poultry industry, coccidiosis is mostly caused by *Eimeria* spp. including *Eimeria acervulina*, *Eimeria maxima*, *Eimeria necatrix* and *Eimeria tenella*. In addition, coccidiosis causes significant economic loss in poultry industry due to negative impact on performance. Anticoccidial in-feed is extensively and widely used as a prophylaxis program in broiler chicken farm in Thailand for years. The anticoccidial in-feed program caused the great concern relating to the incidence of anticoccidial drugs resistance in chicken coccidia. In this study, we determined the Anticoccidial sensitivity test (AST) parameter on broiler chicken challenged with mix of *Eimeria* spp. isolated from Southern part of Thailand. Six groups of 9 broiler chicken were raised in triplicate in this experiment. Uninfected untreated control (UUC) and infected untreated control (IUC) were groups 1 and 2 respectively. Group 3; Maxiban (narasin/nicarbazin 50/50 ppm), group 4; Monimax (monensin/nicarbazin 40/40 ppm), group 5; Sacox (salinomycin 60 ppm) and group 6; Coxidin (monensin100 ppm). Treatment groups were received feed with medication from 12 day old until 22 days old or the end of experiment. All groups excluding group 1 were challenged intraorally at 15 days old with 1 ml of distilled water containing  $1.32 \times 10^5$  oocyst/ml. At 22 days old, all birds were euthanized and necropsied. The intestinal lesion score was blindly evaluated following parameters were set as end points: the total means lesion score (TMLS) and average daily gain. The results showed that all treatment groups had TMLS lower than IUC group ( $p < 0.05$ ). The treatment group 4 had the lowest TMLS and significantly lower than those in group 3. All treatment groups showed the significant average body weight gain higher than IUC group ( $p < 0.05$ ). In conclusion, Monimax (monensin/nicarbazin 40/40 ppm) was efficacious in reducing the overall lesion related to coccidia.

Keywords: AST; Anticoccidial resistance; Anticoccidials; Coccidiosis; *Eimeria* spp; Thailand

## Butyric glycerides act directly and indirectly to protect chicken intestine from pathogens colonization

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Butyrate is a short chain fatty acid naturally produced by microbiota in lower gut. Butyrate enhances gut integrity and maturity and acts as a primary energy source for enterocytes. To extend its benefits in chickens' digestive tract, protected butyrate sources are supplemented in the feed. The current study aims to explore the direct and indirect effects of butyric glycerides mixture of mono-, di-, and triglycerides of butyric acid on avian enterocytes resistance to pathogen colonization. First, we show that butyric glycerides mixture highly enriched with monobutyryl (BG) directly improved the resistance of CHII-8E11 chicken enterocytes to microbial pathogens in vitro. It significantly decreased *C. jejuni* and *S. Typhimurium* adhesion to enterocytes at lower doses than sodium butyrate (SB), 2 vs. 10 mM, respectively. BG also drastically reduced cytotoxicity caused by *C. perfringens*  $\alpha$  toxin at a higher extent than SB. Second, we show that only butyrate released by lipase cleavage, but not butyric glycerides, significantly increased the oxygen consumption rate in chicken enterocytes. In mammals, this metabolic response is known to induce a shift in intestinal ecology to an anoxic profile which favors anaerobic microbiota and disfavors aerobic pathogen growth like *Salmonella* and thus indirectly enhance resistance to pathogens colonization. To further explore this shift, we studied the microbiome of chickens fed or not with the butyric glycerides mix (6 animals/group). Indeed, microbiome analysis revealed a shift in microbiota composition, with a reduction in detrimental facultative aerobes like *Escherichia coli*/*Shigella* and an increase of commensal anaerobes like *Lactobacillus*. In summary, our results suggest that supplementing poultry feed with a butyric glycerides mixture, did not only ensure delivery of butyrate all along the gut but conferred a triple antimicrobial effect. Besides the described antimicrobial effect of  $\alpha$ -monoglycerides, we demonstrate that butyric glycerides act directly on enterocytes, making them more resistant to pathogens adhesion and toxicity. In addition, butyric acid, when released by lipase, acts as a source of energy not only for mammalian but also avian enterocytes and can therefore indirectly drive a microbial ecological shift towards a resistant profile to pathogen invasions. Altogether, we show in vitro how butyric glycerides can enhance animal resilience against pathogenic challenges.

Keywords: butyric glycerides, butyric acid, antimicrobial, enterocytes metabolism, mitochondrial oxygen consumption, microbiota, 8E11 cells.

## **Combining the benefits of postbiotics and phytochemicals to promote early gut microbiota maturation**

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The European ban on the use of antimicrobial growth promoters in 2006 and further restriction of therapeutic antibiotic use resulted in extensive research evaluating alternative solutions to control gut health challenges in poultry. However, it is complex and challenging to understand the link between gut microbiota fitness and broiler performance and health. The gut microbiota of healthy chickens is highly diverse and characterized by a high abundance of butyric acid producing bacteria in the ceca. Therefore stimulating the colonization and growth of these bacteria in the hindgut might help to establish an optimal gut microbiota profile and as a result higher performance. It was hypothesized that postbiotics can promote butyric acid producing bacteria directly or through cross-feeding mechanisms and phytochemicals can control Proteobacteria resulting in higher growth of butyrate producing bacteria. A new solution based on postbiotics and phytochemicals was tested in broiler chickens to evaluate the effect on gut microbiota maturation and its relationship to performance. Four independent trials were conducted comparing broiler birds fed a basal diet with birds fed the new solution on top of the basal diet. Body weight and feed conversion ratio were determined at 42d and the data were analyzed using PROC MIXED model with block nested in study as random effect, and significant difference determined at  $P < 0.05$ . Cloacal samples were collected at 21 and 35d of age for microbiota analysis. The results from the four studies showed that birds fed with the new solution containing postbiotics and phytochemicals showed significant higher body weight and feed conversion ratio compared to control birds. The results of the combined analysis including 4 research trials comparing this solution versus a negative control showed significant higher abundance of butyrate producing bacteria across all ages sampled (cloaca swabs, day 21 and 35) and reduction of *Clostridium perfringens* and *Listeria*. In conclusion, inclusion of the new solution containing postbiotics and phytochemicals resulted in a more developed and balanced gut microbiota leading to improved performance when compared to control birds.

Keywords: gut microbiota, maturation, broiler, postbiotic, phytochemicals

## **Assessment of coccidiosis in broilers in France based on autopsy data from 2018 to 2022**

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Coccidiosis is one of the major digestive pathologies in broiler chickens, and its impact in terms of sustainability is significant, with an average cost estimated at €0.187/chicken. Parasite monitoring is necessary to understand the epidemiological situation and detect sub-clinical forms. Between 2018 and 2022, 12677 batches of 5 conventional broilers between 20 and 30 days old were necropsied using a standardized HTS (Health Tracking System) protocol including coccidial lesion scoring based on the Reid & Johnson reference system, separating 'standard' chickens slaughtered up to 42 days and 'heavy' chickens after 42 days. In 2022, the average lesion score (LS) for *E. acervulina* was 0.94 for heavy chickens (+24% compared with 2018) and 0.65 for standard chickens (-11% compared with 2018). For both types of production, the maximum peak was between 21 and 22 days of age, with a broad risk phase between 20 and 26 days. The prevalence of subjects with *E. acervulina*  $LS \geq 2$ , which could have an impact on performance, is 28% in heavy chickens and 18.5% in standard chickens. In the last two years, higher average LS are observed during autumn and winter. In 2022, the mean LS for *E. maxima* was 0.27 for heavy chickens (+50% compared with 2018) and 0.07 for standard chickens (stable), with a detection possible between 20 and 30 days. The incidence of *E. tenella* is lower, with a mean LS of 0.13 for heavy chickens and 0.04 for standard chickens in 2022. Its detection is more pronounced between 28 and 30 days of age, but possible earlier. Elanco's HTS monitoring highlights the prevalence of coccidiosis in France, which remains significant in its sub-clinical form. *Eimeria acervulina* is the predominant species, followed by *E. maxima* and *E. tenella*. The situation differs according to the type of production and has been evolving over the last 5 years, with an increase in the prevalence of coccidiosis in heavy chickens. This monitoring is important for adapting control programs.

Keywords: coccidiosis ; gut health

## Comparison of the efficacy of different ionophores on the control of *E. acervulina*

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When evaluating the prevalence of the 3 most relevant species (*E. acervulina*, *E. maxima* and *E. tenella*) in broilers, *E. acervulina* has overall the highest prevalence in Europe. The goal of this analysis is to compare 3 of the most widely used ionophores: monensin, salinomycin, and narasin on their efficacy to reduce *E. acervulina* lesions. The data used for this analysis was collected from 14 different European countries in a period between January 2019 and November 2023. Data was collected by independent users in field conditions using Aviapp®, the health and performance software tool of Huvepharma®. *E. acervulina* lesions were scored in broilers according to the system of Johnson and Reid (1970) and an average *E. acervulina* score was calculated for a total of 6,570 flocks: 1,298 flocks on monensin, 4,607 flocks on salinomycin, and 665 flocks on narasin (all full in-feed programmes). The average broiler age at which the lesion scoring was performed was respectively 25.7, 25.1, and 25.8 days. Programmes were evaluated based on their age-lesion score profile. In general, salinomycin flocks had the lowest average score (0.31, 95% CI [0.29, 0.34]), as evaluated by the area under the curve (AUC), followed by monensin (0.44, 95% CI [0.36, 0.53]), and narasin flocks (0.48, 95% CI [0.37, 0.59]). The average maximal score reached throughout a grow-out was the highest for narasin (0.92, 95%CI [0.75, 1.09]) followed by monensin (0.83, 95%CI [0.7, 0.96]) and finally salinomycin (0.66, 95%CI [0.62, 0.69]). The difference between salinomycin and narasin was significant for both measurements (AUC p-value = 0.004, Peak score p-value = 0.003). Finally, as prolonged use of a certain coccidiostat over time can induce reduction of efficacy, a time series was also evaluated. The trends shows a small increase of lesions over time, although for monensin and salinomycin this is much less pronounced as for narasin. In the beginning of 2021, an increase in *E. acervulina* lesion scores in birds receiving narasin and monensin in the feed was seen, while lesions in birds on a programme with salinomycin were stable. In conclusion analysis of field data showed that birds receiving salinomycin have the lowest average *E. acervulina* lesion score, the lowest average peak score and the most stable evolution of lesion scores over time.

Keywords: *Eimeria acervulina*;salinomycin;monensin;narasin

## The Impact of *E. acervulina* on FCR

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Determining the effect of certain health conditions, like coccidiosis, on the performance of broiler flocks in field conditions is very challenging. The primary reason is different variables interacting with each other, having a potential influence or association with performance. In this analysis the association between *E. acervulina* lesion scores and the final feed conversion ratio (FCR) at slaughter age was evaluated. To understand this association, a model (random forest) was developed using health data and performance data collected in field conditions using Aviapp®, the health and performance software tool of Huvepharma®. In total information from 1,785 flocks, distributed across 13 countries worldwide were available for analyses and used to train the model. The breeds included were Ross® 308 and Cobb® 500. The average slaughter age was 38.5 days. The slaughter age and the age at which the monitoring took place were added as background variables to the model. In addition, dysbacteriosis, *E. acervulina*, *E. maxima*, and *E. tenella* lesion scores, and gizzard erosion scores were added to the model to control for potential interactions between different health conditions. The model was used to predict the final FCR for different average *E. acervulina* scores while fixing the scores for *E. maxima* and *E. tenella* at zero, which was the most common scenario in the data set (54% of the records). All results are expressed relative to the baseline which is the average FCR when all health modules are set to zero. In general, the association shows a linear increase in FCR for when the average *E. acervulina* score increases between 0 and 1. A plateau is reached at an average *E. acervulina* score of 1.6. The model revealed that the average loss in FCR associated with a certain *E. acervulina* score is highly dependent on the age at which scoring took place. In general scores observed at older ages, are associated with higher losses (average loss of 6.3 points FCR, 95% CI [1.1, 13.5] at 28 days, vs 3.7 points of FCR, 95%CI [-1.1, 9.3] at 21 days of age for an average *E. acervulina* score of 1. The higher impact in older birds is most likely related to the high feed intake and growth at these ages (28-35 days). A small change in *E. acervulina* scores at this age will result in a big decrease in performance. To conclude, in field conditions, increasing *E. acervulina* scores are associated with a negative impact on the average final FCR.

Keywords: *eimeria acervulina*;FCR



## **Saponin-rich plants mixture supports coccidiosis vaccination program in broiler chickens: a case study of floor pen challenge model.**

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Vaccination against coccidiosis involves the administration of live *Eimeria* spp to chickens to immunize them against the disease. It is a reliable method for preventing coccidiosis; however, it requires a trained professional to administer it correctly. Moreover, the vaccine only provides immunity against the strains it contains. Finally, it takes several weeks for birds to develop a protective immunity. Therefore, it is necessary to protect birds during the development of immunity. Norponin XO is a plant-based solution formulated to support birds and help manage coccidiosis. Its composition is based on saponin-rich plants (*Yucca Schidigera* and *Trigonella foenum-graecum*). The objective of this study was to evaluate the effect of this commercial mixture of saponin-rich plants (Norponin XO) on vaccination against coccidiosis in broiler chickens. 300 one-day-old male Cobb 500 broilers were divided into 30 experimental units, each consisting of 10 chickens. Chickens were randomly divided into 5 groups: UUC: Uninfested Untreated control, IUC: infested untreated control, VACC: infested and vaccinated group at d1, VACC/NPXO: infested, vaccinated, and supplemented with Norponin XO from d1 to d42, NPXO: infested, non-vaccinated and supplemented with Norponin XO from d1 to d42. Birds were orally inoculated at 14 days of age with a 15-fold live strain vaccine. Growth performance (live weight, feed intake, and FCR) was monitored at d42, five birds from each group were randomly selected, and intestinal samples were taken (jejunum) for morphometry. Statistical analyses were performed using analysis of variance (ANOVA) with GraphPad software. The experimental infestation design was successful, as evidenced by the significant reduction in live weight and increase in FCR of the chickens in the IUC group compared to those in the UUC group. All treatments compensated for this loss. Villus length and surface area were reduced by *Eimeria* spp. infestation. While all treatments except vaccination compensated for the loss of villus length, only VACC/NPXO and NPXO were able to compensate significantly for villus surface area. This study demonstrates that NPXO2 supplementation is a valid tool for supporting vaccination programs. Furthermore, the results obtained demonstrate that Norponin XO2 supplementation could be used as a “stand-alone” solution for managing coccidiosis in broiler chicken.

Keywords: Coccidiosis, vaccination, saponins, gut health, broiler chicken

## **Effect of uncoated-buffered sodium butyrate supplementation in diet on small intestinal morphology under high stocking density of broilers**

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Although the effect of high stocking density on the deterioration of microclimate and litter of poultry houses has been studied extensively, its role in the pathogenesis of intestinal diseases and the mechanisms underlying these effects has not received adequate attention from the scientific community. The aim of this study was to evaluate the effect of an uncoated-buffered sodium butyrate (SB) supplementation on duodenum and jejunum histomorphometry of broilers raised from 1 to 42 days under low or high stocking density. A total of 640 day-old male broiler chicks (Cobb-500) were allocated in floor pens at 1 of 2 stocking densities in a completely randomized block design (10 replicates/treatment). Low Stocking Density (LSD) and High Stocking Density (HSD) were 20 and 40 kg of predicted final weight/m<sup>2</sup> floor space, respectively. Pre-starter (1-7 d), starter (8-21 d), grower (22-35 d) and finisher (36-42 d) diets were used for dietary treatments. Control diets (CTRL) were formulated according to Cobb's recommendations (Cobb, 2018). SB (54% uncoated-double buffered sodium butyrate; BUTYLin 54, Dietaxion) was included on-top from 1 to 42 d at 600 ppm in the treated diets. Birds and feed were weighed weekly individually and by pen, respectively, and the mortality recorded daily. At 35 and 42 d of age, one bird per pen was randomly selected and euthanized. Duodenum and jejunum were used to examine histomorphological measures: villus height (VH) and crypt depth (CD) of each animal was the mean of 10 determinations. Parametric data were analyzed using ANOVA and non-parametric data through the Chi-square Test. Between 27 and 42 d the relative weight gain and FCR was better for LSD than for HSD ( $P < 0.05$ ), with no difference between diets, but a tendency for SB to improve FCR ( $P > 0.05$ ). Histomorphological trait VH/CD (VCR) were different between diet treatments at any time and in the two intestinal segments ( $P < 0.05$ ). At 35 and 42 d SB broilers showed higher VCR than CTRL birds ( $P < 0.05$ ). At 35 d HSD animals obtained a higher VCR than LSD for duodenum and jejunum ( $P < 0.05$ ), but at 42 d there were a better VCR in duodenum for LSD ( $P < 0.05$ ) and no differences in jejunum ( $P > 0.05$ ). It can be concluded that HSD affects unfavorably duodenum health of broilers at 42 days of age. Dietary supplementation of broilers from 1 to 42 d with an uncoated-buffered SB improved the development of the upper part of the GIT increasing intestinal health of duodenum and jejunum.

Keywords: chicken; butyrate; villus height/crypt depth ratio; relative weight gain

## **Supplementation of a synbiotic and a precision biotic improves the growth performance of broiler chickens submitted to coccidiosis vaccination**

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In commercial poultry practices, coccidiosis vaccination may be included in coccidia control programs to induce immune response, generate protection against subsequent *Eimeria* challenges, and reduce the severity of coccidiosis. However, coccidiosis vaccination may induce intestinal damage that could impair the growth performance of chickens. Therefore, the objective of the present study was to evaluate the impact of the supplementation of a combination of Synbiotic (SBT) and Precision Biotic (PB) on the growth performance of fast-growing broiler chickens vaccinated against coccidiosis. A total of 720 one-day-old Ross 308 broiler chicks were placed in 24 floor pens (3 treatments, 8 replicates, 30 birds/replicate). The three treatment groups were: unvaccinated control, vaccinated control, and vaccinated birds fed diets supplemented with a combination of SBT (PoultryStar® meEU) and PB (Symphiome™). The coccidiosis vaccination was applied, to the respective groups, by spray at the hatchery. Body weight (BW), daily weight gain (dWG), feed intake (FI), and feed conversion ratio (FCR) were calculated for each feeding phase (starter, 1-14d; grower, 15-27d; finisher, 28-42d). Cumulative performance was calculated for the entire growth cycle. At 21 and 42 d, cecal content samples were collected from two birds per pen for microbiome and metabolome analysis (results will be included in the poster or oral presentation). Growth performance data were subjected to a one-way ANOVA with blocks and, when appropriate, means were separated by Tukey's test. It was observed that the coccidiosis vaccination significantly impaired the BW ( $P = 0.04$ ) and FCR ( $P = 0.03$ ) of the birds at d 28 by 3.7, and 2.4%, respectively, but the supplementation of SBT+PB counteracted this effect. Considering the entire experimental period (1-42d), the coccidiosis vaccination impaired ( $P = 0.02$ ) the FCR by 3 points compared with the nonvaccinated group (1.593 vs 1.623), but the supplementation of SBT+PB improved the FCR by 4.5 points (1.578 vs 1.623). In conclusion, the combination of dietary SBT and PB improves the growth performance and should be considered as an effective solution when adopting vaccination as the program of choice for the control of coccidiosis in broiler chickens.

**Keywords:** Broilers, coccidiosis vaccination, synbiotic, precision biotic

## Evaluation of the effects of a bacterial probiotic in experimentally induced necrotic enteritis

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Necrotic enteritis (NE) is a frequent disease in poultry. Bacterial probiotics can be good candidates to alleviate its negative effects in broilers. Previous work carried out by ANSES and INNOZH defined an experimental model to evaluate the efficacy of additives on *Clostridium perfringens* (Cp). This study was carried out to evaluate the effect of mix of *Bacillus amyloliquefaciens*, *B. licheniformis* and *B. pumilus* (Microsaf®, called the mix) on NE. A total of 160 Ross 308 broilers were divided into 4 groups: NINT (not inoculated, not supplemented); IccNT (inoculated with coccidia and clostridia, not supplemented); IcoT (inoculated with coccidia, supplemented with the mix at 500g/t) and IccT (inoculated with coccidia and clostridia, supplemented with the mix at 500g/t). Comparisons between the test group and the control groups allowed evaluation of the effect of the probiotic on mortality, lesions induced by Cp and compensatory growth after the challenge. At D10, the animals from groups IccNT, IcoT and IccT received an oral inoculation of 1mL containing 10,000 oocysts of *Eimeria maxima*. At D14 and D15, the animals from groups IccNT and IccT received an oral inoculation of 2mL of a mix of strains A and B of Cp at 108 bacteria/mL. During the two days following the first bacterial infection, a clinical examination was carried out to assess morbidity and mortality. An evaluation of the specific lesions due to the inoculated pathogens was done on 25 birds per group which were sacrificed at D16, and the remaining animals were kept to study compensatory growth. Body weight was measured at D10, D14, D16 and D20. The mix showed a significant impact on mortality (40% for IccNT vs 12.5% for IccT) and on the number of birds displaying NE lesions (median score 6 for IccNT vs 3 for IccT). Animals surviving the Cp inoculations showed a decrease in growth significantly more severe in the unsupplemented group than the supplemented group in the 2 days following the challenge (ADWG 15g/d for IccNT vs 27g/d for IccT at D16). Those unsupplemented animals remained more impacted by the disease in the 5 days following the challenge (ADWG 28g/d for IccNT vs 38g/d for IccT at D20).

Keywords: Probiotic, Necrotic enteritis, Broiler

## **Standardized workflow for microbiome analysis – methodology and application in commercial broiler farms**

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The microbiome research, especially through high throughput ‘omics technologies, has exploded in recent years, revealing the intricate communities of microorganisms that reside within and on our bodies and in the environment. Advances in DNA sequencing technologies, data storage and analysis have provided drastic improvements in microbiome analysis, for example, in taxonomic resolution, antibiotic resistance gene profiling (AMR), etc. However, the lack of standardization of methodologies prevents comparing results from one study to another, and thereby, the exact understanding of the microbiome community, its role, and its dynamic. Therefore, we established and validated a standardized workflow from sample collection to data analysis and have been applying this exact same workflow on more than 2000 cecal content samples from 25 commercial broiler farms. By doing so, we ensure consistency and comparable data. Our purpose is to provide repeatable protocols to guide the sample collection, handling, processing procedures and data analysis, which are the basis for reliable results. The details of our standardized workflow for microbiome analysis is as follows, (1) RNeasy preservation, (2) PowerFecal Pro DNA kit, (3) DNA shearing, (4) ONT ligation sequencing kit with expansion 96 barcoding kit, (5) 500 Mbp of sequencing data per sample, (6) internal bioinformatics platform with own developed pipelines and databases for mapping to curated microbial genomes, (7) similar settings for downstream analysis through internal App visualization. By applying that workflow, this allowed pooling all our sequencing data from cecal content, and defining the baseline and dynamic for most of the microbes present in the gut of broiler chickens. Remarkable findings have been made on the dynamic of pathogens such as *Campylobacter jejuni*, showing undetectable to very low levels in young birds followed by an impressive rise after day 24-26 in several trials. Many trials showed close association between *Enterococcus*, especially *E. cecorum*, and leg disorders (e.g. FHN). Similarly, we validated our risk threshold established for *Eimeria* based on subclinical lesions seen for coccidiosis. Since our microbiome database is not biased toward one specific country or one type of chicken production, we found out that age has the strongest effect on the microbial composition in the gut followed by litter & antibiotic usage, and to a lesser extent breed and sex.

**Keywords:** Microbiome analysis; Standardization; Commercial farms; broilers

## **Effects Of Dietary Polyphenol Extracts And Zinc Bacitracin On The Caecal Microbiome Of Young Broilers**

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Phytogenics have gained interest for their potential to enhance gut health and animal performance, positioning them as a sustainable alternative to antibiotic growth promoters (AGPs) in poultry nutrition. However, the comparative effects of phytogenics and AGPs on the gut microbiome of broilers are seldom explored. To address this, a study was conducted with broiler chickens housed in pens and fed a control (CTR) diet, supplemented in water with either a subtherapeutic level of the antibiotic zinc bacitracin (BAC, 50 mg/L, from d 0 - 14) or a proprietary blend of pomegranate and tea polyphenol extracts (PP, 0.8% from d 0 - 2, 0.2% from d 3 - 14). At 14 days of age, the composition and function of the caecal microbiome of the birds were analyzed using a shotgun metagenomic approach. The results revealed that PP increased the species richness (Chao1) compared to BAC, which is desirable for ecological stability and functional diversity. The microbial community composition of PP birds differed from those fed CTR and BAC, as evaluated by Bray-Curtis dissimilarity. At the phylum level, PP compared to CTR increased the relative abundance of Bacteroidetes and tended to have a lower ratio of Firmicutes to Bacteroidetes, which have been shown to be associated with high body weight and efficient fat metabolism in broilers. Compared to the CTR and BAC groups, birds fed PP exhibited a significantly higher abundance of genera *Blautia* and *Butyrivibrio*. Bacteria belonging to *Blautia* are known to be involved in the biotransformation of polyphenols and potentially exhibit probiotic functions while *Butyrivibrio* are prominent butyrate producers and implicated in maintaining gut barrier integrity. Heatmap of functional annotations based on Clusters of Orthologous Genes revealed that treatments exert differential effects on the relative abundance of several gene pathways. Notably, dietary PP increased the abundance of gene pathways involved in secondary metabolites biosynthesis, transport, and catabolism, which may be partly due to the inherent response of the microbiome to metabolize the polyphenols. These results suggest that dietary PP exerts a beneficial influence on the caecal microbiome of young broilers, potentially offering an alternative to AGPs for improving the intestinal health and performance of broiler chickens.

Keywords: polyphenols; AGP; gastrointestinal; microbiome

## **Effect of a triple-strain bacillus-based probiotic on in vitro inhibition of *Enterococcus cecorum* isolated from clinical broilers**

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*Enterococcus cecorum* (EC) is usually recognized as a harmless commensal of the gut of broiler chickens. However, dysbacteriosis conditions can allow this opportunistic pathogen to overgrow and benefit from loosening of tight junctions to translocate from a leaky gut to the bloodstream. EC can then be transported to specific tissues (e.g.: joints) or organs (e.g.: heart) where it can induce significant damage. The clinical picture of pathogenic EC infection in a flock is high mortality due to septicemia early in the growing period followed weeks later by lameness due to osteomyelitis of the femoral head. In this context, 22 different EC isolates collected from broiler chickens, 12 isolates from West European farms and 10 isolates from US farms to assess the in vitro inhibitory capability of a triple-strain *Bacillus*-based probiotic, consisting of *B. subtilis* (DSM 32325), *B. subtilis* (DSM 32324), and *B. amyloliquefaciens* (DSM 25840). A pathogen inhibition assay was conducted to determine the ability of the probiotic supernatant to inhibit the growth of the different EC isolates. The inhibitory effect of the supernatant against bacterial growth was evaluated using optical density (OD 600 nm) by accessing the turbidity and all the experiments were conducted in triplicates. Results revealed that the triple-strain probiotic had a very high magnitude of inhibition against EC ranking from 80% and 96% for the 12 West European and 10 US isolates. This strong in vitro capability for direct inhibition of clinical EC isolates can be explained by the ability of the triple-strain *Bacillus*-based probiotic to release specific inhibitory lipopeptides such as surfactins and fengycins able to inhibit pathogenic bacteria and fungi through the formation of pores in cell membranes as biosurfactants. In this trial, direct inhibition impact on several isolates of EC was observed in vitro after application of a specific triple-strains probiotic supernatant.

## Exploring the antimicrobial potential of glycerides of medium chain fatty acids in a simulated poultry gastrointestinal environment

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Glycerides of medium chain fatty acid (MCFA-G) such as C8, C10 (C8C10-G) and C12 (C12-G) are used as feed material to enhance animal resilience against intestinal infections and improve overall animal performance due to their proven antimicrobial activity in vitro. C8C10-G display a higher activity against Gram-negative bacteria (G-) while C12-G mainly has the strongest activity against Gram-positive bacteria (G+). However, their antimicrobial capacity is poorly described in gut conditions. Here, we simulated the chicken gastrointestinal tract (GIT) to assess the behavior of C8C10-G and C12-G throughout the entire GIT, aiming to evaluate their antimicrobial activity as they pass through each section. For this purpose, C8C10-G and C12-G were subjected to serial in vitro digestions mimicking crop, gizzard, and upper gut, followed by microbial fermentation simulating chicken ileum and caeca. After each section, their antimicrobial activity against *Enterococcus faecalis* ATCC 29212 (G+) and *Escherichia coli* APEC DSM 103262 (G-) was analyzed. The MCFA-G tested exhibit different antimicrobial activity patterns. C8C10-G presented activity against *Ent. faecalis*, throughout the GIT remaining significantly higher averaging 3.8-fold increase when compared to the control. Its activity decreased as it passed through the GIT. Regarding *E. coli*, C8C10-G were substantially active all over the GIT keeping their activity at levels of 1.7-fold. C12-G exhibited strong antimicrobial activity against *Ent. faecalis*. This activity persisted throughout GIT simulation at levels of 3.2-fold average. Surprisingly, C12-G have significantly higher antimicrobial activity against *E. coli* in the duodenum and cecum, resulting in a 1.3-fold increase, in both sections, compared to the control ( $p = 0.001$ ). We hypothesized that the antibacterial activity might be due to a change in the cecal microbiota induced by C12-G, favoring the production of metabolites inhibiting G- bacteria. Our findings showed distinct antimicrobial activity profiles of MCFA-G within the mimicked chicken GIT, which were not evident through conventional antimicrobial activity test, and demonstrated the cecal microbiota modulation by C12-G. Further research would be needed to investigate whether the differences in activity can be explained by metabolic processing of the glycerides and to confirm the different activity levels with an in vivo experiment.

Keywords: Antimicrobial activity; Glycerides of Medium Chain Fatty Acid; Glycerides of C8C10; C12; *E. coli*; *Ent. faecalis*; GIT simulation



## **Intestinal microbiota responses to the dietary supplementation of bacillus sp. pb6 and fiber level in broiler chickens**

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The importance of beneficial microbes in maintaining intestinal health in broiler chickens is increasingly acknowledged. Dietary interventions are perhaps the most practical strategies to steer the microbiota under commercial conditions, either by feed additive supplementation or nutritional modulation. Microbiota responses to the supplementation of *Bacillus* sp. PB6 (PB6) have already been partly shown. In addition, the level of dietary fiber is another important factor that shapes intestinal microbial communities. The objective of this study was to evaluate the microbiota responses to PB6 supplementation in the drinking water and to dietary fiber. The experiment was a 2 x 2 factorial design of PB6 supplementation or not at 1 x 10<sup>8</sup> CFU/liter (PB6+ and PB6-), and of dietary fiber level (low fiber, LF; high fiber, HF). LF and HF diets respectively had crude fiber levels of 2.57% vs 3.57% (d0-10), 2.47% vs 3.66% (d10-21), and 2.39% vs 3.83% (d21-35). 400 male Ross 308 day-old chicks were randomly allocated to the 4 groups consisting of 10 pens of 40 birds each. Cecal content was collected from 2 birds per pen on d28 for DNA extraction. Library preparation was performed after full-length 16S rRNA gene amplification followed by sequencing on a MinION Flow Cell using a GridION device (Oxford Nanopore Technologies, UK).  $\alpha$ -diversity was higher for LF PB6+ birds at both genus ( $p=0.03$ ) and species ( $p=0.04$ ) level vs LF birds. PB6 addition increased  $\alpha$ -diversity of LF birds to comparable levels to that of HF birds. On phylum level, LF PB6+ birds had more Firmicutes than LF birds ( $p=0.02$ ). LF birds had more Proteobacteria than HF birds ( $p=0.03$ ), LF PB6+ birds ( $p=0.01$ ) and HF PB6+ birds ( $p=0.02$ ). LF birds had less Bacteroidetes than LF PB6+ ( $p<0.01$ ), HF ( $p<0.01$ ) and HF PB6+ birds ( $p=0.04$ ). At family level, fiber degraders Christensenellaceae and Rikenellaceae, were less abundant in LF compared to LF PB6+ (C  $p=0.02$ ; R  $p<0.01$ ), HF (C  $p=0.02$ ; R  $p=0.01$ ) and HF PB6+ birds (C  $p=0.01$ ; R  $p=0.01$ ). PB6 supplementation brought these 2 families to similar levels as HF groups. Lachnospiraceae were more abundant in HF than LF birds ( $p=0.04$ ) and did not significantly differ from LF PB6+ birds. This showed again that adding PB6 to the LF diet favored a cecal microbiota resembling that of HF-fed birds. This study showed that HF diet improved the diversity and composition of the cecal microbiota and that the changes due to LF could, at least partly, be restored by PB6 supplementation.

Keywords: *Bacillus* sp. PB6; probiotic; microbiota; dietary fiber

## **Effects of phytobiotics based on tannins, saponins, and organic acids on performance and intestinal health of broiler chickens infected with different *Eimeria* species**

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Coccidiosis is one of the most common parasitic diseases of poultry. Herbal preparations such as tannin and saponin extracts, which have known antiparasitic and antimicrobial properties, can be used for the prevention of disease. This study aimed to investigate the effects of one anticoccidial phyto-genic preparation consisting of extracts of tannins, saponins, and organic acids, applied in feed and drinking water. The study was conducted on 300 both genders broiler chickens, divided into 6 groups of 50 chickens each: 2 control (negative and positive) and 4 treated groups. Chickens of all treated groups and positive control were infected with coccidia on the 14th day by oral inoculation of a hundredfold dose of the vaccine strain (*E. acervulina*, *E. maxima*, *E. tenella*, and *E. necatrix*). Chickens of group P1 were treated with phytobiotic in powder form at a dose of 1000 g/t for 35 days, and chickens of group L2 were treated with a liquid form of phytobiotic at a dose of 2mL/L/24h for 10 days (days 16-25) in drinking water. Chickens of group P1+L2 were treated with phytobiotic in powder at the same dose for 35 days and in liquid form at the same dose for 5 days (days 16-20) in drinking water. Chickens of the L1 group received lasalocid at a dose of 750 g/t feed. The highest average body weight (2052.4g) was recorded in chickens that received the liquid form (L2) for 10 days. The lowest average body weight (1495.9g) was observed in chickens in the positive control. The feed conversion ratio (FCR) in chickens in the negative control was the lowest, while in the group that received the powdered form (P1), it was the highest. In chickens in the negative control and those receiving lasalocid, the lesion score was the lowest. The highest number of oocysts was found in chickens from the positive control (3.75). In the treated groups of chickens, the highest number of oocysts was in L1 (3.29) and P1 (3.17). The lowest number of oocysts was in L2 (2.93). By analyzing the results obtained on the number of oocysts, no significant ( $p < 0.05$ ) difference was observed between all treated groups. Oxidative stress parameters showed that catalase activity was the lowest in P1 and P1+L2. Superoxide dismutase activity among the treated groups was the highest in L1. Glutathione-S-transferase was the lowest in L2 and malondialdehyde in L1. It was concluded that the use of these herbal extracts gives good results in the prevention of coccidiosis and oxidative stress in chickens.

Keywords: tannin; saponin; coccidia; organic acid; chickens

## **Safety and immunogenicity of the associated administration of an in ovo coccidiosis vaccines, an infectious bursitis disease immunocomplex vaccine and a rHVT-ND vaccine in commercial broilers**

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In ovo (IO) vaccination is a widely adopted method in broiler poultry business. A common platform for IO vaccination is the use of recombinant herpesvirus (rHVT), which incorporates genetic material insertion(s) to stimulate immunity against various diseases. Furthermore, more IO vaccines are being developed such as coccidiosis or immune complex Infectious Bursal Disease (ICX-IBD) ones. Co-administering IO vaccines could optimize and minimize hatchery processes without compromising vaccine immunogenicity. In this study, the safety and immunogenicity of the co-administration of EVANOVO®, a live attenuated vaccine against coccidiosis for IO administration (HIPRA), GUMBOHATCH®, an ICX-IBD vaccine (HIPRA) and a rHVT vaccine expressing Newcastle disease genes (rHVT/ND) when mixed before IO administration to 18-day-old broiler embryonated eggs was evaluated. This was compared with the joint IO administration of the HVT-ND and the ICX-IBD vaccines followed by a coarse spray vaccination with a coccidiosis vaccine (EVANT®, HIPRA) on day-old chicks. Additionally, single IO administrations of the HVT-ND and the ICX-IBD vaccines were included for comparison. The study included three broiler batches, each with 105 individuals. The vaccines were prepared and co-administered within the shelf life after reconstitution according to their summary of product characteristics. After hatching, 90 chicks from each group were monitored in separate pens under similar conditions for 47 days. The safety of co-administration was evaluated through hatching rates, viability and adverse reactions. ND and IBD specific antibody levels were measured 47 days after hatching to determine the immunogenicity of both HVT-ND and ICX-IBD vaccines. Weekly oocyst counts (OPG) were performed until 35 days after hatching to evaluate vaccination with both coccidiosis vaccines. No adverse reactions or clinical signs were observed. In addition, there were no statistically significant differences in the hatching rates as well as in the rate of seroconversion of IBD and ND antibodies during the entire study. Additionally, the OPG profile in the coccidia vaccinated groups indicated proper administration and replication of the Eimeria vaccine strains. These results suggest that co-administering the IO coccidiosis, ICX-IBD and HVT-ND vaccines is as efficient as individually administering vaccines in protecting birds against coccidiosis, Marek's disease (MD), Newcastle disease (ND) and Gumboro disease (IBD)

Keywords: Coccidiosis; Marek; Gumboro; Newcastle; in ovo vaccination; Eimeria

## **Evaluation of the productive performance of commercial broilers vaccinated against coccidiosis with an in ovo vaccine in comparison with a coccidiosis coarse spray vaccine**

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Coccidiosis remains a significant challenge in the poultry industry, needing continuous advancements in preventive strategies to mitigate its impact on the productive performance of commercial broilers. The development of balanced vaccines against the main *Eimeria* challenges in broilers, coupled with the emergence of resistances by *Eimeria* field strains to anticoccidial drugs, is making coccidiosis vaccination increasingly popular. The most common methods for administering coccidiosis vaccines include coarse spray, gel, or drinking water, among others. With the poultry sector facing increasing pressure to enhance efficiency, reduce mortality, and promote sustainable practices, understanding the comparative benefits of these vaccination methods becomes crucial for informed decision-making and improved overall flock management. Recently, EVANOVO®, a live attenuated vaccine against avian coccidiosis (HIPRA, S.A.) for in ovo (IO) administration, has been launched into the market. This study evaluated the productive performance of commercial broiler flocks vaccinated with EVANOVO®, comparing it to a traditional coarse spray coccidiosis vaccine available in the European market. The study included 35 commercial flocks per group, with 1,686,570 birds vaccinated with EVANOVO® and 1,399,746 birds vaccinated with the coarse spray coccidiosis vaccine, following the guidelines of both vaccines. The same genetic line, hatchery and management guidelines were implemented in both groups, and vaccinations were performed in the same season. The birds were monitored until slaughter. The productive performance of the birds was evaluated through mortality, 1st-week mortality, average daily gain (ADG), Feed Conversion Rate (FCR) and European Performance Efficiency Factor (EPEF). Additionally, antibiotic consumption was measured, and an economic evaluation based on the results of these parameters was conducted. No coccidiosis outbreaks were reported in either group. The group administered with the IO coccidiosis vaccine showed a significant decrease in mortality, as well as a significant reduction in 1st-week mortality. No significant differences in ADG, FCR, and antibiotic consumption were detected, although numerical differences were observed favouring the IO coccidiosis vaccine. Finally, a higher increase in the EPEF was observed in the group administered with the IO coccidiosis vaccine. These results suggest that the administration of EVANOVO® was able to reduce first-week and overall mortality compared to the traditional administration of coccidiosis coarse spray vaccines, as well as increase the EPEF of the flocks. This study represents the first comprehensive analysis of the performance of an IO coccidiosis vaccine, specifically EVANOVO®, in large-scale and commercial conditions.

**Keywords:** Coccidiosis; broilers; in ovo vaccination; *Eimeria*; performance

## Floor pen study to compare the effects of different in-feed treatments against a mixed eimeria spp. inoculum challenge

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The study was designed to determine the efficacy of the combination of PoultryStar and Symphiome Poultry associated to Paracox™5 against an Eimeria spp inoculum in floor pen conditions. Paracox™5 is for active immunization of chickens to reduce infection and clinical signs of coccidiosis caused by different Eimeria strains. PoultryStar is a well-defined, poultry-specific, multi-species symbiotic product. Symphiome Poultry is a complex glycan mixture technically defined as Precision Biotic. Combination of PoultryStar and Symphiome with Paracox™5 vaccination was tested at different levels: 1) PoultryStar 500g+ Symphiome 250g (PS1); 2) PoultryStar 500g+Symphiome 500g (PS2). 2100 male Ross 308 were used in the trial, divided in 7 groups, 15 replicates per group. Paracox™5 were administered to birds of vaccinated groups. Group 1) Unvaccinated unchallenged 2) Unvaccinated challenged, 3) Vaccinated unchallenged, 4) Vaccinated challenged, 5) Vaccinated+PS1 challenged, 6) Vaccinated+PS2 challenged, 7) nicarbazin+narasin challenged. Base feed was the same for all groups. Blank feed was used in untreated and untreated vaccinated groups; supplemented feed in treated and vaccinated treated groups. Except 1 and 3 birds, all were inoculated orally with an Eimeria laboratory strain on D21. Birds of groups 1 and 3 received sham inoculum. Birds of group 5 had significant higher BW compared to birds of group 1 at D35 and at the D42. Group 5 had significant better DWG and FCR compared to birds of group1 during period D20-27(acute phase) and for the overall period D0-35 and D0-42; considering EPEF group 5 had significant better EPEF compared to group 1 at D35 and D42. Direct comparison of group 5 vs group 7 didn't show any significant differences considering mean BW at D35 and D42 neither considering DWG for periods D20-27, D0-35, D0-42 and FCR for periods D20-27, D0-42; no significant differences were observed on EPEF for group 5 compared to group 7 nor at D35 neither at D42. The direct comparison of group 5 and group 6 vs group 7 showed that the Symphiome treated groups had a significant lower total mean Is on D27. E. tenella Is were significant lower compared to group 7 for both groups 5 and 6 on D27 and for group 6 also on D35. Group 7 showed a significant lower E. maxima score on D27. Overall mortality was higher for group 6 compared to both group 5 and 6; this difference was statistically significant when interval 0-35D was considered.

Keywords: "coccidiosis", "control", "feed additives", "lesion score", "probiotic", "performance"

## **Bacillus licheniformis DSMZ 28710 to mitigate gut health challenges in high-producing broilers**

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Gut health plays a major role in animal performance, with tools to manage gut health experiencing a rapid rise. From the multiple options available, probiotics have been proven to be one of the most effective. There are multiple reasons to apply probiotics. Mitigating challenges on-farm, even before these challenges reach a clinical stage, is one of the most important ones. A good example is necrotic enteritis (NE): *Clostridium perfringens* plays a massive role in the onset and development of NE, but there are multiple predisposing factors that need to be present before it reaches a clinical stage. These factors can already negatively affect performance, without presenting themselves as clinical issues. The development of dysbiosis is one of such factors, often described as a disruption of the microbiota's homeostasis. Symptoms range from increased wet litter to a reduced gut functioning, impacting animal performance negatively. Probiotics can help to prevent and/or mitigating a situation like this, both directly and indirectly. *Bacillus licheniformis* DSMZ 28710 – the active strain in Huvepharma's B-Act® range – is a good example thereof, taking on NE with a two-pronged approach. The first is based on preventing dysbiosis taking hold in the first place, making sure gut health is secured and the motor keeps running. However, if NE has developed, *B. licheniformis* DSMZ 28710 is capable of mitigating the situation by mitigating *C. perfringens* directly. The above was recently confirmed again in peer-reviewed research, where broilers were subjected to an induced NE challenge. Health and performance parameters were recorded for three groups: a control, a B-Act® group and an antibiotic treatment group. Even under the induced challenge, animals receiving the probiotic scored significantly better than the control in terms of technical performance (body weight and FCR) as well as health parameters (mortality and lesion scores). These results were similar as those recorded in the antibiotic treatment group. The trial also confirmed *B. licheniformis* DSMZ 28710's two-pronged approach: even before the NE challenge, animals supplemented with the probiotic already outperformed the control in terms of technical performance - simply by supporting and maintaining proper gut health. The trial highlighted once more that *B. licheniformis* DSMZ 28710 has a place in broiler diets, combining economics with health standards in the most rewarding way.

Keywords: probiotics; *Bacillus licheniformis*; B-Act; NE; necrotic enteritis; broilers; Huvepharma; DSMZ 28710

## Identification of different Eimeria species presence and enumeration of coccidia oocysts in layer poultry flocks in Greece

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Coccidiosis stands out as one of the most significant diseases in poultry, having an impact on birds health and productivity, adding an important cost to the poultry industry. The objective of the study was to investigate the burden of coccidiosis and identify different Eimeria species in layer poultry flocks in Greece. Additionally, we aimed to assess the impact of factors such as vaccination, production system, flock age and size. 300 g of fresh fecal material were collected per sample from 19 flocks, encompassing different ages, production systems, flock sizes and vaccination statuses. Among these, 9 flocks were vaccinated using a live vaccine containing oocysts of Eimeria acervulina, E. maxima, E. tenella, E. mitis and E. praecox (EVANT®, HIPRA). All vaccinated flocks were reared in barn system. To determine the burden of coccidiosis, the samples were tested through the Oocyst Count per Gram (OPG) technique. In order to identify the different Eimeria species present, a real-time polymerase chain reaction (PCR) method was performed. Results indicated that 57.8% of the flocks tested positive for at least one Eimeria species. In unvaccinated flocks, E. acervulina, E. maxima, E. brunetti, E. necatrix, E. tenella and E. mitis were confirmed while E. necatrix and E. acervulina were identified in 2 of the vaccinated flocks, being E. necatrix a field strain. Regarding OPG numbers, statistically higher levels ( $p < 0.05$ ) were observed in non-vaccinated animals compared to vaccinated ones. Statistically significant differences were also noted between flocks with size  $< 20,000$  and those  $> 20,000$  individuals, with the latter exhibiting higher OPG levels ( $p < 0.005$ ). Furthermore, flocks housed in cages showed significantly higher ( $p < 0.05$ ) OPG values than those in barns. Data analyses were performed with the use of a paired t-test and an one-way analysis of variance with post-hoc tests. The study revealed the different effect of the investigated factors to the OPG measurements. Vaccinated flocks exhibited a statistically significant lower OPG values compared to non-vaccinated ones, indicating a reduced pressure of the field Eimeria strains after vaccinal immunization. The isolation of Eimeria species like E. necatrix and E. brunetti reveals the need of using vaccines with a broader spectrum against challenging Eimeria species, enhancing vaccine protection in layer poultry.

Keywords: Eimeria species; Greece; layer poultry; oocyst; vaccination

## Broiler performance and health feeding different sources of minerals

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Trace minerals are essential nutrients for health and productivity in broilers. To determine the effect of different sources of trace minerals on the immune response and performance, a total of three different trials were designed, two with an LPS challenge and one in commercial conditions. The sources tested were sulphate vs IntelliBond, a hydroxy trace mineral (HS). In the first trial 60 1-d-old Ross 708 broilers were distributed to one of 6 treatments: 50 ppm Zn+45 ppm Mn, 100 ppm Zn+90 ppm Mn from 2 sources or only 100 ppm Zn or 100 ppm Mn from HS. At 21 days of age, birds were injected with LPS. In the second trial, 1920 1-d-old Cobb 500 birds were randomly distributed to a 2X3 design with Cu at low (15 ppm) or high (150 ppm) levels and Mn fed at 40, 80, or 120 ppm from HS. The two controls contained Cu at 15 or 150 ppm and Mn at 80 or 120 ppm. Birds were challenged with LPS injection at 35 days. In the third trial, 784 Ross 308 broilers were distributed to one of 7 treatments, with the control being fed 50 ppm of ZnO and 50 ppm of ZnSO<sub>4</sub>, and the other 6 treatments were a dose response of 0, 20, 40, 60, 80 and 100 ppm of Zn from HS. Birds were fed a wheat SBM diet. Samples of blood and liver were taken for superoxide dismutase (SOD) analysis and in trial 3 samples of jejunum were taken for gene expression. In the first trial, especially Zn was determined to be driving SOD response, with higher inclusion of HS leading to an equal response as high sulphate ( $P<0.05$ ). Only increasing Mn did not lead to an increase in SOD activity ( $P>0.05$ ). In the second trial, supplementation of 150 ppm of Cu HS resulted in a higher respiratory burst by macrophages ( $P<0.05$ ). The superoxide dismutase response also indicated lower SOD levels with the use of low sulphates ( $P<0.05$ ). In the third trial, performance in birds receiving no additional Zn was the lowest. Inclusion of Zn at 100 ppm from HS improved performance by 3 points ( $P<0.05$ ) and a clear dose response was observed. Gene expression results of jejunal tight junction proteins indicated a trend for an increased expression at 100 ppm. Overall, the results indicate that minerals are critical in broilers under increased immune pressure, which in a commercial setting can increase overall performance. These studies also show that a trace mineral source with higher quality and availability better supports the immune response, reducing the impact of immune challenges.

Keywords: Minerals, Health, LPS



## **In vitro efficacy assessment of sodium dichloroisocyanurate (SDIC) with potassium monopersulphate (PMP) disinfectant against poultry viruses, yeast, and fungi**

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Effective biosecurity measures are critical for preventing the entry and transmission of diseases in poultry farms, a concern accentuated by recent outbreaks in Malaysian poultry production. This study investigates the in vitro virucidal, yeasticidal, and fungicidal activities of a disinfectant containing sodium dichloroisocyanurate (SDIC) and potassium monopersulphate (PMP) against Adenovirus type 5, Influenza A (H3N2), Reovirus type 3, *Candida albicans*, and *Aspergillus brasiliensis*. Conducted in adherence to European Test Methods (EN1657:2016, EN14675:2015, EN16438:2014, EN17122:2019), the evaluation assesses the disinfectant at concentrations of 0.1%, 0.25%, 0.5%, and 1%, with contact times ranging from 0.5 to 30 minutes. Both suspension and surface tests reveal the SDIC/PMP disinfectant's effectiveness, achieving a minimum 4 log<sub>10</sub> reduction (≥99.99%) for all concentrations after 0.5 minutes, except for 0.1%, which requires a 15-minute contact time. Notably, *Candida albicans* is susceptible, with a 4 log<sub>10</sub> reduction after 5 minutes at 1% and 0.5% concentrations in suspension tests and 15 minutes at 0.25% concentration in surface tests. However, *Aspergillus brasiliensis* displays resistance to all concentrations in both suspension and surface tests. While the SDIC/PMP disinfectant demonstrates broad-spectrum protection against viruses and yeast at concentrations of 1% and 0.5%, with a minimum 5-minute contact time, its fungicidal activity is limited. It underscores the importance of thorough cleaning to eliminate organic matter prior disinfectant application for optimal effectiveness. The study recommends further in vitro research, mimicking real poultry settings, incorporating additional poultry pathogens, and potentially advancing to field studies for comprehensive insights into underlying mechanisms.

**Keywords:** sodium dichloroisocyanurate (SDIC), disinfectant, poultry, virucidal, biosecurity

## **In vitro bactericidal activities of sodium dichloroisocyanurate (SDIC)/potassium monopersulfate (PMP) disinfectant against common poultry bacteria**

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In poultry disease control, antimicrobials have long played a pivotal role, but the rising threat of antimicrobial resistance (AMR) has prompted a shift towards reducing their use. This study addresses the urgent need for alternative strategies to combat bacterial pathogens in poultry by evaluating the bactericidal activities of a disinfectant containing sodium dichloroisocyanurate (SDIC) and potassium monopersulfate (PMP). The focus is on five major bacterial pathogens—*Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* subsp. *enterica* ser. Typhimurium, *Pasteurella multocida* subsp. *Multocida*, and *Campylobacter jejuni*. Following European test methods (EN 1656:2019 and EN 14349:2012), the study assesses the disinfectant at concentrations of 0.1%, 0.25%, 0.5%, 1%, and contact times ranging from 0.5 to 30 minutes. Results reveal outstanding bactericidal activities, with a  $\geq 5$  log<sub>10</sub> reduction ( $\geq 99.999\%$ ) in the suspension test and a  $\geq 4$  log<sub>10</sub> reduction ( $\geq 99.99\%$ ) in the surface test against all pathogens at 0.5% and 1% concentrations, particularly with contact times of 15 and 30 minutes. A positive correlation is observed between disinfectant efficacy, concentration, and contact time. Surprisingly, the SDIC/PMP disinfectant exhibits greater bactericidal activities in the surface test compared to the suspension test. Notably, *Campylobacter jejuni* is the most susceptible, requiring only 0.25% concentration for 5 minutes to achieve microbial reduction, while *Salmonella enterica* subsp. *enterica* ser. Typhimurium displays the least susceptibility, requiring 0.5% for 15 minutes. This study underscores the practical potential of the SDIC/PMP disinfectant as a promising solution for minimizing antimicrobial usage in the poultry industry through effective cleaning and disinfection practices. Future research avenues may explore real-world applications in the poultry farm settings, considering variables such as water pH and environmental temperature.

**Keywords:** bactericidal, sodium dichloroisocyanurate, potassium monopersulfate, cleaning, disinfection

**Does essential oils inhibition of Escherichia coli is dependant of cassia oil ?****C. Girard<sup>1</sup>, K. Fayolle<sup>2</sup>, C. Carlu<sup>1</sup>, S. Kerros<sup>1</sup>, T. Chabrilat<sup>1</sup>**<sup>1</sup>Phytosynthese, Mozac, France, <sup>2</sup>VetAgro Sup, Clermont Ferrand, FrancePresenting author: [claire.carlu@phytosynthese.fr](mailto:claire.carlu@phytosynthese.fr)

Essential oils are new alternatives identified to reduce the use of antibiotics in feed with antimicrobial effects in vitro. EFSA evaluates the safety and/or efficacy of these essential oils and their pure compounds, used as additive in animal feed before they can be authorised for use in the EU. Based on the corresponding EFSA opinions, the authorising regulations for Cassia oil, Cinnamon bark essential oil and Cinnamon leaf essential oil may face important restriction in animal nutrition and particularly long living animals. The aim of this study is to investigate the growth inhibition capacity of essential oils mixture with and without Cassia oil against E. coli. The objective was to develop a highthroughput method of in vitro screening and to test two versions of the formula, with cinnamon oil (PhytoCSC) and without cinnamon oil (PhytoCSC-V2). Progressive dilutions of ratio 2:1 from 1/2 (v/v) to 1/16384 of the two formulas (Phyto CSC and PhytoCSC-V2) were faced to E.coli (106 CFU/mL) in 100-wells microtiter plates with Mueller Hinton Broth (MHB) for 300 µl final volume, in triplicate. Microtiter plates were incubated at 37 °C for 24 h in an automated turbidimeter (Bioscreen C, Labsystems, Helsinki, Finland). Optical Density (OD) measurements was performed every 15 min. Growth curves and growth parameters were processed with DMfit curve-fitting software. Minimum Inhibitory Concentration (MIC) was defined as the lowest EO mix concentration inhibiting E. coli growth for 24h at 37°C. PhytoCSC MIC and PhytoCSC-V2 MIC were the same dilution (1/1280). E.coli growth curves at 1/1280 were both constant with Phyto CSC or PhytoCSC-V2. However, PhytoCSC-V2 impacted E. coli growth at MIC/2 (1/2560) as decreasing growth rate max (0,69 H-1 compared to PhytoCSC 1,35 H-1) and decreasing the OD max (0,164 versus 0,470 with PhytoCSC) but E. coli lag phase was increasing compared to PhytoCSC (1H45 compared to PhytoCSC 2H49). The bacteriostatic activity of the both Phyto CSC formula against E.Coli is very close with an equivalent MIC and similar E.Coli growth curves. These results imply that a formulation without Cassia oil can be possible with similar efficiency.

Keywords: cassia oil; regulations; escherichia coli; MIC

## Characterization of the chemical contents of selected insects as food and feed

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The present study aimed to quantify the mineral and phytochemical content in winged termite (*Macrotermes subhylanus*), stinkbug (*Encosternum delegorguei*), and brown locust (*Locusta napardalina*) insects. The mineral contents were analysed using inductively coupled plasma-atomic emission spectroscopy, while the phenolic compounds were determined using liquid chromatography-electrospray ionisation quadrupole time-of-flight mass spectrometry. The results obtained demonstrate that locust recorded the highest potassium ([K]=11.9 mg/g) and sodium ([Na] =3.305mg/g) than in stinkbug (K=1.723 mg/g, Na=0.106 mg/g) and termite (K=1.099 mg/g, Na=1.499 mg/g), respectively. Phosphorus was recorded the highest in termite (3.04 mg/g) than in stinkbug (1.715mg/g) and locust (0.5019mg/g). Furthermore, stinkbug had the highest magnesium ([Mg]=1.056mg/g) whereas the lowest were recorded in termite (0.380 mg/g) and locust (0.634mg/g). Copper (Cu), manganese (Mn), and Zinc (Zn) were recorded higher in termite (Cu=0.159 mg/g, Mn=8.922mg/g, and Zn=0.329 mg/g) than in stinkbug (Cu= 0.056mg/g, Mn=0.075mg/g, and Zn= 0.021mg/g) and locust (Cu= 0.063mg/g, Mn= 0.035mg/g, and Zn= 0.137mg/g). Phenolic compounds such as Rehmaglutin D, Floripavidine, Rosmarinic acid, Acerosin, and Hyacinthacine C1;(+) -Hyacinthacine C were identified in abundance in termite, quantities of 144.7mg/kg, 115.2mg/kg, 231.5mg/kg, 231.5mg/kg, and 197.6mg/kg respectively. Crotanecine (117.2mg/kg), 7beta-Hydroxycatuabine D (222.6 mg/kg), Acerosin (105.5 mg/kg) and N-Hydroxyannomontine (433.2 mg/kg) were detected in abundance in stinkbug. Rhexifoline and Hyacinthacine A1 were the most abundant phenolic compounds in this study. Principal component analysis (PCA) revealed significant differences in the phenolic compounds profile, among insect species studied. Therefore, it can be concluded that all edible insects studied here contain sufficient minerals and exhibit considerable amounts of phenolic compounds that make these insects a potential source of minerals and pharmacology for use in livestock diets.

Keywords: Edible insects, phenolic compounds, phytochemicals, mineral content, sustainable diet

**Elimination of mycoplasma species from the semen of Hungarian native goose****B. Vegi<sup>1</sup>, Á. Drobnyák<sup>1</sup>, N. Pálinkás-Bodzsár<sup>1</sup>, N. Sztán<sup>1</sup>, É. Török<sup>1</sup>, Z. Szabó<sup>1</sup>, É. Kissné Váradi<sup>1</sup>, J. Barna<sup>1</sup>**<sup>1</sup>National Centre for Biodiversity and Gene Conservation, Institute for Farm Animal Conservation, Godollo, Hungary*Presenting author: vegi.barbara@nbgk.hu*

The primary criterion for the establishment of sperm banks is to store samples free from pathogens. Our previous investigations have demonstrated that three mycoplasma species (*M. anserispalgintidis*, *M. cloacalae*, *M. anseris*) can be detected even in asymptomatic gander semen. We have also confirmed that mycoplasma species survive the sperm cryopreservation procedure, posing a potential source of infection during the insemination of frozen/thawed semen. One of the objectives of our current work is to determine the optimal concentration of tiamulin, when mixed with semen extender, which can destroy pathogens without harming spermatozoa. Another goal is to examine whether tiamulin is suitable for mycoplasma clearance during the cryopreservation process. In the first step, we mixed 50, 80, and 100 mg of tiamulin active substance into 100 ml of semen extender. Subsequently, we determined the changes in spermatological parameters in the treated sperm samples half and one hour later. Cell motility was examined using a CASA system, and the ratio of live, intact cells to morphologically abnormal cells was determined with aniline blue-eosin staining. After 1 hour, the percentage of progressive cells significantly decreased at all concentrations compared to untreated semen. The ratio of live, intact cells significantly decreased after 1 hour of application of 80 and 100 mg of tiamulin compared to the control. There was no difference in morphological abnormalities. Based on the results, the cryopreservation of semen was performed with the application of 50 mg of tiamulin in 100 ml of extender. Freezing procedure was carried out in liquid nitrogen vapor according to the professional rules, using 6% DMF as cryoprotectant. After thawing, there was no difference in the percentage of progressive cells or live, intact cells compared to untreated control samples. Mycoplasma was cultured from semen samples in broth and then cultivated on a special medium. Additionally, PCR analysis was performed after DNA extraction using specific primers for the above mentioned three mycoplasma species. After cultivation, mycoplasma colonies were not observed in tiamulin-treated samples, while they were present in control samples. The results of the molecular genetic analysis supported these findings as well. In summary, the use of 50 mg/100ml concentration of tiamulin in semen extender proved effective for elimination of mycoplasma from gander semen.

Keywords: mycoplasma, geese, semen

## Genetic and acoustic characters of Indonesian long crowing chicken for contest purposes

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The pelung chicken is grouped as the longest crowing chickens in Indonesia mainly utilized for contest and exhibition purposes. Since some exotic chicken breeds might influence the genetic diversity of pelung chicken, this study firstly determined the purity of 25 pelung chickens based on the mtDNA displacement loop (D-loop) region. From the first screening of their purity, there were only 20 pelung chickens further assessed their acoustic characters based on chicken age group: 7, 10, 12 and above 15 months (5 birds each). The chicken song was recorded by using the Sennheiser ME66 microphone and stored in the Sony PCM D50. Raven Pro 1.4 program was used to analyze the spectrogram, oscillogram, amplitude, frequency and duration of chicken song. The 10 months' group produced a higher song duration ( $7.62 \pm 1.52$  s) compared to 7, 12, and above 15 months' group. The 7 months' group also produced the lowest song frequency ( $1.745.58 \pm 559.91$  Hz) compared to others. The 10- and 12-months' group produced a higher song amplitude ( $80\ 648.00 \pm 19\ 786.88$  u) and ( $79\ 667.00 \pm 10\ 349.58$  u) compared to others. There were 3 waveforms and oscillogram regions observed in the pelung chicken songs. The 1st waveform was characterized as a long duration with high amplitude and frequency of song. However, the 2nd waveform showed a long duration, a tight and thick oscillogram with high amplitude and low frequency of song. Finally, their 3rd waveform had a short duration, tight and thick oscillogram with low amplitude, and frequency of song. The 10- and 12-months' group, however, had a better song quality for song contest purposes compared to 7 and above 15 months' group.

Keywords: acoustic, age, long crowing chicken, mtDNA D-loop, pelung chicken

## Comparison of four non-linear growth models in free-ranging lohmann dual cockerels

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On livestock farms, especially in meat production, growth models are a fundamental tool for decision making. They are mathematical functions that explain animal growth patterns based on live weight and growth rate. Their understanding and application allow producers to plan feeding, manage space, define slaughter age, and estimate carcass weight. Gompertz is one of the most widely used models to describe growth in animals, especially in poultry, however, it has a limitation: its inflection point is fixed. Nevertheless, Richards, Von Bertalanffy and Morgan, although less widely used, also allow the growth curve of animals to be defined. The present study aims to compare these four alternatives and to identify which of them is most accurately adapted to the growth of Lohmann Dual breed cocks. For this purpose, a sample of 20 male chickens was selected and their live weight was measured weekly from hatching to 16 weeks of age. During this period, they were fed three types of diets: a starter diet, fed from 0 to 30 days; a grower diet, fed from 31 to 60 days of age; and a fattening diet, fed from 61 to 112 days, at which time they were slaughtered. Data were analyzed using SPSS 15.0 software using the non-linear regression option. In this way, the parameters necessary to apply each model were calculated and integrated into the different growth functions. The results indicated significant differences in mature body weight between the models evaluated, showing a higher growth rate when applying Morgan's model. The parameter  $b$ , which determines the sigmoid shape of the curve, was also different in the case of Morgan ( $b$ : 2.051), being very similar in the other three (Gompertz: 0.165; Richards: 0.133; Von Bertalanffy: 0.133). As for the turning point, it was observed that in all models it was reached around week 9, except for the Von Bertalanffy model, which was reached at week 8. Regarding the growth rate at inflection, it was uniform in all cases, ranging between 27 and 30 g/day. Also, the correlation coefficient ( $R^2$ ) was evaluated to compare the goodness of fit.  $R^2$  values were determined (Morgan: 0.962; Gompertz: 0.895; Richards: 0.874; Von Bertalanffy: 0.889). The Morgan function stood out as the most suitable to describe the growth of Lohmann Dual cocks.

Keywords: growth rate; Morgan's model; Von Bertalanffy's model

## **Comparative study on production performance of brown and white variety of Japanese quail (*Coturnix coturnix japonica*)**

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The present study was planned to compare the production performance of brown and white varieties of Japanese quail. A total of 144 (72 each of the brown and white varieties) healthy quail chicks (day old) were randomly selected. Quail chicks were fed standard quail feed as per ICAR (2013) feeding standards. The body weight of brown quail was 7.18 g, 193.67 g, 232.33 g and the body weight of white quail was 6.90 g, 169 g, and 225.17 g at day 1, 6th week and 12th week of age, respectively. The body weight was found to be significantly higher in the brown variety of Japanese quail. The cumulative FCR was 2.04, 2.44, 3.36 and 2.37, 2.88, 3.88 in brown and white varieties during the 2nd, 4th and 6th weeks, respectively, which differ significantly between the varieties. Feed efficiency for one kg of egg mass differs significantly between the varieties. The mortality rate in the white variety quail was higher than that of brown quail. A statistically significant difference was found between the brown and white varieties of Japanese quail in conformation traits (shank length, keel length, and breast angle). Among the haematological parameters, TEC and MCV were significantly different between the varieties. The serum-biochemical parameters showed a significant difference in the values of blood glucose, total protein, BUN and cholesterol between the brown and white varieties of Japanese quail at the 6th week of age and in the values of AST and triglyceride at the 6th and 12th weeks of age. The eviscerated weight (%) in brown Japanese quail was higher than white Japanese quail. The values of dry matter, crude protein and ether extract differ significantly in the brown and white varieties of Japanese quail. The pH and water holding capacity values of breast meat in both varieties were in the normal range. Brown quail laid the first egg at 6.1 weeks, compared to white quail's 6.4 weeks, and their egg production is better. Statistical analysis revealed a non-significant difference between the varieties in egg quality parameters. The net profit up to 12 weeks of age per bird is Rs. 99.82 for brown quail and Rs. 89.92 for white quail, respectively. Considering the above results, the brown variety of Japanese quail may be a better option for both meat and egg production.

**Keywords:** "Japanese quail; growth performance; feed conversion ratio; egg production performance"



## Effects of different housing systems on the performance of laying quails

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In Germany and large parts of the European Union, Japanese quails (*Coturnix japonica domestica*) are commonly kept for egg production in unstructured mesh-wired cages. Due to a lack of current data on structured housing systems for laying quails, this study aimed to investigate the effect of different housing systems on the performance of laying quails. The study evaluated three housing systems: conventional cage system (CCS), alternative housing system (AHS), and littered floor housing system (FHS). Three pens each were available for CCS and FHS, and six for AHS+ (with nest trough) and AHS- (without nest trough). The CCS pen (Type R.V.203, De Rycke Savimat, Chauffry, France) had a 98 cm x 52 cm mesh-wired floor (L x W), a front height of 22 cm and a rear height of 17.5 cm. The AHS pen measured 74 cm x 77 cm (L x W) with a front height of 51 cm, a rear height of 43 cm, and had a plastic grid floor with 1 cm x 1.5 cm mesh size. The AHS pen included a littered box (56 cm x 30 cm x 24 cm L x W x H) divided into a dust bath area and a nesting area (1/3 to 2/3). Six nesting areas each were equipped with (AHS+) and without (AHS-) a nest trough, usually used for pigeons. The FHS pen was bedded with wood shavings and had the same ground area (0.5 m<sup>2</sup>) as AHS and used the same littered box as AHS-. After 7 weeks of rearing in a littered floor pen, 15 randomly picked laying quails each were transferred to the pens. Eggs laid from week 9 to 36 were collected every second day, with box-laid eggs noted separately. Feed consumption was recorded every 28 days. Results showed higher egg production in AHS- (160.0 eggs/hen housed) and FHS (161.9 eggs/hen housed) compared to CCS (155.4 eggs/hen housed) and AHS+ (154.8 eggs/hen housed) ( $p < 0.001$ ). The amount of commercially marketable eggs was higher in CCS (152.7 eggs/hen housed) and AHS- (153.8 eggs/hen housed) compared to AHS+ (146.0 eggs/hen housed) and FHS (146.1 eggs/hen housed) ( $p < 0.001$ ). Overall feed conversion ratio averaged 3.68 kg feed consumed/kg produced egg mass and did not differ between systems ( $p = 0.371$ ). Comparing the systems provided with a nesting box, AHS+ (49%) and AHS- (46%) had more eggs inside the boxes than FHS (34%) ( $p < 0.001$ ). In conclusion, further optimizing of the tested alternative housing systems is crucial to reduce the proportion of non-marketable eggs and eggs not laid into the nesting boxes for economically sustainable quail egg production not produced in conventional cages.

Keywords: laying quail; housing system; performance

## **Characterizing the interface: Seasonal changes in bird communities on poultry farms and house sparrow - wild bird contacts revealed by camera trapping**

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Wild birds, especially waterfowl are considered reservoirs of poultry pathogens. Nevertheless the route for disease transmission between domestic and wild birds in poultry farms has not been established. Here we analyze wild bird community on commercial layer farms and red-legged partridge farms over a one-year timeframe to assess which species visited these farms. We were also interested in the extent of direct and indirect interactions of any other bird species with the house sparrow (*Passer domesticus*), a species considered a potential bridge host due to its abundance, resistance, and ability to enter buildings and enclosures of poultry farms. We conducted eight camera trapping events between January 2018 and October 2019, in two caged layer farms, one free-range layer farm, and two red-legged partridge farms in South-Central Spain. We observed wild bird visits on all types of farms, with the significantly highest occurrence on red-legged partridge farms where food and water are more easily accessible, followed by commercial caged layer farms, and free-range chicken farms. The house sparrow was the most encountered species on all farms, with the highest frequency in caged layer farms followed by spotless starlings. On the partridge farms, the house sparrow accounted for 58% of the wild bird detections, while on the free-range chicken farm, it made up 11% of the detections. Notably, the breeding season saw the highest number of visits to the farms. Our findings confirm that the house sparrow, due to its high abundance and frequent direct and indirect contact with layers and red-legged partridges, is the most likely species to serve as a conduit for the transmission of pathogens between wild and domestic birds independent of the type of farm. Contacts between house sparrows and other bird species were most frequent during the breeding season followed by the spring migration period. The species most frequently involved in interactions with the house sparrow belonged to the order Passeriformes. The study provides a comparative description of the composition and seasonal variations of bird communities in different types of layer/ poultry farms. The study confirms the effectiveness of biosecurity measures that restrict access to food and water. Additionally, it underscores the importance of synanthropic species, particularly the house sparrow, as potential vectors of avian pathogens.

**Keywords:** House sparrow, indirect contact, pathogen transmission, poultry farms, bridge hosts, biosecurity.

## **Comparative analysis of growth performance and economics in various germplasm of chicken for backyard farming**

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The present experiment was conducted to compare the different germplasms being developed for backyard farming. A total of 336 chicks (day old) were used in the study, comprised of 48 chicks of each germplasm namely G1, G2, G3, G4, G5, G6 and G7. The different genotypes are dual purpose breeds developed by crossing of one indigenous breed of chicken i.e. Kadaknath and two strains viz., Jabalpur Colour and Narmadanidhi strain of chicken. The details of different genotype are as follows: G1- Brown plumage Jabalpur Colour chicken (BrJC) Male (M) X (BrJC) Female; G2- BrJC-M X Black plumage Jabalpur Colour chicken (BIJC)-F; G3- BIJC M X BrJC-F; G4- Narmadanidhi chicken (NN)-M X BrJC-F; G5- BIJC-M X BIJC-F; G6- Kadaknath chicken (Kn)-M X BrJC-F; G7- Kn-M X Kn-F. Identical brooding, feeding and watering arrangements were provided to all the groups of birds used for the study. The chicks were fed standard layer ration as per ICAR 2013 during chick phase (0-8 week) and grower phase (9-12 week). The performance of birds was evaluated at weekly interval in terms of body weight, feed intake and feed conversion ratio (FCR). The results of the study showed that the mean body weight at 12th week of age were significantly different ( $p < 0.05$ ) among groups. Highest body weight was recorded for G3 (1403.83g) and lowest was for G7 (960.54g). At 12th week of age, cumulative feed intake for G1 to G6 group was non-significantly different. However, cumulative feed intake for G7 group showed significant ( $p < 0.05$ ) difference from other groups with highest cumulative feed intake for G2 and lowest for G7 germplasm. Similarly, cumulative FCR for G1 to G6 group differ non-significantly at 12th week of age. However, G7 group showed significant ( $p < 0.05$ ) difference from other groups. In numerical terms cumulative FCR was best for G3 (3.52) and poorest for G7 group (5.17). The net profit up to 12th week of age ranged from Rs. 21.37 to 64.90 for different germplasms with highest profit in G3 group and lowest profit in G7 group. This study was conducted to compare the growth performance and economics of various germplasms of chicken that are developed for rural backyard farming. Considering the results, it can be concluded that germplasm G3 was found best among all the germplasms on the basis of different parameters undertaken in the present study.

Keywords: backyard farming, germplasms, cumulative FCR, mean body weight

## **Poultry in vitro digestion model allows a relevant evaluation of raw materials nutritional value**

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A precise evaluation of raw materials nutritional content is crucial to ensure cost and resource efficient poultry feed formulation. It is essential for competitiveness as it promotes the use of a diverse range of feedstuffs, including by-products and new protein sources. Nutrient digestibility of feedstuffs is usually evaluated in vivo, on roosters or chicks, by calculating the difference between the nutrient ingestion and excretion in feces (e.g. gross energy). The use of in vitro digestion models is quite spread in human nutritional research, but is still at an early stage in animal nutrition. Besides reaching out the 3Rs principle (Replacement, Reduction, Refinement) for a responsible use of animal experiments, these in vitro digestion models constitute a very promising tool to conduct faster and less expensive assessment of feedstuffs compared to in vivo experiment. It is also powerful for screening ingredients before in vivo studies. This work aims to develop a poultry-specific semi-dynamic in vitro digestion model, and evaluate its capacity to predict in vivo apparent metabolizable energy (AME). To determine the model parameters, gastrointestinal contents of adult cockerels (n=10) and chicks (n=36) were collected, and enzyme to substrate ratio, pH and enzymatic activities were measured. These values were confronted and completed with scientific literature. Repeatability and reproducibility (RR) of the model for in vitro energy digestibility (IVED) was determined at 1.1% and 1.4% for wheat and 2.5% and 3.5% for sunflower meal, respectively, similar to slightly lower than in vivo RR. Then, 123 raw materials covering a large variability of feedstuffs used in poultry feed (cereals and byproducts, oilseed meals, protein crops, animal proteins), were chosen in order to compare in vivo and in vitro energy digestibility. AME was measured on cockerels following the European reference method from Bourdillon et al. (1990) and varied between 1450 and 4950 kcal/kg DM. IVED was highly correlated to AME ( $R^2 = 0.92$ ), with an absolute mean prediction error of 6.1%. Adding chemical analysis to the model could improve this precision. These results demonstrate the potential of our poultry in vitro digestion model to predict in vivo energy content of a diverse range of raw materials, therefore allowing for a more efficient and reactive feed formulation. It would be of interest to use it for amino acids digestibility evaluation, especially for protein-rich feedstuffs.

Keywords: poultry ; raw materials ; apparent metabolizable energy ; in vitro digestion model

## **Development of a virtual poultry slaughterhouse simulator to train veterinary students in Europe**

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Veterinary surgeons working on farms and at food-processing establishments play a fundamental role in safeguarding public health and animal welfare. As part of the veterinary curriculum, students must acquire the ability to conduct ante-mortem and post-mortem inspection of animals presented for slaughter. Although an essential part of the meat hygiene undergraduate training in Europe involves students undertaking placements within abattoirs, several issues have adversely affected the ability of students to gain such work placements, in particular the closure of many slaughterhouses across Europe and the biosecurity restrictions to give visitors access. For these reasons, a consortium of Veterinary Public Health experts was created, under the UNA Europa umbrella, to inform the development of Virtual Slaughterhouse Simulators (VSSs) as an innovative demand-driven tool that enables students to explore a realistic abattoir work environment that allows them to safely ‘perform’ ante-mortem, post-mortem and auditing procedures to strengthen and enhance meat hygiene teaching. Research conducted on the use of the Bovine VSS showed that the VSS is a useful learning and teaching resource for veterinary students. As such, a Poultry VSS was developed that includes a risk assessment tool to predict the risk of human campylobacteriosis linked to the consumption of poultry meat. A demonstration of the Poultry VSS will be given at the conference. The results of previous studies encourage further investment and collaboration to improve this technology for training veterinary students and other actors of the poultry food industry in Europe. This project has been funded by the European Union - NextGenerationEU under the National Recovery and Resilience Plan (PNRR) - Mission 4 Education and research - Component 2 From research to business - Investment 1.1 Notice “Alma Idea” 2022 (ex D.M. n. 737/2021)– CUP J45F21002000001.

Keywords: “virtual simulator”, “innovative educational tool”, “poultry”, “slaughterhouse”

## **Agripreneurship intentions in the poultry sector of youth in a tertiary institution**

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The Poultry Sector in KwaZulu Natal, South Africa is faced with an inconsistent supply of inputs, loadshedding, climate change or harsh weather, high cost of production inputs and diseases such as the Avian influenza virus. All of these risks have to be minimized in order to obtain a good return on investment in the poultry value chain. To stimulate growth of the poultry industry will require the sector marketed to attract young entrants. Various programmes have been created to promote innovation, entrepreneurship, and self-sustainability amongst youth. One such programme involves a profitable union of agriculture (poultry production specifically) and entrepreneurship: Agripreneurship. The investigation focusses on exploring the Agripreneurship intentions of youth in the poultry sector. To assess the poultry entrepreneurial intentions of youth at the Indumiso campus, the research design embraced an approach with an administered questionnaire to the selected sample. The target population comprised of 500 youth at the Indumiso campus in the Msunduzi Municipality. Responses gathered from participants indicated that their poultry entrepreneurial intentions were influenced by obstacles such as market access, high costs of production inputs, poultry diseases and limited practical production skills. The poultry sector seemed not to be attractive for twenty-five percent of youth due to the associated risks. Offering poultry training in a blended virtual and physical manner was vital. Ninety percent of youth who participated in design thinking sessions and followed a course of instruction, coaching and mentorship were able to develop their own poultry businesses. Poultry working groups were formed, and experiences, skills and knowledge acquired were exchanged through various media platforms amongst the participants. Youth also followed a structured incubation programme making use of a focused Incubation Model. The Model made use of Hackathons, Pre-incubation, incubation, and post-incubation activities. The training, educational background and poultry production experience had a great impact on the entrepreneurial intentions of eighty-nine percent of respondents. Supporting and providing capacity building interventions enhanced the poultry entrepreneurial intentions of youth.

Keywords: Agripreneurship; poultry; youth

## **Empowering consumers to choose safer chicken in informal markets in Ouagadougou, Burkina Faso**

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Food chains in Low and Middle Income Countries (hereafter: LMIC's) are becoming rapidly complex still in development, and therefore consumption of fresh foods sold in urban informal markets is still topical. Particularly in Sub-Saharan Africa (SSA), unsafe poultry meat is a leading cause of foodborne diseases. Consumers can play an important role in demand of safer food, but limited evidence is available on consumer empowerment to improve food safety in SSA. A consumer research based multimedia behavior change campaign (TV, radio, billboards, and social media) aimed to encourage consumers to choose safe ready-to-eat chicken meat at street outlets (koassas) in Ouagadougou, Burkina Faso. In a longitudinal study a panel of 852 regular consumers of ready-to-eat chicken were interviewed, to a) estimate associations between food safety behavior and campaign recall, and b) associations with key behavioral determinants: intentions, knowledge, attitudes, norms, and agency. Data analyses included mixed-effects ordered logistic and linear regression models, adjusting for confounders. After the campaign, 60% of consumers prompted recalled at least one out of four media messages. Significant associations were observed between campaign recall and access to food safety information (OR 1.449), but no associations were found between behavior scores and campaign recall. However, food safety knowledge scores increased significantly (coef 0.132), with recall of a social media message (delivered by an influencer) being particularly linked to better information access (OR 1.449) and increased knowledge scores (coef 0.250). Additionally, billboard recall was associated with an increase in odds of higher perceived health benefits of consistently paying attention to food safety measures when buying chicken (OR 2.046). TV ad recall was associated with a 0.159 unit decrease in the intention-behavior gap, and decreased odds of reporting lower intentions (OR 0.586) and perceived health benefits (OR 0.440). At endline, buying frequency significantly decreased (OR 0.222), but this was connected with increased prices of chicken over time. This study learns that the consumer campaign effectively reached a large group of consumers. Particularly, online advertising and the involvement of key influencers using humorous and emotionally-appealing messages have potential to empower consumers to consistently pay attention to food safety behaviors while purchasing chicken, ultimately leading to enhancing food safety at informal markets.

Keywords: poultry; food safety; LMIC; consumer health;

## Economic efficiency of males of dual-purpose genotypes in organic poultry production in Germany

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The practice of killing male chicks purely for economic reasons is no longer acceptable under animal welfare legislation, and has been banned in Germany since January 1, 2022. Sex determination in the egg is one solution, but is face with concern of the organic sector, and the search is on for sustainable dual-purpose genetics. As part of the European Union Horizon 2020 project called 'PPILOW', trials on novel dual-purpose genotypes (DPG) were conducted. The trials were on three dual-purpose genotypes (DPG) with different performance profiles: (i). Genotype A: a meat-type, (ii). Genotype B: a rustic breed (B) with a balanced performance profile, and (iii). Genotype C: an egg-type. The study focused on how the performance and product characteristics of the males of the genotypes differed and how this was reflected in the economic efficiency. Specifically, to analyse which dual-purpose genotype shows the lowest input with the highest possible output in order to be economically viable. The economic efficiency of the males of the three DPGs is presented on the basis of on-station trials under organic rearing conditions in comparison to a control group (Genotype JA757). The TIPI-CAL (Technology Impact Policy Impact Calculation) model was used for the economic analysis. The results show that the control group (broiler hybrid JA757), is the most efficient in terms of feed-use as it has the lowest feed conversion ratio (FCR). In contrast, Genotypes A, B and C have high feed conversion ratios, which resulted in higher feed costs. Genotypes A and B show the lowest mortality rates and Genotype C the lowest daily weight gain. The differences in performance are reflected in the production costs. Control Genotype JA757 is the most economically efficient as it has lower production costs. Among the dual-purpose genetics studied, genotype A has the lowest production costs. The full cost difference between Genotype A and control Genotype ISA 757 is € 70 per 100 kg live weight and between Genotype C and control group ISA 757 is € 107 per 100 kg live weight. In conclusion, the results of the study show that the more laying-orientated the dual-purpose genetics, the poorer the feed conversion and the higher the production costs for the broiler. Therefore, the higher production costs for the broiler in the case of layer-focused DPGs can only be covered if higher product prices are achieved or if fattening is "cross-subsidised" via a price premium for eggs. This study was carried out in cooperation with partners of the PPILOW project: SYSAAF, Novogen, Hendrix Genetics, Aarhus University, INRAE, ITAB,. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

Keywords: Poultry, Dual purpose genotypes, dual purpose genetics, economic efficiency, production costs, organic production







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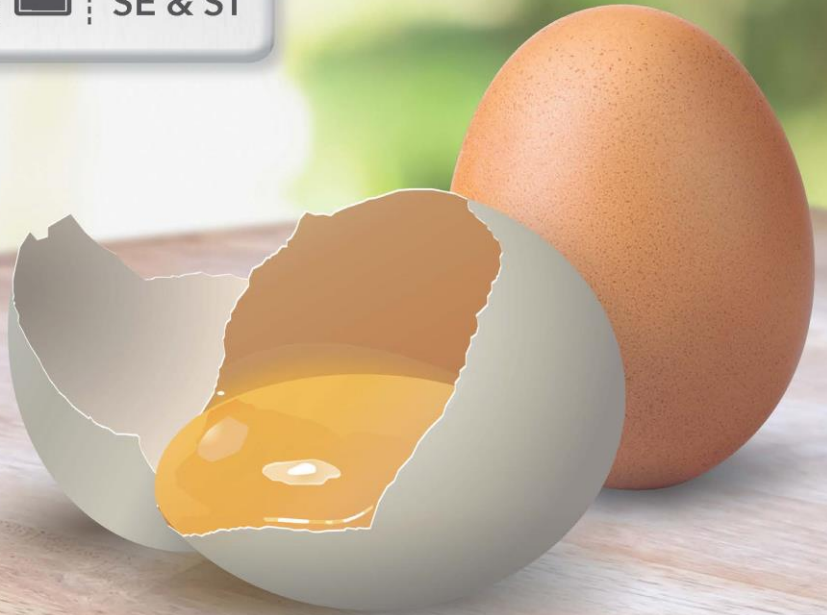
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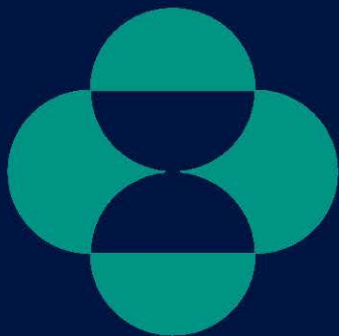
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