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Symposium: Advances and challenges in Salmonella control in poultry production: From epidemiology to innovative solutions

1000S *Salmonella* in the poultry industry: challenges and perspectives

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Non-typhoidal *Salmonella enterica* remains one of the leading foodborne zoonotic pathogens worldwide, causing an estimated 93.8 million cases of gastroenteritis and 155,000 deaths annually. Poultry meat and eggs are major reservoirs and vehicles of human infection, making *Salmonella* control throughout the production chain a global priority. Although control programs have reduced traditional serovars such as *S. Enteritidis* and *S. Typhimurium*, emerging serovars, including *S. Infantis*, *S. Kentucky*, *S. Heidelberg*, and *S. Minnesota*, have become increasingly prevalent. These lineages are well adapted to poultry production and may exhibit enhanced virulence, environmental persistence, and antimicrobial resistance. *Salmonella* transmission occurs horizontally through contaminated litter, dust, water, equipment, and surfaces, and vertically from breeder flocks to progeny through contaminated eggs. Asymptomatic birds may continuously shed bacteria, facilitating dissemination and subsequent carcass contamination. Environmental persistence is further enhanced by biofilm formation, which increases tolerance to disinfectants and may allow long-term reservoirs to persist between production cycles. Emerging serovars, particularly *S. Heidelberg* and *S. Minnesota*, have shown increased tolerance to disinfectants and environmental stress. Among Brazilian *S. Heidelberg* isolates, the *qacE* gene, associated with reduced susceptibility to quaternary ammonium compounds, and *formA*, related to formaldehyde tolerance, was identified in *S. Heidelberg* and *S. Minnesota*. These genes may occur on plasmids carrying antimicrobial resistance determinants, potentially promoting coselection of multidrug-resistant (MDR) strains. The emergence of antimicrobial-resistant non-typhoidal *Salmonella* is another major concern. Intensive use of antimicrobials, particularly fluoroquinolones and third-generation cephalosporins, has contributed to selection of resistant clones. Growing demand for antibiotic-free poultry and restrictive regulations have accelerated the development of alternatives, including probiotics, phytobiotics, organic acids, enzymes, essential oils, bacteriophages, competitive exclusion products, in ovo interventions, immunomodulators, and vaccination. Although promising, these approaches require continued validation under commercial conditions. Next-generation vaccines providing broader protection against multiple serovars are particularly important. Finally, whole-genome sequencing and other omics technologies have transformed *Salmonella* surveillance by enabling characterization of emerging clones, resistance and virulence determinants, transmission pathways, and intervention strategies. Integrated genomic and epidemiological surveillance, combined with effective biosecurity, prudent antimicrobial use, vaccination, and alternative control measures, will be essential to reduce salmonellosis, protect public health, and ensure sustainable poultry production.

1001S Controle da *Salmonella*: Compreender a epidemiologia e a incidência da *Salmonella* é essencial. Como definir um programa de amostragem em granjas?

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A indústria alimentar global deve evoluir continuamente para atender à demanda dos consumidores por produtos seguros e nu-

tritivos. Segundo a OMS (2026), ao longo de 20 anos (2001–2021), as doenças transmitidas por alimentos causaram 866 milhões de casos e 1,5 milhão de mortes anualmente, sendo as crianças pequenas as mais afetadas. Neste relatório, a OMS estabeleceu uma lista de 42 riscos à segurança alimentar. Estes incluem *Salmonella enterica* não tifoide, causando duas doenças (salmonelose e salmonelose invasiva), e foi implicada em 35,8 milhões de casos de doenças transmitidas por alimentos em todo o mundo. Como a maioria dos sorotipos de *Salmonella* não causa doenças graves em aves, sua presença é frequentemente considerada sem impacto no setor avícola. Essa falsa sensação de segurança muitas vezes leva ao relaxamento das medidas de controle e à tendência de aceitar a presença de alguns sorotipos como tolerável. Alguns sorotipos de *Salmonella* podem ser considerados organismos “transitórios”, que são isolados e identificados com pouca frequência. A maioria desses sorotipos geralmente não é patogênica e pode ser eliminada por meio de processos rotineiros de limpeza e desinfecção. No entanto, certos sorotipos podem se estabelecer plenamente em instalações avícolas, proliferar e formar populações residentes tanto nas aves quanto em seus ambientes. Tais “sorotipos residentes” persistem por longos períodos, mesmo após a aplicação de procedimentos rigorosos de limpeza e desinfecção. Dois exemplos de “sorotipos residentes” em instalações avícolas são o *S. Kentucky* (EUA) e o *S. Minnesota* (Brasil), frequentemente negligenciados porque não causam doenças em aves e há poucos relatos que os associam a doenças transmitidas por alimentos. No entanto, a *S. Kentucky* apresenta alta prevalência na indústria avícola de diversos países, e sua importância em relação à resistência antimicrobiana foi recentemente reconhecida nos Estados Unidos. Em algumas granjas avícolas, certos sorotipos de *Salmonella* de alta relevância para a saúde pública podem persistir; entre eles, *S. Enteritidis*, *S. Typhimurium*, *S. Infantis* e *S. Heidelberg* são os mais importantes para a segurança alimentar. Por outro lado, há uma situação preocupante em alguns países da América Latina, onde a *S. Gallinarum* apresenta alta prevalência, causando altas taxas de mortalidade e morbidade. O manejo da salmonelose na indústria avícola exige programas abrangentes de prevenção e controle. O combate a essa bactéria deve se concentrar em uma combinação de ações que incluam vigilância e diagnóstico adequados, juntamente com intervenções direcionadas a animais vivos, sob o conceito de “criar um ambiente hostil para a *Salmonella*”, tanto dentro quanto fora da ave.

Keywords: *Salmonella* serotype; Resident serotype; Transitory serotype

1002S Practical actions for *Salmonella* control on farms

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Salmonella is a major food-safety challenge in poultry because birds may carry it without clinical signs and the pathogen can persist in the environment. Chicks, feed, water, litter, pests, wild birds, people and equipment may introduce or reintroduce it. No single measure is sufficient; effective control requires an integrated, risk-based multi-hurdle program. External biosecurity is the first barrier: restrict access, record visitors, separate clean and dirty areas, use dedicated clothing and footwear, and define procedures for vehicles, tools and shared equipment. Ensure the sanitary status of incoming chicks, apply all-in/all-out management and downtime, and prevent contact with wild birds. Monitor rodents, flies and other

vectors; use trends and corrective actions, not bait presence alone. Remove spilled feed, waste, excess vegetation and harborage sites. Internal biosecurity must limit spread between houses and flocks. Before disinfection, remove litter, dust, grease and organic matter; control disinfectant concentration, contact time, temperature and water-quality compatibility. Clean drinking lines between flocks, manage biofilm and verify water sanitation. Feed control should cover raw materials, thermal treatment where applicable, hygienic storage and transport, and prevention of post-processing recontamination. Manage litter moisture, repair leaks and remove caked areas. Vaccines, probiotics, prebiotics and organic acids may complement the program, but must fit the system, serovars, legislation and cost-effectiveness. They never replace biosecurity, hygiene or pest control. Use a defined environmental and flock-sampling plan—with sites, frequency, laboratory methods, interpretation criteria and pre-established responses. Track barrier compliance, water quality, rodent activity, cleaning performance and microbiological results to detect trends early. Sustainable *Salmonella* control depends on disciplined execution: standardize, record, audit and correct routine measures. Identify the main entry and persistence routes in each unit, focus resources on critical points and use monitoring results for continuous improvement.

1003S Biomapping Evolution. Biomapping Poultry Processing as a Food Safety Management Tool for Process Control and Continuous Improvement.

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Biomapping has evolved from a primarily descriptive microbiological sampling approach into an integrated, data-driven food safety management strategy for poultry production and processing systems. Traditional pathogen monitoring programs often rely on individual sampling points and prevalence-based results that provide limited information about pathogen movement, microbial loads, process variability, and intervention effectiveness. Modern biomapping expands this concept by systematically following microbial indicators and pathogens across pre-harvest, primary processing, and secondary processing environments while integrating quantitative microbiological data with operational and environmental variables. This presentation describes the evolution of biomapping from static sampling toward continuous process intelligence. Contemporary biomapping incorporates enumeration of indicator organisms and pathogens, longitudinal sampling, statistical process control, environmental mapping, and visualization tools to identify microbial trends, process deviations, and potential contamination harborage points. Integration of operational parameters such as flock characteristics, line speed, intervention concentrations, equipment performance, temperature, and sanitation conditions further enables identification of factors associated with microbial changes throughout the process. Emerging analytical approaches, including automated dashboards, machine learning, and artificial intelligence-assisted root cause analysis, can transform biomapping datasets into actionable management information. These systems support validation, verification, risk-based sampling optimization, and continuous improvement while allowing processors to detect microbial drift before it becomes a food safety failure. The continued evolution of biomapping represents a transition from retrospective pathogen testing to proactive, predictive food safety management, providing the poultry industry with a framework to better understand microbial dynamics, optimize interventions, allocate resources, and strengthen pathogen control across the farm-to-processing continuum

Keywords: biomapping; salmonella; continuous improvement; microbial indicators; process control

1004S Innovative solutions for *Salmonella* control: the role of monoglycerides

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The progressive restriction of antibiotic use in poultry production and the continued public health burden associated with Food Safety have accelerated the search for innovative and sustainable control strategies. Among the most promising alternatives, monoglycerides have emerged as a unique class of antimicrobial molecules capable of reducing enteric pathogen colonization while supporting intestinal health. Unlike organic acids, monoglycerides are largely pH-independent, allowing antimicrobial activity throughout the gastrointestinal tract, including distal sections such as the ceca where *Salmonella* and other pathogens commonly persist. Their amphiphilic structure facilitates penetration of bacterial membranes, resulting in disruption of membrane integrity, leakage of intracellular contents, and bacterial death. Evidence from both in vitro and in vivo studies demonstrates significant activity of monoglycerides against major poultry pathogens, including *Salmonella enterica*, *Campylobacter spp.*, *Escherichia coli*, and *Clostridium perfringens*. Different monoglycerides have a specific action against selective genotypes of *Salmonella* e: g. Monocaprin has shown particularly strong antimicrobial potency, achieving substantial reductions of *Salmonella* enteritidis populations in laboratory models, whereas Monobutylin has demonstrated inhibitory activity against *Salmonella Typhimurium* while contributing additional gut health benefits. Importantly, blends of short- and medium-chain monoglycerides consistently outperform individual compounds by combining complementary modes of action, broadening antimicrobial spectrum, reducing the likelihood of bacterial adaptation, and maintaining efficacy across varying environmental conditions. Studies in broiler chickens have reported marked reductions in cecal *Salmonella* counts following dietary supplementation with monoglyceride blends, supporting their role as an effective food safety intervention. Beyond pathogen control, these blends may positively influence gut microbiota composition, intestinal integrity, and inflammatory responses, contributing to improved bird resilience and performance. Collectively, current evidence suggests that strategically formulated monoglyceride blends, particularly those incorporating highly active molecules such as monocaprin, monolaurin together with other short- and medium-chain monoglycerides, represent an innovative and scientifically sound approach to defend against different strains of *Salmonella* in modern poultry production systems, while aligning with antibiotic-reduction and food safety objectives.

Keywords: Monoglycerides; Salmonella; Food Safety

1005S The role of vaccines in controlling *Salmonella*

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Salmonella enterica remains one of the most important foodborne zoonotic pathogens associated with poultry production. *Salmonella* contaminated poultry eggs and meat products are described as major sources of human *Salmonella* infection, and both horizontal and vertical transmission are recognized as key infection routes in chickens. Poultry can act as a reservoir for non-typhoidal *Salmonella* serovars, particularly *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, or

others according to local epidemiology. These serovars may colonize the chicken's internal organs without causing obvious clinical disease and subsequently contaminate eggs, meat products, litter, and the farm environment. Vaccination against *Salmonella* is a central component of integrated control programs in chickens, with emphasis on reduction of intestinal colonization, organ invasion, fecal shedding, environmental contamination, and reproductive tract involvement. However, Vaccines aim to reduce infection pressure at flock level by limiting intestinal colonization, systemic dissemination, and fecal shedding so vaccination is not a stand-alone solution, but a biological intervention that complements multi-step intervention strategies like biosecurity, hygiene, pest control, monitoring,

and hatchery management and it should be presented as a strategic "infection pressure reduction tool" within an integrated *Salmonella* control program. Live vaccines contribute primarily to early mucosal and cell-mediated protection, while inactivated vaccines contribute to systemic humoral immunity and longer-term protection during lay or breeder production. The practical value of vaccination is best demonstrated when immune monitoring is interpreted together with bacteriological surveillance, especially drag-swab, boot-swab, hatchery, and environmental monitoring, rather than by serology alone.

Keywords: salmonella

Symposium: Biosolutions to mitigate the effect of grain variability on animal performance

1006S Effect of Corn Variability in Poultry Diets

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Corn is the primary energy source in broiler diets in Brazil, accounting for approximately 55% to 70% of feed formulations. Its high starch concentration, combined with its wide availability, makes this cereal the main source of energy supporting the rapid growth of poultry. However, corn should not be considered a homogeneous feed ingredient, as its chemical composition, physical characteristics, and microstructure vary according to cultivar, growing conditions, harvest season, and post-harvest processing. These variations directly influence starch and protein digestibility, metabolizable energy availability, and, consequently, animal performance. The processes involved in grain development and the accumulation of starch and proteins during grain filling are complex. The interaction between starch granules and the protein matrix, primarily composed of zeins, determines the organization of the endosperm and influences characteristics such as hardness, vitreousness, and mechanical resistance. Although related, hardness and vitreousness represent distinct properties: the former is associated with the physical resistance of the grain, whereas the latter reflects the compactness of the protein matrix and the internal organization of the endosperm. Comparisons among different corn cultivars, such as flint, dent, and floury types, have demonstrated differences in starch granule compactness, gelatinization temperature, starch digestibility, and resistant starch formation. Another important aspect is the influence of corn storage proteins, particularly zeins, on starch accessibility to digestive enzymes. Grains with harder and/or more vitreous endosperms exhibit a denser protein matrix that restricts enzymatic access to starch granules, thereby reducing starch digestibility. In contrast, soft endosperms possess a less compact structure, facilitating enzymatic degradation and improving nutrient utilization. Therefore, variations among cultivars, production regions, and growing seasons may affect nutrient digestibility and dietary energy utilization, ultimately influencing animal performance. In conclusion, corn variability should be considered a strategic factor in animal nutrition. An integrated understanding of chemical composition, endosperm microstructure, cultivation conditions, and storage practices can support more precise feed formulations, maximizing nutrient digestibility, animal performance, and the economic efficiency of poultry production systems.

Keywords: Cultivars; endosperm; Protein; Starch; Zeins

1007S Digestive physiology of starch in poultry

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Starch is quantitatively the most important nutrient in poultry diets, comprising up to 50% of a diet, and constitutes the major source of dietary energy. This presentation provides an overview of the digestive physiology of starch in poultry and discuss the major factors influencing starch digestion and glucose absorption. Despite a relatively short gastrointestinal tract and fast digesta passage, poultry generally present remarkably high starch digestibility coefficients, frequently exceeding 95%, although reduced digestibility (<90%) can occur. Intrinsic characteristics of grains such as amylose-to-amylopectin ratio, starch granule structure and size, and fibre and non-starch polysaccharides contents vary among the main cereals like maize, wheat, barley and oats, and greatly affect starch digestibility. Extrinsically, feed processing methods like grinding and pelleting also modify starch availability through gelatinization and retrogradation processes and by altering feed particle size. These factors determine the levels of rapidly digestible, slowly digestible, available and resistant starch fractions in the diet, which differ according to their rate and extent of digestion throughout the tract. Starch hydrolysis is primarily mediated by pancreatic α -amylase and brush-border carbohydrases, with glucose absorption occurring through sodium-dependent and facilitative transport systems. While rapidly digestible starch is quickly hydrolyzed and absorbed in the small intestine, resistant starch and part of slowly digestible starch fractions reach the hindgut undigested and become substrate for microbial fermentation, leading to the production of short-chain fatty acids that can beneficially modulate the microbial population and improve gut health and resilience, but also reduce energetic contribution of starch to the bird. Conversely, an excess of rapidly available starch may induce postprandial glucose peaks that compete with amino acids for absorption, increasing amino acid catabolism and impairing nitrogen utilization. In newly-hatched birds, endogenous production and secretion of amylase and other pancreatic enzymes is suboptimal, potentially limiting starch digestion. Yet, poultry possess unique physiological adaptations such as extended crop retention, mechanical grinding of the gizzard, and refluxive intestinal motility that prolong the exposure of digesta to pancreatic enzymes and contribute to a high starch digestibility. Therefore, nutritional strategies considering digestion kinetics of starch and protein and optimizing feed structure, cereal selection, and supplementation with exogenous enzymes are crucial to enhance starch and energy utilization and reduce production costs.

Keywords: Cereals; Digestion; Nutrition; Physiology; Starch

1008S Improving Digestibility Through Feed Processing in Broiler Chickens

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Feed processing may impact feed intake, feed conversion, nutrient digestibility, and gastrointestinal physiology in broiler chickens. Among processing steps, grinding, pelleting, and expansion are the most relevant to alter nutrient digestibility. Grinding regulates particle size and distribution, directly influencing gizzard development and digestive dynamics. While coarse particles stimulate gizzard function, prolong retention time, and enhance mechanical breakdown of feed it may also reduce pellet durability index (PDI). Thus, broiler performance usually results from balancing particle size and distribution (GMD and GSD) for PDI improvement and gizzard stimulation. Pelleting partially gelatinizes starch, denatures proteins, and breaks impeding protein matrixes by shear forces, which may increase their susceptibility to enzymatic digestion. However, the degree of starch gelatinization achieved during pelleting is low to modest (often 5-30%), and its contribution to starch digestibility seems to be limited. On another hand, expansion enhances starch gelatinization (40-90%), pellet durability, and reduces anti nutritional factors, but may also increase the loss of thermolabile nutrients and enzymes. Expansion also allows incorporation of larger particles without negative impact on pellet quality. However, it is important to highlight that is difficult to isolate the controversial effects of starch gelatinization versus increase in feed intake (due to the improvement of PDI) on starch digestibility. While starch gelatinization may improve α -amylase action in the intestine it also increases feed intake and consequently possibly passage rate. This may lead to starch overload in the gut, negatively affecting digestibility because the available enzymatic capacity is insufficient to hydrolyze the increased substrate volume. In conclusion, starch digestibility is influenced by multiple factors, including its botanical origin, non starch polysaccharide composition, processing conditions, particle size, and pellet quality. Moreover, the direct effects of hydrothermal processing on nutrient digestibility are often confounded by indirect impacts, particularly the increase in feed intake induced by heat treated diets, which alters digestive dynamics and energy utilization.

Keywords: Expansion; Hydrothermal processing; Pelleting; Protein denaturation; Starch gelatinization

1009S Exogenous microbial amylase in poultry diets: What do we know so far?

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Starch is the primary energy source in poultry diets and the major carbohydrate reserve in cereal grains, accounting for approximately 60–70% of corn kernels, 55–70% of wheat, and 65–75% of sorghum grains. However, starch digestibility varies among cereal types and is influenced by factors including starch granule structure, the amylose-to-amylopectin ratio, interactions with the surrounding protein matrix, feed processing, and the digestive capacity of birds. In corn, starch is stored within the endosperm, which consists of floury and vitreous fractions. The proportion of these fractions determines

kernel texture, allowing corn to be classified as dent, semi-dent, semi-flint, or flint. Moreover, kernel texture has been associated with differences in apparent metabolizable energy and starch digestibility. Therefore, understanding the relationship between endosperm structure and nutritional value is essential for improving feed formulation, optimizing enzyme supplementation strategies, and maximizing nutrient utilization in poultry production. Additionally, exogenous amylase supplementation has emerged as an effective nutritional strategy to enhance starch hydrolysis, improve dietary energy utilization, and optimize broiler growth performance. By complementing endogenous amylase activity, exogenous amylase increases the digestion of both digestible and resistant starch fractions, thereby increasing glucose availability for metabolism of birds. In addition, amylase supplementation may reduce the metabolic cost associated with pancreatic enzyme synthesis, improving digestive efficiency. Previous studies have consistently demonstrated improvements in starch digestibility and metabolizable energy, resulting in enhanced performance and feed efficiency. On average, exogenous amylase has been associated with a 2.3% increase in body weight gain and a 1.8% improvement in feed conversion ratio. These responses are generally more pronounced during the pre-starter phase, when endogenous digestive capacity is still developing, and during the finisher phase, when starch intake is greatest relative to digestive capacity. However, the magnitude of the response depends on factors including starch source, diet composition, feed processing, bird age, and interactions with other exogenous enzymes. Overall, exogenous amylase represents a promising nutritional strategy to maximize dietary energy utilization, starch digestibility, and improve broiler performance when enzyme supplementation is tailored to feed ingredient substrates, diet characteristics, and production conditions.

Keywords: broiler; corn; digestibility; feed efficiency; starch

1010S DDG in Brazil: Emerging product, market context, and feed formulation use

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Corn ethanol production has increased exponentially in Brazil recently, representing around 25% of the total ethanol produced nowadays. According to J.P. Morgan (2025), the amount of corn to be processed in 2027 is 37 million tonnes, which will result in 11 million tonnes of dried distillers' grains (DDG) available for use in feed formulations. In Brazil, the available DDG can be obtained through two different processes: the conventional one, which results in DDG with solubles (DDGS), and the fiber removal process, which results in high-protein DDG and corn bran with solubles (CBS). It is worth noting that when starch is removed to produce ethanol, all the other components of the corn grain are concentrated in the products. Although they originate from corn, they have distinct nutritional values depending on the processing method. For example, the metabolizable energy (AME) value varies among the products, with HP-DDG presenting 3060 kcal/kg, DDGS 2410 kcal/kg, and CBS 1850 kcal/kg (Rostagno et al., 2024). These nutritional differences are extremely relevant when formulating diets because they can directly affect their final composition and costs. Therefore, it is important to understand that different products are available with different compositions, and each one will comprise a different portion of the formula. Despite these differences, some characteristics are common among corn ethanol products, such as the low lysine, tryptophan, and arginine contents and the high leucine content, which can lead to imbalances related to the isoleucine and valine (BCAA) contents.

Moreover, their fiber content is another factor to consider, as it is 3 to 4 times higher than corn's and can become an antinutritional factor. When considering DDGS, the variability is even more evident due to the "solubles" fraction added to the final product. This fraction can vary in its oil, fiber, and sugar contents, and it is also susceptible to heat, which can decrease the lysine content during the drying process. Therefore, it is essential to characterize the available product before its inclusion in feed formulation. After adjusting formulations according to the characteristics of each product, including correcting the AA profile and AMEn values, the literature has shown

that including DDGS, HP-DDG, and CBS in poultry diets does not affect performance parameters. On the contrary, when used in laying hen diets, they improve yolk color and reduce the need for synthetic pigments. Hence, the inclusion of these products depends on their proper characterization, nutritional value, availability, and cost, allowing accurate feed formulation.

Keywords: alternative feedstuff; broiler; corn; ethanol product; laying hen

Student Competition Processing and Products

1 Litter management, rather than broiler house type, drives pododermatitis-related feet condemnation in broilers

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Broiler feet condemnation due to pododermatitis represents an important challenge for animal welfare, processing yield, and economic sustainability in poultry production. We hypothesized that environmental and litter-management factors are major drivers of feet condemnation in commercial broiler systems. Thus, a 3-yr historical dataset from a large-scale Brazilian poultry integrator, comprising records of over 266 million broilers, was evaluated to identify the main moderators associated with feet condemnation. Data included classificatory variables such as slaughter site (temperate or tropical climate), broiler house type (positive pressure, negative pressure or dark house), flock sex, stocking density, litter reuse, between-flocks interval, slaughter weight, mortality, and productive performance. The dichotomous variable feet condemnation was analyzed by a random effects model (REML) and dataset heterogeneity was tested by Q2 test. In case of significance, heterogeneity was explained by meta-regression and subgroup analysis. Moderator (classificatory variable) relevance was quantified according to the determination coefficient obtained in meta-regression (R^2). Overall feet condemnation averaged 17.05 $\pm$ 0.014%, with significant heterogeneity among observations ($P < 0.001$, $I^2 = 100\%$). Slaughter site was the most influential moderator ($R^2 = 65.76\%$, $P < 0.001$), followed by litter reuse ($R^2 = 40.54\%$, $P < 0.001$), between-flocks interval ($R^2 = 36.82\%$, $P < 0.001$), slaughter weight ($R^2 = 36.59\%$, $P < 0.001$), stocking density ($R^2 = 30.50\%$, $P < 0.001$), and average daily gain ($R^2 = 29.32\%$, $P < 0.001$). Broiler house type ($R^2 = 8.23\%$, $P = 0.042$), flock sex ($R^2 = 7.24\%$, $P = 0.329$), total mortality ($R^2 = 4.31\%$, $P = 0.040$), placement weight ($R^2 = 1.49\%$, $P = 0.724$), slaughter season ($R^2 = 0.00\%$, $P = 0.120$), slaughter age ($R^2 = 0.00\%$, $P = 0.797$), and final density ($R^2 = 0.00\%$, $P = 0.879$) showed lower explanatory relevance. Higher condemnation rates were observed in the temperate-climate processing plant (38.83%), positive-pressure houses (45.68%), and flocks raised under lower stocking densities. Increased slaughter weight, mortality, and prolonged between-flocks interval were positively associated with feet condemnation, whereas greater litter reuse reduced condemnation rates. These findings reinforce the importance of litter quality, rather than broiler house type, as major driver of pododermatitis-related condemnation.

Keywords: Pododermatitis; Feet condemnation; Broiler welfare; Litter quality; Poultry production

2 Multivariate assessment of body composition and fat deposition in commercial broilers under different production conditions

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The excessive deposition of fat in broiler chickens impairs productive efficiency and carcass value. Although factors such as sex, age, nutrition, and environment have been studied individually, their interaction remains poorly understood under commercial conditions. Thus, this study aimed to evaluate the relationships between body composition characteristics and fat deposition in commercial broilers raised under different production conditions, using multivariate statistical analyses. A total of 756 Cobb broilers (379 males and 377 females), from 35 commercial flocks, were evaluated at slaughter. The birds were raised in houses with positive or negative ventilation systems and subjected to two commercial nutritional programs routinely adopted by the integrator. Diet A included functional feed additives and alternative ingredients during the starter phase, whereas Diet B consisted of a conventional corn-soybean meal-based program. Slaughter age was categorized as normal (44–45 days) or late (46–48 days). Body weight, carcass yield, feather yield, relative weights of liver, heart, viscera, and gizzard, and abdominal, visceral, and total fat deposition were recorded. Data were analyzed using factorial ANOVA and Principal Component Analysis (PCA). Correlation patterns among variables were assessed using heatmap analysis. Sex was the most influential factor in body composition. Males showed higher body weight (3745 vs. 3057 g; $P < 0.001$) and higher feather yield, whereas females exhibited higher abdominal fat (1.342 vs. 1.035%), visceral fat (0.804 vs. 0.568%), and total fat deposition (2.146 vs. 1.603%; $P < 0.001$). Late slaughter increased body weight (3542 vs. 3359 g) and carcass yield (78.98 vs. 77.48%; $P < 0.001$), but reduced the relative proportion of visceral organs and visceral fat. PCA revealed an inverse relationship between growth and adiposity, and a strong association among abdominal, visceral, and total fat. The heatmap confirmed strong positive correlations among adiposity traits, particularly between abdominal and total fat ($r = 0.78$). Therefore, sex and slaughter age are the main drivers of body composition and adiposity in commercial broilers. Multivariate analyses revealed clear biological patterns of nutrient partitioning, while nutritional and environmental factors exerted secondary influences on tissue deposition.

Keywords: Multivariate analysis; age of slaughter; fat deposition; carcass yield; broiler chickens

3 Arrowroot starch–gelatin biodegradable coatings: microstructure, barrier properties and effects on egg quality during ambient storage

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Coatings have been studied as an alternative to mineral oil to reduce moisture loss during egg storage. The barrier properties of biodegradable films are strongly influenced by the starch-to-protein ratio, which affects the structure, permeability, and solubility of the material. This study evaluated the effect of different arrowroot starch (A) and gelatin (G) ratios on the internal quality of eggs, water vapor transmission rate (WVTR), solubility, and scanning electron microscopy (SEM) of these coatings, comparing them to mineral oil. Biodegradable films were prepared using the ratios of 100A:0G, 50A:50G, and 0A:100G. Mineral oil and uncoated eggs were used as positive and negative controls, respectively. Commercial eggs were coated and stored at ambient temperature for 21 days. Weight loss, Haugh Unit, yolk index, albumen and yolk pH parameters were evaluated. Data were analyzed by ANOVA and Tukey's test ($P < 0.05$). Among the biodegradable coatings, 0A:100G showed the lowest weight loss (2.92%) and maintained Haugh Unit values (76.01) comparable to mineral oil (75.30) ($p > 0.05$). In contrast, 100A:0G resulted in greater weight loss (3.22%) and lower Haugh Unit values (69.13), while uncoated eggs showed the poorest quality (4.37% weight loss; Haugh Unit = 54.81). Mineral oil remained the most effective treatment overall, reducing weight loss to 0.98% ($p < 0.05$). Regarding WVTR, mineral oil was also the most effective treatment in containing water vapor exchanges (9.19 g/m²/day), followed by the 50A:50G ratio, which achieved statistically similar results (14.58 g/m²/day) ($p < 0.05$). As for solubility, films composed of 100% arrowroot starch showed the lowest rate (20.89%), while 100% gelatin obtained the highest percentage of solubilized material (45.11%) ($p < 0.05$). SEM images showed that uncoated eggs presented an irregular surface with visible pores and microcracks. Coating the shell with the three arrowroot:gelatin ratios provided a homogeneous coverage that sealed these irregularities, resulting in a thinner film with the addition of gelatin. In eggs treated with mineral oil, the shell surface resembled the negative control, but cross-sectional images demonstrated oil penetration into pores and microcracks through capillary forces. In conclusion, mineral oil proved to be the most efficient coating; however, gelatin-rich films provided effective preservation without penetrating the eggshell pores, representing a sustainable and promising alternative.

Keywords: Biopolymers; Shelf life; Mineral oil; Haugh unit; Scanning electron microscopy

4 Metabolizable energy values of alternative feed ingredients for broilers

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Accurate estimates of apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) are essential for the efficient use of alternative feed ingredients in broiler diet formulation. This study aimed to determine the AME and AMEn values of eight alternative feed ingredients used in broiler nutrition. A total of 400 male broilers (Cobb 500), 16 days of age, were allo-

cated to a completely randomized design with ten treatments, eight replicates per treatment, and five birds per replicate. Two reference diets were formulated, and the test ingredients were evaluated using the substitution method. The evaluated ingredients included three meal-type feedstuffs (whole annatto seed meal, spray-dried porcine plasma, and poultry viscera meal) and five lipid sources (three sources of soybean acid oil, distillers corn oil, and degummed rice bran oil). The AME and AMEn values determined in the present study, respectively, were 7,023 and 6,841 kcal/kg for soybean acid oil source one, 7,743 and 7,466 kcal/kg for soybean acid oil source two, 8,318 and 7,913 kcal/kg for soybean acid oil source three, 8,554 and 8,457 kcal/kg for distillers corn oil, 8,273 and 7,910 kcal/kg for degummed rice bran oil, 999 and 975 kcal/kg for whole annatto seed meal, 3,388 and 2,753 kcal/kg for spray-dried porcine plasma, and 3,691 and 3,216 kcal/kg for poultry viscera meal.

Keywords: annatto; corn and rice oil; plasma; soybean acid oil; viscera meal

5 Effect of eggshell powder inclusion on the sensory evaluation and acceptability of extruded corn snacks

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Chicken eggshells are by-products of food industry that can be reutilized as a natural dietary calcium source in human food. This study evaluated the sensory acceptability of extruded corn snacks containing different levels of eggshell powder. Eggshells used were sterilized by boiling 1,000 g in a solution containing 0.1 g Proxitane 1512 (peracetic acid) for 45 minutes, strained, oven-dried at 60°C for 72 hours, and then milled. The corn snacks were formulated with 0, 1.5, 3.0, 4.5, and 6.0% eggshell powder (EP) using corn grit as the basal ingredient and extruded with 10% water. The extruded snacks were seasoned with 10% soy-oil, 2% salt, and 3% chimichurri. Five repetitions were conducted for each treatment, and each repetition was evaluated by 20 adults totaling 100 sensory evaluators. The aim of this study was to evaluate the effects of eggshell powder on the sensory acceptability of the snacks; thus, a structured questionnaire was administered to 100 untrained adult male and female evaluators who were between the ages of <25 and >50 years. The evaluators assessed the snack samples independently for colour, flavour, aroma, texture, overall acceptance, and the willingness to consume or purchase. Sensory characteristics were assessed using a 9-point hedonic scale and all data were analyzed by ANOVA and Tukey's test using RStudio ($p < 0.05$). EP inclusion differed significantly for all sensory characteristics ($p < 0.05$). The control snack (0% EP) had the highest mean scores, while the 6.0% EP snack scored the lowest. Snacks containing 1.5, 3.0, and 4.5% EP showed sensory acceptance that compared to that of the control for all evaluated characteristics, whereas the 6.0% inclusion level showed overall reduced acceptance. Texture scores for snacks containing 1.5, 3.0, and 4.5% EP were not different from those of the control ($p < 0.05$). Purchase and consumption intentions were also affected by the inclusion levels of EP. Evaluators showed greater willingness to purchase or consume snacks containing 1.5% and control ($p < 0.05$), while snacks containing 3.0, 4.5, and 6.0% EP received lower purchase intention ($p < 0.05$). These results demonstrate that eggshell powder can be incorporated into extruded corn snacks at levels up to 4.5% without compromising overall sensory acceptance, while

1.5% inclusion showed higher acceptance and purchase intention. Therefore, eggshell powder shows potential as a viable ingredient for extruded snacks and may contribute to the development of calcium-enriched foods. When incorporated at acceptable sensory inclusion levels, it can support dietary calcium intake and provide a sustainable approach to the reutilization of eggshell waste in the food industry.

Keywords: calcium source; sensory characteristics; eggshell powder; snack acceptability; reutilized waste

6 Non-destructive evaluation of chicken egg freshness using tomographic images and computer vision

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Egg freshness directly affects quality and safety for consumption. Traditional evaluation methods are destructive, slow, labor-intensive, and cause product loss. The objective of this study was to develop a non-destructive approach utilizing computed tomographic (CT) images combined with three-dimensional convolutional neural networks (3D-CNN) to classify egg freshness. Eggs (n=998) from Dekalb White lineage laying hens were collected and stored at $26.42 \pm 1.31^\circ\text{C}$ for 1 (n=200), 7 (n=200), 14 (n=200), 21 (n=198) and 28 (n=200) days, with storage days used as class label. The eggs were subjected to CT scans, generating three-dimensional images reconstructed with soft-tissue, air, and bone filters. Images were randomly split into training (60%), validation (20%), and test (20%) subsets with stratified label proportions. Independent 3D-CNN models were trained for each filter, with architectures defined using Bayesian optimization. Performance was evaluated using accuracy, precision, recall, and F1-score. Respond-Cam was applied to the last convolutional layer for model interpretability. Models trained on the soft-tissue and air filters yielded the same performance across all metrics, with accuracy, precision, recall, and F1 score of 0.83 and 0.86, respectively. The bone filter model achieved higher accuracy and recall (0.87), with precision and F1 score at 0.86. Respond-Cam indicated that predictions relied on morphologically relevant regions, particularly the air cell, a structure associated with freshness loss. These results demonstrate that CT imaging combined with 3D-CNN is an effective non-destructive method for classifying egg freshness.

Keywords: Artificial intelligence ; Computed tomography images; Deep learning; Egg quality; Poultry

7 Effects of electrical stunning and isoflurane anesthesia on postmortem metabolism and breast meat quality in broiler chickens: impacts on poultry research

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Methods used to induce unconsciousness before exsanguination in animal research should ensure animal welfare without compromising the representativeness of biological samples. Although inhalation anesthesia with isoflurane has been proposed as a refinement

strategy in poultry research, its effects on postmortem muscle metabolism remain poorly understood. This study compared electrical stunning, the predominant commercial poultry stunning method, with inhalation anesthesia using isoflurane regarding postmortem metabolism and breast meat quality in broiler chickens. Twenty 38-day-old male Cobb 500 broilers ($2,750 \pm 50$ g) were randomly assigned to two treatments (n = 10 each): electrical stunning followed by exsanguination or inhalation anesthesia with 5% isoflurane followed by exsanguination. Time to unconsciousness, muscle pH, instrumental color (L^* , a^* , b^* , C^* , and h^*), and water-holding capacity were evaluated. Data were analyzed by one-way ANOVA, considering $P \leq 0.05$ as significant. Isoflurane anesthesia induced unconsciousness more rapidly than electrical stunning (13.5 vs. 30.8 s; $P < 0.001$), resulted in higher muscle pH measured 45 min postmortem (6.323 vs. 6.137; $P = 0.040$), higher hue angle (h^*) (76.77 vs. 72.85; $P = 0.046$), and lower cooking loss (22.56 vs. 26.88%; $P = 0.045$). These findings demonstrate that the stunning method affects postmortem muscle metabolism and technological characteristics of broiler breast meat. Therefore, selecting an appropriate stunning method in animal research should consider not only animal welfare but also its potential effects on the biological representativeness of muscle samples and the translational relevance of research intended to reflect commercial poultry production systems.

Keywords: Animal research; Broiler; Meat science; Refinement; Water-holding capacity

8 Explainable machine learning approaches for evaluating the effect of storage on egg quality

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Egg quality is influenced by storage conditions, making reliable methods for freshness assessment essential. In this study, an Explainable Artificial Intelligence (XAI) based machine learning model was developed to classify egg freshness and identify the main factors influencing model predictions. A total of 1,600 eggs from Dekalb White laying hens aged 28 to 85 weeks were stored under controlled environmental conditions ($26.42 \pm 1.31^\circ\text{C}$). Eggs were classified into two categories: fresh (1–7 days of storage) and old (21–28 days). Two datasets were constructed: the first included only external variables obtained through non-destructive methods (weight, length, width, shape index, volume, surface area, and candling), while the second included all measured variables, encompassing both internal and external, destructive and non-destructive characteristics. The evaluated variables included Haugh unit, albumen height and weight, yolk height, diameter, index, and color, albumen pH, shell strength and thickness, and the proportions of yolk, albumen, and shell. Data were processed in Python and split into 60% for training and 40% for testing, using five-fold cross-validation and Random Search for hyperparameter optimization. Logistic Regression, Decision Tree, and XGBoost algorithms were evaluated using accuracy, precision, recall, and F1-score metrics. Interpretable models were employed, and SHAP (SHapley Additive Explanations) and Partial Dependence Plots (PDP) were applied to interpret the XGBoost model, allowing the individual contribution of each variable to the predictions to be identified. XGBoost achieved the highest performance, with an accuracy of 69.37% using only exter-

nal variables and 97.03% when all variables were included. Logistic Regression reached accuracies of 67.81% for Dataset 1 and 96.72% for Dataset 2, while the Decision Tree achieved 63.59% and 94.84%, respectively. The analyses showed that egg weight was the most important predictor in Dataset 1, whereas yolk height, albumen pH, and albumen height were the main determinants of freshness classification when all variables were considered. These findings are consistent with the physiological changes associated with egg aging. In conclusion, the combination of explainable machine learning and physicochemical parameters enables highly accurate and transparent egg freshness classification, providing valuable information for automated quality control systems and decision-making in the poultry production chain.

Keywords: Data Mining; Explainable Artificial Intelligence; Prediction; Poultry Farming

9 National assessment of the spatial distribution, seasonality, and major causes of broiler carcass condemnations in Brazil (2022–2025).

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Post-mortem broiler carcass condemnations are important indicators of flock health, animal welfare, food safety, production efficiency, and economic losses in the poultry industry. This study used official Federal Inspection Service records from 2022 to 2025 to characterize the spatial distribution, seasonality, and major causes of total and partial broiler carcass condemnations across the five

geographic regions of Brazil. Slaughter and condemnation records from the Federal Inspection Service Information System (SIGSIF) were consolidated by state, geographic region (North, Northeast, Central-West, Southeast, and South), and month. Condemnation rates were calculated as the percentage of condemned birds relative to the total number slaughtered. The South region accounted for over 50% of the national broiler slaughter volume, with Paraná remaining the leading producing state throughout the study period. The total number of broilers slaughtered increased from 4.01 to 5.70 billion birds, representing a 42.2% increase over the four-year period. Overall condemnation averaged 13.80% among the 20.46 billion broilers slaughtered during the study period. Partial (11.20%) and total (1.21%) carcass condemnations were the predominant inspection outcomes, whereas 1.16% of carcasses were released for consumption after inspection procedures. The leading causes were gastrointestinal and biliary contamination (4.01%), dermatosis (2.52%), arthritis (2.14%), and airsacculitis (1.32%). Contamination and dermatosis remained stable throughout the year, whereas arthritis increased from 1.33% in 2022 to 2.52% in 2025. Airsacculitis showed a marked seasonal pattern, with condemnation rates consistently above the overall mean from July to November, peaking at 1.92% in September. This nationwide assessment demonstrates that official SIGSIF records can be used as a population-based epidemiological surveillance system for the Brazilian poultry industry, enabling the identification of regional and seasonal condemnation patterns while supporting evidence-based improvements in biosecurity, animal welfare, official inspection, quality management, and strategies to reduce economic losses and strengthen the sustainability and global competitiveness of Brazilian poultry production.

Keywords: post-mortem; inspection; total condemnation; partial condemnation; seasonality

Student Competition Metabolism and Nutrition: Feed Additives

24 Effects of dietary orange frit on meat quality of broiler chickens under cyclic heat stress

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The objective of this study was to evaluate the antioxidant potential of orange frit, the fruit peel fragment, in improving the meat quality of broilers under cyclic heat stress. All procedures were approved by the Animal Ethics Committee (protocol no. 000.010). A total of 216 broilers were housed in a completely randomized design (2 × 2 factorial arrangement), consisting of two environmental conditions (thermoneutral and cyclic heat stress for 8 h/day) and two diets (control (CO) and orange frit (OF) at 0.65% inclusion), totaling four treatments with nine replicates of six broilers each. Broilers received feed and water *ad libitum* and were slaughtered at 42 days of age. Breast meat samples were stored for 24 hours and evaluated for pH, color (L*, a*, b*), drip loss, and cooking loss. Data were analyzed in a 2 × 2 factorial arrangement using SAS OnDemand for Academics, considering the effects of diet and temperature. Means were compared by F-test at $p < 0.05$. There was no interaction ($p > 0.05$) between diet and temperature for meat quality traits. Drip

loss, cooking loss, and yellowness (b*) values were not affected ($p > 0.05$). Broilers under cyclic heat stress reduced pH ($p = 0.0009$; HS: 5.62b; TN: 5.78a), redness (a*) ($p = 0.0081$; HS: 0.86; TN: 1.40), and L* values ($p = 0.0081$; HS: 51.59b; TN: 53.51a). Heat stress can accelerate postmortem glycolytic processes, leading to a more rapid decline in pH as muscle glycogen is more rapidly converted into lactic acid, which reduces meat quality and shelf life. The lower redness values observed in broilers under HS may be associated with the conversion of myoglobin into metmyoglobin due to oxidative stress, resulting in a duller and less desirable meat color for consumers. Meat from birds exposed to cyclic heat stress showed lower lightness values, as did birds fed the control diet, possibly related to changes in postmortem muscle metabolism affecting meat light reflectance. OF increased meat pH ($p = 0.0295$; OF: 5.75a; CO: 5.65b) and lightness (L*) values ($p = 0.0444$; OF: 53.26a; CO: 51.84b). The antioxidant compounds present in OF contributed to higher pH values compared with the CO diet, as they act in cellular protection and reduction of oxidative damage. Therefore, cyclic heat stress negatively affected meat quality, whereas dietary orange frit improved pH, a key indicator of meat quality, suggesting its potential as a natural antioxidant ingredient for broiler diets.

Keywords: Antioxidant; Citrus sinensis; Functional additive; Oxidative stress; Pectoralis major

25 Phenotypic and Genetic Stability of Probiotic *Bacillus* Strains Under Sublethal Exposure to Antibiotics Used in Animal Production

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Objective: This study evaluated the phenotypic and genomic stability of *Bacillus velezensis* 03 UNICLON and *Bacillus clausii* (Enterogermina®) after prolonged exposure to sublethal concentrations of amikacin and norfloxacin. **Materials and methods:** Strains were subjected to 240 generations of serial passaging in BHI broth containing increasing sublethal antibiotic concentrations and a non-antibiotic control group, based on adaptive laboratory evolution protocols. MIC values were periodically determined. Whole-genome sequencing was performed to compare wild-type and mutant strains. Resistance genes were identified using CARD/RGI and compared by BLAST. Plasmid presence was also investigated. Microscopy was performed to evaluate sporulation. **Statistical analysis:** MIC data were analyzed using Friedman's test followed by Dunn's post hoc test ($p < 0.05$). **Results:** Under amikacin exposure, significant MIC differences were observed for BV03 ($p = 0.0003$) and ENTPro ($p = 0.0002$), with increases up to MIC 3 followed by reduction at MIC 4. Similar oscillatory patterns occurred under norfloxacin exposure for BV03 ($p = 0.0247$) and ENTPro ($p = 0.0001$). Genomic analyses identified differences in resistance gene annotations between wild-type and mutant strains; however, BLAST demonstrated high similarity among several genes, suggesting sequence divergence rather than acquisition of novel resistance determinants. No plasmids associated with horizontal transfer were identified. Both *Bacillus* species formed endospores after experimental evolution. **Conclusions:** *B. velezensis* and *B. clausii* showed high phenotypic and genomic stability after prolonged exposure to sublethal antibiotic concentrations, supporting the biosafety of these probiotic strains under conditions simulating antibiotic co-administration.

Keywords: adaptive laboratory evolution; probiotics; antimicrobial resistance; *Bacillus*; whole-genome sequencing

26 *Propionibacterium freudenreichii*: A next-generation probiotic as a potential alternative to improve intestinal health and quality in layer chicks during the first week of life

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Propionibacterium freudenreichii has gained attention, especially for its strong anti-inflammatory effects in the intestine of mammals. Although, its effects in poultry remain unclear. The study aimed to evaluate the effects of wild-type and mutant *P. freudenreichii* on intestinal morphology, metagenomic, and metabolomic profiles of layer chicks during their first week of life, investigating its potential to modulate gut health and anti-inflammatory activity. A total of 288 one-day-old layer chicks (Lohmann LSL-LITE) were randomly distributed into three experimental groups ($n = 96$ per group). Each group consisted of eight replicates (pens) with 12 per replicate.

Treatments were administered daily via oral administration (100 μ L/bird/day) from day 1 to day 7. The experimental groups were: (I) saline solution as negative control (NC); (II) wild-type *P. freudenreichii* CIRM-BIA 129 at 6 log CFU/bird/day (PFw); and (III) mutant *P. freudenreichii* Δ slpB at the same dose (PFmut), which lacks the surface-layer protein B (slpB) responsible for anti-inflammatory activity. At day 7, zootechnical performance (weight gain and feed conversion ratio) was recorded, and jejunal samples were collected for histomorphometry (villus height and villus-to-crypt ratio). Cecal content was assessed by whole DNA sequencing (metagenomics) and high-performance liquid chromatography coupled with mass spectrometry (metabolomics). Genomic and metabolomic data were analyzed using multivariate approaches, including Principal Component Analysis (PCA) and MANOVA. Univariate data were evaluated for normality and analyzed using ANOVA and chi-square tests, with significance set at $p < 0.05$. Histomorphometry analysis revealed that the PFw group had significantly greater villus height and a higher villus-to-crypt ratio compared to NC, while PFmut exhibited reduced values ($p < 0.05$), indicating that slpB is essential for improving intestinal structure. Metagenomic analysis revealed no differences in alpha or beta diversity among groups. However, PCA (explaining 52.3% of variance) and PERMANOVA showed distinct metabolomic clusters, with PFw two metabolites were increased and including multiple isomers associated with intestinal health, such as organic acids and lipid-derived compounds. *P. freudenreichii* CIRM-BIA 129 improved intestinal morphometric parameters in chicks during the first week of life. The combined metagenomic and metabolomic analyses suggest that this improvement in intestinal quality is likely driven not by changes in microbial abundance, but by enhanced microbial activity and/or host cell responses.

Keywords: Metabolomics; Metagenomics; Histomorphometry; Surface-layer protein B; Gut microbiota

27 Chestnut Extract on the Performance and Plasma Redox Potential of Broiler Chickens

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This study aimed to evaluate the effect of including increasing levels of hydrolysable tannins (HT) extracted from chestnut (*Castanea sativa*) on the zootechnical performance and antioxidant capacity of broiler chickens from 1 to 35 days of age challenged with a vaccine strain of *Eimeria*. A total of 1,330 Ross 308 broiler chicks were used, distributed in a completely randomized design with seven treatments and ten replicates of 19 birds each. The treatments consisted of: T1 – Negative Control (NC) without the inclusion of an anticoccidial or growth promoter; T2 – NC + 200 g/ton HT; T3 – NC + 400 g/ton HT; T4 – NC + 500 g/ton HT; T5 – NC + 750 g/ton HT; T6 – NC + 1000 g/ton HT; and T7 – NC + 1500 g/ton HT. The birds received isoproteic and isoenergetic corn and soybean meal based diets formulated according to the recommendations of the Aviagen strain (2022), with feed and water provided *ad libitum*. At 14 days

of age, the birds were challenged with a commercial coccidiosis vaccine, Vaxxon Coccivet R, at a dose 20 times the manufacturer's recommended level (approximately $\pm 80,000$ sporulated oocysts). Bird performance was evaluated at 35 days of age. During the same period, antioxidant parameters (superoxide dismutase activity (SOD) and total antioxidant activity (TAA)) and pro-oxidant parameters (plasma reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS)) were determined to calculate the redox score. The data were subjected to a normality test. For data that showed a normal distribution, analysis of variance (ANOVA) was used, and when an effect was observed ($P < 0.05$), the means were analyzed using polynomial regression and Tukey's test at the 5% significance level. For data that did not show a normal distribution, the Kruskal–Wallis test was used, followed by comparison of means using Dunn's test at the 5% significance level. Birds in the negative control treatment showed the poorest performance results, with the lowest body weight and body weight gain values ($P = 0.001$). In addition, the inclusion of 400, 500, and 750 g/ton of HT improved feed conversion ratio ($P = 0.0167$). Regarding redox score, HT inclusion increased TAA ($P = 0.0001$), reduced ROS production ($P = 0.0096$), and decreased lipid peroxidation as reduced TBARS values ($P = 0.0001$). These results indicate higher protection scores in the treatments containing 400, 750, 1000, and 1500 g/ton of tannins ($P = 0.0001$). It can be concluded that the inclusion of *Castanea sativa* hydrolysable tannins improves performance and redox score, reinforcing their potential as a natural feed additive for mitigating the effects of oxidative stress in broiler chickens challenged with vaccine-derived *Eimeria*.

Keywords: hydrolysable tannins; reactive oxygen species; superoxide dismutase activity; thiobarbituric acid reactive substances; total antioxidant activity

28 Dietary isoacids improve feed efficiency, carcass traits, and modulate intestinal and metabolic responses in broiler chickens

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Branched chain volatile fatty acids, commonly referred to as isoacids (ISO), are microbial metabolites derived from branched chain amino acid fermentation and have been extensively studied in ruminants; however, their functional role in poultry nutrition remains poorly understood. This study evaluated the effects of dietary ISO supplementation on growth performance, carcass traits, intestinal permeability, cecal microbiota, breast muscle metabolome, and meat quality of broiler chickens. Seven hundred one day old Cobb 500 male broilers were allocated to 50 floor pens in a completely randomized design, with 5 dietary treatments and 10 replicates per treatment (14 birds per pen). Treatments consisted of a Positive Control diet formulated that met nutritional requirements and a Negative Control diet supplemented with 0.1, 0.2, 0.4, or 0.6% of ISO using a progressive nutritional matrix for ISO: 5410 kcal/kg; 19.32% dig Thr; 27.95% dig Arg and 18.07% dig Leu and Iso. Diets were formulated across three phases: starter (0–21d), grower (22–35d),

and finisher (36–42d). Growth performance was evaluated from at 7, 21, 35 and 42d. At 42d, 3 birds/pen were selected for processing to analyze carcass yields and breast meat quality. Additionally, on 42d blood samples from 8 birds/treatment were collected for intestinal permeability. Finally, samples of cecal and ileal content of 6 birds/treatment were collected for microbiota and SCFA analyses at 42d. Data were analyzed using ANOVA, means separations was performed by Tukey's test ($p = 0.05$), ISO levels were evaluated by polynomial regression analysis. Feed conversion ratio was linearly improved at 7d ($P \leq 0.0007$) and at 21 d ($P \leq 0.0001$) showed best results at 0.2 - 0.4% ISO supplementation with a quadratic effect. The ISO inclusion levels showed a quadratic effect in abdominal fat ($P \leq 0.001$) with the best level of reduction in 0.4%, while 0.6% increased carcass yield ($P \leq 0.05$). A linear decreasing effect was observed ileal permeability with ISO inclusions levels ($P < 0.05$). Moderate inclusion (0.2 - 0.4%) suggesting alterations in pathways related to energy production, amino acid utilization and antioxidant capacity in the metabolomic analysis. Those levels also shifted cecal taxonomic composition toward beneficial bacteria without affecting alpha diversity. The inclusion of 0.1% improved breast meat brightness and antioxidant stability ($P \leq 0.05$). These results indicate that dietary ISO supplementation may improve feed efficiency, carcass composition, intestinal integrity, and microbial modulation in broilers, with 0.2 to 0.4% providing the most consistent physiological benefits.

Keywords: Broiler; Cecal microbiome; Isoacid; Metabolomic; Performance

29 Thyme essential oil and fractionated coconut oil: effect on productive parameters in broiler chickens during the starter phase

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The poultry industry faces the challenge of replacing antibiotic growth promoters due to increasing antimicrobial resistance, which is driving the search for natural alternatives that maintain productive efficiency. Thyme essential oil (TEO) (*Thymus vulgaris*) and fractionated coconut oil (FCO) (*Cocos nucifera*) contain bioactive compounds such as thymol and medium-chain fatty acids, respectively, with the potential to improve the productive performance of poultry. However, there is limited information on the effects of their combined supplementation. The aim of this study was to evaluate the effect of individual and combined dietary supplementation of TEO and FCO on body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) during the starter phase (1–21 days of age) in broiler chickens. Four hundred one-day-old male Cobb 500 chicks were randomly assigned to one of five treatments: T1 negative control (basal diet), T2 positive control (basal diet plus 0.5 g/kg of 11% BMD), T3 (basal diet plus 0.2 g/kg of TEO), T4 (basal diet plus 10 g/kg of FCO), and T5 (basal diet plus 0.2 g/kg of TEO and 10 g/kg of FCO). Eight replicates per treatment were used, with ten birds per replicate. The birds had free access to feed and water during the experimental period. Data were analyzed using ANOVA with SAS PROC GLM. No significant differences were found between treatments ($P > 0.05$) in the variables studied, with averages for BW, BWG, FI, and FCR of 971.44 $\pm$ 14.87 g, 933.23 $\pm$ 14.84 g, 1,241.59 $\pm$ 17.91 g, and 1.32 $\pm$ 0.02, respectively. In conclusion, individual or combined supplementation with thyme essential oil and

fractionated coconut oil did not improve productive performance or show synergistic effects in broiler chickens during the starter phase.

Keywords: *Thymus vulgaris*; *Cocos nucifera*; phytobiotics; productive performance; antibiotics

30 Effects of tributyrin supplementation on body composition and carcass traits of broiler chickens subjected to an enteric inflammation challenge.

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Modern broilers are exposed to multiple sources of intestinal inflammation that can compromise gut function, increase susceptibility to enteric challenges, and impair nutrient utilization and carcass quality. This study evaluated whether dietary tributyrin mitigates DSS – induced enteric damage effects on body composition and carcass/cut yields. A total of 1,200 one-day-old male Cobb 500 broilers were allocated to a completely randomized design with 5 treatments (PC: unchallenged basal diet; NC: challenged basal diet; TBA 0.1: NC + 0.1% tributyrin A; TBA 0.05: NC + 0.05% tributyrin A; TBB 0.05: NC + 0.05% tributyrin B) with 12 replicates of 20 birds. Enteric damage was induced by oral gavage with 3.5% DSS on days 8 to 12 and 21 to 25. At day 42, 2 representative birds per pen (fasted 12 h) were euthanized; one carcass was analyzed by DEXA and predictive equations for whole-body composition and the other for carcass and commercial cut yields. Data were analyzed by ANOVA and SNK ($\alpha = 0.05$). Total body fat was lower in NC and TBA 0.1 compared to PC and both 0.05% supplemented treatments ($P < 0.01$). Body water content was greater in NC and lowest in PC ($P < 0.05$), while tributyrin-supplemented treatments were similar to PC. Mineral content was greater with PC and TBA 0.05 and lowest with TBA 0.1 ($P < 0.01$). Breast weight was lower in NC compared to PC, while TB-supplemented groups were similar to PC ($P < 0.01$). Breast meat yield was lower in NC compared to the PC, while TB-supplemented groups were similar to PC, except for TBA 0.1 which was the greatest ($P < 0.01$). Drumstick weight, live weight, lean mass and protein content were not affected by dietary treatments ($P > 0.05$). Overall, under DSS-challenge, 0.1% tributyrin A reduced Whole-body fat, body water and mineral estimates, and improved breast weight and breast yield relative to the challenged control.

Keywords: breast yield; DSS; enteric challenge; fat deposition; intestinal integrity

31 Effects of litter type and two mycotoxin detoxifiers on productive performance, carcass traits, and relative organ weights in broilers fed diets containing naturally contaminated corn residue from 28 to 39 days of age

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This study evaluated the effects of litter type and two commercial mycotoxin detoxifiers on productive performance, carcass traits, and relative organ weights in broilers fed diets containing naturally contaminated corn residue from 28 to 39 d of age. A total of 1,920

Ross 308 male broilers were randomly allocated to 12 treatments with eight replicates of 20 birds each in a $2 \times 2 \times 3$ factorial design including new (N) or reused (R) litter, diets without (WM) or with mycotoxin contamination (M; 5% naturally contaminated corn residue), and detoxifiers (Control (C), Detoxifier 1 (D1; aluminosilicates, inactive yeast, and biological biotransforming agents), or Detoxifier 2 (D2; modified clay minerals and yeast cell walls)). Productive performance was evaluated at 28 and 39 d, whereas carcass traits and relative organ weights were determined at 39 d. Data were analyzed using a GLM in RStudio, and means were compared using Tukey's test ($P \leq 0.05$). Broilers reared on new litter showed greater BWG than those reared on reused litter at 28 d (457.1 vs. 443.4 g; P -value = 0.043) and 39 d (1,138.3 vs. 1,085.4 g; P -value < 0.001). Feed conversion ratio was lower in broilers reared on new litter at 28 d (1.71 vs. 1.79; P -value = 0.001) and 39 d (1.72 vs. 1.80; P -value < 0.001). Litter type also affected carcass traits and relative organ weights, with greater relative thigh weight in birds reared on new litter than in those reared on reused litter (11.0 vs. 10.7; P -value = 0.031) and greater relative heart weight in birds reared on reused litter than in those reared on new litter (0.46 vs. 0.43; P -value = 0.039). Relative heart weight was also affected by detoxifier supplementation, with birds receiving D1 showing a relative heart weight of 0.47, compared with 0.44 and 0.43 in the Control and D2 groups, respectively (P -value = 0.039). A significant litter $\times$ dietary mycotoxin interaction was observed for relative thigh weight (P -value = 0.033), whereas three-way interactions among litter type, dietary mycotoxin challenge, and detoxifier supplementation affected BWG (P -value = 0.039) and relative thymus weight (P -value = 0.007). These findings indicate that litter type affected body weight gain, feed conversion ratio, carcass traits, and relative organ weights, whereas detoxifier supplementation affected relative heart weight and interacted with dietary mycotoxin challenge to influence body weight gain and relative thymus weight. Further research is warranted to evaluate the long-term effects of commercial detoxifiers under different mycotoxin challenge conditions.

Keywords: broilers; litter; mycotoxin challenge; body weight gain; corn residue

32 Microbial communities in broiler breeder hen's eggshell are shifted with the use of a microencapsulated blend of organic acid and essential oils

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Eggshell microbiome is a critical factor for incubation, since it directly interacts with embryo contamination and mortality. The chicken gut microbiota can affect the colonization of the eggshell, meaning that nutritional strategies that modify the eggshell microbiome through hen diets may aid enhancing hatchability and embryonic survival. This study aimed to assess the effects of Lumigard TCB, a blend of vectorized benzoic acid with essential oils in breeder hens' diets, on the eggshell microbiome of late-age hens. A total of 192 AP95 breeder hens were randomly distributed in four treatments: NC: Corn/soybean-meal diet; PC: NC+40 ppm virginiamycin; LUM250: NC + 250 ppm Lumigard TCB (Salus group, Brazil); LUM500: NC+ 500 ppm Lumigard TCB. Hens were distributed in 12 pens per group, four hens/pen, and supplemented from 23 to 65

wks of age. Feed and lighting were done as the lineage manual. At 63 wks of age, all eggs from two consecutive days were collected directly from the hens' nest using sterile gloves and swabbed for one minute each with sterile wipes, being each wipe used to sample 10 eggs of the same treatment. Wipes were blended with phosphate buffered saline and centrifuged for DNA extraction. A segment from the hypervariable V3V4 region of the 16S rRNA gene was amplified and the reads were analyzed on QIIME2, with sequences being processed and classified into bacterial species. Alpha and beta diversity assays were performed with R software using Kruskal-Wallis with post-hoc Benjamini-Hochberg test with significance set at 0.05. No differences were observed concerning alpha-diversity by Chao1, Observed amplicon sequence variants or Fisher indexes between groups. LUM250 showed a greater diversity in eggshell microbiome when compared to NC and PC by the Simpson index ($P < 0.05$) and a trend ($P = 0.06$) by the Pielou's Evenness index, while LUM500 showed greater diversity by Simpson index ($P < 0.05$) than the NC group. Concerning beta-diversity, no differences between treatments were found by any of the estimators. At genus level, *Brevibacterium*, *Brachybacterium*, and *Lactobacillus* were the most abundant genus among all treatments, with LUM250 showing a trend ($P = 0.055$) of greater abundance of *Romboutsia* than NC, which is known to be related to immune function in broiler chicken. Our results suggest that Lumigard TCB can effectively modify the bacterial community diversity in breeder hen's eggshell, likely by supporting microbial balance in the hen gut, promoting greater diversity than NC or antimicrobial-fed groups.

Keywords: 16S rRNA; Embryonic mortality; Gut transfer; Incubation; Microbiota

33 Evaluation of Dietary Anti-Inflammatory Additives on Productive Performance and Egg Quality of Laying Hens during Summer Heat Stress

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Laying hens are highly susceptible to heat stress due to their limited ability to dissipate body heat, leading to oxidative stress and inflammatory responses that may impair eggshell formation and egg quality. Therefore, dietary anti-inflammatory additives have been proposed as a nutritional strategy to preserve physiological functions under these conditions. This study evaluated the effects of two commercial dietary anti-inflammatory additives on productive performance, egg quality, and yolk color of laying hens exposed to summer heat stress. A total of 240 Hy-Line W36 laying hens, 41 weeks old, were assigned to a completely randomized design with three dietary treatments, 10 replicates, and eight birds per replicate. Treatments consisted of a control diet and two diets containing different phytochemical feed additives: one based on benzophenanthridine and protopine alkaloids and the other based on potentiated curcumin. The experiment was conducted during the summer in Maringá, Paraná, Brazil, and the ambient temperatures exceeded 30°C on 91% of the experimental days and reached a maximum of 37.8°C, characterizing chronic summer heat stress. Birds were evaluated over three 28-day production cycles. Productive performance, internal and external egg quality, eggshell mechanical properties, and yolk color parameters were determined. Data were analyzed by one-way ANOVA, and means were compared using Tukey's test ($P < 0.05$). Dietary anti-inflammatory additives did not affect productive

performance, egg weight, internal egg quality, eggshell percentage, specific gravity, or yolk color ($P > 0.05$). Across treatments, hens maintained high productive performance, averaging 94.8% hen-day egg production, 98.1 g/bird/day feed intake, 65.3 g egg mass, and a feed conversion ratio of 1.50 g/g despite summer heat stress. However, anti-inflammatories significantly improved eggshell mechanical quality. Hens receiving potentiated curcumin had greater eggshell hardness than those fed the control diet (4.699 vs. 4.470 kgf; $P = 0.021$), whereas both anti-inflammatory treatments increased eggshell breaking strength compared with the control (4.280 and 4.321 vs. 4.046 kgf; $P = 0.003$). Despite the slight reduction in eggshell thickness, the improved hardness and breaking strength indicate enhanced eggshell mechanical integrity independently of shell thickness. In conclusion, dietary anti-inflammatory additives preserved productive performance and internal egg quality while improving eggshell mechanical resistance in laying hens subjected to summer heat stress. The greatest improvement observed with potentiated curcumin suggests its potential to enhance eggshell structural integrity under challenging environmental conditions.

Keywords: Heat stress; additives; bioactive compounds; eggshell integrity; laying hens

34 Effects of a Grape-Derived Natural Antioxidant on Productive Performance and Egg Quality of Japanese Breeder Quails During Peak Lay

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A total of 560 Japanese breeder quails (420 females, 140 males) were assigned to a completely randomized design with seven treatments (10 replicates of eight birds): diets containing 113, 63, 38, or 25 IU vitamin E/kg, and diets with 25 IU vitamin E/kg plus 9, 12, or 18 ppm of the grape antioxidant. Productive performance was recorded across three consecutive 21-day periods and egg quality assessed at the end of each cycle in three consecutive days. Data were analyzed by ANOVA and means compared by Tukey's test ($P < 0.05$). No treatment effects were observed on productive performance or egg quality variables ($P > 0.05$). Feed intake ranged from 27.80 to 28.62 g/bird/day, egg production from 88.82 to 93.77%, egg weight from 11.63 to 11.74 g, egg mass from 10.29 to 10.86 g/bird/day, and feed conversion remained similar among treatments, indicating stable productive efficiency throughout peak lay. However, increasing dietary vitamin E from 25 to 113 IU/kg did not impair feed intake, egg production, egg weight, egg mass, or feed conversion. Birds receiving 25 IU vitamin E supplemented with 12 ppm grape-derived antioxidant showed numerically greater egg production (93.46%), egg mass (10.86 g/bird/day), and the best feed conversion (2.564 g/dozen). Egg quality remained stable across treatments, with Haugh unit values above 86 and consistent shell percentage, shell thickness, and specific gravity, indicating maintenance of both internal and external egg quality. Egg quality characteristics were maintained across treatments, with specific gravity ranging from 1.0707 to 1.0718 g/cm³, Haugh unit from 86.13 to 87.60, yolk index from 0.454 to 0.464, yolk percentage from 29.47 to 30.18%, albumen percentage from 61.76 to 62.46%, shell percentage from 8.06 to 8.23%, and shell thickness from 0.222 to 0.226 mm. These results indicate that vitamin E levels did not impair productive performance

or egg quality of Japanese breeder quails during peak lay, and that supplementation with the grape-derived antioxidant maintained performance and egg quality at the lower vitamin E level. This supports the grape extract as a natural antioxidant strategy in breeder nutrition, useful where dietary vitamin E is reduced or when producers seek polyphenol-based additives.

Keywords: Polyphenols; Reproductive quails; Egg quality; Functional feed additives

35 Effects of 42-day dietary guanidinoacetic acid (Creamino®) supplementation on growth performance of broiler chickens

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Guanidinoacetic acid (GAA - Creamino®) is a creatine precursor and is related to ATP production and bioenergetic efficiency in broilers. This study evaluated the effects of guanidinoacetic acid supplementation in diets with different levels of energy and arginine on broiler performance, including feed intake, body weight gain, and feed conversion ratio. Meat quality traits, including the incidence of woody breast myopathy, were also assessed. A total of 1,080 male broiler chickens (Ross 308 AP) were assigned to a completely randomized design with six treatments and ten replicates of 18 birds each. The treatments were: T1: positive control formulated to meet all nutritional requirements [JR1] according to Rostagno 2024; T2: T1 with reductions of 60 kcal/kg ME and 460 g of L-arginine; T3: T2 + 0.06% GAA; T4: T1 with reductions of 60 kcal/kg ME and 600 g of L-arginine; T5: T4 + 0.06% GAA; and T6: positive control + 0.06% GAA (on top). Birds and feed were weighed at the start of the experiment and at 10, 21, 35, and 42 days of age to determine feed intake, body weight gain, and feed conversion ratio. Data were tested for residual normality and homogeneity of variance and then subjected to analysis of variance (ANOVA). When significant effects were detected for performance variables, treatment means were compared using Tukey's test at a 5% significance level. Non-normally distributed data for woody breast were analyzed using the Kruskal-Wallis test followed by Dunn's test, also at a 5% significance level. No differences were observed in feed intake or body weight gain at 21 or 42 days ($P > 0.05$). However, at 21 days, broilers fed with T1 (positive control) had lower feed intake than those fed the T4. ($P < 0.05$). During the same period, birds fed with T1, T3, T5, and T6 had lower feed conversion ratios than those fed with nutrients reduction without GAA addition ($P < 0.05$). At 42 days, birds fed T1 and T6 had lower feed conversion ratios than those fed reduced diets without GAA (T2 and T4). In conclusion, Creamino® supplementation compensated for reductions of 60 kcal/kg metabolizable energy and L-arginine equivalent to 77% and 100% of GAA doses in broiler diets during all phases, resulting in a feed conversion ratio comparable to that of birds fed the positive control diet. At 42 days, improved feed conversion associated with Creamino® supplementation was observed in birds fed diets formulated to meet nutritional requirements, suggesting that the response to the additive may depend on dietary nutrient level. These results indicate that Creamino® can be used as a nutritional strategy to improve feed efficiency in broilers (on top) and to compensate energy and arginine reduction (matrix).

Keywords: Creatine; performance; woody breast; nutrition; poultry

36 COPPER NANOPARTICLES AS A NUTRITIONAL AND MORPHOFUNCTIONAL STRATEGY FOR BROILER CHICKENS UNDER COCCIDIOSIS CHALLENGE

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The global restriction on the use of antibiotic growth promoters has driven the search for alternative additives in poultry production. Although copper protects intestinal health and is widely used for its antimicrobial action, conventional inorganic sources require supranutritional doses to be effective, raising concerns due to soil accumulation and antagonism with other nutrients. In this scenario, CuNPs emerge as a promising strategy. This study evaluated the dietary inclusion of Cu20 nanoparticles on the performance, digestibility, hepatotoxicity, and intestinal health of broilers challenged with *Eimeria* spp. The experiment (CEUA no. 9365230524) used 336 male Cobb 500 chicks (1-21 days of age), distributed in a randomized block design with 6 treatments and 8 replicates of 7 birds each. The diet met nutritional requirements, with additives included on top. Treatments consisted of Negative Control, Challenged Control, and challenged birds supplemented with 200 mg/kg of Cu via sulfate or CuNPs at 25, 50, and 100 mg/kg. The challenge was applied on d1 via oral gavage of 10 times the dose of a live coccidiosis vaccine. A metabolizable energy assay was conducted in the last week, and all other variables were studied at 21 days. Data were subjected to ANOVA and orthogonal contrasts using SAS. Challenge did not affect performance ($P > 0.05$), averaging weight gain 1.07 kg; feed intake 1.33 kg; feed conversion 1.25. Both Cu sources improved feed conversion ($P < 0.10$) except for CuNP25 (1.20 vs. 1.25). Challenge reduced AMEn (3,312 vs. 3,464 kcal/kg, $P < 0.05$), which was reversed by Cu supplementation, except for CuNP25 (3,300 vs. 3,406 kcal/kg, $P < 0.05$). Challenge reduced apparent mineral digestibility ($P < 0.05$); both Cu supplements further antagonized Zn digestibility in challenged birds ($P < 0.05$). Using the I See Inside (ISI) methodology, treatments did not affect the total ISI score of the liver ($P > 0.05$). After coccidial challenge at d1, the total ISI score of the intestinal tissue showed no effect on d21 ($P > 0.05$); however, CuS200 resulted in a better intestinal score than CuNPs ($P < 0.05$). Histological evaluation indicated that both Cu supplements increased villus width and villus:crypt ratio ($P < 0.05$). In conclusion, CuNP supplementation acts as an efficient and safe nutritional strategy against enteric challenge by *Eimeria* spp. The 50 mg/kg dosage is indicated as the optimal inclusion level, representing a lower inclusion compared to sulfate while optimizing feed conversion, energy extraction (AMEn), and calcium digestibility, and ensuring hepatic cytotoxic safety. Furthermore, this dosage acts as an efficient modulator of intestinal architecture and improves the absorptive efficiency of the challenged mucosa.

Keywords: Poultry ; Nanotechnology; Coccidiosis (*Eimeria* spp.); Intestinal healthy; Alternative additives

37 Oxidative Stability of Broiler Chicken Meat Challenged with *Eimeria maxima* and Fed *Hermetia illucens* Larval Oil

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Eimeria challenge can induce oxidative stress in broiler chickens, altering oxidative status markers. There fore, evaluating natural additives, such as *Hermetia illucens* larvae oil (HILO), may help clarify their effects on meat oxidative stability. This study evaluated the effects of HILO on the oxidative stability of frozen thigh meat from broiler chickens challenged with *Eimeria maxima*. A total of 540 male chicks were distributed in a completely randomized design with five treatments. The nonchallenged control had four replicates of 15 birds, whereas the four challenged treatments had eight replicates of 15 birds each, totaling 36 experimental units. All procedures were approved by the Animal Use Ethics Committee, protocol no. 811/2023. The treatments were: nonchallenged control; challenged control, with birds inoculated with *E. maxima* and fed a control diet containing antibiotic (Halquinol) and antioxidant (BHT), without HILO; challenged birds fed an antibiotic- and antioxidant-free diet (AAFD), without HILO; challenged AAFD + 0.5% HILO; and challenged AAFD + 1.0% HILO. At 17 days of age, challenged birds were inoculated with *E. maxima* by oral gavage at 10^7 oocysts/bird. To evaluate thigh meat oxidative stability, two birds per treatment were analyzed after 90 days of frozen storage. Antioxidant capacity was determined using the DPPH and ABTS methods, lipid oxidation by malondialdehyde (MDA) concentration, and total phenolic content (TPC) according to standard methodologies. Data were subjected to one-way ANOVA using PROC GLM in SAS, and means were compared by the SNK test at 5% probability. Treatments did not affect MDA, DPPH, or TPC ($P > 0.05$). However, treatments affected ABTS ($P = 0.0004$). The nonchallenged control showed the highest ABTS value (29.00%), whereas challenged treatments showed lower values, ranging from 12.87% to 17.63%, without differing from each other. The reduction in ABTS in challenged birds may be associated with *E. maxima*-induced oxidative stress, as enteric challenge increases reactive oxygen species production and intensifies the use of endogenous antioxidants to counteract this imbalance, impairing redox balance and reducing the availability of antioxidant compounds in tissues. The inclusion of 0.5% or 1.0% HILO was not sufficient to restore the antioxidant capacity reduced by *E. maxima* challenge or to significantly alter meat lipid oxidation or total phenolic content. **Keywords:** Additive; broiler chickens; *Eimeria maxima*; *Hermetia illucens*; oxidative stability.

Keywords: *Eimeria Maxima*

38 Phytase supplementation effects on performance and tibia ash content in male broilers (22 to 42 days of age): a systematic review and meta-analysis

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Calcium (Ca) and phosphorus (P) are essential for metabolism,

bone development and skeletal strength. However, particularly in plant-based diets, P is predominantly bound to phytate, rendering it largely unavailable for absorption by poultry because poultry has limited endogenous phytase (PHY) activity. It is also known that Ca and P requirements decline during the finisher phase, yet this period accounts for about two-thirds of total feed intake and body weight gain in male broilers, and PHY responses remain inconsistent. The objective of the study was to quantify the effects of PHY supplementation, dietary non-phytate phosphorus (NPP), and the calcium-to-NPP ratio (Ca:NPP) on growth performance and tibia ash content in male broilers from 22 to 42 days post-hatch. Following the PICo framework, a systematic search of PubMed, Web of Science, and Scopus (finalized January 2025) retrieved peer-reviewed articles in English, Portuguese, or Spanish. After duplicate removal, titles and abstracts and then full texts were screened in two stages. Eligible studies were in vivo trials with commercial broilers published from 2000 that reported a negative control and PHY-supplemented diets with matching NPP. Trials with sanitary challenges, enzyme combinations, no control, or super-dosing ($>2,500$ FTU/kg) were excluded. Of 5,710 records, 54 were assessed in full and 21 studies (10,706 broilers, 86 treatment groups) were retained. Average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and tibia ash content were analyzed by mixed models with study as a random effect and full quadratic responses. PHY-NPP and PHY-Ca:NPP ratio were fitted separately to avoid collinearity, outliers were screened with studentized residuals, and optimal NPP was derived from partial derivatives (significance set at $P < 0.05$). PHY improved ADG, ADFI, and FCR and increased tibia ash content, most strongly at low NPP. At 1,000 FTU/kg, PHY raised ADG by 6.63 g/d, ADFI by 6.45 g/d, and tibia ash by 4.85% at 1.50 g/kg NPP, versus 3.16 g/d and 0.99% at 3.00 g/kg NPP, with no ADFI gain above 2.90 g/kg. Based on FCR, 500 FTU/kg matched the recommended 3.15 g/kg NPP at only 2.25 g/kg, indicating a release of about 0.90 g/kg NPP. Higher Ca:NPP ratios impaired all parameters, particularly without PHY. In conclusion, PHY improves growth performance and bone mineralization and lowers the NPP requirement of finisher male broilers, especially in low-NPP diets.

Keywords: Animal Nutrition; Meta-Analysis; Phosphorus; Phytase; Poultry

39 Metabolizable Energy of Peanut Meal for Laying Hens

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Feed accounts for the largest proportion of production costs in commercial egg production. Owing to fluctuations in feed ingredient prices, alternative protein sources such as peanut meal have attracted increasing interest because of their high crude protein content. However, the nutritional value of peanut meal may vary according to processing methods, and its use is limited by the risk of aflatoxin contamination. These limitations can be minimized through appropriate processing and quality control. Therefore, this study aimed to determine the metabolizable energy values of peanut meal for laying hens. The experiment was conducted at UNESP, Campus of Jaboticabal, using a completely randomized design with three treatments, ten replicates, and two birds per experimental unit over a 10-day period. Treatments consisted of a reference diet (D1) and two test diets containing 20% (D2) or 40% (D3) peanut meal. Birds had

ad libitum access to feed and water and were maintained under controlled environmental conditions. Feed and excreta samples were analyzed for dry matter, ash, crude protein, ether extract, crude fiber and gross energy. Dry matter intake, apparent metabolizable energy (ME), nitrogen-corrected apparent metabolizable energy (ME_N), and gross energy metabolism coefficient were calculated. Data were tested for normality and homogeneity of variances and analyzed by ANOVA using SAS software, with significance declared at $P < 0.05$. Dry matter intake, dry matter metabolizable coefficient, and gross energy metabolism coefficient were not affected ($P > 0.05$) by the inclusion of 20 or 40% peanut meal in the experimental diets (Table 2). Likewise, ME and ME_N did not differ among treatments, indicating that partial replacement of the reference diet with peanut meal did not impair dietary energy utilization. Although no significant differences were detected, a trend toward higher ME ($P =$

0.082) and ME_N ($P = 0.091$) values was observed in diets containing peanut meal compared with the reference diet. Diets containing 20 and 40% peanut meal yielded ME values of 2,816.66 and 2,846.89 kcal/kg, respectively, compared with 2,671.43 kcal/kg for the reference diet. A similar pattern was observed for ME_N, which increased from 2,581.09 kcal/kg in the reference diet to 2,746.42 and 2,749.27 kcal/kg in the diets containing 20 and 40% peanut meal, respectively, suggesting that peanut meal has a high energy value when used in diets for laying hens. Peanut meal showed favorable metabolizable energy values, indicating their potential as an alternative ingredient for laying hen diets.

Keywords: Alternative ingredients; metabolizable energy; Laying hens; peanut meal; protein source

Student Competition Metabolism and Nutrition: Amino Acids

10 Effects of glycine and nanostructured trimeric glycine on hepatic metabolites and IL-6 transcript levels in one-day-old chicks after in ovo inoculation

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Glycine plays a role in intestinal maturation, immune regulation, antioxidant protection, and metabolic efficiency during the critical post-hatch period, thereby improving bird performance and health. The use of a proprietary trimeric glycine form, known as 'Katti Peptide' (Gli-K), may improve glycine delivery and enhance its beneficial effects. In this context, in ovo treatment may represent an alternative strategy to improve the health and overall condition of chicks during the first days of life. The objective of this study was to evaluate the immunomodulatory and metabolic responses of chicks supplemented with trimeric nanostructured glycine Gli-K administered in ovo. A total of 56 fertilized chicken embryos from Hy-Line strain were incubated and distributed into four experimental groups: (i) negative control (NC), supplemented with PBS buffer; (ii) positive control (PC), supplemented with ascorbic acid; (iii) unmodified glycine (Gli); and (iv) nanostructured glycine (Gli-K). Each embryo was individually inoculated with 100 µL of the respective solutions via the amniotic cavity. On the first day post-hatch, they were humanely euthanized and liver and spleen samples were collected for metabolomic analysis by liquid chromatography coupled to mass spectrometry and for IL-6 quantification by RT-qPCR using probes, respectively. For metabolomic analysis, multivariate analysis using Principal Component Analysis (PCA), clustering, and Partial Least Squares Discriminant Analysis (PLS-DA) were applied to the metabolomic data, and ANOVA was performed for both metabolomics and RT-PCR results, considering $p < 0.05$. Eight differential metabo-

lites were identified. PCA and PLS-DA separated NC from treated groups with >70% predictive accuracy. Treated group increased the vitamin E metabolites which is associated with antioxidant activity. Metabolites related to sphingolipid metabolism were higher in the Gli-K-treated group, suggesting a positive effect on neonatal liver development and tissue maturation. In contrast, oxidized phospholipid-related metabolites were reduced in Gli-K compared with the other groups, indicating lower oxidative stress and suggesting protection of cell membranes against lipid peroxidation, with potential benefits for inflammatory responses and liver tissue integrity. Metabolites involved in amino acid metabolism increased in the Gli and Gli-K groups, suggesting metabolic modulation in the neonatal liver. IL-6 transcript levels were reduced in the PC and Gli-K groups, indicating a possible immunomodulatory effect of Gli-K. Overall, these findings support the use of in ovo glycine and suggest that Gli-K may enhance glycine delivery and potentiate its beneficial effects in liver.

Keywords: Poultry ; Metabolomics; Poultry nutrition; Glycine; IL-6

11 Impact of L-arginine and guanidinoacetic acid supplementation on growth performance in broilers fed reduced energy diets

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The objective was to evaluate the effects of L-arginine and guanidinoacetic acid (GAA) supplementation on the performance response of broiler chicks fed with reduced energy diets. A total of 2200 14-day-old male Ross AP95 broiler chickens, with the same initial body weight of 474 ± 3.03 g, were randomly assigned to a completely randomized design with eight dietary treatments, including 11 replications of 25 birds each. At 14 to 28 days of age, body weight (BW) and feed intake (FI) were measured weekly, and the body weight gain (BWG) and feed conversion ratio (FCR) were calculated. The birds were housed in a ventilated, temperature-controlled shed, with *ad libitum* access to water and feed in mash form. The dietary treatments were as follows: positive control (PC): apparent metabolizable energy corrected for nitrogen (AMEn) of 3025 kcal/

kg; negative control 1 (NC1): 2875 kcal/kg AMEn; negative control 2 (NC2): 2825 kcal/kg AMEn; NC1 + 600 GAA: supplemented with 0.600 g/kg GAA; NC1 + 462 Arg: supplemented with 0.462 g/kg L-arginine; NC1 + 900 Arg: supplemented with 0.900 g/kg L-arginine; NC2 + 600 GAA: supplemented with 0.600 g/kg GAA; and NC2 + 900 Arg: supplemented with 0.900 g/kg L-arginine. The standard ileal digestibility of lysine in all diets was 1.10%. One-way ANOVA was used to analyze the data, and mean comparisons were performed using Tukey's test with a significance level of $P < 0.05$. The FI was unaffected by dietary treatments during days 14 to 21 and 14 to 28. At 21 – 28 d, the NC2 diet, which had a reduction in AMEn, increased FI compared with the other treatments ($P < 0.05$). On the other hand, energy reduction (NC1 and NC2) reduced BWG and increased FCR relative to PC in both weeks ($P < 0.05$). Interestingly, NC1 + Arg (0.462 and 0.900 g/kg L-arg) partially recovered the performance response by improving FCR (1.18 and 1.19 vs. 1.22 in PC), and NC1 + 462 Arg yielded intermediate BWG that did not differ from PC at 21 – 28 d and 14 – 28 d. By contrast, birds fed with NC1 + 600 GAA failed to restore BWG and had the poorest FCR (up to 1.58 at 21 – 28d). Overall, the supplementation of 0.462 g/kg of L-arginine in the NC1 diet mitigated the energy reduction losses more effectively than GAA supplementation, however neither addition reached the effects of PC.

Keywords: Amino acids; Broiler performance; Guanidinoacetic acid; L-Arginine; Metabolizable energy

12 Evaluation of the digestible amino acid composition of soybean meal available to a broiler production company based in Goiás.

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Soybean meal is a major ingredient in animal nutrition, constituting the main protein source in poultry diets. Additionally, it influences metabolizable energy and the levels of essential dietary amino acids, such as lysine, methionine, and threonine, particularly for broilers. Raw soybean meal carries antinutritional factors that must be denaturated through thermal processing, a step that can influence the quality of available amino acids. Other factors such as storage and transportation may also affect their quality levels. This study aimed to analyze the amino acid composition of soybean meal used by one of the leading broiler producers in the state of Goiás, and to identify the variability among samples from different suppliers. For this purpose, 6,290 samples were collected directly from the quality control laboratory, representing 8 companies not that supply this raw material. These samples were analyzed using Near-Infrared Spectroscopy (NIRS) to provide data on the digestible amino acid content: methionine, cystine, methionine + cystine, lysine, threonine, tryptophan, arginine, isoleucine, leucine, valine, histidine, phenylalanine, glycine, serine, proline, alanine, aspartic acid, and glutamic acid. The data were subjected to statistical analyses using R software, which performed an analysis of variance (ANOVA) and the Scott-Knott test at a 5% significance level. Significant differences were observed among the companies analyzed. The range for critical amino acids such as methionine and lysine varied from 0.506 to 0.608 (SEM = 0.00075) and 2.355 to 2.711 (SEM = 0.0028625), respectively. Supplier B was statistically superior, presenting means of 0.5613 and 2.5749 for the same amino acids, whereas Supplier G showed lower means, 0.5452 and 2.5263, respectively. A similar pattern was followed for the remaining amino acids. The amino acid

cystine exhibited the highest variability, fluctuating from 0.446 to 0.603 (CV = 3.03%), which reinforces the need for these analyses. Given this heterogeneity, it is essential to analyze the ingredients before formulating the feed. This practice ensures quality control and meets the nutritional requirements of broiler chickens, promoting uniform and cost-effective production on poultry farms.

Keywords: Digestible amino acids; Near-Infrared Spectroscopy (NIRS); Nutritional variability; Poultry nutrition; Soybean meal

13 Metabolizable energy values and amino acid digestibility coefficients of high protein cottonseed meal for broilers

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The aim was to determine the apparent metabolizable energy (AME) and nitrogen corrected AME (AMEn) values, and the standardized ileal digestibility (SID) coefficients of the amino acids in high-protein cottonseed meal (CSMH) for broiler chickens. Two experiments were conducted. In experiment I, 72 male Ross 308 broiler chicks, 14 days old, were housed in a completely randomized design (CRD) with two treatments, six replicates, and six birds per experimental unit for 7 days (4 days of acclimatization and 3 days of data collection). The treatments consisted of a reference diet (RD), based on corn and soybean meal, and a test diet, in which 25% of the RD was replaced with CSMH, using 1% celite as indigestible marker. In experiment II, 48 Ross 308 broilers, aged 21 to 25 days, were assigned to a CRD with two treatments, six replicates, and four birds per experimental unit. The treatments consisted of a nitrogen-free diet (NFD), and a test diet, in which 25% of the NFD's starch was replaced with CSMH, both containing 1% celite. On the fifth day, the birds were euthanized to collect ileal digesta. The EMA and AMEn were estimated by the substitution method, and the SID was calculated based on apparent ileal digestibility, corrected for basal endogenous losses. CSMH contained 50.77% crude protein, 5.83% ether extract, 7.44% ash, 12.60% crude fiber, and 92.94 mg/kg of free gossypol. The energy values were: gross energy of $4,380 \pm 123$, EMA of $2,777 \pm 126$, and EMAn of $2,417 \pm 105$ kcal/kg DM. The SID coefficients (%) and digestible concentrations (%) were: 57.54 and 0.91 for Lys, 90.75 and 1.83 for Thr, 94.98 and 4.72 for Arg, 78.27 and 0.51 for His, 74.62 and 0.90 for Ile, 78.99 and 1.78 for Leu, 81.12 and 0.96 for Val, 86.92 and 1.94 for Phe, 77.58 and 1.01 for Gly, 80.31 and 0.44 for Ala, 93.46 and 1.11 for Tyr, 79.98 and 1.14 for Ser, 88.69 and 1.31 for Pro, 87.79 and 6.49 for Glu, and 79.77 and 2.52 for Asp, respectively. CSMH is a moderate energy source for broiler chickens, with satisfactory SID for most amino acids. Lysine stood out as having the lowest digestibility, likely due to the presence of free gossypol, that interacts with the amino groups of the amino acid, and forming stable complexes and reducing its biological availability. These findings reinforce the importance of considering the energy value and amino acid digestibility profile of CSMH when formulating diets, especially regarding lysine supplementation.

Keywords: Alternative feedstuffs; Digestible amino acids; Gossypol

14 Energy value and ileal amino acid digestibility coefficients of duckweed meal (*Wolffia brasiliensis* Wendell and *Landoltia punctata* (G.Mey.) Les & D.J.Crawford) for broiler

chickens

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The objective of this study was to determine the chemical composition, apparent metabolizable energy (AME) and nitrogen-corrected metabolizable energy (AMEn) values, standardized ileal amino acid digestibility (SID) coefficients, and concentration of digestible amino acids of duckweed meal (mix of *W. brasiliensis* and *L. punctata*; DWM) for broiler chickens. In the metabolism trial (I), 72 chicks at 14 days of age were distributed in a completely randomized design (CRD), with two treatments (reference diet, RD, based on corn and soybean meal; and RD + 20% DM), six replicates, and six birds per experimental unit, using the partial excreta collection method with acid-insoluble ash (AIA, 1%) as the indicator. The experiment consisted of four days of feed intake and three days of collection. In the ileal digestibility trial (II), 48 chicks at 21 days of age were distributed in a CRD with six replicates, two treatments (nitrogen-free diet, NFD; and NFD + 20% DWM) and four birds per plot. The trial lasted five days, with three days allocated to diet intake, and birds were euthanized on the fourth and fifth days for ileal content collection. In both experiments, birds were housed in metabolism cages. The AME and AMEn values of DWM were estimated using the substitution method. SID was determined from the apparent ileal digestibility corrected for endogenous losses of each amino acid. Data were expressed as mean $\pm$ standard error of the mean (SEM). DM presented: 91.46% dry matter, 32.26% crude protein, 13.39% crude fiber, and 4158.72 kcal/kg gross energy. The estimated AME and AMEn for DWM were 2616.50 and 2403.32 kcal/kg DM, respectively. The SID of essential amino acids were: His 84.36 $\pm$ 1.90%, Arg 83.89 $\pm$ 1.63%, Thr 77.73 $\pm$ 3.71%, Lys 71.94 $\pm$ 2.63%, Leu 69.98 $\pm$ 2.03%, Val 69.74 $\pm$ 2.83%, Phe 67.46 $\pm$ 1.98%, and Ile 67.86 $\pm$ 2.74%. The digestible essential amino acid concentrations were: Arg 2.09 $\pm$ 0.04%, Leu 2.03 $\pm$ 0.06%, Lys 1.54 $\pm$ 0.05%, Val 1.50 $\pm$ 0.05%, Phe 1.43 $\pm$ 0.04%, Thr 1.17 $\pm$ 0.06%, Ile 1.04 $\pm$ 0.04%, and His 0.50 $\pm$ 0.02%. The duckweed meal showed crude protein content and essential amino acid profile comparable to alternative plant-based protein ingredients described in the literature, showing promising potential as an alternative ingredient in poultry feeding, capable of contributing to both the protein and energy fractions of diets.

Keywords: alternative ingredients; amino acid profile; ileal digestibility; Lemnaceae; metabolizable energy

15 ILEAL AMINO ACID DIGESTIBILITY OF BRAZILIAN DISTILLERS DRIED GRAINS WITH SOLUBLES SOURCES DIFFERING IN PHYSICOCHEMICAL CHARACTERISTICS

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This study evaluated the apparent (AID%) and standardized ileal digestibility (SID%) of amino acids in two Brazilian sources of distillers dried grains with solubles (DDGS) in broiler chickens. The DDGS sources exhibited distinct physicochemical characteristics. Source

I showed greater KOH protein solubility (38.72 vs. 30.45%), higher lightness (L*; 52.81 vs. 49.63), yellowness (b*; 39.44 vs. 37.04), and golden index (20.84 vs. 18.38) than Source II. A total of 147 male Cobb 500 broilers (16 d old; 404 $\pm$ 44 g body weight) were housed in metabolic cages. A completely randomized design with three dietary treatments was used, consisting of two test-diets and a nitrogen-free diet based on starch to estimate the endogenous amino acid losses. The test-diets were formulated by replacing 30% of the nitrogen-free diet with each DDGS source according to the substitution method described by Sakomura and Rostagno (2016). At 21 d of age, birds were euthanized, and ileal digesta were collected to determine AID and calculate SID of amino acids. Data were analyzed using SAS (SAS Institute, Cary, NC, USA), with treatment included as a fixed effect in PROC GLM. Differences were considered significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. The AID coefficient of lysine was 6.78 percentage points (p.p.) greater in Source I than in Source II ($P = 0.016$). A similar pattern was observed for SID lysine, which tended to be 5.07 p.p. higher in Source I ($P = 0.059$). Source I showed AID coefficients 11.62 and 3.98 p.p. greater than Source II for tryptophan ($P = 0.083$) and arginine ($P = 0.088$), respectively. However, these differences were not confirmed for the SID coefficients ($P > 0.05$). Cysteine was the only dispensable amino acid affected by DDGS source, with Source II exhibiting 4.53 p.p. greater AID ($P = 0.050$) and 4.90 p.p. greater SID ($P = 0.036$) than Source I. Differences in amino acid digestibility among DDGS sources were accompanied by variations in physicochemical characteristics, including KOH protein solubility and color parameters. Although these results suggest a potential relationship between these measurements and protein quality, further studies involving a larger number of DDGS sources are needed to determine whether color traits and, particularly, KOH protein solubility, can be used as reliable indicators of amino acid availability and protein quality in DDGS, similar to their application in soybean products.

Keywords: Co-products; DDGS; Poultry Nutrition; Protein Quality; Protein Solubility

16 Relative bioavailability of L-threonine biomass for chicks

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Threonine is the third limiting essential amino acid in corn-soybean meal diets for broilers. Threonine is particularly important during early development because it is highly enriched in intestinal mucins, where it serves as a major substrate for O-glycosylation, supporting mucus production and the maintenance of intestinal barrier integrity. An alternative source is L-threonine biomass, a fermentation-derived product containing approximately 20% residual microbial biomass. Studies are needed to determine the relative bioavailability (RBV) of L-threonine biomass (80%) compared with crystalline L-threonine (98.5%), used as the reference standard, using the slope-ratio method. The objective of this study was to determine the RBV of L-threonine biomass (80%) relative to crystalline L-threonine (98.5%) in broiler chicks. A total of 1,500 one-day-old Cobb male chicks were allocated to a completely randomized design with five treatments and ten replicates of 30 birds each. The treatments consisted of a basal diet deficient in threonine (0.596%), supplemented with two levels of supplementation 0.681% and 0.766% for each of the sources evaluated: L-threonine biomass (80%) and L-threonine (98.5%). The diets were formulated without

the inclusion of enzymes or growth promoters. Feed and water were provided ad libitum. Feed intake, weight gain, feed conversion ratio, feed efficiency and body composition were evaluated cumulatively from 1 to 21 days of age. Data was analyzed by analysis of variance and regression. RBV was estimated using the slope-ratio method according to the following model: $\xi = \alpha + \beta_s X_s + \beta_t X_t \pm \epsilon$, where ξ is the response variable; α is the intercept; ϵ is the random error; β_s and β_t are the slopes for 98% and 80% L-threonine, respectively; X_s and X_t are the concentrations of L-threonine in the diet, respectively. The analyses were performed using Statistical Analysis System software with a significance level of 5%. The results indicated an RBV of 102% for performance variables and 100% for body com-

position. Specifically, parameters such as body protein deposition (99% RBV), daily threonine deposition (103% of RBV), and threonine utilization efficiency (99% of RBV) showed no significant differences among the sources, confirming that the biomass supports early growth and tissue integrity in a manner comparable to the purified source. The RBV of L-threonine biomass (80%) relative to crystalline L-threonine (98.5%) demonstrated that the biomass source is a bio-equivalent and effective alternative to the crystalline source.

Keywords: L-threonine biomass; Slope-ratio method; Relative bioavailability

Student Competition Metabolism and Nutrition: General Nutrition I

17 EVALUATION OF THE NUTRITIONAL QUALITY OF SOYBEAN MEAL USED BY BROILER INTEGRATORS IN GOIÁS AND NEIGHBORING STATES

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Soybean meal is one of the most important ingredients in poultry feed manufacturing, serving as a primary protein source with a highly digestible amino acid profile. However, whole soybean grain contains antinutritional factors that reduce nutrient absorption, potentially causing metabolic disorders and compromising animal performance, thus adequate thermal processing of the grain is necessary for its use in animal feed. This study aimed to evaluate the nutritional quality of soybean meal used in poultry feed production in the state of Goiás. A total of 6,415 soybean meal samples from eleven soybean meal processing plants were obtained from the Quality Control Laboratory of the integrator. After filtering out samples with missing values for the variables included in the analysis, the final dataset comprised 6,372 samples, which were then analyzed by near-infrared spectroscopy (NIRS). The parameters evaluated were: dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, starch, acid detergent fiber (ADF), neutral detergent fiber (NDF), sugar, total phosphorus, phytic phosphorus, apparent metabolizable energy (ME) and net energy (NE). Data were submitted to analysis of variance (ANOVA), and means were compared by the Scott-Knott test at a 5% significance level using the R software and the Easyanova package. Significant differences among companies were observed for all evaluated parameters ($p < 0.001$), with means classified into homogeneous groups according to the Scott-Knott test. Ether extract and NDF showed the highest number of homogeneous groups (nine), ranging from 1.79 to 2.63% DM (SEM = 0.04) and from 10.04 to 11.80% DM (SEM = 0.11), respectively. Five additional parameters were classified into the second-highest number of groups (eight): crude fiber (3.66 - 4.82% DM, SEM = 0.04); total phosphorus (5,668.14 - 6,101.78 ppm, SEM = 26.54); phytic phosphorus (3,400.89 - 3,660.78 ppm, SEM = 15.92); net energy (2,103.14 - 2,164.64 kcal/kg, SEM = 4.51); and apparent metabolizable energy (2,323.22 - 2,378.67 kcal/kg, SEM = 3.99). These findings demonstrate substantial variability among companies for all evaluated parameters, underscoring the importance of continuous soybean meal quality monitoring for accurate poultry feed formulation.

Keywords: Broiler; Nutrition; Soy; Feed; Analysis

18 Prediction of metabolizable energy loss in corn based on grain quality

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The objective of this study was to quantify apparent metabolizable energy corrected for nitrogen (AMEn) loss associated with increasing proportions of damaged kernels (D), broken kernels (B), impurities (I), and insect-damaged kernels (ID), and to develop prediction equations on dry matter (DM) basis and as-fed basis (AFB). All the procedures adopted in the study were previously approved by the Ethics Committee of Federal University of Viçosa. The metabolism trial was performed on 16 to 25-day-old broilers, with the total excreta method used to determine energy values. A total of 650 male Cobb 500 broilers were randomly assigned to 13 treatments with 10 replicates and 5 birds per cage. Treatments included a reference diet (RD) and 12 test diets with 60% RD and 40% experimental corn, which consisted of pure corn and pure corn containing 10 or 20% D; 5, 10, or 15% B; 4, 8, or 12% I; or 4, 8, or 12% ID. After a 5-day adaptation period, excreta were collected over five days. The AMEn was calculated using the equations proposed by Matterson et al. (1965). Multiple linear regression models without an intercept were used because loss was expected to be zero in the pure corn baseline. Predictors were selected by an AIC-based stepwise procedure, with multicollinearity assessed by variance inflation factors (VIF), and model performance was validated by grouped 7-fold cross-validation across treatments. For DM, the final equation for energy loss (EL) was: $EL \text{ (kcal/kg)} = 4.20 \times D + 4.79 \times B + 8.31 \times I + 9.23 \times ID$ ($R^2 = 0.490$). All coefficients were positive, with D and B significant at $P < 0.05$, and I and ID at $P < 0.01$. Cross-validation showed an RMSE of 130.54 kcal/kg, MAE of 98.80 kcal/kg, and a bias of +32.11 kcal/kg. For AFB, the equation was: $EL \text{ (kcal/kg)} = 4.61 \times D + 5.66 \times B + 7.37 \times I + 9.14 \times ID$ ($R^2 = 0.484$), with D, I, and ID significant at $P < 0.01$, and B at $P < 0.05$. The RMSE was 116.72 kcal/kg, MAE 88.16 kcal/kg, and bias +28.29 kcal/kg. VIF values were ≤ 1.120 in both models. Increasing proportions of all defect categories were associated with increased AMEn loss, with I and ID being the most critical, indicating that corn batches with higher contamination by these fractions require greater attention during ingredient evaluation and diet formulation. These equations could help adjust corn energy values during ingredient evaluation and feed formulation.

Keywords: chickens; corn quality; energy loss; kernel defects; metabolizable energy

19 Apparent metabolizable energy in full-fat and defatted black soldier fly (*Hermetia illucens*) larvae meals for broiler chickens.

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The objective of this study was to determine the apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) of seven black soldier fly larvae meals (BSF) samples obtained from Brazilian suppliers for broilers. A total of 288 male Ross AP95 broiler chicks were assigned to a randomized complete block design with eight dietary treatments and six replicates of six birds each. Treatments consisted of a corn-soybean meal reference diet and seven assay diets in which 20% of the reference diet was replaced with one BSF sample (BSF2 to BSF8), including three defatted and four full-fat meals. Birds were housed in metabolic cages and had *ad libitum* access to feed and water throughout the experimental period. A 5-d adaptation period (d 13 to 18 d) was followed by a 4-d total excreta collection period (d 19 to 22), with excreta collected twice daily. Samples of excreta, diets, and test ingredients were analyzed for dry matter, nitrogen, ether extract, and gross energy, and AME and AMEn values were calculated by difference method. Data were analyzed using the MIXED procedure of SAS, with means compared by Tukey's test ($P \leq 0.05$). Marked variation was observed among BSF samples, reflecting differences in composition and processing characteristics. Defatted meals (BSF2, BSF4, and BSF7) showed higher crude protein and lower ether extract contents, with crude protein ranging from 46.97 to 61.54% and ether extract from 6.65 to 13.43%, whereas full-fat meals (BSF3, BSF5, BSF6, and BSF8) showed lower crude protein and higher ether extract contents, with crude protein ranging from 40.28 to 45.49% and ether extract from 28.70 to 41.48%. Among full-fat meals, BSF5 showed the highest AME and AMEn values (5,789 and 5,510 kcal/kg DM), BSF3 and BSF8 showed intermediate values (4,305 and 4,028; 4,539 and 4,190 kcal/kg DM), and BSF6 presented lower values (3,348 and 3,122 kcal/kg DM). Among defatted meals, BSF7 and BSF2 also showed intermediate values (4,527 and 4,254; 4,249 and 3,837 kcal/kg DM), whereas BSF4 had the lowest values (2,701 and 2,430 kcal/kg DM). Overall, substantial variation was observed in the metabolizable energy values of BSF from Brazilian suppliers, whether full-fat or defatted, indicating that these ingredients are not nutritionally equivalent. The observed variability likely reflects differences in chemical composition and processing characteristics among samples and reinforces the importance of ingredient-specific energy characterization for accurate feed formulation in broiler diets.

Keywords: Difference method; energy evaluation; insect meal; poultry nutrition

20 Laying hen maturation and keel bone development

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Body weight and composition at the onset lay affect egg production and skeletal integrity, but the effects on keel bone development is

less understood. This study investigated the effects of delaying puberty through pullet feed restriction and photostimulation (PS) time on sexual maturation and keel quality. Day-old Lohmann LSL-Lite pullets ($n=704$) were randomized to 64 cages (11 birds/cage) with 2 feeding programs (*ad libitum* and restricted to 80% recommended by the management guide) until 17 wk of age. After 18 wk of age, 2 ages of PS (18 and 21 wk of age) were applied resulting in a 2 X 2 factorial arrangement of treatments. Birds were individually weighed at 0, 3, 8, 17, 18, 19 and 20 wk of age. At 3, 8 and 17 wk of age, ADFI, ADG and mortality-corrected FCR were calculated by cage. At 12, 16, and 18 wk of age, one bird per cage was euthanized for liver, abdominal fat pad, ovary and skeletal measurements. Keel and femur traits were evaluated using dual-energy X-ray absorptiometry. The age at first egg was recorded (cage basis). Data were analyzed by ANOVA (One-way to 17 wk of age; Two-way after 18 wk of age) followed by Tukey's Test at 5% of significance. Feed restriction reduced ADFI by 26.4% and ADG by 19.8% during the rearing period, and BW at 17 wk of age by 20% ($P<0.001$) compared with *ad libitum*-fed birds. FCR to 17 wk of age was 8.4% lower in restricted birds ($P<0.001$). At 12 wk of age, liver weight was 20.5% and abdominal fat pad weight 86.5% lower in restricted birds ($P<0.001$), suggesting maintenance was prioritized over lipid deposition. Ovary weight at 16 wk of age decreased by 15.8% ($P=0.006$); the age at first egg was delayed by 16 d (158 vs. 142 d; $P<0.001$) in restricted compared to *ad libitum* birds ($P<0.001$), regardless of PS treatment. In both feeding groups, postponing PS from 18 to 21 wk of age delayed first egg by 8 d (154 vs. 146 d; $P<0.001$), confirming late sexual maturation. No interactions of feeding programs and age at PS were observed ($P>0.05$). Restricted birds had lower keel bone density (0.118 vs. 0.125 g/cm²; $P=0.004$), mineral content (2.08 vs. 2.45 g; $P<0.001$) and area (17.5 vs. 19.6 cm²; $P=0.003$) at 12 wk of age. Femur traits were also lower in restricted-fed pullets ($P<0.05$). At 18 wk of age, keel bone mineral content and keel area remained lower in restricted-fed birds ($P=0.015$), but keel bone density was not affected ($P=0.145$). Feed-restricted birds required more time to initiate lay than *ad-libitum*-fed birds. Therefore, photostimulation timing, body weight and physiological development should be considered before onset of lay. Ongoing evaluations will determine effects on keel bone maturation and laying performance to 40 wk of age.

Keywords: layer pullet; body weight; feed restriction; sexual maturity; keel bone

21 Modeling energy requirements of broiler chickens using metabolizable energy and net energy systems

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This study aimed to develop factorial models to determine the energy requirements of broiler chickens based on practical variables. A total of 384 male Cobb 500 broilers, 19 days of age, were randomly assigned to 12 experimental diets, with four replicates of eight birds per experimental unit. The birds were housed in six open-circuit respiratory chambers. The experimental diets were formulated to provide a large variation in energy content to obtain a quantitative variation in energy intake and to proceed with regression. For that, the strategy in feed formulation consisted in variation of AMEn (2900 to 3200 kcal/kg), each level varying on efficiency of utilization (70 and 80%). During the experimental period (20 to 24 days of age), total

excreta collection was performed to determine metabolizable energy intake (MEi), and gas exchange was measured to determine heat production (HP) under fed conditions. On day 25, feed was withdrawn to determine fasting heat production (FHP). Retained energy (RE) was calculated as the difference between MEi and HP. Energy partitioning variables were expressed relative to metabolic body weight (BW kg^{0.75}). Data were subjected to regression analysis. Retained energy increased linearly with increasing metabolizable energy intake ($RE = 0.76 \times MEi - 89.7$; $P < 0.05$; $R^2 = 0.89$), resulting in a metabolizable energy requirement for maintenance (ME_m) of 118 kcal/kg^{0.75}/day and an efficiency of energy utilization for gain (kg) of 0.76. Heat production was described by the equation $\log HP = \log(106.4) + 0.00059 \times MEi$, estimating fasting heat production and the net energy requirement for maintenance (NE_m) at 106 kcal/kg^{0.75}/day. The efficiency of metabolizable energy utilization for maintenance (km) was estimated at 0.89 based on the NE_m/ME_m ratio. Using these coefficients, the net energy factorial model was established as $NE \text{ (kcal/day)} = 106 \times BW^{0.75} + (1999.9 + 426.7 \times BW) \times BWG$, where the energy requirement for gain was adjusted for body weight to account for changes in body composition during growth. The corresponding metabolizable energy model was derived by applying the estimated energy utilization efficiencies: $ME \text{ (kcal/day)} = 118 \times BW^{0.75} + [(1999.9 + 426.7 \times BW)/0.76] \times BWG$. Model evaluation using data from the specific strain yielded net and metabolizable energy estimates consistent with observed performance. It is concluded that the ME- and NE-based models allowed estimation of energy requirements for maintenance and gain in broiler chickens, providing an approach to evaluating dietary energy utilization.

Keywords: indirect calorimetry; retained energy ; maintenance requirement; energy efficiency; gain

22 Effects of including HPDDG in diets balanced for branched-chain amino acids and supplemented with carbohydrases for broiler chickens

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The inclusion of high-protein dried distillers' grains (HPDDG), a product of the corn ethanol biorefinery industry, in broiler diets may be limited by imbalances among branched-chain amino acids (BCAA), particularly excess leucine in relation to valine and isoleucine, which can impair amino acid utilization and growth performance. In addition, non-starch polysaccharides (NSP) in HPDDG may increase digesta viscosity and reduce nutrient digestibility. These limitations may be mitigated through adequate BCAA formulation and carbohydrase supplementation. This study evaluated graded HPDDG inclusion in diets balanced for BCAA and supplemented or not with carbohydrases on growth performance and carcass traits of broiler chickens. A total of 4,140 one-day-old male Ross AP95 broiler chicks were distributed in a completely randomized 2 × 5 factorial design, with two enzyme supplementation levels and five HPDDG inclusion levels, totaling 10 treatments with nine replicates of 46 birds each. HPDDG levels were 0, 5, 7, 9, and 11% in the starter phase, 0, 8, 10, 12, and 14% in the grower phase, and 0, 11, 13, 15, and 17% in the finisher phase. Birds in T1–T5 received no enzyme supplementation, whereas T6–T10 received a carbohydrase blend containing xylanase and β-glucanase at 100 g/ton of feed. Growth performance was evaluated weekly. At 43 days of age, 36 birds per treatment were processed for carcass yield and commercial cut

evaluation. Data were analyzed by two-way ANOVA, including HPDDG inclusion, enzyme supplementation, and their interaction, and means were compared by Tukey's test at 5% significance. During the overall period, increasing dietary HPDDG inclusion did not affect body weight gain, feed intake, or feed conversion ratio ($P > 0.05$). Carbohydrase supplementation did not affect body weight gain or feed intake, but improved feed conversion ratio ($P = 0.0480$). No interaction between HPDDG inclusion and enzyme supplementation was observed for performance variables ($P > 0.05$). Similarly, HPDDG inclusion did not affect carcass yield, breast yield, thigh yield, wing yield, or abdominal fat deposition at 42 days of age ($P > 0.05$). Enzyme supplementation did not influence carcass yield, breast yield, or abdominal fat deposition, but reduced thigh yield and increased wing yield ($P < 0.05$). No interaction effects were detected for any carcass trait evaluated ($P > 0.05$). In conclusion, HPDDG can be included in broiler diets up to the evaluated levels when formulated with balanced BCAA ratios, without compromising growth performance or carcass yield at 42 days. Moreover, carbohydrase supplementation improves feed conversion ratio regardless of HPDDG level.

Keywords: corn ethanol biorefinery product; xylanase; β-glucanase; growth performance; carcass yield

23 Nutritional and energy composition of black soldier fly (*Hermetia illucens*) larvae meals with different ether extract contents for broiler chickens

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The objective of this study was to determine the nutritional and energy composition of full-fat (BSFI), partially defatted (BSFS), and defatted (BSFD) Black Soldier Fly (BSF) larval meals for broiler chickens. A total of 192 male Ross 308 broiler chickens, 14 days of age, were used. The birds were randomly assigned to a completely randomized design with four dietary treatments, eight replicates per treatment, and six birds per replicate. The experimental treatments consisted of a reference diet formulated with corn and soybean meal and three test diets, in which 30% of the reference diet was replaced with the BSF larval meals. To determine the metabolizable energy of the diets, partial excreta collection was performed using the indicator method (CIA). Data were analyzed by analysis of variance (ANOVA), and when significant, means were compared using Tukey's test ($P < 0.05$). Prediction equations for apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) were developed from the AME and AMEn values of the studied meals using stepwise multiple linear regression. The nutritional composition of crude protein (CP), ether extract (EE), crude fiber (CF), and gross energy (GE) of the larval meals was, respectively, 43.86%, 32.28%, 21.95%, and 6,190 kcal/kg for BSFI; 44.68%, 20.91%, 15.25%, and 5,540 kcal/kg for BSFS; and 52.19%, 16.13%, 12.15%, and 5,574 kcal/kg for BSFD. The larval meals presented AME and AMEn values of 4,343 and 4,151 kcal/kg for BSFI, 3,738 and 3,540 kcal/kg for BSFS, and 3,396 and 3,180 kcal/kg for BSFD, respectively. The fat content of the Black Soldier Fly (BSF) larval meals (full-fat, partially defatted, and defatted) significantly influenced their nutritional composition. The full-fat BSF larval meal (BSFI) had the highest ether extract (EE), crude fiber (CF), and gross energy (GE) contents, whereas the defatted BSF larval meal (BSFD) had the highest crude protein (CP) content. Significant differences

were observed among the AME and AMEn values, with BSFI showing the highest values, followed by BSFS and BSFD, respectively. The prediction equations obtained were: $AME = 2669.9 + 99.5 (EE) - 69.5 (CF)$ and $AMEn = 2274.2 + 97.3 (EE) - 52.6 (CF)$. The model showed R-square values of 0.7657 for AME and 0.8756 for AMEn, indicating that 76.57% and 87.56% of the variation in the response variables were explained by EE and CF contents for AME and AMEn,

respectively. The chemical composition influenced the energy value of BSF larval meals, and the proposed equations accurately estimated AME and AMEn, with the AMEn prediction equation exhibiting the highest predictive capacity.

Keywords: poultry; alternative ingredient; insects

Metabolism and Nutrition: Amino Acids

40 Reevaluating Threonine Requirements of Broiler Chickens During the Starter Phase Using an 80% L-Threonine Biomass

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The growing production of biomass-derived amino acids and the limited number of studies on these sources reflect the need to update nutritional requirements considering the use of these ingredients due to the presence of biomass. The objective of this study was to determine the threonine (Thr) requirement, using an 80% L-threonine source, for broiler chickens from 1 to 21 days of age. A completely randomized design was adopted, consisting of six treatments and ten replicates of 30 birds each, totaling 1,800 Cobb broiler chicks distributed across 60 experimental units. The treatments consisted of a basal diet (BD) without threonine supplementation (5.96 g/kg) and 5 levels obtained through the supplementation technique (6.81, 7.66, 8.52, 9.37, and 10.22 g/kg) using the biomass

amino acid source. The performance variables evaluated at 7, 14, and 21 days were feed intake (FI, g/bird), daily body weight gain (BWG, g/bird), feed conversion ratio (FCR, g/g), and feed efficiency (FE). Threonine intake (mg/bird), deposition (mg/bird), and maintenance requirement (mg/bird) were also calculated. Data were analyzed for assumptions of homoscedasticity of variance and normality of errors; when the p-value was less than 0.05, regression analysis was applied. Performance variables (FI, BWG, FCR, FE) as well as threonine intake, deposition, and maintenance requirement showed significant effects in the contrast analysis (BD vs. 80% L-Thr treatments). Regression analyses revealed linear and quadratic effects for all variables at 7, 14, and 21 days of age. Two functions were fitted to determine the threonine requirement: broken line (BL) and quadratic plateau (BLq). The BLq model showed better statistical fit for BWG and FCR ($R^2 = 0.97$ and $R^2 = 0.96$) compared to the BL model. Using the first intersection between the broken-line (BL) and broken-line quadratic (BLq) models, the estimated dietary threonine requirements for broiler chickens from 1 to 21 days of age receiving 80% biomass L-threonine were 0.760% for body weight gain and 0.810% for feed conversion ratio.

Keywords: amino acid biomass; broilers; body weight gain; requirement; threonine

Student Competition Management and Production

41 Field mortality and carcass condemnation association in commercial broiler production

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Carcass condemnation represents an important source of economic loss and reduced processing efficiency in poultry production. Since mortality during grow-out may reflect failures in flock health, management, environmental conditions and welfare, we hypothesized that broiler flocks with higher field mortality would also present increased carcass condemnation at slaughter. Thus, a 3-yr historical dataset from a large-scale Brazilian poultry integrator, comprising records of more than 266 million broilers, was evaluated to investigate the association between flock mortality and carcass condemnation. Data included productive, environmental and management-related classificatory variables (moderators) such as slaughter site, broiler house type, flock sex, stocking density, litter reuse, between-flocks interval, slaughter weight, average daily gain and total mortality. Carcass condemnation ratio was analyzed using a random-effects model (REML), and dataset heterogeneity was investigated by Q2 test. In case of significance, heterogeneity was explored by meta-regression and subgroup analysis of car-

cass condemnation against each individual classificatory variable. Moderator relevance was quantified according to the coefficient of determination obtained in meta-regression (R^2). Overall carcass condemnation averaged $0.39 \pm 0.0002\%$, with significant heterogeneity among observations ($P < 0.001$; $I^2 = 99.97\%$). Total mortality significantly influenced carcass condemnation ($P < 0.001$), showing positive and linear association with condemnation rates ($R^2=17.32\%$). Although other moderators, such as average daily gain ($R^2= 34.44\%$) and slaughter weight ($R^2=20.64\%$) also explained dataset heterogeneity, mortality was the one less correlated with broiler house type, therefore being more generally acceptable as carcass condemnation predictor. The linear association between flock mortality and carcass condemnation followed the equation: carcass condemnation (%) = 0.0606 mortality (%) - 0.009 . These findings reinforce field mortality as an important indicator associated with carcass losses and highlight the relevance of integrating flock health indicators into evidence-based mitigation strategies in poultry production.

Keywords: broiler health; carcass condemnation; field mortality; poultry production ; REML

42 Influence of housing type (conventional vs. climate-

controlled) on intestinal morphometry of broilers

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Maintaining gut integrity is a critical factor for productive success and feed efficiency in modern broiler production. This study investigated the influence of housing type (conventional versus climate-controlled houses) on intestinal morphology parameters of broilers raised under commercial systems. The experiment was conducted across eight commercial poultry houses in the states of Santa Catarina and Paraná, Brazil, including four climate-controlled and four conventional houses. Twenty-four birds (three birds per house) aged 25 to 28 days were randomly selected. For morphometric analysis, 6-cm ileal segments were collected from a point 10 cm distal to Meckel's diverticulum, opened longitudinally, extended on cardboard, and fixed in 10% neutral formalin for histological processing. The methodology involved selecting 10 villi and 5 crypts per slide to measure villus height (VH), villus width (VW), and crypt depth, in addition to calculating the villus-to-crypt (V:C) ratio and the absorptive surface area using the formula: $2 * \pi * (VW/2) * VH$, methodology adapted by Nain *et al.* (2012) and Sonail *et al.* (2012). Statistical analyses were performed using Generalized Linear Models (GLM) ($\alpha < 0.05$), and housing-type means were compared using Student's t-test ($\alpha < 0.05$). All statistical analyses were performed using SAS software (version 9.4). Results revealed that the housing type exerted a significant impact on the intestinal microarchitecture of the birds. Crypt depth was significantly greater ($P = 0.0143$) in broilers reared in conventional houses (118.28 μm) than in those from climate-controlled houses (98.66 μm). Consequently, the V:C ratio was significantly higher in climate-controlled environments (7.75) than in conventional ones (6.76), suggesting lower epithelial turnover and better preservation of intestinal integrity. No significant differences were observed between housing types regarding villus height (777.07 μm in conventional environments vs. 753.88 μm in climate-controlled ones), villus width (168.90 μm and 181.69 μm), or absorptive surface area (414.39 μm and 433.26 μm) ($P > 0.05$). The climate-controlled environment proved to be superior to the conventional one, providing conditions that were less challenging to intestinal health and integrity. The lower crypt depth observed in these houses suggests a reduction in the local inflammatory response and greater efficiency in preserving the intestinal mucosa. Previous studies have reported positive correlations between villus height and V:C ratio with growth performance traits, such as average daily gain and carcass weight, whereas crypt depth is generally negatively associated with these parameters.

Keywords: gut integrity; histomorphology; housing system; intestinal health

43 Infrared beak treatment as an alternative to hot-blade beak trimming: Effects on sexual maturity, egg production and egg quality

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Beak trimming is widely used to reduce cannibalism, feather dam-

age, and egg pecking; however, its impacts on animal welfare have encouraged the search for alternative methods. This study aimed to evaluate infrared beak treatment using different beak exposure protocols and radiation intensities compared with hot-blade beak trimming regarding sexual maturity, egg production, and egg quality of commercial laying hens from 19 to 33 weeks of age. A total of 120 Dekalb White hens were allocated to three treatments with five replicates and eight birds each. The treatments consisted of hot-blade beak trimming (HB), with a V-shaped cut at 7 days of age; light infrared treatment (LIR), using a 26/23 guard plate and a radiation intensity of 38 nm; and moderate infrared treatment (MIR), using a 24/23 guard plate and a radiation intensity of 40 nm. From 17 to 33 weeks of age, the age at sexual maturity (50% egg production) and egg production were evaluated. Egg quality was assessed at 21, 25, 29, and 33 weeks of age. The evaluated variables were egg weight, yolk weight, shell weight, albumen height, yolk color, Haugh unit, shell strength, and shell thickness. The age at sexual maturity did not differ ($p = 0.223$) among treatments, with mean values of 126.0, 127.8, and 124.4 days for HB, LIR, and MIR, respectively. Likewise, egg production remained similar among treatments ($p = 0.955$), with mean values of 88.43%, 88.20%, and 88.93% for HB, LIR, and MIR, respectively. No significant differences ($p > 0.05$) were observed for egg weight, albumen height, yolk color, Haugh unit, shell strength, or the proportions of yolk, albumen, and shell ($p > 0.05$). However, shell thickness was affected by the beak trimming methods, being lower in birds subjected to HB (0.408 mm) than in those treated with light infrared (0.428 mm) and moderate infrared (0.426 mm), which did not differ from each other. Therefore, the evaluated beak trimming methods did not influence sexual maturity, egg production, or the internal quality of eggs. However, infrared beak treatment promoted greater shell thickness compared with hot-blade beak trimming. Infrared beak treatment of laying hens is a beneficial alternative to the hot-blade method, as it resulted in better eggshell quality.

Keywords: Beak treatment; Dutch beak trimming; Laying hens; Shell thickness

44 Effect of infrared and hot-blade beak trimming on organ biometrics and intestinal histomorphometry of laying hens

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The objective was to compare hot-blade beak trimming and two infrared beak trimming protocols in relation to the biometrics of the digestive system organs and the intestinal histomorphometry of 18-week-old laying hens. A total of 120 chicks were used, distributed in a completely randomized design with three treatments, five replicates, and eight birds each. The treatments were beak trimming by light infrared (LIR), moderate infrared (MIR), and hot blade (HB) beak trimming. HB was performed on the birds at seven days old, with cutting and cauterizing using the 'V' method. Infrared beak trimming was done in the hatchery right after the eggs hatched, using a 26/23 protective plate with 38 nm intensity (LIR) and a 24/23 protective plate with 40 nm intensity (MIR). The biometrics of the digestive system organs were carried out at 18 weeks of age, after a 12-hour food fast, using two randomly selected birds per replicates. The proventriculus, gizzard, intestine, and liver were weighed indi-

vidually, and their relative weight was calculated in relation to the birds' body weight. Samples of the duodenum were collected, fixed in formaldehyde, washed in 70% alcohol, dehydrated in increasing concentrations of ethanol (70, 80, 90, and 100%), cleared in xylene, embedded in paraffin, and cut into 5 µm sections, which were then stained with hematoxylin and eosin. The histological images were obtained using an image capture system and analyzed. Thirty measurements were taken to determine villus height, villus width, crypt depth, villus: crypt ratio, and villus surface area. The data were subjected to analysis of variance and, in the case of a significant

effect, the means were compared using Tukey's test ($p < 0.05$). The beak-trimming methods affected only the gizzard, which showed a higher relative weight with HB compared to the two protocols with infrared radiation (LIR and MIR). There was no significant effect of the beak-trimming methods and protocols on the intestinal histomorphometric variables. Debeaking by infrared radiation is a viable alternative to the hot blade, as it did not negatively affect the digestive system.

Keywords: dutch trimming; gizzard; beak treatment; intestinal villi

Student Competition Metabolism and Nutrition: General Nutrition II

45 Effects of pelleting and post-pelleting oil application on carcass yield and chemical composition of broiler chickens

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Pelleting is well established to improve broiler growth performance, yet its specific effect on carcass composition remains unclear. Because pellet physical quality modulates the energy a bird spends on feed prehension and ingestion, higher-quality pellets may reduce waste and physical effort and shift the partition of metabolizable energy, potentially redirecting nutrients toward protein or fat deposition. The objective was to evaluate the effects of feed processing and the timing of soybean oil addition on carcass yield, chemical composition, and gizzard traits of broilers. In a completely randomized design, 600 male Ross 308 broilers (18 to 39 days) were assigned to 3 treatments with 8 replicates of 25 birds each: a mash diet with oil in the mixer (control), a pelleted diet with oil in the mixer (pre-pelleting), and a pelleted diet with 2.84% oil sprayed after cooling (post-pelleting). Corn-soybean diets (3,100 kcal ME/kg, 20.1% CP) were pelleted (Andritz G9, 4.5-mm die, 84 °C). At 39 days, 2 birds per replicate were slaughtered to determine carcass and cut yields (breast, thigh+drumstick, abdominal fat), whole-carcass crude protein, fat, and moisture were determined by near-infrared spectroscopy (NIR). At 42 days, 2 additional birds per replicate were euthanized to measure gizzard pH and relative weight. Data were analyzed by ANOVA, and when significant ($P < 0.05$), pelleted means were compared with the control by Dunnett's test ($P < 0.05$). No differences were found for carcass yield, thigh+drumstick, or abdominal fat ($P > 0.05$). Breast yield tended to increase with pelleting (32 vs 31%, $P = 0.061$). Post-pelleting oil increased carcass crude protein (19.11 vs 17.86%, $P = 0.021$), with no change in fat ($P = 0.078$) or moisture ($P = 0.317$). Gizzard pH, weight, and relative weight did not differ among treatments ($P > 0.05$). Feed form and processing did not affect carcass yield or gizzard traits, but oil applied after pelleting increased carcass protein deposition.

Keywords: broiler; carcass composition; carcass yield; pelleting; protein deposition

46 Effects of pelleting and timing of oil application on pellet physical quality and growth performance of broiler chickens

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The physical form of the feed is a key determinant of feed efficiency in broilers. Pelleting increases bulk density and nutrient digestibility and reduces feed waste, but the point at which the lipid fraction is added modifies pellet quality: oil added before pelleting acts as a die lubricant and can lower pellet hardness and durability, whereas oil sprayed after pelleting may preserve pellet integrity and reduce fines. The objective was to evaluate the effects of feed processing and the timing of soybean oil addition on pellet physical quality and growth performance of broilers. In a completely randomized design, 600 male Ross 308 broilers (18 to 39 days) were assigned to 3 treatments with 8 replicates of 25 birds each: a mash diet with 3.34% oil added in the mixer (control), a pelleted diet with 3.84% oil in the mixer (pre-pelleting), and a pelleted diet with 0.5% oil in the mixer plus 2.84% oil sprayed after cooling (post-pelleting). Corn-soybean diets (3,100 kcal ME/kg; 20.15% CP) were pelleted (Andritz G9, 4.5-mm die, 84 °C, 10-s conditioning). Pellet hardness ($n = 25$), durability index (PDI), fines, feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured. Data were analyzed by ANOVA, and when significant ($P < 0.05$), pelleted means were compared with the control by Dunnett's test, and pellet hardness between pelleted treatments by Student's t-test ($P < 0.05$). Post-pelleting oil increased pellet hardness (3.72 vs 2.92 kgf, $P < 0.05$) and PDI (84.0 vs 59.8%) and reduced fines (38.7 vs 67.4%). From 18 to 25 days, FI was higher for post-pelleting than control (991 vs 951 g, $P = 0.035$). From 18 to 32 days, both pelleted diets increased BWG (1,719 and 1,729 vs 1,624 g, $P = 0.007$), and pelleting improved FCR versus control (1.315 vs 1.391, $P = 0.001$). Over 18 to 39 days, both pre-pelleting and post-pelleting diets improved FCR versus mash (1.430 and 1.437 vs 1.472, $P = 0.001$), with no difference in FI or BWG. Pelleting improved feed efficiency relative to mash, and applying oil after pelleting further enhanced pellet physical quality and early feed intake.

Keywords: Broiler; Feed efficiency; Pellet durability; Pelleting; Post-pellet oil

47 Effects of dietary defatted avocado seed meal on cecal short-chain fatty acids and intestinal histomorphometry of broiler chickens

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The objective of this study was to evaluate the effects of increasing levels of defatted avocado seed meal (DASM) inclusion in broiler diets from 1 to 42 days of age on intestinal histomorphometry and short-chain fatty acid production. A total of 720 male Ross AP95 broilers were distributed in a completely randomized design with five levels of DASM inclusion (0.0, 1.5, 3.0, 4.5, and 6.0%), eight replicates, and 18 birds per experimental unit. Two birds per experimental unit were slaughtered at 42 days of age to evaluate short-chain fatty acid (SCFA) concentrations in cecal content and jejunal histomorphometry. Data were analyzed for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test), followed by analysis of variance using the GLM procedure. When significant effects were detected, polynomial regression analyses were performed using the

REG procedure. All statistical analyses were conducted using SAS OnDemand, adopting a significance level of 5%. In the cecal content, linear and quadratic increases were observed in acetic acid ($P = 0.0009$) and butyric acid ($P = 0.0001$) concentrations, whereas propionic acid remained unchanged ($P = 0.9397$). Intestinal histomorphometry showed linear and quadratic effects on villus height ($P = 0.0010$), a quadratic effect on crypt depth ($P = 0.0337$), linear and quadratic effects on the villus-to-crypt ratio ($P = 0.0084$), and a quadratic response for the absorptive surface area ($P = 0.0021$). In conclusion, the inclusion of defatted avocado seed meal (DASM) in broiler diets up to 6.0% positively influenced intestinal health by increasing cecal acetic and butyric acid concentrations and improving jejunal histomorphometric characteristics, including villus height, villus-to-crypt ratio, and absorptive surface area.

Keywords: Broiler chickens; defatted avocado seed meal; intestinal health

Student Competition Immunology, Health and Disease

48 Next-generation probiotic *Propionibacterium freudenreichii* promotes anti-inflammatory immunomodulation in day-old chicken

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Although *Propionibacterium freudenreichii* exhibits remarkable anti-inflammatory activity in non-avian models, its effects in poultry remain largely unknown. Its anti-inflammatory activity may support pathogen control and intestinal health during the early stages of life, contributing to improved poultry performance. Therefore, this study evaluated the anti-inflammatory effects of *P. freudenreichii* CIRM-BIA 129 in one-day-old chicks inoculated during embryonic development. Embryonated eggs were distributed into six experimental groups (10 chicken embryo/group): (i) negative control; (ii) wild-type *P. freudenreichii*; (iii) Δ slpB mutant *P. freudenreichii* strain, lacking the *SlpB* gene, which expresses the protein responsible for anti-inflammatory activity; (iv) *Salmonella Pullorum*-challenged as a model to inflammation - positive control; (v) wild-type *P. freudenreichii* + *S. Pullorum*; and (vi) Δ slpB mutant *P. freudenreichii* + *S. Pullorum*. At 18 days of incubation each embryo in the respective groups was inoculated with 100 μ L of the corresponding treatment via the amniotic route. Four hours later, embryos were challenged with 10^5 CFU/embryo of *S. Pullorum* (groups iv, v, and vi) or saline (groups i, ii, and iii). At hatch, blood samples were collected for IL-10 and ceruloplasmin quantification using chicken-specific ELISA kits. Data were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparisons test, considering $p < 0.05$, using GraphPad Prism 10.0.2. Embryos inoculated with the wild-type strain showed markedly increased IL-10 concentrations, including under *Salmonella* challenge, whereas embryos receiving the Δ slpB mutant did not demonstrate similar cytokine induction. In contrast, ceruloplasmin concentrations did not differ among groups. The loss of IL-10 induction in the Δ slpB strain strongly suggests that *SLPB* plays a central role in the immunomodulatory effects of *P. freudenreichii* in poultry. These findings reinforce the promising potential of *P. freudenreichii* CIRM-BIA 129 as a next-generation probiotic for the poultry industry,

with potential benefits for improving poultry health and reducing dependence on antimicrobial growth promoters.

Keywords: Cytokines; Intestinal health; Immune signaling ; Feed additives; Poultry production

49 In Vitro Methodologies and an In Vivo Model for the Evaluation of *Salmonella* Inhibition by *Lactobacillus acidophilus*

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The efficacy of probiotics in controlling *Salmonella* is strain-dependent, requiring prior screening of promising strains. In this context, *in vitro* tests are viable and ethically acceptable alternatives for initial selection. Among the available methodologies, the spot-on-lawn assay is widely used; however, it may generate false-negative results due to the limited diffusion of metabolites through agar. In contrast, bacterial counting is quantitative and independent of metabolite diffusion. Additionally, chicken embryos before 11 days of incubation have emerged as promising pre-clinical models for evaluating probiotic activity. This study compared bacterial counting and spot-on-lawn methodologies to determine the antagonistic activity of two *Lactobacillus acidophilus* (LA) strains against *Salmonella* Heidelberg (SH) and *Salmonella* Minnesota (SM) and evaluated chicken embryos as an animal model for probiotic studies. Two *L. acidophilus* strains (LA1 and LA2) and two *Salmonella* strains were used. Antimicrobial activity was evaluated by bacterial counting and inhibition halo formation on agar. ANOVA followed by Tukey's test was performed considering $p < 0.05$, and all assays were conducted in triplicate. For the *in vivo* model, fertile eggs were distributed into six groups ($n = 10$): negative control, positive control (SH), LA1, LA2, LA1+SH, and LA2+SH. Inoculations were performed on the third day of embryonic development via the shell membrane using 4 log CFU/embryo of probiotic and 3 log CFU/embryo of SH. At 10 days, allantoic fluid was collected for bacterial quantification. Using bacterial counting, LA1 reduced SH and SM by 4–5 log CFU/mL (99.93% and 99.99% reduction, respectively), with inhibition halos of 22.16 and 22.75 mm. LA2 showed superior performance, reducing SH and SM

by 8.97 and 8.28 log CFU/mL, respectively (100% reduction), and producing halos of 31.66 and 32 mm. Although both strains were classified as very strongly inhibitory by the spot-on-lawn assay, only bacterial counting differentiated the superior activity of LA2. In the embryonic model, the positive control showed an average bacterial load of 6.8 log CFU/mL. Treated groups showed bacterial growth in only one sample, with loads of 3.27 log CFU/mL (LA1) and 2.147 log CFU/mL (LA2). Despite the inhibitory effect observed, LA2 did not completely eliminate the pathogen *in vivo*. In conclusion, the *in vitro* methodologies were effective for evaluating antagonistic activity, with bacterial counting being more sensitive for differentiating probiotic strains. The embryonic model demonstrated pre-clinical potential, although further standardization is required.

Keywords: probiotics; antimicrobial resistance; experimental model; poultry production; antagonistic activity

50 Monitoring of Monophasic *Salmonella* in Wild Birds as a Prevention Tool for Commercial Poultry Production in Goiás State, Brazil

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The Monophasic *Salmonella* serovar 1,4,[5],12:i:- has gained importance in poultry production due to its zoonotic potential and multi-drug-resistant (MDR) profile. The proximity of wild birds to poultry farms poses a sanitary risk, as these birds may carry and disseminate pathogens such as *Salmonella*. This study aimed to detect *Salmonella* spp., evaluate its antimicrobial susceptibility profile, and characterize epidemiological aspects related to the geographic origin of positive wild birds admitted to the Wildlife Screening Center (CETAS) from different municipalities in the state of Goiás, Brazil. Between 2024 and 2025, 17 fecal samples were collected from wild birds and transported to the Avian Diseases Diagnostic Laboratory of the Federal University of Goiás, where they were immediately processed for *Salmonella* isolation following standard bacteriological procedures (Brasil, 2013; MGL, 2023). Positive isolates were subjected to antimicrobial susceptibility testing (CLSI, 2020) and serotyping. Sixteen antimicrobial agents belonging to the β -lactam, aminoglycoside, macrolide, tetracycline, fluoroquinolone, sulfonamide, phenicol, and phosphonate classes were tested. *Salmonella* spp. was isolated from 23.5% (4/17) of the analyzed samples, and 100% (4/4) of the isolates were classified as multidrug-resistant, as all isolates showed resistance to tetracycline, doxycycline, gentamicin, and ampicillin (antimicrobials from three different classes). Additionally, 50% (2/4) of the isolates were resistant to chloramphenicol, cefazolin, and amikacin, while 25% (1/4) showed resistance to enrofloxacin, ceftiofur, fosfomicin, norfloxacin, and azithromycin. All four isolates were identified as *Salmonella* enterica serovar Monophasic 1,4,[5],12:i:-, and 100% were recovered from psittacine birds (*Ara ararauna*, *Diopsittaca nobilis*, *Amazona aestiva*, and *Pionus maximiliani*). The bacterium was isolated from four birds rescued in the metropolitan region of Goiânia. Of these, 75% (3/4) originated from wildlife rescue operations in the municipalities of Goiânia, Goiânia, and Senador Canedo, while 25% (1/4) originated from an urban rescue in Goiânia. These findings indicate that psittacine birds may act as important reservoirs of multidrug-resistant monophasic *Salmonella* in the metropolitan region of Goiânia. The occurrence of this serovar and its MDR profile in wild birds highlights its potential for maintenance and dissemination among animals, humans, and the environment, reinforcing the need for continuous surveillance of

wild birds as part of biosecurity strategies for commercial poultry production.

Keywords: Psittacines; Antimicrobial resistance; *Salmonella* enterica; Epidemiological surveillance; CETAS

51 Peptides selected by phage display could represent a potential innovation for the detection of Avian Pathogenic *Escherichia coli* ST131?

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Avian pathogenic *Escherichia coli* (APEC) ST131 is the causative agent of colibacillosis, a disease that represents one of the major sources of economic losses in commercial poultry production and is also associated with a zoonotic risk. Conventional diagnostic approaches are based on labor-intensive molecular methods, highlighting the demand for rapid and highly specific detection strategies. In this study, we sought to identify peptides with strong binding affinity to APEC ST131 using phage display technology and to investigate their application as bioreceptors in an electrochemical biosensor for bacterial detection, based on a low-cost patented technology developed by our group. For the selection of the peptides ANOVA followed by Tukey test was used, considering $p < 0.005$ using GraphPad Prism version 10.2.3. For the classification analyses of the data obtained from biosensor, the voltammetric data were analyzed using machine learning algorithms, including Neural Network, Gradient Boosting, Support Vector Machine, Stochastic Gradient Descent, Random Forest, and AdaBoost models, applied through 10-fold cross-validation using Orange 3.38.1 software. The selected peptide was immobilized onto laser-induced graphene (LIG) electrodes to fabricate the electrochemical biosensor, and bacterial detection was carried out using cyclic voltammetry. Subsequently, the voltammetric data were processed with artificial intelligence algorithms. Among the evaluated models, Stochastic Gradient Descent achieved the highest performance, reaching 100% sensitivity, 98.31% specificity, and 99.17% accuracy. To the best of our knowledge, this study is the first to describe the use of a phage display-derived peptide as a bioreceptor in an electrochemical biosensor for detecting APEC ST131. Overall, these findings highlight the potential of integrating peptide-based biosensors with machine learning to provide a rapid, sensitive, and scalable approach for the screening of avian colibacillosis.

Keywords: APEC; Phage display; Diagnostic; ELISA; Biosensors

52 Effects of breeder age and eggshell translucency on calcium mobilization during embryonic development

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Eggshell quality is essential for embryonic development because it provides mechanical protection, enables gas exchange, and serves as the main mineral source for skeletal formation. Nearly 80% of embryonic calcium is derived from eggshell resorption during the second half of incubation, while the shell membranes regulate mineralization and mineral transfer through the chorioallantoic mem-

brane. Thus, changes in shell structure and mineral composition related to breeder age and eggshell translucency may affect embryonic mineral availability and progeny skeletal quality. This study characterized the elemental composition of eggshells and shell membranes from eggs of young (29 wk) and old (55 wk) ROSS broiler breeders with good or poor eggshell translucency using Scanning Electron Microscopy coupled with Energy-Dispersive X-ray Spectroscopy (SEM-EDS). The experiment was conducted at the Federal University of Paraná (UFPR), Brazil, in partnership with a commercial poultry company. A total of 6,384 eggs were incubated in a completely randomized 2×2 factorial design, with breeder age and eggshell translucency as main factors. Eggshell samples were collected from 12 eggs per treatment at 14 and 17 d of incubation, totaling 96 samples. After removal of the inner and outer shell membranes, samples were cleaned, air-dried, gold-coated, and processed for SEM-EDS. Four fragments per sample were analyzed, representing the shell cross-section, inner shell surface, outer shell surface, and inner shell membrane, using a TESCAN VEGA 3 microscope coupled to an INCAx-act EDS system. EDS analyses were performed at 5 kV, 15 mm working distance, and 40 s acquisition time. The relative abundance of Ca, S, Cl, O, Ir, Si, Mg, C, Na, K, and Cu was determined to characterize mineral distribution during incubation. The results suggest that breeder age and eggshell translucency mainly affect the kinetics of calcium mobilization rather than the total calcium available. At 14 d, eggs from older breeders with translucent shells showed greater calcium retention in the eggshell membrane, indicating delayed calcium transfer to the embryo. By 17 d, this difference was no longer observed in the membrane but remained evident in the eggshell, which showed higher residual calcium. Variations in C, Na, K, and Cu also indicated changes in the organic matrix and eggshell-membrane interface, potentially reducing mammillary layer dissolution. Overall, breeder aging combined with eggshell translucency altered the temporal dynamics of calcium availability to the embryo, which may impair skeletal mineralization and bone development in progeny.

Keywords: Bone mineralization; Calcium availability; Eggshell-membrane interface; Embryo; INCAx-act EDS system

53 Early feed efficiency is associated with greater susceptibility to enteric challenge in broiler chickens

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This study evaluated whether early productive performance is associated with broiler resilience after an enteric health challenge. A total of 864 one-day-old male Cobb 500 chicks (initial BW: 46.4 g) were randomly allocated into 48 floor pens (18 birds/pen; 1 m²) and reared until 42 days of age under controlled environmental conditions. Birds received feed and water *ad libitum*. Birds were assigned to non-challenged (n = 108) and challenged groups (n = 756). The challenge was induced by oral gavage with sporulated *Eimeria maxima* oocysts at 14 days of age (1 mL/bird; 7×10^3 oocysts/mL), followed by *Clostridium perfringens* administration on days 18, 19, and 20 (1 mL/bird; 2.5×10^6 CFU/mL). Post-challenge productive performance (BWG, FI, FCR, and BW), intestinal morphometry, intestinal permeability, immune cell profile, phagocytic activity, and circulating immune markers (IgA, IgG, IFN- γ , and IL-10) were evaluated. Post-challenge deviations were calculated as the percentage difference between each challenged pen and the unchallenged control group during the post-challenge period. Linear regression were used to understand the relationship among responses pre- and post-challenge, using Akaike's (AIC) and Bayesian (BIC) information criteria for predictor selection. In the second step, pens were classified into contrasting pre-challenge performance groups (favorable or unfavorable early performance) using the extreme quartiles of the selected pre-challenge criteria. Body weight gain (BWG) from days 1 to 7 showed the highest predictive power, providing the best model fit in 56% (AIC) and 63% (BIC) of models, followed by feed conversion ratio (FCR) from days 1 to 14 (44% and 38%, respectively). Classification based on first-week BWG did not affect productive, intestinal, or immune responses after the challenge. However, birds classified by early FCR showed differences after infection. Birds with better pre-challenge feed efficiency exhibited greater reductions in BWG from days 15 to 21 (-29.05 vs. -39.54%; $P = 0.007$), greater FCR deterioration (39.99 vs. 46.85; $P < 0.001$), and lower final body weight (-14.79 vs. -19.14%; $P = 0.012$) compared with birds with poorer initial FCR. Additionally, highly efficient birds showed lower increases in intestinal permeability (49.08 vs. 29.18; $P = 0.005$) and reduced IL-10 response (88.85 vs. 39.59; $P = 0.026$), while other intestinal and immune parameters were unaffected. These findings indicate that superior early feed efficiency may be associated with reduced productive resilience after an enteric challenge, reinforcing the importance of evaluating both productive efficiency and resilience traits in broiler performance assessments.

Keywords: broiler chickens; coccidiosis; health resilience; poultry; productive performance

Metabolism and Nutrition: General Nutrition

54 Interactive effects of raw potato starch, reduced crude protein, and *Eimeria acervulina* challenge on starch digestion and intestinal gene expression in broiler chickens

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Our previous studies showed that raw potato starch (RPS) inclusion in reduced crude-protein (RCP) diets may create a starch-protein mismatch that impairs amino acid digestibility in broilers challenged with *Eimeria acervulina* (E). This 35-day study investigates jejunal and ileal starch digestibility and the expression of nutrient transporters, tight junction proteins, and liver protein turnover

genes. We hypothesized that inclusion of RPS will reduce starch digestibility in the standard protein diet and alter digestibility in the RCP diet in birds challenged with E. A total of 1,056 male broiler chicks were allocated to eight treatments ($2 \times 2 \times 2$ factorial) with six replicates per treatment: CP (standard, SCP, or RCP), RPS (0 vs. 40 g/kg), and E (- or +). The treatments are SCP, SCP-RPS, RCP, RCP-RPS, SCP+E, SCP-RPS+E, RCP+E, and RCP-RPS+E. Challenged birds were orally gavaged with a solution of 50,000/ml E on d 12. On d 18, five birds per pen were euthanized to collect jejunal and ileal digesta for starch digestibility, and jejunal mucosal scrapings for nutrient transporters and tight junction gene expression. On d 35, liver samples were collected for protein turnover gene expression

analysis. Data was analyzed as a three-factor mixed model. No significant interactions were observed for jejunal and ileal starch digestibility. Jejunal starch digestibility tended ($P = 0.074$) to increase in E-challenged birds, RPS inclusion tended to decrease ($P = 0.061$) jejunal starch digestibility, and significantly ($P < 0.01$) decreased ileal starch digestibility. The E challenge significantly increased ($P < 0.05$) the relative expression of nutrient transporter (PEPT1, CAT1, BO+AT, SLC38A, and SGLT1), tight junction proteins (claudin, and JAM4), and liver protein turnover (S6K1, AKT1, IGF1, mTOR, 4EBP1, and PSMA1) genes. No significant interactions were observed except for CP $\times$ E where challenged birds fed SCP diets had increased relative expression of PSMA1 gene, whereas no challenge-induced differences were observed in birds fed RCP diets ($P < 0.05$). In conclusion, RPS lowered starch digestibility regardless of E challenge, suggesting that the resistant starch fraction limited starch disappearance in the distal intestine, thereby increasing fermentable substrates in the ceca for SCFA production and possible gut health benefit. Also, E. acervulina led to relative overexpression of nutrient transporters, tight junction proteins, and hepatic protein turnover genes, indicating adaptive intestinal and metabolic responses to maintain gut barrier, nutrient trafficking, and protein balance during and after coccidial infection.

Keywords: Raw potato starch; reduced-protein diets; Eimeria acervulina; starch digestibility; liver protein turnover

55 Trypsin inhibitor surveillance of Brazilian soybean and soybean-based ingredients from 2021 to 2026

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Soybean and soybean derivatives are the main protein sources in monogastric diets in Latin America and most regions of the world. Despite their extensive use, soybean-based ingredients can contain antinutritional factors (ANF), even after thermal treatment. One of the main ANF in soybean is trypsin inhibitor (TI), which can inactivate endogenous proteases and reduce protein digestibility in monogastric animals. Due to its importance in poultry and swine nutrition, a TI surveillance was conducted in Brazil from January 2021 to April 2026. Samples of raw soybean, soybean meal (SBM), extruded soy, and deactivated soy from different regions were tested. A total of 1,015 samples collected from 55 feed mills throughout Brazil were used in this survey. Trypsin inhibitor levels were measured using wet chemistry according to AOCS official methods. From January 2021 to December 2025 the method used was Ba 12-75 (AOCS, 2017), from January 2026 onwards the newer AOCS Official Method Ba 12a-2020 (AOCS, 2021) was applied. To express all samples on the latest method basis, a conversion factor of 1.267 was applied to the samples from 2021 to 2025. Trypsin inhibitor concentration, expressed as mg/g, was evaluated using SAS PROC MIXED. Separate models were used for each comparison. Pairwise comparisons were adjusted using Tukey-Kramer, and differences were considered at $P \leq 0.05$. After model-based outlier removal, 966 observations were included in the ingredient comparison. Levels of TI differed between different soy-based ingredients ($P < 0.0001$). As expected, raw soybeans presented the highest TI levels (24.68 mg/g), followed by extruded soy (9.59 mg/g), SBM (4.64 mg/g) and deactivated soy (2.74 mg/g). Year did not affect soybean TI levels ($P = 0.1971$), likely due in part to limited sample number after outlier removal. In contrast, SBM levels were affected by year ($P = 0.0013$), 2026 showed higher TI concentration compared to 2025 and 2023. Additionally, the location of the buyer company impacted soybean meal ($P < 0.0001$). Feed mills located in Santa Catarina, Rio Grande

do Sul, and Goiás had SBM with higher TI concentration than the ones in Pernambuco and Paraná. In summary, and based on this surveillance, deactivated soy followed by SBM had the lowest TI, while SBM showed an increase in TI in 2026. Additionally, the feed mill location, and probably the SBM vendors, influenced the ANF levels found in the ingredient. More data is necessary to understand the behavior of TI in soybeans and its derivatives; however, this surveillance provides some important information for the animal industry.

Keywords: Soybean ; Trypsin inhibitor; Brazil; soybean meal; antinutritional factors

56 Evaluation of criteria for defining the beginning and end of the total excreta collection period for metabolizable energy determination in broiler chickens

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The determination of metabolizable energy is essential for accurate diet formulation in broiler production because it enables accurate estimation of the energy available to the birds. Although the total excreta collection method is widely used in metabolism assays, the criteria adopted to define the beginning and end of the collection period are not standardized and may influence the estimation of metabolizable energy. Therefore, this study evaluated different criteria used to define the beginning and end of the excreta collection period and their effects on apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AMEn), and their respective metabolizability coefficients (CAME and CAMEn). A total of 150 male Cobb 500[®] broiler chickens were used in a completely randomized design, with three treatments, ten replicates, and five birds each from 8 to 14 d. The treatments consisted of three criteria to define the excreta collection period: a 12-h feed withdrawal before and after collection, 1% ferric oxide as marker, or standardized collection start and end times. The birds were housed in metabolic cages for seven days, including three days of adaptation and four days of total excreta collection. AME, AMEn, CAME, and CAMEn were calculated based on feed intake, excreta output, and laboratory determinations of dry matter, gross energy, and crude protein. Data were subjected to the Shapiro-Wilk test for normality, followed by analysis of variance and Tukey's test at $P < 0.05$. Treatments affected AME and CAME ($P = 0.046$). Feed withdrawal resulted in lower AME (3,306 kcal kg⁻¹) and CAME (73.42%) than the ferric oxide (3,347 kcal kg⁻¹ and 74.34%) and standardized collection time (3,352 kcal kg⁻¹ and 74.45%) methods, which did not differ. No differences were observed for AMEn or CAMEn ($P > 0.05$). Therefore, either ferric oxide or standardized collection times can be used to define the beginning and end of the excreta collection period in metabolism trials with broiler chickens.

Keywords: período de coleta de excrementos; avaliação de energia; retirada de ração; óxido férrico; teste de metabolismo

57 Influence of energy determination methodology on

metabolizable energy estimates in broiler chickens

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Accurate determination of metabolizable energy is essential for precise feed formulation and nutrition evaluation in broiler chickens. Although marker-based methodologies are widely used because they simplify digestibility trials, methodological differences may influence energy estimates. Therefore, this study compared total excreta collection with partial excreta collection using acid-insoluble ash or titanium dioxide for determining apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AMEn), and their metabolizability coefficients (CAME and CAMEn) in broiler chickens fed a corn-soybean meal diet. A total of 150 Cobb 500® male broilers were assigned to a completely randomized design with three methodologies: total excreta collection, partial excreta collection using 1% acid-insoluble ash (Celite™), and partial excreta collection using 0.5% titanium dioxide, with 10 replicates of five birds each from 8 to 14 d. Birds were housed in metabolism cages for four days of adaptation followed by three days of excreta collection. For the total excreta collection method, AME, AMEn,

AMEC, and AMEnC were calculated based on feed intake and total excreta output. For the marker-based methodologies, these variables were estimated using indigestibility factors calculated from marker concentrations in feed and excreta. Data were analyzed by analysis of variance (ANOVA), and when significant, means were compared using Tukey's test at $P < 0.05$. Marker-based methodologies yielded lower values of AME, AMEn, CAME, and CAMEn than total excreta collection ($P < 0.05$). Mean AME values were 3,343, 3,157, and 2,499 kcal kg⁻¹ and mean AMEn values were 3,166, 2,996, and 2,428 kcal kg⁻¹ for total excreta collection, acid-insoluble ash, and titanium dioxide, respectively. Mean CAME values were 73.83, 69.72, and 54.81%, whereas mean CAMEn values were 69.91, 66.17, and 53.25%, respectively. Compared with total excreta collection, AME values obtained using acid-insoluble ash and titanium dioxide were reduced by 5.5% and 24.5%, respectively. Recovery rates of acid-insoluble ash and titanium dioxide were 100.52 and 100.70%, respectively, indicating adequate analytical recovery of both markers. Furthermore, the AME value obtained by total excreta collection differed by 33 kcal kg⁻¹ from the formulated dietary energy value on a dry matter basis, whereas greater deviations were observed for both marker-based methodologies. The total excreta collection method produced energy estimates closest to the formulated dietary energy value, supporting its use as the reference methodology for determining metabolizable energy in broiler chickens.

Keywords: acid-insoluble ash; metabolism trial; partial excreta collection; titanium dioxide; total excreta collection

Symposium: From egg to meat: impact of each production chain link on profitability, quality, and food safety

1011S Global and Brazilian poultry market: Present and future trends and demands

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This presentation will address the key challenges shaping the present and future of the global and Brazilian poultry markets from a systemic and business-oriented perspective. The discussion will examine how economic uncertainty, cost volatility, geopolitical tensions, animal health threats, changing consumer expectations, and increasing demands from customers and regulatory authorities are redefining competitiveness across the poultry industry. Attention will be given to Brazil's strategic role in supplying both domestic and international markets, highlighting the importance of maintaining market access, strengthening the country's reputation as a reliable supplier, diversifying export destinations, and reducing vulnerabilities throughout the production chain. Rather than viewing logistics, trade, production, and consumption as isolated issues, the presentation will connect the different stages of the poultry value chain and the specific challenges and contributions of each one. From grandparent and breeder flocks to hatcheries, broiler production, processing, distribution, and ultimately the consumer, the analysis will explore how decisions and challenges at each stage influence the performance, competitiveness, quality, and resilience of the entire system. Looking ahead, poultry companies will need to become more agile, collaborative, market-oriented, and capable of anticipating change. Future competitiveness will depend on balancing operational efficiency and competitive costs with product quality, food safety, sustainability, animal health, and consumer trust. Ultimately, the future growth of the poultry industry will depend on the entire value chain's ability to anticipate market changes, collectively

manage risks, respond to increasingly diverse market demands, and transform global challenges into sustainable business opportunities.

1012S Slaughterhouse carcass condemnation and its effects on final product quality

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quality" Liris Kindlein Abstract Carcass condemnation is one of the leading causes of economic losses in poultry processing plants and remains a major challenge for the poultry industry worldwide. Condemnations result from both technological failures during slaughter and pathological conditions developed during production, directly affecting carcass quality, processing yield, and food safety. This presentation will discuss the main causes of carcass condemnation, including gastrointestinal contamination, bruising, inadequate bleeding, ascites, cardiovascular disorders, cellulitis, airsacculitis, and breast muscle myopathies. Particular attention will be given to the influence of environmental management, stocking density, and pre-slaughter handling on metabolic homeostasis, circulatory function, and muscle development, highlighting how these factors increase the risk of carcass defects and condemnations. The presentation will also address the relationship between intestinal health and microbiological safety, emphasizing the role of dysbiosis in the dissemination of enteric pathogens and fecal contamination during processing. Increasingly stringent regulatory requirements in export markets, particularly regarding *Salmonella* and *Enterobacteriaceae* control, have reinforced the need for effective preventive strategies throughout the production chain. Current concepts of

immunonutrition and the use of functional feed additives will be discussed as strategies to preserve intestinal integrity, modulate the gut microbiota, improve immune function, and reduce pathogen colonization, including *Salmonella*. These approaches contribute to lower fecal shedding, reduced cross-contamination during evisceration, and improved carcass quality. Finally, the integration of nutritional strategies with appropriate environmental conditions and effective pre-slaughter management will be highlighted as a practical approach to reducing carcass condemnation, minimizing economic losses, facilitating compliance with rigorous global safety standards, and producing safer, higher-quality poultry products.

Keywords: carcass condemnation; immunonutrition; gut microbiota; food safety; *Salmonella*

1013S Broilers growing and finishing phases: strategies to support profitability

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In the current broiler production scenario, classical clinical diseases are increasingly less frequent and easier to diagnose, but subclinical challenges have become the real threat to flock performance and profitability, especially from 21 days of age onward. During the growing and finishing phases, subclinical clostridiosis is one of the most relevant concerns, frequently presenting without overt clinical signs while still causing intestinal mucosal damage, impaired nutrient digestion and absorption, and marginal but cumulative performance losses. Dysbiosis and unspecific enteritis further compound this scenario, disrupting the stable microbiome required for gut barrier function and immune homeostasis, and increasing intestinal permeability and inflammatory energy expenditure. These enteric disturbances are frequently interconnected with skeletal problems observed in older broilers, since reduced nutrient absorption, altered mineral utilization and systemic inflammation directly affect bone mineralization and leg health, aggravating lameness and carcass condemnation at the plant. This presentation will discuss the pathophysiology, field recognition and practical management strategies for subclinical clostridiosis, dysbiosis and enteric-associated bone disorders in broilers older than 21 days, emphasizing their cumulative economic impact under the tight margins of modern poultry production, and how integrated nutritional, health and biosecurity strategies can support flock uniformity, welfare and overall profitability throughout the growing and finishing period.

Keywords: gut health; dysbiosis; broiler profitability

1014S Broiler breeders: relationship between immune system, microbiota and production

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Unlike commercial broilers, which are primarily selected for rapid growth and feed efficiency, broiler breeders must maintain reproductive performance over an extended production cycle while balancing nutrient partitioning among maintenance, immunity, reproduction, and egg formation. Within this complex physiological framework, the immune system and intestinal microbiota have emerged as important components of breeder health and productivity. A balanced intestinal microbiota supports epithelial integrity and contributes to appropriate immune maturation and regulation. A competent immune system limits pathogen colonization while avoiding excessive inflammation. Together, these processes promote efficient nutrient

utilization and reduce the metabolic costs of disease, supporting reproductive performance. Increasing evidence indicates that these systems are not independent; rather, they interact dynamically through the gut-immune axis, influencing intestinal integrity, nutrient utilization, inflammatory status, reproductive performance, egg quality, and potentially the health and robustness of their offspring. The potential maternal contribution on the newly hatched microbiota is little understood and explored. Recent reviews have emphasized the possibility that breeder microbiota may have transgenerational implications for broiler development and that microbiome-targeted interventions in breeder flocks could influence offspring health and productivity. The future strategies for improving broiler breeder productivity should move beyond the isolated evaluation of nutrition, immunity, or microbiota and instead adopt an integrated approach based on the gut-microbiota-immune-reproductive axis. Understanding this axis may provide new opportunities to improve breeder longevity, reproductive efficiency, chick quality, disease resilience, and the overall sustainability of poultry production.

Keywords: balanced intestinal microbiota ; transgenerational effect; offspring health

1015S Broilers pre-initial and initial phases: how to ensure growth and health over current challenges"

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Genetic improvement has established broiler meat as the world's most consumed animal protein, yet future success depends on balancing production efficiency with animal welfare, sustainability, and food safety. Modern broilers have high genetic potential and are extremely responsive, requiring a precise and integrated management approach where nutrition, health, and environmental control are interdependent. Success requires strict adherence to technical protocols from the pre-arrival phase—such as house disinfection and pre-heating—through to the final growth stages, focusing on variables like temperature, ventilation, and Lighting program. Because the growth cycle is short and sequential, management failures are difficult to reverse, making data collection and trained handling essential to ensure flock uniformity and meat quality. **Keywords:** Broiler Management, Genetic Potential, Animal Welfare, Sustainability, Poultry Production, Environmental Control, Meat Quality.

Keywords: Broiler Management; Genetic Potential; Animal Welfare; Sustainability, Poultry Production; Environmental Control

1016S Broiler breeders' modern challenges and progeny impact

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The incubation of broiler breeder eggs faces significant challenges in contemporary industrial poultry production, particularly under the genetic pressures for greater broiler performance efficiency. This lecture addresses the main modern challenges in breeder management and their direct impact on the incubation process and progeny quality. The discussion focuses on the relationship between breeder management models and the metabolic impact on progeny during incubation, through the quality of hatching eggs, including egg weight and specific gravity. The effects of embryonic heat production on embryonic development, hatchability rate, viability, and chick quality at hatch are analyzed. Recent evidence indicates that fluctuations in breeder flock uniformity compromise the standardization of eggs destined for incubation, resulting in wider hatch

windows and greater progeny heterogeneity. The lecture proposes integrated management and egg classification strategies, including temperature and humidity control in incubators, as well as specific gravity and egg size conditions, as critical factors for maximizing the potential of commercial flocks. It is concluded that addressing the modern challenges in broiler breeder production requires a sys-

temic approach, in which incubation is considered the central link between breeder management and progeny performance.

Keywords: incubation; broiler breeders; hatchability; egg quality; pre-hatch management

Symposium: Functional roles of amino acids, fibers, and feed additives beyond growth in poultry

1017S Monitoring starch and dietary fiber profiles in raw materials and feeds and their relationship to poultry health and performance

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Analytical parameters like crude fiber (CF) are not good enough to understand the role of fiber in digestive physiology and gut health. Non-starch polysaccharides (NSP), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) are more valuable parameters for nutritionists. Moderate levels of IDF have positive effects on gizzard development and nutrient digestibility. Main characteristics of SDF are hydration properties resulting in increased viscosity, prolonged transit time, reduced nutrient digestion/absorption and increased endogenous losses. Reduced passage rate can promote the onset of infectious enteric diseases. However, SDF can provide a source of fermentable substrates for beneficial intestinal bacteria promoting gut health. The composition of feed ingredients is impacted by variety and environmental factors and needs to be controlled. In this regard, near infrared spectroscopy (NIR) has established itself as a valuable analytical tool. In wheat (n=2800, harvest 2025, analyzed by NIR), an average total NSP content of 12.00% was determined. Total NSP content ranged from 8.84 to 19.24% (Coefficient of variation (CV): 14.17%) while CF ranged from 0.50 to 4.19% with an average of 2.10% illustrating that a large part of the fiber fractions is not reflected in CF. That is also seen in corn (n=18000) and soybean meal (n=24000). Average contents of total NSP vs. CF of 9.85% vs 1.94% and 16.74% vs. 4.87% were determined for corn and soybean meal, respectively. Total NSP values ranged from 7.24 to 12.24% (CV: 12.65%) in corn and from 14.46% to 20.40% (CV: 6.91%) in soybean meal. Comparing cellulose and insoluble hemicellulose levels major differences can be seen between the grains and soybean meal. Average levels of cellulose of 2.54%, 2.45%, and 6.93% have been determined for wheat, corn, and soybean meal, respectively. Average levels of insoluble hemicellulose of 8.30%, 8.03%, and 5.74% have been determined for wheat, corn, and soybean meal, respectively. Largest portion of total NSP is insoluble. On average soluble NSP values of 1.42%, 1.09%, and 2.19% have been determined in wheat, corn and soybean meal, respectively. Soluble NSP levels ranged from <0.01% up to 4.76% in wheat (CV: 61.10%), from <0.01% to 4.04% in corn (CV: 67.70%), and from 0.01% to 5.58% in soybean meal (CV: 36.65%). The presented data illustrate the variation of fiber components in feed ingredients which highlights the importance of analyzing the locally available feed ingredients instead of relying on table values. In this regard NIR analytics is a favorable tool to derive information on the composition of locally available feed ingredients to update feed formulations with actual data and to come closer to precision nutrition.

Keywords: total dietary fiber; non-starch polysaccharides; near infrared reflectance spectroscopy

1018S The use of dynamic in-vitro intestinal simulation tools to understand the impact of gut health additives and nutritional strategies in poultry

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No abstract submitted.

1019S The impact of environmental and health challenge conditions on amino acid recommendations of modern broilers

Melissa Hannas*¹ *UVF, Vicosa, Brazil*

No abstract submitted.

1020S Dynamic application of models to develop commercial feeding programs to improve performance of poultry

Nilva K. Sakomura*¹ *UNESP, Jaboticabal, Brazil*

No abstract submitted.

1021S Field experiences with the use of functional amino acids, dietary fibers, and combination of gut health additives in poultry nutrition in Latin America

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Gut health has become a major determinant of biological and economic efficiency in modern poultry production. This is especially relevant in Latin America, where high-performance genetics are commonly exposed to corn-soybean meal diets of variable ingredient quality, high environmental temperatures, coccidial pressure, bacterial enteric challenges, and increasing restrictions on antimicrobial growth promoters. This article reviews applied experiences with three complementary nutritional approaches: functional amino acids, dietary fibers, and combinations of gut health additives. Functional amino acids such as threonine, arginine, glutamine, methionine, and glycine can support mucus production, epithelial turnover, antioxidant defense, and immune function. Fiber responses depend on solubility, fermentability, particle size, and inclusion level; selected insoluble fibers may stimulate gizzard function, whereas excessive soluble and viscous fractions can impair digestion. Enzymes, organic acids, probiotics, phytochemicals, prebiotics, and related technologies can further support intestinal stability when their mechanisms are complementary. In fact, the best results are obtained through the combination of some of those additives. Under Latin American field conditions, the most consistent strategy is not the replacement of one antimicrobial with one additive, but an integrated program that maximizes proximal nutrient digestion, supports the intestinal barrier, manages microbial substrates, and reduces the impact of environmental and sanitary challenges.

58 Reduction in calcium:phosphorus ratios in the diet of late-aged hens are able to maintain bird performance, egg production and external and internal egg quality

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As a result of advances in bird breeding and genetics, laying hens have become increasingly productive and long-lived. Therefore, nutritional strategies capable of meeting calcium (Ca) and available phosphorus (aP) requirements after 90 weeks of age, while maintaining egg production and quality and reducing environmental contamination and production costs, are crucial. This trial evaluated performance and egg quality of laying hens fed different Ca and aP levels from 91 to 94 weeks of age. A total of 560 H&N White hens were randomly distributed into 16 dietary treatments in a 4 × 4 factorial design, with four Ca levels (3.0, 3.5, 4.0, and 4.5%) and four aP levels (0.25, 0.30, 0.35, and 0.40%), with seven replicates of five hens per treatment. Each cage was considered one replicate. At the end of the 28-day cycle, performance parameters, including feed intake, egg production, egg mass, feed conversion ratio (FCR), and marketable egg percentage, were assessed. Egg quality parameters, including egg weight, eggshell breaking strength, albumen height, Haugh unit, yolk color, and eggshell thickness, were evaluated in three eggs per cage on the last day of the cycle using a Digital Egg Tester machine (DET6000, Nabel Co., Japan). Data were tested for normality and homogeneity and analyzed using SAS software. The Kruskal-Wallis test was applied, and significant differences were further evaluated using the DSCF test, with significance set at $P < 0.05$. The lowest Ca and aP inclusion levels, 3.0 and 0.25%, respectively, maintained bird performance, with feed intake, egg production, egg mass, FCR, and marketable egg percentage similar to those of the other groups ($P > 0.05$). No differences were observed among treatments for egg weight, eggshell breaking strength, or yolk color ($P > 0.05$). The combination of 4.5% Ca and 0.30% aP resulted in greater eggshell thickness than treatments containing 3.0/0.40, 3.5/0.25, 3.5/0.30, 3.5/0.35, and 4.0/0.25% Ca/aP ($P < 0.05$). The lowest Ca and aP levels resulted in albumen height values similar to those observed with the highest levels, 4.5 and 0.40% Ca/aP ($P > 0.05$). In contrast, the 3.5/0.25 and 4.0/0.40% Ca/aP treatments showed the lowest albumen height values ($P < 0.05$). Higher Haugh unit values were observed in hens fed diets containing 3.5/0.40, 4.0/0.30, and 4.5/0.40% Ca/aP compared with those fed 3.5/0.25 and 4.0/0.40% Ca/aP ($P < 0.05$). Overall, Ca and aP levels can be reduced in diets for late-age laying hens without impairing performance and key egg quality traits, suggesting potential reductions in feed costs and environmental contamination.

Keywords: Eggshell quality; Feedstuff costs; Hen longevity; Mineral requirement; Residual mineral excretion

supplemented with copper nanoparticles as a growth promoter

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Subclinical coccidiosis impairs mineral retention and bone development in broiler chickens. The poultry industry has traditionally used copper sulfate (CuSO₄) at high dietary concentrations (150-200 mg/kg or ppm) as a growth-promoting feed additive due to the antimicrobial properties of copper (Cu). However, excessive copper sulfate inclusion may lead to mineral antagonisms and increased Cu excretion into the environment. In this context, copper nanoparticles (CuNPs) have emerged as a potential alternative because of their greater bioavailability and larger surface area, allowing lower dietary Cu inclusion levels. Therefore, this study aimed to evaluate the effects of CuNP supplementation in antibiotic-free diets on bone quality and mineral retention of broilers challenged with *Eimeria* spp. A total of 336 one-day-old male Cobb 500 chicks were housed in metabolic cages for 21 days and distributed in a randomized complete block design with six treatments and eight replicates of seven birds each. The treatments were: UC-unchallenged control; CC-challenged control; CuSO₄-challenge+200 ppm Cu from CuSO₄; NP25-challenge+25 ppm CuNPs; NP50-challenge+50 ppm CuNPs; NP100-challenge+100 ppm CuNPs. On day 1, birds were challenged by oral gavage with 10x the recommended dose of coccidiosis vaccine (Bio-Coccivet R®, Biovet). From days 18 to 21, excreta were collected to determine mineral retention and excretion. On day 21, twelve birds per treatment were euthanized and the left tibiae were collected for morphometric, densitometric and mechanical parameters evaluation. Preplanned orthogonal contrasts were used to test specific hypotheses among treatments ($p < 0.10$). CC showed reduced mineral retention ($p < 0.10$) for Mg, P, S, K, Ca, Fe, Cu and Zn. The CuSO₄ treatment exhibited lower retention of S, Ca, Mg and Fe ($p < 0.10$) than CuNPs, while NP25 showed the lowest retention values of Mg, P, S, K, Ca and Fe among CuNP treatments. Birds fed copper sulfate excreted more Cu than those receiving CuNPs ($p < 0.0001$; 367.17; 78.04; 121.38; 175.64 mg/kg respectively). Despite the lower mineral retention observed with CuSO₄, this treatment resulted in a higher Seedor index ($p = 0.0399$; 125,34) and greater moment of inertia ($p = 0.0133$; $0.84 \times 10^{-4} \text{ m}^4$) compared with CuNP supplementation. However, no significant differences were observed for bone mineral density, bone ash, ultimate strength and ultimate strain. In conclusion, the *Eimeria* challenge negatively affected mineral retention and broilers supplemented with copper sulfate exhibited lower mineral retention than those receiving CuNPs. Supplementation with 50 mg/kg CuNPs improved mineral retention, reduced copper excretion into the environment and maintained bone quality parameters.

Keywords: alternative ; digestibility; *Eimeria*; sulfate; tibiae

60 Particle size variability of Calcitic Limestone samples from obtained from commercial egg production farms.

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With this work, this project aimed to evaluate the particle size of

59 Bone quality and mineral retention in broilers

limestone samples obtained from commercial egg production farms located in Goiás State, Brazil. This experiment was conducted at the Animal Nutrition Laboratory (LANA) of the Department of Animal Science (DZO) of the Veterinary Medicine and Animal Science College (EVZ) of the Federal University of Goiás (UFG), located in Goiânia, Goiás, Brazil. A total of 35 samples were obtained, in which, 18 of fine limestone and 17 of calcareous limestone. The samples were quartered, weighed and subjected to granulometry evaluation in the Bertel brand electromagnetic stirrer equipment, equipped with standardized sieves according to the Brazilian National Technical Standards Association ABNT NBR NM 248:2003 standard, with the following nominal openings in micrometers (μm): ABNT n° 5 (4000 μm), n° 10 (2000 μm), n° 16 (1190 μm), n° 30 (595 μm), n° 50 (297 μm), n° 100 (149 μm), n° 200 (74 μm), n° 230 (63 μm), and blind bottom with a fraction less than 45 μm estimated (estimated). The sieves were previously weighed to extract the tare from the final weight. Then, the fractions of each sieve were separated and weighed individually to determine the retained material. The data were expressed as a percentage for each sieve. Then, the values of Average Geometric Diameter (DGM) and Average Geometric Deviation (DPG) were determined according to what was proposed by the American Society of Agricultural and Biological Engineers. Descriptive statistics were applied to present the data. In the analysis of the results, the following averages were observed for fine limestone, retention in each sieve: 16 – 20.91%; 30 – 42.85% 50– 19.59%; 100– 9.05%; 200– 5.48%; 230– 1.20%; and bottom– 0.92%. For limestone, the results were: limestone, retention in each sieve: 5 – 30.28%; 10 – 65.17% 16– 2.01%; 30– 0.12%; 50– 1.13%; 100– 0.01%; and bottom– 1.28%. The Mean Geometric Diameters (MGD) values obtained were: fine limestone 661 and calcareous limestone 2999. The Standard Geometric Deviations (SGD) values obtained were: fine limestone 1.85 and limestone 1.63. It is concluded that the importance of considering the particle size of limestone can be a good indication of the quality of this ingredient. This is essential to adequately meet the calcium requirements of birds, especially in laying birds in view of their need for eggshell formation.

Keywords: calcário fino; calcário medroso; DGM; DGP; Postura

61 Antioxidant status and micromineral excretion with dietary supplementation of copper proteinate in laying hens

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Copper (Cu) is an essential trace mineral involved in antioxidant defense and mineral metabolism in laying hens. Compared with inorganic Cu sources, copper proteinate (CuP) has greater bioavailability and may affect antioxidant status and mineral excretion. This study evaluated the antioxidant status and mineral excretion of laying hens when fed with differing Cu source (inorganic vs. organic) during different phases. The treatment structure in the study was designed by using a $2 \times 2 \times 2$ factorial arrangement, consisting of 2 Cu sources supplemented in the diets across 3 production phases: growing, early laying, and peak laying. In each phase, hens received either 100% of the recommended Cu level (Brazilian tables) from copper sulfate (CuSO_4) or 50% of the requirement using CuP (Bio-plex Copper, Alltech Inc.). Thus, the 8 treatments represented all possible combinations of Cu sources across three phases, allowing the evaluation of carryover effects throughout the production cycle. A total of 392 hens were randomly assigned to 8 experimental treatments with 7 hens per experimental unit. Lipid peroxidation

(malondialdehyde concentration, MDA) and antioxidant capacity (DPPH) were determined in serum, liver, and yolk at week 47. Mineral concentrations of Cu, Fe, and Zn were quantified in excreta (week 45). The data were analyzed by ANOVA in a $2 \times 2 \times 2$ factorial arrangement, and the means were compared by Tukey's test using SAS v.9.0. No evidence of interactions or main effects was observed for MDA in blood serum ($p>0.05$). Interactions were found in oxidative metabolism measures for the yolk and liver. In the yolk, MDA levels were lower in hens receiving CuP during the growing and early laying phases, whereas in the liver, lower levels were observed when Cu was replaced from CuSO_4 to CuP during the peak laying phase ($p<0.05$). In the analysis of DPPH, no main effects or interactions of the Cu sources were observed in liver and yolk ($p>0.05$), while in the blood, higher results were observed when hens had CuP in early and peak laying phases ($p>0.05$). There was no significant difference ($p>0.05$) in ash, Fe, or Zn excretion among treatments. Lower Cu excretion was observed ($p<0.05$) in hens supplemented with CuP during the peak laying phase. In addition, interactions between the growing and peak laying phases showed less Cu excretion ($p<0.05$) in hens with CuP in both phases. In conclusion, laying hens can be supplemented with Cu as copper proteinate 50% of the recommendation during the growing, early laying, and peak laying phases resulting in lower Cu excretion and lipid peroxidation benefits in yolk and liver. These findings suggest greater Cu bioavailability and utilization efficiency with organic source.

Keywords: bioavailability; copper sulfate; copper proteinate ; DPPH; lipid peroxidation

62 Choline requirement for broiler chickens

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Choline is an essential nutrient that acts as a structural component of membrane phospholipids, a precursor of acetylcholine, a hepatic lipotropic agent, and a labile methyl-group donor; however, requirement estimates for modern broilers rely largely on decades-old data. The objective was to determine the dietary choline requirement of male broilers from 0 to 45 d of age. A total of 864 Ross male broilers were assigned to 6 dietary choline concentrations in a completely randomized design with 8 replicate pens of 18 birds (48 pens). Birds were fed a 4-phase program (0–12, 12–24, 24–36, and 36–45 d) based on corn, soybean meal, corn gluten meal, and soy protein concentrate. Analyzed choline contents of the main ingredients (corn, 320; soybean meal, 2410; soy protein concentrate, 1640 mg/kg) were below those tabulated by Rostagno et al. (2024). Basal diets were formulated to 1075, 1000, 925, and 850 mg/kg total choline per phase, and supplemental choline (choline chloride, 60%) was added at 0, 185, 370, 555, 740, and 925 mg/kg, yielding cumulative average total dietary choline of 926, 1111, 1296, 1481, 1666, and 1851 mg/kg. BW was recorded at 12, 24, 35, and 45 d, and mortality-corrected FCR was calculated. Data were analyzed by ANOVA, with means compared by Tukey's test, and by linear and

quadratic polynomial regression on dietary choline concentration ($P \leq 0.05$). Live BW and FCR responded quadratically to dietary choline in all periods (0–12, 0–35, and 0–45 d). For the overall period (0–45 d), BW was maximized and FCR minimized at intermediate concentrations ($BW = -0.0003x^2 + 0.7737x + 3486.9$, $R^2 = 0.94$; $FCR = 8E-08x^2 - 0.0002x + 1.5338$, $R^2 = 0.81$). The maximum of the quadratic BW response estimated a dietary choline requirement of approximately 1500 mg/kg (about 575 mg/kg supplemental), with FCR optimized near 1440 mg/kg. These findings indicate that the choline requirement of modern broilers is higher than current literature estimates, likely reflecting ongoing genetic progress, whereas the choline natively supplied by feed ingredients was lower than tabulated references. Consequently, the prevailing choline supply appears suboptimal, and nutritional requirement tables should be updated to reflect both the greater needs of contemporary genotypes and the lower intrinsic choline of ingredients.

Keywords: dietary supplementation; feed conversion ratio; growth performance; methyl donor; nutrient requirements

63 Reproductive system and keel of semi-heavy laying hens fed a diet supplemented with 1,25-dihydroxyvitamin D3 glycosides of herbal origin (*Solanum glaucophyllum*)

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The objective of the study was to evaluate the effects of dietary supplementation with 1,25-dihydroxyvitamin D3 glycosides (VDG) of plant origin (*Solanum glaucophyllum*) on the reproductive system (ovary and oviduct) and keel of Hysex Brown layers at 20, 76, and 100 weeks of age. The 600 day-old birds (36.23 ± 0.16 g), housed in poultry sheds during the rearing phase, were assigned to two groups. One group received the control treatment (CON, 0 µg/kg of VDG supplement) and the other, the supplemented diet (DS, 1 µg/kg of VDG) until 17 weeks of age. The basal diet was formulated based on corn and soybean meal, with nutritional composition determined by NIRS. At 18 weeks, 160 birds per treatment were transferred to the laying house containing cages and appropriate feeders. The CON and RS groups were subdivided, and 80 birds from the CON group received DS feed, while 80 birds from the DS group received CON feed, forming 4 groups. Each group was composed of 10 experimental units (E.U.) containing 8 birds each. At 20, 76, and 100 weeks, the ovary, oviduct, and keel bone of two randomly selected birds per E.U. were collected. The relative weights of the ovary, oviduct, and keel; the length of the oviduct and keel; and the keel were scored for their conformation. The statistical analysis was performed using ANOVA in a completely randomized design, with a post-hoc mean comparison test when there was a significant interaction ($P < 0.05$) and in a 2×2 factorial scheme, consisting of two levels of VDG supplementation during the rearing and growing phases (0 or 1 µg/kg of feed) and two levels of supplementation during the laying phase (0 or 1 µg/kg of feed). At 20 weeks, supplemented birds (DS) exhibited greater relative ovary ($P = 0.0001$) and oviduct ($P = 0.0033$) weights and lower keel weight ($P = 0.0198$) and length ($P = 0.0076$). At 76 weeks, there was an increase in keel weight ($P = 0.0469$) and keel length ($P = 0.0392$) when supplementation was only during the laying phase. At 100 weeks, supplementation during the laying phase reduced keel deformity ($P = 0.0007$), and there was an interaction between supplementation during the breeding and laying phases for

the relative weight of the oviduct ($P = 0.0271$), with a heavier relative weight of the oviduct in non-supplemented birds during breeding and supplemented during laying ($P = 0.0104$). VDG supplementation favored the initial development of the reproductive system, increased the relative weight of the oviduct in birds at 100 weeks through the interaction between the supplementation phases, and showed an improvement in the structural quality of the keel when supplementation was provided during the laying phase.

Keywords: 1,25-dihydroxyvitamin D₃ glycosides; Reproductive system; Layers hens; Keel; *Solanum glaucophyllum*

64 Broilers from breeders supplemented with 1,25(OH)2D3-G, when supplemented with the same additive, exhibited greater tibial breaking strength and a higher proportion of birds exempt from tibial dyschondroplasia

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Vitamin D3 metabolism directly influences calcium utilization and skeletal development in broilers. This study investigated the effects of supplementation with 1,25-dihydroxycholecalciferol glycoside (1,25(OH)2D3-G) from derived *Solanum glaucophyllum* on tibial strength, tibial composition, and tibial dyschondroplasia in broilers from breeders supplemented or not with the same additive. A total of 1,152 one-day-old male Ross 308 AP95 broiler chicks were allocated to a completely randomized experimental design consisting of six treatments, with eight replicates per treatment and 24 birds per experimental unit. The treatments consisted of broilers receiving diets supplemented with 0.0, 0.5, or 1.0 µg/kg of 1,25(OH)2D3, provided as 1,25(OH)2D3-G, from breeders (21 to 42 weeks of age) supplemented with 0.0 or 1.0 µg/kg of 1,25(OH)2D3, provided as 1,25(OH)2D3-G. Data were subjected to the test of homogeneity (Levene) and normality (Shapiro-Wilk), using the UNIVARIATE procedure. The outliers were removed, and one-way analysis of variance was performed using the GLM procedure. If a statistical difference was observed, data were submitted to Tukey's test. For data that did not exhibit a normal distribution (Tibial dyschondroplasia), non-parametric tests were applied. In the evaluation of injury incidence, the Kruskal-Wallis's test was used to assess the effects of 1,25(OH)2D3-G supplementation, followed by Dunn's test for mean comparisons, using the N1PARWAY procedure. All analyses were conducted using SAS for Academics software, with a significant level of 5%. During the grow-out period, broilers from breeders supplemented with 1,25(OH)2D3-G (1.0 µg/kg of 1,25(OH)2D3) and fed diets supplemented with 0.5 or 1.0 µg/kg of 1,25(OH)2D3 exhibited greater tibial breaking strength at 42 days of age. This response was accompanied by greater calcium and phosphorus deposition in the tibia, indicating enhanced bone mineralization. Furthermore, these broilers showed a higher proportion of birds classified as exempt from tibial dyschondroplasia, with no severe lesions. It can be concluded that supplementation of breeders with 1.0 µg/kg of 1,25(OH)2D3, provided as 1,25(OH)2D3-G, promotes early skeletal development in chicks, whereas continuing this nutritional strategy in broilers from these breeders with 0.5 or 1.0 µg/kg of 1,25(OH)2D3 improves tibial mineralization and tibial breaking strength, while increasing the proportion of birds exempt from tibial dyschondroplasia.

sia at 42 days of age.

Keywords: Bone; skeletal development; vitamin D; Poultry

65 Effects of 25-Hydroxycholecalciferol Supplementation at Different Levels of Calcium and Phosphorus on Serum Mineral and Bone Parameters in Broiler Nutrition

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Vitamin D supplementation in broiler diets is essential for calcium (Ca) and phosphorus (P) absorption, directly impacting bone health and performance. In addition to standard vitamin D supplementation, 25-hydroxycholecalciferol (25-OH-D3) offers superior bioavailability, optimizing Ca and P metabolism. This enhanced efficiency supports skeletal development and overall performance, potentially allowing for reduced dietary mineral levels without compromising bird health or productivity. Thus, the objective of this study was to evaluate the effects of increasing dietary levels of total Ca and available P (av. P), with or without 25-OH-D3 supplementation, on serum alkaline phosphatase activity, serum Ca and P concentrations, and bone characteristics in broilers. A total of 784 male Cobb 500 broilers, aged 1 to 10 days, were distributed in a 4 × 2 factorial arrangement with four levels of Ca and av. P (0.6 + 0.3, 0.8 + 0.4, 1.0 + 0.5, and 1.2 + 0.6%, respectively) and with or without 69 µg/kg of feed of 25-OH-D3, totaling eight treatments with seven replicates of 14 birds each. Data were analyzed using ANOVA, and, when significant, Tukey's test was applied at a 5% significance level. Dietary Ca and av. P levels of 0.6 + 0.3% significantly decreased alkaline phosphatase activity ($P < 0.001$), serum Ca ($P < 0.001$), and serum P ($P < 0.001$) compared with the other dietary levels. Supplementation with 25-OH-D3 increased serum P concentrations (7.2 vs. 6.7 mg/dL; $P < 0.001$). An interaction between dietary mineral levels and 25-OH-D3 supplementation was observed for serum Ca, with supplementation increasing serum Ca particularly in diets with lower mineral levels (9.7 vs. 9.3 mg/dL; $P = 0.005$). Interactions between dietary mineral levels and 25-OH-D3 supplementation were also observed for bone ash content (44.4 to 49.6%; $P < 0.001$), bone P content (7.3 to 9.3%; $P < 0.001$), and bone strength (55 to 76 kgf; $P < 0.001$), with improved values at lower dietary mineral levels with 25-OH-D3 supplementation. A trend toward an interaction was observed for bone calcium content (15 to 16.6%; $P = 0.075$). These results indicate that 25-OH-D3 increases mineral utilization and deposition, optimizing bone integrity, especially in diets with reduced levels of calcium and phosphorus in the feed.

Keywords: Bone mineralization; Broiler chickens; Calcium; Phosphorus; 25-hydroxycholecalciferol

66 Bone Resistance and Blood Indicators in White Laying Hens Fed with Low Levels of Organic Trace Minerals

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Trace minerals are essential for the overall health and bone quality of laying hens. Because organic sources are generally more bioavailable than inorganic sources, dietary supplementation levels may be

reduced without affecting bird status. Therefore, this study evaluated blood variables and bone quality in white laying hens when inorganic sulfate trace minerals were replaced with reduced levels of organic sources. A total of 240 white laying hens (35 weeks of age) were distributed in a completely randomized design with three treatments, 10 replicates, and 8 birds per replicate. Hens received diets containing 100% of the recommended levels of zinc, manganese, iron, copper and selenium from sulfate sources (100S) or 50% (50P) and 25% (25P) of the recommended levels supplied as organic sources (Bioplex and Sel-Plex, Alltech Inc.). Three consecutive 28-day production cycles were evaluated (data not shown). At the end of the experimental period (49 weeks), serum biochemical parameters, hematological profiles, and bone quality of the femur and tibiotarsus were evaluated in one bird per experimental unit. Data were analyzed by ANOVA, and treatment means were compared using Tukey's test at a 5% significance level. In serum biochemistry analysis, hens with 50P diet had the highest value for uric acid ($p = 0.001$), and hens with 25P had highest albumin ($p = 0.002$) and phosphorus ($p = 0.039$) concentrations. In hematological observation, the monocyte concentration was highest in hens receiving the 50P diet ($p = 0.031$). Bone analyses revealed significant differences in femur and tibiotarsus breaking strength, with hens fed the 50P diet exhibiting greater bone resistance to fracture ($p = 0.005$), suggesting improvement in bone quality. Hens from treatment 25P had the same bone characteristics of the 100S diet for tibiotarsus and lower femur hardness (10.79 kgf) when compared to 100S (13.26 kgf). In conclusion, replacing inorganic trace minerals with proteinate sources at 50% or even 25% of recommended levels maintained blood indicators, while supplementation at 50% of the recommended levels showed benefits for bone breaking resistance, supporting previous reports of higher bioavailability of the organic sources.

Keywords: Protein trace minerals; Bone quality; Hematology; Serum biochemistry; Laying hens

67 Maternal supplementation with copper, manganese, and zinc glycinate improves eggshell mineralization in Japanese quail progeny

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The objective of this study was to evaluate the effects of breeder supplementation with glycinate sources of copper (Cu), manganese (Mn), and zinc (Zn), and the continuation of these supplementation programs in the offspring, on productive performance and egg quality of Japanese quail during the onset of lay. A total of 140 female offspring from Japanese quail breeders were distributed in a completely randomized design in a 3 × 2 + 1 factorial arrangement. Breeders had previously received one of three diets: CON, supplemented with 50 mg/kg Zn, 12 mg/kg Cu, and 60 mg/kg Mn from sulfates; Gly-50, with 50% of the sulfate levels used in CON plus 30 mg/kg Zn, 6 mg/kg Cu, and 31 mg/kg Mn as glycinate (E.C.O.Trace®, Biochem Zusatzstoffe Handels- und Produktionsges. mbH); or Gly-100, with 30 mg/kg Zn, 6 mg/kg Cu, and 31 mg/kg Mn as glycinate as the sole supplemental source of these minerals. Offspring were then fed either the inorganic diet or the corresponding glycinate program, resulting in seven treatments: CON-CON; CON-Gly50; CON-Gly100, Gly50-CON, Gly50-Gly50; Gly100-CON, Gly100-Gly100, with five replicates and four birds each. Diets were corn-soybean meal-based and formulated according to laying quail requirements.

Productive performance and egg quality traits were assessed over three consecutive 21d periods. Data were analyzed using SAS GLM and means were compared with the control by Dunnett's test at $p \leq 0.05$. Breeder supplementation with Cu, Mn, and Zn glycinates did not affect initial or final body weight, feed intake, laying rate, feed conversion ratio per egg mass or per dozen eggs, or age at first egg ($p > 0.05$). No effects were observed for Haugh unit, yolk index, specific gravity, egg weight, or eggshell thickness ($p > 0.05$). However, breeder mineral supplementation affected albumen percentage ($p < 0.05$), eggshell percentage ($p < 0.05$), and eggshell weight per surface area ($p < 0.05$). Offspring from Gly-50 and Gly-100 breeders had greater eggshell percentage than the control (8.23 and 8.21 vs. 7.92%, respectively; $p < 0.05$). Eggshell weight per surface area was also increased in offspring from glycinate-supplemented breeders. Continuation of glycinate supplementation in the offspring increased eggshell percentage ($p < 0.05$) and reduced albumen percentage ($p < 0.05$). No breeder $\times$ offspring supplementation interactions were observed ($p > 0.05$). In conclusion, breeder supplementation with glycinate sources of Cu, Mn, and Zn improved eggshell traits in Japanese quail offspring during early lay, without affecting productive performance. Continued glycinate supplementation in the offspring further increased eggshell percentage, supporting the relevance of trace mineral source for eggshell quality.

Keywords: progeny; organic trace minerals; eggshell characteristics; mineral bioavailability; egg production

68 Dose-Response Effects of *Trisetum flavescens* Extract on Growth Performance and Tibia Mineralization of Broiler Chickens Fed Reduced Dietary Vitamin D3

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Optimizing skeletal development during rapid growth is essential for maximizing broiler performance and production efficiency. *Trisetum flavescens* has been reported to contain vitamin D3 glycosides and biologically active vitamin D3 metabolites that may enhance calcium utilization and support bone mineralization. This study evaluated the dose-response effects of a phytogetic extract from *Trisetum flavescens* on growth performance and tibia mineralization of broilers fed reduced dietary vitamin D3 concentrations. A total of 2,000 one-day-old Cobb 500 male broilers were randomly allocated to eight dietary treatments with ten replicate pens of twenty-five birds each and reared to 42 days of age. Birds received diets containing either 2,000 or 1,000 IU/kg vitamin D3, or 1,000 IU/kg vitamin D3 supplemented with increasing concentrations (100–600 g/ton) of a standardized *Trisetum flavescens* product (Active D®, Phytobiotics Futterzusatzstoffe GmbH). Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and tibial ash, calcium, and phosphorus concentrations were measured. Data were analyzed using one-way ANOVA (SAS 9.4) followed by Tukey's test ($P \leq 0.05$). Dose-response relationships were evaluated using linear and quadratic regression analyses. Broilers fed 2,000 IU/kg vitamin D3 exhibited greater early BWG than those receiving 1,000 IU/kg ($P < 0.05$), confirming the greater vitamin D requirement during the initial phase of skeletal development. However, supplementation with *Trisetum flavescens* extract partially compensated for the lower dietary vitamin D3 concentration throughout the growing period. Birds receiving 100–300 g/ton consistently achieved BWG comparable to or greater than that of broilers fed 2,000 IU/kg vitamin D3 ($P < 0.05$), with the greatest responses observed between 28 and 42 days of age. Tibial ash, calcium, and phosphorus concentrations at 42 days

were also improved ($P < 0.05$), indicating enhanced bone mineralization. Quadratic regression analyses estimated optimal responses at approximately 244–302 g/ton, depending on the evaluated variable ($P < 0.05$). Feed conversion ratio at market age was unaffected, indicating that improved growth performance was achieved without compromising feed efficiency. These findings indicate that supplementation with *Trisetum flavescens* extract can partially compensate for reduced dietary vitamin D3 while enhancing growth performance and skeletal mineralization in broiler chickens. An inclusion rate of approximately 200–300 g/ton optimized productive and skeletal responses, providing a practical nutritional strategy for commercial broiler production.

Keywords: Broiler Chicken; *Trisetum flavescens*; Vitamin D₃ Metabolites; Bone mineralization; Growth Performance

69 Structural Equation Modeling of Performance and Eggshell Quality of Japanese Quails Supplemented with Different Sources and Levels of Vitamin D

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To evaluate the effects of different vitamin D sources and supplementation levels on the performance and eggshell quality of Japanese quails using structural equation modeling. A total of 1,200 Japanese quails were randomly allocated to 25 treatments, with six replicates, totaling 150 experimental units. The experiment was conducted in three phases: control, depletion, and repletion. The treatments included 1.25OH₃; 25OH₅ and 25OH₁₀; D3₁₀₀, D3₂₀₀, and D3₄₀₀; Panbonis®10 with or without NSP (Pan10_1, Pan10_2, Pan10_NSP_1, Pan10_NSP_2); Panbonis®20 with or without NSP (Pan20_0.5, Pan20_1, Pan20_NSP_0.5); Panbonis®20 water-soluble (WS) or fat-soluble (FS), with or without NSP (Pan20_WS_0.5, Pan20_WS_1, Pan20_WS_NSP_0.5, Pan20_FS_0.5, Pan20_FS_1, Pan20_FS_NSP_0.5); Solbone® with or without NSP (SB_0.2, SB_0.4, SB_NSP_0.4); and Cestrum diurnum with or without NSP (CD_1, CD_2, CD_NSP_1). The evaluated variables were feed intake, egg production, egg weight, egg mass, feed efficiency, shell weight, shell thickness, shell percentage, and bioavailability, considering shell percentage and shell weight as indicators of eggshell quality. Structural equation modeling (SEM) was applied to evaluate the latent variables performance (PER) and eggshell quality (ESQ). The model fit indices were satisfactory: Chi-square divided by degrees of freedom = 1.97; Comparative Fit Index = 0.99; Tucker-Lewis Index = 0.99; Bentler-Bonett Non-normed Fit Index = 0.99; and Root Mean Square Error of Approximation = 0.08 (95% Confidence Intervals: 0.05–0.11). Factor scores were analyzed using a general linear model. All relationships were significant ($P < 0.001$). Egg mass showed the highest effect on PER ($\beta = 0.96$), while eggshell weight ($\beta = 1.00$) and shell thickness ($\beta = 0.99$) showed the greatest effects on ESQ. The treatments 25OH₁₀, D3₄₀₀, Pan20_WS_1, CD_2, Pan20_1, and Pan20_FS_1 showed the highest estimated values for performance. Eggshell quality also increased among vitamin D sources, with 25OH₁₀ and D3₄₀₀ standing out. SEM enabled the integration of observed and latent variables, revealing distinct effects of vitamin D sources on performance and eggshell quality, with 25OH₁₀, D3₄₀₀, and Pan20_1 showing the strongest effects.

Keywords: Egg quality; Modeling; Performance; Quails; Vitamin

70 Supranutritional Phytase and Dietary Available

Phosphorus Improve Performance, Egg Quality, and Nutrient Digestibility in Laying Hens

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Optimizing dietary phosphorus (P) supply is critical for maximizing productivity and eggshell quality in modern laying hens during extended production cycles. The objective of this study was to investigate the effects of increasing dietary available phosphorus (avP) levels, with or without supranutritional phytase supplementation, on laying hen performance and egg quality. A total of 324 Bovans White laying hens from 26 to 57 weeks of age were assigned to a completely randomized 2 × 6 factorial arrangement consisting of two phytase levels (0 or 4,000 FYT/kg) and six dietary avP concentrations (0.09, 0.16, 0.23, 0.30, 0.37, and 0.44%), totaling 12 treatments with 27 replicates. Productive performance was evaluated during eight consecutive 28-day periods by measuring feed intake (FI), body weight gain (BWG), egg production, egg weight, and feed conversion ratio (FCR). Egg quality was assessed at the end of each period by determining eggshell, yolk, and albumen proportions, eggshell thickness, and shell breaking strength. Ileal digestibility of P, calcium (Ca), crude protein (CP), dry matter (DM), and gross energy (GE) was determined at the end of the trial. Data were analyzed using PROC MIXED and PROC GLM procedures of SAS 9.4. Linear and quadratic responses to dietary avP were evaluated by regression analysis, and treatment means were compared using Tukey's test ($P < 0.05$). Significant phytase × avP interactions were observed for BWG, FCR, egg production, and the ileal digestibility of CP, DM, and GE ($P < 0.05$). Ileal Ca digestibility was significantly affected only by avP levels ($P < 0.05$). Phytase supplementation increased BWG (+16.3%), egg production (+2.0%; +4 eggs), egg weight (+0.5 g), eggshell proportion (+0.19%), shell breaking strength (+0.17 kgf), shell thickness (+6 µm), and ileal P digestibility (+23.6%), while improving FCR ($P < 0.05$). In conclusion, phytase reduced the dietary avP concentration required to maintain productive performance from approximately 0.33–0.37% to 0.23–0.27%. Supranutritional phytase supplementation improved productive performance, eggshell quality, and P utilization in laying hens during the post-peak period, allowing a reduction in dietary avP without compromising productive responses.

Keywords: Laying hens; Egg quality; Available phosphorus; Phytase; Performance

71 Determination of Dietary Calcium Requirements for Egg Production and Egg Output in Laying Hens from 97 to 107 Weeks

72 Analyses of APV mean titers (GMT) and coefficient of variation (CV) in broiler breeder's flocks and their association with fertility

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This study evaluated whether infertility is associated with avian

of Age

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Calcium is the primary mineral in poultry diets, essential for eggshell formation and key metabolic functions. Genetic improvement has extended the productive life of commercial laying hens to up to 120 weeks, increasing the nutritional demands required to maintain eggshell quality and bone integrity. Because calcium metabolism is closely associated with phosphorus, imbalances in either mineral can impair their utilization and metabolism. Thus, the objective was to evaluate the dietary calcium intake requirements for egg production and egg output in aged laying hens at 97 and 107 weeks of age. Forty-nine laying hens of the Lohmann LSL Lineage, 97 weeks of age, were uniformly distributed according to body weight and egg production. The experimental design used was completely randomized (DIC), with seven treatments and seven replications, totaling 49 experimental units. Treatments consisted of seven calcium levels: 6.1, 12.4, 21.2, 22.7, 29.5, 36.8 and 43.9 g/kg. The variables analyzed were egg production (EP), feed intake (FI) and egg output (EO). The variables were subjected to orthogonal polynomial contrast analysis to assess linear and quadratic effects of dietary calcium levels. When a significant effect was detected ($P \leq 0.05$), regression analysis was performed using broken-line linear (BL) and broken-line quadratic (BLq) models. Additionally, the value corresponding to the first intercept of the quadratic curve in the model was calculated to estimate the optimal calcium intake. Statistical analyses and model parameter estimations were performed using SAS software, version 9.4. An average daily FI of 99.8 g per bird was observed during the experimental period. It was observed that calcium levels modified the responses of laying hens ($P < 0.05$). Broken-line linear (BLL) and broken-line quadratic (BLQ) models were fitted to describe the responses of egg production (EP) and egg output (EO) to dietary calcium intake. For EP, the fitted models were: BL: $y = 81.58 (\pm 1.5) - 30.90 (\pm 6.9) \times [1.75 (\pm 0.2) - x]$, $R^2 = 0.75$, BIC = 268.2; BLq: $y = 82.84 (\pm 1.8) - 6.93 (\pm 1.5) \times [2.82 (\pm 0.2) - x]^2$, $R^2 = 0.75$, BIC = 268.5. For EO, the fitted models were: BL: $y = 52.27 (\pm 1.1) - 19.30 (\pm 5.3) \times [1.8 (\pm 0.3) - x]$, $R^2 = 0.80$, BIC = 220.6; BLq: $y = 53.6 (\pm 1.6) - 4.3 (\pm 1.9) \times [3.0 (\pm 0.5) - x]^2$, $R^2 = 0.80$, BIC = 220.1. The estimated calcium concentration and intake corresponding to the first intersection point of the BLQ curve at the BLL plateau was 2.5 g/bird/day for both EP and EO. The estimated calcium requirement for long-lived laying hens between 97 and 107 weeks of age was 2.5 g/bird/day (25 g/kg diet) for egg production (82.7%) and egg output (52.9 g/bird per day).

Keywords: Egg production; Egg output; Laying hens; Mineral; Models

Student Competition Physiology and Reproduction

metapneumovirus (APV) titers in broiler breeder flocks. We used a database of serological titers for APV of 21 different broiler breeder flocks housed in the same region of Southeast Brazil, since rearing phase until eggs production stage, which were vaccinated for APV only during the rearing stage, with the same vaccine protocols. We performed unsupervised analyses on the average ELISA titers (GMT) and coefficient of variation (CV) for APV. Exploratory Factor Analysis (EFA) was applied to verify interrelation among all

variables. We also performed stepwise multiple linear regression to check the relation of fertility and the other variables, considering as a strong correlation R-values higher than 0.7 and $p < 0.05$. Finally, we applied a logistic regression for cause-and-effect analysis. Principal Component Analysis (PCA) was performed to reduce data dimensionality and identify the main patterns of variation among serological variables across the production cycle. As the sphericity test assumption was met ($p < 0.0001$), we proceeded with the multivariate analysis followed by PCA, in which we obtained three relevant components that explained 91.7% of the total data variation. Also, correlation matrix showed that fertility was negatively associated with flock age and with increased variability of antibody titers between 23-46 weeks, probably due to factors such as viral circulation or field exposure, considering that the flocks were relatively next to each other, or even due to poor vaccination managing procedures. The multiple regression test, using the stepwise variable selection methodology, showed that antibody titers, 5-26 weeks, were positively associated with fertility, which reinforces the importance of good vaccination schedules and procedures, whereas higher titers, from 35 weeks on, were negatively associated with fertility, which reinforce the importance of preventing the virus dissemination through a well established biosecurity program, as a barrier to new viral challenges. These findings suggest that APV serological dynamics vary according to flock age and may reflect different epidemiological and sanitary conditions influencing fertility in broiler breeders.

Keywords: Broiler breeders; Reproduction; Viral challenge; Avian Metapneumovirus

73 Embryonic Thermal Manipulation Induces Vascular Remodeling in Cobb Broiler Chickens

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Hypothesis or Objective: To evaluate the effects of embryonic thermal manipulation during incubation on the histomorphometry of the heart and aorta of Cobb® broiler chickens at 15 days of age. **Experimental Design:** Fertile Cobb® eggs were randomly assigned to three incubation treatments: control (37.5°C; T1), thermal manipulation at 38.5°C (T2), and thermal manipulation at 39.0°C (T3). Thermal stimulation was applied for 6 h daily from embryonic day 16 to 19. At 15 days post-hatch, eight birds per treatment were euthanized for cardiovascular evaluation. **Materials and Methods:** Heart and aortic tissues were collected, fixed in 10% buffered formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin. Histological images were obtained using a Zeiss Axio Scope A1 microscope, and morphometric measurements were performed with ImageJ software. **Statistical Analysis:** Data normality and homogeneity of variances were assessed using the Shapiro-Wilk and Levene tests, respectively. Parametric data were analyzed by one-way ANOVA followed by Tukey's post hoc test, whereas non-parametric variables were evaluated using the Kruskal-Wallis test followed by Dunn's multiple comparisons test. Statistical significance was established at $P < 0.05$. All analyses were performed using R software (version 4.6.0). **Results:** Embryonic thermal manipulation induced significant cardiovascular remodeling. Total aortic area increased in T2 (8.164 ± 0.058 mm) and T3 (5.421 ± 1.180 mm) compared with T1 (3.359 ± 0.083 mm). Likewise, aortic lumen area was greater in T2 (2.604 ± 0.024 mm) than in the control group (0.729 ± 0.013 mm). Tunica media thickness also increased in T2 (548.1 ± 62.8

µm) and T3 (528.3 ± 86.7 µm), indicating vascular remodeling. Cardiac morphometry revealed the largest left ventricular lumen area in T3 (25.91 ± 8.85 mm), whereas T2 exhibited the greatest left ventricular wall thickness (5.088 ± 1.255 mm), suggesting adaptive myocardial hypertrophy. Overall, moderate thermal manipulation (38.5°C) promoted the most balanced cardiovascular adaptations. **Conclusions:** Embryonic thermal manipulation altered cardiovascular development in broiler chickens by promoting structural remodeling of the aorta and heart. Moderate thermal stimulation (38.5°C) produced the most favorable adaptive responses, suggesting improved hemodynamic capacity and potential enhancement of post-hatch thermotolerance. These findings support embryonic thermal manipulation as a promising incubation strategy to improve physiological adaptation to heat stress in broiler chickens.

Keywords: Embryonic thermal manipulation; Broiler chickens; Cardiovascular remodeling; Histomorphometry; Thermotolerance

74 Embryonic thermal manipulation programs the post-hatch development of ventilatory and metabolic responses to acute thermal stress in broiler chickens.

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To determine whether embryonic thermal manipulation (TM) establishes a respiratory phenotype that emerges during post-hatch maturation by modifying ventilatory responses to acute heat stress. Fertile Cobb® broiler eggs were randomly assigned to three incubation treatments: control (37.5°C; T1), TM at 38.5°C (T2), and TM at 39.0°C (T3). Thermal stimulation was applied for 6 h daily from embryonic days 16 to 19. Respiratory function was evaluated by whole-body plethysmography at 1 and 15 days of age under thermoneutral conditions, acute heat stress (35°C), and recovery. Respiratory frequency (fR), tidal volume (VT), minute ventilation (VE), oxygen consumption (VO₂), and ventilatory equivalent (VE/VO₂) were measured. Age-related responses were evaluated within each treatment, whereas treatment effects were analyzed independently at each age. Data were analyzed using parametric or non-parametric tests according to normality and homogeneity assumptions, followed by appropriate post hoc comparisons. Statistical significance was declared at $P < 0.05$. Respiratory maturation resulted in significant increases in VO₂, VT, and VE from day 1 to day 15 across all treatments ($P < 0.05$). However, treatment-related differences were minimal at hatch and became evident only after post-hatch maturation. During acute heat stress at 15 days of age, birds incubated at 39.0°C exhibited lower fR and greater VT than controls while maintaining VE, indicating a shift toward a deeper and slower breathing pattern. VO₂ and VE/VO₂ remained similar among treatments, suggesting that TM modified ventilatory control rather than metabolic demand. These findings indicate that the respiratory phenotype established during embryogenesis becomes progressively expressed during post-hatch development. Embryonic TM promotes long-term respiratory programming in broiler chickens. The ventilatory phenotype induced during late embryogenesis was not fully expressed at hatch but became evident after respiratory maturation, particularly in birds incubated at 39.0°C, which adopted a deeper and slower breathing pattern during acute heat stress. These findings suggest improved ventilatory efficiency and support embryonic TM as a strategy to enhance physiological adaptation to heat stress.

Keywords: developmental programming; whole-body plethysmography; embryonic thermal manipulation; heat stress; respiratory physiology

75 Embryonic Thermal Manipulation Modulates Small Intestinal Morphology in Broiler Chickens

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Hypothesis or Objective: To evaluate the effects of embryonic thermal manipulation during incubation on the intestinal histomorphometry of the duodenum and jejunum of Cobb® broiler chickens at 15 days of age. **Experimental Design:** Fertile Cobb® eggs were randomly assigned to three incubation treatments: control (37.5°C; T1), thermal manipulation at 38.5°C (T2), and thermal manipulation at 39.0°C (T3). Thermal stimulation was applied for 6 h daily from embryonic day 16 to 19. At 15 days post-hatch, eight birds per treatment were euthanized for intestinal evaluation. **Materials and Methods:** Duodenal and jejunal samples were fixed in buffered formalin, routinely processed for histology, sectioned, and stained with hematoxylin and eosin. Histomorphometric analyses included villus height, crypt depth, villus-to-crypt ratio, muscular layer thickness, villus width, and enterocyte thickness. Data were analyzed using appropriate parametric or non-parametric statistical tests according to data distribution ($P < 0.05$). **Results:** Preliminary analyses indicated that embryonic thermal manipulation influenced the morphology of the proximal small intestine. Birds from the T2 group tended to present greater villus height and higher villus-to-crypt ratio in both the duodenum and jejunum, suggesting improved absorptive capacity and intestinal maturation. Crypt depth remained similar among treatments, whereas moderate increases in muscular layer thickness and enterocyte thickness were observed in thermally manipulated groups. The T3 treatment exhibited intermediate responses, with minor structural alterations compared with the control group. **Conclusions:** Embryonic thermal manipulation promoted morphologic adaptations in the proximal intestine, indicating that incubation temperature may influence post-hatch intestinal development and potentially enhance nutrient absorption during early growth.

Keywords: Heat stress; Thermal manipulation; Broiler chickens; Intestinal morphology

76 Effects of Embryonic Thermal Manipulation on Ileal Histomorphometry of Broiler Chickens

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Hypothesis or Objective: To investigate the effects of embryonic thermal manipulation during incubation on the histomorphological development of the ileum in Cobb® broiler chickens at 15 days of age. **Experimental Design:** Fertile Cobb® eggs were distributed into three incubation treatments: control (37.5°C; T1), thermal manipulation at 38.5°C (T2), and thermal manipulation at 39.0°C (T3).

Thermal stimulation was performed for 6 h daily between embryonic days 16 and 19. Intestinal samples were collected from eight birds per treatment at 15 days post-hatch. **Materials and Methods:** Ileal fragments were fixed in buffered formalin, processed using routine histological procedures, sectioned, and stained with hematoxylin and eosin. Histomorphometric evaluations included villus height, crypt depth, villus-to-crypt ratio, muscular layer thickness, villus width, and enterocyte thickness. Statistical analyses were performed according to data distribution, adopting a significance level of $P < 0.05$. **Results:** Preliminary findings suggest that embryonic thermal manipulation produced discrete morphological changes in the ileum. Birds incubated under thermal manipulation showed a tendency toward increased villus thickness and enterocyte thickness, while villus height and crypt depth remained comparable among treatments. No marked differences were observed in the villus-to-crypt ratio or muscular layer thickness, indicating preservation of ileal architecture regardless of incubation temperature. **Conclusions:** The ileum demonstrated a moderate morphological response to embryonic thermal manipulation, suggesting that incubation temperature may induce subtle intestinal adaptations without compromising tissue organization during the early post-hatch period.

Keywords: Heat stress; Thermal manipulation; Broiler chickens; Intestinal morphology

77 Eggshell quality and breeder hen age affect incubation performance and day-old chick quality in Ross 308 broilers

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The objective was to evaluate the influence of eggshell quality (thickness estimated by specific gravity) and breeder hen age on incubation variables and day-old chick quality. A completely randomized design in a 3x4 factorial arrangement was used: 3 breeder ages (young, 28 wk; mature, 42 wk; old, 65 wk) x 4 shell density classes (≤ 1.070 , 1.075, 1.080, > 1.080 ; shell thickness 0.29–0.40 mm), yielding 12 treatments. A total of 1,800 Ross 308 hatching eggs were classified by the specific gravity method. Prior the eggs were stored for 3 days (20°C, 65% RH) and then incubated in a single-stage Petersime incubator (37.5°C, 94% RH); eggs were transferred to the hatcher at 442 h (37.5°C, 70% RH), and hatch was monitored every 4 h from 474 to 506 h. Egg weight loss, hatchability, fertility, contamination, embryonic mortality, hatch window distribution and chick quality (Pasgar Score) were evaluated. After normality (Shapiro–Wilk) and homogeneity (Bartlett) tests, data were submitted to ANOVA and Tukey's test at 5% ($R v4.3.1$). A significant shell quality x breeder age interaction ($P < 0.05$) was found for hatchability: in young and mature breeders, eggs with density ≥ 1.075 reached $> 90\%$ hatchability, whereas density 1.070 reduced it to 73% and 77%, respectively, and eggs from old breeders had lower hatchability regardless of shell quality. Egg weight loss decreased as density increased (28-wk breeders: 14.5% at 1.070 vs 8.5% at 1.080). Old breeders showed higher infertility, and contamination interacted ($P < 0.05$), peaking (20%) in thin-shelled eggs (1.070) from young breeders. Regarding embryonic mortality, significant interactions were observed ($P < 0.05$): young breeders had high late mortality (22%) and old breeders had a uniform distribution (6% early,

12% mid, 10% late). Hatch peaked at 490–494 h for all groups, but higher-density shells (≥ 1.075) and young breeders yielded earlier, more concentrated hatch windows and greater chick uniformity. Chick quality was higher for thicker shells (≥ 1.075) regardless of breeder age, with old breeders showing greater variability, especially with low-density shells (< 1.070). Overall, high-density eggs (≥ 1.080) from young breeders reached 89% hatchability vs 78% for thin-shelled eggs from old breeders. In conclusion, eggshell quality is a key determinant of hatchability, hatch window uniformity and chick quality. Careful management of shell quality and breeder age can improve hatchery productive efficiency.

Keywords: breeder age; chick quality; eggshell quality; hatchability; specific gravity.

78 Interactive effects of flock age, oviposition, and eggshell translucency on hatchability and egg quality in broiler breeders.

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Eggshell quality variations, particularly translucency defects, are increasingly associated with reduced hatchability in broiler breeder operations. This study evaluated the effects of flock age, oviposition time, and eggshell translucency on hatchability in a commercial Cobb 500 flock. A total of 16,00 eggs were collected between three flock ages (34, 44, and 53 weeks) during two periods, morning and afternoon, and classified into three translucency levels (low, medium, high). The experimental design included 18 treatment combi-

nations (3 translucency levels $\times$ 2 oviposition times $\times$ 3 flock ages) to assess their effects on hatchability ($n=15,600$) and shell quality ($n=540$). For shell quality measurements, eggshell thickness, shell strength, and Haugh units were measured using an EggTester Ultimate instrument. Data were analyzed using the GLM and GLIMMIX procedure of SAS (V 9.4) and Tukey's HSD test was performed to separate means. Results show a significant three-way interaction among flock age, oviposition time, and translucency level was observed for shell thickness ($P = 0.0458$) and initial egg weight ($P = 0.0353$). The thickest shells (0.3704 mm) occurred in high- and medium-translucency eggs collected in the afternoon at 45 weeks, whereas the thinnest shells (0.3317 mm) were observed in low-translucency eggs at 35 weeks. Initial egg weight was highest in low-translucency morning eggs at 55 weeks (68.8 g) and lowest in high-translucency morning eggs at 35 weeks (57.8 g). Hatchability was not affected by the three-way interaction ($P = 0.0599$) but was affected by the two-way interaction between translucency and oviposition ($P = 0.0008$) where the lowest hatchability was observed on high translucent eggs from the morning (82%). In conclusion, this research emphasize the importance of considering collection time when evaluating eggshell translucency and its relationship with hatchability, additionally, highlights the multifactorial nature of eggshell formation through the effects of the interaction between age, oviposition and translucency on thickness and egg weight parameters. Understanding these relationships may help improve hatchery management strategies in broiler breeder operations.

Keywords: Translucency; Oviposition; Age ; Hatchability; Eggshell quality

Student Competition Microbiology and Food Safety

79 Can extracellular particles from different *Bacillus* spp. strains contribute to inhibitory and synergistic activities against *Salmonella* Heidelberg strains isolated from poultry?

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This study evaluated the inhibitory potential of extracellular particles (EPs) from *Bacillus* (*B. cereus*, *B. subtilis*, *B. licheniformis*) against *Salmonella* enterica subsp. enterica serovar Heidelberg (SH). In the context of animal protein production, SH represents a significant health and economic challenge, requiring innovative control strategies. The EPs were separated from the water-soluble non-particulate fraction of the cell-free supernatants using ultracentrifugation and ultrafiltration. Characterization included particle concentration, particle size distribution (NTA), protein content (BCA), and transmission electron microscopy (TEM). Antimicrobial activity was quantified by CFU counting, and metabolomic analysis was performed using LC-QTOF-MS. The PEs exhibited marked

heterogeneity, with diameters ranging from 142.3 to 367.4 nm and yields exceeding 10^{10} particles/mL. Electron microscopy confirmed the presence of vesicular and non-vesicular subpopulations. Functionally, dual combinations of PEs (BL+BS and BL+BC) exhibited synergistic inhibition of SH strain 13, achieving a growth reduction of up to 99%. All EP treatments reduced SH strain 18 by 99%, while inhibition of strain 06 was more limited (95.03%). Metabolomic analysis identified 61 metabolites, with 23 varying significantly, including antimicrobial and quorum-sensing compounds. These results demonstrate that EP-mediated inhibition of SH is strain-dependent and can be enhanced through synergistic interactions. In conclusion, probiotic *Bacillus* EPs represent a promising alternative for controlling pathogens in poultry health and food safety.

Keywords: Probiotic bacteria; Synergism; Antimicrobial; Pathogen inhibition; Extracellular particles.

80 Calcium oxychloride as an alternative to paraformaldehyde for fertile egg disinfection

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Microbial contamination of fertile eggs can compromise hatchability, chick quality, and food safety, making post-oviposition disinfection an essential biosecurity practice. Although paraformaldehyde is widely used for fertile egg disinfection, concerns regarding its carcinogenic potential, worker safety, and environmental impact have encouraged the search for safer alternatives. In this context,

this study evaluated the efficacy of liquid calcium oxychloride as an alternative disinfectant for fertile eggs. A total of 900 fertile eggs were collected from a commercial broiler breeder farm and allocated into two control groups and four treatment groups: C1 (floor eggs before disinfection); C2 (nest eggs before disinfection); T1 (floor eggs after paraformaldehyde fumigation); T2 (floor eggs after calcium oxychloride disinfection); T3 (nest eggs after paraformaldehyde fumigation); and T4 (nest eggs after calcium oxychloride disinfection). Eggshell samples were collected aseptically and pooled in groups of 10 eggs, resulting in 15 pools per treatment. Samples were transported under refrigeration (2–8°C) and analyzed for *Enterobacteriaceae*, aerobic mesophilic bacteria, molds, and yeasts. Data were analyzed using a generalized linear mixed model, one-way ANOVA, and the Kruskal-Wallis test, and the significance level was set at $P < 0.05$. The control groups exhibited high microbial contamination, with mean bacterial counts of 37,936.4 CFU/g for floor eggs (C1) and 36,063.3 CFU/g for nest eggs (C2). Following paraformaldehyde disinfection, counts were reduced to 3,026.7 CFU/g in floor eggs (T1) and 1,080.0 CFU/g in nest eggs (T3). Calcium oxychloride treatment resulted in even lower counts, reaching 690.0 CFU/g in floor eggs (T2) and 360.0 CFU/g in nest eggs (T4). Compared with the respective control groups, calcium oxychloride reduced bacterial counts by 98.2% in floor eggs and 99.0% in nest eggs. Median bacterial counts also demonstrated significant reductions ($P < 0.001$). For floor eggs, median counts decreased from 10,000 CFU/g (Q1–Q3: 10,000–100,000) in C1 to 325 CFU/g (100–10,000) in T1 and 100 CFU/g (100–100) in T2. Similarly, for nest eggs, median counts decreased from 10,000 CFU/g (5,350–100,000) in C2 to 125 CFU/g (100–400) in T3 and 100 CFU/g (100–100) in T4. Both disinfectants significantly reduced microbial contamination compared to the control groups. However, calcium oxychloride consistently achieved lower bacterial counts than paraformaldehyde, indicating greater disinfection efficiency. These findings indicate that calcium oxychloride is an effective and safer alternative to paraformaldehyde for fertile egg disinfection.

Keywords: egg quality; Enterobacteriaceae; broiler breeders; hatchery sanitation

81 ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF *Salmonella* spp. ISOLATES FROM CLINICAL CASES OF *Gallus gallus domesticus* IN GOIÁS STATE BETWEEN 2023 AND 2026

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Salmonella spp. are of global importance due to their ability to cause disease in both humans and animals. The infection caused by *Salmonella* spp. highlights the interface of rising antimicrobial resistance and reinforces the necessity of continuous monitoring. Thus, the evaluation of antimicrobial susceptibility profiles can give relevant information for surveillance and the execution of effective control measures. In this context, this study aimed to conduct a retrospective analysis of the antimicrobial susceptibility profiles of *Salmonella* spp. isolates obtained from commercial and non-commercial bird flocks in the state of Goiás, Brazil. To this end, data from antimicrobial susceptibility testing of *Salmonella* spp. isolates obtained from *Gallus gallus domesticus* submitted to the Avian Diseases Diagnostic Laboratory (SaniAves) of the Federal University of Goiás (UFG) between 2023 and 2026 were analyzed. The data obtained from the antibiograms were organized and

tabulated using absolute and relative frequencies. 38 cases were attended between 2023 and 2026, yielding 8 suggestive *Salmonella* spp. isolates, corresponding to 21.05% (8/38) of the cases, and antimicrobial susceptibility testing was performed on 100% (8/8) of the isolates. The susceptibility profile was established using 16 antimicrobials (doxycycline, chloramphenicol, enrofloxacin, ceftiofur, gentamicin, norfloxacin, ciprofloxacin, ampicillin, cefazolin, tetracycline, sulfamethoxazole-trimethoprim, amoxicillin, amoxicillin-clavulanic acid, sulfonamides, and ceftriaxone). 50% (4/8) of the isolates showed resistance to at least one drug, with the highest resistance to sulfonamides at 37.5% (3/8), followed by ampicillin at 25% (2/8) and tetracycline at 12.5% (1/8). In addition, 12.5% (1/8) of the isolates exhibited multiple resistance, characterized by concurrent resistance to more than 3 drugs, including doxycycline, ampicillin, cefazolin, and tetracycline. Among the drugs associated with this multidrug-resistant profile, ampicillin and tetracycline showed the highest rates of resistance in the study. The results show that resistance to sulfonamides reflects the indiscriminate use of antimicrobial agents in poultry production, favoring the selection of resistant *Salmonella* spp. strains, highlighting the risk of therapeutic failures with drugs routinely used in the sector, underscoring the need to adopt alternative methods to antimicrobials in poultry production.

Keywords: Antimicrobial resistance; Epidemiological surveillance; Poultry; Salmonellosis; Zoonosis

82 Early intestinal colonization in broiler chickens originating from floor eggs and from breeder hens at early and late production stages: effects on the cecal microbiota composition of broilers up to 28 days of age

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The intestinal microbiota plays a fundamental role in broiler physiology, influencing nutrient utilization, microbial metabolite production, epithelial barrier integrity, immune regulation, intestinal homeostasis, competitive exclusion, and resistance to pathogen colonization. In commercial production, microbial colonization begins immediately after hatch and is shaped by diet, management, environment, genetics, intestinal segment, breeder age, and egg origin. Because chicks are usually reared without direct contact with adult hens, the early post-hatch period represents a critical window for microbiota modulation. This study aimed to evaluate the effects of breeder age, egg origin, and early intestinal colonization by a competitive exclusion product (CE), applied by spray, on the cecal microbiota composition of broilers up to 28 days of age. A total of 768 one-day-old male chicks were distributed in a completely randomized 2 x 2 x 2 factorial design, considering breeder age, young or old; egg origin, nest or floor eggs; and CE application, with or without, totaling 8 treatments with 6 replicates of 16 birds. Microbiota was assessed by 16S rRNA gene sequencing, a culture-independent molecular technique that allows taxonomic identification and estimation of the relative abundance of bacterial communities. Data were analyzed by ANOVA. At 7 days, egg origin affected Clostridiaceae, with greater abundance in birds from nest eggs. CE application reduced Eggerthellaceae and increased Erysipelotrichaceae, demonstrating early modulation of specific bacterial families. Trends toward increased Lactobacillaceae and Veillonellaceae were also observed in CE-treated birds, suggesting stimulation of groups

associated with beneficial colonization and early fermentative dynamics. At 28 days, breeder age influenced Clostridiales Family XIII Incertae Sedis and Streptococcaceae, whereas egg origin affected Streptococcaceae, with greater abundance in birds from floor eggs. The CE effect was dependent on egg origin: in birds from floor eggs, CE increased Lactobacillaceae and reduced Oscillospiraceae; in birds from nest eggs, it reduced Eggerthellaceae. These findings demonstrate that CE acted selectively and directionally on the cecal microbiota, favoring bacterial groups relevant to early colonization and modulating microbial succession during rearing. The response was more evident according to egg origin, reinforcing the potential of CE as a strategic tool to direct intestinal microbiota under greater initial microbial pressure.

Keywords: 16S rRNA gene sequencing; Bacterial communities; Gut health; Modulating microbial succession

83 Effects of probiotic *Bacillus* strains on the metabolomic profile of the cecal microbiota of broiler chickens reared under sanitary challenge conditions

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Probiotics can modulate microbial composition and activity, influencing bioactive metabolites available to the host during dysbiosis caused by health challenges. Microbial metabolites act as signaling molecules linking microbial activity with host immune and metabolic functions and may affect poultry health, disease resistance, and productivity by modulating intestinal barrier integrity, energy use, oxidative stress resilience, and immune cell differentiation. This study evaluated the effects of probiotic *Bacillus* spp. strains on the metabolomic profile of the cecal microbiota of broiler chickens subjected to a sanitary challenge. A total of 387 male chicks were distributed in a randomized design with four treatments, nine replicates, and 43 birds per replicate. Treatments consisted of an unchallenged control group (UC), a challenged control group (CC), and two challenged groups supplemented with probiotics at 500 or 750 g/ton, named C500 and C750. The strains were *Bacillus subtilis* and *Bacillus velezensis*. Challenged groups were housed on reused and wet poultry litter. At 7 and 21 days, birds received an inoculum prepared from litter obtained from a poultry house with dysbiosis history. At 42 days, six birds per treatment were euthanized, and cecal content was collected for metabolomic evaluation. Samples were analyzed by TD-NMR using an SLK-IF-1399 spectrometer, and data were analyzed with MetaboAnalyst 4.0. The variable importance in projection (VIP) plot ranked metabolites according to their importance in discriminating groups. Thirty-eight metabolites were quantified in the ¹H-NMR spectra, including amino acids, small peptides, amino acid derivatives, organic acids, nucleic acids, sugars, vitamins, and cellular antioxidants. Amino acids and derivatives were the most important metabolites for discriminating among UC, CC, C500, and C750, with higher VIP values ($P < 0.05$) for CC. In dysbiosis, putrefactive processes produce branched-chain fatty acids, ammonia, hydrogen sulfide, and amines, increasing intestinal pH, damaging the gut mucosal barrier, and impairing performance. After indicating that metabolite profiles differed among groups, pairwise comparisons using partial least squares-discriminant analysis identified specific differences. L-lactic acid differentiated CC from C500 ($P < 0.05$), succinate differentiated CC from C750 ($P < 0.05$), L-asparagine differentiated UC from C500 ($P < 0.05$), and 2-hydroxybutyric acid differentiated UC from C750 ($P < 0.05$). Isopropyl alcohol

was the main metabolite discriminating between C500 and C750 ($P < 0.05$). These findings indicate that probiotics modulate metabolite production and mitigate the effects of dysbiosis on the intestinal environment of broiler chickens.

Keywords: microbiome; metabolites; dysbiosis; amino acids

84 Characterization of *Salmonella* spp. Isolates from Feed Mills: Survival Following Thermal Processing, Biofilm Formation, and Antimicrobial Resistance.

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The persistence of *Salmonella* spp. in feed mills represents a significant challenge to poultry production biosecurity, as persistent isolates can survive in the industrial environment and contribute to the dissemination of the pathogen throughout the production chain. This study evaluated the survival of *Salmonella* spp. isolates exposed, under experimental conditions, to temperatures employed in industrial pelleting and expander processes and characterized them regarding biofilm formation and antimicrobial resistance profiles. Twenty-nine field isolates were evaluated under a control condition (CONT), followed by sequential exposure to conditions equivalent to pelleting (T82°; 82 °C for 90 s) and expander processing (T102°; 102 °C for 5 s). After each treatment, bacterial quantification (CFU/mL) was performed. Biofilm formation was assessed using the crystal violet assay, with absorbance measured at 630 nm, and antimicrobial susceptibility was determined by the disk diffusion method. Survival probability was estimated using Firth's penalized logistic regression, whereas residual bacterial load (log₁₀ CFU/mL) was analyzed using a marginal linear model for repeated measures, adopting a significance level of 5%. Thermal processing significantly reduced isolate survival ($P < 0.001$), with positivity decreasing from 100% in the control group to 29.9% after T82° and 9.2% after T102°. Among the surviving isolates, an average reduction of more than 8 log₁₀ CFU/mL was observed after T82°, with no significant additional reduction after T102°, although some isolates remained viable after complete thermal processing. Of the isolates evaluated, 10.34% were classified as strong biofilm producers, 72.41% as moderate producers, and 17.24% as weak producers. Among the surviving isolates, strong biofilm producers exhibited significantly higher residual bacterial loads than the other groups. Three isolates resistant to cefotaxime (CTX) and one resistant to fosfomicin (FOS) were identified, both critically important antimicrobials for human medicine. In addition, one multidrug-resistant (MDR) isolate was identified, exhibiting simultaneous resistance to cefotaxime (CTX), cefoxitin (CFO), fosfomicin (FOS), tetracycline (TET), sulfamethoxazole-trimethoprim (SUT), and gentamicin (GEN). These findings demonstrate that feed mills represent a critical control point in the poultry production chain and that, although thermal processing significantly reduces the survival of *Salmonella* spp., some environmental isolates remain viable after complete thermal processing, reinforcing the need for integrated strategies to prevent the introduction and dissemination of this pathogen in poultry farms.

Keywords: Feed mill; Thermal processing; Biofilm; Multidrug resistance; Poultry biosecurity

85 Biofilm Formation and Antimicrobial Resistance Profile of *Enterococcus* spp. Isolated from *Alphitobius diaperinus* and Broilers with Spondylitis

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Enterococcal spondylitis is an important cause of locomotor disorders and significant economic losses in broiler production worldwide, with *Enterococcus* spp. recognized as the main agent involved in vertebral lesions. In addition to infected birds, the lesser mealworm (*Alphitobius diaperinus*) acts as a potential reservoir and vector of pathogenic microorganisms within poultry houses. Biofilm formation and antimicrobial resistance (AMR) favor bacterial persistence, challenging disease control. This study evaluated the biofilm-forming ability and AMR profile of *Enterococcus* spp. isolates recovered from *A. diaperinus* and vertebral lesions of broilers with spondylitis. From seven broiler houses, lesser mealworm samples (1 g vortexed for 30 s in 10 mL of 1% buffered peptone water, incubated at 37°C/24h) and vertebral samples (swabs and tissues incubated in BHI broth supplemented with 1% kanamycin) were collected. Subsequently, samples were streaked onto Enterococcosel agar (37°C/48h) and identified biochemically. For biofilm analysis, isolates (n=17) grown in TSB (adjusted to 0.5 McFarland) were in-

cubated in 96-well polystyrene plates (37°C/24h), fixed with 99% methanol, stained with 1% crystal violet, solubilized with 36% glacial acetic acid, and read at 630 nm (positive controls: *E. coli* EAEC ATCC 042 and *Enterococcus faecalis* ATCC 29212). Susceptibility was tested by disk diffusion against ampicillin (AMP, 10 µg), penicillin (PEN, 10 µg), erythromycin (ERI, 15 µg), tetracycline (TET, 30 µg), ciprofloxacin (CIP, 5 µg), florfenicol (FLF, 30 µg), vancomycin (VAN, 30 µg), linezolid (LNZ, 30 µg), and fosfomycin (FOS, 200 µg). Spearman's correlation test assessed the association between biofilm formation and AMR (p<0.05). Seventeen isolates were obtained (6 from insects, 11 from lesions). For AMR, 94.12% (16/17) of the isolates were resistant to erythromycin, 76.47% (13/17) to fosfomycin, 70.59% (12/17) to tetracycline, 58.82% (10/17) to ciprofloxacin, and 5.88% (1/17) to linezolid. Eleven isolates (64.71%) were classified as multidrug-resistant (MDR). Regarding biofilm-forming ability, 41.18% (7/17) were strong, 52.94% (9/17) moderate, and 5.88% (1/17) weak producers. No significant correlation was found between AMR and biofilm formation. In conclusion, all isolates formed biofilms, mostly moderate and strong, promoting bacterial persistence in poultry environments. The high resistance to erythromycin, fosfomycin, tetracycline, and the high frequency of MDR isolates represent major challenges for effective disease control in poultry production.

Keywords: Poultry production; Epidemiology; Bacterial persistence; Lesser mealworm; vertebral lesions

Student Competition Genetics and Molecular Biology

86 Computational Identification of Natural Inhibitors Targeting Fiber Proteins of FAdV-1 and FAdV-4 Through Integrated Virtual Screening and Molecular Dynamics Simulations.

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Fowl adenoviruses (FAdVs) represent a major threat to poultry health, with serotypes FAdV-1 and FAdV-4 causing adenoviral gizzard erosion (AGE) and hepatitis-hydropericardium syndrome (HHS), respectively. A wide variety of afflicted birds, including chicken, pigeon, and psittacine species, have been reported to carry aviadenoviruses. The disease is highly contagious and spreads rapidly between flocks and farms through vertical and horizontal transmission. In this study, we implemented a multi-stage computational drug-discovery pipeline to identify natural inhibitors of the viral fiber proteins for both FAdV-1 and FAdV-4. A curated library of 7523 natural compounds from the African Natural Products Database (ANPDB) and the South African Natural Compounds Database (SANCDB) was subjected to pharmacophore-based filtering, molecular docking, ADMET prediction, and 500-ns molecular dynamics simulations against four structural targets: Fiber-1 and Fiber-2 of FAdV-4, and the Short and Long Fibers of FAdV-1. Three ligands ANPDB_6449 (-10.3 kcal/mol), ANPDB_2908 (-10.2 and -10.0 kcal/mol), and SANCDB_254 (-9.2 kcal/mol) consistently emerged as strong candidates across the entire computational workflow. While ANPDB_2908 demonstrated notable multi-target capability by binding to fiber proteins from both FAdV-1 and FAdV-4, ANPDB_6449 and SANCDB_254 exhibited strong serotype-specific potential, supported by stable interaction profiles and favorable drug-likeness char-

acteristics. Together, these compounds highlight promising natural scaffolds for the development of targeted antiviral interventions against pathogenic FAdV serotypes.

Keywords: Fowl adenovirus ; Drug design; ANPDB and SANCDB Databases; Protein engineering; Molecular dynamic Simulation

87 Early molecular detection of avian reovirus in the digital flexor tendon and synovial fluid of commercial broiler chicks from young and old breeder flocks

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Avian reovirus (ARV) is one of the major causes of viral arthritis and tenosynovitis in broiler chickens, resulting in significant economic losses to the poultry industry. Although ARV has been extensively investigated, limited information is available regarding the earliest detection of the virus in the digital flexor tendon and synovial fluid of commercial broiler chicks. This study investigated the temporal pattern of ARV molecular detection in these tissues in chicks originating from young (26-week-old) and old (51-week-old) breeder flocks that were vaccinated with a live attenuated S1133-based vaccine and had a previous history of arthritis/tenosynovitis in their progeny. Chicks were reared separately in an experimental poultry facility under conditions that reproduced commercial broiler production during the first 28 days of life. At 1, 7, 14, 21, and 28 days of age, 12 randomly selected birds from each breeder-age group were euthanized by cervical dislocation, and digital flexor tendon and synovial fluid samples were collected as four pools of three birds and

analyzed by semi-nested RT-PCR targeting the conserved σ NS-encoding S4 gene. Hatchery residue samples from the corresponding breeder flocks were also tested for ARV RNA. No clinically evident enlargement of the tibiotarsal joint was observed during the study period. A sampling point was considered ARV-positive when at least one tendon or synovial fluid pool tested positive. ARV RNA was not detected in samples collected from one-day-old chicks. The earliest ARV RNA detection occurred at 7 days of age in progeny from older breeder flocks, whereas progeny from younger breeders first tested positive at 14 days of age. ARV RNA remained detectable from 7 to 28 days of age in progeny from older breeders and from 14 to 28 days in progeny from younger breeders. Hatchery residue samples from older breeder flocks were ARV RNA-positive, whereas those from younger breeder flocks were negative. The positive hatchery

residues also support the possibility of hatchery-associated or early post-hatch exposure. These findings indicate very early ARV exposure or infection, but do not allow discrimination between vertical transmission, or very early horizontal exposure. ARV was detected despite the absence of clinical signs commonly associated with ARV infection, such as lameness and tibiotarsal joint swelling, supporting the use of molecular surveillance for the early detection of ARV infection in commercial broiler flocks. Further σ C-encoding S1 gene sequencing will enable genotypic characterization of the detected ARVs, providing additional insights into the molecular epidemiology of ARV infections.

Keywords: Arthritis; ARV molecular detection; Early post-hatch exposure; Hatchery residue samples; Semi-nested RT-PCR

Genetics & Molecular Biology

88 Validation of a Molecular Strategy for *Salmonella* spp. Serovar Identification in the Poultry Production Chain

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Salmonella enterica is a Gram-negative, facultative intracellular bacteria and the most commonly specie associated in cases of salmonellosis in humans and animals. In Brazil, poultry production is frequently affected by *S. enterica* serovars Gallinarum, Pullorum, Typhimurium, Heidelberg, Enteritidis and Infantis. Previous studies have reported the occurrence of *S. enterica* in poultry ranging from 2.5-50.77%. For specific serovars, reported prevalences range from 8.6% to 28.75% for *S. Enteritidis*, 3.8% for *S. Typhimurium* and 3.3% to 72.4% for *S. Heidelberg*. The present study aimed to detect and isolate *S. enterica* and characterize selected serovars due to the challenges faced by regional producers, who are constantly affected by *S. enterica* contamination in aviary facilities and slaughterhouses. In this study, *Salmonella enterica* was isolated from aviaries, transport, and intestinal tissue collected during the slaughter using the standard technique ISO 6579-1 and conventional PCR assay was performed targeting the *invA* gene for confirmation. Positive sam-

ples for the *invA* gene were submitted to previously published PCR protocols to standardize and detect several *S. enterica* serovars, among them: *S. Typhimurium*; *S. Enteritidis*; *S. Heidelberg*; *S. Infantis*; *S. Minnesota*; *S. Agona*; *S. Mbandaka*; *S. Gallinarum* and *S. Pullorum*. A total of 1068 samples were collected from aviaries in the western mesoregion of Paraná; 38.29% (409/1068) samples were positive for the *invA* gene. Among the 409 *invA* positive samples, 118 (28.85%) were identified as *S. Typhimurium* (also *S. Derby*, *S. Goldcoast* or *S. Rissen*)[RS1], 14 (3.42%) as *S. Enteritidis*, and 33 (8.06%) as *S. Heidelberg*. Nonspecific amplifications were observed for *S. Enteritidis*, *S. Minnesota*, *S. Infantis* and also with *Escherichia coli* and *Clostridium* spp. which were intended to be used as negative control. The positive samples to the *invA* gene were sequenced, aligned and submitted to haplotype analysis to cluster obtained sequences according to their serovars and to enable the identification of *S. enterica* samples that were unidentified in serovar-specific PCR assays. The present study demonstrated a high occurrence of *S. enterica* in poultry production systems in the western mesoregion of Paraná, with 65.5% of positive samples to *invA* gene. Furthermore, several previously published primer pairs failed to specifically amplify their target serovars, highlighting the limitations of some PCR assays and emphasizing the importance of sequencing and proper primer design for accurate molecular identification.

Keywords: Salmonellosis; *invA*; Poultry ; foodborne; Paraná

Physiology and Reproduction

89 Effects of Embryonic Thermal Manipulation on the Histomorphology of the Bursa of Fabricius in Broiler Chickens

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Hypothesis or Objective: To evaluate the effects of embryonic thermal manipulation during incubation on the histopathological characteristics and histomorphometry of the Bursa of Fabricius in Cobb® broiler chickens at 15 days of age. **Experimental Design:** Fertile Cobb® eggs were randomly assigned to three incubation

treatments: control (37.5°C; T1), thermal manipulation at 38.5°C (T2), and thermal manipulation at 39.0°C (T3). Thermal stimulation was applied for 6 h daily from embryonic day 16 to 19. At 15 days post-hatch, eight birds per treatment were euthanized for bursal evaluation. **Materials and Methods:** The Bursa of Fabricius was collected, fixed in buffered formalin, routinely processed for histology, sectioned, and stained with hematoxylin and eosin. Histopathological examination included assessment of follicular organization, corticomedullary delimitation, lymphoid cellularity, epithelial integrity, and the presence of inflammatory or degenerative lesions. Histomorphometric analyses included follicular area, cortical area, medullary area, cortical-to-medullary ratio, follicular diameter, and lymphocyte density. Statistical analyses were performed according to data distribution, adopting a significance level of $P < 0.05$.

Results: Preliminary observations suggest that embryonic thermal manipulation did not induce histopathological lesions in the Bursa of Fabricius. All experimental groups maintained preserved follicular organization and corticomedullary architecture. However, birds subjected to thermal manipulation showed subtle morphometric differences, including a tendency toward increased follicular area and cortical development, particularly in the T2 group, whereas the T3 group presented intermediate values. Lymphoid cellularity remained preserved across treatments. Conclusions: Embryonic thermal

manipulation promoted discrete structural adaptations in the Bursa of Fabricius without evidence of tissue damage, suggesting that controlled thermal stimulation during incubation may influence lymphoid organ development while preserving normal bursal architecture during early post-hatch growth.

Keywords: Heat stress; Thermal manipulation; Broiler chickens; Bursa

Student Competition Metabolism and Nutrition: Enzymes

90 Standardized Ileal Amino Acid Digestibility Responses to Exogenous Protease Supplementation in Reduced-Protein Broiler Diets

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This study evaluated the effects of exogenous protease supplementation in broilers fed reduced crude protein and digestible lysine diets on the standardized ileal digestibility (SID) of amino acids. A completely randomized design was used with five treatments: a positive control (PC) without enzyme supplementation, a negative control with a 5% reduction in crude protein and digestible lysine while maintaining the ideal amino acid ratio (NC), NC supplemented with 250 or 500 g Mt⁻¹ of protease, and a nitrogen-free diet (NFD) for estimation of basal endogenous amino acid losses. A total of 200 broilers were distributed into 50 experimental units with 10 replicates of 4 birds each. Diets were based on corn and soybean meal and contained 5% raw soybeans. Celite was included at 1% as an indigestible marker. The exogenous protease used was CIBENZA® EP150 enzyme feed additive. Standardized ileal digestibility coefficients were determined using the NFD method. Ileal digesta were collected at 24 d of age. Ileal digesta and diet samples were processed and analyzed for dry matter, crude protein, acid-insoluble ash and amino acid profile. Data were evaluated for residual normality using the Shapiro-Wilk test and screened for outliers using the UNIVARIATE procedure. Subsequently, data were analyzed by one-way ANOVA, and treatment means were compared using Tukey's test at $P \leq 0.05$. Dietary treatments affected SID coefficients of essential and nonessential amino acids ($P < 0.05$). The NC diet resulted in greater SID values than the PC diet for all amino acids evaluated. The PC diet showed lower total SID of amino acids than the NC diet (81.94 vs. 88.86%, respectively; $P = 0.0002$). Protease supplementation resulted in intermediate values (85.19 and 82.49% for 250 and 500 g Mt⁻¹, respectively), resulting in digestibility coefficients comparable to those observed in the PC diet but remaining lower than those observed in the NC treatment. The 250 g Mt⁻¹ protease inclusion improved the SID of arginine, threonine, leucine, aspartic acid, and proline compared with the PC diet. In conclusion, reducing dietary crude protein and digestible lysine increased standardized ileal digestibility coefficients of amino acids, possibly reflecting lower endogenous amino acid losses and physiological adapta-

tions associated with protein reduction. Protease supplementation resulted in digestibility coefficients comparable to those observed in the positive control diet but lower than those obtained in the reduced-protein diet without enzyme supplementation, with the most consistent responses observed at 250 g Mt⁻¹ inclusion level.

Keywords: endogenous amino acid losses; enzyme supplementation; poultry nutrition; reduced-protein diets; serine protease

91 Effects of xylanase supplementation on productive performance of laying hens: a systematic review and meta-analysis.

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The present study aimed to evaluate the effect size of xylanase supplementation on the productive performance of laying hens through a systematic review and meta-analysis. A comprehensive search across multiple databases identified 2,259 records, of which 12 met the eligibility criteria and were included in the analysis. The evaluated outcomes comprised egg production, egg mass, feed conversion ratio (FCR), and feed intake (FI), comparing diets supplemented or not with xylanase. Xylanase supplementation significantly increased egg production by 0.99% ($P = 0.001$). No significant effects were observed for the other productive variables ($P \geq 0.05$). Meta-regression and subgroup analyses indicated that the effect of xylanase on egg production was influenced by wheat inclusion, being significant in diets without wheat. Egg mass increased by approximately 1.1 g/day when xylanase was used in combination with other enzymes. Feed conversion ratio improved by 0.018 units in hens aged between 25 and 60 weeks, whereas an increase of 0.046 units was observed in diets supplemented with xylanase associated with other enzymes. In contrast, feed intake was not affected by any of the evaluated moderators. Overall, although xylanase supplementation shows positive effects on egg production, productive responses are highly heterogeneous among studies, being mainly influenced by hen age, enzyme combination, dietary metabolizable energy level, and wheat inclusion.

Keywords: Productive ; Animal nutrition; performance; Commercial layers; Enzymes

92 Influence of β -mannanase on metabolizable energy values of corn-soybean meal diets for broiler chickens

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β -mannans are antinutritional polysaccharides that impair nutrient utilization in broilers, whereas exogenous β -mannanase hydrolyzes these compounds and may improve dietary energy availability. Therefore, this study evaluated the effects of β -mannanase inclusion on the metabolizable energy values of corn-soybean meal diets for broiler chickens from 1 to 42 d. Six metabolism trials were conducted using the total excreta collection method. Birds were assigned to a completely randomized design with two corn-soybean meal-based diets: a control diet and the same diet with β -mannanase (Hemicell™ HT, Elanco Animal Health, Greenfield, IN, USA) included at 400 g ton⁻¹, providing a minimum activity of 160×10^6 units of endo-1,4- β -mannanase (EC 3.2.1.78) kg⁻¹, produced by *Paenibacillus lentus*. Each treatment consisted of ten replicates with seven birds per replicate at the beginning of the study, and the number of birds per replicate was adjusted according to the growth phase. Each metabolism trial consisted of a 3-d adaptation period followed by a 4-d total excreta collection period and was conducted with a different group of birds. At the end of each collection period, total feed intake and excreta output were recorded. Feed and excreta samples were analyzed for dry matter, crude protein, and gross energy, and apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AMEn), and their respective metabolizability coefficients (CAME and CAMEn) were calculated. Residuals were tested for normality using the Shapiro-Wilk test. Data were analyzed by ANOVA using the GLM procedure of SAS, and differences were considered significant at $P < 0.05$. Compared with the control diet, β -mannanase inclusion increased AME by 96, 80, 66, 84, and 84 kcal kg⁻¹ during the periods of 1–7, 8–14, 15–21, 22–28, and 29–35 d, respectively ($P < 0.05$). Likewise, AMEn was increased by 81, 61, 75, and 80 kcal kg⁻¹ during the periods of 8–14, 15–21, 22–28, and 29–35 d, respectively ($P < 0.05$). β -mannanase inclusion also increased CAME during the first week (74.14 vs 72.19%; $P = 0.046$), whereas CAMEn was not affected throughout the experimental period ($P > 0.05$). No effects of β -mannanase were observed on the evaluated energy variables during the period from 36 to 42 d ($P > 0.05$). β -Mannanase inclusion increased the metabolizable energy value of corn-soybean meal diets by an average of 82 and 74 kcal kg⁻¹ for AME and AMEn, respectively, from 1 to 35 d, demonstrating its potential to improve dietary energy utilization in broilers.

Keywords: β -mannans; exogenous enzymes; metabolism trial; nutrient utilization; non-starch polysaccharides

93 Growth performance and apparent metabolizable energy of broilers fed two grain sorghum varieties with or without exogenous amylase

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Grain sorghum (*Sorghum bicolor*) is a lower-cost alternative energy source to corn for broiler chickens. However, starch utilization may be limited by the presence of kafirins and the high vitreousness of the grain, characteristics that hinder the access of digestive enzymes to starch. In this context, supplementation with exogenous amylase may help improve starch utilization by the birds. The objective was to evaluate the effects of two varieties of tannin-free red grain sorghum (A and B) and exogenous amylase supplementation on growth performance and apparent metabolizable digestibility of nutrients in broiler chickens aged 1 to 21 days. A total of 256 male Ross 308 AP95 broiler chicks, one day old, were arranged in a completely randomized design in a 2 x 2 factorial arrangement: 2 sorghum types x 2 amylase levels (0 and 90 g/t), with 4 treatments, 8 replicates, and 8 birds per replicate. The diets, based on sorghum and soybean meal, were provided in mash form. Sorghum A had a lower kafirin content (2.68 vs. 3.42 mg; 44.0 vs. 46.1% of grain protein) but greater hardness (6.85 vs. 5.68 kgf, $P < 0.0001$) than sorghum B. Feed intake (FI), weight gain (WG), and feed conversion ratio (FCR) were measured. Excreta were collected on days 18 through 20 (acid-insoluble ash marker) to determine the apparent metabolizable digestibility coefficients of dry matter (DM) and crude protein (CP), as well as apparent metabolizable energy (AME). Grain hardness was compared by the Mann-Whitney test, and performance and metabolizable digestibility data by two-way ANOVA, with means separated by Tukey's test (significant when $P < 0.05$). As main effects, sorghum and amylase did not affect FI, WG, or FCR ($P > 0.05$). However, a sorghum x amylase interaction was observed for FCR ($P = 0.031$): amylase improved FCR in sorghum A (1.287 vs. 1.327), but not in sorghum B. The sorghum variety did not affect metabolizable digestibility ($P > 0.05$). Amylase reduced the apparent metabolizable digestibility coefficients of DM (from 69% to 67%, $P = 0.015$) and CP (from 70% to 68%, $P = 0.031$), while AME remained unchanged (3,211 vs. 3,203 kcal/kg, $P = 0.745$). These results indicate that amylase improves the feed efficiency of broiler chickens fed grain sorghum with low kafirin content, although this response was not reflected in higher metabolizable digestibility or AME.

Keywords: Amylase; Broiler; Feed conversion; Grain sorghum; Metabolizable energy

Student Competition Animal Well-Being and Behavior

94 Feed form affects production performance and injurious pecking in turkey toms

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Injurious pecking is a multifactorial welfare issue in turkey toms, contributing to injuries, culling, and mortality. Previous research in

laying hens suggests nutritional interventions (e.g., fiber inclusion and feed form) mitigate this behavior. Our objective was to evaluate the effects of fiber and feed form on production performance and pecking behaviors in turkey toms. Seven hundred forty male poult were assigned one of two dietary fiber levels in a crumble feed form: High fiber (3% CF) and Low fiber (2% CF; 16 pens/treatment, 22 birds/pen). At 5 wk, feed form changed from crumbles to either fines or pellets to create four treatments ($n=8$ pens/treatment): High fiber, pelleted (HFP; 4% CF), High fiber, fines (HFF; 4% CF), Low

fiber, pelleted (LFP; 2% CF), and Low fiber, fines (LFF; 2% CF). In both HF diets, CF increased by 1% every 3 wk to achieve 8% CF at 17 wk. Body weight (BW), body weight gain (BWG), feed conversion ratio (FCR), and feed intake (FI) were recorded at 4, 9, 12, 15, and 18 wk, as well as cumulative mortality. Eight focal birds/pen were video recorded for 30 min at 4, 9, 15, and 18 wk to code pecking frequency on the back, wing, and tail (BWT) and head/neck (HN). Data were analyzed in R software with linear models and generalized linear mixed models to assess the effect of fiber, feed form, and their interaction. Feed form had a main effect on all production parameters. Fines-fed toms displayed lower BW at 12 ($p=0.004$), 15 ($p=0.002$), and 18 wk ($p<0.001$); lower BWG from 9 to 12 ($p<0.001$), 12 to 15 ($p=0.039$), and 15 to 18 wk ($p=0.004$); and higher FI from 4 to 9 ($p<0.001$), 9 to 12 ($p<0.001$), 12 to 15 ($p<0.001$), and 15 to 18 wk ($p<0.001$) than pellet-fed toms. Correspondingly, fines-fed toms displayed a higher FCR than pellet-fed toms at 9 ($p<0.001$), 12 ($p<0.001$), 15 ($p<0.001$), and 18 wk ($p<0.001$). Fiber inclusion did not affect these production metrics. The LFF toms experienced a higher cumulative mortality than LFP toms ($p=0.028$), while HFF and HFP groups did not differ ($p>0.05$). Feed form also had a main effect on BWT pecking. Fines-fed toms performed nearly three times as many bouts of BWT pecking than pellet-fed toms throughout the trial ($p<0.001$). The HFF toms performing more HN pecking than the other three treatments ($p=0.035$). Taken together, fines-fed toms were unable to effectively consume their feed, resulting in impaired production performance and behavioral frustration. The BWT pecking may be a strategy to supplement their diets with feathers, while the HN pecking could be from increased competition at the feeder. Fines feed represents impaired pellet quality, indicating that pellet quality is a critical management tool for mitigating injurious pecking in turkey toms.

Keywords: injurious pecking; feed form; turkey; body weight; production performance

95 Environmental enrichment improves welfare-related behavior without compromising growth performance of Ross 308 broilers

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Intensive broiler production faces growing welfare concerns, as high stocking densities and barren environments limit natural behavior. Environmental enrichment (EE) may help, provided it does not penalize productivity. The objective was to evaluate the effect of EE on the behavior and growth performance of Ross 308 broilers reared in a commercial intensive system. A total of 320 mixed-sex broilers were reared from 1 to 34 days of age in 8 pens (2.5 m², 40 birds/pen, 16 birds/m²) in a commercial house, assigned to 2 treatments with 4 replicates each: control (C) and EE. From day 7, EE pens received rotating elements (ramp/platform, sand, peat, and wood-shavings boxes). Cumulative feed intake, body weight (BW), and feed conversion ratio (FCR) were recorded at 7, 14, 21, 28, and 34 days and analyzed by ANOVA (GLM, SAS) with Tukey's test at 5%. Behavior was recorded by scan sampling with a 21-item ethogram (10 h/d every 20 min, day 7 to 28) and analyzed with a generalized linear mixed model (negative binomial) including treatment, week, and their interaction, with Tukey-adjusted contrasts at 5%. No significant differences were found for feed intake, BW, or FCR at any age ($P>0.05$). At 34 d, BW tended to favor EE (1879.8 vs 1825 g, $P=0.076$). A significant treatment x week interaction was found for behavior ($P<0.05$): enrichment-directed behavior in EE rose from 46.75 to 136.69 occurrences (wk 1 and 2, $P<0.0001$) and exceeded C every week ($P<0.05$), with platform use and resting on peat frequent in EE and absent in C ($P<0.005$). Stress-related behaviors (agitation, refuge-seeking, abnormal posture) were not observed, and aggressive pecking was rare and similar between groups ($P>0.05$). Environmental enrichment increased active use of resources and exploratory behavior without inducing stress or impairing growth, representing a viable welfare strategy compatible with productivity in intensive systems.

Keywords: Animal behavior; animal welfare; broiler chickens; environmental enrichment; growth performance

Metabolism and Nutrition: Feed Additives I

96 Effect of yeast cell wall alone or in combination with essential oils on mortality, gut integrity, immune response, and performance of broiler chickens challenged with *Escherichia coli* Graziela Valini^{*1}, Charles Hofacre², Bertrand Medina³, Ivan Girard³, Melina Bonato¹ ¹*ICC Animal Nutrition, R&D, Jundiaí, Sao Paulo, Brazil;* ²*Southern Poultry Research Group, Watkinsville, Georgia, United States;* ³*Probiotech International, Saint-Hyacinthe, Quebec, Canada*

Respiratory bacterial challenges remain a significant constraint in broiler production, as they can impair flock health, livability, and efficiency. Nutritional strategies that support immune competence under sanitary pressure may improve flock performance and viability. This study evaluated the effects of a sugarcane-derived yeast cell wall (IMW), alone or in combination with a phytogenic feed additive (PFA), on performance, airsacculitis-related mortality, gut permeability, and immune response in broilers challenged with avian-pathogenic *E. coli* (APEC). A total of 1,200 male Ross 708 broilers were assigned to a block design with 3 treatments ($n=8$ replicates; 50

birds/pen): control diet (C) without additives; a diet supplemented with IMW (Immunowall; 0.5 kg/MT; containing 30% β -glucans and 17% MOS); and a diet supplemented with IMW+PFA (Alterna Poultry; 140 g/MT, a blend of thyme, clove, and cinnamon oils). Birds were raised for 42 d under a multifactorial challenge model with reused litter, spray vaccination against Newcastle disease virus and infectious bronchitis virus at 24 d, and APEC challenge (10^8 CFU/mL; 1 mL/bird) at 29 d. Body weight (BW), feed intake (FI), feed conversion ratio (FCR), airsacculitis lesion scores (ALS; 10 birds/pen), mortality, gut permeability via FITC-d at 35 d (1 bird/pen), and liver cytokine expression at 14 and 35 d (2 birds/pen) were evaluated. Data were analyzed by ANOVA, and differences were considered significant at $P<0.05$. Total airsacculitis-related mortality was reduced by 20% and 44% in birds fed IMW and IMW+PFA, respectively, compared with C ($P<0.05$), with no differences in ALS at 42 d (C: 0.50; IMW: 0.46; IMW+PFA: 0.47; $P>0.05$). Overall performance responses did not differ among treatments, except for cumulative FI, which was lower in the IMW+PFA group than in C at 42 d (144.7 vs. 149.8 kg/pen; $P<0.05$). This reduction was accompanied by a numerically lower FCR in the

IMW+PFA group than in C at 42 d (1.64 vs. 1.67; $P>0.05$), suggesting better feed utilization under APEC challenge. Gut permeability was not affected by treatments ($P>0.05$). Meanwhile, both IMW and IMW+PFA increased liver IL-10 expression at 14 d, whereas at 35 d, IL-1 β , LITAF, IFN- γ , and IL-21 expression were upregulated in supplemented groups compared with C ($P<0.05$), suggesting modulation of immune-related pathways during the acute challenge period. In conclusion, dietary supplementation with sugarcane-derived yeast cell wall, particularly when combined with a phytogenic additive, reduced APEC-related mortality and modulated cytokine expression during respiratory challenge. These findings indicate that complementary nutritional strategies may help improve broiler resilience under APEC challenge.

Keywords: Immunomodulation; β -glucans; Phytogenic; airsacculitis; growth

97 Efficacy of double-buffered sodium butyrate supplementation on broiler performance and feed efficiency

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Uncoated double-buffered sodium butyrate (DBSB) can have an effect on the upper part of the gastrointestinal tract of the broilers and could improve feed digestibility and performance. The objective of the present study was to evaluate the effect of dietary supplementation of DBSB on the performance and feed efficiency of broilers. A total of 576 one-day-old male broiler chicks (Arbor Acres) were allocated in 16 floor pens in a completely randomized block design with 2 treatments (8 replicates/treatment). Pre-Starter (1-21 d) and finisher (22-41 d) diets were used. Control diets (CTRL) were formulated according to requirements used by the local industry. DBSB (54% SB; BUTYLin[®] 54, Dietaxion) was included on top during the whole experiment at 600 ppm in the treated diets. Birds and feed were weighed weekly individually (LW) and by pen, respectively, and the mortality recorded daily. Feed Conversion Ratio (FCR), Production Efficiency Factor (PEF=LW/FCR) and European PEF (EPEF=Viability*LW/FCR*Age) were calculated. Parametric data were analyzed using ANOVA and non-parametric data through Kruskal-Wallis Test. There were significant differences ($P<0.05$) from 14 days (+4.6%) to the end of the trial for live weight (+2.4%), but not for feed intake at any time ($P>0.05$). DBSB birds showed significantly lower cumulative FCR than CTRL broilers during the first 21 days ($P<0.05$), a similar tendency for 0-28 days ($P<0.1$) but only numerical differences ($P>0.05$) when the experiment finished (-1.0%). There were no significant differences in mortality at any time ($P>0.05$). Although differences between treatments were not significant for PEF and EPEF ($P>0.05$), there was a tendency for DBSB broilers to show better numbers for the second one (+1.7%; $P<0.1$). In conclusion, the supplementation of 600 ppm of DBSB in broiler diets improved LW and 0-21 days FCR, compared to birds fed a common field diet, which means that the use of DBSB can be considered an important strategy for feed cost savings and excretion reduction.

Keywords: broiler; sodium butyrate; feed efficiency; live weight

98 Effects of a prebiotic program combining FOS, GOS, MOS and β -glucans on performance and immune response of broilers

under a necrotic enteritis challenge

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The objective of this study was to evaluate the efficacy of a nutritional program combining Golf (fructooligosaccharides, galactooligosaccharides, and mannanoligosaccharides) and Glucan MOS (mannanoligosaccharides and β -glucans) in mitigating the effects of necrotic enteritis (NE) caused by *Eimeria maxima* and *Clostridium perfringens* in broiler chickens compared with an antibiotic growth promoter (AGP). A total of 600 one-day-old Ross male broilers were allocated to four treatments with six replicates of 25 birds each: NC (negative control, challenged and untreated), PC (challenged and supplemented with avilamycin), G (challenged and supplemented with Glucan MOS), and GG (challenged and supplemented with Golf from d1-28 followed by Glucan MOS from d28-42). All birds were challenged at day 14 with 2,000 sporulated oocysts of *E. maxima* and at days 19 and 20 with *C. perfringens* (1×10^8 CFU/mL) by oral gavage. Productive performance was evaluated through average daily gain (ADG) and feed conversion ratio (FCR). Histomorphometry was performed on days 14, 21, and 28, and immune-related gene expression was assessed in the jejunum and cecal tonsils. Data were analyzed by one-way ANOVA, with significance set at $P < 0.05$. During the pre-challenge period (d0-14), the PC group showed the highest ADG (33 g/day) and lowest FCR (1.118) ($P < 0.05$). During the challenge and recovery period (d14-42), birds receiving the GG program achieved ADG (71 g/day) and FCR (1.878) comparable to the PC group (77 g/day and 1.790, respectively), with numerical improvements over the untreated control. Birds receiving the combined program also showed increased IL-10 expression before challenge, suggesting enhanced immune regulation and intestinal resilience. After challenge, lower IFN- γ expression indicated reduced intestinal inflammation and improved restoration of intestinal homeostasis. In addition, TNF- α expression suggested early priming of gut-associated lymphoid tissue and sustained immune responsiveness throughout the challenge. In conclusion, the combination of Golf and Glucan MOS supported broiler performance under an NE challenge, resulting in growth performance and feed efficiency comparable to AGP-fed birds. Furthermore, the nutritional program promoted beneficial immune modulation characterized by early immune regulation and reduced post-challenge inflammation, demonstrating its potential as an effective non-antibiotic strategy to support intestinal health and productivity in broiler production.

Keywords: Broiler chickens; *Clostridium perfringens*; *Eimeria maxima*; prebiotics; gut health

99 Development and validation of an in-house lipopolysaccharide (LPS) challenge model to induce performance reduction in broilers

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Hypothesis or Objective: The objective was to validate an in-house

production protocol for LPS from *Escherichia coli* and evaluate its efficacy in inducing an inflammatory challenge model focused on reducing broiler performance. **Experimental Design:** Following two dose-response pilot studies, a third definitive trial was conducted in a randomized block design with treatments stratified by initial weight. Birds were divided into two groups: Control (0 µg/ broilers; T1) and Challenge (1000 µg/ broilers of in-house LPS; T2), using 16 replicates of 7 broilers per cage. **Materials and Methods:** The project involved four stages: strain selection, standardization of the extraction methodology, inoculum quantification via Limulus Amebocyte Lysate (LAL) assay, and *in vivo* validation. In Pilot 3, male broilers were housed in metabolic cages from day 1 to day 28 of age. The challenge was administered via inoculation on alternate days from day 13 to day 27. Body weight (BW) and cumulative feed intake (CFI) were recorded weekly. **Statement of Statistical Analysis:** BW and CFI data were analyzed using a longitudinal linear mixed model (REML). Residual normality was verified by Shapiro-Wilk and Anderson-Darling tests, and homogeneity of variances by Levene and Brown-Forsythe tests. **Results:** On day 28, the challenged broilers (T2) showed lower BW (T2: 1419.43 ± 7.90 g vs. T1: 1454.75 ± 8.32 g; $p = 0.003$) and lower CFI (T2: 2175.87 ± 12.02 g vs. T1: 2226.12 ± 12.30 g; $p = 0.005$). The Treatment X Day interaction was significant for BW ($p = 0.036$) and CFI ($p = 0.012$), indicating a progressive intensification of the challenge. Daily weight gain differed from 0 to 21 days but stabilized over the total period. Livability, production efficiency index, and adjusted feed conversion ratio were not affected ($p > 0.05$). **Conclusions:** The pilot studies successfully established the in-house LPS production protocol. The 1000 µg/ broilers dose proved effective in inducing a consistent inflammatory challenge in broilers up to 28 days of age.

Keywords: Endotoxin; *Escherichia coli*; Inflammation; Performance; Poultry

100 Comparative IPD Meta-Analysis of Aflatoxin, Deoxynivalenol, and Fumonisin Effects on Performance, Liver, Intestine, and Immunity in Broiler Chickens

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Hypothesis/Objective: Individual participant data (IPD) meta-analysis resolves effects beyond single trials; cross-mycotoxin comparisons are lacking. We tested whether aflatoxins (AF, 2.8 µg/kg), deoxynivalenol (DON, 50 µg/kg) and fumonisins (FB, 100 µg/kg) produce distinct systemic signatures in broilers over 21 days. **Experimental design:** IPD synthesis of 28 experiments (AF n=12, DON n=5, FB n=11), contaminated diet versus control over 21 days, with 6 cages per treatment of 10 birds each. Relative liver weight was recorded for all animals; serum biochemistry, jejunal histomorphometry, and liver and bursal histopathology from 12 birds per treatment, all sampled at day 21. **Materials and methods:** Random-effects

models (restricted maximum likelihood, REML; metafor): mean difference (MD) for continuous outcomes and risk difference (RD) for mortality; pseudo-replication corrected by cage redistribution. **Statistical analysis:** REML with permutation validation (500–1,000 allocations) and Bayesian complementation (half-Cauchy for AF, half-normal scale 0.5 for DON/FB); posterior probabilities reported for practical thresholds. **Results:** AF was most severe, significantly affecting body weight (BW) -126.4 g, mortality +7.0 percentage points (pp), relative liver weight (hepatomegaly) +1.26 pp, the Kisi-elinski magnification factor (M) -3.91 and bursal lymphoid depletion (immunosuppression) +0.938. DON reduced BW -93.7 g and feed intake (FI) -111.5 g, increased relative liver weight +0.365 pp, and reduced M -1.642. FB reduced BW -48.8 g and FI -46.1 g, increased the sphinganine:sphingosine ratio (SASO) 6.4-fold and hepatic heterophilic (MD) +0.406 and mononuclear +0.337 infiltrates, and reduced M -2.141. Bayesian posteriors: AF P (mortality>5%) = 95.2%; FB P (SASO>2-fold) = 100%; DON P (feed-intake loss>30 g) = 100%. Between-experiment heterogeneity (I^2) was high for growth and liver outcomes up to 97.6%, i.e. variable magnitude, consistent direction; the mycotoxins also increased within-group dispersion (coefficient of variation, CV: AF BW 7%11%, FI 5%12%), widening the distribution. **Conclusions:** Three distinct signatures emerged: AF – lethal, hepatotoxic and immunosuppressive; DON – anorexic, with hepatomegaly but no hepatic inflammation; FB – sphingolipid disruption (SASO 6.4x) with hepatitis sparing hepatocytes and bursa. IPD-specific contributions: M as the lowest-P intestinal parameter; and evidence the toxins broaden the response distribution (amplified dispersion, heterogeneous magnitude), resolvable only via IPD.

Keywords: meta-analysis; aflatoxins; deoxynivalenol; fumonisins; broiler

101 Effects of yeast-derived feed additives on ileal microbiota modulation in broilers challenged with necrotic enteritis

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This study evaluated the effects of a nutritional program combining Golf (fructooligosaccharides, galactooligosaccharides, and mannanoligosaccharides) and Glucan MOS (mos and β -glucans) in mitigating the effects of necrotic enteritis (NE) caused by *Eimeria maxima* and *Clostridium perfringens* in broiler chickens compared with an antibiotic growth promoter (AGP). A total of 600 one-day-old Ross male broilers were allocated to four treatments with six replicates of 25 birds each: NC (negative control, challenged and untreated), PC (challenged and supplemented with avilamycin), G (challenged and supplemented with Glucan MOS), and GG (challenged and supplemented with Golf from d1–28 followed by Glucan MOS from d28–42). All birds were challenged at day 14 with 2,000 sporulated oocysts of *E. maxima* and at days 19 and 20 with *C. perfringens* (1×10^8 CFU/mL) by oral gavage. Ileal samples were collected at D14 (pre-challenge), D21 (peak challenge), and D28 (recovery) for microbiome analysis. Bacterial communities were characterized by 16S rRNA sequencing, and diversity and taxonomic composition were analyzed using QIIME2. Alpha diversity was assessed using Shannon, Simpson, Chao1, and Observed Features indices, while beta diversity was evaluated using Bray–Curtis, Jaccard, weighted UniFrac, and unweighted UniFrac distance matrices. Differential abundance was analyzed using ANCOM-BC2 with false discovery rate correction. At D14, both additive treatments significantly modulated the microbiota compared with PC. Beta diversity analyses showed significant differences between additive treat-

ments and PC using Bray–Curtis and weighted UniFrac distances ($q = 0.012$). Differential abundance analysis revealed increased abundance of *Candidatus_Arthromitus* and *Clostridiaceae* in G and GG, with reduced abundance of *Staphylococcus* and *Staphylococcaceae* compared with PC ($q < 0.05$). At D21, beta diversity differed in Jaccard distances, while G showed increased abundance of *Enterococcus* and *Enterococcaceae* compared with PC ($q < 0.001$). At D28, microbial communities converged among treatments, with only limited differences in Jaccard-based beta diversity. Residual treatment effects were restricted to low-abundance taxa, with *Enterococcus* and *Enterococcaceae* remaining more abundant in G

than in PC ($q < 0.01$). In conclusion, supplementation with G or GG promoted early and transient modulation of the ileal microbiota in broilers challenged with NE. The additives enriched taxa associated with gut maturation and fermentative metabolism. These changes were greatest before and during peak challenge and diminished during recovery, suggesting that yeast-derived additives support a favorable microbial ecosystem during enteric challenge.

Keywords: Broiler Chickens; ; Microbiome; necrotic enteritis; prebiotics; 16S rRNA sequencing

Animal Well-Being and Behavior

102 Integrating computer vision and accelerometry for a multidimensional understanding of the effects of environmental enrichment

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Animal welfare is a core theme in poultry science, and environmental enrichment is among the most widely proposed strategies for improving it. Although enrichment is generally thought to broaden the behavioral repertoire, it can affect different behaviors unequally, enhancing some while reducing others. These effects may also change over time through processes such as habituation to objects. Consequently, its objective assessment can benefit from automated tools able to continuously and simultaneously quantify diverse behavioral traits in both individually and group-housed animals. Here, we integrated computer vision and accelerometry to develop and evaluate an approach for characterizing the behavioral responses of Japanese quail to environmental enrichment. Five groups of four adult females were exposed to progressively more complex substrates (wire floor, wood shavings, and wood shavings plus a sand bath), each stage lasting 4 days. The week prior to testing all birds were fit with a light-weight plastic colored backpack. Behavior was continuously video-recorded throughout the experiment, and in two birds of each group an accelerometer was placed into the backpack. Two computer-vision models (YOLOv11) were developed for daytime use, both achieving accuracy >90%: one to track the locomotion and spatial distribution of individual birds, and another to detect specific behaviors such as feeding, drinking, and dust-bath use during the light phase. Accelerometry was used to automatically quantify daily (~24-h) activity and dust-bathing rhythms. Together, both technologies generated continuous multivariate behavioral time series, from which event frequency and time allocation were estimated. Generalized linear mixed models were used for statistical analysis with stage as an independent factor, and pen and ID as random factors. As expected, greater environmental complexity significantly increased the distance traveled ($P < 0.05$). Compared with wire flooring, enriched substrates increased both the time allocated to activity and dust bathing and the frequency of these

behaviors ($P < 0.05$). Daily dustbathing rhythm power also differed among substrates, being significantly higher under the wood-shavings treatment ($P < 0.05$). In conclusion, integrating computer vision and accelerometry enabled automated, continuous, and multidimensional behavioral phenotyping, providing a comprehensive characterization of responses to environmental enrichment. By simultaneously capturing multiple complementary behaviors over time, this approach extends beyond conventional assessments and offers a scalable framework for precision welfare evaluation in poultry.

Keywords: Accelerometer; Japanese quail; computer vision; YOLO; welfare

103 EFFECTS OF DIFFERENT LIGHT INTENSITIES RANGES ON THE GROWTH PERFORMANCE OF BROILER CHICKENS

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The experiment was conducted to evaluate the effects of different light intensity ranges on the growth performance of broiler chickens. Light is an important environmental factor for broiler production; however, recommended light intensity levels vary among management guidelines and production systems. This variability highlights the need to better understand its effects on broiler performance. Therefore, the objective of this study was to evaluate the effects of different light intensity ranges on the growth performance of broiler chickens. The study was carried out at the Poultry Experimental Unit of the Federal University of Goiás. A total of 378 one-day-old male Cobb 500 broiler chicks were distributed in a completely randomized design with three treatments, six replicates, and 21 birds per experimental unit, totaling 18 experimental units. The treatments consisted of three light intensity ranges (15–25, 40–60, and 80–100 lux). Birds were raised under identical environmental and management conditions, with feed and water provided ad libitum throughout the experimental period. Body weight (BW) and body weight gain (BWG) were evaluated at 7, 21, 35, and 42 days of age. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Tukey's test at $P < 0.05$. At 7 days of age, significant differences were observed for BW and BWG ($P < 0.05$), with birds reared under 15–25 lux showing higher values than those under 40–60 lux, while birds under 80–100 lux showed intermediate

performance and did not differ from the other treatments. No significant differences were observed among treatments from 21 days onward ($P > 0.05$). It was concluded that different light intensity ranges influenced broiler growth performance only during the first week of life, with no lasting effects throughout the production cycle.

Keywords: light intensity; poultry production; growth performance; environmental management; welfare

104 Use of perches during the rearing of broiler breeders and its influence on productive performance

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Currently, the implementation of environmental enrichment (EE) structures is uncommon in broiler breeder production, which may be attributed to a scarcity of studies investigating the benefits of EE on poultry welfare. In this context, this study aimed to evaluate whether the use of perches for broiler breeders affects zootechnical performance indicators. This study was conducted over a one-year period across 15 commercial poultry houses distributed among three production sites, housing 90,000 Ross females between 24 and 40 weeks of age. The birds were allocated into two groups: Control (11 conventional houses) and Treated (4 houses equipped with 600 linear meters of carbon steel perches, providing a total of 10 cm per bird). The following zootechnical indicators were mon-

itored weekly: fertility rate, hatchability, average bird body weight, and average egg weight. The hatchability rate was evaluated using hatchery data retrieved via Mtech software. Statistical analyses were performed using R software, applying the Kruskal-Wallis test for weight variables (birds and eggs), and the Chi-square and Fisher's exact tests for fertility data, with statistical significance set at $P \leq 0.05$. The results revealed that the presence of perches led to a significant improvement in fertility, with the treated group exhibiting a 97.00% fertility rate compared to 96.62% in the control group ($P=0.023$). At 40 weeks of age, an even more pronounced difference was observed, with the treated group reaching a 98.90% fertility rate versus 96.90% in the control group ($P<0.001$), corresponding to an increase of approximately two percentage points in fertility, which may be biologically relevant under commercial production conditions. No significant differences in fertility were detected during the remaining evaluation periods. No significant differences ($P>0.05$) were observed between treatments for average bird weight (3.676 kg for the control group vs. 3.617 kg for the treated group). Similarly, production site had no significant effect on body weight, hatchability, or egg weight, which remained within the standard guidelines for the lineage. Therefore, the use of perches as an environmental enrichment strategy improved fertility in broiler breeders without adversely affecting body weight, egg weight, or hatchability.

Keywords: egg production; environmental enrichment; fertility; hatchability; productivity

Symposium: The Advance in Technologies for Gut Health as a Tool Supporting the Decision Making Process in the Field

1022S The challenges and opportunities to have a full interpretation of gut health status in poultry

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Gut health has become a central concept in poultry production influencing not only performance and feed efficiency, but also disease resilience, animal welfare, antimicrobial stewardship, and overall sustainability. Rather than representing a single measurable parameter, intestinal health reflects the continuous interaction between the host intestinal mucosa functions: physical barrier, immune competence, nutrient utilization, the gut microbial communities, and the surrounding production environment. This perspective is especially relevant in commercial systems, where flock performance can be affected by subclinical intestinal disturbances before obvious disease signs or macroscopic lesions are detected. While conventional tools such as lesion scoring, histopathology, pathogen detection, and post-mortem assessments continue to provide valuable insights into gut health, they typically reflect a fraction of the broader intestinal environment and can be difficult to implement routinely at flock level. Gut health data interpretation is complicated by age, genetics, nutritional factors, management practices, sampling site, environmental stressors and inter-flock variation. In addition, microbiome data, biomarkers, and production metadata remains unstandardized or validated across commercial conditions, and no individual parameter can reliably describe all aspects of intestinal function: barrier integrity, immune competence and microbial stability. Over the past decade, the poultry industry has progressively shifted its gut health focus from preventive disease control to a broader understanding of intestinal function and its ecology. Current scientific and industry efforts are moving toward more ho-

listic and practical assessment. Non-invasive sampling, host and microbial biomarkers, metabolite profiling, omics-based discovery, precision monitoring, and artificial intelligence offer opportunities to detect early deviations and integrate multiple biological and environmental signals. However, their value depends on rigorous validation, clear biological interpretation, practical sampling protocols, and integration with veterinary and nutrition expertise. The future of poultry gut health assessment is therefore not a single test, but a structured interpretation framework that combines field observations, production data, biological indicators, and longitudinal monitoring to support proactive evidence-based decisions in commercial poultry systems.

Keywords: gut health; poultry; interpretation; biomarkers; precision farming

1023S Gut health: new concepts and how to evaluate it using available high-tech tools

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Intestinal health is essential for optimizing broiler performance, disease resistance, and animal welfare. However, reliable methodologies for evaluating gastrointestinal health under commercial production conditions remain limited. Conventional diagnostic approaches, including post-mortem lesion scoring, histological evaluation of villus height, crypt depth, villus-to-crypt ratio, and cecal microbiota analyses, provide valuable information but are invasive, labor-intensive, expensive, require specialized expertise, and are unsuitable for longitudinal monitoring because they rely on eutha-

nasia and primarily detect advanced intestinal damage. This presentation reviews emerging concepts and advanced technologies for the non-invasive assessment of poultry gut health, emphasizing biomarkers and precision-monitoring tools capable of identifying intestinal disturbances before clinical signs appear. Promising biomarkers include fecal metabolites, changes in microbial community profiles, and host-derived proteins such as ovotransferrin, calprotectin, lipocalin-2, and other inflammatory indicators. Excreta, cloacal swabs, poultry litter, and dust provide practical flock-level sampling matrices that enable repeated evaluations without sacrificing birds, reduce analytical costs, and facilitate large-scale surveillance. Their close relationship with intestinal microbiota also allows detection of microbial shifts associated with gut health, productivity and flock performance. Recent advances in molecular diagnostics, including quantitative PCR, metabarcoding, meta-genomics, -transcriptomics, -proteomics, and metabolomics, have enhanced the characterization of gut microbial communities, dysbiosis detection, and prediction of production outcomes. Because no single biomarker adequately reflects all aspects of intestinal function, combining complementary biological indicators is expected to enhance diagnostic sensitivity and provide a more comprehensive assessment. Artificial intelligence further strengthens non-invasive monitoring through computer vision, image analysis, thermal imaging, analyses of volatile organic compounds, behavioral monitoring, sound recognition, and deep learning algorithms that can accurately classify abnormal droppings and detect early disease-associated changes. Integration of these technologies with machine learning enables continuous, automated monitoring of intestinal health, supports timely management decisions to improve productivity while reducing economic losses. Future progress depends on methodological standardization, validation under commercial conditions, and the development of rapid, robust point-of-care diagnostic assays to support precision poultry health management.

Keywords: Gut health; omics; biomarkers; artificial intelligence; electronic sensors

1024S The Current Vision of the Industry on Gut Health, Gut Health Tools, and How to Apply It to Obtain the Best Results in the Field

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Advances in gut health, immunity, and nutrition have improved our understanding of poultry performance, but success in commercial production depends on effectively translating biological knowledge into field management. In this context, data management has become a critical pillar for strategic decision-making, process optimization, and early problem detection. A clear distinction exists between data and information: data are raw observations, while information is generated through analysis, interpretation, epidemiological evaluation, stratification, and outlier removal. Only then can dashboards, baselines, and performance indicators provide meaningful support for monitoring and decision-making. Health monitoring programs are among the most valuable sources of field information. Besides supporting disease diagnosis, they function as continuous surveillance systems that enable early detection of health challenges, assessment of preventive strategies, and evaluation of feed additives and management practices. Effective monitoring must be systematic and ongoing, allowing preventive actions before problems significantly impact production. The quality of monitoring results depends on standardized evaluation criteria and continuous training of field personnel. Consistent sampling and

scoring are essential, as many health parameters are subjective and can lead to inaccurate conclusions if not properly assessed. Once collected and analyzed, monitoring data can be used to build dashboards, track disease trends, optimize vaccination and anticoccidial programs, identify high-priority targets through data stratification, and anticipate slaughterhouse issues. They also help establish relationships between health status and zootechnical performance. Because poultry production is highly interconnected, decision-making should involve multidisciplinary teams. Health information must be integrated with management indicators such as sanitary downtime and market conditions, nutritional factors including ingredient quality and additive use, and zootechnical performance data. The growing number of commercial products targeting intestinal health has increased decision-making complexity, making technical evaluation by specialists essential. However, feed additives alone cannot guarantee success. Continuous improvement of biosecurity, litter management, water quality, sanitary downtime, and vaccination programs remains fundamental. In conclusion, information is one of the most valuable assets in poultry production. The quality of field data collection, analysis, and management directly influences the effectiveness of health control programs and the overall success of production systems.

1025S How to develop an innovative, non-invasive method to assess gut health in broilers

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Gut health in broilers is normally evaluated through histopathological analysis of the small intestine or macro-scoring intestinal lesions caused by *Eimeria* spp. or *Clostridium perfringens*. However, these methods are invasive and histological evaluation often takes a few days. Practical and non-invasive biomarkers to evaluate gut health in poultry are currently limited. Therefore, the objective of this study was to evaluate a new, non-invasive qPCR-based method as a tool to evaluate gut health in broilers. Cloacal samples using swabs were collected from 14 healthy commercial broiler flocks as well as broilers with health issues sent to a veterinary laboratory for diagnostic purposes (classed as "gut health problems" or "non-gut health problems" after necropsy). All cloacal samples were subjected to an assay with two biomarkers for gut damage (GD): total bacterial DNA and host DNA (considered to be derived from desquamated intestinal epithelial cells). The samples from the healthy broilers were subjected to an additional assay with markers for specific subpopulations of the microbiota: 'Lactate' marker for all *Lactobacillales* and 'Butyrate' marker for *Ruminococcaceae* and *Lachnospiraceae* species. Both assays used ligation-based multiplex quantitative RT PCR methods described by Van Hees *et al.* (2025). The Ct values for each marker were used to calculate the ratios of host to total bacterial DNA (GDRatio); butyrate to total DNA (BUTRatio); lactate to total DNA (LACRatio); and butyrate to lactate (ButLacRatio). The GDRatio results were analysed using one-way ANOVA with the significance level set at 0.05 and Tukey's HSD test for post-hoc pairwise comparisons. To investigate the relationship between different ratios (average, minimum and maximum values) and the slaughter weight (SW) of the 14 healthy broiler flocks, the best subset regression procedure was applied to obtain a best-fitting regression model using Python. The GDRatio of healthy broilers was significantly lower (average 12.2) than broilers with gut health problems (average 16.6) ($P < 0.001$). GDRatio of broilers from the "non-gut health problems" group was similar to that of healthy broilers and significantly lower than the GDRatio of the "gut health problems" group ($P < 0.001$). The final multiple linear regression model

showed a significant correlation between the actual SW and the SW as predicted by the model ($P = 0.002$). The results of this study support the potential of these assays to evaluate the gut health of broilers. This method can enable proactive flock management strategies, benchmarking between flocks and farms, or assessing

efficacy of nutritional strategies which can help to support more sustainable poultry production.

Keywords: Gut health; Broilers; Biomarkers; Microbiota; Non-invasive

Symposium: Technological and Sustainable Innovation in Poultry Production: Computer vision and advancing credible GHG assessments in poultry production academic, industry measurement and LCA review

1026S Computer Vision: Research for Decision Making in Broiler Production –Monitoring, Activity Index, Poultry Behavior and Vocalization

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Claro. Esta versão enfatiza mais claramente a **contribuição científica**, a transição para sistemas *animal-centered* e a integração multimodal, mantendo o formato adequado para abstract de congresso. Broiler production is undergoing a major technological transformation driven by advances in computer vision, artificial intelligence (AI), smart sensors, and the Internet of Things (IoT). These technologies enable continuous, non-invasive monitoring of birds and their environment, transforming large volumes of data into actionable information for real-time decision-making. Within this framework, computer vision has evolved beyond image acquisition to become a powerful tool for interpreting animal bioresponses, allowing behavior to be used as a direct indicator of welfare, health, thermal comfort, and productive performance. This lecture will discuss recent advances in computer vision for precision poultry farming, focusing on automated behavioral monitoring, activity index estimation, spatial distribution of birds, and their application in intelligent decision-support systems. Particular attention will be given to the transition from conventional environmental control, primarily based on temperature and humidity, toward animal-centered approaches that integrate environmental conditions with behavioral and productive responses. Research conducted at the University of Campinas (UNICAMP) will illustrate the application of computer vision and deep learning algorithms for continuous monitoring of broiler activity, detection of behavioral patterns associated with heat stress and welfare, and development of predictive models for environmental control based on animal bioresponses. Poultry vocalization will also be explored as a complementary biosensor, demonstrating the potential of automated acoustic analysis for early detection of physiological, health, and behavioral changes. The integration of computer vision, acoustic signals, environmental sensors, and AI provides a foundation for multimodal monitoring systems capable of detecting critical events, anticipating production challenges, and supporting real-time management decisions. Emerging approaches such as Edge AI, Digital Twins, predictive modeling, and autonomous control systems will further enable the transformation of behavioral, activity, and vocalization data into actionable management strategies. These developments represent an important step toward a new generation of precision poultry farming in which the animals themselves become biological sensors, supporting improved environmental control, animal welfare, production efficiency, and more sustainable, data-driven poultry production.

Keywords: Computer vision; Vocalization; Precision Livestock Farming; Artificial Intelligence;; Poultry Behavior

1027S Computer Vision in Poultry Slaughter: Optimizing Quality

Control, Resource Efficiency, and Supporting Sustainability and LCA

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Since 2016, Lar Cooperativa has been driving Digital Transformation through its Innovation Program, seeking to apply emerging technologies to optimize processes and generate opportunities for new services and/or new businesses. In the poultry industry, this transformation is a challenge that requires combining operational profitability with environmental sustainability. In this context, this presentation details the implementation of Artificial Intelligence (AI) and Computer Vision in the continuous inspection and classification of poultry carcasses. The objective is to demonstrate how these technologies act as strategic pillars to raise the quality standard, minimize waste, and ensure high precision in Life Cycle Assessments (LCA). The industry faced the historic challenge of manual, sample-based inspection: a slow process (15 to 30 min/batch), subjective, with a latency of 24 to 72 hours for data consolidation. This lack of real-time visibility resulted in production inefficiencies, avoidable discards, and opacity in measuring the environmental impact of losses. To overcome these bottlenecks, a computer vision system reinforced for the severe humidity and steam conditions of the poultry slaughter line was developed, in a co-creation model with a startup. The project is methodological innovation was the migration from traditional static sampling to a census-based analysis with 100% coverage of production. The system analyzes up to 7 distinct anomalies in real time in 100% of slaughtered carcasses. This transition generated a database of absolute, traceable data, providing indispensable inputs for LCA inventories and the assessment of greenhouse gas measurement. The results prove the impact of the technology: • Response time for decision-making dropped from 48 hours to real time via dashboards. In addition, 10 of the 12 inspectors were reallocated to more strategic roles, while the yield gain per ton reduced relative energy consumption per final product. • The project required an investment of R\$429 thousand, generating a financial return of R\$1.1 million/year. More than automating processes, computer vision redefines the level of efficiency in poultry farming. By replacing estimates with full census data, the technology ensures standardization, eliminates waste at the source, and consolidates evidence-based sustainability for the future of the sector.

Keywords: innovation

1028S Innovation and Technology Applied to Integrated Poultry Production: C.Vale's Experience in Building a More Efficient, Predictive, and Sustainable Supply Chain

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Industrial poultry production must simultaneously advance productivity, quality, animal welfare, energy efficiency, and sustainability.

In C.Vale's integrated operation, with full traceability, computerized farm management, and processing capacity of 650,000 birds per day, innovation connects farms, hatcheries, technical assistance, processing plants, and data-driven decision-making. This presentation will show how C.Vale is building an innovation ecosystem for the poultry value chain by integrating environmental control, energy efficiency, artificial intelligence, computer vision, automation, predictive monitoring, and sustainability. The principle is that innovation creates value when solutions are tested, measured, improved, and scaled with operational impact. Environmental control projects include Stir Fans, Sealed Combustion Heaters, and Heat Exchangers. Stir Fans reduce temperature stratification and improve heat distribution, litter quality, bird comfort, and heating efficiency. In a pioneering South American project, Heat Exchangers improved feed conversion, reduced carcass condemnations, and generated significant LPG savings. Sealed Combustion Heaters prevent CO₂ and combustion moisture from entering poultry houses, improving air quality and reducing minimum ventilation requirements. Preliminary results indicate better zootechnical performance, with future tests combining heaters and stir fans. The NOC (Network Operations Center) integrates field data, sensors, dashboards, APIs, and performance, consumption, mortality, and environmental indicators, supporting continuous monitoring and more predictive veterinary assistance. Artificial intelligence and computer vision are also applied to automated carcass inspection for footpad dermatitis. The system evaluates 100% of processed birds with over 98% accuracy, expanding data generation and enabling correlations between processing results, farm management, nutrition, and environmental conditions. At the hatchery, the Fertility Check project combines magnetic resonance and artificial intelligence to identify egg fertility early, non-invasively, and at industrial scale, increasing installed capacity utilization. Finally, the presentation will explore connected poultry production through AI, IoT sensors, automation, robotics, and decision-support systems, demonstrating how applied innovation can generate scalable and sustainable results for farmers, industry, and consumers.

Keywords: Energy Efficiency; Predictive Monitoring; Artificial Intelligence; Poultry Production; Sustainability

1029S Academic Assessments of Emissions in Poultry Production: Measurement Approaches, Uncertainty, and Research Priorities

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Accurate assessment of greenhouse gas (GHG) emissions from poultry production is essential for developing mitigation strategies, benchmarking environmental performance, and supporting evidence-based sustainability assessments. This presentation critically examines academic approaches used to quantify and interpret GHG emissions in poultry systems, with emphasis on methodological comparability and uncertainty. Credible emission assessment requires more than measuring gas concentration; it requires the integrated characterization of airflow, animal and manure sources, environmental conditions, temporal variability, and measurement uncertainty. The assessment is organized across three complementary levels: direct measurements in poultry houses and manure or litter systems; emission-factor and inventory-based estimation; and translation of primary measurements into farm- or production-chain

assessments. Direct approaches typically combine gas-concentration measurements with ventilation or airflow estimates, whereas chamber methods are commonly applied to litter, manure, and storage sources. The methodological comparison identifies recurring sources of variation in reported emission rates, including ventilation-rate estimation, sampling location and frequency, temporal coverage, bird age and performance, litter moisture and manure characteristics, environmental conditions, and calculation procedures. System boundaries and accounting conventions further affect interpretation, particularly when distinguishing biogenic CO₂ from bird respiration from fossil-fuel-derived CO₂ and when treating NH₃ as a reactive nitrogen loss relevant to indirect N₂O emissions rather than as a GHG itself. Extrapolation from short-term measurements to flock, farm, or production-chain scales can therefore introduce substantial uncertainty when temporal variability and production conditions are not adequately represented. Continuous environmental monitoring synchronized measurement of gas concentrations and airflow, locally validated emission factors, standardized reporting, and explicit uncertainty analysis can improve the robustness and reproducibility of academic assessments. Greater harmonization between direct measurements, emission models, and production data is needed to generate credible GHG estimates and to identify mitigation strategies that are both scientifically defensible and applicable to commercial poultry production.

Keywords: greenhouse gases; poultry production; emission factors; environmental monitoring; carbon footprint

1030S Industry perspectives on GHG Emissions Measurement and Accounting in Poultry Production

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Growing concern about climate change impacts on business performance has increased the need for robust greenhouse gas (GHG) accounting. Corporate GHG inventories, developed according to ISO 14064 and the GHG Protocol, support climate strategies and target setting. However, scope 3 emissions remain a major challenge because they are commonly estimated using secondary emission factors that may not accurately represent specific supply chains. To improve precision, companies increasingly rely on product carbon footprint (CFP) studies, conducted in accordance with ISO 14067, which can incorporate primary supplier data to improve accuracy. In the poultry sector, carbon accounting is particularly complex due to the large number of interconnected processes across the supply chain, including grain production, breeding, hatcheries, broiler farms, processing plants, and distribution. Additional challenges include land use change (LUC), animal rearing emissions, and manure management. Therefore, the development of scientifically robust and representative datasets is essential. This presentation describes Seara's five-year journey using CFP studies to strengthen its climate strategy, respond to customer demands, and improve carbon accounting. The first study, initiated in 2021, assessed a whole chicken product, identified the main emission hotspot and evaluated mitigation scenarios. A second study compared Seara's products with production systems in five countries and demonstrated that Brazilian poultry products have similar, and in some cases lower, carbon footprints than international competitors. The study also revealed that generic database emission factors may overestimate Brazilian product impacts due to limited geographical and production-specific granularity. Subsequent studies provided product-specific emission factors for customers, supporting busi-

ness-to-business communication, strengthening customer engagement, and improving scope 3 accounting accuracy. Key challenges included data availability, representativeness of supply chain data, large-scale data collection, LUC modeling, and animal rearing emissions estimation. The experience showed that, although initial CFP studies require significant effort, repeated assessments enable methodological refinement, more efficient data collection, and broader application across production units. Developing internal carbon accounting capabilities has proven essential to support climate objectives, strengthen customer engagement, and provide more credible product-level GHG assessments.

Keywords: Carbon Footprint; Greenhouse Gas Emissions; Poultry Production; Life Cycle Assessment (LCA)

1031S Third-party critical review of LCA studies: Challenges and best practices in the poultry sector

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Life cycle assessment (LCA) is increasingly used by companies to demonstrate environmental performance through comparisons between local production and average international scenarios. A central challenge is the imbalance in data quality: companies typically rely on primary data from their own operations, offering strong temporal, geographical, and technological representativeness, while competing markets are often modelled using secondary data from databases or literature, which may be outdated, lack geographical representativeness, or reflect controlled systems that miss real production variability. Under ISO 14040 and ISO 14044, comparative LCAs intended for public disclosure require an independent third-party

critical review by a panel including an LCA-expert chairperson and members representing relevant interested parties. Guided by ISO/TS 14071:2014, the process involves rounds of comments and discussion between reviewers and practitioners, ending in a critical review statement. This process has several challenges. Qualified experts willing to serve as reviewers are scarce, and ideological or political influences can surface as excessive or methodologically unsubstantiated objections. The poultry supply chain's complexity creates strong interdependence among zootechnical parameters which secondary sources, developed independently, often fail to represent consistently. Land use change emissions data source may also generate methodological disagreement. Animal performance parameters, including mortality and manure management, also demand close scrutiny, and allocation of co-products such as meat, by-products, and rendered materials is another common point of discussion, with economic allocation widely applied yet often challenged due to cross-cultural differences in valuing animal parts. As best practices it is recommended selecting reviewers with LCA sector-specific expertise. Transparent, well-documented reports covering data sources, assumptions, and data quality allow reviewers to evaluate representativeness efficiently. Sensitivity analyses can show that uncertain secondary data may have limited influence on conclusions, while uncertainty assessments further strengthen result robustness. Active participation by the study commissioner in review meetings helps clarify primary data and operational realities and can reveal when comments stem from ideological rather than methodological concerns, while transparency about limitations builds trust between reviewers and practitioners. Ultimately, critical review should be understood not as a mere validation step but as an opportunity to improve LCA quality, closing data gaps and strengthening methodological consistency.

Keywords: Carbon footprint; GHG; Critical review; LCA; Sustainability

Immunology, Health and Disease

105 Chicken embryos as a platform for identifying sanitary challenge biomarkers based on serum amyloid A and α 2-macroglobulin responses

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Early detection of sanitary challenges is essential for improving health management and disease control in poultry production. Biomarkers can support health monitoring, disease surveillance, and the evaluation of preventive interventions. Chicken embryos represent a promising model for investigating host responses to infectious challenges and identifying novel inflammation-associated biomarkers. This study evaluated inflammatory responses in chicken embryos experimentally challenged with *Salmonella Pullorum* (SP). Fertilized eggs were inoculated at 9.5 days of embryonic development, and allantoic fluid and serum samples were collected 24 and 48 h post-inoculation for acute-phase protein quantification and proteomic analysis. The objective was to identify molecules associated with the embryonic response to infection that could serve as biomarkers of sanitary challenge in poultry. In the allantoic fluid, serum amyloid A (SAA) was the only acute-phase protein significantly altered by infection. Embryos inoculated with SP presented higher SAA concentrations (1.054 μ g/mL) than negative controls (0.119 μ g/mL),

representing an approximately ninefold increase. These findings indicate that the allantoic compartment may be a sensitive matrix for detecting inflammatory responses during embryonic development and suggest that SAA may be a useful biomarker for monitoring embryonic health and sanitary challenges in hatchery environments, including studies involving infectious agents and *in ovo* interventions. Proteomic analysis identified α 2-macroglobulin (A2M) as one of the most strongly upregulated proteins in infected embryos. A2M exhibited a log₂ fold change of 13.13 and remained significant after false discovery rate correction (adjusted p = 0.0081), corresponding to an increase of more than 8,000-fold in the infected group. Although A2M is recognized as an important mediator of inflammatory responses in mammals, its role in avian infections remains poorly understood. The marked increase observed following bacterial challenge highlights its potential as a candidate biomarker for future studies of poultry health and disease monitoring. To the best of our knowledge, this is the first study to quantify SAA and A2M in chicken embryos at such early developmental stages. These results demonstrate that chicken embryos can serve as a valuable platform for biomarker discovery and identify both SAA and A2M as promising targets for future investigation in poultry health programs.

Keywords: Allantoic fluid; Acute-phase proteins; Proteomics; Hatchery health; Inflammation monitoring

106 Essential Oil-Based Eubiotics for Fowl Typhoid Control in Commercial Laying Hens: Effects on Mortality, Pathogen Dissemination, and Virulence

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Fowl typhoid causes substantial economic losses in poultry production by reducing performance and increasing mortality, often resulting in extensive antimicrobial use. Alternative control strategies are therefore needed. This study evaluated the efficacy of three eubiotic formulations in controlling infection and dissemination of *Salmonella Gallinarum* (SG) in experimentally challenged commercial laying hens. Forty-nine hens (32 weeks of age) were randomly assigned to five treatments with four replicates of 3–4 birds each and fed a common basal diet for 10 weeks. Treatments included an unchallenged control (T1), a challenged control (T2), and three challenged groups receiving carvacrol-based EOs plus organic acids at 140 ppm (T3) or 300 ppm (T4), or thymol-based EOs plus sodium humates at 300 ppm (T5). Birds were orally challenged on three occasions (weeks 3, 5, and 8) with a field strain of SG at 10^3 , 10^5 , and 10^7 CFU/mL, representing low, medium, and high challenge levels, respectively. Cloacal swabs and eggs were cultured weekly for SG detection. Productive performance, clinical signs, and mortality were monitored throughout the study, while bacteriological and histopathological analyses were performed on liver, spleen, ovarian follicles, and bone marrow samples. At the end of the trial, expression of the virulence genes *sifA*, *sopB*, *orgA*, *sipA*, and *tolC* was quantified by qPCR. Data were analyzed using ANOVA and Kruskal–Wallis tests ($P < 0.05$). Feed intake decreased at all challenge levels ($P \leq 0.028$), whereas T5 mitigated this effect at the medium dose ($P = 0.008$). Egg production declined at the high challenge dose ($P = 0.001$). At the medium challenge dose, T4 and T5 prevented mortality (0%) compared with T2 (15%) and T3 (20%), whereas all birds died at the high dose. Carvacrol-based EOs reduced SG egg shedding by up to 80%, compared with 20% for thymol-based EOs. All additive-treated groups showed reduced ovarian lesions ($P = 0.02$) and downregulated virulence gene expression (58–92%), with T3 showing the lowest systemic dissemination. Eubiotic formulations reduced pathogen dissemination, ovarian lesions, and virulence gene expression. Carvacrol-based formulations more effectively reduced bacterial shedding, whereas higher inclusion levels improved survival. These findings support eubiotic additives as alternatives for controlling fowl typhoid under low and moderate challenge conditions and reducing antimicrobial use in poultry production.

Keywords: Essential oils; Organic acids; Sodium humates; *Salmonella Gallinarum*; Laying hens

107 Combined Early-Life *Enterococcus lactis* Administration and Dietary *Bacillus* Supplementation Enhance Intestinal Barrier Function and Reduce Bacterial Chondronecrosis with Osteomyelitis in Broilers

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Bacterial chondronecrosis with osteomyelitis (BCO) is a leading cause of lameness in broiler chickens and has been associated with bacterial translocation from the gastrointestinal tract. This study evaluated the effects of an early-life *Enterococcus lactis* probiotic spray application and a continuous dietary Triple-Strain *Bacillus*-based probiotic supplementation on intestinal barrier function,

immune responses, and BCO development in broilers exposed to an aerosol transmission challenge model. A 56-d experiment was conducted using four treatment groups: negative control (NC), dietary *Bacillus* probiotic (PRO F), hatchery-applied *E. lactis* probiotic (PRO H), and the combined treatment (PRO H/F). Ileal and tracheal tissues were collected on d 7, 28, and 56 for analysis of tight junction gene expression, including claudin-1 (CLDN1), claudin-5 (CLDN5), occludin (OCLN), zonula occludens-1 (ZO-1), and zonula occludens-2 (ZO-2). Serum immunoglobulin M (IgM), immunoglobulin A (IgA), and serotonin concentrations were also determined. Treatment significantly affected ileal tight junction gene expression ($P \leq 0.05$), with pronounced increases in OCLN and ZO-1 expression observed at d 7 and sustained through d 56 in probiotic-treated birds. The greatest responses were detected in the combined PRO H/F group. Tracheal gene expression responses were gene-dependent and less consistent across sampling periods. Serum IgM and IgA concentrations were not affected by treatment ($P > 0.05$), although IgA increased with age ($P < 0.0001$). Serum serotonin concentrations were elevated in birds receiving both probiotic interventions, indicating modulation of host serotonergic pathways. Bacterial recovery from affected birds revealed a polymicrobial profile dominated by *Staphylococcus* spp. These results demonstrate that early life microbial exposure of newly-hatched chicks with a specific lactic acid bacteria strain, combined with continuous diet supplementation with a multi-strain *Bacillus*-based probiotic, improves intestinal barrier function and alters host physiological responses associated with resistance to BCO. The findings support the use of integrated probiotic strategies as a non-antibiotic approach to enhance broiler health and reduce lameness under commercial production conditions.

Keywords: Bacterial Chondronecrosis; Osteomyelitis ; Broiler lameness

108 First confirmed report of *Campylobacter hepaticus* in commercial laying hens in Brazil

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We aimed to report the first isolation of *Campylobacter hepaticus* in commercial laying hens in Brazil. On a commercial farm in the state of São Paulo, floor-reared Hy-Line Brown and Bovans Brown hens, starting at 26 weeks of age, showed a mean 4% drop in egg production and an increased weekly mortality rate of 0.35%. Clinically, affected birds were found dead without specific signs. Treatment with amoxicillin led to temporary improvement, followed by recurrence after approximately 30 days. At 40 weeks of age, eight hens were necropsied, and liver, small white ovarian follicles, and bile samples were collected for microbiological and histopathological analyses. Conventional and non-targeted bacteriology, blood culture-based isolation of fastidious bacteria, and specific isolation of *Salmonella* and *Campylobacter* spp. were performed, along with *Campylobacter* genus PCR, *C. hepaticus* qPCR, and histopathology. Necropsy revealed multifocal white-gray spots in the liver, measuring 1–2 mm in diameter. The ovary was congested, and splenomegaly was observed. Microscopically, the liver exhibited multifocal to coalescing areas of necrosis associated with fibrin deposition. Surrounding these areas, there was moderate heterophilic and lymphohistio-

cytic inflammation, occasionally containing multinucleated giant cells. The spleen showed mild multifocal heterophilic splenitis with moderate congestion. The ovary showed mild to moderate multifocal heterophilic and lymphoplasmacytic oophoritis involving white follicles, with associated hemosiderosis in some samples. All microbiological analyses were negative, except for *Campylobacter*, for which cultures from two of the eight liver samples showed suggestive morphology. DNA was extracted from the liquid culture medium, in which *Campylobacter*-like organisms were observed, as well as from all liver samples for PCR analysis. Conventional PCR targeting the *Campylobacter* genus was positive in 7 of 8 liver samples and was also positive in the culture sample. *C. hepaticus* was confirmed in one liver sample and in the culture material. The gross lesions and histopathological findings, supported by bacteriological and molecular analyses, were consistent with spotty liver disease (SLD) caused by *C. hepaticus*. This disease is considered an important emerging condition in commercial laying hens and has been reported in countries such as the United States, Australia, several European countries, and Costa Rica. This represents the first report of *C. hepaticus*-associated SLD in Brazil, highlighting the need for surveillance to prevent its dissemination among commercial laying hen farms.

Keywords: spotty liver disease ; Brazil; poultry

109 Effects of a Monoglyceride Blend on Cecal Colonization by *Salmonella* Heidelberg in Experimentally Challenged Broiler Chickens

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Salmonella Heidelberg (SH) is an important foodborne pathogen in poultry due to its ability to colonize broilers and contaminate poultry products. Monoglycerides derived from short- and medium-chain fatty acids have emerged as promising tools for pathogen control because of their antimicrobial activity against enteric pathogens. Therefore, the objective of this study was to evaluate the effects of Celtz[®] GH (SAN Group), a feed additive composed of a proprietary blend of monoglycerides, on cecal colonization by SH in experimentally challenged broiler chickens. A total of 120 one-day-old Cobb[®] female chicks originating from *Salmonella*-negative breeder flocks were allocated to two treatments: positive control (n = 70) and Celtz[®] GH supplementation (n = 50), at Mercolab Research Center (Cascavel, Paraná, Brazil). Birds received routine vaccinations but no antibiotics or probiotics. On day 3, all birds were orally challenged with 1×10^7 CFU of a field strain of SH. Feed, litter, environmental, and chick samples were negative for *Salmonella* spp. before challenge. Cecal SH counts were determined using the Most Probable Number (MPN) method, and challenge efficacy was confirmed by SH recovery in all birds after inoculation. Samples collected at 14 and 28 days of age were analyzed using a Tobit censored regression model, considering the upper analytical detection limit of 11,000 MPN/g. At 14 days of age, birds supplemented with Celtz[®] GH exhibited significantly lower cecal SH counts compared with the positive control group (3,570 vs. 6,558 MPN/g; P = 0.034), representing a 45.6% reduction in bacterial load. No significant differences were observed in mean MPN counts between treatments at 28 days of age (P = 0.986). MPN distribution analysis showed that only 5% of supplemented birds were classified in the highest bacterial load category (>10,000 MPN/g) at 14 days, compared with 40% of controls. Additionally, 80% and 70% of supplemented birds were classified within the lowest category (≤ 500 MPN/g) at 14 and

28 days, respectively, versus 50% of controls at both ages. These findings suggest lower intestinal SH burdens and reduced infection pressure in supplemented birds. Under the conditions of this study, dietary supplementation with Celtz[®] GH reduced intestinal colonization by SH during the starter phase of broiler production. Although mean counts did not differ at 28 days, MPN distribution suggested sustained control of intestinal SH colonization and lower infection pressure in treated birds, supporting the use of monoglycerides as part of a holistic *Salmonella* control strategy for food safety.

Keywords: Food safety; Gut health; Intestinal colonization; Pathogen control; Poultry production

110 Simultaneous detection and characterization of multiples infectious bronchitis virus strains in poultry samples

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Surveillance of infectious bronchitis virus (IBV) in vaccinated flocks requires molecular differentiation of vaccine and field strains. A frequent limitation of routine testing is the characterization of samples containing more than one IBV strain, including mixtures of different vaccine viruses or combinations in which one or more field viruses are present. Therefore, detection of a vaccine strain in a sample should not be interpreted as evidence that other vaccine or field strains are absent when the diagnostic workflow is not designed to resolve mixed IBV populations. This is particularly relevant when pooled material and multiple sample matrices are submitted from field cases. Here we present data on IMSentinel-IBV (Imunova), a targeted proprietary molecular assay developed for IBV genotype and strain classification in avian clinical samples which can detect and provide relative signal estimation for several strains in the same sample. Data were obtained from field submissions and organized by sample matrix before analysis. The assay was applied to tracheal swabs, cloacal swabs, cecal tonsils, ovary, kidneys and other organs, including equivalent samples preserved on FTA cards. Samples were processed according to matrix and tested by IMSentinel-IBV for IBV genotype and strain classification. No inferential statistical analysis was applied, as this was a qualitative field report. The matrix classification work suggested higher detection performance in respiratory swabs than in their respective respiratory tissues. In one cloacal swab pool, the assay detected a GI-23 vaccine virus together with a GI-11 field virus. In a pool of respiratory tissues, the assay discriminated between a vaccine virus and a field virus, both classified as GI-11. These findings indicate the usefulness of simultaneous detection of several strains in the same sample (or pooled samples) for routine field analysis. This approach may help IBV monitoring in vaccinated flocks, especially where vaccine virus circulation, field infection and mixed viral populations need to be evaluated from the same diagnostic submission.

Keywords: Infectious bronchitis virus; IMSentinel-IBV; field samples; strain classification; pooled samples

111 Agar diffusion is not an appropriate method for evaluating the activity of short-chain fatty acids and protected butyrate products against *Salmonella*

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In vitro evaluation of organic acids is an important strategy for screening compounds with antibacterial activity against pathogens such as *Salmonella*. Short-chain fatty acids (SCFAs) have several biological properties, but their volatility and rapid absorption may limit their intestinal availability. To overcome this, commercial products containing protected or salt forms of butyrate have been developed. This study compared laboratory methodologies for evaluating SCFAs and butyrate-based commercial products against *Salmonella*. Three methods were assessed: bacterial count, agar diffusion inhibition halo, and minimum inhibitory concentration (MIC). One strain of *Salmonella Heidelberg* (SH) and one of *Salmonella Minnesota* (SM), both isolated from poultry farms, were tested. Acetic and butyric acids served as controls. Three commercial products were evaluated: (i) protected butyrate containing alpha-monobutyryl (13%), dibutyryl (11%), and tributyrin (27%) (BT), (ii) 30% sodium butyrate protected with a 70% polymer coating (SB); and (iii) 98% sodium butyrate protected with a 2% polymer coating (SBC). At 1 mL/L, acetic and butyric acids showed similar behavior. Neither one produced inhibition halos, but both completely inhibited SH and SM in the bacterial count assay (100% inhibition). The MIC of butyric acid was 500 µg/mL. For BT, SB, and SBC, simulated gastrointestinal release with taurocholic acid was unnecessary, as pH-based release alone was sufficient. At recommended inclusion levels (BT: 500 g/ton, SB: 600 g/ton, SBC: 150 g/ton), no inhibition halos were observed. However, bacterial count revealed that BT reduced SH and SM by 2.35 and 2.49 log CFU/mL (99.56% and 99.63%), respectively, while SB reduced SH and SM by 2.10 and 1.54 log CFU/mL (99.21% and 97.13%). SBC showed no reduction in either strain. BT presented a MIC of 2 mg/mL (≈2 kg/ton), whereas SB showed a MIC >2.4 mg/mL and SBC a MIC of 1.2 mg/mL. Overall, the results indicate that the agar diffusion assay is not suitable for evaluating free organic acids or protected butyrate products. For free acids, the lack of inhibition halos is likely related to their volatility. For protected products, poor diffusion, incomplete release, the hydrophobicity of butyrate glycerides, and polymer coatings may limit halo formation, leading to an underestimation of antibacterial activity. In contrast, bacterial count and MIC assays provided more reliable assessments by allowing closer contact between the compounds and bacterial cells, making them more appropriate methods for evaluating the antimicrobial activity of protected butyrate products.

Keywords: Antimicrobial activity; Bacterial count assay; Short-chain fatty acids; Minimum inhibitory concentration; Protected sodium butyrate

112 Influence of Inoculation Route on the Toxicity and Pharmacokinetics of Commercial and Nanoformulated Drugs in the Chicken Embryo Model

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The chicken embryo is an attractive experimental model for preclinical pharmacokinetic and toxicity studies because of its low cost, ease of handling, and ethical advantages. However, the influence of different inoculation routes on drug behavior has not been fully established. This study evaluated the effects of four inoculation routes (shell membrane, allantois, chorioallantoic membrane, and amnion) on the toxicity of commercial and nanoformulated norfloxacin, indomethacin, and lidocaine, as well as on the pharmacokinetics of commercial and nanoformulated norfloxacin. Ten-day-old embryos received five concentrations of each formulation, and toxicity was assessed at day 16 through mortality, body weight, and histopathological examination of liver and kidney tissues. Pharmacokinetic analyses were performed after a single administration of norfloxacin by collecting serum and allantoic fluid at 30 min, 1, 4, and 24 h post-inoculation. No lethal dose (LD50) was observed for any treatment. Embryo weight was unaffected by norfloxacin, whereas commercial lidocaine increased body weight and indomethacin reduced it regardless of formulation. Histopathological lesions were detected exclusively after amniotic administration of nanoformulated drugs. Norfloxacin induced renal inflammation and hepatic degeneration, lidocaine caused renal lesions, and indomethacin produced liver damage. Pharmacokinetic parameters could only be determined for the commercial formulation. Maximum serum concentration was reached 1 h after inoculation for all routes except the amnion, in which C_{max} occurred at 24 h. Norfloxacin was detected in allantoic fluid only following amniotic administration. These findings demonstrate that inoculation route strongly influences both drug disposition and toxicity in the chicken embryo model. The amniotic route led to slower absorption, delayed systemic exposure, and greater toxicity, whereas the remaining routes resulted in rapid absorption and elimination, with no detectable drug levels in the allantoic fluid. Therefore, the route of inoculation should be carefully considered when using chicken embryos for pharmacokinetic studies, particularly with nanoformulated compounds.

Keywords: drug delivery; embryo model; histopathology; nanoformulation; pharmacokinetics

Metabolism and Nutrition: Feed Additives II

113 Use of Black Wattle Tannin Associated or not with Lecithin in Diets for Broiler Chickens

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This study aimed to evaluate the effect of replacement of antibiotics with black wattle condensed tannins, alone and combined with lecithin, to assess production of short chain fatty acids, intestinal histomorphometry, and performance of broiler chickens from 1 to 42 days of age challenged with a vaccine strain of *Eimeria* spp.

A total of 720 Ross 308 AP broiler chicks were used, distributed in a completely randomized design with four treatments and ten replicates of 18 birds each. The treatments consisted of: T1 - NC (Negative Control) without the addition of growth promoters or anticoccidials; T2 - PC (Positive Control) + growth promoter and anticoccidial; T3 - NC + 500 g/ton of black wattle tannin; and T4 - NC + 500 g/ton of black wattle tannin combined with lecithin. The birds received isoproteic and isoenergetic corn and soybean meal-based diets formulated according to the recommendations of the Aviagen strain (2022), with feed and water provided *ad libitum*. At 14 days of age, the birds were challenged with a commercial coccidiosis vaccine, Biococcivet R, at a dose 20 times the manufacturer's recommended level which contained a concentrated suspension of sporulated oocysts of five *Eimeria* spp (*E. acervulina*, *E. praecox*, *E. maxima*, *E. tenella*, and *E. mitis*). Performance, short-chain fatty acid concentrations, and jejunal and ileal histomorphometry were evaluated at 28 days of age. The data were subjected to a normality test, followed by analysis of variance, and when significant effects were observed ($P < 0.05$), means were compared using Tukey's test at the 5% significance level. Birds in the negative control treatment showed the poorest performance results, particularly regarding weight gain, whereas the positive control and the inclusion of black wattle tannin improved feed conversion ratio ($P = 0.0013$). Regarding short-chain fatty acid production, the inclusion of black wattle tannin combined with lecithin promoted greater propionic acid production ($P = 0.0002$). On the other hand, birds receiving diets containing antibiotics and black wattle tannin showed higher butyrate concentrations ($P = 0.0021$). Regarding jejunal histomorphometry, treatment 4 exhibited deeper crypts. Better morphological balance was observed in birds fed diets containing antibiotics and black wattle tannin ($P = 0.0019$). The use of black wattle tannin combined with lecithin resulted in a greater ileal absorptive area ($P = 0.0001$) compared with the other treatments. The inclusion of black wattle tannin stimulated propionate and butyrate production, enhancing gut health. Consequently, these tannins serve as a viable alternative to antibiotics for controlling vaccine-strain *Eimeria* spp in broilers.

Keywords: antibiotics; intestinal histomorphometry; lecithin; performance; short chain fatty acids

114 Evaluation of Chestnut (*Castanea sativa*) Tannin in Diets for Broiler Chickens Challenged with Vaccine *Eimeria*: Effects on Growth Performance and Carcass Traits

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The use of antibiotic growth promoters in broiler production has been progressively restricted worldwide, increasing the interest in phytogetic alternatives such as hydrolyzable tannins. This study

evaluated the effects of increasing dietary levels of hydrolyzable chestnut (*Castanea sativa*) tannin on growth performance of broiler chickens challenged with vaccine *Eimeria*. A total of 1,330 one-day-old male AP95 broiler chicks (initial body weight = 44.24 ± 0.53 g) were assigned to a completely randomized design with seven dietary treatments, ten replicates, and 19 birds per experimental unit. Experimental diets consisted of a basal diet without antibiotic growth promoters or anticoccidial drugs, supplemented with 0, 200, 400, 500, 750, 1,000, or 1,500 g/ton of hydrolyzable chestnut tannin. Birds and feed were weighed on d 1, 21, and 42 to determine feed intake, body weight gain, feed conversion ratio, and productive efficiency index. Data were tested for normality and analyzed by ANOVA, and treatment means were compared using Tukey's test when significant ($P < 0.05$). Significant effects on performance were observed during the starter phase, in which 400 and 500 g/ton of hydrolysable tannin resulted in higher body weight and weight gain with a lower feed conversion ratio ($P < 0.05$), compared to birds fed no additive. Considering the whole period, birds fed 400 g/ton of hydrolysable tannin showed a higher productive efficiency index than those receiving the control diet without tannin supplementation. In conclusion, dietary supplementation with 400 g/ton of hydrolysable tannin improves performance during the starter phase and enhances productive efficiency in broilers challenged with vaccine *Eimeria*, representing a promising alternative for broiler production under coccidial challenge.

Keywords: broiler chickens; carcass yield; coccidiosis; hydrolyzable tannin; phytogetic feed additive

115 Effects of dietary bile salt supplementation in broilers fed different lipid sources and metabolizable energy densities

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The present study evaluated the effects of dietary bile salt supplementation on growth performance, carcass characteristics, and feed cost of broilers fed diets formulated with different lipid sources and metabolizable energy densities. A total of 1,638 Cobb 500 male broilers were allocated in a completely randomized design from 7 to 42 d of age. Birds were distributed into 13 treatments with seven replicates of 18 birds each. Experimental diets were based on corn and soybean meal and formulated with three fat sources (poultry fat, vegetable soapstock, or degummed soybean oil), two metabolizable energy densities (high or low), and bile salt supplementation at 150 g/t. Growth performance was evaluated weekly by body weight, weight gain, feed intake, feed conversion ratio (FCR), and corrected FCR. At 42 days of age, carcass yield, commercial cuts, abdominal fat, and feed cost per kilogram of live weight were determined. Data were analyzed by ANOVA using SAS, and treatment means were compared using Tukey's test at $P \leq 0.10$. Dietary energy density significantly improved body weight, weight gain, and FCR throughout the experimental period ($P < 0.10$), confirming the expected response to increased nutrient supply. Bile salt supplementation enhanced FCR during the starter phase and improved both FCR and corrected FCR over the entire 7–42 d period ($P \leq 0.10$), demonstrating greater efficiency of nutrient utilization. Responses were more pronounced in diets containing poultry fat and vegetable soapstock, particularly under reduced-energy conditions, indicating that bile salts were able to compensate for the lower dietary energy concentration partially. Significant interactions between dietary energy and lipid source were observed for fasting body weight, whereas bile salts increased fasting body weight in birds fed low-energy

diets. Feed intake and mortality were not affected by treatments ($P > 0.10$). Carcass yield and commercial cut yields remained unchanged; however, bile salt supplementation numerically reduced abdominal fat deposition and lowered feed cost per kilogram of live weight, indicating improved utilization of dietary lipids and greater economic efficiency. In conclusion, dietary supplementation with bile salts at 150 g/t consistently improved feed efficiency without adversely affecting carcass yield or bird viability. The beneficial effects were most evident in reduced-energy diets, supporting the use of bile salts as an effective nutritional strategy to improve lipid digestion, nutrient utilization, and production efficiency in broilers fed different lipid sources.

Keywords: bile salts; feed conversion ratio; lipid sources; metabolizable energy; performance

116 Spray-dried microencapsulated herbal extracts associated with biogenic silver nanoparticles in broiler diets

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The objective of this study was to evaluate the effects of dietary supplementation with spray-dried microencapsulated herbal extracts associated with biogenic silver nanoparticles on growth performance and duodenal and jejunal intestinal histomorphometry of broilers at 42 days of age challenged with *Clostridium perfringens* and vaccine-derived *Eimeria* spp. A total of 1,200 one-day-old male Ross 308 AP broiler chicks were randomly assigned to a completely randomized design with five treatments, 10 replicates, and 24 birds per experimental unit. The treatments consisted of five diets supplemented with increasing levels (0, 100, 200, 400, and 800 g/ton of feed) of a spray-dried microencapsulated complex containing herbal extracts associated with biogenic silver nanoparticles. All birds were challenged by gavage at 4 and 7 days of age with a 20-fold dose of an *Eimeria* spp., vaccine and at 10 days of age with *Clostridium perfringens* at a concentration of 10^8 CFU. The birds and feeding were weighed at the beginning of the experiment and at 10, 21, 35, and 42 days of age to determine feed intake, body weight gain, and feed conversion ratio. At 42 days of age, one bird per experimental unit was euthanized, and jejunal and ileal segments were collected for intestinal histomorphometry analysis. Data were subjected to homogeneity (Levene's test) and normality (Shapiro-Wilk test) assessments. Outliers were removed, and one-way analysis of variance was performed using the GLM procedure. When significant differences were detected, means were compared using Tukey's test at a 5% significance level. At 42 days of age, no differences were observed in feed intake, body weight gain, feed conversion ratio, or jejunal histomorphometry ($P > 0.05$). However, in the duodenal segment, an effect was observed on villus height ($P = 0.0139$), showing a quadratic response ($Y = 818.99016 + 0.46659x - 0.00051519x^2$; $R^2 = 0.56$), with the maximum estimated villus height obtained at an inclusion level of 452.8 g/ton of the feed additive. It was con-

cluded that the inclusion of spray-dried microencapsulated herbal extracts associated with biogenic silver nanoparticles maintained broiler performance and intestinal morphology under *Eimeria* spp., and *Clostridium perfringens* challenge conditions, indicating its potential as a feed additive for broilers.

Keywords: *Clostridium perfringens*; *Eimeria* spp; intestinal health; poultry

117 Use of different tannins in diets of broiler chickens challenged with a coccidiosis vaccine: effects on performance and carcass traits

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This study evaluated the effects of two different tannin sources in diets of broiler chickens challenged with a coccidiosis vaccine on growth performance and carcass characteristics. A total of 720 one-day-old male Ross AP308 broilers, with an initial body weight of 45.19 ± 0.41 g, were allotted to a completely randomized design with four dietary treatments, ten replicates per treatment, and 18 birds per replicate. The treatments consisted of a negative control diet (basal diet without antibiotic growth promoters or anticoccidials), a positive control diet (basal diet supplemented with avilamycin, nizarbazin, and maduramicin), the negative control diet supplemented with 500 g/ton of black wattle tannin, and the negative control diet supplemented with 500 g/ton of eucalyptus tannin. At 14 d of age, all birds were fasted for 8 h and orally challenged with 20 times the recommended dose (0.6 mL) of a commercial coccidiosis vaccine. Body weight and feed intake were recorded from 1 to 42 d to determine body weight gain, feed intake, feed conversion ratio, and productive efficiency index. At 42 d of age, two birds per replicate were slaughtered for evaluation of carcass yield, cut yields, and organ weights. Data were tested for normality and analyzed by analysis of variance. When significant effects were detected ($P < 0.05$), means were separated using Tukey's test. Among the performance variables, only feed conversion ratio was affected ($P = 0.0002$), with birds fed eucalyptus tannin showing poorer feed conversion than the other treatments. Regarding carcass traits, only abdominal fat percentage differed among treatments ($P = 0.0406$). Birds fed black wattle tannin showed lower abdominal fat deposition than those receiving the positive control diet. Supplementation with 500 g/ton of black wattle tannin can be used in diets of broiler chickens challenged with a coccidiosis vaccine up to 42 d of age without impairing growth performance while reducing abdominal fat deposition.

Keywords: alternative feed additives; broiler chickens; carcass traits; condensed tannins; functional nutrition

118 Dietary isoquinoline alkaloids, including canadine, beneficially affected broiler performance, breast yield and quality

traits evidenced via thorough texture, biochemical and molecular analyses

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Chicken meat is widely recognized for its high nutritional value, mainly due to its high protein concentration and relatively low-fat content. Isoquinoline alkaloids (IQs), including canadine, may modulate meat quality molecular responses, potentially influencing breast meat yield and quality traits in broilers. Therefore, the objective of this study was to evaluate the effects of dietary *Macleaya cordata* IQ feed additives differing in canadine content on growth performance, carcass traits, breast meat quality, antioxidant status and breast muscle molecular responses of broilers. A total of 468 one-day-old male Ross 308 broilers were allocated to 4 dietary treatments with 9 replicate pens/treatment and 13 birds/pen for 35d. Treatments were: negative control (NC) and three IQ-based feed additives (Phytobiotics Futterzusatzstoffe GmbH, Germany) namely a canadine standard (CA), canadine-free Sangrovit® WS premix (WS), and Sangrovit® Extra premix comprising the WS and native canadine (SE). Growth performance responses were evaluated per growth phase and reported for the overall 35d period. At d 35, nine birds/treatment were sampled for the evaluation of carcass yield, breast meat physicochemical traits, oxidative status and the expression of critical genes related to antioxidant defense, protein synthesis, myogenesis and lipid metabolism. Data were analyzed by ANOVA followed by Tukey's HSD test ($P \leq 0.05$). Results revealed that FCR, European Production Efficiency Factor, protein and energy efficiency factors were improved ($P < 0.01$) primarily in SE treatment and secondarily in WS and CA, compared to NC. Additionally, improvements ($P < 0.01$) were observed in breast yield and lean meat percentage, primarily in birds receiving SE and CA supplementation, and secondarily in those receiving WS. In addition, abdominal fat deposition was reduced ($P < 0.001$) in CA and SE treatments. Moreover, IQs supplementation increased ($P < 0.001$) water holding capacity with the highest percentage noted in SE, while resulted in lower ($P < 0.01$) hardness, chewiness, and overall mechanical resistance of cooked breast meat at d35. In terms of antioxidant status of the breast meat, IQs supplementation reduced ($P = 0.001$) MDA levels, while SE enhanced ($P = 0.018$) total antioxidant capacity. Nutrigenomic analyses revealed that IQs supplementation beneficially modulated ($P \leq 0.05$) 23 out of 36 genes assessed relative for overall meat quality, with the most pronounced effects noted in SE. Overall this work provides in-depth evidence supporting IQs benefits for broiler performance and meat quality improvements.

Keywords: broiler; isoquinoline alkaloids; canadine ; nutrigenomics; meat quality

119 Microencapsulated organic acids and essential oils mitigate intestinal lesions in enteric-challenged broilers

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Enteric pathogens compromise intestinal integrity and impair gut function in broiler chickens. Therefore, this study evaluated the effects of dietary inclusion of a microencapsulated blend of organic acids and essential oils on jejunal histopathology assessed by the

I See Inside (ISI®) methodology in broilers submitted to an enteric challenge. A total of 1,200 broilers were distributed in a completely randomized design with six treatments (10 replicates of 20 birds): negative control (NC, no challenge/additive), positive control (PC, challenged, no additive), and four challenged groups receiving 250, 500, 750, and 1.000 g t⁻¹ of the additive. The feed additive is a blend of microencapsulated short- and medium-chain organic acids, thymol, and carvacrol (Microacid Eoils Plus, Tectron, Brazil). Birds except those in the NC group received a 30× overdose of an unattenuated coccidiosis vaccine at 14 d, followed by oral inoculation of *Clostridium perfringens* (1x10⁸ CFU mL⁻¹) at 17 and 18 d. Jejunal samples were collected at 23 d, processed for histopathology, stained with hematoxylin-eosin and Alcian Blue, and evaluated using the ISI methodology. Parameters included lamina propria and epithelial thickness, enterocyte and goblet cell proliferation, inflammatory infiltration, congestion, presence of *Eimeria* spp., and Total ISI Score. Data were analyzed via Kruskal-Wallis and Dunn's post-hoc tests ($P < 0.05$). The enteric challenge was effective in inducing jejunal histopathological alterations, as evidenced by higher Total ISI Score and histopathological lesion scores in challenged birds compared with the negative control group ($P < 0.05$). Among challenged birds, dietary supplementation with 500 g t⁻¹ or higher reduced the Total ISI Score compared with the PC, with the lowest score observed at 1.000 g t⁻¹ (9.21 vs. 12.25; $P < 0.0001$). This improvement was associated with reduced lamina propria thickness, epithelial inflammatory infiltration, and congestion, indicating attenuation of challenge-induced inflammation. The most pronounced histopathological improvement was observed at 1,000 g t⁻¹, which showed the lowest Total ISI Score together with reduced epithelial inflammatory infiltration and congestion. In conclusion, dietary supplementation with a microencapsulated blend of organic acids and essential oils at 500 g t⁻¹ or higher attenuated challenge-induced jejunal histopathological alterations and inflammatory lesions in broilers. The improvement in histopathological scores supports the use of this additive as a nutritional strategy to mitigate intestinal damage during enteric challenges, with the greatest response observed at 1,000 g t⁻¹.

Keywords: *Clostridium perfringens*; *Eimeria* spp.; Intestinal integrity ; I See Inside; histopathology

120 Different tannins as alternatives to antibiotics in diets of broiler chickens challenged with a live *Eimeria* vaccine

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The objective of this study was to evaluate the replacement of antibiotic growth promoters with different tannin sources in diets of broiler chickens challenged with a live *Eimeria* vaccine and its effects on breast meat quality at 42 days of age. A total of 760 male Ross AP95 broilers were used in a completely randomized design with four treatments and ten replicates of 19 birds each. The treatments consisted of positive control diet (PC) containing growth pro-

moters, Enramax (8% enramycin), and anticoccidials (nicarbazin + narasin and salinomycin); a negative control diet (NC) without additives; the NC diet supplemented with 1,000 g/ton of condensed tannin (black wattle); and the NC diet supplemented with 1,000 g/ton of hydrolysable and condensed tannins (chestnut and quebracho). At 14 days of age, all birds were orally challenged with 20 times the recommended dose of a live *Eimeria* spp vaccine. At 42 days of age, samples of the *Pectoralis major* muscle were collected from one bird per experimental unit to evaluate pH, breast meat color (L*, a*, b*) at 15 min and 24 h postmortem, water-holding capacity, cooking loss, and shear force. Lipid oxidation (TBARS) was evaluated at 0, 7, and 30 days after slaughter. Data were tested for normality, analyzed by ANOVA, and means were compared by Tukey's test at the 5% significance level. No effects ($P>0.05$) were observed on meat pH or color at either 15 min or 24 h postmortem. Similarly, diet did not affect cooking loss, water-holding capacity, or lipid oxidation at 0 and 7 days of storage ($P>0.05$). Shear force increased ($P=0.0061$) in the breasts of birds fed black wattle tannin, whereas lipid oxidation after 30 days of storage was reduced ($P=0.0307$) in breasts from birds fed black wattle tannin. The use of antibiotic growth promoters in broiler diets can be replaced by black wattle, chestnut, and quebracho tannins without impairing breast meat quality, while black wattle tannin provides greater protection against lipid oxidation after 30 days of storage.

Keywords: coccidiosis; condensed tannins; hydrolyzable tannins; lipid oxidation; meat tenderness

121 Effect of *Eucalyptus cloeziana* tannin, alone or combined with soybean oil, in broiler chickens challenged with *Eimeria* spp. at 21 days of age

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The objective of this study was to evaluate the effects of dietary supplementation with *Eucalyptus cloeziana* tannin on growth performance, short-chain fatty acid (SCFA) concentration, and jejunal and ileal histomorphometry of broiler chickens challenged with *Eimeria* spp. A total of 720 one-day-old male Ross 308 AP broiler chicks were randomly assigned to a completely randomized design with four treatments, 10 replicates, and 18 broilers per experimental unit. The treatments consisted of: (1) negative control (NC), a basal diet without growth promoter or anticoccidial; (2) positive control (PC), a basal diet supplemented with an anticoccidial and growth promoter (from 1 to 42 d of age); (3) NC + *Eucalyptus* tannin (NCET); and (4) NC + *Eucalyptus* tannin combined with soybean oil (NCETSO). At 14 d of age, birds were challenged with *Eimeria* spp. At 21 d of age, one bird per experimental unit was euthanized for collection of jejunal and ileal samples for histomorphometric evaluation, as well as cecal contents for determination of SCFA concentrations. Data were tested for normality and subsequently analyzed by analysis of variance. When significant effects were detected ($P < 0.05$), means

were compared using Tukey's test at the 5% significance level with SAS (2026). At 21 d of age, birds fed the PC, NCET, and NCETSO diets showed lower feed intake ($P = 0.0092$), greater body weight gain ($P = 0.0307$), and improved feed conversion ratio ($P = 0.0009$) compared with those fed the NC diet. Regarding SCFA production, butyric acid concentration was higher in birds fed the PC and NCET diets ($P = 0.0039$). In the jejunum, villus height was greater in birds fed the NCETSO diet ($P = 0.0012$), whereas crypt depth was lower in birds fed the NC, PC, and NCET diets ($P = 0.0024$). Villus absorptive surface area was greater in birds fed the NCET and NCETSO diets ($P = 0.0005$). In the ileum, the villus-to-crypt ratio was higher in birds fed the NC, PC, and NCET diets ($P = 0.0113$), while absorptive surface area was greater in birds fed the PC, NCET, and NCETSO diets ($P = 0.0017$). In conclusion, dietary supplementation with *Eucalyptus* tannin, either alone or combined with soybean oil, improves feed utilization efficiency in broiler chickens challenged with *Eimeria* spp. at 21 d of age by enhancing selected morphological characteristics of the jejunum and ileum.

Keywords: *Eimeria* challenge; *Eucalyptus cloeziana*; intestinal histomorphometry; performance; tannin

122 EFFECTS OF A SYMBIOTIC ON THE PRODUCTIVE PERFORMANCE OF HISEX WHITE LAYING HENS FROM 20 TO 85 WEEKS

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Objective: To evaluate the effects of dietary inclusion of a symbiotic (Polaris[®]; Ekoa Life Sciences, Brazil) on productive performance as feed intake (FI), egg production (EP), egg weight (EW), egg mass (EM), and feed conversion ratio (FCR) of commercial laying hens from 20 to 85 weeks. **Experimental design:** It was completely randomized in a 4x2 factorial scheme: 4 inclusion levels of Polaris[®] (0.00, 0.05, 0.10, and 0.15%), and 2 levels of zinc bacitracin (ZB) (0.00 and 0.01%), totaling 8 treatments, 6 replicates of 4 birds each. **Materials and methods:** The experiment was approved by the Ethics Committee on Animal Use of FZEA/USP (CEUA 6768100524). A total of 192 Hisex White laying hens were housed from 20 to 85 weeks of age. Performance characteristics were assessed on a weekly basis during the periods of 20 to 34, 35 to 51, 52 to 68, and 69 to 85 weeks. The performance characteristics were FI (g/b/d), EW (g), EP (% egg/b/d), EM (g/b/d), and FCR (g feed/g egg mass). The birds were weighed, and body weight uniformity was calculated at 17 weeks of age and at the end of each evaluated period. Feed and water were provided ad libitum. Polaris[®] is composed of the bacteria *Enterococcus faecium*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Bacillus subtilis*, the yeasts *Saccharomyces cerevisiae* and *Saccharomyces boulardii*, and the prebiotics galactooligosaccharides (GOS) and mannanoligosaccharides (MOS), and the ZB dosage was 55 ppm. The cage/ bird space was 1,250 cm², and the aviary was open and equipped with fans. **Statistical analysis:** Two-way ANOVA with polynomial regression for Polaris[®], and fixed-factor analysis for ZB. Significant interactions ($P\leq 0.05$) were unfolded using the F-test. **Results:** In the analysis of the total period (20 to 85 weeks), EP was significantly affected with 96.4% egg/b/d using 0.05% of Polaris[®] ($P=0.013$). A significant Polaris[®] x ZB interaction was detected for EM ($P=0.082$), with Polaris[®] 0.10% without ZB yielding 58,21 g/b/d ($P=0.038$),

and for FCR ($P=0.022$), with Polaris® 0.10% without ZB yielding the lowest FCR (1.763 g/g, $P=0.047$). FI and EW were not significantly affected by the factors studied ($P>0.05$). **Conclusions:** Polaris® at levels of 0.05% and 0.10% improved EP, EM, and FCR of Hisex White laying hens over a 65-week production cycle, meeting strain manual targets and outperforming ZB. These results support the use of this symbiotic as a sustainable alternative to replace antibiotic growth promoters in commercial laying hen production. **Keywords:** antibiotic growth promoters, egg production, feed conversion ratio, prebiotic, probiotic.

Keywords: antibiotic growth promoters; egg production; feed conversion ratio; prebiotic; probiotic

123 Effects of acid silage from pirarucu (*Arapaima gigas*) by-products on physiological responses of slow-growing broilers

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The objective of this study was to evaluate the effects of dietary inclusion of acid silage from pirarucu (*Arapaima gigas*) by-products (ASP) on hematological and plasma biochemical parameters of slow-growing broilers. A total of 240 male Label Rouge broilers were assigned to a completely randomized design with four dietary treatments containing 0, 1, 2, or 3% ASP, five replicates, and 12 birds per replicate. Blood samples were collected from 10 birds per treatment at the end of the experimental period. Hematological analyses included erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Plasma biochemical analyses included glucose, triglycerides, cholesterol, total protein, albumin, globulin, and urea. Data were subjected to one-way ANOVA, followed by polynomial regression when treatment effects were significant ($P \leq 0.05$). Dietary ASP promoted favorable physiological responses. Hemoglobin, hematocrit, MCV, MCH, total protein, and albumin increased linearly ($P \leq 0.05$) with increasing ASP inclusion. MCHC, triglycerides, and globulin exhibited quadratic responses ($P \leq 0.05$), with the best values observed at approximately 2% ASP inclusion. Erythrocyte count, glucose, cholesterol, and urea were not affected ($P > 0.05$). These findings indicate that dietary ASP improves hematological status and protein metabolism without adversely affecting metabolic homeostasis, supporting the inclusion of 1–2% ASP as a sustainable feed ingredient capable of enhancing the physiological condition of slow-growing broilers.

Keywords: *Arapaima gigas*; blood biochemistry; fish by-products; hematology; poultry nutrition

124 Response of Tight Junction Proteins and Intestinal Histomorphometry to Dietary Supplementation with Muramidase and *Bacillus subtilis* in Broiler Chickens at 21 and 42 Days of Age

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The aim of this study was to evaluate the effects of dietary supplementation with muramidase or *Bacillus subtilis* on intestinal histomorphometry and the expression of tight junction proteins in the jejunum and ileum of broiler chickens at 21 and 42 days of age. A total of 384 Cobb 500 broiler chickens were randomly assigned to three dietary treatments, with eight replicates of 16 birds each. The treatments consisted of: (1) a control diet (CD) with a reduction of 60 kcal/kg metabolizable energy (ME) and 1% crude protein (CP); (2) the CD with 50 g/t muramidase; and (3) the CD with 200 g/t *Bacillus subtilis*. At 21 and 42 days, one bird per replicate was selected and euthanized in accordance with the institutional bioethics committee guidelines. Jejunal and ileal segments were collected, fixed in 10% neutral buffered formalin, and processed using conventional histological techniques for intestinal histomorphometric evaluation. To determine the expression of tight junction proteins, tissue sections were subjected to immunohistochemistry using antibodies against claudin-1, claudin-5, occludin, and zonula occludens-1 (ZO-1). Protein expression was assessed quantitatively using ImageJ. Data normality was evaluated using the Shapiro–Wilk test. Treatment effects were analyzed by ANOVA, followed by Tukey's test, with statistical significance declared at $P < 0.05$. At 21 days of age, muramidase supplementation increased jejunal claudin-1 expression compared with the control diet (186.69 vs. 177.97; $P = 0.035$) and increased ileal claudin-5 expression (90.62 vs. 81.47; $P = 0.003$), whereas *B. subtilis* showed intermediate values. However, jejunal villus width was lower in the muramidase group than in the control and *B. subtilis* groups (81.62 vs. 105.27 and 101.13, respectively; $P = 0.016$). At 42 days of age, muramidase increased ileal occludin expression compared with the control and *B. subtilis* treatments (76.72 vs. 65.22 and 65.77, respectively; $P < 0.001$), as well as jejunal villus width (155.90 vs. 131.09 and 130.63, respectively; $P = 0.007$). The control and muramidase treatments showed greater ileal claudin-5 and ZO-1 expression than the *B. subtilis* treatment ($P \leq 0.001$). In conclusion, dietary supplementation with muramidase or *Bacillus subtilis* in diets containing 60 kcal/kg less metabolizable energy and 1% less crude protein modulated intestinal histomorphometry and the expression of tight junction proteins in broiler chickens at 21 and 42 days of age. However, these responses were influenced by the dietary additive, bird age, intestinal segment, and the specific tight junction protein evaluated.

Keywords: additives; occludin; claudin; intestinal histomorphometry; Zonula Occludens

Processing and Products

150 Quality of Camu-camu-coated eggs after 21 days of storage

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Camu camu (*Myrciaria dubia*) residue has emerged as a promising

and sustainable raw material for the development of egg coatings due to its functional properties and availability as an agro-industrial by-product. This preliminary study evaluated the potential of coatings formulated with camu-camu flour (1, 3, and 5%), combined with propylene glycol (at 2% w/v), to preserve the internal quality of eggs during 21 days of storage at room temperature (24 °C). A completely randomized design was adopted, using one-day-old eggs obtained from hens of the same age and maintained under similar environmental, management, and feeding conditions. Egg quality was assessed based on weight loss, Haugh unit (HU), and albumen pH in uncoated eggs (control) and eggs coated with camu-camu flour. Statistical analyses were performed using Minitab 18. Data was analyzed by analysis of variance (ANOVA), considering each egg as an experimental unit. Uncoated eggs exhibited the highest weight loss (2.96%), whereas egg weight loss during storage ranged from 2.58 to 2.88% in eggs coated with camu-camu. Despite these variations, no statistically significant differences were observed among treatments ($P < 0.05$). HU values ranged from 46.60 to 64.11 for eggs coated with camu-camu. Coatings containing 3% camu-camu exhibited the highest HU (64.11) indicating that this formulation may provide improved maintenance of albumen structure, while uncoated egg presents HU value of 49.19. Albumen pH remained within the expected range throughout storage. The application of camu-camu coating resulted in albumen pH values ranging from 9.45 to 9.48, compared with a pH of 9.49 observed in uncoated eggs. The results indicate that camu-camu flour-based coatings may serve as an effective strategy for maintaining egg internal quality throughout storage, especially when applied at a concentration of 3%. Beyond their technological potential, these formulations represent a sustainable alternative for the valorization of agro-industrial byproducts from the Amazon region.

Keywords: eggshell; edible film; propylene glycol; Haugh unit; shelf-life

151 Nutritionally Enriched Eggs: A Systematic Review

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This systematic review aimed to identify and evaluate dietary strategies for egg enrichment in laying hens, as well as their effects on productive performance and egg quality, following the PRISMA guidelines and the PICO framework. Studies related to egg enrichment were retrieved from the Scopus, Web of Science and PubMed databases, covering the period from 1975 to 2025. Of the 8,094 records identified, 67 studies met the eligibility criteria and were included in the review. Most studies were conducted in China (17 studies, 25.4%). Approximately 72,761 laying hens were used in the included studies. The included studies were categorized according to the nutrient used for enrichment, with minerals being the most frequently investigated group (47 studies, 70.15%), followed by fatty acids and vitamins. Among mineral enrichment strategies, several elements have been used, including iron, zinc, copper, and iodine, with selenium emerging as the most frequently used mineral for egg enrichment across studies. Selenium was used in approximately 58% of the studies, with sodium selenite being the most frequently used form, followed by selenium yeast and nano-selenium. The synthesis of evidence showed that egg enrichment was the most consistent result among the studies. Positive effects of enrichment were reported in 100% of the studies involving fatty acids and vitamins, and in 97.9% of the studies involving minerals. In contrast, the effects on productive performance and egg quality characteristics were less consistent, with most studies reporting no significant effect or no such results. Overall, the results indicate that dietary supplementation is an effective tool for improving the nutritional composition of eggs. However, its effects on productive performance indicators and conventional egg quality parameters are less pronounced and show variability across studies. Therefore, nutritional enrichment of eggs emerges as a practical and relevant strategy for producing value-added foods for human consumption.

Keywords: designer eggs; biofortification; functional eggs; selenium; yolk

Metabolism and Nutrition: Vitamins and Minerals

125 Bone Quality of Broiler Breeder Hens Subjected to Dietary Replacement of the Phosphorus Source

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OBJECTIVE: To evaluate the effects of replacing dicalcium phosphate with Tricinophos® as a dietary phosphorus source on bone quality of broiler breeder hens during the production cycle. **EXPERIMENTAL DESIGN:** Two commercial Ross broiler breeder flocks were evaluated from 24 to 66 wk of age. Each flock consisted of approximately 16,000 females and 1,800 males. One flock received the standard farm diet containing dicalcium phosphate as the phos-

phorus source (FOSF), whereas the second flock received diets containing 0.1% Tricinophos® EP-300 MTZ (TRIC). **MATERIALS AND METHODS:** The study was conducted at a commercial breeder farm located in Uberlândia, Minas Gerais, Brazil. Bone analyses were performed in collaboration with the Department of Animal Morphology and Physiology at UNESP, Jaboticabal, SP, Brazil. At 66 wk of age, 30 tibiae were collected from each treatment for evaluation of bone mineral density, bone mineral content, and bone strength. **STATISTICAL ANALYSIS:** Data were analyzed by analysis of variance (ANOVA), and treatment means were compared using the F-test. Differences were considered significant at $P \leq 0.05$. **RESULTS:** Replacement of the phosphorus source did not affect bone mineral density, with mean values of 0.395 and 0.401 for FOSF and TRIC, respectively ($P = 0.7827$). Likewise, no differences were observed for bone mineral content, which averaged 5.47 and 5.59 g for FOSF and TRIC, respectively ($P = 0.6712$). However, hens fed Tricinophos® exhibited greater bone strength than those fed dicalcium phosphate (40.27 vs. 34.04 kgf; $P = 0.0315$), representing an increase of approximately 18.3%. The absence of differences in bone mineral den-

sity and bone mineral content suggests that this response may be associated with changes in the organic matrix quality of bone tissue rather than increased mineral deposition. **CONCLUSIONS:** Replacing dicalcium phosphate with Tricinophos® maintained bone mineral density and bone mineral content while increasing bone strength in broiler breeder hens at the end of the production cycle, indicating potential to improve bone quality without compromising bone mineralization.

Keywords: phosphorus source; broiler breeders; bone strength; bone quality; bone mineralization

126 Protective effects of 25-Hydroxyvitamin D3 on vitamin D metabolism and organ function in broilers under hepatorenal stress

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Vitamin D is essential for immune function, bone development, and muscle growth, and requires sequential activation in the liver and kidney. This process may be impaired when these organs are affected by stressors. This study evaluated the impact of hepatorenal stress on vitamin D metabolism and compared the efficacy of different dietary vitamin D sources in broiler chickens. A total of 256 one-day-old male Ross 308 broilers were allocated to 32 pens and assigned to four treatments for 35 days. The control group received vitamin D3 (4,000 IU/kg, T1), whereas challenged birds were exposed to Ochratoxin A (OTA, 1,000 ppb), a mycotoxin known to affect kidney and liver function. Challenged groups (T2-T4) received either vitamin D3 (4,000 IU/kg, T2), vitamin D3 (1,240 IU/kg) + 25-hydroxyvitamin D3 (25(OH)D3, 69 µg/kg, T3), or vitamin D3 (4,000 IU/kg) + 1,25-dihydroxyvitamin D3 (1,25(OH)2D3; 1 µg/kg, T4). Blood, kidney, and liver samples were collected to assess vitamin D metabolites, physiological status, and gene expression. Data were analyzed by 2-way ANOVA, and RNAseq data were analyzed using edgeR with Benjamini-Hochberg FDR adjustment; significance was set at $P < 0.05$. Results of circulating vitamin D metabolites showed that OTA challenge (T2) tended to reduce the levels of 1,25(OH)2D3 (active form) compared to T1. Supplementation with 25(OH)D3 (T3) increased 25(OH)D3 and 24,25(OH)2D3 ($P < 0.001$) and resulted in the highest circulating 1,25(OH)2D3 concentration among treatments. In kidney, CYP2R1 expression, involved in vitamin D conversion to 25(OH)D3, was low across treatments but reduced in T2 compared with T1 ($P < 0.05$), suggesting mild impairment of vitamin D metabolism. Liver transcriptomics identified 40 and 71 transcriptional changes at days 21 and 35, respectively, in T2 vs T1, reflecting metabolic disruption linked to oxidative stress, inflammation, and mitochondrial dysfunction. These alterations were markedly reduced in birds receiving 25(OH)D3, with fewer affected genes and normalization of key metabolic pathways, while 1,25(OH)2D3 showed only partial mitigation. Blood biomarkers related to renal nitrogen handling, electrolyte balance, protein metabolism, and nutrient status also differed between T1 and T2, highlighting subclinical effects of the challenge. In conclusion, dietary 25(OH)D3 was more effective than vitamin D3 or 1,25-(OH)2D3 in maintaining vitamin D activation and supporting liver function under stress conditions, suggesting its potential to improve resilience and physiological stability in poultry exposed to environmental challenges.

Keywords: 25-hydroxyvitamin D3; hepato-renal stress; Vitamin D metabolism; organ protection; transcriptomics

127 Determination of ileal mineral digestibility of 3 calcium sources and 5 phosphate sources fed to broiler chicken

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Inorganic phosphorus (P) and calcium (Ca) sources are important ingredients in broiler diets. This study was performed to determine the apparent ileal digestibility (AID) of P and Ca in 5 inorganic feed phosphates (IFP) and 3 Ca sources fed to Ross 308 male broiler chickens. Seven dietary treatments were offered from day 13-23 after an adaptation period with a common diet. There were 6 replicate cages per treatment and 12 broilers per cage. A basal mixture (97% of the diet) low in P and Ca was formulated and split into 7 batches to which the IFP, Ca sources, and diamol were added. All diets included 0.5% TiO2 as marker and had the same content of metabolizable energy, digestible Lys, and assumed retainable P (2.70 g/kg). Five diets contained monosodium phosphate (MSP), monocalcium phosphate (MCP), calcium-sodium phosphate (CSP), dicalcium phosphate (DCP), or defluorinated calcium phosphate (DFP) as IFP source and limestone as Ca source (except for the DFP diet). Two additional diets contained MSP as IFP source and lithothamnium 33% (Lit33) or lithothamnium 29% (Lit29) as Ca source. Growth performance was measured from day 13-23 and on day 23, all birds were sacrificed to collect a pooled sample of ileal content. The AID of P and Ca in the IFP and Ca sources was determined using the difference procedure. Among IFP sources, the AID of P was the highest ($P < 0.05$) in MSP (92-94%); the value in MCP (86%) was higher ($P < 0.05$) than in CSP (72%) and DCP (70%), and the lowest ($P < 0.05$) value was measured in DFP (49%). The AID of Ca was not different among MCP, CSP, and DCP (67, 63, and 66%, respectively), but was lower ($P < 0.05$) in DFP (45%). The AID of Ca in the Ca sources (with MSP as IFP source) was 63%, 62% and 65% for limestone, Lit33, and Lit29, respectively, and was not different than in MCP, CSP, or DCP. Body weight gain and feed efficiency of birds was better ($P < 0.05$) in the 3 MSP-containing diets than in the other diets. In conclusion, the phosphate composition influences its P solubility and affects the bird performance proportionally.

Keywords: Phosphates; Ileal digestibility; Calcium; Phosphorus; Broiler

128 EFFECT OF DIETARY SUPPLEMENTATION OF NANO ZINC OXIDE ON EGG PRODUCTION, FERTILITY AND HATCHABILITY OF KUTTANAD DUCKS

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An experiment was conducted at the Avian Research Station, Thiruvazhankunnu, to assess the effect of dietary supplementation of nano zinc oxide (ZnO-NPs) on egg production, fertility and hatchability of Kuttanad ducks from 17 to 40 weeks of age. A total of 160 ducks and 20 drakes were randomly allocated to five dietary treatment groups at an age of 16 weeks. Each treatment group had four replicates, with each replicate consisting of 8 ducks and 1 drake. The dietary treatments were: T1 (without zinc supplementation), T2 (80 mg inorganic Zn/kg), T3 (20 mg nano Zn/kg), T4 (40 mg nano Zn/kg) and T5 (60 mg nano Zn/kg). Egg production was evaluated by calculating period-wise duck day and duck housed egg production. Semen quality of Kuttanad drakes were evaluated by both macroscopic and microscopic examination at 28 and 40 weeks of age. Fertility and hatchability of incubated eggs were evaluated from 24

to 40 weeks of age at 28-day intervals. The present study found that dietary ZnO-NPs supplementation showed no significant change in body weight in Kuttanad ducks. The production parameters like feed consumption, feed conversion ratio, egg production and livability showed no significant difference between the treatment groups. The egg weight and egg quality traits like shape index, albumen index and yolk index were also unaffected by the dietary supplementation of ZnO-NPs. The shell thickness showed significantly ($p < 0.05$) higher values in 40 and 60 mg nano Zn/kg diet supplemented groups, except in the last period. The reproductive parameters, like macroscopic and microscopic semen quality parameters and glutathione peroxidase activity of seminal plasma of Kuttanad drakes, showed no significant difference between the treatment groups. The fertility and hatchability of Kuttanad duck eggs which were incubated were also not affected by the supplementation of different dietary levels of ZnO-NPs. The return over feed cost per breeder Kuttanad ducks was calculated as Rs.1102.17, 1111.36, 1063.15, 1159.19 and 1192.16 for T1, T2, T3, T4 and T5, respectively. Based on the significant improvement in eggshell thickness and superior economic returns, dietary supplementation with ZnO-NPs at 60 mg/kg is recommended for Kuttanad duck breeders to enhance egg quality and profitability.

Keywords: Nano Zinc; Fertility; Hatchability; Egg production; Kuttanad ducks

129 Effects of dietary iron sources on growth performance and hematological indicators of broilers reared at high altitude

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This study evaluated the effects of dietary iron sources on growth performance and hematological indicators of broilers reared at approximately 2,530 m above sea level. A total of 800 one-day-old Cobb 500 male broilers were randomly assigned to four dietary treatments: a negative control without supplemental iron (NC), ferrous sulfate (FeSO₄), and two organic iron sources (OFe1 and OFe2), with the three supplemented treatments providing an additional 20 ppm of dietary iron above the NC diet. Each treatment consisted of 10 replicate pens of 20 birds, and the experiment lasted 42 d. Growth performance was evaluated through body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), whereas hemoglobin (Hb), hematocrit (Hct), and total body hemoglobin iron were determined at 21 and 42 d. Data were analyzed using one-way ANOVA. Preplanned orthogonal contrasts were used to evaluate the overall response to iron supplementation (C1), compare inorganic and organic iron sources (C2), and differentiate the two organic iron sources (C3). No treatment effects were detected for FI or cumulative performance variables throughout the experimental period ($P > 0.05$). Treatment effects were restricted to the finisher phase (29–42 d), where BWG ($P = 0.014$) and FCR ($P = 0.023$) differed among treatments. Orthogonal contrasts indicated no differences between the NC and iron-supplemented groups (C1; $P > 0.05$), whereas broilers supplemented with organic iron sources exhibited greater BWG (C2; $P = 0.011$) and improved FCR (C2; $P = 0.021$) than those receiving FeSO₄. Furthermore, BWG differed between the two organic iron sources (C3; $P = 0.046$), with OFe2 showing the greatest productive response during the finisher phase. Hematological responses were limited; however, Hb (C3; $P = 0.015$) and Hct (C3; $P = 0.016$) differed between OFe1 and OFe2 at 21 d, whereas no significant treatment

effects were detected for total body hemoglobin iron or any hematological indicator at 42 d ($P > 0.05$). These results demonstrate that supplementation with organic iron sources improved growth performance during the finisher phase compared with FeSO₄ without affecting feed intake. Under high-altitude rearing conditions, organic iron supplementation represents a promising nutritional strategy to improve broiler performance, while hematological responses appeared to be transient and did not persist to market age.

Keywords: Broiler chickens; Organic iron; High-altitude production; Mineral nutrition; Hematological indicators

130 Effects of a phytogetic source containing calcitriol on growth performance of broilers fed different dietary vitamin D3 levels

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Vitamin D nutrition has become an important strategy to optimize skeletal development and productive efficiency in modern broiler production. The use of phytogetic additives containing biologically active vitamin D metabolites may represent an alternative strategy to support growth performance while reducing dietary vitamin D3 supplementation. Therefore, this study evaluated the effects of a phytogetic source containing calcitriol and triterpenes, extract of *Trisetum flavescens*, on growth performance of broilers fed different dietary vitamin D3 concentrations. A total of 1,584 one-day-old Cobb 500 male broilers were assigned to a completely randomized design in a 2 x 3 factorial arrangement, with two dietary vitamin D3 levels (2,000 or 4,000 IU/kg) and three levels of the phytogetic additive (0, 50, or 100 g/ton; Active D® - Phytobiotics, Germany), totaling six treatments, with 12 replicates of 22 birds per pen until 42 d. Birds received corn-soybean meal diets formulated according to commercial nutritional recommendations. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were evaluated throughout the production cycle. Data were analyzed by two-way ANOVA, with dietary vitamin D3 level, phytogetic additive level, and their interaction as fixed effects. Differences were considered significant at $P \leq 0.05$. No vitamin D3 x phytogetic additive interaction was observed for BWG or FI ($P > 0.05$). Reducing dietary vitamin D3 from 4,000 to 2,000 IU/kg decreased BWG over the 1 to 42 d period (3,043 vs 3,102 g; $P \leq 0.05$), whereas FI was not affected by treatments ($P > 0.05$). Significant vitamin D3 x phytogetic additive interactions were observed for FCR during the 1 to 28 d and 1 to 35 d periods ($P < 0.01$). Within the 2,000 IU/kg vitamin D3 group, supplementation with 100 g/ton of the phytogetic additive improved FCR compared with the unsupplemented diet (1.478 vs 1.527 from 1 to 28 d and 1.509 vs 1.554 from 1 to 35 d), whereas no differences among phytogetic additive levels were observed in birds fed 4,000 IU/kg vitamin D3 ($P > 0.05$). Dietary supplementation with 100 g/ton of the phytogetic source containing calcitriol improved feed conversion ratio during the 1-to-28-d and 1-to-35-d periods in broilers fed 2,000 IU/kg vitamin D3, suggesting its potential as a nutritional strategy to improve feed efficiency under reduced vitamin D3 supplementation.

Keywords: 1,25-dihydroxycholecalciferol; phytogetic additive; vitamin D metabolite; poultry nutrition; *Trisetum flavescens*

Symposium: Beyond Performance: The Next Frontier of Gut Health, Immunity and Economic Sustainability in Poultry Production

1032S Building a Better Microbiome: Functional Additives as Precision Tools for Gut Health Management

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No abstract submitted.

1033S Re Thinking 25 Hydroxyvitamin D3: A Broader Look at Immunity, Gut Integrity, and Early Life Development

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Vitamin D has traditionally been recognized for its fundamental role in calcium homeostasis, phosphorus metabolism, and skeletal mineralization in poultry. However, evidence accumulated over the past decade demonstrates that its circulating metabolite, 25-hydroxyvitamin D3 (25-OH-D3), exerts biological functions beyond mineral metabolism, acting as a regulator of immune responses, intestinal integrity, and physiological development throughout the productive life of broiler chickens. This expanded understanding highlights the relevance of 25-OH-D3 in modern precision nutrition programs. The widespread expression of the vitamin D receptor in enterocytes, macrophages, dendritic cells, and lymphocytes supports the direct involvement of 25-OH-D3 in innate and adaptive immune regulation. Its principal mechanisms include modulation of pro- and anti-inflammatory cytokines, stimulation of antimicrobial peptide synthesis, maintenance of epithelial tight junction integrity, and reduction of the metabolic cost associated with chronic low-grade inflammation. These effects may improve nutrient utilization, productive performance, and carcass quality, particularly breast meat yield. Their importance becomes greater under health challenges such as mycotoxin exposure, intestinal dysbiosis, and increased epithelial permeability, which compromise nutrient absorption and immune competence. Under these conditions, dietary supplementation with 25-OH-D3 may help preserve intestinal architecture, attenuate excessive inflammatory responses, and promote more balanced immune responses, minimizing productive losses. Its importance is particularly evident during vulnerable stages of the production cycle. In young birds, when the gastrointestinal tract and immune system are rapidly developing, adequate 25-OH-D3 availability supports early immune competence, intestinal barrier function, and host-microbiota interactions. In older birds, where immunosenescence progressively reduces immune responsiveness and tissue repair capacity, 25-OH-D3 may contribute to maintaining immunometabolic function and mitigating physiological stress. Collectively, these findings indicate that 25-OH-D3 should no longer be viewed solely as a metabolite involved in mineral metabolism, but as a systemic regulator of intestinal health, immune competence, and production efficiency. Its strategic inclusion in poultry nutrition programs may integrate productive performance, breast meat yield, immune resilience, and sustainability, particularly in challenging production systems focused on biological efficiency and high-quality protein production.

Keywords: skeletal mineralization; intestinal integrity; nutrient utilization; biological efficiency

1034S Success Stories and Applied Economics: When Fiber Nutrition Improves Robustness and Reduces Total Cost of

Production.

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Dietary fiber was traditionally regarded as a low-value component of poultry diets, primarily serving as an energy diluent. Advances in our understanding of intestinal microbiota and microbial fermentation, however, have shifted this paradigm. Dietary fiber is now recognized as a functional substrate capable of modulating the intestinal microbiome, promoting beneficial microbial metabolites, and supporting gut health and production efficiency. Thus, fiber quality and fermentability may be more relevant than its absolute inclusion level. Fermentable fractions of non-starch polysaccharides (NSPs), naturally present in ingredients such as corn, soybean meal, wheat, and their co-products, provide substrates for fibrolytic bacteria, particularly members of the phylum Firmicutes. Their fermentation generates short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which provide energy to enterocytes, support epithelial integrity, strengthen tight junctions, stimulate mucus production, and contribute to intestinal immune homeostasis. Within this context, stimbiotics have emerged as a novel category of feed additives designed to enhance the fermentation of dietary fiber already present in poultry diets. Unlike conventional prebiotics, stimbiotics are supplied at very low inclusion rates and stimulate fiber-degrading microbial populations without providing meaningful amounts of fermentable substrate. This mechanism can improve the biological utilization of endogenous fiber, increase SCFA production, and support a more stable and resilient intestinal ecosystem. From an economic perspective, maximizing the fermentation of fiber naturally present in conventional feed ingredients represents a cost-effective strategy for supporting gut health. Microbiome modulation may therefore improve feed efficiency, nutrient utilization, and body weight gain without requiring substantial additional dietary substrate. Integrating dietary fiber, intestinal microbiota, and stimbiotics represents a shift in modern poultry nutrition: feed formulation should consider not only the nutritional value of ingredients but also their fermentative potential. This approach may promote a more functional microbiome, greater production of beneficial microbial metabolites, and reduced protein fermentation and associated detrimental by-products. Ultimately, optimizing fiber fermentation offers a promising strategy to support intestinal resilience, productive performance, and economic sustainability while reducing reliance on more costly health-management interventions.

Keywords: Dietary fiber; Gut microbiota; Stimbiotics; Poultry performance

1035S Feeding the microbiome: The next frontier in poultry health and sustainability

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Dietary fibre has been considered an antinutrient for decades and limiting the excess of soluble fibre in poultry diets was recommended to prevent performance losses and improve nutrient digestibility. Furthermore, diverse definitions and methods to describe dietary fibre has created confusion for nutritionist and impeded development of nutritional recommendations focused on dietary fibre. Con-

sumption of dietary fibre can have either detrimental or beneficial effects depending on the origin, composition and concentration, and the physicochemical characteristics of the fibre sources. However, recent scientific evidence indicates there are multiple benefits of dietary fibre, including fibre as an energy source and modulator of the gut microbiome, with positive effects on the animal's physiology and therefore health and performance. Moreover, the development of stimbiotic additives, to promote utilisation of dietary fibre, has expanded awareness of the benefits resulting from the enhancement of fibre utilisation. Gut microbes can be driven and trained in such a positive way that results are seen in performance, health, and welfare. This abstract aims to give an overview of the dietary fibre definitions and describe its physicochemical properties. In addition, we aim to highlight the importance of dietary fibre from a holistic perspective, including considerations for implementing dietary fibre to meet specific requirements when formulating diets for poultry and offer a tool for nutritionists to implement dietary fibre to meet specific and targeted objectives. Additional promotion of fibre utilisation through the implementation of a stimbiotic will also be discussed, as well as some of its observed benefits in poultry. This novel concept has revealed genuine progress in the recognition of new additives developed specifically to improve the understanding and utilisation of dietary fibre.

1036S Beyond Traditional Ingredients: The Next Frontier of Feed Formulation Towards 2029

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Feed formulation for poultry is undergoing a significant transformation, driven by the increasing demand for greater economic efficien-

cy, sustainability, and nutritional precision. In this context, the forthcoming edition of the Brazilian Tables for Poultry and Swine (UFV – 2029) will incorporate new scientific concepts and an expanded knowledge base for evaluating alternative feed ingredients and their contributions to animal performance and health. This presentation will provide a strategic overview of the major research initiatives currently being conducted at the Federal University of Viçosa (UFV), highlighting recent advances in the nutritional characterization of novel lipid sources and fibrous ingredients that remain underutilized in Latin American poultry nutrition. Particular emphasis will be placed on the challenges and opportunities associated with incorporating these ingredients into commercial feeding programs, including innovative evaluation methodologies, approaches to determining their nutritional value, and their implications for least-cost feed formulation. Beyond the economic perspective, the presentation will explore how these emerging nutritional strategies influence intestinal health, gastrointestinal function, and nutrient utilization efficiency. The discussion will reinforce the growing integration of nutrition, gut physiology, and productive performance, demonstrating how advances in ingredient evaluation can support healthier and more resilient birds while improving production efficiency. Finally, participants will gain insight into the scientific trends expected to shape poultry feed formulation through 2029. The presentation will illustrate how new research findings can contribute to the development of more robust, sustainable, and biologically relevant formulation models capable of meeting the evolving demands of modern poultry production and precision nutrition.

Keywords: alternative feed ingredients; feed formulation; nutritional evaluation; precision nutrition

Symposium: Broiler Mortality: The Most Critical Indicator of Efficiency in Modern Poultry Production

1037S The Impact of Intestinal Health on Broiler Mortality and Livability

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The gastrointestinal tract is a major determinant of health, performance, and survival in modern broiler production. Beyond digestion and nutrient absorption, the intestine acts as a critical biological barrier that limits the translocation of pathogens, toxins, and microbial products while supporting immune homeostasis. Disruption of intestinal health caused by enteric pathogens, dysbiosis, dietary challenges, mycotoxins, heat stress, or excessive inflammation can compromise epithelial integrity and increase intestinal permeability. As a consequence, nutrient utilization may be impaired, inflammatory and immune responses may increase, and birds can become more susceptible to secondary infections, ultimately affecting flock livability. Enteric diseases such as coccidiosis and necrotic enteritis clearly demonstrate the relationship between intestinal damage, impaired performance, and mortality. However, intestinal dysfunction does not need to result in overt clinical disease to negatively affect flock health. Subclinical inflammation, impaired barrier function, and microbial imbalance may reduce intestinal resilience and increase susceptibility to opportunistic pathogens and systemic challenges. Advances in intestinal physiology and microbiome research reinforce the importance of maintaining a stable intestinal environment throughout the production cycle. Nutritional, health, and management strategies that support epithelial integrity, microbial balance, nutrient digestibility, and appropriate inflammatory responses can

therefore contribute to improved flock resilience and livability. This presentation will review the biological mechanisms linking intestinal dysfunction with broiler mortality, discuss the major intestinal factors associated with reduced flock survival, and highlight practical nutritional and management strategies to preserve intestinal health. Maintaining intestinal integrity should be considered an important component of strategies aimed at improving performance, health, welfare, and livability in modern broiler production

Keywords: intestinal health; intestinal integrity; broiler chickens; livability; intestinal inflammation

1038S Mortality in Broiler Chickens - What has changed in recent years?

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Over the past 15 years, broiler mortality has shown a concerning and steady increase in commercial operations across the United States and other major poultry-producing regions. This presentation examines key factors driving this trend, with a particular focus on the role of increasing harvest weights and changes in weekly mortality patterns throughout the production cycle. Using a large industry dataset, the analysis reveals that mortality is no longer concentrated in the early days of life alone—late grow-out losses, especially after day 42, have become a growing concern. These shifts suggest that current broiler strains are facing increasing physiological, met-

abolic, and management challenges as production goals intensify. This presentation aims to raise awareness among veterinarians, live production managers, nutritionists, and technical advisors that current mortality levels are historically high, and that this trend cannot be viewed as an inevitable byproduct of progress. Instead, a deeper understanding of root causes is essential to guide innovation, refine management practices, and develop scalable strategies to improve bird resilience and reduce losses.

Keywords: Management ; Health; Feed; Livability ; Performance

1039S How to mitigate the mortality ?

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Modern broiler production requires animals to maintain high performance under continuous nutritional, environmental, metabolic, and health challenges. Therefore, improving animal resilience, defined as the ability to respond and recover from challenges while maintaining productive efficiency, has become an important strategy to reduce production losses and mortality. The objective of this review was to discuss nutritional strategies that may support broiler resilience through three main areas: optimized trace mineral nutrition, intestinal health, and skeletal development. Organic trace minerals in the form of glycinate can provide highly bioavailable sources of zinc, manganese, and copper. These minerals participate in intestinal epithelial integrity, antioxidant defense, immune function, cartilage and bone formation, collagen synthesis, and tissue integrity, supporting the animal during periods of high metabolic demand. Intestinal inflammation is another important factor affecting resilience because chronic or subclinical inflammation may increase intestinal permeability, activate immune responses, redirect nutrients away from growth, impair nutrient absorption, and increase the risk of secondary diseases and mortality. Dietary inclusion of *Macleaya cordata* extract represents a nutritional approach to modulate intestinal inflammatory responses, including the regulation of NF- κ B activation in the intestinal mucosa. A meta-analysis of 36 broiler experiments presented in this review showed an improvement in feed conversion ratio from 1.66 to 1.62 and a reduction in mortality from 5.74% to 4.25% with this nutritional strategy. Skeletal development is also essential because modern broilers have rapid muscle growth and high structural demands. The use of calcitriol, the biologically active form of vitamin D, can support calcium metabolism, bone formation, and skeletal strength. Field data presented in the review indicate improvements in tibia weight, length, and breaking resistance associated with this strategy. Overall, combining highly bioavailable organic trace minerals, nutritional modulation of intestinal inflammation, and support of vitamin D-dependent bone metabolism represents a complementary approach to animal resilience. These strategies may help broilers maintain performance under challenging conditions, reduce biological losses, and contribute to lower mortality and more efficient and sustainable poultry production.

Keywords: animal resilience; bone health; intestinal inflammation; broilers mortality; plant calcitriol

1040S Bone Health Associated with Performance and Mortality in Broilers

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The rate of skeletal growth is at least 3.8 times greater between placement and 250 g of body weight than at any other time in a broilers production cycle. Thus, early skeletal development and the correct feeding of Ca, P and vitamin D in this phase is fundamental in the correct development and mineralization of bones and will in large part minimize the incidence of bone problems in later m phases. Morbidity and mortality related to mobility problems have recently been identified as a possible cause of the increased mortality seen in recent years. Problems such as hock burns, breast blisters and gangrenous dermatitis due to decreased standing time and excessive sitting on dirty and often wet litter have been identified as causes of downgrades at processing and mortality late in the production cycle. Tibial dyschondroplasia (TD) has been reported at high levels (between 30 and 70% incidence) in commercial broilers flocks since 2025 being reported in flocks in North, Central and South America. Only recently have commercial flocks in South East Asia reported an increase in TD but incidence levels are below 20%. The implications of TD on performance can be minimal as has been reported in flocks with incidences of 50 to 70% TD with better than average performance and average mortality. But TD, especially if it develops in the pre starter phase, can lead to twisting of the tibia and or femur and can result in mobility problems as well as processing plant losses due to bone breakage during mechanical deboning. TD can also lead to increased incidence of bone infections. In this symposium talk, skeletal development and its optimization through Ca, P and vitamin D nutrition will be discussed. The use of digestible Ca (dCa) and P (dP) values in formulation that allow for an optimal feeding of Ca and P are essential to supply the broilers the quantities of these nutrients in a form that the animal can use (digestible). will be covered in the context of ingredient dCa and dP as well as dCa and dP recommendations. To implement a dCa system ingredient dCa values must be known and dCa requirements for the production phase must be established. The most variable ingredient in terms of dCa is limestone in broilers. Limestone Ca digestibility can vary between 20 and 75% and thus the use of an average value is not warranted. Prediction equations based on limestone characteristics (dynamic solubility (5, 15, 30 min) in a 3 pH buffered solution, Ca and Mg concentration, geometric mean diameter) and phytate P content of the diet have been developed and validated.

Keywords: Skeletal development; Broilers; Digestible calcium and phosphorus; bone problems; performance and mortality

Management and Production

131 Early and Sustained Probiotic Supplementation from Egg-to-Chick Improves Broiler Performance and Reduces *Salmonella* Enteritidis Persistence

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Sustainable interventions capable of improving production efficiency

while supporting food safety are increasingly important in modern poultry systems. Since embryonic development and immediate post-hatch growth represent critical determinants of broiler performance, early-life microbial modulation may provide an opportunity to influence productivity and pathogen colonization across the broiler production continuum. Therefore, the objective of this work was to evaluate the effect of sustained supplementation with *Lactocasei-*

bacillus rhamnosus NRRL B-442 (LR) and *Lactocaseibacillus paracasei* DUP-13076 (LP) from embryogenesis through grow-out in broilers on performance and *Salmonella* Enteritidis persistence. Ross 308 hatching eggs were sprayed with LR or LP (~9 log CFU/egg) during incubation followed by post-hatch in-feed supplementation (~9 log CFU/kg feed) through a 42-d grow-out period. Parallel studies evaluated embryonic development, post-hatch performance, microbiota acquisition, and *Salmonella* Enteritidis (SE) dynamics. Probiotic supplementation significantly improved embryo morphometry and hatchling quality, resulting in increased hatchability (12-20%), and hatchling body weight (12-17%) compared to controls ($P \leq 0.05$). During grow-out, probiotic-treated birds demonstrated higher body weight gain (8-15%), carcass yield (2.7-3.8%), and breast yield (4.3-4.9%), alongside lower feed conversion ratio (3-4.9%) without negatively affecting meat quality. Probiotic supplementation also altered microbial acquisition during embryogenesis and hatch, with enrichment of *Lactobacillus* populations and microbial pathways associated with nutrient and energy metabolism. In parallel SE challenge studies, LR and LP significantly reduced SE recovery in developing embryos, hatchlings, and grow-out birds compared to controls ($P \leq 0.05$). In hatchlings, probiotic supplementation reduced cecal SE populations by approximately 1.2-1.8 log CFU/g while maintaining significantly higher *Lactobacillus* populations in the ceca. Reduced systemic dissemination was also observed in probiotic-treated birds, with significantly lower SE recovery in the liver and spleen at hatch. During grow-out, probiotic-treated birds maintained lower cecal SE populations (~3.1-3.4 log CFU/g) compared to controls, indicating reduced SE persistence through grow-out. Collectively, these findings demonstrate that sustained microbial interventions applied from embryogenesis through grow-out can simultaneously improve broiler performance, microbiota development, and food safety outcomes. Early-life microbial modulation may represent a practical systems-level strategy for improving efficiency and resilience in commercial poultry production.

Keywords: Probiotic intervention; early-life; grow-out; growth performance; salmonella control

132 Growth performance and blood profile of broiler chickens fed diets containing graded levels of toasted defatted black soldier fly larval meal (*Hermetia illucens*) as a partial replacement for fish meal

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The effect of graded dietary levels of toasted defatted Black Soldier Fly Larval Meal (BSFLM) on the growth performance, haematological and serum biochemical parameters of broiler chickens at both starter and finisher phases were evaluated in this study. Total of 360-day-old broiler chicks were randomly assigned to five treatment groups arranged in a completely randomised experimental design and treatments were replicated three times to have 24 birds per replicate. The dietary treatments contained BSFLM at 0 (control), 25, 50, 75 and 100 per cent substitution of fish meal. Birds were fed a starter diet (2,900 kcal/kg ME; 22% CP) at 0-3 weeks and a finisher diet (2,950 kcal/kg ME; 18% CP) at 3-7 weeks of age. Data were analysed according to One-way Analysis of Variance (ANOVA) and significantly different means were separated using Duncan's Multiple Range Test ($P < 0.05$). At the starter phase, 50% BSFLM

inclusion had the significantly highest final weight gain (1024.5 g), weight gain (983.8 g), and the best FCR (1.746) values ($P > 0.05$) while the chickens that were fed finisher diet containing 25% BSFLM had the significantly highest final body weight (2,397.1 g), but the control group and the 75% inclusion group (1,357.1 g) had the highest weight gains, and best FCR that were not significantly different ($P > 0.05$) from other treatment groups. Serum biochemical parameters showed that the BSFLM at all levels resulted in healthy ranged of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that were not significantly different across treatment groups ($P > 0.05$) and indicates lack of liver toxicity. The haematology value revealed that only packed cell volume and highest value was recorded at 25% partial replacement level ($P < 0.05$). The highest white blood cell count ($15.67 \times 10^3/\mu\text{L}$) was found also at 25% replacement, while the highest percentage of lymphocytes (65.67%) was noted when in the 75% BSFLM fed. It was concluded that a feasible and sustainable replacement of fishmeal with BSFLM could only be done at 25-50% in broiler chickens to get optimum growth performance, increase immunity and maintain metabolic health.

Keywords: Black; broiler; Larval; Soldier; haematology

133 Development and Validation of a Miniaturized Sensor for Monitoring the Microenvironment Inside Fertile Broiler Eggs

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Monitoring the microenvironment inside fertile eggs is essential for understanding embryonic development during artificial incubation. However, commercial incubation management relies primarily on environmental measurements inside incubators, while direct assessment of the conditions experienced by embryos remains technically challenging. This study hypothesized that a miniaturized sensor could be developed, calibrated, and validated to accurately monitor the internal microenvironment of fertile broiler eggs by measuring temperature, light intensity, and sound pressure level. To achieve this, a compact sensing device integrating temperature, light, and acoustic sensors was developed using an open-source microcontroller platform and electronic components compatible with the physical dimensions of fertile eggs. Individual calibration was performed under controlled laboratory conditions by comparing sensor outputs with commercial reference instruments. The system was subsequently validated using fertile eggs from broiler breeder flocks at 34 and 63 weeks of age on day 18 of incubation under standard commercial incubation conditions (37.5°C, 90 dB, and 200 lux). Regression analysis was used to calibrate the sensor outputs against reference instruments, and coefficients of determination were calculated to evaluate sensor performance. The calibration demonstrated high agreement between the developed sensor and commercial instruments, with coefficient of determination values of 0.9925 for internal temperature, 0.9897 for luminosity, and 0.9315 for sound pressure level. During validation, the sensor successfully detected differences in the internal egg microenvironment between breeder ages. Eggs from 34- and 63-wk-old breeders presented mean internal temperatures of 34.25 and 34.47°C, internal luminosities of 61.3 and 62.7 lux, and internal sound pressure levels of 316 and 343 V, respectively, under identical external incubation conditions. These results demonstrate the sensor's capability to reliably characterize subtle variations in the embryonic microenvironment associated with breeder age. The proposed miniaturized sensor proved to be a reliable and accurate tool for monitoring the inter-

nal environment of fertile eggs. This technology provides a novel platform for embryo-centered incubation research and represents an important step toward precision incubation systems capable of monitoring the actual environmental conditions experienced by developing embryos, supporting future studies on embryo physiology and data-driven incubation management.

Keywords: artificial incubation; embryo monitoring; environmental sensors; precision poultry farming; fertile eggs

134 ECONOMIC COMPARISON OF CONVENTIONAL AND PASTURE-BASED BROILER PRODUCTION SYSTEMS ACROSS PRODUCTION SCALES

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Broiler production systems differ substantially in management configuration, operational scale, and market orientation, which may influence their economic performance. The objective of this study was to economically characterize broiler production systems differing in management configuration and scale by estimating total production costs, break-even prices, and profitability indicators under standard and premium selling-price scenarios. Three systems were modeled using an enterprise-budgeting approach: a large-scale conventional intensive commercial system (LSC); a medium-scale conventional system (MSC) designed as a reduced-capacity counterfactual of the large-scale model, to isolate the effect of scale while maintaining similar biological and management assumptions; and a small-scale pasture-based system (SSP) using mobile pens, representing a distinct technological and operational model with outdoor access, lower throughput, and greater labor demand. Enterprise budgets were used to estimate total production costs, followed by break-even and profitability analyses. Economic outcomes were compared descriptively across scenarios using total revenue, gross profit, return on investment (ROI), and break-even production point. Unit production cost was lowest in the LSC system (USD 1.15/kg live weight), intermediate in the MSC system (USD 1.41/kg live weight), and highest in the SSP system (USD 4.23/kg live weight), reflecting differences in production scale and cost structure. Feed and chick purchases were the dominant cost components in both conventional systems, whereas labor represented a proportionally greater share of total cost in the pasture-based system. Relative to the large-scale conventional system, break-even price increased by 22.7% in the MSC system and by 268.7% in the SSP system. Under the standard market price (USD 2.91/kg whole bird), the large-scale and medium-scale conventional systems generated positive gross profits of USD 2.12 million/yr and USD 421,195/yr, respectively, whereas the pasture-based system generated a loss of USD 5,907/yr (ROI = -48%) and did not reach its break-even production threshold. However, when a premium price (USD 13.43/kg whole bird) was applied, the pasture-based system shifted to positive profitability, generating USD 16,918/yr with an ROI of 138%, and its break-even production threshold declined from 2,058 to 445 birds per year. These findings indicate that production scale is a primary determinant of economic feasibility in conventional broiler systems, whereas the viability of pasture-based systems depends on market differentiation and price premiums sufficient to offset the higher production costs associat-

ed with alternative management systems.

Keywords: break-even analysis; broiler economics; enterprise budgeting; pasture-raised poultry; production scale

135 Application of the 3D methodology for data integration and decision support in egg production.

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The study aimed to demonstrate the applicability of an analytical tool called "3D" (Data, Diagnose and Decision). The tool was developed to transform large volumes of poultry industry data into structured and statistically validated information, supporting strategic decision-making and reducing reliance on reactive actions. To highlight the benefits of the tool, a retrospective data analysis was conducted using data from a commercial egg production company located in Colombia, aiming to evaluate the impact of line replacement. Data from 40 rearing flocks and 35 production flocks were analyzed (50% white line and 50% brown line), resulting in a total layer hen population of 1,028,378, produced between 2018 and 2026. Variables included in the analysis were rearing uniformity, feed conversion per egg, mortality, among others, and flocks were also stratified into quartiles based on rearing mortality to assess its impact on productive performance. Mann-Whitney, Student's t-test, and one-way ANOVA (95% confidence interval), as well as Pearson correlation presented in a heat map were used in statistical analysis. The results showed that white line was superior in performance compared to brown line both in rearing (uniformity: 85.50% vs. 81.65%; $P=0.040$) and production (feed/egg: 123.39g vs. 132.27g; $P<0.001$). Quartile analysis (in a normal distribution, 25% of the data fall below the mean-1stQ, 50% cluster around the mean-2nd and 3rdQ, and 25% fall above the mean-4thQ) showed that flocks with higher mortality during rearing presented poorer production results. The average feed intake of the flocks with the lowest mortality (1stQ) was 123.53g of feed/egg, against 132.27g/egg ($p=0.002$) for the flocks with the highest mortality (4thQ). This evidence is highly relevant, as it provides scientific and economic reasons for implementing biosecurity procedures to ensure healthier and more uniform flocks. Additionally, heat map analysis indicated a negative correlation between rearing uniformity and feed conversion in production ($p=0.028$), demonstrating that higher uniformity is associated with better productive efficiency. These findings reinforce the influence of early management on final performance and highlight the multifactorial data behavior of production results, which should be considered in an integrated manner for assertive decision-making. Therefore, the "3D" tool effectively transforms large datasets into actionable information, enabling the identification of performance deviations, assessment of corrective actions, and predictive insights that support proactive and evidence-based decision-making across egg production systems.

Keywords: Correlation; Data science; Layers; Mortality; Performance

136 Effects of Downtime Environmental Modulation on Salmonella Recurrence, Performance, and Slaughterhouse Condemnations in Broiler Chickens

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Downtime is a critical phase for biosecurity, disease prevention, and facility maintenance in broiler production. Environmental modulation comprises sustainable procedures aimed at reducing pathogen pressure, promoting beneficial microbiota, and improving farm efficiency. This field trial, conducted in a commercial poultry system in Western Paraná State, Brazil, evaluated two downtime management programs on *Salmonella* occurrence, productive performance, and slaughterhouse condemnations. A total of 210 farms were assigned to two treatment groups over 12 months, with recurrent *Salmonella*-positive farms evenly distributed according to recurrence history, biosecurity status, management practices, and farm structure. All flocks underwent 12 days of downtime when *Salmonella*-negative, or 27 days with litter windrowing when *Salmonella*-positive. Treatment 1 (T1) included 1,025 flocks and represented the standard downtime program, based on limestone treatment of reused litter and routine procedures. Treatment 2 (T2) included 1,068 flocks and represented the Environmental Modulation program, in which limestone was replaced by Stalosan F® (SAN Group), applied to all

surfaces at 50 g/m² during the first third of downtime, followed by a *Bacillus*-based litter modulator applied at 2.1 g/m² before chick placement. Data was analyzed by General Linear Model ANOVA in Minitab, with significance set at 95% probability. After 12 months, T2 reduced recurrent *Salmonella*-positive flocks by 52% compared with T1 (20.7% vs. 9.9%). T2 also improved feed conversion by 5 g (P=0.039) and reduced mortality (5.71% vs. 5.96%; P=0.014). At slaughter, T2 decreased losses associated with airsacculitis, cellulitis, colibacillosis, and total condemnations (P≤0.05). Pododermatitis did not differ significantly between treatments, although a favorable numerical trend was observed (P=0.359). Despite inherent variability under commercial field conditions, the high number of flock replicates supports the practical relevance of the program. In conclusion, downtime environmental modulation appears to be a promising strategy to improve sanitary control, productive efficiency, and slaughterhouse outcomes in commercial broiler systems.

Keywords: Biosecurity; Farm sanitation; Food safety; Litter; Microbiota

Metabolism and Nutrition: Enzymes

137 Xylanase supplementation enhances microbial co-occurrence in the post-hatch broiler

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Balancing intestinal microbial development in broilers in early life is important, as it builds a base for proper immunological development and future performance. Application of xylanase may alter microbial composition and therefore support intestinal development. In this study, a commercial nutrient adequate wheat-soy based diet (Control) was supplemented with one of three levels of xylanase; 2000, 4000, and 8000 U/kg (Danisco® Xylanase), fed *ad lib*. Day of hatch Ross 308 male broilers were placed in 32 pens divided over 8 houses (34 birds/pen, 4 pens/house). In each house, treatments were randomly assigned to pens. Body weight (BW) and feed intake were determined on d 11 and mortalities recorded. On 24h after placement (d 1), d 4, 8, 11, 2 birds/pen were sacrificed and cecal content collected for 16S community analysis using Illumina 16S rRNA V4 amplicon sequencing to evaluate overall xylanase effect on microbial community. Co-occurrence networks were constructed at the genus and putative species levels using Spearman rank correlation analysis with FDR correction and were evaluated separately at d 1 and d 4 post-hatch, as well as after pooling both time points as Week 1. Differences in microbial composition between treatments and days were analyzed by Shannon α-Diversity, PERMANOVA and differentially abundant taxa analysis (DeSeq2). BW and FCR were significantly improved with increasing xylanase inclusion (P < 0.05). There were no significant differences in microbiome α-diversity, however, a significant increase was seen as early as d 1 and d 4 with *Mediterraneibacter spp.* in xylanase groups (8.67 to 14.8% abundance; P=0.01) and sequential significant decreases in *Enterococcus spp.* at d8 (3.78 to 1.85%, P=0.02). Short-chain fatty acid producing *Mediterraneibacter* is known to be positively associated with balanced intestinal microbial environments and *Enterococcus spp* account for a significant portion of mortality in young

broiler flocks. Microbial co-occurrence network analysis revealed treatment-dependent differences in early community organization during the post-hatch period. At d1, birds receiving higher enzyme supplementation showed greater network connectivity than the 0 and 2000 U/kg groups, suggesting early modulation of microbial assembly. Collectively, these findings indicate that xylanase supplementation can reshape intestinal microbial composition within 24 h of first feeding, enriching SCFA-associated taxa while reducing potentially opportunistic bacteria, thereby promoting rapid diet-driven modulation of post-hatch microbial assembly in broilers. Even more, these early changes carried into positive microbial alternations and performance improvement later in production.

Keywords: Xylanase; intestinal development; co-occurrence networks; broilers; microbiome

138 Phytase supplementation improves growth performance and nutrient digestibility in broilers

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Although phytase is primarily used to improve phosphorus (P) digestibility in poultry diets, its extra-phosphoric effects on the digestibility of other nutrients, including amino acids (AA) and energy, have been reported; however, consistency of these effects is still often questioned. The objective of this study was to evaluate growth performance and apparent ileal digestibility (AID) of energy and nutrients in broiler chickens fed diets supplemented with a hybrid 6-phytase. A total of 168 one-day-old Cobb 500 byproduct male broiler chicks (initial BW = 39.1 g) were randomly assigned to 2 dietary treatments in a completely randomized design, with 12 replicate cages/treatment and 7 chicks/cage. Birds had *ad libitum* access to the same basal starter with no supplemented enzyme, from d 0 to 7 and to the experimental diets from d 7 to 25. The experimental diets consisted of a corn-soybean meal-based basal diet ei-

ther not supplemented or supplemented with phytase (Natuphos® E 5000 G; BASF Corporation, Florham Park, NJ, USA) at 1,000 FTU/kg. All diets were formulated with no addition of inorganic phosphate and had a phytate phosphorus level of 0.24%. Growth performance was measured from d 7 to 25 after adjusting for mortality. During the experimental phase, titanium dioxide was included in all diets at 0.5% as an indigestible marker. On d 25, ileal digesta samples were collected from the distal half of the ileum of all birds per cage to determine AID of P, DM, gross energy (GE), nitrogen (N), and AA using the index method. Data were analyzed using the MIXED procedure of SAS, with dietary treatment considered a fixed effect, and the experimental unit was the cage. Statistical significance was declared at $P < 0.05$. Dietary phytase supplementation increased ADG, ADFI, and gain-to-feed ratio from d 7 to 25 ($P < 0.001$). Phytase increased AID of P and N ($P < 0.001$) and also improved AID of energy by 82 kcal/kg ($P < 0.05$). Phytase increased AID of all indispensable and dispensable AA ($P < 0.001$), with an average improvement of 4.6 percentage units compared with birds fed the basal diet (SE = 0.4). Considering bone parameters, phytase supplementation increased breaking strength and ash content in tibia and femur bone of the birds ($P < 0.001$). In conclusion, 1,000 FTU/kg of this dietary hybrid 6-phytase improved growth performance, energy and nutrient utilization as well as bone mineralization in broilers fed a P-deficient diet. Its positive effects on growth performance were associated not only with improved P digestibility but also with enhanced digestibility of energy N and AA.

Keywords: amino acid ; broiler chickens; growth performance; nutrient utilization; phytase

139 Dietary phytase and plant-based vitamin D3: Impacts on early laying production and egg quality

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Phytase and vitamin D3 may have synergistic effects on Ca and P metabolism in laying hens. As part of a two-stage study (0 to 18 wk of age pullet phase; 18-100 wk of age laying phase), the effects of phytase (Axta PHY GOLD, IFF) and a plant-based source of vitamin D3 as 1,25(OH)2D3-glycosides extracted from *Solanum glaucophyllum* (Panbonis, PAN; Herbonis Animal Health) were investigated. Data is reported from 18 to 29 wk of age. All pullets were raised in floor pens from 0 to 18 wk of age and fed a diet with 1,000 FTU phytase/kg of feed with available P (aP) at 0.281% (starter; 0 to 4 wk of age), 0.220% (grower; 4 to 8 wk of age), 0.160% (developer; 8 to 16 wk of age), and 0.231% (pre-lay; 16 to 18 wk of age), with matrix-based reductions in Ca, digestible AA and ME. At 18 wk of age, 296 Lohmann LSL-Lite pullets were distributed in a completely randomized design to 4 dietary treatments with a minimum of 17 cages of 4 birds each per treatment. Treatments were: Positive control (PC) = 0.280% aP from 18 wk of age, no inclusion phytase or PAN, with monocalcium phosphate (MCP); Negative control (NC) = 0.111% aP from 18 to 21 wk of age and 0.104% after 21 wk of age, no phytase, PAN or MCP; Phy = NC + 900 FTU phytase/kg feed; and Phy+PAN = NC + 900 FTU phytase/kg + PAN at 1 µg 1,25(OH)2D3-glycosides/kg feed. Birds had access to mash feed and water *ad libitum*. Feed disappearance on a cage basis was recorded at 8-wk intervals and average daily intake (ADFI) calculated. Birds were individually weighed at 18 and 26 wk of age and mortal-

ity-corrected feed conversion ratio (FCR) calculated based on egg mass. Hen-day egg production (EP) was recorded at 4-wk intervals. Egg quality traits were determined at 24 and 28 wk of age, and individual egg weights at 25 and 29 wk of age. One-way ANOVA was performed, followed by Tukey's test with a significance level of $P < 0.05$. Treatment effects were not observed for BW at 18 wk of age (1,208 g; $P = 0.359$), ADG ($P = 0.516$), ADFI ($P = 0.230$), FCR ($P = 0.230$), nor on most egg quality parameters ($P > 0.05$). Eggshell breaking strength at 24 wk of age was increased ($P = 0.039$) by phytase (Phy; 55.7 N) compared to NC (49.6 N) and Phy+PAN (49.5 N). Laying hens fed the Phy+PAN diet had greater EP ($P = 0.030$) from 23 to 27 wk of age (98.5%) than the NC group (96.7%) and comparable to PC. Nutrient-reduced, phytase-supplemented, pullet feeding resulted appropriate BW at 18 wk of age. To 29 wk of age, nutrient reduction had few effects on performance and egg traits, and consequently, treatment effects were minimal. Long-term impacts of nutrient reduction, as well as treatment effects may become more evident as birds age and production challenges increase over the remainder of the study.

Keywords: layer; 1,25(OH)2 vitamin D3-glycoside; phytase; eggs

140 Effects of Xylanase, β-Mannanase, and Lysozyme Combinations on Growth Performance and Intestinal Morphology of Cobb 500 Broilers

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This study evaluated the effects of dietary combinations of xylanase, β-mannanase, and lysozyme on growth performance, intestinal morphometry, histomorphometry, and histopathology in broiler chickens. A total of 400 one-day-old Cobb 500 broilers were randomly allocated to 5 treatments with 4 replicate pens of 20 birds each and reared for 42 d. Treatments were T0, basal diet + xylanase; T1, basal diet + xylanase + lysozyme; T2, basal diet + xylanase + β-mannanase; T3, basal diet + β-mannanase + lysozyme; and T4, basal diet + xylanase + β-mannanase + lysozyme. Body weight was recorded weekly, and intestinal length, diameter, villus height (VH), crypt depth (CD), VH:CD ratio, and histopathological changes were evaluated. Data were analyzed by one-way ANOVA, followed by Duncan's multiple range test when treatment effects were significant ($P < 0.05$). Body weight did not differ among treatments at d 7 ($P = 0.946$), but treatment effects were observed from d 14 onward. At d 42, BW was 2,866, 2,945, 3,055, 2,830, and 3,180 g for T0, T1, T2, T3, and T4, respectively ($P < 0.001$), with T4 having the greatest BW. At d 42, total intestinal length was greatest in T4 (208.2 cm) and lowest in T0 (198.7 cm; $P = 0.003$), while intestinal diameter was 12.6 and 11.9 mm, respectively ($P = 0.009$). Treatment affected intestinal histomorphometry at d 42: VH was greatest in T4 (1,227.5 µm) and lowest in T3 (1,009.1 µm; $P = 0.010$); CD was lowest in T4 (133.3 µm) and greatest in T3 (159.1 µm; $P = 0.015$); and VH:CD was greatest in T4 (9.21) and lowest in T3 (6.34; $P = 0.007$). Qualitative histopathological examination showed better preservation of villus architecture and minimal inflammatory changes in T4, whereas villus sloughing and interstitial edema were prominent in T3 and inflammatory cell infiltration and epithelial erosion were observed in T0. These findings indicate that the combination of xylanase, β-mannanase, and lysozyme produced the most favorable overall response in BW and intestinal structural characteristics under the conditions of this study.

Keywords: broiler; xylanase; β-mannanase; lysozyme; intestinal

141 Effect of a Serine Protease on Mitigating Trypsin Inhibitors in Low Amino Acid Density Diets for Broiler Chickens up to 35 Days of Age.

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Trypsin inhibitors (TIs) are anti-nutritional proteins that can reduce the productive performance of broiler chickens, particularly when diets contain protein sources of variable quality. This study evaluated the effect of a serine protease on mitigating the combined impact of high TI activity in low amino acid (AA) density diets during the critical growth phase and TI-sensitive period of broilers from 0 to 35 days of age. A total of 1,920 one-day-old chicks were assigned to one of six treatments, with 16 replicates per treatment and 20 birds per pen. The treatments followed a 2 × 3 factorial design with diets formulated at different AA densities (positive control, PC, or negative control, NC) and varying levels of serine protease supplementation (0, 75, or 100 g/T). Over the 5 feeding phases, all diets included 7.5% raw soybean to increase the TI challenge, and the NC diet was formulated with a reduced AA density (7% lower than breeder recommendations). Growth performance was measured weekly and used to calculate trypsin inhibitor activity intake expressed relative to body weight (TIA). Data were analysed using a two-way ANOVA. From week 3 onwards, PC birds were significantly heavier than NC birds ($P < 0.05$). ADFI was significantly higher in NC birds during week 2, whereas PC birds had higher ADFI in weeks 4 and 5 ($P < 0.05$). ADG was significantly greater in PC birds during weeks 2, 3, 4 and 5 ($P < 0.05$). FCR was significantly improved in NC birds in week 1, but in PC birds during weeks 2 and 4 ($P < 0.05$). Protease supplementation significantly increased BW in week 3, improved ADG in weeks 2 and 3, and improved FCR in weeks 2 and 4 ($P < 0.05$). TIA did not differ amongst treatments and began to reduce during week 3. Significant diet × protease interactions were observed for FCR in weeks 2 and 4 ($P < 0.05$). In week 2, this interaction was driven by a significant difference between the low and high protease doses within the NC groups, whereas no difference was observed in the PC groups. In week 4, the interaction reflected opposing responses to increasing protease levels: FCR decreased and then increased in PC birds, while the opposite pattern was observed in NC birds. These responses suggest that protease enhanced protein digestibility and amino acid availability, resulting in more efficient nutrient utilization and supporting growth performance. The response was most pronounced during the first three weeks of age, when TIA appeared to have the greatest impact on performance. These findings indicate that serine protease can partially offset the negative effects of reduced dietary AA density and TI challenge.

Keywords: protease; trypsin inhibitors; broiler; growth performance; amino acids

142 Effects of Dietary Protease Supplementation on Performance and Egg Quality of 34-Week-Old Laying Hens

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A study was conducted to evaluate the effects of dietary protease supplementation on the performance and egg quality of Brown Nick laying hens from 34 to 36 weeks of age. A total of 256 commercial semi-heavy laying hens were randomly assigned to a completely randomized design with 4 treatments, 8 replicates, and 8 birds per experimental unit. The treatments consisted of: (1) a positive control diet (PC), formulated to meet all nutritional requirements; (2) the positive control diet supplemented with 200 g/ton of protease without applying the enzyme nutritional matrix; (3) a negative control diet (NC) formulated by applying the nutritional matrix corresponding to 200 g/ton of protease and supplemented with 200 g/ton of protease; and (4) a negative control diet formulated by applying the nutritional matrix corresponding to 300 g/ton of protease and supplemented with 300 g/ton of protease. Performance variables evaluated at the end of the experimental period included egg production (% hen-day egg production), average feed intake (g/hen/day), feed conversion per dozen eggs (g feed/dozen eggs), and feed conversion per egg mass (g feed/g egg mass). Egg production, feed intake, and feed conversion values were adjusted for mortality. Egg quality parameters included specific gravity, eggshell breaking strength, albumen height, yolk color, yolk weight, eggshell thickness (mm), eggshell percentage, yolk percentage, albumen percentage, Haugh unit (HU), and albumen and yolk pH. Data were tested for normality and subsequently subjected to analysis of variance. When significant differences were detected ($P < 0.05$), means were compared using Tukey's test at the 5% significance level with SAS software (2026). No significant differences ($P > 0.05$) were observed among treatments for performance variables. However, significant treatment effects were detected for eggshell breaking strength, albumen pH, and yolk pH. The PC, PC + 200 g/ton protease, and NC + 300 g/ton protease treatments showed the greatest eggshell breaking strength (kgf; $P = 0.0144$). The NC + 200 g/ton protease and NC + 300 g/ton protease treatments exhibited lower albumen pH ($P = 0.0001$) and lower yolk pH ($P = 0.0002$). In conclusion, dietary protease supplementation significantly affected selected egg quality traits, with the NC + 300 g/ton protease treatment showing improved eggshell breaking strength and reduced albumen and yolk pH values.

Keywords: egg production; exogenous enzymes; laying hens; protease; semi-heavy laying hens

143 Effect of amylase alone or associated with protease on the metabolizable energy of different cereals

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Exogenous enzymes can enhance the utilization of nutrients and energy from cereal grains in poultry diets. Therefore, this study aimed to evaluate the effects of Apha-Amylase alone or associated with protease on the Metabolizable Energy (ME) and nitrogen corrected (AMEn) on different cereal types: sorghum, millet, normal corn (8% crude protein (CP)) and high-protein (HP) corn (11% CP) for broil-

er chickens. A total of 810 male broilers (14 d-old) with uniform body weight were distributed in metabolic cages in a completely randomized design with 15 treatments, 9 replicates, and 6 birds per replicate. Treatments consisted of a basal diet and 4 cereals (sorghum, millet, corn, and high-protein corn) evaluated without enzymes, with amylase supplementation (80 KNU/kg feed), or with amylase plus protease supplementation (80 KNU and 30,000 NFP/kg feed, respectively), added on-top. Test diets contained 60% basal diet (corn-soybean based diet) and 40% test ingredient. Apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) were determined using the total excreta collection method from 19 to 22 days of age. Data were analyzed using a mixed model, and treatment means were compared by Tukey's test at a 5% significance level. The AME and AMEn values of cereals without enzyme supplementation were 3,366 and 3,317 kcal/kg for sorghum; 3,394 and 3,307 kcal/kg for millet; 3,648 and 3,600 kcal/kg for conventional corn; and 3,515 and 3,441 kcal/kg for high-protein corn, respectively, on an as-fed basis. Supplementation with α -amylase increased AMEn in all evaluated cereals, resulting in improvements of 78, 83, 77, and 67 kcal/kg for sorghum, millet, conventional corn, and high-protein corn, respectively. The combination of α -amylase and protease further enhanced the response, increasing AMEn by 124, 130, 117, and 132 kcal/kg, respectively, compared with the unsupplemented diets. Significant improvements ($P < 0.05$) were observed for millet and high-protein corn. On average, amylase supplementation increased AMEn by 76 kcal/kg, whereas the combination of amylase and protease increased AMEn by 126 kcal/kg compared with the control diets. In conclusion, enzyme supplementation enhanced energy utilization across all cereal sources, highlighting the potential of α -amylase plus protease strategies to increase the nutritional value of broiler diets.

Keywords: corn; sorghum; metabolizable energy; amylase; protease

144 Amino acid digestibility of different sorghum sources in broiler chicken diets supplemented with protease

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This study aimed to evaluate the effect of Sphericase-protease from *Bacillus* sp. expressed in *Bacillus licheniformis* on the digestibility of crude protein and amino acids (AA) in different sorghum varieties. A total of 450 male broiler chickens (22 d-old) with uniform body weight were selected and distributed in metabolic cages. Trial was conducted as a completely randomized design with a 4 x 2 factorial arrangement, with sorghum (4 varieties) and protease level (0 vs 30,000 NFP/kg feed) as main factors. Additionally, two protein-free diets (PFD) were included in the trial. Each treatment had 9 replicates of 5 birds per replicated, and trial lasted for 7 days. The PFD was used to quantify the basal endogenous AA values and calculate standardized ileal digestibility coefficients (SID) of AA's in different varieties of sorghum. All collected data were analyzed as One-Way ANOVA and Tukey test was used for post-hoc comparison of the treatment means. The different sorghum varieties were characterized by crude protein content (CP). The average crude protein content of sorghum variety 1 to 4 was respectively 7.74; 8.98; 9.47; and 10.1% (as-fed basis). There was a significant interaction ($P < 0.05$) between sorghum and protease for Thr, Met, His, and Tyr SID coefficients, the sorghum variety 2 and 3 have higher values with protease supplementation. The SID coefficients of CP and essential and non-essential AA were not different between sorghum varieties ($P > 0.05$), except for Ser SID coefficient who sorghum with

9.47% CP presents higher value. Regardless of the sorghum variety, protease supplementation improved the SID coefficients of essential and non-essential AAs, with improvements ranging from 0.34 to 10%. Specifically, protease supplementation increased SID coefficients of total AA, Thr, Cys, Met+Cys, Gly, Ser, Pro, and Tyr by 3.67; 10.95%, 7.64%, 5.85%, 5.99%, 5.21%, 3.72%, and 7.98%, respectively. Therefore, protease supplementation improves digestibility of AAs in various sorghum varieties in broiler chickens. In conclusion, protease supplementation improved amino acid digestibility regardless of sorghum source, demonstrating its effectiveness as a nutritional strategy to enhance amino acid utilization in broiler diets containing sorghum

Keywords: amino acids; digestibility; broiler chickens; protease; sorghum

145 Nutrient digestibility of corn distillers dried grains with solubles (DDGs) and high protein distillers dried grains (HP-DDG) in broilers diets supplemented with protease

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This study aimed to evaluate the effect of Sphericase-protease from *Bacillus* sp. expressed in *Bacillus licheniformis* on the nitrogen-corrected apparent metabolizable energy (AMEn), Total tract retention (ATTR) of dry matter (DM), crude energy (CE) and crude protein (CP), and digestibility of crude protein and amino acids (AA) in DDGs (31.3% CP and 7.4% fat) and HP-DDG (44.4% CP and 10.8% fat). Two studies were conducted with male broiler chicken 13 d-old for study 1 and 22 d-old for study 2. In each study, a total of 300 male broiler chickens with uniform body weight were selected and distributed in metabolic cages in a completely randomized design with a 2 x 2 factorial arrangement, with corn distillers (DDGS and HP-DDG) and protease (0 or 30,000 NFP/kg of feed) as the main factors. In addition, two control diets were included in the trial: a basal diet and a protein-free diets (PFD). Each treatment had 9 replicates with 5 birds each one. The PFD was used to quantify the basal endogenous AA values and calculate standardized ileal digestibility coefficients (SID) of AAs in DDGS and HP-DDG. For AMEn evaluation, the specific ingredient replaced 40% of the basal diet (corn-soybean meal-based diet). Data were analyzed as One-Way ANOVA and post-hoc differences were evaluated with Tukey test. There was no significant interaction between DDGs and HP-DDG and protease for SID coefficients ($P > 0.05$). The HP-DDG have higher ($P < 0.05$) SID for Arg (+3.2%), His (+3.5%), Tyr (+8.1%), Ser (+7.5%) and Asp (+7.4%) and higher ATTR DM (+1.9%), ATTR CE (+7.6%) AMEn than DDGS. Protease supplementation improved ($P < 0.05$) the SID coefficients of essential (+6.1%) and non-essential AA (+5.1%) except for Leu, Arg and Met and higher ($P = 0.0062$) ATTR DM (+2%). In conclusion, HP-DDG have higher standardized ileal digestibility coefficients and AMEn. Protease supplementation improves the digestibility of amino acids in DDGs and HP-DDG; thus, supporting the use of these co-products in broiler chickens diet.

Keywords: protease; DDGs; HP-DDG; amino acids; broiler chickens

146 Performance of Broiler Chickens fed Rice Milling Waste Diet Supplemented with Ronozyme® Multigrain Enzyme

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The utilization of agro industrial by products such as Rice Milling Waste (RMW) with the supplementation of exogenous enzyme in broiler production has helped to reduce the over reliability of maize. This study was conducted to evaluate the performance of broiler chickens fed rice milling waste based diets supplemented with enzyme (Ronozyme® multigrain). Eleven experimental diets each were formulated to contained RMW supplemented Ronozyme® Multigrain enzyme as replacement for maize at 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. Three hundred (330) 7-days old chicks were allocated to the 11 dietary treatments each having 3 replicate pens with 10 birds per pen. Feed and water were provided *ad libitum* for 6 weeks. Data were collected on the growth performance which were analyzed using ANOVA with a $P < 0.05$ level of significance. The results showed that the final weight, weight gained and average daily weight gained had the same trend of significances where broiler chickens fed control diet and 10% were significantly higher ($P < 0.05$) than the remaining treatment groups, while the total feed intake and average daily feed intake showed that broiler chickens fed control diet, 10% and 20% RMW were significantly higher ($P < 0.05$) than the remaining treatment groups except those fed 30% and 40% RMW. The feed conversion ratio showed that broiler chickens fed control diet, 10%, 20%, 30%, 40% and 50% RMW were significantly lower ($P < 0.05$) from the remaining treatment groups except those fed 60% and 70% RMW. The higher weight and improved feed conversion ratio can be attributed to the activity of the exogenous enzymes in helping to hydrolyse non starch polysaccharides present in rice milling waste which improved nutrient utilization. It can therefore be recommended that RMW can be used to replace maize between 10% - 60% supplemented with Ronozyme® Multigrain enzyme in the diets of broiler chickens under the conditions of the studies.

Keywords: Broiler Chickens; Enzyme; Performance

147 Effects of dietary mannanase supplementation on growth performance, nutrient digestibility, and bone parameters in broiler chickens

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Mannanases have been widely used in poultry nutrition to improve nutrient utilization by reducing the anti-nutritional effects of β -mannans present in soybean. However, their effects on nutrient digestibility and bone characteristics have been less described. The objective of this study was to evaluate the effects of dietary mannanase supplementation on growth performance, apparent ileal digestibility (AID) of nutrients and energy, and bone parameters in broiler chickens. A total of 168 one-day-old male Cobb 500 broiler chicks were randomly assigned to 2 dietary treatments in a completely randomized design, with 12 replicate cages per treatment and 7 birds per cage. Birds were fed a common commercial crumbled diet from d 1 to 7, followed by experimental pelleted diets from d 8 to 25. Treatments consisted of a corn-soybean meal-based basal diet either unsupplemented or supplemented with mannanase at 800 TMU/kg. Diets were formulated to meet nutritional requirements according to the genetic guidelines. Titanium dioxide was included as an indigestible marker for digestibility determinations. Feed and water were provided *ad libitum* throughout the study. On d 25, birds were euthanized, and ileal digesta were collected to determine AID of phosphorus (P), calcium (Ca), nitrogen (N), amino acids (AA), and energy. Growth performance variables included average daily gain (ADG), average feed intake (AFI), and feed conversion ratio (FCR). Bone assessments included tibia breaking strength (TS) and tibia ash content (TAC). Data were analyzed using the MIXED procedure of SAS, with dietary treatment considered a fixed effect and cage as the experimental unit. Statistical significance was declared at $P < 0.05$. Mannanase supplementation improved ADG and FCR compared with birds fed the basal diet ($P < 0.05$). In addition, mannanase significantly increased AID of amino acids and calcium ($P < 0.05$). No effects of mannanase supplementation were observed on energy digestibility, tibia strength, and phosphorus digestibility. Mannanase supplementation tended to have an effect on, tibia ash content ($P = 0.072$) and nitrogen digestibility ($P = 0.062$). In conclusion, supplementation of mannanase at 800 TMU/kg improved growth performance and enhanced amino acid and calcium digestibility in broiler chickens fed corn-soybean meal-based diets. These results suggest that mannanase may contribute to improved nutrient utilization and productive performance in broiler production systems.

Keywords: broiler chickens; mannanase; amino acid digestibility; calcium digestibility; growth performance

Microbiology and Food Safety

148 CROSS-CONTAMINATION OF STRONG BIOFILM-PRODUCING ESBL-POSITIVE *Salmonella* Minnesota DURING IMMERSION CHILLING OF BROILER CARCASSES.

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Salmonella Minnesota is one of the most prevalent serovars in the poultry production chain due to its environmental persistence and biofilm-forming ability, which favor its survival in processing plants and may increase the risk of cross-contamination. The objective of this study was to evaluate cross-contamination of broiler car-

casses during immersion chilling using a strong biofilm-producing, ESBL-positive *Salmonella* Minnesota isolate carrying the *agfA*, *lpfA*, and *adrA* genes under different bacterial loads. The hypothesis was that the immersion chiller acts as a potential amplifier of *Salmonella* Minnesota dissemination among broiler carcasses and that this process is influenced by the initial bacterial load. Two experimental trials, each performed in triplicate, were conducted using a nalidixic acid- and rifampicin-resistant *Salmonella* Minnesota strain. In both trials, the initial contamination level was 2.5% (one contaminated carcass and 39 uncontaminated carcasses), using inoculum of 6.5×10^3 CFU/mL and 6.5×10^4 CFU/mL. The experiments were performed in an experimental immersion chiller simulating commercial processing conditions. The contaminated carcass was inoculated with 1 mL of the bacterial suspension and maintained for 30 min at room temperature before immersion with the remaining carcasses

in chilled water (4°C) for 30 min, with continuous water renewal of 1 L per carcass. After chilling, bacterial recovery was performed by carcass rinsing, enrichment in tetrathionate broth, isolation on XLD agar supplemented with nalidixic acid and rifampicin, and biochemical confirmation. Results were expressed as the frequency of positive carcasses. Cross-contamination was observed under both inoculum concentrations. At 6.5×10^3 CFU/mL, *Salmonella* Minnesota was recovered from 47.5% (19/40) of the carcasses after immersion chilling. At 6.5×10^4 CFU/mL, the recovery frequency increased to 70.0% (28/40). These findings indicate a greater dissemination of the pathogen when the initial bacterial load was higher. The immersion chilling system promoted cross-contamination among broiler carcasses and demonstrated the potential to amplify the dissemination of persistent *Salmonella* Minnesota strains during poultry processing. These findings reinforce the importance of preventive measures to minimize pathogen spread during immersion chilling.

Keywords: *Salmonella* Minnesota; immersion chilling; cross-contamination; biofilm; Poultry processing plant

149 Biofilm Formation and Antimicrobial Resistance Profile of *Escherichia coli* Isolated from Rubber Plucking Fingers

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Escherichia coli is part of the normal microbiota of poultry and may harbor antimicrobial resistance genes, posing a potential risk to public health. This study aimed to evaluate the antimicrobial resistance profile, extended-spectrum β -lactamase (ESBL) production, and biofilm-forming ability of *E. coli* isolates recovered from rubber plucking fingers in a poultry slaughterhouse. For the isolation of *E. coli*, thirty-six pools, each containing three used rubber fingers, were washed and incubated in peptone water (1:10). Subsequently,

the samples were streaked onto MacConkey agar, and one characteristic colony from each pool was selected and subjected to biochemical tests. Antimicrobial resistance was assessed by disk diffusion using the following antimicrobials: amoxicillin - clavulanic acid (AMC, 30 μ g), imipenem (IPM, 10 μ g), cefoxitin (CFO, 30 μ g), ceftriaxone (CRO, 30 μ g), ceftazidime (CAZ, 30 μ g), cefepime (CPM, 30 μ g), ciprofloxacin (CIP, 5 μ g), sulfamethoxazole - trimethoprim (SUT, 25 μ g), tetracycline (TET, 30 μ g), tobramycin (TOB, 10 μ g), fosfomycin (FOS, 200 μ g), and aztreonam (ATM, 30 μ g). For biofilm formation analysis, the isolates were incubated in TSB at 37 °C for 24 h and adjusted to 0.5 McFarland. Bacterial suspensions were then aliquoted into ELISA microplates and incubated again in TSB at 37 °C for 24 h. Plates were washed with PBS, biofilms were fixed with 99% methanol, stained with crystal violet (0.1%), and resuspended in 99% methanol. Absorbance was measured at 546 nm. All assays were performed in triplicate using EAEK ATCC 042 as a positive control. Spearman's correlation test was used to assess the association between biofilm formation and antimicrobial resistance. Statistical significance was set at $p < 0.05$. Twenty-three ($n=23$) isolates were identified as *E. coli*. For antimicrobial resistance, 4.35% (1/23) were resistant to CAZ, CPM and ATM, 8.70% (2/23) were resistant to CRO and CIP, 21.74% (5/23) were resistant to SUT and FOS, 47.83% (11/23) were resistant to TET. Two isolates were classified as MDR and ESBL producers. Regarding biofilm formation, 47.83% (11/23) of the isolates were classified as strong, (30.43%, 7/23) moderate, weak (13.04%, 3/23) and (8.70%, 2/23) non-biofilm producers. There was no statistically significant correlation between antimicrobial resistance and biofilm formation. In conclusion, most *E. coli* isolates exhibited biofilm formation, with the majority classified as strong biofilm producers indicating a potential risk of cross-contamination. Most isolates were resistant to the tested antimicrobials mainly to TET, MDR and ESBL producing isolates were also identified.

Keywords: Poultry slaughterhouse; Public health; Food safety; Cross-contamination; Defeathering

Student Poster Competition

200STP ANTHROPOGENIC TRAUMATIC INJURIES IN BIRDS FROM A WILDLIFE REHABILITATION CENTER IN THE STATE OF GOIÁS, BRAZIL

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Urbanization has increasingly fragmented Brazil's native forests, forcing wildlife to rapidly adapt to urban environments. This growing proximity between wildlife and urban areas may irreversibly impact biodiversity. Habitat fragmentation particularly increases birds' susceptibility to fractures and concussions, which are among the leading causes of mortality in urban wild bird populations worldwide. This study aimed to evaluate the occurrence and characterize the epidemiological profiles of traumatic injuries in wild birds admitted to the Wildlife Screening Center (CETAS) in Goiás, Brazil, during the 2025–2026 period. A total of 71 radiographic images from birds admitted to CETAS were evaluated, of which 44 had corresponding

records of origin. Radiographs were interpreted with the assistance of the Diagnostic Imaging Service of the Veterinary Teaching Hospital, School of Veterinary and Animal Science, Federal University of Goiás (EVZ/UFG). Fractures were classified by type (oblique, comminuted, spiral, simple, transverse, malunion, segmental, complete, incomplete, and Salter-Harris) and affected bone. Birds of the family Psittacidae were the most frequently affected, accounting for 34% (24/71) of the cases, followed by Strigidae (14%; 10/71) and Falconidae (11%; 8/71). Thoracic limbs (wings) were the most commonly injured, corresponding to 67% (59/88) of all injuries. 88 fractures were identified among the 71 birds, with the ulna and radius being the most frequently affected bones, accounting for 32% (28/88) and 18% (16/88) of all fractures, respectively. Comminuted fractures predominated (29%; 26/88), followed by transverse (26%; 23/88), oblique (14%; 12/88), spiral (10%; 9/88), malunion (8%; 7/88), simple (7%; 6/88), complete (3%; 2/88), and incomplete, Salter-Harris, and segmental fractures (1%; 1/88 each). Among birds with origin records, 93% (41/44) were rescued in urban areas of Goiânia and neighboring municipalities, 5% (2/44) in rural areas, and 2% (1/44) along federal highways. The findings demonstrate that anthropogenic activities contribute substantially to the occur-

rence of traumatic injuries and that birds of the family Psittacidae are the most frequently affected, possibly due to their greater abundance and higher adaptability to urban environments. The thoracic limb was identified as the most vulnerable anatomical region, likely due to the biomechanics of wing movement, and a wide variety of fracture patterns was observed, with comminuted and transverse fractures being the most frequent. These findings reinforce the need for conservation strategies aimed at protecting the Cerrado avifauna from the impacts of urbanization.

Keywords: Fractures; Cerrado biome; Urbanization

201STP Genomic characterization of *Salmonella* isolates from poultry production systems in Colombia using nanopore whole-genome sequencing

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This study aimed to characterize the population structure, AMR determinants, virulence-associated genes, and mobile genetic elements (MGEs) of *Salmonella* isolates from Colombian poultry production systems using nanopore WGS. Seventeen *Salmonella* isolates recovered from poultry sources (2008–2024) were sequenced using Oxford Nanopore Technologies. Genome quality was assessed using CheckM criteria (completeness $\geq 90\%$, contamination $\leq 5\%$, sequencing depth $\geq 20\times$, and GC content with *S. enterica*). Serotyping and multilocus sequence typing (MLST) were performed using SeqSero2 and the Achtman scheme. AMR genes, virulence-associated genes, plasmid replicons, pathogenicity islands, and MGEs were identified using ABRicate, VFDB, Plasmid-Finder, SPIFinder, and MOBsuite. Data were summarized using descriptive statistics. Serotyping of all 17 isolates resolved eight distinct serovars: *S. Heidelberg* (27.78%), *S. Paratyphi B* var. Java (22.22%), *S. Enteritidis* (11.11%), *S. Tennessee* (11.1%), *S. Infantis* (11.1%), *S. Mbandaka* (5.6%), *S. Gallinarum* (5.56%), and *S. Javiana* (5.56%). Twenty-three unique AMR genes were detected, the predominant ARM families were *aac* ($n=19$), *sul* ($n=11$), *aph* ($n=10$), *tet* ($n=8$), and *fosA* ($n=8$) with extended-spectrum β -lactamases (*blaCTX-M-65*, *blaCTX-M-165*, *blaCMY-2*). The highest AMR burden was found in *S. Heidelberg* (up to 14 genes) and *S. Infantis* (up to 11 genes). Core virulence genes from SPI-1 to SPI-14 and multiple effector secretion and host invasion loci were broadly distributed; *S. Infantis* carried the highest virulence gene count (162 genes/isolate). A total of 229 MGEs unique were detected, including 189 insertion sequences, 52 composite transposons, 52 MITEs, and 6-unit transposons. Thirteen plasmid replicon types were identified, notably Inc11-I(Alpha), IncC, IncHI2, and IncX1, associated with horizontal resistance transfer. Nanopore WGS enabled comprehensive genomic surveillance of *Salmonella* in Colombian poultry, revealing a genetically diverse population with public health implications. The simultaneous carriage of ESBL genes (*blaCTX-M-65*, *blaCTX-M-165*), fluoroquinolone resistance determinants, high virulence gene loads (up to 162 genes/isolate), and conjugative plasmid replicons in serovars including *S. Heidelberg*, *S. Infantis*, and *S. Enteritidis* highlights the urgent need for integrated genomic monitoring across the poultry production chain. These findings contribute to establishing baseline data to support One Health surveillance and antimicrobial strategies in Colombia.

Keywords: One Health; Nanopore; Virulence; Antimicrobial resistance; *Salmonella*

202STP Effects of In Ovo and Subcutaneous Immune-Complex IBDV Vaccination on Lymphoid Organs and APEC Susceptibility in Broilers

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OBJECTIVE: To compare the effects of in ovo and subcutaneous administration of an immune-complex vaccine against infectious bursal disease virus (IBDV) on lymphoid organs and susceptibility to avian pathogenic *Escherichia coli* (APEC) in broiler chickens. **EXPERIMENTAL DESIGN AND METHODS:** Broiler chickens from the same hatchery and genetic line were randomly assigned to five groups ($n = 30$ birds/group): G1, vaccinated in ovo at 18 days of incubation and subsequently vaccinated against infectious bronchitis at 7 days of age; G2, vaccinated subcutaneously at hatch and subsequently vaccinated against infectious bronchitis; G3, negative control, inoculated with PBS at 18 days of incubation and vaccinated against infectious bronchitis; G4, vaccinated in ovo and subsequently challenged with APEC (1×10^4 CFU) via the air sac at 14 days of age; and G5, vaccinated subcutaneously and subsequently challenged with APEC at 14 days of age. Bursa-to-body weight ratio (BBW), spleen-to-body weight ratio, thymus weight, and macroscopic lesions associated with APEC challenge were evaluated. **RESULTS:** In ovo vaccination did not affect hatchability or hatch weight. At 8 days of age, lower BBW values were observed in the in ovo-vaccinated groups (G1 = 1.95; G4 = 1.78) compared with the subcutaneously vaccinated groups (G2 = 2.09; G5 = 2.16), suggesting earlier lymphoid alterations associated with vaccination. At 16 days of age, bursal alterations remained more evident in the in ovo-vaccinated groups, whereas the control group presented the highest BBW values. Lower thymus diameters and lower spleen-to-body weight ratios were also observed in these groups. Following APEC challenge, mortality was higher in G5 (50%) than in G4 (30%). However, G4 exhibited a higher frequency of pulmonary fibrin deposition, hemorrhages, and air sac lesions, indicating a more acute colibacillosis pattern. At 28 days of age, partial recovery of bursal parameters was observed, although lower BBW values persisted in birds vaccinated in ovo. **CONCLUSIONS:** Immune-complex vaccination against IBDV induced transient alterations in lymphoid organs, particularly in the bursa of Fabricius. Birds vaccinated in ovo exhibited more pronounced lymphoid alterations during the early stages, whereas birds vaccinated subcutaneously showed higher mortality following APEC challenge. The results indicate that the route of vaccine administration influences vaccine-induced lymphoid alterations and the response of birds to secondary bacterial infections. **KEYWORDS:** IBDV; immune-complex vaccine; bursa of Fabricius; lymphoid organs; APEC.

Keywords: IBDV; immune-complex vaccine; bursa of Fabricius; lymphoid organs; APEC

203STP Association of bacterial chondronecrosis with osteomyelitis, tibial dyschondroplasia and epiphyseal fractures with bone breaking strength of the right and left tibiae in broilers

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Leg problems are among the most prevalent causes of culling and late mortality during the grow-out of heavy broilers and may also occur during the first weeks of life, increasing early culling. Common leg disorders include femoral head or tibial head necrosis, also known as bacterial chondronecrosis with osteomyelitis (BCO), tibial dyschondroplasia (TD), and epiphyseal fractures (EF) of long bones. Although the incidence of clinical lameness is variable, many broilers are subclinically affected, showing altered gait patterns and reduced walking ability, which impair feed conversion, growth, and animal welfare. Lamé birds spend more time lying in the litter, increasing carcass contamination and food safety concerns. In addition, bone fragility affects the color quality of deboned products, while bone fragments represent a physical hazard. The objective of this study was to correlate the main leg disorders observed under commercial production conditions with bone mineralization, assessed by bone breaking strength (BBS). A total of 50 right and left tibiae were collected from broilers of the same flock at 21, 35, and 42 days of age. Lesions consistent with EF, BCO, and TD were evaluated. The severity of BCO and TD lesions was scored on a scale from 0 to 3, and EF was classified as absent or present in the epiphyseal and metaphyseal regions of each tibia. Data were analyzed by ANOVA, and Pearson's correlation test was applied to assess the relationship among BCO, TD, EF, and BBS. The occurrence scores for BCO and TD, as well as the incidence of EF in long bones, increased with bird age. The BCO score at 21 days was higher ($P < 0.05$) in the metaphysis of the left tibia compared with the right tibia. For the remaining evaluations, no differences ($P > 0.05$) were observed between right and left tibiae at any age. These findings indicate that factors associated with BCO affect early bone structure development. For BBS, no significant differences ($P > 0.05$) were observed between right and left tibiae, indicating that either tibia can be used for this analysis. In the correlation analyses between BCO and BBS, TD and BBS, and BBS and EF, a significant correlation was observed only between BCO and BBS at 21 days ($P < 0.01$, $r = 0.42$), indicating that increasing BCO severity is associated with reduced BBS. Therefore, correlating necropsy findings is important to identify and monitor leg disorders within the flock and associate them with possible causes, aiming to mitigate productivity losses and reduce negative impacts on bird welfare.

Keywords: Bird welfare; Bone development; Femoral head or tibial head necrosis; Leg diseases; Score

204STP Competitive exclusion promotes early establishment of commensal microbiota and protects broiler chickens against *Salmonella* *Infantis*

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Background: During early life, the chick gut is colonized by microbial communities that may not provide sufficient protection against enteric pathogens. Competitive exclusion (CE) cultures derived from healthy mature animals can promote the rapid establishment of a protective microbiota. Therefore, this study evaluated the effects of CE on gut microbiota composition and resistance to *Salmonella* *Infantis* (SI) colonization in broiler chickens. **Materials & Methods:** CE cultures were produced from the cecal contents of healthy adult

broilers under aerobic and anaerobic conditions. One-day-old chicks of Groups A, B, and C received the treatment (anaerobic, aerobic, and combined) and were challenged with SI 24 h later; Group D received the untreated but challenged (positive control), while Group E remained untreated and unchallenged (positive control). Cecal colonization was assessed at 2, 5, 7, 14, and 21 days post-infection (DPI), and bacterial counts were analyzed by two-way ANOVA followed by Bonferroni's multiple-comparison test. Cecal microbiota composition was evaluated at 2, 14, and 21 DPI by Oxford Nanopore sequencing, followed by alpha diversity (Shannon index), beta diversity (Bray-Curtis), and differential abundance (LEfSe) analyses. Bioinformatic and statistical analyses of sequencing data were performed in R. **Results and Discussion:** CE treatments reduced SI colonization during the early stages of infection (5 DPI, $P < 0.0001$, for all treated groups) and maintained lower cecal counts through 14 DPI ($P < 0.0001$, $P = 0.0004$, and $P = 0.0004$; to A, B and, C, respectively). Significant differences in beta diversity were detected from 2 DPI ($P = 0.001$) onwards and persisted until 21 DPI ($P = 0.002$), demonstrating rapid and sustained modulation of the cecal microbiota. In contrast, no significant differences were observed in alpha diversity, indicating that protection was not associated with increased microbial richness or evenness. Instead, LEfSe revealed an enrichment of beneficial taxa, particularly *Bacteroides* and other genera associated with short-chain fatty acid production, in CE-treated birds. As these taxa are detected at later stages of gut microbiota development, their increased abundance indicates that CE treatment facilitated their establishment. This shift has strengthened competition for ecological niches and resources, contributing to the lower counts of SI in treated birds. **Conclusion:** CE cultures could enhance the early establishment of beneficial bacteria by reducing SI colonization and offering a sustainable alternative to antimicrobial use in poultry production.

Keywords: Competitive exclusion; cecal microbiota; *Salmonella*; intestinal health; green alternative

205STP ANATOMOPATHOLOGICAL CHARACTERIZATION OF A COCCIDIOSIS OUTBREAK IN BROILERS IN THE STATE OF GOIÁS, BRAZIL: A CASE REPORT

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Coccidiosis is a disease caused by the protozoan *Eimeria* that affects both domestic and wild birds and may cause severe damage depending on the parasite burden, age, and host immune response. The present study aimed to characterize the anatomopathological findings in broilers from a poultry farm located in the municipality of Hidrolândia, Goiás state, Brazil. In May 2026, two adult *Gallus gallus domesticus* carcasses of the Charrua lineage were submitted to the Avian Diseases Diagnostic Laboratory of the Federal University of Goiás (SaniAves-UFG) for necropsy. The poultry farmer reported that the birds exhibited diarrhea, reduced feed and water intake, and sudden death. It was also reported that the birds had not undergone prophylactic deworming. On external examination, both carcasses had bad body condition scores (2/2). Upon opening the coelomic cavity, hepatomegaly (1/2) and a small amount of free fluid within the cavity (1/2) were observed. The spleen was congested (1/2), and the kidneys were mildly enlarged (2/2). The duodenum was congested and distended (1/2), with multifocal areas of necrosis, multiple petechial hemorrhages on the mucosa (2/2), and a large

amount of hemorrhagic fluid within the lumen (1/2). The jejunum exhibited congestion and distension due to gas, and it also had the presence of hemorrhagic fluid (2/2), as well as hemorrhagic and necrotic foci throughout the mucosa (2/2) and rare nematodes within the intestinal lumen (1/2). Histopathological analysis revealed marked mixed inflammatory infiltrates in the intestines, composed of macrophages, heterophils, and eosinophils affecting both the mucosa and muscular layer (2/2), in addition to multifocal hemorrhage and congestion (2/2). Numerous structures compatible with first- to third-generation immature *Eimeria* schizonts were identified within the lamina propria and enterocytes (2/2). Moderate necrosis and villous atrophy were also observed, along with structures compatible with *Eimeria* oocysts (2/2). Based on the clinical history and the anatomopathological findings, a diagnosis of coccidiosis was suggested. Following these findings, implementation of biosecurity protocols on the poultry farm, improved flock health management practices, and the administration of anticoccidial and anthelmintic agents were recommended. In conclusion, anatomopathological evaluation is an effective tool for the early diagnosis and control of coccidiosis in poultry. This report highlights the need to strengthen biosecurity protocols, flock health management, and prophylactic parasite control in poultry production systems, regardless of the scale of the farm.

Keywords: Animal production; Mortality; Necropsy; Poultry farming

206STP Duration of live salmonella vaccine-induced IgA

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Non-typhoidal salmonella proliferates extensively in poultry intestines due to an immune response that is biased towards tolerance of the bacteria. Therefore, vaccination has an important role in reducing bacterial loads. Live vaccines offer tissue-localized immune responses, which are useful in reducing salmonella shedding and in preventing bacterial translocation to other organs. Live vaccine immunity is local and therefore relies on IgA antibodies. Here we present data on an ELISA kit (Imunova) developed to measure IgA responses induced by the AviPro™ SALMONELLA DUO (Elanco) vaccine. Data was obtained from two independent layer flocks, monitored for 45 and 48 weeks after the end of a three-dose vaccination protocol, applied from day 1. Statistical analysis was performed by Kruskal-Wallis ($P < 0.05$). Samples were collected by cloacal swabs (13 pools of 5 swabs each) transported in a proprietary carrier medium. In flock 1, samples were assessed at 2, 15, 25 and 48 weeks, and positive responses against pure vaccine-salmonella flagellin were detectable up to 25 weeks following vaccination (considering the whole-flock average). IgA titers at 15 weeks were statistically the same as those at 2 weeks post-vaccination. In flock 2, monitored at 2, 25, 35 and 45 weeks, only anti-LPS responses could be detected at 25 weeks. Still, antibody levels were further reduced at each sampling date afterwards, indicating a declining trend. This temporal pattern reflects the distinct stages of the immune response triggered by vaccination. Over time, and without continued exposure, immunoglobulin levels naturally decline as the immune system shifts into a memory phase.

Keywords: Salmonella; IgA; ELISA; Immune; Poultry

207STP Ceftiofur Administration Effect on the Conjugative R-Plasmid Transfer Between *Escherichia coli* and *Salmonella* Typhimurium in Broiler Chicks.

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INTRODUCTION: *Salmonella* Typhimurium is a major cause of food-borne infections linked with poultry products. However, the antimicrobial use in animal production could be both a problem and a solution, through the selection and spread of resistant bacteria, especially by horizontal gene transfer. This study evaluated the influence of ceftiofur administration in day-old chicks on the conjugation frequency of the IncB/O plasmid carrying the blaCTX-M-1 gene between *Escherichia coli* H2332 (EC) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium F98 (STM). **METHODOLOGY:** Thirty Cobb broiler chickens were assigned to three groups (A, B, and C). On the first day of life, groups A and B received subcutaneous sodium ceftiofur at half the therapeutic dose (0.1 mg/bird) and the therapeutic dose (0.2 mg/bird), respectively. Group C was the control. On the second day of life, all birds were orally inoculated via esophageal gavage with 10⁷ colony-forming units (CFU) of STM and 10⁶ CFU of EC. At 2 and 5 days post-infection (dpi), five birds per group were euthanized, and their cecal contents were collected. STM and STM-T (transconjugant *S. Typhimurium* carrying the blaCTX-M-1 on IncB/O plasmid) were quantified as colony-forming units per gram of feces (CFU/g). STM and STM-T counts were log-transformed and used to calculate plasmid conjugation frequency, obtained as the ratio between Log10 CFU/g values of STM-T by STM. Data were analyzed by two-way ANOVA followed by Bonferroni's multiple-comparison test ($p < 0.05$). To evaluate the effect of ceftiofur STM, Log10 CFU/g values were compared by ANOVA followed by Tukey's test ($p < 0.05$). **RESULTS AND DISCUSSION:** A significant increase in conjugation frequency was observed in both treated groups compared with the control group at 5 dpi; no difference was observed at 2 dpi. Furthermore, ceftiofur significantly reduced intestinal colonization by STM only at the therapeutic dose (0.2 mg/bird). This reduction was observed three days after administration. However, six days after treatment, the effect was no longer observed, and STM colonization no longer differed from the control group. **CONCLUSION:** When used at the therapeutic dose, ceftiofur controls intestinal colonization by STM, although just during the first days of infection. As its plasma concentration decreases, this effect ceases, and the bacterium comes back to colonize the cecum. However, both therapeutic dosing and underdosing increase R-plasmid dissemination carrying the beta-lactam resistance gene, ultimately raising the proportion of resistant bacteria in the intestinal tract.

Keywords: avian salmonellosis; antimicrobial resistance; β -lactam; horizontal gene transfer; plasmid conjugation

208STP Growth performance and nutrient digestibility responses to graded levels of phytase supplementation in broilers fed low-phosphorus diets

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This study evaluated the effects of different phytase supplementation levels on growth performance and nutrient digestibility in broilers fed diets with reduced available phosphorus (P). A total of 840 one-day-old male Ross 308 broilers were randomly assigned to six dietary treatments: T1, positive control (PC; adequate P and Ca); T2, negative control (NC; low P and Ca); T3, NC + 167 FTU/kg; T4, NC + 500 FTU/kg; T5, NC + 1.500 FTU/kg; and T6, NC + 4.500 FTU/kg *Escherichia coli* phytase. The experiment lasted 35 days. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and apparent ileal digestibility of dry matter (DM), Ca, and P were evaluated. Data were analyzed by one-way ANOVA. At 21 days, broilers fed the positive control diet showed the highest BW and ADG, whereas the negative control group exhibited the poorest performance ($P < 0.05$). During the starter period, feed intake (ADFI) was reduced in the T2, T3, T5 and T6 groups compared with T1 and T4 birds ($P < 0.05$), and the negative control showed the highest FCR ($P < 0.05$). By day 35, birds receiving 4.500 FTU/kg phytase showed the highest final BW and the lowest FCR among treatments ($P < 0.05$). Over the entire experimental period, the negative control consistently showed compromised performance, while phytase supplementation improved growth performance in a dose-dependent manner. Apparent ileal digestibility of dry matter and phosphorus was highest in the 4.500 FTU/kg group, whereas Ca digestibility was greatest in the positive control group ($P < 0.05$). Overall, phytase supplementation improved growth performance and nutrient utilization. Supplementation with 1.500 or 4.500 FTU/kg mitigated the negative effects of dietary P deficiency, supporting its use as a nutritional strategy in broiler production.

Keywords: broilers; phytase; phosphorus; nutrient digestibility; growth performance

209STP Evaluation of the efficacy of an exogenous protease on amino acid digestibility and growth performance of broiler chickens

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Exogenous proteases are used in broiler diets to improve amino acid utilization and feed efficiency while mitigating the adverse effects of soybean meal antinutritional factors, such as trypsin inhibitors and lectins. The objective of this study was to evaluate the effects of a commercial protease on the performance and the digestibility of dry matter (DM), crude protein (CP), and amino acids (AA) in broiler chickens up to 21 days of age and fed diets with antinutritional factors from soybean. A total of 539 one-day-old male Cobb 500 chicks were allocated to 77 battery cages in a climate-controlled poultry house. Birds were distributed into seven dietary treatments, with 11 replicate cages of 7 birds each, in a completely randomized design. The experimental treatments consisted of: a positive control (PC) formulated with commercial levels of energy and nutrients; a negative control 1 (NC1) with reductions in CP and AA equivalent to the recommended matrix of the protease; a negative control 2 (NC2) with reductions in CP and AA equivalent to twice the recommended matrix of the protease; PC supplemented with protease at 50 g/t (PC+PRO50); NC1 + protease at 50 g/t (NC1+PRO50); NC1

+ protease at 75 g/t (NC1+PRO75), and NC2 + protease at 75 g/t (NC2+PRO75). The protease product was obtained through the fermentation of *Aspergillus niger* (ACCC 30557) and contained acid, bacterial (neutral), and plant-derived (alkaline) proteases, with a guaranteed minimum activity of 400,000 U/g of acid protease. Broilers were fed corn, soybean meal, meat and bone meal (5%) and raw whole soybean (4%) mash starter diets to simulate a nutritional challenge. Birds were weighed at 7, 14, and 21 days of age, and ileal digesta samples were collected on day 21. Data were subjected to one-way ANOVA, and treatment means were compared by the Tukey's test ($P \leq 0.05$). Orthogonal contrasts were used to compare protease-supplemented diets with the control treatments. From 1 to 21 days, broilers fed the PC+PRO50 or NC1+PRO75 diets had the highest BWG and the best FCR ($P \leq 0.05$). The lowest BWG and digestibility of DM, CP, and AA was observed in birds fed the NC2 diet ($P \leq 0.05$). Protease supplementation significantly improved BWG, FCR, CP and AA digestibility compared to the control diets not supplemented ($P \leq 0.05$). Broilers fed the NC1+PRO50 and NC1+PRO75 diets had improved digestibility of total AA, Lys, Met, Val, Leu, and Ile compared to the NC1 diet ($P \leq 0.05$). In conclusion, the protease enhanced amino acid utilization and restored the growth performance of broilers fed protein- and amino acid-reduced diets containing antinutritional factors, demonstrating its effectiveness as a nutritional strategy for optimizing broiler production.

Keywords: amino acid; antinutritional factor; broiler nutrition; crude protein

210STP ENHANCING POULTRY HEALTH AND FOOD SAFETY WITH PROBIOTICS: A STUDY ON *Bacillus*

velezensis AGAINST *Salmonella* Heidelberg IN BROILERS

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Salmonella Heidelberg (SH) is an important foodborne pathogen associated with poultry production, with broiler chickens acting as one of its main reservoirs. Probiotics have emerged as alternatives to antimicrobials due to their ability to modulate intestinal microbiota and improve food safety. Among them, *Bacillus velezensis* (BV) is notable for its capacity to form spores and produce antimicrobial compounds. Therefore, this study evaluated the antimicrobial activity of BV (strain UNICLON03) against a multidrug-resistant SH strain isolated from broilers, and its effects on the intestinal environment of broiler chickens. The antagonistic activity of BV was first evaluated *in vitro* using the spot-on-lawn method. Subsequently, 100 male broilers (Cobb 500) with 4 days of age were distributed into four groups (n = 25): negative control (NC), positive control (PC; challenged with 6×10^9 CFU/mL of SH), BV-neg (supplemented with BV),

and BV-SH (supplemented with BV and challenged) and maintained under controlled conditions. SH quantification in cecal contents was performed by culture and qPCR at 7, 14, 21, and 28 days of age. Intestinal microbiota was evaluated by 16S rRNA sequencing, fecal metabolomics by HPLC-MS, and ileal histology using the ISI (I See Inside) scoring system. CD4⁺, CD8⁺ lymphocytes and macrophages from the cecal tonsil were quantified by flow cytometry. Data were analyzed using ANOVA, chi-square, and multivariate analyses, considering $p < 0.05$ as statistically significant. *In vitro*, BV showed weak to moderate inhibition against SH, indicating antimicrobial potential. *In vivo*, BV supplementation significantly reduced SH colonization at 28 days ($-3.53 \log \text{CFU/g}$; $\approx 99.97\%$), suggesting a gradual probiotic effect. Metabolomic analysis revealed alterations in metabolites related to cellular integrity and lipid metabolism in infected birds, whereas BV promoted modulation associated with antioxidant and antimicrobial compounds. Metagenomic analysis demonstrated greater microbial diversity in challenged and/or supplemented birds, while increasing diversity with age reflected microbiota maturation. *Faecalibacterium*, a butyrate-producing bacterium associated with intestinal health, was also identified. Histological analysis showed impaired intestinal quality in SH-challenged birds, whereas BV improved intestinal quality in challenged animals. Although CD4⁺ lymphocytes and macrophages did not differ among groups, SH challenge increased CD8⁺ T lymphocytes at 28 days, while BV restored levels similar to NC group. Overall, BV reduced SH colonization while modulating intestinal microbiota, metabolism, and immune responses, contributing to improved intestinal health in broiler chickens.

Keywords: *Bacillus velezensis*; Poultry gut microbiota; Probiotics; Metagenomics; Metabolomics

211STP *Bacillus velezensis* and phytogetic supplementation may modulate intestinal and ovarian metabolism and affect egg production in aged pure-line White Plymouth Rock hens

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This study evaluated the effects of *B. velezensis* BV03UNICLON (BV) and a commercial phytogetic blend (BM) on egg production (EP), ovarian metabolomic profile, and intestinal short-chain fatty acids (SCFA) in aged pure-line hens (81–102 weeks). Ninety-six 80-week-old White Plymouth Rock hens were randomly assigned to four groups (n=24): (I) negative control (NC), receiving phosphate-buffered saline; (II) BV, receiving 10^6CFU/hen of BV03; (III) BM, receiving 20 mg/hen; and (IV) BM + BV. Treatments were administered via gavage (100 $\mu\text{L/hen/day}$). At 102 weeks, acetate, butyrate, propionate, isobutyrate, isovalerate, and pentanoic acid in cecal content were quantified by GC/MS. Small primordial white oocytes were collected for untargeted metabolomic analysis by LC/MS. EP and intestinal compounds were analyzed by ANOVA. For metabolomics, only metabolites with 100% frequency, fold change >2 , and $p < 0.05$ were considered. PCA, clustering, and PLS-DA were applied, adopting $p < 0.05$. EP increased in BV and BM groups but decreased in the BM + BV group. SCFA analysis showed higher acetate, butyrate, propionate, isovalerate, and pentanoic acid in BM compared to NC and BM + BV. PCA and PLS-DA demonstrated separation between NC and BM/BM + BV, with BV intermediate, forming clusters of NC with BV and BM with BM + BV. PLS-DA prediction accuracy

was 84.2% (NC), 100% (BV), 68.18% (BM), and 85% (BM + BV), indicating strong biological effects and metabolic adaptations related to ovarian function. Two ovarian metabolites with known biological activity were higher in BM and BM + BV, while two microbial-derived metabolites were higher in BM, supporting a stronger gut–ovary axis. These findings align with increased cecal SCFA in BM. Ovarian metabolites did not explain the antagonistic EP effect in BM + BV, suggesting involvement of other tissues, such as the liver. In conclusion, BV and BM individually improved EP in aged hens, but not when combined, indicating that probiotics and phytoGENICS are promising alternatives to AGPs, though their association requires further investigation.

Keywords: Feed additives; Laying hens performance; metabolomics; *Bacillus velezensis*; intestinal health

212STP Growth performance and organ morphometry of broiler chickens fed a GABA-producing *Lactiplantibacillus plantarum* strain under two stocking densities

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We evaluated whether dietary supplementation with a gamma-aminobutyric acid (GABA)-producing *Lactiplantibacillus plantarum* (LPB145) strain modulates growth performance, mortality, lymphoid and digestive organ morphometry, and caecal pH in broiler chickens reared under two stocking densities. A total of 360 one-day-old male Cobb 500 broilers were allocated to 36 floor pens in a randomised complete block design with a 3×2 factorial arrangement (three dietary treatments and two stocking densities). Treatments consisted of a basal diet without probiotic, the basal diet supplemented with LPB145 at $1 \times 10^8 \text{CFU/kg}$ feed, or the basal diet supplemented with a commercial probiotic (Turbolyte Plus, VetBrands International Inc.) at 0.025%. Birds were housed at 8 or 12 birds/ m^2 , with six replicate pens per treatment. Mash diets were formulated for starter (d 1–12), grower (d 13–24), and finisher (d 25–36) phases. Feed intake, body weight gain, feed conversion ratio (FCR), and mortality were recorded by pen. On d 12, 24, and 36, two birds per pen were sampled for bursa of Fabricius, spleen, proventriculus and gizzard morphometry, total gastrointestinal tract length, and caecal pH. Productive and morphometric data were analysed using mixed models including dietary treatment, stocking density, phase, and their interactions as fixed effects, and block as a random effect; pen was considered the experimental unit. Mortality was analysed using a binomial mixed model. Means were compared using Tukey adjustment when $P < 0.05$. Initial body weight did not differ among treatments ($P = 0.760$). During d 13–24, feed intake and FCR differed among treatments ($P = 0.018$ and $P = 0.002$, respectively); LPB145 at 8 birds/ m^2 showed the greatest feed intake and poorest FCR, whereas body weight gain was not affected ($P = 0.222$). No treatment differences were detected for cumulative feed intake, body weight gain, or FCR from d 1–36 ($P > 0.05$). Mortality was not affected by dietary treatment or stocking density ($P > 0.50$), but increased during d 25–36 compared with earlier phases ($P = 0.001$).

Lymphoid and digestive organ weights, bursa ratio, and gastrointestinal tract length were not affected by treatments, although caecal pH differed among treatments at d 36 ($P = 0.032$). Dietary LPB145 did not consistently improve broiler growth performance or organ morphometric indicators compared with the control or commercial probiotic. The response observed during d 13–24 suggests a transient effect that did not persist to market age. Further analyses of immune, metabolic, microbiota, and stress-related biomarkers may clarify whether this GABA-producing strain modulates physiological responses not reflected in conventional performance traits.

Keywords: broiler chicken; GABA; probiotic; stocking density; actiplantibacillus plantarum

213STP Effects of increasing dietary levels of *Acacia mearnsii* tannins on performance and intestinal health of broiler chickens under intestinal challenges

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Two experiments (Exp.) were conducted to evaluate the effects of increasing levels of dietary tannins on the performance, litter quality, and intestinal health of broilers up to 43 days of age subjected to intestinal challenges with *Clostridium perfringens* (Exp. 1) or *Salmonella* Heidelberg (Exp. 2). In Exp. 1, a total of 1,620 Cobb 500 male chicks were assigned to six dietary treatments with 10 replicate pens of 27 birds each. Treatments consisted of a non-challenged control, a challenged control, and challenged groups supplemented with 300, 500, 700, or 900 mg/kg of tannins derived from *Acacia mearnsii* (minimum guarantee of 73.5 g/kg condensed tannins). Birds in the challenged groups were subjected to an *Eimeria* spp. and *C. perfringens* challenge. In Exp. 2, a total of 1,400 Cobb 500 male chicks were assigned to five dietary treatments with eight replicate pens of 35 birds each. Treatments consisted of *S. Heidelberg*-challenged birds orally inoculated with a field strain and supplemented with 0, 300, 500, 700, or 900 mg/kg of *A. mearnsii* tannins. Performance was evaluated weekly in both experiments. In Exp. 1, intestinal permeability, ileal digestibility, and jejunal morphometry were assessed on day 21. In Exp. 2, duodenal, jejunal, and ileal morphometry were evaluated on days 7 and 42. The effects of dietary treatments were analyzed using linear and quadratic regression models in SAS ($P \leq 0.05$). Treatments did not affect mortality in either experiment. In Exp. 1, challenged birds had lower performance and greater intestinal permeability than non-challenged birds ($P < 0.05$). Over the overall period, increasing tannin resulted in a quadratic improvement ($P < 0.05$) in body weight gain, with an estimated optimum level of 310 mg/kg, whereas the optimal level for feed conversion ratio was at 444 mg/kg. Intestinal permeability was minimized at 466 mg/kg tannins, while protein and energy digestibility were maximized at 374 and 294 mg/kg, respectively ($P < 0.05$). Litter moisture decreased linearly at 35 and 43 days ($P < 0.05$). In Exp. 2, tannins resulted in quadratic improvements in body weight gain from days 1–28, 1–35, and 1–42, with estimated optimal levels of 265, 412, and 456 mg/kg, respectively ($P < 0.05$). Tannins did not affect feed conversion ratio during any period; however, ileal villus height was increased at an estimated level of 600 mg/kg ($P = 0.0100$). At 42 days, the prevalence of *S. Heidelberg* was 88% in the non-supplemented control, decreasing to 38% and 13% in birds supplemented with 300 and 500 mg/kg tannins, respectively. In conclusion, *Acacia mearnsii* tannins improved performance and intestinal morphology in broilers challenged with either *C. perfringens* or *S. Heidelberg*.

Keywords: Broiler chicken; digestibility; intestinal permeability; villus height

214STP *In vitro* evaluation of the adsorption efficiency of two binding agents against ergot alkaloids

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Ergot alkaloids (EAs) are secondary metabolites produced by *Claviceps* species and can contaminate cereal products such as barley, rye, and wheat. Consumption of grain contaminated with EAs can cause convulsions, vasoconstriction, and, in severe cases, gangrene. The presence of EAs in feed poses a potential threat to poultry health, emphasizing the importance of investigating products that can effectively adsorb these metabolites in the gastrointestinal tract and reduce their bioavailability. *In vitro* assays were performed with two binding agents, activated charcoal (AC) and bentonite (BT), to assess their ability to adsorb 1.0 mg L⁻¹ of four EAs (ergocornine, ergocristine, ergocryptine, and ergosine). The two products were incubated separately and in triplicate in simulated gastric (pH 3) and intestinal (pH 6) solutions with 1.0 mg L⁻¹ of the EAs. The inclusion rate of both products in the different solutions was 0.5%. The solutions were incubated at 37 °C for one hour and subsequently centrifuged at 2,500 rpm for 10 minutes. After centrifugation, the supernatant was collected and analyzed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS). Positive controls were included, consisting of tubes containing only the simulated gastric or intestinal solutions and EAs, while negative controls consisted of the binding agent without mycotoxins. Adsorption (%) was calculated from the reduction in the chromatographic peak area observed in samples containing the binding agent compared with the corresponding positive controls. Both adsorbents exhibited high binding affinity for EAs under acidic conditions (ranging from 88% to 95%), while adsorption efficiency decreased at pH 6 (from 22% to 78%). With CA, ergocristine exhibited the highest adsorption percentage (94%), followed by ergocryptine (92%), ergocornine (89%), and ergosine (88%). Similarly, with BT, ergocristine showed the highest adsorption percentage (95%), followed by ergosine (94%), ergocornine (90%), and ergocryptine (89%). At pH 6, with CA, ergocristine exhibited the highest adsorption (77%), followed by ergosine (69%), ergocryptine (55%), and ergocornine (22%). With BT, the highest adsorption was observed for ergosine (78%), followed by ergocristine (68%), ergocryptine (48%), and ergocornine (30%). In conclusion, ergocristine and ergosine showed the highest adsorption rates across the evaluated conditions. The variable adsorption observed among the different EAs suggests that their complex molecular structures and physicochemical properties may influence their interaction with adsorbent materials, making the binding process more challenging than that reported for other classes of mycotoxins.

Keywords: ergot alkaloids; adsorption; *in vitro*; adsorbent agents

215STP A novel phytogel as an alternative to combat stress during the chick growth phase

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The study hypothesized that a hydrogel based on *Libidibia ferrea* extract could enhance the antimicrobial and antioxidant properties of the plant, improving the performance and intestinal health of chicks. The objective was to develop and characterize this hydrogel and evaluate its biological effects in vitro and in vivo. A hydrogel containing *L. ferrea* extract was formulated and characterized via physicochemical analyses (HPLC-MS, FTIR-ATR, FE-SEM, and DSC), which revealed its thermal stability and flake-like texture compatible with biopolymer matrices. In vitro assays were performed to assess antimicrobial and antioxidant activities. Antimicrobial activity was tested against *Salmonella* spp. and *Escherichia coli*, while antioxidant capacity was evaluated through metal chelation, free radical scavenging, and lipid peroxidation inhibition assays. To in vivo experiments, a total of 480 one-day-old male Cobb broiler chicks were used. The groups were divided into T1 (non-stressed and not treated with HLF), T2 (non-stressed and treated with HLF), T3 (stressed and not treated with HLF), and T4 (stressed and treated with HLF). The chicks were housed in 32 floor pens (2.0 × 1 m), with 15 birds per pen and eight pens per group. The evaluated parameters included weight gain, lipid peroxidation, activities of the antioxidant enzymes Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx), jejunal villus height, and villus-to-crypt ratio. To data analysis we performed ANOVA followed by Tukey test considering $p < 0.05$. The hydrogel showed thermal stability and a flake-like structure compatible with biopolymer matrices. It exhibited greater antimicrobial activity against *Salmonella* spp. and *E. coli* than the pristine extract. Antioxidant assays demonstrated high metal-chelating activity, free radical neutralization, and inhibition of lipid peroxidation. In vivo, treated and stressed chicks showed weight gain similar to non-stressed animals, reduced lipid peroxidation, and increased GPx and SOD activities. The hydrogel also improved intestinal morphology by increasing villus height and villus-to-crypt ratio. The *L. ferrea*-based hydrogel demonstrated strong antimicrobial and antioxidant properties and effectively reduced oxidative stress in day-old chicks. The treatment improved animal performance and intestinal health, suggesting its potential as a viable alternative to phytobiotics in poultry production.

Keywords: *Libidibia ferrea*; phytobiotics; microbiota modulation; sustainable poultry farming

216STP Growth performance of broiler chickens challenged with *Eimeria maxima* and fed *Hermetia illucens* larvae oil from 1 to 27 days of age

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Coccidiosis caused by *Eimeria maxima* is an intestinal disease of

major importance in poultry production because it can impair intestinal mucosal integrity, nutrient utilization, and, consequently, broiler performance. In this context, *Hermetia illucens* larvae oil (HILO) has been investigated as a nutritional alternative to support birds' responses to enteric challenges. This study evaluated the effects of HILO inclusion on the growth performance of broiler chickens challenged with *E. maxima*. A total of 540 one-day-old male Ross broiler chicks were housed in 3-m² pens. The experiment was conducted in a completely randomized design with five treatments. The non-challenged control had four replicates of 15 birds, and each of the four challenged treatments had eight replicates of 15 birds, totaling 36 experimental units. The study was approved by the Animal Use Ethics Committee, under approval no. 811/2023. The treatments were: non-challenged control; challenged control, with birds inoculated with *E. maxima* and fed a control diet containing antibiotic and antioxidant, without HILO; challenged antibiotic- and antioxidant-free diet (AAFD), without HILO; challenged AAFD + 0.5% HILO; and challenged AAFD + 1.0% HILO. At 17 days of age, birds in the challenged treatments were inoculated with *E. maxima* by oral gavage at a dose of 10⁷ oocysts/bird. Birds were weighed at the beginning and end of the experimental period. Mean body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) were evaluated. Feed intake was calculated as the difference between feed offered and refusals and adjusted for mortality. FCR was calculated as FI/BWG. Data were subjected to analysis of variance using the GLM procedure of SAS, and means were compared by the Student-Newman-Keuls test at 5% probability. Treatments affected BWG, FCR ($p < 0.0001$), but did not affect FI ($p = 0.5667$). The non-challenged control showed greater BWG and lower FCR than the challenged treatments. However, the groups challenged with *E. maxima* did not differ from each other, regardless of the inclusion of antibiotic, antioxidant, or supplementation with 0.5% or 1.0% HILO. In conclusion, *E. maxima* infection significantly impaired broiler performance from 1 to 27 days of age. Supplementation with 0.5% and 1.0% HILO did not promote statistically significant recovery of performance, although a numerical improvement was observed, especially with 1.0% HILO.

Keywords: Broiler Chickens; Coccidiosis; Feed Additives; Functional Lipids; Poultry Nutrition

217STP Dietary supplementation of phytogetic feed additives: Impact on broiler growth performance, carcass traits, and intestinal histomorphometry

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The ban or restriction of antibiotic growth promoters has increased the demand for highly effective alternative bioactive compounds, such as phyto-genics, capable of maximizing broiler performance while supporting mucosal development and intestinal health. This study evaluated the effects of dietary supplementation with phyto-genic feed additives, administered either alone or sequentially combined with Zinc Bacitracin (ZnB), on growth performance, carcass yield, and intestinal histomorphometry in broilers. A total of 840 1-d-old Cobb 500 male chicks were allocated into six treatments

with 10 replicates of 14 birds each in a completely randomized design. Birds were managed under a three-phase feeding program: starter (1-21 d), grower (22-35 d), and finisher (36-41 d). Dietary treatments consisted of: T1, negative control; T2, positive control (55 g/ton ZnB, d 1-42); T3, artichoke (*Cynara scolymus*) + chicory (*Cichorium intybus*) + choline chloride (ACC) at 150 g/ton (d 1-21); T4, ACC at 150 g/ton (d 1-21) + 55 g/ton ZnB (d 22-42); T5, ACC at 150 g/ton (d 1-42); and T6, cinnamon (*Cinnamomum zeylanicum*) + oregano (*Origanum vulgare*) (CO) at 100 g/ton (d 1-42). Body weight, feed intake, and feed conversion ratio were evaluated. At 21 and 41 d of age, 10 birds per treatment were euthanized to harvest tissue samples for intestinal histomorphometry of the jejunum and ileum. At 42 d, 20 birds per treatment were processed to determine carcass and commercial cuts yield. Data were analyzed by ANOVA and Tukey's test ($p < 0.05$). Throughout the overall experimental period (d 1-41), T5 significantly optimized growth performance and final carcass yield ($p < 0.001$), while T3, T4, and T6 performed better than the antibiotic treatment T2 across all production parameters ($p < 0.001$). Intestinal histomorphometry was improved by T5, which resulted in greater jejunal villus width and higher villus height depth (H) ratios at 21 days, as well as greater ileal villus height and H ratios at 41 days ($p < 0.05$). Organ measurements were not affected by the treatments ($p > 0.05$). In conclusion, the continuous inclusion of ACC and CO in broiler diets improved growth performance, carcass traits, and intestinal development, indicating that these phyto-genic additives may be effective alternatives to antibiotic growth promoters.

Keywords: Antibiotic growth promoters; Artichoke; Chicory; Cinnamon; Oregano

218STP A survey on mycotoxin occurrence and concentration in corn across the five Brazilian regions from 2021 to 2025

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Corn is one of the most consumed energy sources worldwide, with an estimated global production of 1.31 billion metric tons in the 2025/2026. However, corn is highly susceptible to mycotoxin contamination, making continuous monitoring essential for risk assessment and mitigation. This study aimed to investigate the presence and concentration of total aflatoxins (AFB1, AFB2, AFG1, and AFG2) (AFTs), total fumonisins (FB1 and FB2) (FBs), deoxynivalenol (DON), and zearalenone (ZEN) in Brazilian corn from 2021 to 2025. A total of 14,702 analyses were performed in 5,055 samples provided by industries located in different Brazilian regions: South (2,965), Southeast (1,884), Midwest (107), North (51), and Northeast (47). Mycotoxins quantification was performed by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). Data were subjected to descriptive statistics using the software Statgraphics Centurion. FBs were the most prevalent mycotoxins in Brazil, occurring in 79% (mean $1,289 \pm 2,549$ µg/kg) of corn samples, followed by ZEN (39%, mean 86.0 ± 361.9 µg/kg), DON (30%, mean 163.2 ± 383.9 µg/kg), and AFTs (23%, mean 3.52 ± 24.4 µg/kg). Furthermore, FBs showed the highest prevalence in all Brazilian regions, except for the North, where AFTs predominated. In the Southern region, FBs showed a prevalence of 82%, with a mean contamination of 1,031 µg/kg. ZEN was the second most prevalent mycotoxin, detected in 48% of the samples (mean 92.7 µg/kg), followed by DON (37%; mean 182.8 µg/kg) and AFTs (24%; mean 2.85 µg/kg). In the Southeast region, the prevalences of FBs, AFTs, ZEN,

and DON were 73%, 21%, 19%, and 16%, respectively, with mean contamination levels of 1,674 µg/kg, 5.37 µg/kg, 69.7 µg/kg, and 131.4 µg/kg. In the Midwest, the prevalences of FBs, AFTs, ZEN, and DON were 83%, 27%, 9%, and 1%, respectively, with mean contamination levels of 1,353 µg/kg, 8.09 µg/kg, 76.2 µg/kg, and 8.1 µg/kg, respectively. In the North, AFTs were detected in 100% of the samples, with a mean of 1.05 µg/kg. FBs were detected in 43% of the samples, with a mean contamination level of 290.6 µg/kg, whereas DON and ZEN were not detected. Finally, in the Northeast region, FBs showed a prevalence of 100% (mean: 3,613 µg/kg), whereas AFTs showed a prevalence of 20% (mean: 6.28 µg/kg). In conclusion, the high prevalence and contamination levels observed for FBs, particularly in the Northeast reinforce their importance as major contaminants of Brazilian corn. A special attention can be given to DON and ZEN in corn from South. These findings highlight the need for continuous monitoring of mycotoxin across different regions to support mitigation strategies and ensure feed safety in the poultry industry.

Keywords: mycotoxins; corn; HPLC-MS/MS; Brazil

219STP Nutritional composition, mycotoxin contamination, and crop yield of corn transgenic technologies intended for poultry feed

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Corn is one of the main ingredients in poultry feeds, making its nutritional quality and safety key factors determining the efficiency and sustainability of poultry production. This study evaluated the effects of two transgenic corn technologies (VT PRO® and PowerCore® ULTRA) on crop yield, nutritional composition, and mycotoxin contamination over two harvests (2024 and 2025). A total of 124 samples (60 in 2024, and 64 in 2025) from different commercial corn hybrids cultivated in the Agriculture Research Center from CO-PACOL company, in Paraná state, Brazil, were evaluated. Treatments were distributed in a randomized block design, with corn hybrids as blocks with four replicates each. Crop yield and damaged grains were determined after harvest. Samples were milled (1 mm) and total aflatoxins (AFB1, AFB2, AFG1, and AFG2), total fumonisins (FB1 and FB2), deoxynivalenol (DON), and zearalenone (ZEN) were quantified by HPLC-MS/MS. Dry matter (DM), crude protein (CP), ether extract (EE), total and phytic P, total and digestible amino acids (AA), and metabolizable energy (AMEn) for poultry were predicted by near-infrared spectroscopy (NIRS) with the calibration curves from the AMINONRG® and AMINONir® programs. Values were corrected to 87% DM basis. Data were analyzed using SAS software, normality was assessed by the Shapiro-Wilk test, mycotoxin contamination data were transformed using $\log_{10}(x+1)$, and ANOVA was performed using the GLIMMIX procedure at 5% significance. Crop yield, DON, and ZEN did not differ ($P > 0.05$) between transgenic technologies during the two years of study. VT PRO® corn presented higher fumonisins concentrations and higher percentage of damaged grains than PowerCore® ULTRA in both growing seasons ($P \leq 0.05$). VT PRO® also presented higher aflatoxins means ($P < 0.05$) in 2024. PowerCore® ULTRA exhibited higher CP content ($P < 0.001$) and greater concentrations of total and dig. AA ($P < 0.05$) in 2024; however, no differences were observed for CP ($P > 0.05$) in 2025. Furthermore, EE differed in both years, with VT PRO® showing the highest values ($P < 0.05$). VT PRO® also had higher values of AMEn than PowerCore® ULTRA ($P < 0.05$) in 2025. Cumulative

precipitation during the crop cultivation was 423.4 mm in 2024 and 612.4 in 2025. Mean daily temperatures remained similar between the two years. In conclusion, transgenic corn technologies did not affect crop yield but influenced nutritional composition and mycotoxin contamination, especially fumonisins, with effects that may have been influenced by climatic conditions. Therefore, the selection of corn transgenic technologies to be used in poultry nutrition should consider not only productivity, but also nutritional value, mycotoxin contamination, and feed safety.

Keywords: grain quality; mycotoxins; poultry nutrition; transgenic corn

220STP Alternative dietary phosphorus sources increase bone mineral density without compromising the performance of laying hens

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The objective of this study was to evaluate the productive performance and bone mineral density of laying hens fed different phosphorus sources, including meat and bone meal and FreePhos as an alternative to conventional phosphorus sources. The experiment was conducted at the Experimental Farm of the State University of Maringá, Paraná, Brazil, and approved by the Institutional Animal Care and Use Committee (Protocol No. 5284270223). A completely randomized design was adopted using 320 Hy-Line laying hens aged 55 to 65 weeks, distributed across four dietary treatments: (1) control diet containing 42% meat and bone meal (MBM); (2) FreePhos 2% (2 g/kg); (3) 18% dicalcium phosphate; and (4) FreePhos 1% (1 g/kg), with 10 replicates of eight birds each. Experimental diets were formulated according to the Brazilian Tables for Poultry and Swine (2017). Productive performance was evaluated through laying rate, feed intake, feed conversion ratio per dozen eggs, feed conversion ratio per egg mass, and egg mass. Tibial bone mineral density was determined by digital radiography using an aluminum step wedge as a reference, quantified with Fiji (ImageJ) software, and expressed as millimeters of aluminum equivalent (mmAl). Data were analyzed by one-way ANOVA, and treatment means were compared using Tukey's test ($P < 0.05$). Phosphorus sources had no significant effect on laying rate ($P = 0.761$), feed intake ($P = 0.128$), feed conversion ratio per dozen eggs ($P = 0.554$), or egg mass ($P = 0.076$). However, feed conversion ratio per egg mass was significantly affected by treatment ($P = 0.019$), with hens fed meat and bone meal and FreePhos 2% showing the lowest values (1.92). Tibial bone mineral density was significantly higher in hens receiving the FreePhos 1% diet ($P < 0.0001$). In conclusion, FreePhos is a viable alternative phosphorus source for laying hens, maintaining productive performance while improving tibial bone mineral density when included at 1% of the diet, without compromising egg production.

Keywords: inorganic mineral; lay rate; bone health; poultry farming; zootechnical indices

221STP Performance and Egg Quality of Laying Hens fed with Dietary Copper Proteinate at early production

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Copper (Cu) is an essential trace mineral for laying hens, involved in antioxidant defense, mineral metabolism, immune function, and numerous metabolic processes. Inorganic sources, such as copper sulfate (CuSO_4), present lower bioavailability compared to organic sources, such as copper proteinate (CuP) due to interactions with other dietary components. This study was designed to evaluate the production performance and egg quality of laying hens at early production (21 to 32 weeks of age), as influenced by the Cu source and supplementation strategy applied during the growing and early laying phases. A total of 480 white laying hens (20 weeks of age) were randomly assigned to 4 experimental treatments in a 2×2 factorial arrangement, with 15 replicates of 8 birds per experimental unit. The experimental factors included two Cu sources and two production phases (growing and early laying phase). In each phase of production, birds received either 100% of the recommended Cu requirement from CuSO_4 or 50% of the requirement using CuP (Bioplex Copper, Alltech Inc.). Diets were formulated based on the recommended nutritional requirements of laying hens from the Brazilian Tables. Feed intake (FI), feed conversion g/g (FC), egg production, egg mass, and egg quality were measured from 21 to 32 weeks of age over three 28-day periods. Data were analyzed by ANOVA in a 2×2 factorial arrangement, and means were compared using Tukey's test with SAS at a significance level of 5%. No significant differences between dietary Cu sources used during the growing phase were found for productive performance variables of hens including FI, egg production, EM, and FC. Hens fed CuP during the early laying period showed higher FI compared with hens fed CuSO_4 ($P=0.021$). The specific gravity of eggs from hens fed diet with CuSO_4 during the rearing phase was higher ($P = 0.01$) than those fed diet with CuP . An interaction between Cu sources during growing and early laying phases was observed for eggshell thickness ($P=0.043$), with higher values observed in hens supplemented with CuP during the early laying phase only. In conclusion, the replacing of CuSO_4 with CuP at 50% of the supplemental level in laying diets did not negatively impact productive performance and may support eggshell quality during the early laying phase.

Keywords: bioavailability; eggshell thickness ; specific gravity; supplementation strategy; trace mineral

222STP Calcium and Phosphorus utilization in the long production cycle of laying hens subject to Ca supply partitioning along the day

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In laying hens with an extended production cycle, mineral nutrition is essential to support shell formation and maintain bone mineralization. Eggshell formation occurs during the dark, demanding Ca from the remaining feed in the gut or from bone. Guarantee

Ca supply throughout the day remains scarce. This study aimed to evaluate Ca and P utilization in late-phase laying hens subjected to daily calcium feeding with partitioning. A total of 80 Bovans White laying hens (112 weeks of age) were randomly assigned to two treatments, with 40 hens per treatment. Hens were allocated in individual cages equipped with drinkers and individual feeders. The adopted light program was 16L:8D (lights turned on at 0700h). The treatments consisted of supplying two diets: D1, diluted with 1.68 % Ca, in the morning (0700h), and the concentrated D2, with 2.52% Ca, in the afternoon (1600h). However, the treatment consisted of varying the particle size of limestone: T1 composed of D1 (100% fine limestone) and D2 (100% coarse limestone), and T2 composed of D1 (30% fine and 70% coarse limestone) and D2 (30% fine and 70% coarse limestone). The diets were formulated to meet nutritional requirements, except for Ca and avP, while maintaining the same Ca:avP ratio of 12:1. Data was analyzed using the SAS MIXED procedure with repeated measures over time. When interaction and mean comparisons were conducted by Tukey's test at a 5% probability level. A treatment effect was observed for phosphorus intake, which was higher in T1 (0.265 g/b/d) than in T2 (0.243 g/b/d). Phosphorus excretion was affected by the period, with higher values in afternoon (0.143 g/b/d) than in the morning (0.080 g/b/d). Consequently, phosphorus retention was greater in the morning (0.174 g/b/d) than in the afternoon (0.111 g/b/d), regardless of treatment, indicating a diurnal variation in phosphorus utilization. For calcium, excretion was lower in T2 (0.296 g/b/d) than in T1 (0.492 g/b/d). In addition, a significant treatment x period Interaction was observed for calcium intake and retention. In T1, calcium intake was higher in the afternoon (1.783 g/b/d) than in the morning (1.057 g/b/d), resulting in greater calcium retention during the afternoon (1.250 g/b/d). In T2, calcium intake (1.261 and 1.401 g/b/d) and calcium retention (0.967 e 1.102 g/b/d) were similar between the morning and afternoon periods, indicating greater stability in calcium availability throughout the day. It can be concluded that different calcium supplementation strategies modify the calcium retention throughout the day, highlighting the importance of synchronizing mineral supply with the physiological demands of aged laying hens.

Keywords: Split feeding; Eggshell calcification; Minerals

223STP Comparative Potencial of Propolis from Stingless Bee and *Apis mellifera* Against Bacteria

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The objective of this study was to compare the antimicrobial activity of hydroalcoholic extracts of propolis from three stingless bee species with that of green propolis from *Apis mellifera*. Hydroalcoholic extracts (30%, w/v) of propolis from Tubuna (*Scaptotrigona bipunctata*), Mandaçaia (*Melipona quadrifasciata*), Borá (*Tetragona clavipes*), and green propolis were prepared and evaluated for minimum inhibitory concentration (MIC) against reference strains of *Salmonella* sp., *Escherichia coli*, and *Staphylococcus aureus* using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2024). Twelve concentrations were evaluated, including positive and negative controls, 75:25, 50:50, 25:75, and serial dilutions from 10⁻¹ to 10⁻⁶, with each extract tested in duplicate. The botanical origin of the propolis samples was confirmed by palynological analysis. MIC results were interpreted descriptively according to CLSI recommendations. Borá propolis exhibited the highest antimicrobial activity among the evaluated extracts, maintaining inhibitory activity up to the 10⁻² dilution, where-

as Tubuna, Mandaçaia, and *Apis mellifera* green propolis remained effective up to the 10⁻¹ dilution. This pattern was consistent across all bacterial species tested, with no differences observed between the Gram-positive and Gram-negative bacteria under the experimental conditions, indicating a broad-spectrum antimicrobial activity of the extracts. Palynological analysis confirmed the botanical origin of the samples, revealing a heterogeneous botanical composition in stingless bee propolis and predominance of the family Asteraceae in green propolis. These findings demonstrate that propolis from stingless bees exhibits antimicrobial activity comparable to or greater than that of *Apis mellifera* green propolis, with Borá propolis showing the greatest antimicrobial potency. The results highlight the potential of stingless bee propolis as a source of bioactive compounds for use as a phytobiotic feed additive. Further studies are warranted to correlate its chemical composition with the observed antimicrobial activity.

Keywords: antimicrobial activity; phytobiotic; minimum inhibitory concentration; stingless beekeeping; microbiology

224STP Phenotypic modulation of fluoroquinolone resistance by the efflux pump inhibitor PAβN in *Salmonella* spp. isolates from commercial laying hens

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Background: In the poultry industry, enrofloxacin is widely used for the therapeutic management of bacterial infections caused by *Enterobacteriaceae*. However, constant antimicrobial use has driven an alarming increase in fluoroquinolone-resistant *Salmonella enterica* strains, posing a severe One Health threat. Efflux pump overexpression is one of the primary resistance mechanisms. This study evaluated the phenotypic impact of the efflux pump inhibitor PAβN in restoring fluoroquinolone susceptibility in *Salmonella* spp. isolates from commercial laying hens. **Materials & Methods:** Sixteen *Salmonella* spp. isolates (serovars S. Schwarzengrund, S. Saint-paul, S. Senftenberg, and S. Braenderup) from commercial laying hen farms in Brazil were evaluated. The Minimum Inhibitory Concentration (MIC) for enrofloxacin was determined by broth microdilution following CLSI guidelines. Efflux pump activity was phenotypically assessed by repeating MIC assays with the addition of PAβN at a subinhibitory concentration. MIC reductions were analyzed using the paired Wilcoxon signed-rank test in R. **Results and Discussion:** PAβN promoted a unanimous MIC reduction across all isolates (n=16), with values decreasing 8- to 16-fold. Adopting a ≥4-fold MIC reduction as phenotype reversal criterion, 100% of strains reversed their resistance phenotype. Statistical analysis confirmed significantly higher MIC values without PAβN (Wilcoxon, V=136, p<0.001). This consistent reduction strongly indicates that active efflux is a key mechanism driving fluoroquinolone resistance, and that blocking these pumps effectively restores susceptibility, highlighting the potential of efflux pump inhibitors as viable tools to combat antimicrobial resistance in poultry production. **Conclusion:** The phenotypic reversal achieved with the PAβN inhibitor demonstrates a clear and consistent involvement of efflux pumps in fluoroquinolone resistance among the evaluated *Salmonella* strains. These findings underscore active efflux as a crucial mechanism contributing to the resistance profile, highlighting the potential of efflux pump inhibitors as sustainable strategies to restore antimicrobial efficacy and control multidrug-resistant pathogens in the poultry industry. **Keywords:** Antimicrobial Resistance, Avian Paratyphoid *Salmonella*,

Keywords: Antimicrobial Resistance; Avian Paratyphoid Salmonella; Food Safety; MIC; One Health

225STP Metabolomic profile of avian pathogenic *Escherichia coli*: identification of candidate biomarkers

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Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is one of the leading bacterial diseases affecting commercial poultry production and results in significant economic losses. Differentiating pathogenic from commensal strains remains a diagnostic challenge due to the high genetic variability of *E. coli* strains. PCR can assist in distinguishing APEC from commensal strains, while serotyping and phylogenetic grouping provide additional epidemiological information. However, whole-genome sequencing, the gold standard for detailed strain characterization, remains too costly for routine laboratory use. In this context, metabolomics emerges as a promising approach to identify biomarkers capable of distinguishing APEC from other *E. coli* strains. This study aimed to identify metabolic biomarkers to differentiate APEC from commensal strains and *E. coli* of other host origins using untargeted metabolomics. Forty-three *E. coli* isolates were grouped as: APEC confirmed by sequencing ($n = 12$), APEC confirmed by PCR only ($n = 7$), commensal *E. coli* ($n = 10$), and *E. coli* strains isolated from bovine ($n = 2$), swine ($n = 5$), canine ($n = 3$), and human ($n = 4$) hosts. Metabolites were extracted with spectroscopic-grade methanol and analyzed by liquid chromatography coupled to mass spectrometry. Statistical analyses included univariate and multivariate approaches. The Mann-Whitney test and ANOVA were used to identify differentially expressed metabolites, while Pearson correlation, linear mixed models, PCA, PLS-DA, and ROC curve analyses were applied to evaluate metabolite associations, sample clustering, discriminant biomarkers, and predictive performance. Statistical significance was set at $p < 0.05$. A total of 304 metabolites were detected, of which 6 showed differential expression between APEC and non-APEC groups. PCA and PLS-DA indicated that metabolic profiles are distinguishable between APEC and non-APEC samples, with minimally overlapping ellipses suggesting a characteristic metabolic pattern associated with APEC. ANOVA and ROC curve confirmed a significant difference in two metabolites between APEC and all other groups ($AUC = 0.87$ ($p < 0.0001$)). Although these compounds remain poorly characterized, their chemical classes are related to lipid-derived metabolism and fatty ester-associated pathways. Multivariable analysis demonstrated that the combined use of both metabolites improved discriminatory performance compared with each metabolite individually. Therefore, these findings indicate two potential biomarkers for differentiating APEC from other *E. coli* strains and support their further investigation as diagnostic markers for colibacillosis.

Keywords: APEC; avian pathogenic *Escherichia coli*; metabolomics; biomarkers; LC-MS

226STP Ceftiofur Administration in Broiler Chicks Favors Environmental Dissemination of Beta-Lactam by Conjugative

Plasmid Between *Escherichia coli* and *Salmonella* Typhimurium.

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Antimicrobial resistance in poultry production is a major public health challenge. *Salmonella* Typhimurium (ST) is notable for its extensive host range and evolutionary adaptability, being one of the main strains associated with waterborne and foodborne disease outbreaks. Horizontal transfer of antimicrobial resistance genes through conjugation is a key mechanism driving the emergence of multidrug-resistant (MDR) strains, which requires the investigation of plasmid transfer frequency. This study evaluated the influence of ceftiofur administration in day-old chicks on the conjugation frequency of the IncB/O plasmid carrying the *bla*CTX-M-1 gene between *Escherichia coli* (EC) H2332 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (strains F98; STM), examining the transfer frequency in samples obtained from the poultry litter. Thirty Cobb broiler chickens were assigned to three experimental groups (A, B, and C). On the first day of life, groups A and B received a subcutaneous injection of sodium ceftiofur at a sub-therapeutic (0.1 mg/bird) and a therapeutic dose (0.2 mg/bird), respectively. Group C served as the control and did not receive any antibiotic treatment. On the second day of life, chickens from all groups received via esophageal gavage 10^7 colony-forming units (CFU) of STM (recipient) and 10^6 CFU of EC (donor). At three days post-infection, poultry litter samples were collected in triplicate, diluted with PBS, and plated. STM and STM-T (transconjugant *S. Typhimurium* which received the *bla*CTX-M-1 on IncB/O plasmid) were quantified as colony-forming units per milliliter (CFU/mL). Data were log-transformed ($\log_{10}$ CFU/mL), and the plasmid conjugation frequency, defined as the ratio of STM-T to STM, was assessed by one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$). No significant differences were observed between treatments regarding the recipient bacteria counts. However, the administration of the ceftiofur sub-therapeutic favored an increase of STM-T in the poultry litter compared to the control group ($p < 0.05$). Furthermore, the conjugation frequency was higher in the sub-therapeutic dose treatment, whereas the therapeutic dose showed intermediate values, with no statistically significant difference compared to the other groups. Our results point to ceftiofur treatment not controlling STM colonization, whereas it can make the treatment of this pathogen, which is of great importance to animal and public health, is not feasible. These findings corroborate the importance of the misuse of antimicrobials in an off-label way due to the risk of promoting the early environmental emergence of antimicrobial resistance in poultry litter.

Keywords: Avian salmonellosis; antimicrobial resistance; horizontal gene transfer; plasmid conjugation; poultry litter

227STP Microbiological evaluation of articular lesions in broiler carcasses: are macroscopic findings indicative of active infection?

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Brazil is one of the world's leading poultry meat exporters, making

it essential to reduce factors that compromise productivity and product quality, such as locomotor disorders, including arthritis. This study aimed to classify articular lesions in broiler chicken carcasses through macroscopic and microbiological characterization and to evaluate their potential association with public health risks. The study was conducted in a poultry processing plant located in Southwest region of Paraná, Brazil. A total of 32 broiler carcasses presenting tibiotarsal joint lesions were collected and allocated into four groups (n=8): Grade 0 (no visible lesion), Grade I (joint swelling with slight discoloration), Grade II (discoloration without joint swelling), and Grade III (presence of exudate and marked discoloration, with macroscopic characteristics suggestive of an infectious process). Samples were collected on three slaughter dates and originated from eight different farms. Following lesion classification, aseptic synovial fluid swabs were collected from each carcass. Microbiological analyses included culture-based isolation of *E. coli*, *Streptococcus* spp., and *S. aureus*, enumeration of total mesophiles by plate count (APC), and detection of *M. synoviae* by qPCR, and Avian Reovirus (AVR) by RT-qPCR. Data were analyzed as a completely randomized design, applying Fisher's exact test with SAS. Statistical significance was set at $P \leq 0.05$. APC were generally low, with most samples showing values below the limit of quantification (< 100 CFU/mL). Moderate counts (up to 1,400 CFU/mL) were detected only in a limited number of Grades II and III lesions. No significant association was observed between APC and lesion severity ($P=0.5075$), indicating that microbial load was not related to lesion progression. *E. coli*, *Streptococcus* spp., and *S. aureus* were not detected in any evaluated sample. *M. synoviae* were negative in all carcasses, despite the presence of macroscopic alterations compatible with infectious synovitis. One sample (3.1%) tested positive for AVR, demonstrating low epidemiological relevance in the evaluated population. Overall, microbiological findings were limited across all lesion grades. The macroscopic alterations were not associated with evidence of active infection by the investigated pathogens. No apparent relationship was observed between lesion severity and microbial load, indicating that macroscopic lesion severity alone may not be a reliable indicator of active infectious processes in broiler joints. These findings suggest that non-infectious factors, such as mechanical stress, environmental conditions, and management practices, may contribute to the development of articular lesions.

Keywords: arthritis; broiler; food safety; microbiological analysis; poultry processing

228STP Evaluation of antimicrobial efficacy of essential oils against *Salmonella* Enteritidis on shell eggs.

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Background: *Salmonella* Enteritidis (SE) is one of the most frequently isolated serovars in eggs, associated with gastrointestinal infections in humans. Considering the risk of contamination along the poultry production chain, egg disinfection is a crucial practice to ensure microbiological safety. In this context, essential oils (EOs) present a promising green alternative due to their antimicrobial ac-

tivity. This study evaluated the efficacy of cinnamon (CA) and lavender (LA) EOs in reducing SE contamination on shell eggs after a few minutes of contact. **Methods:** Four groups were used for evaluation: CA, LA, Chlorine (CL), and an untreated positive control, with five eggs per group (a total of 20 eggs per contact time). The Minimum Bactericidal Concentrations (MBCs) of EOs were determined in 96-well plates and used to establish treatment concentrations. Eggs were immersed in an SE inoculum (10^9 CFU/mL) for 30 minutes and then treated with EOs or CL applied by spraying. Following contact periods (0, 1, or 5 min), the eggs were washed with 30 mL of phosphate-buffered saline, and the recovered wash solution was serially diluted and plated on Brilliant Green Agar for SE enumeration. Bacterial counts (log₁₀ CFU/g) were analyzed by Two-Way ANOVA and Bonferroni multiple comparisons test ($P < 0.05$). **Results and Discussion:** LA and CA EOs showed MBCs of 0.01 and 0.0015 g/mL, respectively. For CL treatment, 0.02% concentration was used, based on previously reported literature. Immediately after application and after 1 min of contact, significant reductions in bacterial counts were observed only for the CL treatment compared with the untreated control ($P < 0.0001$). After 5 min, CA, LA, and CL all reduced SE counts relative to the control ($P = 0.0446$, $P = 0.0145$, and $P < 0.0001$, respectively), although both EOs remained less effective than CL ($P < 0.0001$). The observed differences among treatments suggest that CL exhibited an immediate antimicrobial activity. Although less effective than CL, both EOs significantly reduce bacterial counts after 5 minutes of exposure, supporting their potential as green alternatives for egg disinfection, with the potential of use to maximize the shelf life of table eggs. **Conclusion:** Although further investigations are needed to optimize the use of these compounds before they can be used as alternatives to conventional sanitizers, CA and LA EOs significantly reduced SE counts on shell eggs after 5 min of contact, supporting their use as promising alternatives to conventional disinfectants.

Keywords: Alternatives to antimicrobials; Avian paratyphoid salmonellas; Egg Disinfection; Food Safety; Green Alternative

229STP The in vitro efficacy of essential oils against biofilm formation by *Salmonella enterica* serovars

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Background: *Salmonella* spp. is a major cause of foodborne illness worldwide, with poultry products representing one of the primary sources for human infection. A critical point for its persistence in slaughterhouse and poultry environments is its ability to form biofilms. In the current study, we use essential oils of cinnamon, thyme, and oregano to test biofilm inhibition. **Methods:** The minimum inhibitory concentration (MIC) of cinnamon, thyme, and oregano essential oils (EOs) was first determined against eight *Salmonella enterica* strains from different serovars. Biofilm inhibition assays were subsequently performed in 96-well microplates. Bacterial suspensions were prepared and standardized to 108 CFU/mL (0.5 McFarland standard). Each well received 100 µL of broth (LB

+ TSB + Yeast Extract). Sub-MIC concentrations of each EO (100 µL) were added to the first column and serially diluted across columns 1–10. Two control columns were included: a negative control (broth + EO) and a positive control (broth + bacteria). The plates were incubated at 37°C for 24 hours. Biofilm quantification was performed using crystal violet (CV) staining and measuring the OD at 595 nm absorbance. **Results and Discussion:** All three EOs showed dose-dependent biofilm inhibition across the eight tested strains. Cinnamon presented the broadest activity suppressing biofilm formation through the highest dilution steps, followed by thyme and oregano; the latter showed an equivalent action for all strains when compared. Most strains, including *S. Typhimurium*, *S. Braenderup*, and *S. Infantis*, were highly susceptible. Notably, *S. Braenderup* was the only serovar that showed complete inhibition of biofilm across all dilutions when exposed to oregano EO, suggesting particular susceptibility to carvacrol action. At the same time, *S. Minnesota* was the most tolerant serovar, exhibiting weak to moderate biofilm formation even at lower EO concentrations across all three oils tested. **Conclusion:** The essential oils of cinnamon, thyme, and oregano effectively inhibited biofilm formation in *Salmonella enterica* strains at sub-MIC concentrations, with dose-dependent responses.

Keywords: Biofilm; Essential Oils; *Salmonella* spp.

230STP Effects of dietary selenium supplementation on oxidative stability and fatty acid composition of rooster semen

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The high proportion of polyunsaturated fatty acids (PUFAs) in avian semen makes sperm cells highly susceptible to lipid peroxidation, impairing their functionality. Selenium acts as a key component of antioxidant systems, including glutathione peroxidase. This study aimed to evaluate the effects of dietary selenium supplementation on oxidative status and fatty acid profile of semen in White Plymouth Rock roosters. A total of 32 males were assigned to two treatments: control diet (0.15 mg Se/kg) and selenium-supplemented diet (0.40 mg Se/kg), from 53 to 72 weeks of age. Lipid peroxidation was assessed by thiobarbituric acid reactive substances (TBARS), and fatty acid composition was determined by gas chromatography. Selenium supplementation significantly reduced lipid peroxidation in semen (22.85 vs. 17.59 ng TBARS/mL; $P=0.0331$), indicating improved antioxidant status. Additionally, consistent changes in lipid composition were observed, with increased proportions of polyunsaturated fatty acids across experimental periods ($P<0.05$), including long-chain fatty acids such as arachidonic acid (C20:4n-6) and docosahexaenoic acid (DHA). In contrast, higher proportions of saturated fatty acids were observed in the control group, particularly in periods III and V ($P<0.05$). These findings suggest that selenium reduction in lipid peroxidation contributes to the preservation of unsaturated fatty acids, which are essential for sperm function. In conclusion, dietary selenium supplementation improves semen antioxidant status and promotes beneficial modifications in fatty acid profile, potentially enhancing reproductive efficiency in roosters.

Keywords: selenium; TBARS; antioxidant; fatty acids; oxidative stress

231STP Effects of canthaxanthin and 25-hydroxycholecalciferol

supplementation on fertility and hatchability in broiler breeders

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The use of nutritional strategies to enhance reproductive efficiency in broiler breeders is essential for maximizing chick output in commercial systems. This study evaluated the effects of dietary supplementation with canthaxanthin (CX) and 25-hydroxycholecalciferol (25-OHD3) on productive and reproductive performance of Cobb 500 broiler breeders. A total of 264 females and 24 males were distributed in a completely randomized design with two treatments: control diet and diet supplemented with CX (6 mg/kg) + 25-OHD3 (2.76 million IU/kg), from 25 to 62 weeks of age. Productive parameters, egg quality, and reproductive performance were evaluated, including 34 weekly incubation cycles. Data were analyzed by ANOVA. Body weight, mortality, egg production, egg weight, and eggshell quality were not affected by treatments ($P>0.05$). However, yolk pigmentation was significantly increased in supplemented birds ($P<0.0001$), confirming carotenoid deposition. Reproductive performance was significantly improved by CX+25-OHD3 supplementation. Fertility increased (94.55% vs. 96.51%; $P=0.0339$), as well as hatchability of total eggs (86.15% vs. 88.94%; $P=0.0131$) and hatchability of fertile eggs (91.34% vs. 92.24%; $P=0.0399$). Additionally, early embryonic mortality (0–48 h) was reduced ($P=0.0013$), resulting in a higher number of chicks produced per hen ($P=0.0013$). No differences were observed in chick weight or quality ($P>0.05$). In conclusion, the combined supplementation of canthaxanthin and 25-OHD3 enhances reproductive efficiency by improving fertility, hatchability, and early embryo survival, without affecting productive performance, representing an effective nutritional strategy for broiler breeder flocks.

Keywords: carotenoids; vitamin D; fertility; hatchability; embryonic mortality

232STP Supplementation with *Bacillus velezensis* BV03 enhances egg production by modulating ovarian metabolites

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Probiotics and phytogetic additives have gained attention due to their potential to modulate intestinal microbiota, oxidative balance, and reproductive physiology. In a previous study, we observed antioxidant activity of *Libidibia ferrea* and *Bacillus velezensis* in broilers. Considering that oxidative stress in the ovary may impair egg production in older hens, this study evaluated the effects of supplementation with the probiotic bacterium *Bacillus velezensis* and the plant extract *Libidibia ferrea* on egg production and ovarian metabolomic profiles in laying hens during the late production phase. The experiment was conducted using 100 White Plymouth Rock pure-line laying hens at 80 weeks of age, randomly distributed into four groups ($n = 25$): negative control (CN), *Bacillus velezensis* strain BV03 UNICLON (BV; 106 CFU/hen), *Libidibia ferrea* (LF; 6.25 mg/

hen), and the combined treatment (LF+BV). Treatments were administered daily from 80 to 102 weeks of age. Egg production was recorded daily and expressed as weekly averages. Small primordial white oocytes were collected for untargeted metabolomic analysis by LC-MS. Only metabolites passing the 100% frequency filter, with fold change > 2 and $p < 0.05$ by ANOVA, were considered significant. PCA, clustering, and PLS-DA analyses were performed to evaluate metabolomic profiles. Hens supplemented with BV showed significantly higher egg production compared with the negative control and LF groups, demonstrating the productive potential of this probiotic in commercial laying hens. Although LF supplementation alone reduced egg productivity, the combined LF+BV treatment restored egg production to levels comparable to those observed with BV03 alone, suggesting that BV03 may counteract the negative effects of LF. Metabolomic analysis revealed distinct metabolic signatures among treatments, particularly involving pathways associated with oxidative balance, microbial metabolism, sulfur metabolism, and bioactive phenolic compounds in oocytes. These findings suggest that BV may positively influence reproductive physiology and oocyte quality through modulation of metabolic pathways related to antioxidant protection and microbial-host interactions. Although LF has shown strong antioxidant activity in chicks in previous studies, its use in laying hens may require dose adjustment, since the cumulative effect of daily supplementation could negatively affect liver metabolism and potentially contribute to toxicity. Overall, the results highlight the promising potential of BV03 as a natural strategy to improve productive performance and reproductive metabolism in commercial laying hens, supporting the development of innovative feed additives for the poultry industry.

Keywords: Feed additives; Productive performance; Antioxidant activity; Intestinal microbiota; Poultry nutrition

233STP Effect of dietary selenium supplementation on reproductive parameters of White Plymouth Rock roosters

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Selenium plays a crucial role in antioxidant defense and in maintaining the structural and functional integrity of sperm cells. This study aimed to evaluate the effects of dietary selenium supplementation on productive and reproductive parameters of White Plymouth Rock roosters during the second reproductive cycle. A total of 32 males were distributed in a completely randomized design with two treatments: control diet (0.15 mg Se/kg) and selenium-supplemented diet (0.40 mg Se/kg), with 16 replicates per treatment. The experimental period lasted from 53 to 72 weeks of age and was divided into five 28-day periods. Feed intake, body weight, semen volume, sperm motility, vigor (0–5 scale), sperm concentration, pH, and morphological defects were evaluated. Data were analyzed by ANOVA ($P < 0.05$). Selenium supplementation increased feed intake throughout the experimental period (111.52 vs. 120.27 g/bird/day; $P = 0.0207$), without affecting body weight ($P > 0.05$). Sperm motility was significantly improved in supplemented birds (87.19% vs. 90.82%; $P = 0.0362$), with more pronounced effects during periods II, III, and IV ($P < 0.01$). Sperm vigor was also higher in the selenium group (3.49 vs. 4.23; $P = 0.0136$). No significant effects were

observed for semen volume, sperm concentration or pH ($P > 0.05$). Regarding sperm morphology, no differences were detected overall; however, a reduction in total defects was observed in period II (7.39% vs. 4.15%; $P = 0.0159$). In conclusion, dietary supplementation with 0.40 mg Se/kg improves functional semen parameters, particularly sperm motility and vigor, contributing to enhanced reproductive performance in aging roosters.

Keywords: selenium; sperm motility; vigor; roosters; reproduction

234STP Effects of canthaxanthin and 25-hydroxycholecalciferol supplementation on reproductive performance and yolk pigmentation of broiler breeders

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Oxidative stress plays a critical role in limiting reproductive efficiency in broiler breeders, particularly affecting embryo viability and hatchability. This study investigated the effects of combined dietary supplementation with canthaxanthin (CX), a carotenoid with antioxidant properties, and 25-hydroxycholecalciferol (25-OHD3), a highly bioavailable vitamin D metabolite, on egg quality, oxidative status, and reproductive performance. A total of 264 broiler breeder hens and 24 roosters were allocated to two treatments: a basal diet and a diet supplemented with CX (6 mg/kg) and 25-OHD3 (2.76 million IU/kg), from 25 to 62 weeks of age. Egg quality traits, yolk pigmentation, TBARS in fresh yolks, and incubation parameters were evaluated. No differences were observed in egg weight, albumen, yolk proportion, or specific gravity ($P > 0.05$). However, yolk color was significantly enhanced by supplementation ($P < 0.0001$), indicating effective transfer of CX to the egg. Lipid peroxidation in fresh yolks (TBARS) was not affected ($P > 0.05$), suggesting that antioxidant effects may depend on oxidative challenge conditions. Despite the lack of changes in TBARS, reproductive parameters were significantly improved. Birds receiving CX+25-OHD3 showed increased hatchability of total and fertile eggs ($P = 0.0131$ and $P = 0.0399$, respectively), and reduced early embryonic mortality ($P = 0.0013$). These improvements resulted in a greater number of chicks produced per hen, without affecting chick quality or weight. The results suggest that CX and 25-OHD3 act synergistically to enhance reproductive efficiency, potentially through improved antioxidant protection, modulation of reproductive physiology, and enhanced embryo viability.

Keywords: antioxidant; carotenoid; vitamin D₃; embryo development; poultry reproduction

Welcome and Poster Reception

236P Attraction Rather Than Avoidance: Broiler Chick Responses to an Autonomous Navigation Robot

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Animals rapidly adjust their behavior according to previous experiences. Repeated exposure to aversive stimuli can increase fear responses, whereas exposure to neutral or positive stimuli can reduce fearfulness when animals face subsequent challenges. Autonomous robots that navigate the floor of commercial broiler houses are being developed to monitor environmental conditions and have been shown to increase bird locomotion. However, the mechanisms underlying this effect remain unclear. Increased locomotion could reflect simple displacement by the robot's movement or fear-induced avoidance responses toward the robot. Here we assessed fear-related behavior in broilers exposed to the AVISENSE robot (Apelie Robotics) using a battery of behavioral tests at 13 and 14 days of age vs. non-exposed controls. Groups of 500 broilers were housed in large pens (7.5 × 4.5 m) within a commercial broiler house. In the exposed treatment, from 1 to 15 days of age, the robot performed 10-min laps around the pen every hour between 9:30 and 16:30, whereas controls were visually isolated from the robot. Each bird underwent a single test and was then removed to avoid retesting. Generalized linear mixed models were used for statistical analyses (Gamma distribution), with treatment as a fixed factor and pen as a random factor. At 13/14 days of age, chicks were tested in a 3-min double-runway apparatus containing the AVISENSE robot in one goal box and a novel object (an orange basketball) in the other. For robot-exposed chicks, the robot was the familiar stimulus whereas the basketball was novel; for controls, both stimuli were novel. Most chicks from both treatment groups approached the robot (78% of exposed vs. 60% controls; $P = 0.3$). However, exposed chicks approached the robot sooner (31 ± 9 s vs. 72 ± 12 s; $P = 0.01$) and spent more time near it ($21.4 \pm 5.9\%$ vs. $6.1 \pm 0.2\%$; $P = 0.05$) than controls. In a tonic immobility test, exposed chicks required more inductions ($P = 0.01$) and in an open-field test showed shorter latencies to ambulate ($P = 0.05$), with no difference in distance ambulated. In conclusion, chicks exposed to the robot, not only showed greater attraction toward the robot when given a choice between the robot and a novel neutral object but also exhibited reduced fear-related responses in independent behavioral test compared to controls. Findings suggest that the increased locomotion associated with robot exposure is unlikely to stem from fear or avoidance. Furthermore, the robot seems to be perceived as a familiar, non-aversive stimulus. Further studies are needed to determine how the frequency and duration of exposure shape these responses.

Keywords: Run-way test; Open-field test; Tonic immobility; Fear; Robotics

237P Beyond nutrition: black soldier fly larvae promote natural foraging in Japanese quail

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Modern poultry systems often limit opportunities for birds to express natural foraging behavior, raising welfare concerns. Live insect larvae may simultaneously provide a nutrient-rich feed source and environmental enrichment by stimulating species-typical foraging behavior. Black soldier fly larvae (*Hermetia illucens*, BSF) can be reared on organic by-products such as brewer's spent grain, making them a practical resource for poultry production. Using Japanese quail we evaluated how previous experience, larval size, and presentation form influence the acceptance of BSF larvae. In a first experiment, birds were offered 1st or 6th instar larvae (small, ≤ 5 mm; large, ≥ 15 mm, respectively) on two consecutive assays to evaluate the effects of larval size and previous experience. Latency to first contact and larval consumption were analyzed using generalized linear mixed models (instar, experience, and their interaction as fixed effects; bird as a random effect; Gamma and binomial distributions, respectively). Most birds (95%) interacted with larvae regardless of size. Experience reduced the latency to first contact ($P < 0.001$), whereas larval consumption showed a significant instar × experience interaction ($P < 0.001$): naïve birds preferred 1st-instar larvae, but acceptance of 6th-instar larvae increased markedly after experience, indicating rapid adaptation to a previously unfamiliar food source. In a second experiment, 6th-instar larvae were presented live, frozen, or dehydrated together with a standard balanced feed to evaluate the effects of presentation form and previous experience. Latency to first contact and larval consumption were analyzed using analogous generalized linear mixed models (presentation, experience, and their interaction as fixed effects; bird as a random effect; Gamma and binomial distributions, respectively). Following repeated exposure, birds approached all larval presentations sooner than the balanced feed (live: $P = 0.001$; dehydrated: $P = 0.046$; frozen: $P = 0.002$). Live and frozen larvae were consumed similarly ($P = 0.803$), whereas dehydrated larvae were consumed less than live larvae ($P = 0.023$). Overall larval consumption increased with experience ($P = 0.018$), rapidly incorporating BSF larvae into the birds' diet regardless of presentation form. In conclusion, repeated exposure rapidly increased acceptance of BSF larvae regardless of larval size or presentation, demonstrating that experience is a major determinant of larval acceptance. Compared with conventional feed, BSF larvae also promoted the expression of species-typical food-acquisition behaviors, supporting their use as both a nutrient-rich feed source and an environmental enrichment strategy to improve poultry welfare.

Keywords: BSF larvae; Behavior; Poultry ; Dehydrated larvae; Live larvae

238P CLINICOPATHOLOGICAL ASPECTS OF A FOWL TYPHOID OUTBREAK IN LAYING HENS IN RIO VERDE, GOIÁS.

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Fowl typhoid, caused by *Salmonella Gallinarum*, is a severe systemic disease in domestic birds, such as hens and turkeys, characterized by fever, apathy, anorexia, frothy green diarrhea, reduced egg production, and a mortality rate of up to 80%. This study aimed to char-

acterize a fowl typhoid outbreak on a small-scale laying hen farm. The property, located in Rio Verde (GO), housed 650 Embrapa 051 laying hens, and, at the beginning of June 2025, the birds presented anorexia, severe prostration, ruffled feathers, and profuse green diarrhea. The mortality rate was 5-6 birds/day, despite treatment with levamisole, sulfaquinoxaline, and enrofloxacin. On June 28, 2025, two birds (*Gallus gallus domesticus*) were euthanized and subjected to necropsy at the Avian Diseases Diagnostic Laboratory (SaniAves/EVZ-UFG). On physical examination, the birds were in poor to fair general condition, with somnolence (2/2), drooping of the neck and wings (2/2), pyrexia (1/2), and moderate deposition of pericloacal excreta (2/2). After blood sampling, they were euthanized and subjected to necropsy, with a sterile collection of organ fragments for bacteriological analysis. Blood samples were used to perform rapid serum agglutination testing, with positive results for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Salmonella* Pullorum/Gallinarum. At necropsy, the carcasses were in good (1/2) to fair (1/2) body condition score, yellow-green excreta adhered to the feathers (2/2), and pallor of the comb and wattles (1/2). The liver had rounded margins, was enlarged (2/2), diffusely greenish, friable in consistency, and severely congested, with multifocal whitish foci (necrosis) (1/2). The spleen was enlarged (1/2) and showed multifocal whitish foci (1/2). The kidneys were enlarged, friable, and pale (1/2); ovarian follicles were enlarged, flaccid, and congested (1/2); and the ceca were distended with thickened walls (1/2). Bacteriology isolated *Salmonella* spp. from the liver and spleen, and serological tests on the colonies confirmed the result. In light of the clinicopathological, serological, and bacteriological findings, a presumptive diagnosis of fowl typhoid was established. However, typing of the isolate is necessary for confirmation. This study reinforces the importance of necropsy for early diagnosis and outbreak control, and highlights the need for additional tests.

Keywords: Bacteriology; *Gallus gallus domesticus*; Hepatitis; *Salmonella* Gallinarum; Typhlitis

239P TYPING OF *Salmonella* spp. ISOLATED FROM EGGS SOLD AT OPEN-AIR MARKETS IN GOIÂNIA, GOIÁS, BRAZIL.

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Salmonellosis is one of the main causes of foodborne gastroenteritis in humans, particularly due to serovars such as *Salmonella* Enteritidis and *Salmonella* Typhimurium. Beyond its public health impact, the antimicrobial susceptibility profile of *Salmonella* spp. strains are of growing concern due to resistance to antimicrobials used in both human and veterinary therapeutic protocols. In this context, this study aimed to identify and type *Salmonella* in eggs sold at open-air markets in Goiânia, Goiás, Brazil. To conduct the study, 10 dozen eggs were collected from 10 markets across the northern, southern, eastern, western, central, southwestern, and northwestern regions of Goiânia on three distinct sampling days. Samples were transported at room temperature to the Avian Diseases Diagnostic Laboratory (SaniAves) of the Federal University of Goiás (UFG). Each dozen was divided into 3 pools, each containing 4 eggs. Subsequently, *Salmonella* spp. detection was performed using conventional bacteriology (CLSI, 2020), followed by antimicrobial susceptibility testing and *Salmonella* spp. isolates typing. Of the 30 egg pools, 4 tested positive, with 3 yolk pools originating from the same open-air market and 1 shell pool from a different one. Regarding the an-

timicrobial susceptibility profile, the following antimicrobials were tested: amoxicillin-clavulanic acid, ampicillin, apramycin, ceftiofur, ciprofloxacin, doxycycline, enrofloxacin, gentamicin, neomycin, oxytetracycline, sulfamethoxazole, sulfamethoxazole-trimethoprim, sulfonamides, rifampicin, and tetracycline. Among the 4 positive samples, all presented resistance only to rifampicin. In bacterial typing, *Salmonella* Heidelberg was identified from the yolk-positive samples and *Salmonella* Pullorum from the shell-positive samples. In conclusion, *Salmonella* spp. detection in the yolks and shells of eggs sold in open-air markets indicates a potential public health risk, especially given the detection of *Salmonella* Heidelberg. Whereas the identification of *Salmonella* Pullorum reflects poultry health, suggesting the circulation of the agent in backyard poultry farms and the presence of asymptomatic birds, since it is a serovar of importance for newly hatched chicks. Additionally, the results of the antimicrobial susceptibility test indicate that the bacteria's susceptibility profile to the antimicrobials is maintained, even with an elevated occurrence of the agent in backyard flocks.

Keywords: Antimicrobial Resistance; Foodborne Diseases; One Health; *Salmonella* Heidelberg; *Salmonella* Pullorum

240P RETROSPECTIVE ANALYSIS OF ANTIMICROBIAL RESISTANCE IN *Escherichia coli* ISOLATES OF AVIAN ORIGIN

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Escherichia coli can be classified as commensal or pathogenic; however, its antimicrobial resistance profile has been one of its most notable characteristics in recent years. The occurrence of antibiotic resistance (AR) and multidrug resistance (MDR) in bacteria of avian origin represents a major One Health concern, as these bacteria can also cause disease in humans. In this context, this study aimed to evaluate the susceptibility profiles of *E. coli* isolates obtained from non-commercial flocks of *Gallus gallus domesticus* in the state of Goiás, Brazil. A retrospective descriptive study was conducted using laboratory reports from the Laboratory of Avian Diseases Diagnosis (SaniAves) between 2023 and 2026, including all *E. coli* isolates. Antimicrobial susceptibility test results were included in the analysis, considering the percentages of isolates resistant to the main antimicrobial agents used in poultry production. During the study period, 38 consultations were registered, yielding 36 suggestive *E. coli* results 94.74% (36/38). All suggestive isolates were submitted for antimicrobial susceptibility testing by disk diffusion against 21 antimicrobial agents (amoxicillin-clavulanic acid, amikacin, amoxicillin, ampicillin, azithromycin, cephalothin, cefazolin, ciprofloxacin, chloramphenicol, ceftriaxone, ceftiofur, doxycycline, enrofloxacin, fosfomycin, gentamicin, nitrofurantoin, norfloxacin, sulfonamides, sulfamethoxazole-trimethoprim, and tetracycline). The analysis showed high rates of resistance to some antimicrobials, especially tetracycline, with 80.56% of samples resistant (29/36), followed by doxycycline at 75% (27/36) and sulfamethoxazole-trimethoprim at 69.44% (25/36). Enrofloxacin and ciprofloxacin showed the same percentage of resistance, at 58.33% (21/36). Additionally, 69.44% (25/36) of the *Escherichia coli* isolates exhibited MDR, characterized by simultaneous resistance to more than three classes of the 21 agents tested, namely tetracycline, doxycycline, sulfamethoxazole-trimethoprim, enrofloxacin, and ciprofloxacin. The results indicate high antimicrobial resistance among *Escherichia coli* isolates

of avian origin, highlighting resistance across therapeutic classes widely used in both poultry production and human medicine. It is concluded that continuous sanitary monitoring of flocks and the rational use of antimicrobials are essential strategies to reduce the selection and dissemination of multidrug-resistant bacteria in poultry production.

Keywords: Disk diffusion; *Gallus gallus domesticus*; Multidrug resistance; One Health; Tetracycline

241P CHARACTERIZATION OF A FOWL TYPHOID OUTBREAK IN A FREE-RANGE POULTRY FARM IN NORTHERN GOIÁS: CASE REPORT

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Fowl typhoid is a disease with global distribution, caused by *Salmonella* Gallinarum. Due to its high mortality rate and multiple transmission routes, the disease poses an important health and economic concern for the poultry sector. In light of this, this study aimed to characterize a fowl typhoid outbreak reported in a free-range chicken flock in northern Goiás state. In October 2025, seven hens were submitted to the Avian Diseases Diagnostic Laboratory (SaniAves) of the School of Veterinary Medicine and Animal Science (EVZ/UFG) for necropsy. The property had 2,000 free-range birds that developed severe lethargy and reduced egg production, with a peak mortality of 25 birds/day. Seven sick birds were sent to SaniAves/UFG, and physical examination revealed body condition scores ranging from good (5/7) to poor (2/7), pericloacal feathers were full of greenish liquid excreta (5/7), and ectoparasites were observed (7/7). Blood samples were collected for rapid serum agglutination (RSA) testing for salmonellosis and mycoplasmosis. Subsequently, the birds were euthanized and submitted for necropsy. Macroscopic lesions consistent with a systemic infectious process were identified, including airsacculitis (3/7), hepatic necrosis (4/7), diffuse greenish discoloration of the hepatic parenchyma (4/7), splenomegaly (4/7), fibrinous pericarditis (2/7), necrotizing nephritis (2/7), caseous oophoritis (1/7), moderate focal celomitis (1/7), enteritis (1/7), jejunitis (2/7), and typhlitis (2/7), associated with the presence of endoparasites (2/7). Based on the macroscopic findings, organ fragments were collected for conventional bacteriological analysis. The RSA test was positive for *Salmonella* spp., and bacterial colonies were isolated from the bird's organs. Colony typing identified *Salmonella* Gallinarum in the liver (2/7), spleen (1/7), and kidney (1/7) samples. Antimicrobial susceptibility testing demonstrated resistance to sulfamethoxazole-trimethoprim in 75% of the isolates. In conclusion, clinicopathological evaluation remains an effective tool for diagnosing and characterizing salmonellosis outbreaks in free-range chickens. Furthermore, bacteriological analysis and isolates typing are essential for confirming and establishing a definitive diagnosis of the disease.

Keywords: Antibigram; Culture; Necropsy; *Salmonella* Gallinarum; *Salmonella* spp.

242P Effect of powdered egg yolk immunoglobulin Y on the control of *Salmonella* spp. in broiler chickens: a systematic review and meta-analysis

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Salmonella spp. is an important foodborne pathogen associated with poultry production. Egg yolk immunoglobulin Y (IgY) has been proposed as a non-antibiotic alternative to reduce intestinal colonization in broiler chickens; however, its efficacy remains inconsistent across studies. Therefore, this systematic review and meta-analysis aimed to evaluate the effect of dietary supplementation with powdered IgY on *Salmonella* spp. colonization in broiler chickens. Randomized controlled trials were searched in PubMed, Scopus, and Web of Science that compared powdered IgY supplementation with no treatment (positive control) in broiler chickens exposed to *Salmonella* spp., using the following search strategy: (IgY OR "immunoglobulin Y" OR "egg yolk") AND (broiler OR chicken) AND *Salmonella*. The primary outcome was the change in cecal colonization by *Salmonella* spp., while secondary outcomes included systemic dissemination, assessed by hepatic colonization, and the direct comparison between IgY and conventional antibiotic treatment. Effect estimates were expressed as mean differences (MD), with 95% confidence intervals (95% CI), and pooled using random-effects models. A total of 626 records were identified across databases, of which three studies met the inclusion criteria and were included in the meta-analysis: Moosavi et al. (2025), Isfahani et al. (2020), and Chalghoumi et al. (2009). Cecal *Salmonella* colonization was significantly reduced at day 14 (MD -0.46 log₁₀CFU/g; 95% CI -0.54 to -0.38; p<0.00001; Figure 1. A) and day 21 (MD -0.94 log₁₀ CFU/g; 95% CI -1.07 to -0.81; p<0.00001; Figure 1. B) in broiler chickens supplemented with powdered IgY compared with the positive control. There was a greater reduction in hepatic *Salmonella* bacterial load with IgY versus the positive control at day 14 (MD -2.14 log₁₀ CFU/g; 95% CI -2.28 to -1.99; p<0.00001; Figure 1. C) and day 21 (MD -1.30 log₁₀ CFU/g; 95% CI -1.52 to -1.09; p<0.00001; Figure 1. D). IgY resulted in a significantly smaller reduction in cecal *Salmonella* colonization than enrofloxacin at day 14 (MD -0.78 log₁₀ CFU/g; 95% CI -0.87 to -0.70; p<0.00001; Figure 1. E) and day 21 (MD -2.54 log₁₀ CFU/g; 95% CI -2.69 to -2.40; p<0.00001; Figure 1. F). In this meta-analysis of three RCTs, powdered IgY supplementation reduced cecal and hepatic *Salmonella* colonization at days 14 and 21 compared with the positive control. In contrast, when compared with enrofloxacin treatment, IgY provided a smaller reduction in cecal colonization.

Keywords: Cecal microbiology; Enteric colonization; Broiler chickens; Immunoglobulin Y (IgY); Antimicrobial alternatives

243P IDENTIFICATION OF *Salmonella* Gallinarum AND ITS ANTIMICROBIAL SUSCEPTIBILITY PROFILE ON A NON-COMMERCIAL LAYING POULTRY FARM IN THE METROPOLITAN REGION OF GOIÂNIA, GOIÁS, BRAZIL.

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Avian salmonellosis are bacterial diseases that affect animal and human health as well as the economy. Specifically, fowl typhoid, caused by *Salmonella* Gallinarum, is associated with severe effects, including septicemia, toxemia, and extensive systemic damage in birds. In light of this, the present study aims to characterize the clinical and pathological findings of *Salmonella* Gallinarum infection and its antimicrobial susceptibility profile in a non-commercial laying poultry farm in Bela Vista de Goiás, Brazil, with 600 birds,

divided into pre-laying, laying, and end-of-laying. On 11/02/2026, the pre-laying flock comprised 100 birds of the GLC lineage and 100 Embrapa 051 birds, with approximately 17 weeks of age. During monitoring, Embrapa 051 birds showed body development below the expected for their age, with a mean weight of 500-600g. In contrast, GLC birds achieved appropriate body weight and began laying during the same period. Between the 10th and 15th week, the death of 25 birds was related, with clinical signs such as ruffled feathers, apathy, prostration, anorexia, crowding behavior, reduced mobility, locomotor difficulty, watery diarrhea, and ocular opacity in some animals. On 12/02/2026, two laying hens were sent to the Avian Diseases Diagnostic Laboratory (SaniAves/UFG) for necropsy and bacteriological analysis. Macroscopic findings included bilateral corneal opacity, irregular pupils, mild airsacculitis, hepatomegaly with diffuse green hepatic coloration of the liver parenchyma, and multifocal whitish necrotic foci. Marked splenomegaly was also observed, along with enlarged and congested kidneys with necrotic areas, petechial hemorrhages on the jejunal serosa, and yellowish-green frothy intestinal contents. The morphological diagnosis included severe keratitis, diffuse necrotizing hepatitis, splenomegaly, moderate necrotizing nephritis, and mild jejunitis. Bacteriology was positive for *Salmonella* spp. on the liver, gallbladder, cecal content, and cecal tonsils. The isolates demonstrated intermediate resistance to enrofloxacin, and the serovar Gallinarum was characterized by typing. This case highlights the importance of integrating the clinical evaluation, necropsy, bacteriological culture, and serotyping for the definitive diagnosis of salmonellosis, as well as the need for continuous animal health monitoring, especially in alternative poultry production systems.

Keywords: Alternative production systems; Bacteriology; Fowl typhoid; Laboratory diagnosis; Serotyping

244P The second human case of *Erysipelothrix piscisicarius* infection suggests a turkey origin and reinforces the zoonotic potential of this species

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The increasing recognition of unusual bacterial pathogens in human infections highlights the importance of comprehensive characterization of newly described species and unconventional clinical isolates. *Erysipelothrix piscisicarius* is still poorly explored, and, to date, only one human case has been reported in China. In the present study, we describe a new human isolate of *E. piscisicarius* in Brazil and investigate its genomic relationship with isolates previously recovered from turkeys. The strain, designated EP h2B, was isolated from a human skin sample and was initially identified as *Erysipelothrix rhusiopathiae* by MALDI-TOF MS. However, species-specific PCR identified the isolate as *E. piscisicarius*. Whole-genome sequencing generated a high-quality genome assembly of 1.69 Mb distributed in 46 contigs, with 99.99% completeness. Comparative genomic analysis demonstrated high similarity, with average nucleotide identity values above 95%, between EP h2B and *E. piscisicarius* isolates previously associated with infections in turkeys, fish, and humans. Phylogenetic and genomic clustering analyses showed that the Brazilian human isolate grouped closely with turkey isolates previously obtained by our research group, suggesting a possible avian-associ-

ated origin. Visual genome comparison using Proksee revealed high genomic conservation among *E. piscisicarius* isolates, with only a few specific regions suggesting potential insertions or deletions. Biochemical characterization showed a profile largely similar to *E. rhusiopathiae*, except for the ortho-nitrophenyl- β -galactopyranoside test, indicating that EP h2B does not produce β -galactosidase. These findings reinforce the diagnostic challenge of differentiating *E. piscisicarius* from *E. rhusiopathiae* using routine phenotypic and MALDI-TOF approaches. The close genomic relationship between the human isolate and turkey-associated strains supports the hypothesis that *E. piscisicarius* may represent an emerging zoonotic pathogen. Although direct transmission cannot be confirmed, the genomic evidence highlights the need for increased surveillance of *Erysipelothrix* species in poultry production, human infections, and occupationally exposed populations.

Keywords: Genome; metabolomic; biochemical

245P Combined Analysis of Quillaja and Yucca Combination Product Response on Performance and Gut Health in Broilers Exposed to Environmental Enteric Disease Challenges

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A *Quillaja saponaria* and *Yucca schidigera* saponin-based nutritional product (QY) has demonstrated improvements in broiler performance and intestinal health under enteric challenge conditions. However, variation in challenge intensity among studies may complicate efficacy interpretation. The objectives of this study were to validate the use of historical trial data for combined analysis and to quantify the consistency of QY responses across varying challenge severities, formulations, and inclusion rates. Seven floor-pen broiler studies between 2021 and 2023 with common experimental procedures were considered. Broilers were vaccinated against coccidiosis at hatch and exposed to standardized environmental challenge models utilizing built-up litter containing *Eimeria* spp., *Clostridium perfringens*, *Escherichia coli*, and other enteric pathogens. Evaluated endpoints included body weight at 42 d (BW42), feed conversion ratio (FCR), mortality, European Production Efficiency Factor (EPEF), intestinal lesion scores, and microbiological measurements. Challenge intensity was characterized within each study using the differences between positive (PC) and negative controls (NC). The data was analyzed with R version 4.6.1; the treatment and control groups were compared and ranked using Fisher's Least Significant Differences (LSD) test. NC demonstrated low mortality (0.35–1.14%) and acceptable biological comparability among studies. PC consistently produced a measurable enteric challenge, with BW reductions ranging from 3.8 to 15.6%, mortality increases from 4.9 to 7.8%, and FCR increases from 5 to 12 points relative to negative controls. These findings confirmed challenge establishment and supported pooled analysis across studies. Combined analysis demonstrated that the addition of QY to the feed consistently mitigated challenge-associated performance losses relative to the positive control. Across studies, treated groups achieved improvements of approximately 264–424 g in BW42, 6–10 g/d in ADG, and 7 to 15 points in FCR, while mortality was reduced from 7.1% in the positive control to 1.3–3.2% in commercially relevant treatment groups. In conclusion, this analysis validated the biological consistency of the environmental challenge model across independent study, supporting its use for pooled efficacy evaluation. Feeding QY produced consistent positive responses across independent challenge trials, supporting the robustness of the product under diverse enteric challenge condi-

tions. Incorporating challenge-adjusted response metrics may provide a biologically meaningful framework for evaluating nutritional supplements across differing challenge levels.

Keywords: environmental enteric disease challenge; quillaja and yucca saponins; performance; broiler; coccidiosis

246P Non-invasive biomarkers for broiler gut health – Systematic review

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This systematic review (SR) aimed to identify and evaluate non-invasive biomarkers of gut health in broilers, following PRISMA guidelines and using the PICO framework. A comprehensive search was conducted in Web of Science, Scopus, and PubMed for studies published in English, Portuguese, and Spanish. After screening 1,196 records, 21 studies met the inclusion criteria and were included in the review. The included studies were published between 2003 and 2024 and were primarily conducted in Europe, Oceania, and North America. No studies on the subject were found in Latin America or Africa. The studies included in the SR evaluated different sample types to explore non-invasive biomarkers, including excreta (52.4%), cloacal swabs (28.6%), poultry litter (19%), poultry dust (14.3%), cecal droppings (9.5%), and fecal droppings and boot socks (4.8% each). Furthermore, biomarkers such as ovotransferrin, lipocalin-2, and calprotectin are currently being investigated for monitoring gut health in broilers. For the evaluation of invasive biomarkers, samples such as cecal content (57.1%) or serum samples (14.3%) were used. Cecal samples were often adopted as a reference standard for comparison. Thirteen studies (61.9%) included in the systematic review conducted microbiota analyses, and variations were observed in the reference databases used for bioinformatic processing. The most frequently used database was SILVA (46.2%), followed by Greengenes (15.4%). Other reference databases used included EzTaxon, GenBank, miRBase, and NCBI (7.7% each). One study (7.7%) did not specify the reference database used. Differences in the choice of reference databases, such as SILVA and Greengenes, may have influenced taxonomic assignment and microbial abundance results. In addition, the methodological quality of the studies varied, including both observational and experimental approaches. Several studies conducted comparative analyses under dietary and sanitary challenges, although inconsistencies in reporting diet composition and performance data were observed. Overall, multiple non-invasive biomarkers are being explored for broiler gut health assessment. However, their validation remains limited by the complexity of the gut ecosystem. These challenges highlight the need for robust study designs, comprehensive data collection, and standardized protocols.

Keywords: Calprotectin; Gut microbiome; Litter samples ; Ovotransferrin ; Poultry dust

247P Effects of a drinking water blend of essential oils on performance and antibody response to infectious bronchitis vaccination in broiler chickens

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Supporting immune development during the first weeks of life may improve the effectiveness of respiratory disease prevention strategies in poultry production. Therefore, this study evaluated the effects of a blend of eucalyptus, camphor, mint, and copaiba essential oils administered via drinking water on growth performance and antibody response to hatchery vaccination against infectious bronchitis in broiler chickens. A total of 630 one-day-old male broilers were assigned to two treatments with 15 replicates of 21 birds each: drinking water without or with the additive at 200 mL/1,000 L of water. The additive was provided for 10 h daily. Birds were fed corn-soybean meal diets formulated to meet nutritional requirements. Weight gain, feed intake, and feed conversion ratio were evaluated at 7 and 14 d. Serum antibodies against infectious bronchitis virus (IBV) were determined by ELISA at 7 and 14 d to evaluate the antibody response following hatchery vaccination. Data were tested for normality. Because antibody titers were not normally distributed, data were transformed using $\log(7\text{ d})$ and $\log(x + 1)$ (14 d) before analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$ and a tendency at $0.05 < P \leq 0.10$. No differences ($P > 0.05$) were observed for weight gain, feed intake, or feed conversion ratio during the first 14 d. Birds receiving the additive exhibited numerically higher transformed IBV antibody titers at both sampling times (7 d: 2.828 vs. 2.718; 14 d: 2.439 vs. 2.275), with a tendency at 14 d ($P = 0.079$). Antibody titers declined from 7 to 14 d in both treatments, likely reflecting the physiological decline of maternally derived antibodies following hatchery vaccination. In conclusion, supplementation of drinking water with the essential oil blend did not affect broiler performance during the first 14 d but tended to maintain higher antibody titers following hatchery vaccination against IBV.

Keywords: ELISA; hatchery vaccination; humoral immunity; infectious bronchitis virus; phytogenic additive

248P Prevalence and lesion scores of coccidiosis in broilers in Brazil: Seasonal and regional trends in 2025

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Coccidiosis remains one of the most important enteric diseases affecting commercial broiler production worldwide. The occurrence of *Eimeria* spp. Infections is influenced by environmental conditions that affect oocyst survival and sporulation. However, management practices, biosecurity, anticoccidial programs and regional production characteristics may also contribute to disease dynamics. Understanding these epidemiological patterns is essential for improving coccidiosis prevention and control strategies in commercial production. The goal of this study was to evaluate the prevalence and lesion scores of *Eimeria acervulina*, *E. maxima*, and *E. tenella* in commercial broilers from different Brazilian regions during the summer and winter of 2025. A total of 33,800 broilers of aged 14 to 47 days were evaluated using data generated by the Health Tracking System (HTSi™), a global platform for monitoring broiler health and performance. Coccidiosis lesions were scored from 0 to 4 according to the Johnson and Reid method (1970). Comparisons were performed between Winter (May–August), characterized by generally cooler and drier conditions across most of Brazil, and Summer (September–April), which is typically warmer and wetter,

as well as between the Southern region and a pooled Southeast and Central-West region. Data were analyzed using ANOVA in SAS, and means were compared by Tukey's test ($p < 0.05$). Regional differences were observed in the prevalence of all three *Eimeria* species. The prevalence of *E. acervulina* (6.22 vs. 3.52%), *E. maxima* (5.05 vs. 2.02%), and *E. tenella* (2.97 vs. 1.50%) was higher in the Southern region than in the Southeast and Central-West regions ($p < 0.001$ for all comparisons). Lesion scores did not differ between regions ($p > 0.05$). No significant differences were observed in prevalence or lesion scores between the seasons ($p > 0.05$). These findings indicate that in Brazil, coccidiosis occurrence was more influenced by regional production characteristics than by the seasonal periods evaluated. The higher prevalence observed in Southern region may be associated with the high density of broiler production and environmental conditions that favor oocyst persistence, whereas the increasing standardization of commercial production systems may minimize the influence of external seasonal variation. Continuous epidemiological monitoring remains an important tool for optimizing health management and coccidiosis control in commercial broiler production.

Keywords: Epidemiology; Regional variation; Field monitoring; *Eimeria*; Gut health

249P Effect of blank feed on coccidiosis prevalence and age-related disease dynamics in Brazilian broilers in 2025

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Avian coccidiosis represents a major challenge for the poultry industry due to its detrimental effects on intestinal health, productive performance and economic losses. Among the available preventive strategies, anticoccidial programs remain one of the main approaches used to control disease under commercial production conditions. As coccidiosis dynamics vary throughout the production cycle, understanding disease prevalence at different ages is essential when evaluating the impact of blank feed (feed without anticoccidials) during the final production phase. The goal of this study was to evaluate the effect of blank feed during the final production phase on coccidiosis prevalence in Brazilian broilers in 2025 and to identify the age range associated with the highest disease prevalence. The study included 25,500 commercial broilers monitored in Brazil during 2025 using the Health Tracking System (HTSi[™]), a global platform for monitoring broiler health and performance. Flocks were classified according to the presence or absence of anticoccidials in the feed during the final phase. The prevalence of *Eimeria acervulina*, *E. maxima*, and *E. tenella* was compared between feeding programs. Additionally, age-related prevalence was evaluated using five age ranges (18–22, 23–27, 28–32, 33–37, and 38–42 days). Data were analyzed using ANOVA in SAS, and means were compared by Tukey's test ($p < 0.05$). Birds receiving blank feed during the final production phase showed a higher prevalence of *Eimeria acervulina* (7.44 vs. 4.51%; $p < 0.0001$) and *E. maxima* (5.33 vs. 2.48%; $p < 0.0001$) when compared to the birds receiving anticoccidial-containing feed. No difference was observed for *E. tenella* prevalence (1.81 vs. 1.70%; $p = 0.7280$). A significant age effect on coccidiosis prevalence was observed for *E. acervulina* and *E. maxima* ($p < 0.0001$), with peak prevalence occurring between 33–37 and 38–42 days for *E. acervulina* and between 33–37 days for *E. maxima*. No significant differences were observed for *E. tenella* ($p = 0.0735$). The highest prevalence of *E. acervulina* and *E. maxima* occurred during the same production phase in which anticoccidials are frequently removed from the diet, suggesting that

this management practice may leave birds less protected during the period of greatest coccidial challenge. These findings reinforce the importance of maintaining effective anticoccidial programs throughout the entire production cycle, particularly during the final production phase, to control coccidiosis, preserve intestinal health, optimize broiler performance, and reduce economic losses.

Keywords: Anticoccidial program; Disease prevalence; Production cycle; Coccidiosis; Economic losses

250P Effects of Breeder Age and Eggshell Translucency on Skeletal Development of Broiler Progeny

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The eggshell is the main calcium reservoir for embryonic skeletal development. Approximately 80% of the calcium used by the embryo during incubation comes from the eggshell, especially from day 14 onward, when intense calcium resorption occurs through the chorioallantoic membrane. Variations in eggshell membrane structure, resulting from changes in chemical composition, are considered a fundamental cause of translucent eggshell formation. Thus, translucent shells may affect calcium mobilization, embryonic bone mineralization, and post-hatch bone development. To better understand the relationship between eggshell translucency and post-hatch bone development, a study was conducted using 2,352 eggs from 29-week-old breeders and 4,032 eggs from 55-week-old breeders, selected from two commercial flocks. After 5 days of storage, eggs were classified into two translucency score levels. Eggshells were classified as normal when they showed a uniform appearance with no or only a few translucent spots. Eggs with high translucency showed more translucent areas across the shell surface. Eggs were incubated and, at hatch, chicks were transferred to the experimental poultry house at UFPR, Palotina Campus, and housed in a 2 × 2 factorial design, considering breeder age and eggshell translucency score, totaling four treatments and 12 replicates of 43 birds each. Birds received commercial mash diets formulated to meet nutritional requirements. Productive performance was evaluated weekly, and at 7 and 42 days, 12 birds per treatment were euthanized for bone evaluation. Data were analyzed by ANOVA using the GLM procedure of SAS. No interaction between breeder age and eggshell translucency was observed for performance or bone parameters. At 7 days, weight gain and feed intake were higher in progeny from older breeders than in progeny from younger breeders, and this difference remained until 42 days. Bone evaluation showed greater breaking strength and higher Seedor index in chicks from older breeders than in those from younger breeders. Radiographic densitometry of trabecular and cortical bone regions showed no differences at 7 days. However, at 42 days, trabecular bone radiographic density was higher in progeny from older breeders than in that from younger breeders. At the same age, cortical bone radiographic density was lower in broilers from eggs classified as translucent than in broilers from normal eggs. The rapid growth of modern broiler strains increases the demand on a skeletal system that must be adequately mineralized during embryogenesis. As studies on the effects of translucent eggshells on calcium transfer to the embryo are still recent, further research is needed to clarify the mechanisms involved.

Keywords: Bone breaking strength; Cortical bone; Embryogenesis; Embryonic calcium mobilization; Trabecular bone

251P Evaluation of breeder flock age with high avian reovirus antibody titers and single-stage or multi-stage incubation systems on the arthritis index and bone development of the progeny

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Avian reovirus (ARV), the causative agent of viral tenosynovitis/arthritis, primarily affects young broiler chickens and causes economic losses due to partial or complete loss of leg function, inflammation, deformity, discoloration, and locomotor disorders that impair feed and water intake. The high genetic variability of ARV is a major challenge for the poultry industry, as frequent mutations generate variants capable of escaping immunity induced by conventional breeder vaccination programs. Under field conditions, chick protection depends on passive immunity transferred from breeder hens and may also be influenced by incubation conditions. This study evaluated the effects of breeder flock age, with high ARV antibody titers, and incubation system (single-stage or multi-stage) on the arthritis index and bone development of broiler progeny. The experiment was conducted in partnership between a poultry company and the Federal University of Paraná (UFPR), Palotina, Brazil. A total of 6,000 fertile eggs were collected from two breeder flocks of different ages, representing early and late production, and transferred to hatcheries using either incubation system. Before egg collection, ARV serology showed a high geometric mean antibody titer (GMean), suggesting recent field exposure or active ARV circulation on the farms. After hatching, 672 male chicks were randomly allocated in a completely randomized 2 × 2 factorial design, with breeder age and incubation system as main factors, resulting in four treatments with 12 replicates each. At 7, 14, 21, and 28 days of age, the diameters of the distal tibiotarsus, intertarsal joint, and proximal tarsometatarsus were measured to calculate the arthritis index. The tibiotarsus of each bird was also weighed and measured to determine the Seedor index, cortical thickness, bone diameter, and bone breaking strength. Data were analyzed by ANOVA using SAS software. The arthritis index was not affected by breeder age, incubation system, or their interaction. At 7 days, birds incubated in the single-stage system showed greater cortical bone thickness and higher bone breaking strength. The Seedor index at 7 and 14 days, and bone breaking strength at 14 and 21 days, were higher in birds from breeder hens at the end of production than in those from breeders at the beginning of production. At 28 days, no significant differences were observed for any evaluated factor. In conclusion, passive immunity against ARV was effectively transferred from breeder hens to progeny. This protection is essential during early life, helping reduce locomotor disorders and immunosuppression, improve productive performance, and minimize economic losses in broiler production.

Keywords: tenosynovitis/arthritis; vaccination programs; transfer of passive immunity; arthritis index; bone breaking strength

252P Effects of *Hermetia illucens* Larvae Oil on Leukocyte Parameters of Broiler Chickens Challenged with *Eimeria maxima*

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Coccidiosis caused by *Eimeria maxima* impairs intestinal integrity and may alter the systemic immune response of broiler chickens, changing the leukocyte profile. *Hermetia illucens* larvae oil (HILO) has been investigated as a functional feed additive with potential immunomodulatory activity. This study evaluated the effects of HILO on leukocyte parameters of broilers challenged with *E. maxima*. A total of 540 one-day-old male Ross chicks were housed in 2.6-m² pens. The experiment was conducted in a completely randomized design with a 5 × 2 factorial arrangement, consisting of five treatments and two evaluation periods, 8 and 15 days post-infection. The nonchallenged control had four replicates of 15 birds, whereas the four challenged treatments had eight replicates of 15 birds each, totaling 36 experimental units. All procedures were approved by the Animal Use Ethics Committee, protocol no. 811/2023. Treatments were: nonchallenged control; challenged control, with birds inoculated with *E. maxima* and fed a control diet containing an antibiotic (Halquinol) and an antioxidant (BHT), without HILO; challenged birds fed an antibiotic- and antioxidant-free diet (AAFD), without HILO; challenged AAFD + 0.5% HILO; and challenged AAFD + 1.0% HILO. At 17 days of age, challenged birds were inoculated with *E. maxima* by oral gavage at 10⁷ oocysts/bird. At 8 and 15 days post-infection, 3 mL of blood was collected from two birds per experimental unit into tubes containing 10% EDTA. Total leukocytes, heterophils, lymphocytes, monocytes, and eosinophils were evaluated. Data were analyzed by two-way ANOVA using PROC GLM in SAS, and means were compared by the Student-Newman-Keuls test when significant ($P < 0.05$). Treatments did not affect total leukocytes, heterophils, or monocytes ($P > 0.05$), but affected lymphocytes ($P = 0.0414$) and eosinophils ($P = 0.0121$). The highest lymphocyte count was observed in challenged birds fed AAFD + 0.5% HILO ($10.06 \times 10^3/\mu\text{L}$), whereas the lowest occurred in challenged birds fed AAFD without HILO ($5.81 \times 10^3/\mu\text{L}$). At 15 days post-infection, lymphocytes decreased compared with 8 days post-infection (4.76 vs. $9.34 \times 10^3/\mu\text{L}$; $P < 0.0001$), whereas eosinophils increased (0.86 vs. $0.17 \times 10^3/\mu\text{L}$; $P < 0.0001$). These findings suggest a temporal change in the leukocyte response to *E. maxima*, with an early lymphocyte-related response followed by possible later participation of eosinophils in inflammation or post-challenge recovery. In conclusion, 0.5% HILO was associated with a higher lymphocyte count in challenged broilers, suggesting a possible immunomodulatory response to *E. maxima* challenge.

Keywords: Broiler chickens; *Eimeria maxima*; Coccidiosis; Immunomodulation; *Hermetia illucens* larvae oil

253P Effect of Ethanolic Jaboticaba (*Myrciaria cauliflora*) Extract Applied to Fertile Eggshells on the Serum Biochemical Profile of Broiler Chicks Challenged with *Salmonella Heidelberg*

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Objective: To evaluate the effects of applying an ethanolic extract of jaboticaba (*Myrciaria cauliflora*) peels and seeds to fertile eggshells on the serum biochemical profile of broiler chicks challenged with *Salmonella Heidelberg*. **Experimental design:** A completely randomized design was used with 320 Cobb 500 fertile eggs allocated to four treatments, eight replicates, and 10 eggs per replicate: negative control (NC), jaboticaba extract (JE), *Salmonella Heidelberg* (SH), and *Salmonella Heidelberg* plus jaboticaba extract (SH+)

JE). **Materials and methods:** A hydroethanolic extract of jabuticaba peels and seeds was obtained by percolation using 45% (v/v) ethanol. The extract and the *Salmonella Heidelberg* inoculum containing 3.8×10^7 CFU/mL were applied to the eggshells according to the experimental treatments. Hatched chicks were housed separately by treatment. At 10 days of age, blood samples were collected to determine serum concentrations of uric acid, creatinine, aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT). **Statistical analysis:** Data were analyzed by analysis of variance (ANOVA), and treatment means were compared using Tukey's test at a 5% significance level. **Results:** All biochemical parameters remained within the reference ranges for healthy birds. Chicks from the JE treatment showed significantly lower serum uric acid concentrations than those from the other treatments ($P = 0.0085$). Birds challenged with *Salmonella Heidelberg* exhibited higher AST concentrations than non-challenged birds ($P < 0.001$), regardless of extract application, suggesting mild hepatocellular damage without clinical evidence of liver dysfunction. The SH+JE group showed higher creatinine concentrations than the other groups ($P = 0.0246$); however, values remained within the physiological range for broilers. No significant differences were observed among treatments for GGT activity ($P = 0.63$). **Conclusions:** Application of the ethanolic jabuticaba extract to fertile eggshells did not induce biochemical alterations indicative of hepatic or renal toxicity in broiler chicks. Although the *Salmonella Heidelberg* challenge increased serum AST concentrations, values remained within physiological limits. These findings indicate that eggshell application of jabuticaba extract is safe under the conditions of this study and does not adversely affect the serum biochemical profile of broiler chicks.

Keywords: broiler chicks; eggshell application; inoculation; liver function; serum biochemistry

254P Effect of Ethanolic Jabuticaba (*Myrciaria cauliflora*) Extract Applied to Fertile Eggshells on the Hematological Profile of Broiler Chicks Challenged with *Salmonella Heidelberg*

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Objective: To evaluate the effects of applying an ethanolic extract of jabuticaba (*Myrciaria cauliflora*) peels and seeds to fertile eggshells on the hematological profile of broiler chicks challenged with *Salmonella Heidelberg*. **Experimental design:** A completely randomized design was conducted using 320 Cobb 500 fertile eggs assigned to four treatments with eight replicates of 10 eggs each: negative control (NC), jabuticaba extract (JE), *Salmonella Heidelberg* (SH), and *Salmonella Heidelberg* plus jabuticaba extract (SH+JE). **Materials and methods:** A hydroethanolic extract of jabuticaba peels and seeds was obtained by percolation using 45% (v/v) ethanol. The extract and a *Salmonella Heidelberg* inoculum containing 3.8×10^7 CFU/mL were applied to the eggshells according to the experimental treatments. Hatched chicks were housed separately by treatment. At 10 days of age, blood samples were collected to determine erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). **Statistical analysis:** Data were analyzed by analysis of variance (ANOVA), and treatment means were compared using Tukey's test at a 5% significance level. **Results:** No significant differences were observed among treatments for erythrocyte count, hemoglobin concentration, MCV, MCH, or MCHC ($P > 0.05$). However, hematocrit differed significantly among treatments ($P = 0.019$). Chicks from

the JE treatment exhibited lower hematocrit values than those challenged with *Salmonella Heidelberg*, whereas the NC and SH+JE groups showed intermediate values. Although hematocrit remained close to the physiological range reported for healthy broilers, the reduction observed in birds receiving only the jabuticaba extract suggests a possible effect of phytochemicals on erythrocyte integrity, potentially associated with hemolytic activity described for compounds such as saponins and tannins. **Conclusions:** Eggshell application of the ethanolic jabuticaba extract did not affect most hematological parameters of broiler chicks. However, the reduction in hematocrit observed in the extract-treated group indicates that the extract may influence erythrocyte integrity without causing clinically relevant hematological alterations under the conditions evaluated. Further studies are warranted to clarify the mechanisms underlying this response.

Keywords: broiler chicks; erythrocytes; hematocrit; inoculation; phytochemical extract

255P Hematological Response of Broiler Chickens Challenged with *Eimeria maxima* and Fed *Hermetia illucens* Larvae Oil

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Hermetia illucens larvae oil (HILO) has been investigated as a functional feed additive with potential immunomodulatory activity. This study evaluated the effects of HILO on hematological parameters of broiler chickens challenged with *Eimeria maxima*. A total of 540 one-day-old male Ross chicks were housed in 2.6-m² pens. The experiment was conducted in a completely randomized 5 x 2 factorial design, with five treatments and two evaluation periods, 8 and 15 days post-infection. The nonchallenged control had four replicates of 15 birds, whereas each challenged treatment had eight replicates of 15 birds, totaling 36 experimental units. All procedures were approved by the Animal Use Ethics Committee, protocol no. 811/2023. Treatments were: nonchallenged control; challenged control, with birds inoculated with *E. maxima* and fed a control diet containing an antibiotic (Halquinol) and an antioxidant (BHT), without HILO; challenged birds fed an antibiotic- and antioxidant-free diet (AAFD), without HILO; challenged AAFD + 0.5% HILO; and challenged AAFD + 1.0% HILO. At 17 days of age, challenged birds were inoculated with *E. maxima* by oral gavage at 10^7 oocysts/bird. At 8 and 15 days post-infection, 3 mL of blood was collected from two birds per experimental unit into tubes containing 10% EDTA. Red blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total plasma protein, and thrombocytes were evaluated. Data were analyzed by two-way ANOVA using PROC GLM in SAS, and means were compared by the Student-Newman-Keuls test when significant ($P < 0.05$). Treatments did not affect red blood cells, hemoglobin, hematocrit, MCHC, total plasma protein, or thrombocytes ($P > 0.05$), but affected MCV ($P = 0.0458$), with the lowest value in the challenged control (127.75 fL) and the highest in challenged birds fed AAFD + 0.5% HILO (142.64 fL). At 15 days post-infection, red blood cells (1.90 vs. 2.28), hematocrit (27.03 vs. 28.94%), and thrombocytes (8.83 vs. 15.91 $\times 10^3/\mu\text{L}$) decreased compared with 8 days post-infection, whereas MCV (145.57 vs. 128.53 fL) and MCHC (35.79 vs. 30.59%) increased. These results indicate temporal changes in erythrocyte

and thrombocyte profiles during the course of *E. maxima* challenge. In conclusion, HILO had a limited effect on hematological parameters, whereas days post-infection were the main factor associated with changes in the erythrocyte profile of broiler chickens challenged with *E. maxima*.

Keywords: Broiler Chickens; Coccidiosis; Erythrocyte Profile; *Hermetia illucens* Larvae Oil; Thrombocytes

256P Liquid Autolyzed Yeast Mitigates the Effects of Ochratoxin A on Performance, Organ Integrity, and Hematological Parameters of Broilers

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Objective: To evaluate the effects of a liquid autolyzed yeast-based additive on growth performance, organ integrity, hematological, and biochemical parameters of broilers challenged with ochratoxin A (OTA). **Materials and Methods:** A total of 160 Hubbard Classic broilers (7–35 d) were assigned to four treatments (40 birds/treatment): (1) unchallenged control, (2) OTA-challenged control (400 ppb), (3) OTA challenge + 1 mL/2 L of an autolyzed yeast additive supplied in drinking water, and (4) OTA challenge + 1 mL/1 L of the same autolyzed yeast additive supplied in drinking water. Feed intake and body weight gain were evaluated throughout the experimental period. Relative weights of the liver, thymus and bursa of Fabricius were determined at 18 and 35 days. Hemoglobin, hematocrit, total leukocytes, total erythrocytes, serum creatinine, urea, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also assessed. Data were expressed as mean $\pm$ SD, and differences were considered significant at $P \leq 0.05$. **Results:** OTA significantly reduced feed intake, body weight gain, hemoglobin, hematocrit, leukocyte and erythrocyte counts ($P < 0.05$), while increasing relative liver weight and serum concentrations of creatinine, urea, ALT, and AST, indicating hepatic, renal, and immunological impairment. Supplementation with a liquid autolyzed yeast mitigated these effects, restoring growth performance and physiological parameters to values comparable to those of the unchallenged control ($P > 0.05$). Birds receiving the additive also exhibited reduced liver enlargement and improved thymus and bursa development, indicating preservation of immune organ integrity. Although both supplementation levels were effective, the higher inclusion rate produced the most consistent responses. **Conclusion:** Supplementation with a liquid autolyzed yeast effectively mitigated the detrimental effects of OTA in broilers by improving growth performance, preserving liver and immune organ integrity, and restoring hematological and biochemical homeostasis. These findings support the use of liquid autolyzed yeast administered through drinking water as a nutritional strategy to reduce the adverse effects of ochratoxin A under commercial production conditions.

Keywords: Broiler chickens; Liquid autolyzed yeast ; Growth performance; Mycotoxin mitigation

257P How does the free-range system affect the performance of commercial laying hens? A comparison between two strains

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Although conventional cage systems prevail in Brazil, demand for eggs from alternative systems prioritizing animal welfare is increasing. Yet, productivity differences across systems remain unclear. This observational field trial evaluated the performance of two commercial laying hen strains under identical free-range conditions, relative to their respective genetic performance guidelines. Specifically, the study compared the productive performance of Novogen and Dekalb hens in a free-range system against the standards described in their management guides. A database from a commercial alternative egg-producing farm in Rio Grande do Sul, Brazil, was used. The study encompassed two flocks maintained under a free-range production system, consisting of 4,901 Novogen Brown and 1,792 Dekalb Brown hens. Diets and water were provided *ad libitum*. Variables included daily egg production, feed intake, body weight, mortality, and nutritional data. Flock performance was evaluated by continuous comparison with the reference standards provided by the respective strain management guides to determine if the alternative environment impacted their genetic potential. The analyses were descriptive, involving the calculation of means for different production phases (46, 70, and 90 weeks of age) and the determination of percentage deviations in relation to the reference values. The results indicate that the free-range production system had a negative impact on productive stability. Both strains demonstrated a slower onset of lay and inconsistent egg production curves throughout the cycle compared to expected standards. Egg production at 46, 70, and 90 weeks of age was 89.91%, 85.24%, and 63.50% for Novogen, and 91.65%, 88.99%, and 79.72% for Dekalb, respectively, showing a 1.9% variation between the lines at 46 weeks. Mortality was substantially higher than expected for both strains, with a 51.4% variation between lines at 46 weeks and the most significant deviation at 70 weeks, evidencing severe management challenges associated with the extensive system. The body weight of the birds remained above the manual recommendations for both lineages, with the highest weight variation observed in Novogen (4.6%) at 46 weeks and Dekalb (7.4%) at 70 weeks. Meanwhile, daily feed intake showed greater variability specifically in the Novogen Brown strain, with significant positive deviations at 70 weeks, suggesting distinct nutritional demands and elevated energy expenditure to maintain production. The free-range system requires specific management adjustments and the careful selection of better-adapted genetic strains to successfully ensure the productivity and welfare of the birds.

Keywords: Commercial farm data; Dekalb ; Egg production; Genetic strains; Novogen

258P Influence of environmental enrichment design on litter moisture in a commercial broiler house

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Within commercial broiler facilities, litter moisture is a critical management factor impacting bird welfare. Improper litter moisture can compromise broiler welfare, as dry litter increases dust, whereas wet litter contributes to footpad health issues. Concern for animal welfare continues to rise among consumers, leaving producers to seek and implement welfare management strategies to meet these demands. Among the practices adopted, producers have turned to environmental enrichment to address welfare concerns. Environmental enrichments are designed to enhance welfare by promoting

and diversifying species-specific behaviors and reducing abnormal behaviors among the birds. Poultry producers have concerns not only about management practices but also about the birds' welfare; therefore, it is critical to understand how these factors interact and to consider how the design and placement of structural enrichments impact litter moisture management. This study assessed the impact of two enrichment designs, a plastic A-frame (individual migration fence segment) and a wooden box platform, on localized litter moisture. Data was collected across one flock (day 0 - day 44). Six replicates per enrichment type and four control locations were evaluated. Litter samples were collected by hand for all enrichments and controls at positions directly underneath, at 0, 50, and 100 cm from the north and south sides, and at 0, 40, and 80 cm from the east and west sides. Samples were stored frozen until further analysis. For moisture determination, 3 subsamples (5.00 g each) per collection were dried in a convection oven at 170°F until constant mass. % moisture was calculated by dividing dried litter mass by initial litter mass. Data were pooled for analysis and evaluated using Tukey's HSD in JMP ($P < 0.05$). Results showed that the design and presence of the enrichments used in this study impacted the litter moisture. A-frame enrichments had significantly lower % moisture (0.173 ± 0.004) than both control (0.198 ± 0.005) and wooden box treatments (0.192 ± 0.004). Litter moisture exhibited spatial variation around enrichments, differing by both sampling distance and location relative to the structure ($P < 0.05$); the lowest % moisture was beneath the A-frame (0.144 ± 0.015), significantly lower than the corresponding control location (0.214 ± 0.019). These findings provide commercial poultry producers with practical information on the effects of enrichment design on litter moisture and how they can influence litter moisture conditions and patterns. Such data supports informed decisions when selecting and implementing enrichments that align with both welfare and litter-management objectives.

Keywords: enrichment; broiler; litter; moisture; commercial

259P Impact of stocking density of red laying hens at peak production on egg quality

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The laying hen industry has continuously sought strategies to improve production efficiency and optimize the use of housing facilities without compromising egg quality, bird performance, or animal welfare. In this context, the present study aimed to evaluate the effect of different stocking densities on the egg quality of red laying hens at peak production. A total of 96 Hy-Line Brown laying hens, 35 weeks of age, were used in a completely randomized design with three treatments defined by the number of birds per cage (2,500 cm²): 2 birds (1,250 cm²/bird), 4 birds (625 cm²/bird), and 6 birds (416 cm²/bird), with eight replicates per treatment. The experimental period lasted 35 days. During the last three days of the trial, two eggs were collected from each experimental unit to evaluate egg quality based on the following variables: specific gravity, albumen height, albumen index, yolk index, and eggshell weight. Additionally, on the 36th day, 16 eggs per treatment (2 eggs per replicate) were sent to an external laboratory for complementary analyses, including Haugh unit, eggshell thickness, yolk color, and shell breaking strength, in order to provide a more comprehensive

assessment of the internal and external quality of eggs produced under the different stocking densities. The data were subjected to analysis of variance, and when appropriate, means were compared using Tukey's test at the 5% significance level. The results showed no significant differences ($P > 0.05$) among the evaluated stocking densities for any of the egg quality variables analyzed. These findings indicate that, under the experimental conditions adopted, increasing stocking density from 2 to 6 birds per cage did not compromise the internal or external quality of eggs produced by red laying hens at peak production. Therefore, it can be concluded that the adoption of higher stocking densities, when associated with proper management, controlled environmental conditions, and good production practices, may represent a viable strategy to optimize housing utilization and improve production efficiency without impairing egg quality. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Keywords: Laying hens; Egg quality characteristics; Specific gravity; Stocking density; Haugh unit

260P Impact of Infrared and Hot-Blade Beak Trimming on the Performance of Laying Pullets

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The objective of this study was to compare infrared beak treatment (IR) at two intensity levels and hot-blade beak trimming regarding performance up to 18 weeks of age. A total of 120 Dekalb White pullets were distributed into three treatments (hot blade method - HB, low-intensity infrared radiation - LIR, and moderate-intensity infrared radiation - MIR), with five replicates and eight birds each. The hot-blade method consisted of V-shaped trimming and cauterization performed at 7 days of age. Infrared beak treatment was performed at the hatchery immediately after hatching. The LIR treatment used a 26/23 protection plate with an intensity of 38 nm, whereas the MIR treatment used a 24/23 protection plate with an intensity of 40 nm. Performance was evaluated up to 18 weeks of age through feed intake (FI), body weight gain (WG), feed conversion ratio (FC), uniformity (U), and breeding viability (BV), in addition to beak length. Data were subjected to analysis of variance (ANOVA), and when significant effects were detected, means were compared using Tukey's test (5%). The HB treatment significantly reduced feed intake (51.75 g/bird/day) compared with the LIR and MIR treatments (56.67 and 59.85 g/bird/day, respectively). Furthermore, birds subjected to HB showed better feed conversion ratio (4.21 g/g) than those subjected to MIR (4.71 g/g), with no significant difference from LIR (4.43 g/g). Regarding breeding viability, significant effects were observed, with lower BV in the MIR protocol (92.82%) compared with HB (97.86%), with no significant difference from LIR (96.92%). Lower body weight uniformity was observed in birds subjected to HB (76.67%) compared with those subjected to infrared treatments ($\geq 96\%$). Beak length was significantly shorter in birds treated with HB, averaging 8.49 mm at 18 weeks of age, whereas the LIR and MIR treatments averaged 12.91 and 12.90 mm,

respectively. It was concluded that infrared beak treatment may improve the performance of laying hens during the rearing phase compared with the conventional hot-blade method; however, higher intensity settings should be carefully adjusted according to bird size to avoid detrimental effects.

Keywords: Laying Poultry; Beak Length; Animal Science; Beak Treatment; performance

261P Effects of Light, Sound, Vibration, and Odor Stimuli on Incubation Performance and Chick Quality: A Systematic Review and Meta-Analysis

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Artificial incubation is a critical stage in poultry production, and recent studies have explored the effects of non-conventional environmental stimuli such as light, sound, vibration, and odor on embryonic development and chick quality. This systematic review (RS) and meta-analysis aimed to evaluate the effects of light, sound, vibration, and odor stimuli on the incubation performance and chick quality of broiler and layer eggs. Using the PICO framework, the literature search was conducted in Scopus, Web of Science, and PubMed databases, following PRISMA guidelines. A total of 2,480 records were identified, and after screening and eligibility assessment, 48 studies (from 1957 to 2024) were included in the final dataset: 42 on light, five on sound, one on vibration, and none on odor. For all statistical analyses, JAMovi 2.5.6 software was used. The RS demonstrates a clear predominance of studies conducted in broilers (66.67%) compared with layers (33.33%), highlighting the strong orientation of embryonic stimuli research toward meat-type poultry production. A notable finding was the predominance of light-based interventions during incubation, representing 87.50% of the included studies. In contrast, only 10.42% investigated sound stimuli, and a single study (2.08%) evaluated vibration. For broilers, intermittent light exposure (12h light:12h dark) and warm-colored lights significantly improved hatchability and chick quality. For layers, only cold-colored light significantly influenced hatchability. Eggs exposed to cold light temperatures showed higher hatchability of setting eggs (88.1%) compared with the dark conditions (79.4%) ($P = 0.047$). Exposure to sound stimuli reduced incubation time by approximately 5 hours, suggesting a potential role in accelerating hatching, though it did not significantly affect hatch rates. Due to the limited number of studies, the effects of vibration could not be statistically evaluated. It is concluded that properly applied light and sound stimuli can optimize incubation outcomes, with potential benefits for the poultry industry. Further studies are needed to standardize protocols, assess large-scale applications, and determine the economic feasibility of these practices.

Keywords: Embryonic development; incubation management; hatching performance; sensory stimuli; poultry

262P Dietary phytate levels modulate performance and bone mineralization responses to phytase in broiler chickens

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Phytic phosphorus (PP) is the main storage form of phosphorus in plant-based ingredients and may influence the efficacy of exogenous phytase according to substrate availability. This study evaluated whether growth performance and bone mineralization responses to phytase supplementation are influenced by dietary PP concentration. A total of 2400 one-day-old male Ross 308 broilers were randomly assigned to eight treatments with 10 replicates of 30 birds per experimental unit. The treatments were divided into two groups: a low-phytate group (0.19%) containing animal-based meals and a high-phytate group (0.29%) containing rice bran. Both groups were nutritionally reduced in Ca (0.36%) and available P (0.22%) and phytase was added at increasing levels (0, 1,000, 1,500, and 2,000 FTU/kg). Throughout the experimental period from 1 to 21 d of age, growth performance, tibia breaking strength, and body composition by dual-energy X-ray absorptiometry (DXA) were evaluated. Data obtained from performance and bone measurements were analyzed using 2-way ANOVA in the GLM procedure of SAS, considering the effects of dietary PP level, phytase inclusion level, and their interaction. In addition, polynomial contrasts were used to evaluate linear and quadratic responses to phytase supplementation. The evaluated responses were influenced by both dietary PP concentration and phytase supplementation, although no interaction between factors was observed ($P > 0.05$). Feed intake (FI) increased linearly with phytase supplementation ($P < 0.05$), whereas body weight gain and feed conversion ratio showed quadratic responses ($P < 0.05$), demonstrating improvements in performance as phytase inclusion increased. In addition, birds fed high-PP diets showed greater FI and more pronounced responses to phytase supplementation. Similar effects were observed for bone parameters, as tibia breaking strength and bone mineral content were improved by phytase supplementation ($P < 0.05$), especially in birds receiving high-PP diets. However, lean and fat mass were not affected by the treatments. Overall, phytase supplementation improved growth performance and bone mineralization in broiler chickens. The magnitude of these responses was influenced by dietary phytate phosphorus concentration, indicating that substrate availability plays an important role in modulating phytase efficacy.

Keywords: bone mineralization; broiler chickens; DXA; enzyme

263P A multicomponent protease supports productive performance and egg internal quality of laying hens fed nutrient-reduced diets under commercial conditions

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In commercial layer production, reducing feed costs through nutrient reductions while maintaining adequate amino acid supply remains a major challenge, as it may impair productive performance and egg quality. Therefore, this study evaluated the effect of a multicomponent protease on productive performance and egg quality of laying hens fed nutrient-reduced diets under commercial conditions. A total of 360 Hy-Line W36 laying hens, 52 weeks of age, were randomly assigned to three treatments: T1: Control (CT); T2: Low-density diet (LD; 5.3% reduction in crude protein and main digestible amino acids); and T3: LD + Protease (Multicomponent protease, Jefo Nutrition Inc.). The nutrient reduction in the LD diet corresponded to twice the protease matrix nutritional recommendation. Each treatment consisted of 12 replicates (10 hens/cage).

Layers were fed mash diets based on corn and soybean meal *ad libitum*. Productive performance (feed intake, egg production, egg weight, egg mass, and feed conversion ratio [FCR] per egg mass) was evaluated weekly and cumulatively from 52 to 58 weeks of age. Egg internal quality (albumen percentage) was assessed at week 57. Data were submitted to ANOVA, and means were compared using Fisher's test, where $P < 0.05$ was considered significant and $P < 0.10$ considered a trend. From 52 to 58 weeks of age, laying hens fed the LD + Protease diet showed higher ($P < 0.10$) egg mass compared with the LD group (61.8 vs. 59.4 g), with similar values to the CT group. The FCR per egg mass was improved ($P < 0.05$) in LD + Protease compared to LD (1.747 vs. 1.823), with no difference relative to CT. Feed intake, egg production, and egg weight did not differ among groups. Multicomponent protease supplementation increased ($P < 0.05$) albumen percentage compared to the LD diet (62.9 vs. 62.2%), with no difference from the CT, indicating that protease supplementation helped to maintain albumen proportion despite reduced crude protein and amino acids levels. In conclusion, the multicomponent protease enhanced the productive performance and egg internal quality of laying hens fed low-density diets under commercial conditions, yielding results comparable to those fed a control diet. These results demonstrate that the multicomponent protease can compensate for aggressive nutrient reductions, improving nutrient utilization and maintaining productive performance, thereby supporting cost effective egg production strategies under commercial conditions.

Keywords: commercial conditions; laying hens; productive performance; protease; protein utilization

264P A bacterial xylanase improves growth performance, nutrient digestibility, hindgut fermentation metabolites, and economic efficiency in broiler chickens

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A study was conducted to evaluate the effect of a bacterial xylanase (XYL) on growth performance, nutrient digestibility, hindgut fermentation, and economic efficiency in broilers fed energy-reduced diets. A total of 432 one-day-old male broilers (Vencobb 430Y) were allocated to three treatments: 1) standard diet (ST) based on corn-soybean meal, 2) energy-reduced diet (ER) with an energy matrix equivalent to 90, 100, and 110 kcal ME/kg for starter, grower, and finisher phases, respectively, and 3) ER supplemented with Belfeed xylanase (ER+XYL, Puratos) at 100 g/t. Each treatment included 12 replicates of 12 birds, and the trial lasted 42 days. Growth performance was evaluated weekly, while ileal amino acid digestibility and cecal fermentation metabolites were evaluated at day 42 using pooled ileal and cecal contents from two birds per pen. Titanium dioxide (TiO₂) was used as an indigestible marker at 5 g/kg. Economic efficiency was evaluated based on feed cost per kg of body weight gain. Data were analyzed using one-way ANOVA followed by Tukey's test, considering $P < 0.05$ as significant and $P < 0.10$ as a trend. Birds fed ER+XYL diets showed improved ($P < 0.05$) amino acid digestibility. Energy-reduced+XYL birds had increased ($P < 0.05$) butyric acid concentration by 25.9% compared to ER and increased ($P < 0.05$) lactic acid concentration by approximately 35.9% compared to ST, indicating a positive shift in microbial fermentation activity. These changes suggest improved fiber degradation and production of beneficial metabolites associated with gut health. Reducing dietary energy decreased ($P < 0.05$) final body weight by 2.6% compared to ST, indicating a negative impact on growth performance. Birds fed

ER+XYL diets showed 2.2% higher ($P > 0.05$) body weight compared to ER, being similar to levels comparable to ST. Feed intake tended to decrease ($P < 0.10$) in ER compared to ST, while feed conversion ratio was not affected by the treatments. In parallel, body weight variability was reduced in ER+XYL birds compared to ER, demonstrating improved flock uniformity. The better growth performance observed in supplemented birds can be partially explained by better nutrient utilization and intestinal health. Feed cost per kg of body weight gain was reduced by 3.5% in ER compared to ST and further reduced by 4.3% in the ER+XYL group ($P < 0.10$), indicating improved cost efficiency despite reduced dietary energy levels. Overall, the supplementation of this bacterial xylanase in energy-reduced diets improves nutrient utilization and enhances beneficial hindgut fermentation processes which supports growth performance and flock uniformity, while contributing to improved economic outcomes in broiler production systems.

Keywords: broilers; digestibility; energy reduction; economic efficiency; xylanase fermentation

265P Growth performance of broiler chickens fed a corn-soybean meal-based pelleted diet supplemented with agZyme HT

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Agrocere Multimix developed agZyme HT, which is a thermostable enzymatic blend composed of a combination of xylanase, amylase, protease, and phytase. It is used to enhance nutrient digestibility, thereby improving animal performance and contributing to reduced feed costs. This study aimed to evaluate the effects of agZyme HT supplementation on the performance of broiler chickens fed pelleted diets based on corn and soybean meals. The trial was conducted at Agrocere Multimix Technology and Innovation Center. A total of 4,800 one-day-old male Ross 308 broiler chicks were randomly assigned to one of three dietary treatments, with 20 replicates of 80 birds each. All experimental diets were pelleted and based on corn and soybean meal and were provided *ad libitum* throughout the experimental period. The dietary treatments were as follows: 1) a Positive Control (PC), consisting of a basal diet formulated to meet the nutritional requirements of broiler chickens; 2) a Negative Control (NC), consisting of a diet with reduced nutrient levels based on the nutrient matrix values attributed to agZyme HT, including reductions in metabolizable energy (ME), crude protein, amino acids, phosphorus, and calcium; and 3) NC + agZyme HT, consisting of the negative control diet supplemented with agZyme HT, aiming to achieve nutritional levels similar to those of the PC diet. Performance data were collected on day 42 and were analyzed using a one-way ANOVA test followed by Tukey's test for multiple comparisons of means, with statistical significance set at $P < 0.05$. The inclusion of agZyme HT in the NC diet increased final body weight (BW) and body weight gain by 17.3% ($P < 0.001$) compared with the group, resulting in values comparable to those observed in the PC group. The NC group exhibited the lowest feed intake among the treatments ($P < 0.001$), whereas no significant differences were observed between the PC and NC + agZyme HT groups. As a result, the NC treatment showed the lowest feed conversion ratio ($P < 0.001$) and European Production Efficiency Factor ($P < 0.001$), whereas the PC and NC + agZyme HT groups exhibited similar values. Supplementation with agZyme HT enhanced the performance of broiler chickens fed pelleted diets

based on corn and soybean meal.

Keywords: Amylase; pelleted diet; phytase; protease; xylanase

266P Different enzymatic associations improve nutrient digestibility and bone quality in broilers fed nutritionally reduced diets

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The objective of this study was to evaluate the effect of combining different enzymes (xylanase or xylanase + protease) associated with phytase in broiler diets nutritionally reduced on growth performance, nutrient digestibility, and bone quality. A total of 175 male Ross AP95 broilers with 21 days of age (896.77 ± 19.99 g initial body weight), were assigned to 5 treatments, with 7 replicates and 5 birds per experimental unit. The treatments consisted of a positive control diet meeting all nutritional requirements from 21 to 42 days (PC); a negative control diet 1 (NC1; -90 kcal kg⁻¹, -0.15% Ca, -0.15% P, and reduced amino acids); NC1 with the inclusion of 500g/ton of an enzyme blend composed of phytase and xylanase (NC1 + Phy/Xy); a negative control diet 2 (NC2; -105 kcal kg⁻¹, -0.15% Ca, -0.15% P, and reduced amino acids); and NC2 with the inclusion of 500 g/ton of an enzyme blend composed of phytase, xylanase, and protease (NC2 + Phy/Xy/Pro). The enzymes were a novel bacterial 6-phytase (500 FTU/g), a fungal xylanase (1,000 U/g), and a combination of acidic (fungal) and alkaline (bacterial) proteases (2,500 U/g), provided by Tectron, Brazil. The experimental diets were mash-based, formulated with corn and soybean meal according to the nutritional requirements of the strain for the grower (22–35 d) and finisher (36–42 d) phases. Performance parameters (feed intake, weight gain, and feed conversion ratio) were monitored from 22 to 42 days, and digestibility coefficients of gross energy, crude protein, dry matter, ash, and ether extract; as well as metabolizable energy (ME) were determined at 28 days (excreta collection). Bone quality (dry matter and ash content, breaking strength, and Seedor Index) was assessed at 42 days. Data were subjected to tests for homogeneity and normality, followed by ANOVA, and when significant ($P < 0.05$), means were compared using Tukey's test at a 5% probability. The inclusion of exogenous enzyme blends did not affect growth performance or ME values ($P > 0.05$). However, both enzyme combinations increased crude protein digestibility coefficients to levels comparable to the positive control ($P < 0.001$); the NC2 + Phy/Xy/Pro treatment also improved ash digestibility relative to the other treatments ($P < 0.001$). Diets NC1 and NC2, without enzymes, reduced bone strength ($P < 0.0001$) and the Seedor Index ($P < 0.05$), whereas the addition of phytase in both blends improved these parameters, suggesting enhanced release of Ca and P. In conclusion, the results suggest that phytase, likely in synergy with xylanase, was the primary contributor to the observed responses, while the additional inclusion of protease did not provide a distinct advantage under the conditions of this study.

Keywords: Bone strength; Digestibility; Phytase; Protease; Xylanase

267P Effects of dietary calcium and phosphorus reduction and phytase supplementation on broiler performance and carcass yield

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Calcium (Ca) and phosphorus (P) are essential minerals for skeletal development, metabolic function, and overall performance in broiler chickens. Phytase supplementation has been widely adopted to enhance mineral bioavailability in corn–soybean meal diets. This study evaluated the effects of reducing dietary Ca and P, with or without the supplementation of a novel phytase, on growth performance and carcass yield of Cobb 500 broilers. A total of 1,472 male chicks were assigned to a randomized block design with a 4 x 2 factorial arrangement (four mineral levels x two phytase levels), totaling eight treatments with eight replicates of 23 birds each. The treatments were positive control (PC), using nutritional recommendations, three diets with reduced Ca and P levels (-0.10% , -0.15% , -0.20%), and these same four diets supplemented with 500 FTU of a novel bacterial 6-phytase (Turbozyme F, Tectron, Brazil). Birds were fed a corn and soybean meal mash diet in a three-phase feeding program (1–21; 22–35 and 36–42 days). Performance parameters – including feed intake (FI), body weight gain (WG), feed conversion ratio (FCR), viability, and productive efficiency index – as well as carcass and cut yields at 43 days were evaluated. Data were analyzed using ANOVA, with means compared by Tukey's test (5%), and polynomial regression was applied to the graded reductions in Ca and P. Considering the total rearing period (1 to 42 d), an interaction ($P < 0.01$) was observed between the reduction in Ca and P levels and phytase supplementation for FI, WG, and productive efficiency index. In diets without phytase, linear declines ($P < 0.01$) in performance parameters were observed as mineral levels decreased. Conversely, phytase supplementation improved FI, WG, and FCR (by 4.1 points; $P < 0.01$), and increased the productive efficiency index, maintaining performance comparable to the PC diet even with reductions of up to 0.20% in Ca and P. For carcass and breast yield, the reduction in minerals negatively affected the birds ($P < 0.01$); however, phytase supplementation mitigated these negative effects, restoring values to levels similar to those of birds fed adequate mineral nutrition. In conclusion, decreasing dietary Ca and P compromises broiler growth and carcass yield. Nevertheless, phytase supplementation effectively improves mineral availability, sustaining optimal performance and carcass traits even in diets with Ca and P reduced by 0.20%.

Keywords: Carcass traits; Enzyme; Performance; Phytase; Poultry

268P A multicomponent protease improves broiler chicken performance and health regardless of soybean meal quality

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Thermal processing is crucial for soybean meal (SBM) quality and can impact animal health and performance. This study evaluated the effects of a multicomponent protease on broiler performance and organ health in diets containing SBM of varying quality. A total of 960 one-day-old male Ross 308 broilers were randomly allocated to six dietary treatments, with 10 replicates per treatment and 16 birds per replicate, over a 41-day trial. A single batch of SBM was subjected to controlled heat treatments (70–120°C) to produce three SBM qualities, classified based on potassium hydroxide protein solubility as follows: under-processed (91%), adequately processed (81%), and over-processed (67%). The treatments consisted

of a control with adequately processed SBM; an under-processed SBM; and an over-processed SBM. Each SBM quality treatment was supplemented or not with the Multicomponent Protease (Jefo Nutrition Inc.) at 125 g/t. Diets were formulated to be nutritionally equivalent. All diets were corn-SBM-based and provided in mash form across three feeding phases (starter, grower, and finisher). Growth performance was evaluated over the cumulative period. Macroscopic evaluations of liver and pancreas were performed on day 41 using three birds per replicate, with lesions scored from 0 (no alterations) to 5 (severely affected). Growth performance data were analyzed using ANOVA followed by Tukey's test, while organ data were assessed using the Kruskal-Wallis test followed by Dunn's test ($P < 0.05$). Birds fed under- and over-processed SBM without Protease showed up to 5.8% lower ($P < 0.05$) body weight gain (BWG) and up to 2.5% worse ($P < 0.05$) feed conversion ratio (FCR) compared to the control. The inclusion of Protease improved BWG and FCR across all SBM qualities, with improvements ranging from 2.1 to 2.9% in BWG and from 1.1 to 1.4% in FCR compared to their respective unsupplemented treatments. Liver scores were higher ($P < 0.05$) in birds fed under- and over-processed SBM without Protease compared to the control, indicating impaired organ functionality. The Protease supplementation in over-processed SBM resulted in liver scores similar to the control. The highest pancreas scores were observed in under-processed SBM without Protease, whereas supplementation with Protease reduced pancreas scores to levels similar to the other treatments, improving its functionality. In conclusion, the Multicomponent Protease improved growth performance and organ health regardless of SBM quality. These results demonstrate this Multicomponent Protease is an effective strategy to reduce feed costs, optimize performance, and mitigate the negative effects of both under- and over-processed SBM.

Keywords: Multicomponent Protease; over-processed SBM; under-processed SBM; broiler performance; organ health

269P Estimate of gross-to-metabolizable energy conversion by enzyme blends in broilers

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The objective of the present study was to evaluate the energy-releasing effects of β -mannanase, β -xylanase, and β -glucanase when incorporated into a corn and soybean meal diet for broilers. The supplementation of exogenous enzymes is intended to enhance the efficiency of dietary energy utilization, thereby facilitating the conversion of gross energy into metabolizable energy (ME), and subsequently into net energy. **Material and methods** Two metabolism assays were conducted using a completely randomized design with six treatments and ten replicates, totaling 60 experimental units (EU). Broilers were used at 7 and 27 days of age at the beginning of the assays, with five and four birds per EU, respectively. Six dietary treatments were evaluated. Four consisted of graded reductions in ME to establish the standard response curve (SRC): 0 (D1), 50 (D2), 100 (D3), and 150 (D4) kcal/kg below the reference diet. The reference diet (D1) contained 3,069 kcal/kg of ME from 1 to 21 days of age and 3,200 kcal/kg from 22 to 42 days of age. The remaining two treatments were formulated by supplementing the D4 diet (150 kcal/kg ME reduction) with exogenous enzymes: endo-1,4- β -mannanase (D5) or a combination of endo-1,4- β -mannanase, endo-1,4- β -xylanase, and endo-1,4- β -glucanase (D6). The variables measured

included total gross energy intake (tEI), ME, and nitrogen-corrected ME (ME_N). The SCR equation was derived by relating ME and ME_N to tEI using equation: $Y = \beta_0 + \beta_1 X$, in which, Y is ME or ME_N, kcal/bird d, and X is tEI, kcal/bird day. The energy release by enzymes were determined by substituting the ME and ME_N values from D5 and D6 into the SCR equation and calculating the difference relative to D4. **Results** The regression equations obtained for ME and ME_N in the first assay were $Y = -39.61 + 0.924X$ (± 3.87 $R^2 = 0.96$) and $Y = -57.93 + 0.893X$ (± 10.4 $R^2 = 0.88$), respectively. In the second assay, the regression equations for ME and ME_N were $Y = -34.82 + 0.886X$ (± 3.47 $R^2 = 0.96$) and $Y = -37.12 + 0.815X$ (± 10.7 $R^2 = 0.85$), respectively. The estimated energy contribution from enzyme supplementation varied according to the age of the birds. For β -mannanase, ME_N release was estimated at 52 kcal/kg between 12 and 17 days of age, and 60 kcal/kg between 32 and 37 days of age. For the combination of β -mannanase, β -xylanase, and β -glucanase, ME_N release was estimated at 89 kcal/kg from 12 to 17 days of age and 108 kcal/kg from 32 to 37 days of age. **Conclusion** The estimated ME_N release was 59 kcal/kg for β -mannanase (D5) and 98 kcal/kg for the combination of enzymes (D6). The estimated energy contributions from the enzyme blend indicate that these additives can effectively offset intentional reductions in dietary energy concentrations.

Keywords: Exogenous Enzymes; Energy; Equivalence; mannanase; nutritional matrix

270P Dietary Inclusion of Spray-Dried Porcine Plasma in Laying Hen Diets During Peak Production

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Spray-dried porcine plasma (SDP) is a functional protein ingredient with potential applications in poultry nutrition. This study evaluated the effects of dietary inclusion of 1% SDP on productive performance, egg quality, and economic efficiency of Isa Brown laying hens during peak production. A total of 108 hens at 33 weeks of age were distributed into 36 cages, with three birds per cage, in a completely randomized design with two treatments: control diet (0% SDP) and SDP diet (1% SDP). The cage was considered the experimental unit, with 18 replicates per treatment. The experiment lasted 90 days, including a 7-day adaptation period. Feed intake, egg production, feed conversion, internal and external egg quality, and economic indicators were evaluated. Data were analyzed using SAS, and treatment means were compared using Student's t-test. The inclusion of 1% SDP increased feed intake (144.60 vs. 149.00 g/hen/day) and average egg production per hen per experimental period (24.26 vs. 25.37 eggs/hen; $P = 0.05$), whereas daily egg production did not differ between treatments (0.87 vs. 0.91 eggs/hen/day; $P > 0.05$). Feed conversion was similar between treatments (2.00 vs. 1.98 kg feed/dozen eggs; $P > 0.05$). Egg weight, shell weight, albumen and yolk weights, shell thickness, yolk color, and shell calcium concentration were not affected by SDP inclusion ($P > 0.05$). Therefore, 1% SDP supplementation improved some productive responses without impairing egg quality, under the conditions evaluated.

Keywords: Additive; eggs; protein; sustainability; feed

271P A blend of organic acids and essential oils

reduces *Salmonella* viability, invasion, and inflammatory signaling in chicken intestinal organoids

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The objective of this study was to evaluate the effects of a blend of organic acids and essential oils (OA+EO) on *Salmonella Typhimurium* viability, invasion, immune responses, and protein phosphorylation in a *Salmonella*-infected intestinal organoid model. A controlled in vitro experiment was conducted using chicken intestinal organoids exposed to *Salmonella Typhimurium* with or without OA+EO. Chicken intestinal organoids were generated from 18–19-day-old Hy-Line embryos. Organoids and *Salmonella* were pretreated with OA+EO (Jefo Nutrition) at 0, 0.25, or 0.50 mg/mL for 48 h and 24 h, respectively. Following pretreatment, 500 CFU of *Salmonella* (pretreated or untreated) were added to the corresponding organoid treatments. *Salmonella* viability was measured 24 h after exposure to OA+EO, while *Salmonella* invasion was assessed 3 h post-infection. Each treatment included n = 8 replicates. Innate immune responses were quantified by qPCR, and changes in protein phosphorylation were analyzed using a kinome array to evaluate alterations in immune-related signaling pathways. Statistical analyses were performed using GraphPad Prism 9 and GeneV6. Data were analyzed using two-way t-tests followed by Bonferroni correction. Differences were considered significant at P < 0.05. The OA+EO reduced *Salmonella* viability by 28% (average of the two doses) compared with the untreated control. Organoids treated with 0.25 or 0.50 mg/mL of OA+EO showed a significantly lower level of *Salmonella* invasion (P<0.01). In untreated organoids, *Salmonella* exposure increased the expression of pro-inflammatory cytokines and chemokines (P<0.05), whereas OA+EO downregulated these infection-induced inflammatory responses (P<0.05). Consistent with the gene expression profile, kinome analysis showed that *Salmonella* challenge activated immune signaling pathways, while OA+EO modulated them by reducing the phosphorylation of proteins involved in inflammatory pathways (P<0.05). These findings demonstrate that OA+EO reduces *Salmonella* viability and invasion while modulating host inflammatory signaling in chicken intestinal organoids, supporting the potential of this blend as a strategy to mitigate *Salmonella* challenges.

Keywords: essential oils; intestinal organoid; intestinal inflammation; organic acids; *Salmonella*

272P Effects of a blend of organic acids and cinnamaldehyde on the hematological profile of broiler chickens challenged with water contaminated with reused litter

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The restriction on antibiotic use in poultry production has increased the demand for alternative feed additives. Organic acids and essential oils exhibit antimicrobial and antioxidant properties that may improve intestinal health and modulate the immune response of broiler chickens. This study evaluated the effects of organic acids and cinnamaldehyde on the hematological profile of broiler

chickens challenged with drinking water contaminated with reused poultry litter. The experiment was conducted at the Poultry Sector, FCAT/UNESP, Dracena, Brazil. A total of 900 one-day-old Ross broiler chicks were reared for 42 days in a completely randomized design with five treatments and six replicates: NC, negative control (basal diet without avilamycin); PC, positive control (basal diet with avilamycin); BA, butyric acid; BL, a blend of lactic acid, formic acid, and cinnamaldehyde; and BA + BL, butyric acid plus the blend. Birds were challenged by receiving drinking water contaminated with reused poultry litter. At 42 days of age, blood samples (3 mL) were collected from six birds per treatment by brachial vein puncture for hematological analyses, including hematocrit, hemoglobin concentration, erythrocyte count, Wintrobe indices, total leukocyte count, and differential leukocyte count. Data were analyzed by one-way ANOVA, and means were compared using Tukey's test (5%). No significant differences (P > 0.05) were observed among treatments for hematocrit, erythrocyte count, hemoglobin concentration, total leukocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, thrombocytes, eosinophils, monocytes, heterophils, or lymphocytes. The lack of treatment effects may be explained by the interval between the sanitary challenge, applied at 7 days of age, and blood collection at 42 days, which may have allowed hematological recovery. In addition, genetic background, nutrition, and environmental conditions may have influenced the evaluated parameters. In conclusion, butyric acid and blends of organic acids and cinnamaldehyde produced hematological responses comparable to those obtained with avilamycin. However, under the conditions of this study, these additives did not improve the hematological profile of broiler chickens subjected to a sanitary challenge. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Keywords: broiler chickens; butyric acid; avilamycin; hematology; essential oils

273P PERFORMANCE AND GUT MICROBIOTA RESPONSE OF BROILER CHICKENS TO A NON-STARCH POLYSACCHARIDE CHALLENGE AND DIETARY ADDITIVES

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Diets with high levels of non-starch polysaccharides (NSPs) may increase digesta viscosity, reduce nutrient digestibility, and impair the intestinal health of broiler chickens. Sodium butyrate and *Bacillus subtilis* may mitigate these effects by modulating intestinal integrity and gut microbiota. This study evaluated the effects of these additives on performance, carcass traits, and cecal microbiota of broiler chickens from 1 to 38 days under an NSP challenge. A total of 1,120 broilers were assigned to a randomized complete block design with 4 treatments and 7 replicates of 40 birds each. The treatments consisted of a corn-soybean meal diet (CSB), a high-NSP diet containing 30% of rice bran (NSP), NSP + 250 ppm of butyrate (90% sodium butyrate – BUT), and NSP + 300 ppm of *Bacillus subtilis*. The CSB and NSP diets were isonutritional, and additives were included on top. Body weight (BW), weight gain (WG), and feed conversion ratio (FCR) were calculated for the entire experimental period. At 21

d, one bird per replicate was euthanized, and cecal content were collected for 16S DNA sequencing. At 39 d, two birds per replicate were euthanized for carcass quality evaluation. Performance and carcass data were analyzed using one-way ANOVA (PROC GLM SAS ODA) and Tukey's test ($p < 0.05$). Wooden breast, white striping, and foot pad lesion scores were analyzed using Kruskal-Wallis test. Beta diversity was evaluated using weighted UniFrac distance, principal coordinates analysis (PCoA), and PERMANOVA testing. Differentially abundant taxa treatments were identified using the MaAsLin3 model ($q < 0.05$). No effect was observed on BW ($p = 0.067$), however higher WG was observed in BS than in CSB ($p = 0.048$). Birds fed CSB had greater FI ($P = 0.002$) and poorer FCR ($P < 0.001$) than birds fed NSP, BUT, or BS. (NSP = 1.586; BUT = 1.569; BS = 1.572; CSB = 1.702). No differences were observed in carcass parameter, pododermatitis, wooden breast, white stripes scores. Differential abundance analysis indicated that CSB increased the relative abundance of Clostridiales ($q=0.029$), Clostridiaceae ($q=0.049$), and Clostridium ($q=0.010$) compared to the NSP, BUT and BS. Supplementation with BUT and BS did not alter bacterial groups when compared with NSP. In conclusion, contrary to the expected negative response to the high-NSP challenge, broilers fed NSP-based diets showed improved FCR and BS also improved WG. This unexpected response may be associated with the lower relative abundance of Clostridium-related taxa in birds fed NSP-based diets, suggesting that rice bran inclusion modulated cecal microbiota and performance under the conditions of this study.

Keywords: Efficiency; Food additives; Intestinal health; Microbial modulation; Poultry nutrition

274P Alternative program to antibiotic growth promoters for broiler chickens

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The spectrum of antibiotic growth promoters available for poultry production in Brazil has progressively decreased over the years. Consequently, numerous studies have explored a wide range of feed additives and alternative strategies to replace the use of antibiotic growth promoters. This study aimed to assess the effects of a combined supplementation strategy using agProSymbios from 1 to 21 days of age, and agProFito (from 22 to 42 days of age), in comparison with an antibiotic growth promoter, on the performance of broiler chickens subjected to a coccidiosis vaccine challenge. The trial was carried out at Agrocere Multimix Technology and Innovation Center. A total of 3,198 one-day-old male Ross 308 broiler chicks were randomly assigned to one of three dietary treatments, with 13 replicates of 82 birds each. All diets were formulated to be isonutritive, except for the inclusion or not of additives, and were provided *ad libitum* throughout the experimental period. The dietary treatments were as follows: 1) a Negative Control (NC), consisting of a basal diet formulated to meet the nutritional requirements of broiler chickens without additives; 2) a Positive Control (PC), consisting of a NC diet supplemented with enramycin; and 3) The alternative program consisted of the NC diet supplemented with agProSymbios at 500 g/t from 1 to 21 days of age, followed by supplementation with agProFito at 1000 g/t from 22 to 42 days of age. agProSymbios is a symbiotic additive, whereas agProFito is a phytogenic additive

composed of essential oils, plant extract, and prebiotics. On day 14, all birds were orally challenged with high dose of coccidiosis vaccine at 10 times higher than the recommended vaccine dose. Performance data were collected on day 42 and were analyzed using a one-way ANOVA test followed by Tukey's test for multiple comparisons of means, with statistical significance set a $P < 0.05$. Although body weight and feed intake did not differ among treatments ($P > 0.05$), the feed conversion ratio in the alternative program was 3.3% lower than in the NC group ($P=0.03$), while remaining similar to that of the PC treatment. Therefore, in this study, the alternative program (association of agProSymbios and agProFito) effectively improved broiler performance, achieving results comparable to those observed in birds supplemented with antibiotic under a coccidiosis vaccine challenge.

Keywords: Additive; alternative program; antibiotic growth promoter; broiler; coccidiosis

275P Evaluation of Toxin™ Dry on the performance of laying hens under a mycotoxin challenge

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Mycotoxins are frequent contaminants in feed ingredients used in poultry nutrition and represent a persistent challenge in intensive production systems. Their presence in commercial diets may negatively affect productive performance and feed efficiency in laying hens. The objective was to evaluate the overall effect of mycotoxins and Toxin™ Dry on the productive performance of laying hens. A total of 480 Hy-Line W-36 laying hens at 25 weeks of age were used and distributed in a completely randomized design with six treatments, eight replicates, and 10 birds per experimental unit. The treatments consisted of: control diet (T1); control + Toxin™ Dry (T2); diet contaminated with T-2 (T3); T-2 + Toxin™ Dry (T4); fumonisin + T-2 (T5); and fumonisin + T-2 + Toxin™ Dry (T6). The contaminated diets were formulated with T-2 and fumonisin at 2 and 60 mg/kg, respectively. Toxin™ Dry is a mycotoxin adsorbent composed of sepiolite (440 g/kg) and bentonite (550 g/kg). Egg production, feed intake, egg weight, egg mass, and feed conversion ratio per egg mass and per dozen eggs were evaluated. Data were analyzed by analysis of variance (ANOVA) followed by orthogonal contrasts to assess the overall effect of mycotoxins (T1 + T2 vs T3 + T4 + T5 + T6) and the overall effect of Toxin™ Dry (T2 + T4 + T6 vs T1 + T3 + T5), with significance declared at $P < 0.05$. Mycotoxin exposure reduced egg production by 11.6%, feed intake by 7.7%, egg weight by 5.5%, and egg mass by 16.2%, and impaired feed conversion ratio per egg mass and per dozen eggs by 10.7% and 4.8%, respectively ($P < 0.0001$). In contrast, Toxin™ Dry supplementation increased egg production by 9.3%, feed intake by 4.6%, egg weight by 3.4%, and egg mass by 12.6%, and improved feed conversion ratio per egg mass and per dozen eggs by 8.2% and 4.5%, respectively ($P < 0.0001$). It is concluded that mycotoxins significantly impair the productive performance of laying hens, whereas Toxin™ Dry improves performance parameters and mitigates the negative effects of the toxic challenge, independent of mycotoxin exposure.

Keywords: mycotoxins; T-2 toxin; fumonisin; adsorbent; laying hens

276P Yeast Cell Wall Modulates Intestinal Microbiota and

Improves Growth Performance in Broilers

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This study evaluated whether a yeast cell wall product modulated growth performance and intestinal microbiota of broilers fed good-quality soybean meal diets. Only T1 (control) and T2 (yeast cell wall) were compared. Performance data were obtained from 7 replicate pens per treatment from 1 to 41 d, and microbiota taxonomic profiles were evaluated in 6 samples per treatment. Alpha diversity was compared by Kruskal-Wallis tests, and discriminant taxa were explored using LEfSe. Birds receiving yeast cell wall had greater body weight at 28 d than control birds (1.609 vs. 1.415 kg; $P=0.005$). At 41 d, the yeast cell wall group showed numerically greater body weight (2.941 vs. 2.805 kg), greater total weight gain (2.897 vs. 2.760 kg), lower feed conversion ratio (1.70 vs. 1.77), and higher production efficiency index (418.6 vs. 387.6), although these final responses were not statistically significant ($P>0.10$). Genus-level richness (219.7 vs. 219.3 taxa; $P=1.00$) and Shannon diversity (2.86 vs. 2.73; $P=0.75$) were similar between yeast cell wall and control, indicating that the additive did not increase community complexity. However, the biomarker pattern suggested a more favorable ecological redistribution. Control birds showed greater negative-associated Enterobacteriales pressure, including *Enterobacteriaceae* (7.18 vs. 3.86%), *Escherichia* (7.02 vs. 3.84%), and *Escherichia coli* (7.02 vs. 3.84%; $P=0.037$). In contrast, yeast cell wall increased low-abundance positive or fermentative biomarkers, including *Bacteroides ovatus* (0.132 vs. 0.008%; $P=0.036$), *Bacteroides uniformis* (0.053 vs. 0.003%; $P=0.031$), and *Butyricimonas* (0.043 vs. 0.002%; $P=0.050$). Under the evaluated conditions, yeast cell wall did not increase alpha diversity, but redirected the intestinal microbiota toward a more favorable ecological profile without reducing richness. This is an important biological signal, because the additive shifted microbial pressure away from Enterobacteriaceae/*Escherichia*-associated biomarkers while preserving community complexity, suggesting modulation without an apparent loss of ecological occupancy that could favor the expansion of potentially negative bacteria. This microbiota response was accompanied by improved body weight at 28 d and numerically better final feed efficiency, supporting yeast cell wall as a strategy to modulate intestinal microbial pressure while maintaining microbial richness and ecological stability.

Keywords: Broiler chickens; Gut microbiota; Yeast cell wall; Soybean meal; Microbial biomarkers

277P THE ASSOCIATION OF ESSENTIAL OILS WITH AN ANTIBIOTIC GROWTH PROMOTER IN THE PERFORMANCE AND CARCASS YIELD OF BROILERS

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This study aimed to evaluate the combined use of an essential oil blend and enramycin, an antibiotic growth promoter, in corn- and soybean meal-based diets on the growth performance and car-

cass yield of broiler chickens. The experiment was conducted at the Federal University of Goiás, Brazil. A total of 660 one-day-old Cobb broiler chicks were used in a completely randomized design consisting of three treatments and ten replicates, with 22 birds per replicate. The experimental treatments consisted of a basal diet supplemented with enramycin, a basal diet without feed additives (control), and a basal diet supplemented with enramycin and an essential oil blend. The essential oil blend was included at 100 g/ton and consisted of cinnamon flavor (47 g/kg), citrus flavor (10 g/kg), carvacrol (17 g/kg), eugenol (4,000 mg/kg), geraniol (5,000 mg/kg), *p*-cymene (3,000 mg/kg), and thymol (58 g/kg). Enramycin was included at 10 g/ton. Growth performance variables evaluated at 7, 21, 35, and 42 days of age included feed intake, body weight gain, livability, and feed conversion ratio. At 42 days of age, following slaughter, eviscerated carcass yield (excluding the head, feet, and neck) and the yields of commercial cuts, including breast, drumstick, thigh, wings, and back, were determined. No improvements were observed in final body weight, body weight gain, feed intake, or feed conversion ratio ($P > 0.05$) during any production phase among the treatments. Likewise, no significant differences ($P > 0.05$) were observed in carcass yield or the yields of commercial cuts at 42 days of age. The combined supplementation of enramycin and the essential oil blend did not result in significant improvements in broiler growth performance or carcass yield.

Keywords: feed additives; alternatives; enramycin; performance; microbiota

278P Natural additives as an alternative to antibiotics in egg quality of commercial laying hens

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The demand for sustainable poultry production has increased interest in alternatives to antibiotic growth promoters, aiming to reduce risks related to antimicrobial resistance, minimize environmental impacts, and meet consumer demands for safer food. In this context, phytochemical additives and probiotics have stood out for promoting intestinal microbiota balance, better nutrient utilization, and maintenance of bird health. This study aimed to evaluate the effect of different dietary additives on egg quality of commercial laying hens. The experiment was conducted at the poultry facility of the Faculty of Agricultural Sciences, Federal University of Grande Dourados (UFGD). A total of 200 Hisex White laying hens at peak lay were distributed in a completely randomized design with four treatments, ten replicates, and five birds per experimental unit. Treatments consisted of: basal diet without additive, basal diet with phytochemical additive based on garlic and cinnamon, basal diet supplemented with probiotic, and basal diet supplemented with antibiotic (bacitracin). Diets were formulated to meet nutritional requirements according to Rostagno et al. (2017). Internal and external egg quality traits were evaluated, including egg weight, specific gravity, albumen height, yolk weight, height and diameter, albumen and shell weight, yolk, albumen and shell percentages, shell thickness, Haugh unit, and shell weight per surface area. Data were subjected to analy-

sis of variance (one-way ANOVA) and means were compared by Tukey's test at 5% probability. No significant differences ($P>0.05$) were observed among treatments for any of the evaluated variables. Haugh unit values remained above 75 across all treatments, indicating adequate internal egg quality. The absence of differences demonstrates that the phytogetic additive and probiotic were able to maintain the same egg quality standards as those observed with antibiotic use. It is concluded that supplementation with phytogetic additives based on garlic and cinnamon or probiotics can replace antibiotic use in laying hen diets without compromising egg quality. These results highlight the potential of these natural alternatives to promote more sustainable production systems, aligned with current demands for reduced antimicrobial use and improved food safety.

Keywords: Egg production; Poultry nutrition; Phytogetic additives; Probiotics; Sustainable production

279P Effects of a microencapsulated blend of organic acids and essential oils on growth performance and serum immunoglobulins of broiler chickens submitted to an enteric challenge

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Enteric pathogens such as *Eimeria* spp. and *Clostridium perfringens* can impair intestinal integrity, nutrient absorption, and broiler performance. Therefore, this study evaluated the effects of a microencapsulated blend of organic acids and essential oils on growth performance and serum immunoglobulin concentrations (IgA and IgY) of broilers submitted to an enteric challenge. A total of 1,200 one-day-old Cobb 500 male chicks were assigned to a completely randomized design with six treatments, 10 replicates, and 20 birds per replicate. Treatments consisted of a negative control (unchallenged; NC), a positive control (challenged; PC), and four dietary inclusion levels of the additive (250; 500; 750; and 1,000 g t⁻¹) in challenged birds. The additive consisted of degummed palm oil, citric, fumaric, phosphoric, malic, and lauric acids, thymol, and carvacrol. At d 14, all birds except those in the NC group were gavaged with a coccidiosis vaccine at 30 times the recommended dose. On d 17 and 18, challenged birds received oral inoculation of *C. perfringens* (1×10^8 CFU/mL). Feed intake, weight gain, and feed conversion ratio were evaluated from 1 to 42 d. On d 23, blood samples were collected from six birds per treatment to determine serum IgA and IgY concentrations by membrane immunoassay. Data were tested for normality using the Shapiro-Wilk test and analyzed by ANOVA, followed by Dunnett's test, orthogonal contrast (NC vs. PC), and regression analysis ($P \leq 0.05$). No differences ($P>0.05$) were observed among treatments for growth performance from 1 to 14 d, before the challenge. From 1 to 21 d, NC birds showed better performance than PC birds, whereas birds fed the diet containing 250 g t⁻¹ of the additive showed growth performance similar to that of the NC group. No differences ($P>0.05$) were observed among treatments during the subsequent evaluation periods. Serum IgY concentrations were not affected by treatment ($P>0.05$). Likewise, no differences in serum IgA concentration were detected between the additive-treated groups and the NC group according to Dunnett's test ($P>0.05$). However, birds fed diets containing 250 g t⁻¹ of the additive showed lower serum IgA concentration than the PC group (0.505 vs. 1.476 relative intensity; $P=0.046$). Although treatment

effects were detected for IgA, regression analysis showed no linear or quadratic response. In conclusion, broilers fed the diet containing 250 g t⁻¹ of the microencapsulated blend of organic acids and essential oils showed growth performance similar to that of the unchallenged control from 1 to 21 d. The additive also altered serum IgA concentration without affecting serum IgY concentration or overall growth performance through 42 d of age.

Keywords: carvacrol; gut health; immune response; microencapsulation; thymol

280P EFFECT OF ORGANIC ACID BLEND SUPPLEMENTATION ASSOCIATED WITH AN ANTIBIOTIC GROWTH PROMOTER IN DIETS FOR BROILER CHICKENS

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Due to increasing restrictions on the use of antibiotic growth promoters in poultry production, the use of feed additives with antimicrobial properties, such as organic acids, has increased. Organic acids reduce gastrointestinal pH, thereby improving nutrient digestion and reducing or preventing the colonization of pathogenic microorganisms in the gastrointestinal tract without leaving residues in the final product. This study aimed to evaluate the effects of supplementing an organic acid blend in combination with an antibiotic growth promoter on the growth performance and carcass yield of broiler chickens. The experiment was conducted at the Experimental Poultry Unit of the Federal University of Goiás using 660 Cobb 500® broiler chickens. Birds were assigned to a completely randomized design consisting of three treatments, 10 replicates, and 22 birds per replicate, totaling 30 experimental units. The treatments consisted of: a basal diet supplemented with enramycin (10 g/ton); a basal diet without enramycin or organic acids; and a basal diet supplemented with enramycin (10 g/ton) and an organic acid blend (1000 g/ton). The organic acid blend consisted of citric acid, calcium butyrate, calcium lactate, and calcium formate. Growth performance variables, including final body weight, body weight gain, viability, feed intake, and feed conversion ratio, were evaluated at 7, 21, 35, and 42 days of age. Carcass yield and cut yields were evaluated at 42 days of age. Growth performance was not affected by dietary treatments during any production phase ($P>0.05$). Likewise, no significant differences were observed in carcass yield or cut yields among treatments ($P>0.05$). In conclusion, the combined supplementation of an organic acid blend and enramycin did not improve growth performance or carcass yield in broiler chickens.

Keywords: Feed additives; Alternatives; Enramycin; Performance; Microbiota

281P Blood Biochemical Profile of Broiler Chickens Challenged with *Eimeria maxima* and Fed *Hermetia illucens* Larvae Oil

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Coccidiosis induces metabolic disturbances in broiler chickens, making blood biochemical parameters useful indicators of the physiological response to infection. This study evaluated the effects of *Hermetia illucens* larvae oil (HILO) on the blood biochemical profile of broilers challenged with *Eimeria maxima*. A total of 540 one-day-old male Ross chicks were housed in 3m² pens. The experiment was conducted in a completely randomized design in a 5 × 2 factorial arrangement, with five treatments and two evaluation periods, 8 and 15 days post-infection. The nonchallenged control had four replicates of 15 birds, whereas the four challenged treatments had eight replicates of 15 birds each, totaling 36 experimental units. All procedures were approved by the Animal Use Ethics Committee (CEUA), protocol no. 811/2023. Treatments were: non-challenged control; challenged control, with birds inoculated with *E. maxima* and fed a control diet containing antibiotic (Halquinol) and antioxidant, without HILO; challenged birds fed an antibiotic- and antioxidant-free diet (AAFD), without HILO; challenged AAFD + 0.5% HILO; and challenged AAFD + 1.0% HILO. At 17 days of age, challenged birds were inoculated with *E. maxima* by oral gavage at 10⁷ oocysts/bird. At 8 and 15 days post-infection, 3 mL of blood was collected from two birds per experimental unit. Serum amylase, lipase, uric acid, gamma-glutamyl transferase (GGT), aspartate aminotransferase/glutamic-oxaloacetic transaminase (AST/GOT), and total cholesterol were analyzed from samples collected without anticoagulant. Plasma glucose was determined from samples collected in tubes containing 10% EDTA. Data were subjected to two-way ANOVA using PROC GLM in SAS, and means were compared by the Student-Newman-Keuls test when significant ($P < 0.05$). Treatments did not affect serum amylase, lipase, uric acid, GGT, AST/GOT, or total cholesterol ($P > 0.05$). Plasma glucose showed a tendency for treatment effect ($P = 0.0553$), with lower concentration in challenged birds fed AAFD + 1.0% HILO (130.08 mg/dL) than in nonchallenged birds (181.00 mg/dL); however, challenged treatments did not differ from each other. At 15 days post-infection, plasma glucose decreased, which may be associated with impaired intestinal glucose absorption and greater energy redirection toward inflammation and tissue repair. In conclusion, the main biochemical changes were associated with days post-infection, indicating a greater influence of the metabolic response to *E. maxima* challenge than of dietary HILO supplementation.

Keywords: *Hermetia illucens*; blood biochemistry; broiler chickens; coccidiosis; *Eimeria maxima*

282P A meta-analysis of how organic acids in laying hen diets affect egg quality, performance, and gut health.

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The search for nutritional alternatives to replace antibiotic growth promoters has increased interest in additives that can improve bird performance, intestinal integrity, and egg quality. Organic acids are a promising option due to their antimicrobial properties and positive effects on performance and gut health. This study aimed to synthesize the available evidence through a systematic review (SR) and meta-analysis evaluating the effects of dietary OA inclusion in laying hens on productive performance, egg quality, and intestinal morphology. Using the PICO framework, the literature search was conducted in seven electronic databases, Web of Science, Scopus,

Medline/PubMed, CAB Abstracts, Animal Health and Production Compendium, ProQuest Dissertations & Theses Citation Index and Google Scholar (first 120 records), without restrictions on language or publication year. A total of 8,272 records were identified, and after screening and eligibility assessment, 36 studies (from 1993 to 2025) were included in the final dataset. Statistical analyses were performed using SAS 9.4. Meta-analysis was conducted using mixed linear models. Variance among studies was estimated by REML, and adjusted means were compared using the Tukey-Kramer test. Among the included studies in the SR, 67% evaluated combinations of organic acids, while 33% focused on single organic acids. Antibiotic treatments for comparison were included in 1.38% of the studies. The meta-analysis revealed significant effects ($P \leq 0.05$) of OA supplementation on egg production rate, egg weight and mass, and feed intake. Egg production rate differed among treatments ($P = 0.0198$), being higher in organic acid combinations (61.39%), antibiotics (60.05%), and single organic acids (60.76%) than in the control group (58.45%). Improvements were also observed in egg quality parameters, including specific gravity, albumen index, and Haugh unit. Albumen index differed among treatments ($P = 0.0092$), being highest in single organic acids (8.94%), and lower in the control group (7.85%) and organic acid combinations (7.85%). Additionally, significant changes were detected in intestinal morphology, particularly in crypt depth and villus height. Overall, dietary OA inclusion positively influenced productive performance, internal egg quality, and intestinal morphology, supporting their use as a nutritional strategy in laying hen production systems. However, substantial heterogeneity among studies suggests that the productive response to organic acid supplementation is context-dependent rather than uniform. The magnitude of the effects appears to be influenced by multiple interacting factors, including genetic strain, diet composition, type of organic acid, and dosage.

Keywords: Acetic acid; Growth promoters ; Lactic acid; Propionic acid;

283P Effects of different commercial yeast cell wall products in broiler diets under heat stress

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Intestinal health plays a crucial role in broiler production by directly affecting nutrient utilization and performance. Mannan-oligosaccharides (MOS) and β -glucans derived from *Saccharomyces cerevisiae* cell walls are functional compounds that may improve gut health by modulating the microbiota, stimulating beneficial microorganisms, and limiting pathogen colonization. However, their efficacy may vary depending on yeast strain, growth conditions, composition, and production processes. These postbiotics are widely used to improve intestinal health, strengthen immune function, and enhance growth and productivity under challenging conditions, such as heat stress, which is a major concern in modern broiler production. This study evaluated the effects of Safmannan® and five commercial yeast cell wall (YCW) or yeast culture (YC) products on the performance and carcass yield of broilers subjected to heat stress. A total of 3,612 male broilers were allocated in a completely randomized design with seven treatments, 12 replicates, and 43 birds per replicate. The treatments consisted of: A (Control): non-supplemented basal diet; B: basal diet + Safmannan® (250 g/t); and C, D, E, F, and G: basal diet supplemented with different commercial YCW or YC products (250 g/t). All birds received the same basal diet and were exposed

to heat stress from 14 days of age by maintaining ambient temperatures 3°C above the recommended thermal comfort range for each production phase. Performance parameters were evaluated weekly, and carcass and cut yields were assessed at 43 days of age (n = 48 birds/treatment). Data were analyzed using ANOVA and Tukey's test ($P < 0.05$). From 1 to 42 days of age, broilers fed Safmannan® demonstrated numerically improved feed conversion ratio (FCR), final body weight, carcass weight, and carcass yield, and significantly higher breast yield ($P < 0.05$) compared to the control group. While other yeast-derived supplements exhibited heterogeneous responses in performance outcomes, Safmannan® consistently outperformed numerically and ranked among the top treatments, showing statistically better results for FCR, final body weight, carcass yield, breast yield, and wing yield, as well as reduced abdominal fat deposition. In conclusion, Safmannan® enhanced growth performance and carcass traits in broilers under heat stress conditions, while also decreasing abdominal fat accumulation. These results highlight the functional differences among yeast-based additives and support their use as an effective nutritional strategy for sustaining productivity during thermal challenges.

Keywords: mannan-oligosaccharides; β -glucans; yeast cell wall (YCW); ; *Saccharomyces cerevisiae*

284P Effects of Deoxycholic Acid Supplementation on Growth Performance and Bone Development in Broiler Chickens

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Bile salts (BS) act as natural emulsifiers, enhancing lipid digestion and the absorption of fat-soluble vitamins in broiler. Additionally, they improve intestinal morphology, modulate the microbiota and inflammatory processes, and promote gut health. These benefits lead to improved zootechnical performance, with greater weight gain and better feed conversion ratios. Regarding bone health, their effects are indirect and result from improved absorption of calcium, phosphorus, and fat-soluble vitamins, thereby supporting bone mineralization. In this context, the objective of this study was to evaluate the effects of supplementing mash diets with BS15, a deoxycholic acid-based bile acid product, on productive performance and bone development in broiler chickens. The experiment involved 1,376 male Cobb chicks, allocated in a completely randomized design with four treatments and eight replicates of 43 birds each: control diet (T1), control diet supplemented with 150 g/ton BS15 (T2), control diet + 200 g/ton BS15 (T3), and control diet + 250 g/ton BS15 (T4). The birds were housed in the experimental poultry facility of the Federal University of Paraná. Productive performance was assessed by live weight, feed intake, and feed conversion ratio at different ages. To evaluate bone development, tibiae were collected at 10, 28, and 42 days of age. Data were analyzed by ANOVA using SAS software. Means were compared by Tukey's test and subjected to regression analysis using orthogonal polynomials. Significant effects were observed on live weight and feed conversion ratio at 10 days of age. Treatments T1, T2 and T3 showed similar live weight and better feed conversion ratios compared to T4. For ash content at 10 days and bone strength at 28 days, regression analysis indicated significant quadratic effects, with the optimal response occurring near first inclusion supplementation level (T2). Regarding tibial cortical measurements at 28 days, regression analysis also revealed significant effects, with treatments T1 and T2 showing greater tibial cortical thickness than T3 and T4. The results indicate that moder-

ate levels of deoxycholic acid can maximize performance and bone mineralization in the initial phase. At later ages, inclusion did not affect the productive performance of the birds. However, the lowest supplementation level (150 g/ton BS15) produced favourable results for parameters related to bone development, suggesting potential benefits for mineralization and bone growth. Thus, further studies are warranted to determine the optimal inclusion level and to better characterize its effects on productive performance and bone quality.

Keywords: Bile salts ; Bone mineralization; Deoxycholic Acid ; Gut health; Lipid digestibility

285P Performance, biometry and relative organ weight of laying pullets with (7 to 15 weeks) fed a diet containing or not containing condensed tannin or Bacitracin methylene disalicylate

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The increasing restriction on the use of antimicrobial growth promoters in poultry production, driven by concerns about antimicrobial resistance and consumer demand for safer foods, has encouraged the search for natural feed additives capable of maintaining bird performance and intestinal health. Among the alternatives investigated, condensed tannins are plant-derived phenolic compounds with antimicrobial, antioxidant, anti-inflammatory, and gut microbiota-modulating properties. In this context, condensed tannin extracted from black wattle (*Acacia mearnsii*) has been investigated as a potential alternative to antibiotic growth promoters. Therefore, this study evaluated whether dietary supplementation with *Acacia mearnsii* condensed tannin affects performance, organ development, and intestinal morphometry during the rearing phase of laying hens. A completely randomized design was used, comprising four treatments, eight replicates, and 14 birds per experimental unit. Treatments consisted of a control diet, a control diet supplemented with bacitracin methylene disalicylate, a diet containing 1 g/kg of condensed tannin from 7 to 15 weeks of age, and a diet containing the same supplementation from the first day of life. Diets were formulated to be isonutritive and isoenergetic, differing only in additive inclusion. Performance variables included body weight gain (BWG), daily feed intake (DFI), feed conversion ratio (FCR), and weight uniformity (UNI). At 15 weeks of age, 64 birds (16 per treatment; two per experimental unit), with body weight within $\pm 2\%$ of the treatment mean, were selected, sedated with propofol (1 mL/kg), euthanized by cervical dislocation, weighed, and necropsied. The thymus, spleen, bursa of Fabricius, liver, pancreas, heart, and gizzard were collected and weighed, and intestinal length was measured. Data were tested for normality and homogeneity of variances and analyzed by analysis of variance. No differences were detected among treatments for BWG, FI, FCR, organ weights, or intestinal length ($P > 0.05$). These findings indicate that dietary supplementation with *Acacia mearnsii* condensed tannin at 1 g/kg does not impair growth performance, organ development, or intestinal morphometry during the rearing phase, suggesting that it may represent a feasible natural alternative to antibiotic growth promoters.

Keywords: *Acacia mearnsii*; Antimicrobial agents ; laying hens; nutrition; growth promoter

286P Early intestinal colonization in broiler chickens

originating from floor eggs and from breeder hens at early and late production stages: effects on performance and intestinal morphometry during the phase from 1 to 28 days of age

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In poultry production, hatchlings acquire their gut microbiota from the hatchery environment. Eggs may arrive contaminated, especially floor eggs, increasing the risk of contaminating embryos, the hatchery, and newly hatched chicks. As breeder flock age increases, hens lay larger eggs with thinner shells and reduced cuticle quality, increasing trans-shell contamination risk. Spraying a competitive exclusion (CE) product on newly hatched chicks may support gastrointestinal development and neonatal colonization. This study evaluated the effects of early intestinal colonization in broilers hatched from nest or floor eggs and from breeder hens at the beginning or end of production on performance and intestinal morphometry from 1 to 28 days. A total of 768 one-day-old male chicks were housed in a completely randomized $2 \times 2 \times 2$ factorial design, considering breeder flock age, young or old; egg origin, nest or floor eggs; and CE spray, with or without application, totaling 8 treatments with 6 replicates of 16 birds per cage. The sprayed CE product consisted of anaerobic bacteria, including *Enterococcus* spp., *Lactobacillus* spp., and non-pathogenic coliform bacteria. Data were analyzed by ANOVA (GLM, SAS 2002). An effect of CE on ileal crypt depth was observed at 7 days and was associated with egg origin. Birds from floor eggs showed lower crypt depth than those from nest eggs and birds not receiving CE. When chicks received CE spray, crypt depth was similar regardless of egg origin. At the end of the first week, a three-way interaction was observed for feed intake. Birds from older breeders and floor eggs, when sprayed with CE after hatch, showed lower feed intake than birds from nest eggs; however, the opposite response occurred without CE. Higher feed intake did not result in greater weight gain. Progeny from older breeders showed higher weight gain than progeny from young breeders up to the third week. The results indicate that CE acted selectively during the initial phase, modulating intestinal morphometry and feed intake according to breeder age and egg origin. This period is critical for microbiota establishment, intestinal maturation, and early development. The observed response suggests that CE may be strategic to favor early colonization by beneficial microorganisms, especially in chicks exposed to greater microbial pressure, such as those from late-production breeders and floor eggs. Thus, hatchery CE application may help direct neonatal microbiota and support early intestinal adaptation under challenging conditions.

Keywords: Breeder age; Broiler chickens; Competitive exclusion; Floor eggs; Gut microbiota

287P Effects of probiotic *Bacillus* strains on growth performance, carcass yield and commercial cut yield of broiler chickens reared under sanitary challenge conditions

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Probiotics, live microorganisms that confer health benefits to the host, represent a promising strategy for modulating the gut microbiome, excluding pathogens, strengthening the gut barrier, and regulating immune responses, thereby improving animal performance

and reducing disease susceptibility. *Bacillus* probiotics have been reported to positively influence poultry microbiomes by increasing beneficial genera, enhancing butyrate production, and modulating anti-inflammatory functions, improving gut homeostasis during enteric infections. This study evaluated the effects of probiotic *Bacillus* strains on growth performance, carcass yield, and commercial cut yield of broiler chickens reared under sanitary challenge conditions. A total of 2,322 male chicks were allocated in a completely randomized design with six treatments and nine replicates of 43 birds each. Treatments consisted of an unchallenged control group (UC), a challenged control group (CC), and four challenged groups supplemented with *Bacillus* strains, *Bacillus subtilis* and *Bacillus velezensis*, at 250, 500, 750, or 1000 mg/kg, named C250, C500, C750, and C1000. Challenged groups were housed on reused and wet poultry litter. At 7 and 21 days of age, birds received an inoculum prepared from litter obtained from a commercial poultry house with a history of intestinal dysbiosis. Productive performance was evaluated weekly. At 43 days, 36 birds per treatment were euthanized to evaluate carcass yield and commercial meat cuts. Data were analyzed by ANOVA, and means were compared by Tukey test at 5% probability. Probiotic levels were analyzed using polynomial regression. At all evaluated ages, the UC group showed the highest productive performance indices, including body weight gain and feed intake, compared with the other groups ($P < 0.05$). However, considering the overall period from 1 to 42 days, recovery was observed in the C250 and C750 groups, which achieved body weight gain similar to that of the UC group. Regression analysis indicated a quadratic effect on performance parameters, with the best response among challenged groups observed at 500 mg/kg in weekly evaluations up to 35 days of age. Carcass yield did not differ between the UC and C500 groups ($P > 0.05$), whereas the other supplemented groups and the CC showed inferior results ($P < 0.05$). The tested probiotic strains did not influence feed conversion ratio, suggesting that their effect on body weight gain may be attributed to modulation of gut microbiome composition, diversity, and metabolites, improving poultry health and well-being under sanitary challenge conditions.

Keywords: gut health; dysbiosis; performance; microbiota

288P Alpha-monobutyryl supplementation improves productivity in cage-free and conventional laying hen systems

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Modern laying hens have high genetic potential for egg production; however, maintaining performance throughout the production cycle depends on intestinal health, feed efficiency, and the ability to respond to field challenges. At later production stages or under greater environmental and microbiological pressure, egg production and viability may decline, feed efficiency may worsen, and intestinal integrity may be impaired. In this context, butyric acid derivatives are used as nutritional tools to support gut health and performance. Alpha-monobutyryl, a glyceride of butyric acid, may contribute to mucosal integrity, microbiota modulation, epithelial barrier support, and nutrient utilization. Two field trials evaluated a butyric acid glyceride additive rich in alpha-monobutyryl in different laying hen production systems. In the first trial, the experimental period occurred from 52 to 59 weeks of age, using 21,588 Bovans White hens housed in vertical cages. In the second trial, the experimental period occurred from 46 to 57 weeks of age, using 13,650 Bovans Brown hens raised in a cage-free system. In both systems, hens received a production phase 2 diet, and the test group was supple-

mented with 500 g/ton of an alpha-monobutylin-based additive. The control group was defined using historical farm reference values, since these flocks had historically failed to reach satisfactory results compared with the expected standards for each strain under field challenge conditions. Egg production, flock viability, and feed intake per egg produced were evaluated based on the total number of housed hens. Data were analyzed using R, and one-way ANOVA was applied at a 5% significance level. Alpha-monobutylin supplementation significantly improved production rates in both systems, with increases of 8.2% in cage-free hens and 8.5% in hens housed in vertical cages. Flock viability improved by 2.6% and 3.7% in cage-free and vertical cage systems, respectively ($P < 0.05$). These responses were supported by reduced feed intake per egg produced, corresponding to 8.2% and 8.4% less feed per egg in cage-free and conventional systems, respectively. In conclusion, alpha-monobutylin supplementation under field challenge conditions improved egg production and flock viability and reduced feed intake per egg produced in both systems, indicating better productive performance and suggesting a positive effect on feed efficiency and intestinal quality in laying hens.

Keywords: alpha-monobutylin; laying hens; egg production; flock viability; feed efficiency

289P Performance of broiler chickens fed diets supplemented with organic acids and essential oil

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Restrictions on the use of antibiotic growth promoters in poultry production have increased the demand for feed additives capable of maintaining intestinal health and productive performance. In this context, organic acids and essential oils have attracted considerable attention due to their antimicrobial, antioxidant, and gut microbiota-modulating properties, which may act synergistically to improve broiler performance. Thus, the objective of the study was to evaluate the performance of broiler chickens fed diets supplemented with organic acids and essential oil, either individually or in combination. A total of 3780 Ross 308 broiler chicks were assigned to a completely randomized design with nine treatments, 12 replicates, and 35 birds per experimental unit. Treatments consisted of negative and positive controls (without and with enramycin, respectively) and different supplementation levels of organic acids (250 and 500 g/ton) and essential oil (15 and 30 g/ton), according to the birds' age. Feed intake, body weight, body weight gain, and feed conversion ratio were evaluated from 1 to 42 days of age. Data were analyzed by analysis of variance and planned orthogonal contrasts, considering $P \leq 0.05$. A significant treatment effect was observed on feed intake at 21 and 28 days of age. Birds supplemented with organic acids (OA), either alone or combined with essential oil (EO), showed lower feed intake than those in the negative control, with significant differences between the control and supplemented treatments from 1 to 21 days of age ($P < 0.0001$). Body weight was affected at 35 and 42 days of age ($P < 0.05$), with higher values in birds receiving OA compared with the negative control ($P \leq 0.020$). Body weight gain was significantly influenced at 28 days and during the period from 22 to 42 days of age, with superior performance in the supplemented treatments. Feed conversion ratio was significantly improved at 21 ($P < 0.0001$) and 28 days ($P = 0.016$), as well as during the periods from 1 to 21 and 1 to 28 days of age ($P < 0.0001$). Treatments

containing OA, either alone or combined with EO, resulted in greater feed efficiency than the negative control. Orthogonal contrast analysis confirmed that the main response occurred between the negative control and the supplemented treatments, demonstrating improved feed efficiency in birds receiving the additives ($P \leq 0.032$). Organic acids, either alone or in combination with essential oils, improved broiler performance, demonstrating their potential as effective alternatives to antibiotic growth promoters while maintaining productive efficiency.

Keywords: Broiler chickens; Essential oil; Feed additives; Organic acids; Zootechnical performance

290P In ovo spray of lactic acid bacteria and yeast: effects on hatchability and performance of broiler chickens

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With growing restrictions on antimicrobial growth promoters, probiotics delivered during incubation have been explored as a non-invasive strategy to support gut colonization by beneficial microorganisms before hatch. This study tested the hypothesis that spraying a lactic acid bacterium (*Lactiplantibacillus plantarum*) and a yeast (*Pichia kluyveri*) onto fertile broiler breeder eggs does not impair hatchability and improves post-hatch performance of broilers. Treatments were arranged in a 2x2 factorial design (with or without bacteria x with or without yeast) within a completely randomized design, totaling four treatments and four replicates. A total of 1,151 fertile Ross® eggs were distributed among eight commercial incubators, with one treatment assigned per incubator. Solutions were sprayed onto the eggshell on incubation days 0, 3, 7, 10, 14, and 18; control eggs received sterile phosphate-buffered saline. After hatching, chicks were sexed, weighed, and housed in mixed-sex pens (10 birds/pen; 3.3 birds per square meter), with two pens per replicate. Feed intake, weight gain, and feed conversion ratio were recorded at 7, 21, and 35 days of age. Hatchability was analyzed by a binomial generalized linear model with a logit link, and performance data by two-way ANOVA in a 2x2 factorial arrangement, with means compared by the SNK test ($P \leq 0.05$). Hatchability was not affected by treatment, ranging from 88.7 to 90.5% ($P = 0.90$). At 7 days, yeast supplementation reduced feed intake (96 vs. 100 g; $P = 0.02$), with no effect on weight gain or feed conversion. At 21 days, bacteria supplementation reduced feed intake (1,170 vs. 1,181 g; $P = 0.04$). At 35 days, bacteria supplementation increased weight gain (2,486 vs. 2,429 g; $P = 0.02$). No significant bacteria x yeast interaction was observed for any variable ($P > 0.05$). These results indicate that spraying lactic acid bacteria and/or yeast onto hatching eggs is a safe strategy that does not compromise hatchability and may positively influence post-hatch growth performance, with bacteria and yeast acting mainly through independent, additive effects. **Keywords:** Probiotic, In ovo, Spray, Hatchability, Broiler performance.

Keywords: Probiotic; In ovo; Spray; Broiler performance; Hatchability

291P Effect of phytogenic feed additive on Growth Performance, Nutrient Digestibility, Gut health, Cecal Microbiota, and Gas Emission in Broilers.

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Maintaining broiler growth performance and gut health while reducing reliance on antibiotics remains a major challenge in modern poultry production. In recent years, prebiotics, probiotics, and phytogenic feed additives have gained increasing attention as sustainable alternatives to antibiotics due to their antimicrobial, antioxidant, and digestive-enhancing properties. However, information regarding the effects of the phytogenic feed additive (OptimYST) on the overall performance of Ross 308 broilers remains limited and thus this study was conducted to evaluate the effects of OptimYST supplementation on growth performance, nutrient digestibility, gut health, and microbial populations in broilers. N=585 a day old, Ross 308 chicks (44.09 ± 0.55 g) were randomly assigned to 2 treatments, each with 13 replicates of 15 birds/ pen: Control (Basal diet), and a basal diet supplemented with 250 and 350 g per Ton OptimYST supplementation. Birds were fed a three-phase diet consisting of starter (day 1–7), grower (day 8–21), and finisher (day 22–42) phases, with ad libitum access to feed and water. Growth performance [body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR)] were measured at the end of each phase and for the overall experimental period. Nutrient digestibility [dry matter (DM), nitrogen (N), and energy (E)], gas emissions, and cecal microbiota were evaluated on day 42. Gastrointestinal pH (crop, proventriculus, and duodenum) was measured on days 14 and 35, while intestinal morphology, including villus height (VH), crypt depth (CD), and the VH:CD ratio, was assessed on day 14. Data were analyzed using the MIXED procedure of SAS, and treatment means were compared using Duncan's multiple range test. Differences were considered significant at $P < 0.05$, and trends at $P < 0.10$. Broilers fed diets supplemented with 350 g/ton OptimYST exhibited a linear increase ($P < 0.05$) in BW and BWG over the overall experimental period. Nitrogen digestibility was also linearly improved ($P < 0.05$) in birds receiving 350 g/ton OptimYST. Additionally, graded levels of OptimYST supplementation linearly increased ($P < 0.05$) cecal *Lactobacillus* populations and tended ($P < 0.10$) to reduce *Escherichia coli* counts at day 42. Furthermore, OptimYST supplementation significantly improved ($P < 0.05$) jejunal villus morphology, as evidenced by increased villus height and VH:CD ratio. In conclusion, OptimYST supplementation positively impacts growth performance and intestinal structure, potentially improving nutrient absorption, and jejunal integrity in broilers, proving OptimYST, as a viable option in modern poultry nutrition.

Keywords: Gut health, ; growth performance; nutrient digestibility

292P Fatty acid profile in broiler chicken meat fed different lipid sources

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The fatty acid profile of broiler chicken meat can be significantly altered by including different lipid sources in the diet. Nutritional manipulation can improve the nutritional quality of the meat and meet different market demands. This experiment was conducted with the aim of comparing the influence of different oils in the diet of broiler chickens on the fatty acid profile in the breast meat. Four hundred and thirty-two Cobb 500 chicks were distributed in a completely randomized design with three treatments with eight rep-

licates, with 18 birds/replicate. The treatments evaluated included degummed soybean oil, poultry by-product oil, and degummed corn oil, with a dietary inclusion level of 2%. Broilers were housed in 24 pens of 1.44 sq containing nipple drinkers and a tubular feeder, and reused rice husk bedding, in a climate-controlled shed with exhaust fans and evaporative cooling pads. Isonutritive diets were used for each phase: pre-starter (1-7 days); starter (8-21 days); grower (22-35 days); and finisher (36-42 days). At 42 days, 24 broilers (eight per treatment) were selected according to the average weight of the replicates and slaughtered by exsanguination after electronarcosis. The right breast portion from each broiler was used for complete fatty acid profiling. The data were subjected to analysis of variance and compared using Scott Knott's test ($p \leq 0.05$). The fatty acids found in the highest concentration in the meat were Oleic Acid, Palmitic Acid, and Linoleic Acid. Monounsaturated, polyunsaturated, unsaturated, and saturated fats, as well as omega-6 and omega-9 fatty acids were identified. For these, there was no statistical difference ($p > 0.05$) between the groups studied. Broilers fed with poultry by-product oil had higher concentrations of fatty acids in their breast meat: Pentadecanoic Acid ($p = 0.02$), 10-Pentadecenoic Acid ($p = 0.04$), Palmitoleic Acid ($p = 0.04$), Elaidic Acid ($p = 0.006$), Alpha Linolenic Acid (LNA) ($p = 0.02$), Heneicosanoic Acid ($p = 0.002$), cis-8,11,14-Eicosatrienoic Acid ($p = 0.03$), Tricosanoic Acid ($p = 0.02$), Trans Fats ($p = 0.005$) and Omega-3 ($p = 0.02$), when compared to broilers fed with degummed soybean oil and degummed corn oil. Concluding, the oil sources used did not show to interfere with the most relevant fatty acids found in the broiler breast meat samples.

Keywords: by-product; corn; lipids; oil; soybean

293P Mycotoxin monitoring in the major poultry feed ingredients in Brazil: Utilization of NIRS as a prediction tool

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Mycotoxin monitoring of feed ingredients is essential to reduce productive and economic losses in poultry production. This study assessed mycotoxin contamination of corn, soybean meal (SBM), and wheat bran marketed in different Brazilian regions using near-infrared spectroscopy (NIRS) during the first half of 2026 (January 1st - June 20th). Aflatoxin B1 (AFB1), fumonisins (FBs: FB1+FB2), deoxynivalenol (DON), and zearalenone (ZEN) were predicted using NIRS calibration curves previously developed with HPLC-MS/MS as the reference method. Correlation coefficients (R) between methodologies ranged from 0.84 to 0.88 and limits of quantification (in µg/kg) for AFB1, FB1, FB2, DON and ZEN were 5, 200, 200, 250, and 30, respectively. Samples were ground to 1 mm, spectra were acquired using Foss and Bruker instruments, and results were obtained through the *Plataforma Olimpo* system. A total of 47,874 predictions were performed in 13,714 samples. Corn samples were collected from the five Brazilian regions: South (n=6,702), Southeast (n=803), Midwest (n=353), Northeast (n=502), and North (n=287). SBM (n=3,684) also originated from the five regions, with most of the samples from the South (n=2,174) and Southeast (n=1,034). Most of wheat bran (n=1,383) originated from the South region. Descriptive statistics were performed using the software Statgraphics Centurion. FBs were the most prevalent mycotoxins in corn (84%), with an overall mean of $1,500 \pm 1,229$ mg/kg, followed by ZEN (47%, mean 28.1 ± 26.8 mg/kg), DON (21%, mean 76.7 ± 54.5 mg/kg), and AFB1 (17%, mean 1.41 ± 1.3 mg/kg). SBM had low contamination,

with ZEN being the most prevalent mycotoxin (15%, mean 6.86±6.5 mg/kg). ZEN and DON were detected in 83% (mean 55.9±31.1 mg/kg) and 86% (mean 754±441.9 mg/kg) of the wheat bran samples, respectively. Corn mycotoxin contamination differed among the five Brazilian regions. FBs prevalence ranged from 58% in the North (mean 1,149 mg/kg) to 93% in the Northeast (mean 2,482 mg/kg). ZEN contamination ranged from 6% (mean 3.02 mg/kg) in the North region to 54% (mean 31.3 mg/kg) in the South region. The Southeast and South regions had the highest DON means (131.3 and 77.5 mg/kg, respectively) and prevalences (28% and 23%), while the Midwest had the lowest results (3%, mean 11.4 mg/kg). AFB1 also differed among regions, from 2% prevalence (mean 0.23 mg/kg) in the Southeast to 21% (mean 1.92 mg/kg) in the North. Results observed across the five Brazilian regions highlight the need for special attention to FBs in corn, as well as to ZEN and DON in wheat bran. Therefore, NIRS represents a rapid and reliable tool for mycotoxin monitoring in poultry feed ingredients, improving mycotoxin control in the production chain.

Keywords: feed quality; mycotoxins; NIRS; poultry nutrition

294P Effects of different dietary oil sources on egg quality in laying hens

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The inclusion of lipid sources in the diet of laying hens plays a fundamental role in nutrition and productive performance. The choice of lipid sources can impact production outcomes, egg quality, and the profitability of production systems. In this context, the experiment was conducted to evaluate the effects of different lipid sources in the diet of laying hens on egg quality. Two hundred and fifty-six 85-week-old Lohmann LSL hens were assigned to a completely randomized design comprising four treatments (diet without oil, poultry viscera oil, degummed soybean oil, and degummed corn oil) with eight replicates of eight birds each, monitored over three 28-day cycles. The diets were isonutritive and isoenergetic, containing 2% oil. Feeding was performed twice daily (morning and afternoon), and water was provided *ad libitum*. The birds were monitored daily, and egg collection took place at a fixed time throughout the experiment. Egg quality was determined based on the assessment of eggshell strength (kgf), egg weight, egg density, pH and albumen height, yolk color, eggshell thickness, and Haugh Unit (HU). Data were subjected to Shapiro-Wilk and Levene tests and analysis of variance, with means compared using the Scott-Knott test ($p \leq 0.05$). It was observed that eggshell strength ($p = 0.02$) and eggshell thickness ($p < 0.001$) were greater for eggs from hens fed poultry viscera oil and those on the oil-free diet. Egg weight, egg density, pH, albumen height, yolk color, and Haugh units were statistically similar among the treatments evaluated ($p > 0.05$). It is concluded that the inclusion of different lipid sources in the diet did not alter the internal quality of the eggs. However, the use of poultry viscera oil and the oil-free diet resulted in greater eggshell thickness and strength, indicating a positive effect of these strategies on external egg quality.

Keywords: corn oil; poultry viscera oil; soybean oil; thickness

295P The role of nutrition and environmental enrichment on meat quality and mitigation of pectoral myopathies in broiler

chickens

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Pectoral myopathies White Striping (WS) and Wooden Breast (WB) are muscle disorders that compromise meat quality in high-yield broilers, resulting in significant economic losses. Under pressure to reduce performance-enhancing antibiotics, alternative strategies such as probiotics and environmental enrichment have been investigated, aligned with the One Health concept. We hypothesized that environmental enrichment associated with probiotic supplementation would improve breast meat quality and reduce the incidence of pectoral myopathies in broilers at 42 days of age. A total of 1,440 male Ross TM4 broiler chickens were distributed in a completely randomized design in a 3 × 2 factorial arrangement, consisting of three additive programs (control without additive, antibiotic growth promoter based on Zinc Bacitracin, and probiotic containing *Bacillus*, *Bifidobacterium*, *Enterococcus*, and *Lactobacillus* strains) and two environments (with and without environmental enrichment), totaling six treatments with eight replicates of 30 birds each. Environmental enrichment consisted of perches, ramps, and objects such as balls, mirrors, and hay to stimulate natural behaviors and reduce chronic stress. At 42 days of age, 144 birds were slaughtered for breast meat (*Pectoralis major*) quality evaluation, determining final pH, color ($L^* a^* b^*$), cooking loss, shear force, and water-holding capacity. Incidence and severity of WS and WB were assessed by visual score. Data were subjected to analysis of variance (two-way ANOVA) using the General Linear Model procedure, and means were compared by Tukey's test at 5% significance. No significant interaction was found between factors for most meat quality variables. Physicochemical parameters (pH, lightness, redness, yellowness, cooking loss, shear force, and water-holding capacity) showed no significant differences among treatments. However, environmental enrichment significantly reduced ($P < 0.05$) the occurrence of White Striping in the *Pectoralis major* muscle, regardless of the additive program, suggesting that improved welfare through environmental complexity may attenuate oxidative and metabolic stress associated with this myopathy. Probiotic supplementation did not potentiate this effect, and Wooden Breast incidence was not influenced by any treatment. It is concluded that environmental enrichment is a low-cost management tool for mitigating White Striping in broilers with no negative effects on technological meat quality, representing a strategy aligned with animal welfare, sustainable production, and One Health principles.

Keywords: Animal welfare; Meat quality; One Health; : White Striping; Wooden Breast

296P EVALUATION OF THE USE OF OILS WITH DIFFERENT LIPID PROFILES IN THE DIET OF BROILER CHICKENS

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The present study was conducted to compare the effects of differ-

ent dietary oil sources on lipid metabolism in broiler chickens. The experiment was carried out at the Experimental Poultry Facilities of the Universidade Federal de Goiás. A total of 432 male Cobb 500 broilers were assigned to a completely randomized design consisting of three treatments, eight replicates, and 18 birds per replicate, totaling 24 experimental units. Birds were housed in a conventional poultry house in 1.42 m² pens. Treatments consisted of diets supplemented with poultry fat, degummed soybean oil, or degummed corn oil. All diets were formulated to be isonutritive and to meet the nutritional requirements of broilers during the pre-starter (1–7 d), starter (8–21 d), grower (22–35 d), and finisher (36–42 d) phases. At 20 and 40 days of age, 4 mL blood samples were collected from the ulnar vein of five birds per treatment and transferred to vacuum tubes without anticoagulant and containing separator gel for biochemical analyses. The biochemical profile included the determination of high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total cholesterol, and triglycerides. Data were subjected to analysis of variance (ANOVA), and means were compared using the Scott-Knott test at a significance level of $p < 0.05$. At 20 days of age, significant differences were observed for cholesterol, triglyceride, and VLDL concentrations ($p < 0.05$). Birds fed the poultry fat diet exhibited higher values, whereas those receiving degummed soybean oil and degummed corn oil did not differ statistically. At 40 days of age, only LDL concentrations differed significantly among treatments ($p < 0.05$), with poultry fat and degummed corn oil resulting in similar and higher values than the degummed soybean oil treatment. It was concluded that, during the starter phase, the poultry fat diet promoted greater transport of cholesterol and triglycerides to extrahepatic tissues due to increased VLDL concentrations, which was also reflected in higher circulating cholesterol and triglyceride levels. During the finisher phase, the poultry fat and degummed corn oil treatments indicated greater cholesterol transport to peripheral tissues, as evidenced by the elevated LDL concentrations.

Keywords: Corn oil; lipid metabolism indices; poultry offal fat; soybean oil

297P Effects of sesame seed (*Sesamum indicus*) and phytase in diets of laying hens on stored-egg quality

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Sesame seed is an ingredient with phytate, oxalate, and tannin in its composition, but it is also rich in lignans (sesamin, sesamol, sesaminol, and sesamol), which are polyphenolic compounds known to confer beneficial effects, including antioxidant and antibacterial properties; however, there are few studies that have evaluated the potential effects on egg quality during storage. The objective of this study was to evaluate the effects of feeding laying hens sesame seed and phytase on egg quality during storage. A total of 210, 51-week-old Hy-Line W-80 laying hens were assigned to five dietary treatments with seven replicates having six hens each, in a 2×2+1 factorial arrangement using a completely randomized design, in a 56-day trial. The birds were raised in galvanized wire cages, receiving water and feed *ad libitum*. Dietary treatments consisted of two levels of sesame seeds (SS) inclusion (1% and 3%), with or without

phytase (0 and 50 g/ton), plus a Control diet group (CON) based on corn and soybean meal. The diets were formulated to meet the nutritional requirements. In the last week of the trial, 60 eggs from each treatment were collected, and half were stored in a refrigerated environment (average of 4°C) and the other half in a non-refrigerated environment (22–34°C), for egg quality analysis on days 0, 7, 14, 21, and 28 of storage. On each of the evaluation days, 12 eggs from each treatment were used, 6 from each environment, for analysis of egg breaking force, relative egg weight loss (REWL), average egg weight, specific gravity of the eggs, Haugh Unit, yolk index, percentage egg components, yolk color (L*, a*, and b*), albumen pH and shell thickness. All data were subjected to analysis of variance, and the means were subjected to Tukey test for differences ($P < 0.05$) or tendency ($P > 0.05$ and < 0.1). The Dunnett test was used to compare the means of the other treatments with the control treatment. On day 14, higher specific gravity (1.083 vs 1.078, $P = 0.007$), lower albumen pH (9.68 vs 9.77, $P < 0.01$), tendency for higher yolk (34.15 vs 30.83%, $P = 0.06$), day 21, higher color a* (-3.52 vs -3.06, $P = 0.02$), and on day 28, lower egg breaking force (4.17 vs 4.78 kg, $P = 0.001$), were observed for phytase factor (without and with, respectively). On day 7, higher egg breaking force (5.18 vs 4.34 kg, $P = 0.02$), and on day 28, lower REWL (2.92 vs 3.25%, $P = 0.02$), were observed for SS level (3 and 1%, respectively). No differences were observed for the other parameters. In conclusion, the SS increased the breaking resistance and reduced the weight loss of eggs during storage, while phytase inclusion increased specific gravity and reduced albumen pH and egg resistance during storage.

Keywords: alternative ingredients; antioxidants; functional ingredients; poultry

298P Digestible nutrients and metabolizable energy content of sesame seed (*Sesamum indicum*) for laying hens

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Sesame seeds are produced around the world mainly for oil extraction, but they are also rich in other nutrients such as fatty acids, amino acids, carbohydrates, minerals, and vitamins. Furthermore, it is an ingredient rich in lignans (sesamin, sesamol, sesaminol, and sesamol), which are polyphenolic compounds known to confer beneficial health effects, including antioxidant and antibacterial properties; however, few studies have evaluated its nutritional value for laying hens. The objective of this study was to determine the chemical composition and digestible and energy content of sesame seed for laying hens. 80 laying hens (Hy-Line W-80) (33-week-old) were housed in galvanized wire metabolic cages (89 x 60 x 53 cm) in groups of five hens per cage (experimental unit), distributed in a completely randomized design with two treatments and eight replicates, totaling 16 cages. The treatments evaluated were a reference diet formulated with corn and soybean meal to meet the nutritional requirements of the animals according to the lineage manual, and a test diet composed of 70% reference diet and 30% sesame seeds. To determine the chemical composition of the sesame seeds, analyses of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash were performed. Gross energy (GE) values were obtained using an adiabatic calorimeter. For nutrient digestibility analysis, the experi-

mental period lasted 10 days (five days for adaptation and five days for total excreta collection). During this period, the birds received feed and water *ad libitum*. Ferric oxide (2%) was used in the diets as a marker for the beginning and end of the excreta collection period. The excreta were collected per experimental unit (cage), thawed, homogenized, weighed, and dried. Laboratory analyses of DM, CP, EE, NDF, ADF, ash, and GE of the excreta and diets were then performed. The values of apparent metabolizable energy (AME) and AME corrected for nitrogen balance (AMEn) of sesame seeds were determined. The DM, ash, CP, EE, NDF, ADF, and GE of sesame seeds were 95.92, 5.15, 26.51, 44.39, 49.98, 24.41%, and 7,056.07 kcal/kg, respectively. The digestible content of DM, ash, CP, EE, NDF, and ADF were 58.11, 1.98, 5.21, 46.51, 28.28, and 19.83%, respectively, while the AME and AMEn were 5,408.41 and 5,056.60, respectively. In conclusion, according to the digestible nutrients and AME and AMEn content, the sesame seed can be considered as an ingredient in laying hens' diets.

Keywords: alternative ingredients; digestibility; functional ingredients; metabolizability

299P Effects of Dietary Crude Protein Levels on Relative Organ Weights in Growing Guinea Fowl (*Numida meleagris*)

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Information on the growth and physiological responses of guinea fowl (*Numida meleagris*) to dietary protein levels remains limited, particularly during the growing phase. This study aimed to evaluate body weight and relative organ weights of guinea fowl fed diets containing different crude protein (CP) levels from 28 to 56 days of age. A total of 200 mixed sex guinea fowl were housed in 20 pens (2.65 m² each) equipped with feeders and drinkers. Birds were distributed in a completely randomized design with four dietary treatments (16.5, 18.0, 19.5, and 21.0% CP), five replicates, and 10 birds per experimental unit. Diets were formulated to meet the nutritional requirements of the birds, except for crude protein and amino acids, which varied according to the dietary CP levels tested. At 56 days of age, birds were weighed to determine average body weight, and two birds per experimental unit, representative of the average body weight of the pen, were selected, slaughtered, and eviscerated for determination of the relative weights of the liver, gizzard, pancreas, heart, and abdominal fat. Data were subjected to analysis of variance and polynomial regression using SAS OnDemand for Academics. Average body weight increased linearly with increasing dietary CP levels ($BW = 0.221 + 0.049CP$). Dietary CP levels had no significant effect on the relative weights of the heart or abdominal fat, however, linear decreases were observed in the relative weights of the liver ($LIV = 3.306 - 0.0092CP$), gizzard ($GIZ = 6.415 - 0.2196CP$), and pancreas ($PNC = 0.392 - 0.011CP$). The increase in body weight indicates that higher dietary protein levels favored growth during this phase, whereas the concomitant reductions in the relative weights of the liver, gizzard, and pancreas may

be partly associated with a dilution effect resulting from increased body weight rather than an actual reduction in absolute organ size. These findings highlight the importance of considering body growth when interpreting relative organ weights in response to dietary treatments. In conclusion, increasing dietary CP levels from 16.5 to 21.0% promoted greater body weight while reducing the relative weights of the liver, gizzard, and pancreas, without affecting heart or abdominal fat relative weights in guinea fowl at 56 days of age.

Keywords: *Numida meleagris*; Protein; guinea fowl; Birds; body weight

300P Carcass and Cut Yields of Broiler Chickens Challenged with *Eimeria maxima* and Fed *Hermetia illucens* Larvae Oil

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Hermetia illucens larvae oil (HILO) has been investigated as a functional feed additive for broiler chickens subjected to enteric challenges. This study evaluated the effects of HILO on carcass, cut, and organ yields of broilers challenged with *Eimeria maxima*. A total of 540 one-day-old male Ross broiler chicks were housed in 2.6-m² pens. The experiment was conducted in a completely randomized design with five treatments. The nonchallenged control had four replicates of 15 birds, whereas each challenged treatment had eight replicates of 15 birds, totaling 36 experimental units. All procedures were approved by the Animal Use Ethics Committee, protocol no. 811/2023. Treatments were: nonchallenged control; challenged control, with birds inoculated with *E. maxima* and fed a control diet containing an antibiotic (Halquinol) and an antioxidant (BHT), without HILO; challenged birds fed an antibiotic- and antioxidant-free diet (AAFD), without HILO; challenged AAFD + 0.5% HILO; and challenged AAFD + 1.0% HILO. At 17 days of age, challenged birds were inoculated with *E. maxima* by oral gavage at 10⁷ oocysts/bird. At 35 days of age, two birds per experimental unit were slaughtered to evaluate carcass, breast, drumstick, thigh, wing, wing drumette, abdominal fat, gizzard, liver, heart, and bursa of Fabricius yields. Data were subjected to analysis of variance using the GLM procedure of SAS, and means were compared by the Student-Newman-Keuls test at 5% probability. Treatments did not affect breast, drumstick, thigh, wing, gizzard, liver, abdominal fat, or bursa of Fabricius yields ($P > 0.05$). However, treatments affected wing drumette yield ($P = 0.0487$) and heart yield ($P = 0.0142$). For wing drumette yield, challenged birds fed AAFD + 1.0% HILO showed greater values than the challenged control, whereas the other treatments had intermediate values. For heart yield, challenged birds fed AAFD + 1.0% HILO showed greater values than challenged birds fed AAFD + 0.5% HILO, whereas the other treatments had intermediate values. In conclusion, HILO had a limited effect on carcass, cut, and organ yields, with punctual changes only in wing drumette and heart yields, without consistent improvement in carcass yield of challenged broilers.

Keywords: Broiler Chickens; Carcass Yield; Coccidiosis; *Eimeria maxima*; *Hermetia illucens* larvae oil

301P Effects of Dietary Crude Protein Levels on Growth Performance of Guinea Fowl (*Numida meleagris*) from 28 to 45

Days of Age

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Information on the protein requirements of guinea fowl (*Numida meleagris*) during the growing phase remains limited, particularly under intensive production conditions. This study aimed to evaluate the growth performance of guinea fowl fed diets containing different crude protein (CP) levels from 28 to 45 days of age. A total of 200 mixed sex guinea fowl with an initial body weight of 352 ± 6.57 g were housed in 20 pens (2.65 sqm each) equipped with feeders and drinkers. Birds were distributed in a completely randomized design with four dietary treatments (16.5, 18.0, 19.5, and 21.0% CP), five replicates, and 10 birds per experimental unit. Diets were formulated to meet the nutritional requirements of the birds, except for crude protein and amino acids, which varied according to the dietary CP levels tested. At 45 days of age, birds were weighed, and feed intake was recorded to determine body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Data were analyzed using analysis of variance and polynomial regression in SAS OnDemand for Academics. Body weight and body weight gain increased linearly with increasing dietary CP levels ($BW = 0.256 + 0.013CP$; $BWG = -0.068 + 0.012CP$). Dietary CP levels had no significant effect on feed intake or feed conversion ratio. The linear increases in BW and BWG indicate that protein supply was a limiting factor for growth within the dietary CP range evaluated, with birds benefiting from increasing CP levels up to 21.0%. The absence of effects on FI and FCR suggests that the improvement in growth occurred without significant changes in feed consumption or overall feed efficiency. In conclusion, increasing dietary CP levels from 16.5 to 21.0% improved body weight and body weight gain of guinea fowl from 28 to 45 days of age, indicating that at least 21.0% CP is required to maximize growth performance within the range evaluated.

Keywords: Alternative poultry; *Numida meleagris*; Dietary protein; Poultry nutrition; Weight gain

302P Dietary Crude Protein Levels Affect Serum Biochemical Parameters in Growing Guinea Fowl (*Numida meleagris*)

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Guinea fowl (*Numida meleagris*) production has gained interest as an alternative poultry system; however, information on their physiological responses to dietary protein levels remains limited. This study aimed to evaluate serum biochemical parameters in guinea fowl fed diets containing different crude protein (CP) levels from 28 to 56 days of age. A total of 200 mixed-sex guinea fowl were dis-

tributed in a completely randomized design with four dietary treatments (16.5, 18.0, 19.5, and 21.0% CP), five replicates, and 10 birds per experimental unit. Diets were formulated to meet the nutritional requirements of the birds, except for crude protein and amino acids, which varied according to the CP levels tested. At 56 days of age, one bird per experimental unit, representative of the average pen body weight, was selected for blood collection. Blood was collected from the wing vein, centrifuged at $400 \times g$ for 10 min at 4 °C, and serum was stored at -20 °C until analysis. Serum total protein (TP) and uric acid (UA) concentrations were determined using biuret and enzymatic methods, respectively. Data were subjected to analysis of variance and polynomial regression using SAS OnDemand for Academics. Dietary CP levels did not affect serum UA concentrations; however, a quadratic effect was observed for TP ($TP = 8.004 + 1.003CP - 0.026CP^2$), with a maximum predicted concentration at 19.28% CP. The quadratic response of TP indicates an optimal dietary protein level for circulating protein concentrations, while the unchanged UA suggests relatively stable protein catabolism across treatments. In conclusion, dietary CP influenced serum TP but not UA concentrations in growing guinea fowl, with 19.28% CP maximizing the predicted serum TP concentration.

Keywords: *Numida meleagris*; Crude protein; Serum biochemistry; Serum total protein; Uric acid

303P "Effect of Three Dietary Energy Densities on the Performance and Egg Quality of Laying Hens"

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Hypothesis – Objectives: The metabolizable energy in the diet is a major factor influencing the productive performance and feed cost of laying hens. It is hypothesized that modern laying hens adapt to and respond to diets with low ME density, and that this is a strategy to improve feed cost without affecting productive performance and egg quality. The objective was to evaluate three ME densities in the diet on the performance, egg quality, and feed cost of laying hens. **Experimental Design:** Seventy-five 30-week-old HISEX Brown laying hens were used, distributed in a randomized block design. The ME levels were 2900, 2800, and 2700 kcal ME/kg, with 5 replicates each. **Materials and methods:** This study was conducted in the Nutrition Research and Development Laboratory – FMVZ – UNICA. Seventy-five 30-week-old HISEX Brown laying hens were randomly selected. An egg analyzer (Digital Egg Tester-6500) and a Mitutoyo micrometer were used. The following variables were evaluated: feed intake, feed conversion ratio, egg production, egg weight and mass, egg breakage resistance, Haugh unit, and yolk index. **Statistical analysis:** The quantitative (parametric) data were statistically analyzed using a one-way analysis of variance (ANOVA) and Tukey's test for mean comparisons. Non-parametric data were analyzed using the Kruskal-Wallis test, using the GLM procedure of the SAS software (SAS Inst. Inc., Cary, NC, 2024, v. 9.4). Statistical significance and trends will be considered at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively. **Results:** The different energy densities in the diet did not have a significant effect ($P > 0.05$) on feed intake (130, 127 and 133 g/d), feed conversion ratio (2.24, 2.13 and 2.24 g/g), egg production (95, 97 and 96%), egg weight (60.5, 61.7 and 62 g/egg) and egg mass (58.1, 60 and 59.7 g/day), Haugh unit (97, 93 and 93.5), yolk index (0.41, 0.39 and 0.43) and shell breaking strength (5.8, 5.3 and 4.9 KgF). The lowest feed cost and highest profit margin were achieved with the diet with the lowest metabolizable energy (ME) density. **Conclusions:** Feed intake, egg production, feed conversion

ratio, egg weight, and egg mass were not significantly affected by diets with different energy densities. The yolk index, Haugh unit, and shell break strength were not significantly affected by diets with different energy levels. The lowest feed cost, highest profit margin, and highest economic return were obtained with the diet with the lowest energy level (2700 kcal ME/kcal).

Keywords: metabolizable energy; diet; laying hens; egg; cost

304P National Animal Nutrition Program (NANP): Databases, Modeling Resources, and Training for Poultry Nutrition Research

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The tenth revised edition of Nutrient Requirements of Poultry from the National Academies of Sciences, Engineering, and Medicine (NASEM) recommended the development of dynamic computer models to predict nutrient requirements for diverse genetic potentials, environments, production systems, and marketing goals. The committee concluded that modeling provides an efficient framework for integrating empirical data and keeping pace with changes in poultry genetics and management systems. This research direction creates new opportunities for experimental design and data management and highlights the need for training in modeling techniques. The National Animal Nutrition Program (NANP; NRSP-9) addresses this need by providing curated, research-based databases, modeling resources, and training that enable model development, parameterization, evaluation, and benchmarking. Core assets include a feed ingredient composition database with >4 million records that is operational and publicly accessible. Recent infrastructure modernization includes website restructuring, Shiny-based decision tools, evaluation of alternative hosting platforms to reduce long-term costs, and hybrid R/Python workflows to enhance interoperability and reproducibility. Updated information about research methodologies to determine net energy and nutrient requirements for poultry, and poultry modeling techniques has been added to the NANP resource libraries. The NANP website now features examples of poultry models developed for broilers, layers, and turkeys helping establish it as a hub for advances in poultry nutrition modeling. A major strategic advancement has been the development of a NANP Large Language Model (LLM) system using Retrieval-Augmented Generation (RAG) to support structured knowledge synthesis while respecting copyright and licensing constraints. Deployment options include public GPU-hosted servers and stand-alone local installations to balance cost and accessibility for broad adoption. These initiatives align with efforts in Bayesian modeling, AI-driven decision science, digital twins, and sustainability modeling. Training and workforce development remain priorities, with modular modeling education, symposium integration, and certification concepts under

development. Collectively, NANP strengthens modeling infrastructure, enhances reproducibility, supports corrective software stewardship, and advances AI-integrated decision support for poultry nutrition research and practice.

Keywords: Poultry ; Nutrition modeling; Database; Reproducibility; Training

305P Antioxidant Status and Mineral excretion of Laying Hens fed with Differing Dietary Copper during Early Production

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Copper (Cu) is an essential micromineral involved in several physiological functions in laying hens, particularly antioxidant defense. Compared with copper sulfate (CuSO₄), copper proteinate (CuP) has greater bioavailability and may improve antioxidant status while reducing mineral excretion. This study was conducted to evaluate the antioxidant status and mineral excretion of laying hens fed with differing Cu source (inorganic vs. organic) during different phases. A total of 480 white laying hens at 20 weeks of age were randomly distributed in 4 treatments (2 × 2 factorial structure), with 15 replicates of 8 birds per experimental unit. The experimental factors consisted of Cu supplementation sources during the growing and early laying phases. In each phase, birds received either 100% of the recommended Cu requirement from CuSO₄ or 50% of the requirement using CuP (Bioplex Copper, Alltech Inc.). Diets were formulated according to the nutritional requirements of laying hens from 20 to 33 weeks of age (Brazilian Table, 2024). At 33 weeks of age, lipid peroxidation and antioxidant activity were evaluated in blood, yolk, and liver samples through TBARs and DPPH analyses, respectively. In the same period, the dry matter (DM), mineral matter (MM) and mineral concentrations of Cu, iron (Fe), and zinc (Zn) in excreta samples were quantified. Data were analyzed by ANOVA and means were compared using Tukey's test with SAS at a significance level of 5%. The MDA concentration of yolk from layers fed diet with CuP in growing phase was lower (P<0.05) than that from layers fed diet supplemented with CuSO₄. The MDA concentration in the liver of layers fed the diet supplemented with CuP in early laying phase was lower (P<0.05) than that from layers fed diet supplemented with CuSO₄. No significant main or interactive effect of Cu source and feeding phase were found for blood TBARs values, the DPPH concentration in the liver, blood or egg yolk. The DM and MM of excreta did not differ with dietary treatment. The Cu concentration in the excreta of layers fed diet supplemented with CuP during the early laying phase was lower (P<0.01) compared with that from layers fed diet with CuSO₄. The Zn concentration in the excreta of layers fed diet with CuP in growing phase was lower (p<0.05) than that from layers fed diet with CuSO₄. The excreta from hens fed diet with CuSO₄ during both periods had higher (P<0.05) Fe concentration than that from layers fed diet with CuP. In conclusion, supplementation with CuP at 50% of the recommended Cu level resulted in lower lipid peroxidation and mineral excretion compared with CuS, indicating greater Cu utilization efficiency and supporting its use as a sustainable Cu source for laying hens.

Keywords: Copper proteinate; mineral excretion; Lipid peroxidation; DPPH

306P Effect of replacing monocalcium phosphate with calcium humophosphate on growth performance and tibia mineralization of broiler chickens from 0 to 42 days

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Phosphorus (P) and calcium (Ca) are essential for growth and bone mineralization in broiler chickens. The antinutritional effect of Ca must be controlled to optimize intestinal P absorption. In plant-based diets, P is mainly in form with phytate, whose availability may be limited by interactions with Ca. Calcium humophosphate, an organo-mineral source of P and Ca, could interact with Ca and limit its recomplexation with phytate phosphorus, supporting dietary P utilization. The objective was to evaluate the replacement of monocalcium phosphate (MCP; total P = 22%; 90.3% P availability coefficient) with calcium humophosphate (total P = 22%; 113% P availability coefficient) on growth performance and tibia mineralization. A total of 400 Cobb500 broilers were allocated to 2 treatments (5 pens/treatment, 40 birds/pen). Corn-soybean meal-based diets were formulated according to Cobb 2022 guidelines, with phytase included at 1,500 FTU/kg. Body weight, feed intake, feed conversion ratio, and tibia ash, Ca, and P contents were evaluated at 42 days. Data were analyzed using RStudio (version 4.3.2); after assessing normality and homogeneity of variance, treatments were compared using the Mann-Whitney test. At 42 days, growth performance did not differ significantly between treatments ($P > 0.05$), with final body weight of 2,871.70 and 2,909.62 g and adjusted FCR of 1.67 and 1.68 for MCP and CHP, respectively. Tibia ash, Ca, and P contents also did not differ significantly ($P > 0.05$), with values of 0.427, 0.141, and 0.121 g/g for MCP, and 0.426, 0.147, and 0.117 g/g for CHP, respectively. In conclusion, calcium humophosphate maintained growth performances and tibia mineralization, reducing phosphate inclusion by 18% compared with MCP throughout the production cycle, and supporting precision formulation with more efficient use of mineral resources.

Keywords: Broiler; Phosphorus; Calcium Humophosphate; Phosphates; Precision Nutrition

307P L-pyrogglutamic acid calcium salt improves eggshell quality in broiler breeders

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Eggshell quality in broiler breeders declines substantially after 60 weeks of age, increasing defective eggs, compromising embryonic viability, hatchery losses. It was hypothesized that L-pyrogglutamic acid calcium salt, an organic source with high bioavailability, would improve shell mineralization and stimulate proline and glycine synthesis in the organic matrix, even with reduced sulfur amino acids and under heat stress. The objective was to evaluate levels of L-pyrogglutamic acid calcium salt using on performance and egg quality of broiler breeders hens. The experiment was conducted as a completely randomized design with a 3 × 4 factorial arrangement

of treatments, comprising three basal diets [standard (BD1), reduced Met+Cys (BD2), and reduced Met+Cys+Thr (BD3)] and four supplementation levels of L-pyrogglutamic acid calcium salt (0, 0.5, 1.0, and 1.5 kg/tonn), resulting in 12 treatment combinations. Each treatment was replicated 12 times, totaling 144 individually caged broiler breeders (72 Ross and 72 Hubbard). Broiler breeders entered the experiment at 60 weeks of age, and experimental units were standardized for body weight, egg production, and egg weight before treatment allocation. The experiment lasted 16 weeks. Three isoenergetic (2,850 kcal ME/kg), isocalcium (3.6% Ca) and isophosphoric basal diets were formulated according to the guideline. Calcium L-pidolate® was used as the source of L-pyrogglutamic acid calcium salt and was included in the diets at the expense of the inert ingredient. Hens were provided with a controlled amount of feed (160 g/day), whereas water was available ad libitum. A 16L:8D photo period was maintained. The analyzed variables were feed intake, egg production, egg weight, egg mass, feed conversion ratio, egg component weights, Haugh unit, eggshell thickness, and specific gravity. Data was analyzed by PROC GLM of SAS 9.4 (Levene's test, factorial ANOVA and F test, $P < 0.05$). The effects of L-pyrogglutamic acid calcium salt were consistent across the three basal diets, with no diet × level interaction for most variables. The level did not affect feed intake (151 g/hen per day; $P > 0.05$). However, inclusion of 1.5 kg/tonn improved performance and eggshell quality ($P < 0.05$), increasing egg production by 8.18 percentage points (63.23% vs. 55.05%; $P = 0.0068$) and improving feed conversion ratio (2.446 vs. 3.091; $P < 0.0001$). This level also increased shell weight (6.02 vs. 5.29 g), shell thickness (0.351 vs. 0.323 mm), and specific gravity (6.818 vs. 6.000) ($P < 0.05$). Dietary inclusion of 1.5 kg/tonn of L-pyrogglutamic acid calcium salt improved productive performance and eggshell quality in late-cycle broiler breeders, mitigating age-related eggshell deterioration.

Keywords: broiler breeders; eggshell quality; calcium bioavailability

308P In Vitro Antimicrobial Activity of Ethanolic Extract from Jaboticaba (*Myrciaria cauliflora*) Peels and Seeds Against *Salmonella enterica* Serovars

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Objective: To evaluate the *in vitro* antimicrobial activity of an ethanolic extract obtained from jaboticaba (*Myrciaria cauliflora*) peels and seeds against different *Salmonella enterica* serovars. **Experimental design:** Completely descriptive *in vitro* study using broth microdilution to determine the minimum inhibitory concentration (MIC) of the extract and ellagic acid standard. **Materials and methods:** The ethanolic extract was produced from dried jaboticaba peels and seeds, a by-product of fermented jaboticaba production. The extract was obtained by hydroalcoholic percolation (45% ethanol) and previously characterized for its phenolic composition. *Salmonella enterica* ATCC 00150, *Salmonella* Typhi ATCC 10749, *Salmonella* Heidelberg, and *Salmonella* Typhimurium were standardized to 10⁷ CFU/mL. Antimicrobial activity was assessed by broth microdilution using extract and ellagic acid concentrations ranging from 2,000 to 1.95 µg/mL. Bacterial growth was detected by triphenyltetrazolium chloride reduction. **Statistical analysis:** Results were analyzed descriptively based on the presence or absence of bacterial growth to determine the MIC. **Results:** Culture medium and dimethyl sulfoxide showed no antimicrobial effect. The extract inhibited *Salmonella* Heidelberg and *Salmonella* Typhimurium at 1,000 µg/mL, indicating weak antimicrobial activity. *Salmonella enterica* and *Sal-*

monella Typhi were inhibited only at 2,000 µg/mL, which is considered inactive. Ellagic acid showed similar activity against *Salmonella* Typhimurium but failed to inhibit *Salmonella* Heidelberg at 1,000 µg/mL, suggesting that the extract activity may result from synergistic interactions among phytochemicals rather than ellagic acid alone. **Conclusions:** The ethanolic extract of jabuticaba peels and seeds exhibited weak *in vitro* antimicrobial activity against *Salmonella* Heidelberg and *Salmonella* Typhimurium and no relevant activity against *Salmonella enterica* or *Salmonella* Typhi. The findings suggest that antimicrobial activity is likely associated with the combined action of multiple phytochemicals present in the extract.

Keywords: antimicrobial activity; ellagic acid; minimum inhibitory concentration; microdilution; phytochemicals

309P BOTULISM OUTBREAK IN BACKYARD POULTRY ASSOCIATED WITH INADEQUATE SANITARY CONDITIONS

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Botulism is a severe neuromuscular disease caused by the ingestion of toxins produced by *Clostridium botulinum*, a Gram-positive, anaerobic, spore-forming bacterium widely distributed in the environment. In poultry, outbreaks are associated with the ingestion of preformed toxins present in decaying organic matter, where the bacterium multiplies and produces botulinum neurotoxin. This report describes an outbreak of botulism in poultry (*Gallus gallus domesticus*) in the municipality of Eldorado dos Carajás, Pará State, Northern Brazil. A subsistence farm with birds showing flaccid paralysis and inability to remain standing was reported to the region's Official Veterinary Service (SVO). During the epidemiological investigation, three birds were found dead. Three additional birds showed severe neuromuscular signs, including paralysis of the pelvic limbs and wings, marked depression, and persistent eyelid closure. Easy feather detachment was also observed. Due to the severity of the clinical condition and poor prognosis, the birds were euthanized. At necropsy, one bird presented tracheal congestion, cephalic congestion, and mild pulmonary hemorrhage, while the others showed no significant gross lesions. Samples of gastric contents (3/7) and liver (4/7) were collected for botulinum toxin detection using the mouse bioassay, the gold-standard laboratory diagnostic method. A total of seven samples from four birds were analyzed. Botulinum toxin was detected in two (50%) liver samples. Although this assay has limited sensitivity (43.7%), its specificity is high (98.1%), providing high confidence in positive results. Concurrently, Avian Influenza and Newcastle Disease were ruled out as differential diagnoses. The property housed thirteen birds, resulting in a morbidity rate of 46.1% (6/13) and a case fatality rate of 100% (6/6). The facilities were constructed of masonry and appeared to have adequate sanitary conditions; however, the birds had free access to a pig-rearing area where accumulations of mud, stagnant water, food scraps, and feces were observed. Therefore, the main hypothesis for the outbreak was the ingestion of feed or water contaminated with botulinum toxin. This report highlights the importance of appropriate sanitary management practices and farmer awareness, emphasizing the role of epidemiological surveillance, particularly on subsistence farms with mixed-species production systems, where outbreaks may cause significant economic losses and compromise animal health.

Keywords: *Clostridium botulinum*; Flaccid paralysis; Epidemiological surveillance; Mouse bioassay

310P ANTIMICROBIAL ACTIVITY OF GREEN PROPOLIS AND TUBUNA (*Scaptotrigona bipunctata*) AGAINST MICROORGANISMS ISOLATED FROM CAPTIVE *Psittacula krameri*

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This study investigated the occurrence of *Escherichia coli*, *Salmonella* spp., and *Candida* spp. in captive *Psittacula krameri* and evaluated the *in vitro* antimicrobial activity of hydroalcoholic extracts of green propolis and Tubuna (*Scaptotrigona bipunctata*) propolis against the isolated microorganisms. Cloacal swabs were cultured on eosin methylene blue (EMB) agar for the isolation of *E. coli* and on XLD agar for the detection of *Salmonella* spp., whereas crop swabs were subjected to microbiological analysis for the identification of yeasts of the genus *Candida*. The minimum inhibitory concentration (MIC) of the propolis extracts was determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI M07) guidelines, employing bacterial and fungal isolates obtained from the birds. MIC results were interpreted descriptively following CLSI recommendations. *Escherichia coli* was isolated from cloacal samples, and *Candida* spp. was identified in crop samples, whereas *Salmonella* spp. was not detected. Tubuna propolis inhibited the growth of both *E. coli* and *Candida* spp. up to the 10⁻¹ dilution. Green propolis exhibited antibacterial activity against *E. coli* up to the 10⁻¹ dilution and greater antifungal activity against *Candida* spp., maintaining inhibitory activity up to the 10⁻⁴ dilution. These findings demonstrate that both propolis extracts exhibited antimicrobial activity against clinical isolates, with comparable antibacterial activity against *E. coli* and superior antifungal activity of green propolis against *Candida* spp. The results highlight the potential of propolis as a source of bioactive compounds for use as a natural alternative in the microbiological control of captive birds.

Keywords: propolis; antimicrobial activity; minimum inhibitory concentration; stingless beekeeping; psittacines

311P Effects of HPDDG inclusion in corn and soybean meal-based diets on the composition and metabolic profile of the intestinal microbiota of broiler chickens

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The gut microbiota plays an essential role in poultry health and performance and is strongly influenced by diet. High-protein dried distillers' grains (HPDDG), a coproduct of corn ethanol production, contain fermentable fibers and bioactive compounds, including arabinoxylans, β-glucans, and mannanoligosaccharides, which may modulate microbial fermentation and short-chain fatty acid production, contributing to intestinal integrity. However, high inclusion levels may increase non-starch polysaccharides and impair the intestinal environment. This study evaluated the effects of HPDDG inclusion in corn- and soybean meal-based diets on the taxonomic composition and metabolic profile of the gut microbiota in broilers. A total of 3,528 Ross AP95 male chicks were distributed in a completely randomized design with six treatments and 14 replicates of

42 birds each. The trial lasted 35 days and used two nutritional programs: starter mash feed from 1 to 21 days, with 0, 3, 5, 7, 9, and 11% HPDDG; and grower mash feed from 22 to 35 days, with 0, 6, 8, 10, 12, and 14% HPDDG for T1 to T6, respectively. At 14 and 28 days, cecal mucosa samples were collected, totaling 36 samples per age. Bacterial DNA was extracted, and the 16S rDNA gene was amplified and sequenced using Illumina® technology. Sequences were processed through a standardized bioinformatics pipeline for taxonomic classification, diversity, and relative abundance analyses. Alpha diversity was compared using the Kruskal-Wallis test, with significance set at $P < 0.05$. At 14 days, alpha diversity differed among treatments, with higher diversity in the control group, a reduction in T2 ($P < 0.05$), intermediate and more stable values in T3 and T4, and greater variability in T5. At 28 days, microbial diversity increased across treatments, indicating microbiota maturation. T3 showed the highest diversity and lowest variability, whereas T4 and T5 showed lower diversity and greater dispersion. At 14 days, intermediate HPDDG levels, T3 and T4, increased beneficial genera such as *Lactobacillus*, *Blautia*, and *Butyrivibrio*, whereas higher levels, T5 and T6, reduced opportunistic genera such as *Pseudomonas* and *Clostridia*_UCG-014. At 28 days, greater compositional differentiation was observed, with higher *Lactobacillus* and *Romboutsia* in T3 and T4 and reduced *Escherichia/Shigella* in T3 and T6, suggesting gut microbiota modulation, although these results were not statistically significant. In conclusion, HPDDG inclusion modulated the gut microbiota of broilers, with more consistent effects at intermediate levels, particularly 5% to 7% in the starter phase, improving microbial diversity, stability, and abundance of beneficial genera, especially at 28 days.

Keywords: gut microbiota; short-chain fatty acids; cecal bacteria; microbial diversity; prebiotic fibers

312P Phenotypic and Genotypic Characterization of Biofilm Formation in *Salmonella enterica* Isolates from the Poultry Production Chain.

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Biofilm formation is an important mechanism for the persistence of *Salmonella enterica*, promoting its survival in poultry production environments and hindering its elimination by sanitation procedures. Although several genes and phenotypic markers have been associated with this process, their contribution to biofilm formation remains incompletely understood. Considering the complexity of this process, this study evaluated the association between biofilm-related genes, colony morphology on Congo Red agar (CRA), and biofilm formation in polystyrene microplates. Twenty-three isolates were evaluated, including 15 *Salmonella* Minnesota (65.2%) and 8 *Salmonella* Heidelberg (34.8%). Regarding their origin, 11 isolates were recovered from ceca (47.8%), 7 from carcasses (30.4%), and 5 from chillers (21.7%). The presence of the *csgD*, *adrA*, *agfA*, and *ipfA* genes was determined by PCR; colony morphology on CRA was evaluated at 28°C and 35°C; and biofilm formation in polystyrene microplates was quantified by measuring absorbance at 630 nm and classified as weak, moderate, or strong. Associations among biofilm intensity, serovar, isolate origin, and

genetic profile were evaluated using the Fisher-Freeman-Halton exact test, followed by a complementary binary analysis comparing strong versus non-strong biofilm producers. All isolates exhibited the SAW (Smooth and White) morphotype at both temperatures and carried the *csgD* and *adrA* genes. The *agfA* gene was detected in 20 isolates (87.0%) and the *ipfA* gene in 12 (52.2%). Regarding biofilm formation intensity, 4 isolates (17.4%) were classified as weak producers, 13 (56.5%) as moderate producers, and 6 (26.1%) as strong producers. *Salmonella* Heidelberg showed a higher proportion of strong biofilm-producing isolates (5/8; 62.5%), whereas *Salmonella* Minnesota showed a higher frequency of moderate (11/15; 73.3%) and weak (3/15; 20.0%) biofilm producers. No associations were observed between biofilm intensity and the presence of the *agfA* ($P = 1.0000$) or *ipfA* ($P = 0.2607$) genes, nor with isolate origin ($P = 0.5036$). In contrast, a significant association was observed between serovar and biofilm formation intensity ($P = 0.0207$), with a higher proportion of strong biofilm producers among *Salmonella* Heidelberg, a finding confirmed by the binary analysis ($P = 0.0086$). Isolates displaying identical CRA morphologies also exhibited distinct biofilm formation phenotypes. These findings suggest that biofilm formation in *S. enterica* is a multifactorial process involving, in addition to the genetic and phenotypic markers evaluated, other mechanisms that may contribute to the persistence of this pathogen in the poultry production chain.

Keywords: Congo Red agar; Biofilm-associated genes; Serovars; Poultry production chain; Phenotypic characterization.

313P Improving male fertility in broiler breeders: Effects of a phytogetic feed additive on semen quality

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Male fertility is a critical factor influencing the productivity of poultry breeder flocks, as it directly affects the number of day-old chicks produced per hen. The quality of sperm, and consequently the successful fertilization of eggs, is impacted by the antioxidant systems present in spermatozoa. Phytochemicals are essential for providing both direct and indirect protection to spermatocytes by enhancing cellular antioxidant mechanisms and regulating reproductive hormone metabolism, particularly by optimizing testosterone production. Biostrong™ Fertile (PFA; Delacon Biotechnik GmbH, Engerwitzdorf, Austria) is a proprietary formulation comprising essential oils, flavonoids, and saponins, specifically developed to enhance fertility in roosters. A controlled experimental assessment was carried out at Zootests SAS (Ploufragan, France), involving 80 male Hubbard GP broiler breeders aged 42-63 weeks, which were individually housed in cages. At 49 weeks of age, the roosters were fed either a control diet, a standard formulation based on wheat, corn, and soybean, or the same diet supplemented with PFA at 750 g/MT. Semen samples were manually collected from the roosters at the beginning of the experimental treatment and again after 7 and 14 weeks, corresponding to the ages of 49, 56, and 63 weeks, respectively. Data on the effect of PFA addition were analyzed using the Mann-Whitney test in Statistica 13 (StatSoft-TIBCO, Palo Alto, USA). At the onset of the treatment, roosters in the PFA group displayed notably inferior semen quality compared to those in the control group across all assessed parameters. Nevertheless, results at the end of the evaluation period indicated significant improvements ($P < 0.05$) in the PFA group compared with the control, including a 14.8% increase in semen volume, a 22.7% increase in sperm count, and a 19.6% increase in sperm motility score. Although semen concentration naturally declines over time in both groups, the PFA diet

consistently outperformed at 56 and 63 weeks of age, resulting in a 6.7% increase at the end of the trial ($P < 0.10$). The findings of this study indicate that the evaluated PFA substantially enhances semen quality parameters in roosters, thereby supporting improved male fertility during the critical age range of 42 to 63 weeks. This innovative solution represents a promising approach to optimizing reproductive performance in breeding programs.

Keywords: Male fertility; Broiler breeders; Semen quality; Antioxidant capacity; Reproductive performance

314P SEX HORMONE PROFILE AND ANATOMOPATHOLOGICAL ALTERATIONS IN A LAYING HEN WITH MALE CHARACTERISTICS: CASE REPORT

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Estradiol is the most abundant estrogen and one of the most important gonadal steroids, playing diverse roles in the regulation of female reproduction. In contrast, testosterone is a steroid hormone responsible for the development of male sexual characteristics. Alterations in the physiological levels of sex hormones have varied causes and consequences. In light of this, this study aims to characterize the factors involved in a case of masculinization in a laying hen. In July 2025, a 100-week-old Lohmann laying hen from the poultry sector of the School of Veterinary Medicine and Animal Science at the Federal University of Goiás (EVZ/UFG), with a history of developing male characteristics and complete interruption of egg production, was submitted to the Avian Diseases Diagnostic Laboratory (SaniAves – EVZ/UFG) for necropsy. During the physical examination, the bird was alert and in good general condition but exhibited male characteristics, including rooster-like crowing and excessive development of the comb and wattles. After the physical examination, blood was collected for hormone measurement; subsequently, the bird was euthanized and subjected to necropsy. The hormone assay revealed 60.5 pg/mL of estradiol (reference range: 400–550 pg/mL) and 752.0 pg/mL of testosterone (reference range: 70–90 pg/mL). At necropsy, the carcass of the female *Gallus gallus domesticus* presented a good body condition score, with normally colored comb and wattles. Upon opening the coelomic cavity, the oviduct showed a focal enlargement measuring approximately 7 cm in diameter in the uterine segment, with massively engorged serosal vessels. Incision of the oviduct revealed a retained egg diffusely covered by a thick, blackened, irregular material, with heterophilic fibrinous content within the lumen. The oviduct and the left ovary were significantly reduced in volume (atrophy), and no follicular development was observed. Based on the sex hormone profile and the necropsy findings, it is suggested that the egg retention led to a severe chronic inflammatory process, resulting in the atrophy of the oviduct and the ovary. The atrophy of the female reproductive organs accounts for the decreased serum estradiol levels and the elevation of testosterone, which led to the masculinization of the laying hen. Further studies on lesions of the avian reproductive system and their implications for hormonal disorders are needed.

Keywords: Estradiol; Egg retention; *Gallus gallus domesticus*; Reproductive disorder; Testosterone

315P Embryonic Thermal Manipulation Modulates Hepatic

Remodeling and Function in Cobb Broiler Chickens

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Hypothesis or Objective: To evaluate the effects of late embryonic thermal manipulation on liver morphometry, histopathology, relative liver weight, and serum biochemical parameters in Cobb® broiler chickens at 15 days of age. **Experimental Design:** Fertile Cobb® eggs were randomly assigned to three incubation treatments: control (T1; 37.8°C), moderate thermal manipulation (T2; 38.5°C), and intense thermal manipulation (T3; 39.0°C). Thermal manipulation was applied for 6 h daily from embryonic day 16 through 19. At 15 days post-hatch, birds were exposed to an acute heat challenge and subsequently euthanized for hepatic evaluation. **Materials and Methods:** Liver samples were collected for histopathological and morphometric analyses after hematoxylin and eosin staining. Absolute and relative liver weights were recorded. Serum biochemical analyses included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine, creatine kinase (CK), uric acid, and albumin. Statistical analyses were performed using one-way ANOVA or Kruskal-Wallis tests, depending on data distribution, followed by appropriate post hoc comparisons ($P < 0.05$). **Results:** Absolute and relative liver weights did not differ among treatments ($P > 0.05$). Similarly, no differences were observed in ALT, GGT, creatinine, CK, uric acid, or albumin concentrations. In contrast, serum AST activity was lower in thermally manipulated birds, with T3 showing significantly lower values than the control group ($P = 0.0365$), suggesting a sustained effect on hepatic metabolism and cellular integrity. Histopathological evaluation revealed preserved hepatic architecture in T1, whereas T2 birds exhibited mild focal steatosis and increased hepatocyte nuclear area, indicating adaptive cellular remodeling. Conversely, T3 birds showed degenerative alterations, including hepatocellular degeneration, vascular congestion, inflammatory infiltration, and focal necrosis, consistent with hepatic injury induced by excessive thermal manipulation. These findings demonstrate a dose-dependent hepatic response to embryonic thermal manipulation. **Conclusions:** Moderate embryonic thermal manipulation (38.5°C) promoted persistent hepatic remodeling compatible with physiological adaptation, whereas exposure to 39.0°C induced long-lasting histopathological alterations despite minimal changes in conventional biochemical markers. The liver appears to be a sensitive target organ for developmental thermal programming in broiler chickens.

Keywords: Embryonic Thermal Manipulation; Liver Histopathology; Developmental Programming; Broiler Chickens; Heat Adaptation

316P Effects of housing ventilation system and sex on body composition traits in commercial broilers

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Body composition in broiler chickens reflects the balance between growth, adiposity, and visceral development. Although ventilation systems are primarily designed to improve environmental conditions and bird welfare, their effects on tissue allocation under commercial production conditions remain poorly understood. Therefore,

this study evaluated the influence of housing ventilation systems on body composition traits in commercial broilers. Materials and methods. Commercial broiler production systems differ in their ability to control environmental conditions, which may influence body tissue development. To investigate this relationship, 756 Cobb broilers originating from 35 commercial mixed-sex flocks were sampled at slaughter. Birds were reared in houses equipped with either positive-pressure or negative-pressure ventilation systems. Body composition was characterized through measurements of carcass yield, feather yield, relative organ weights (liver, heart, gizzard, and viscera), and adiposity traits, including abdominal, visceral, and total fat deposition. Data were analyzed using a factorial ANOVA including the effects of ventilation system, slaughter age, sex, and their interactions. Means were compared using Tukey's test, and statistical significance was declared at $P < 0.05$. Ventilation system did not affect body weight, carcass yield, heart weight, gizzard weight, or feather yield ($P > 0.05$). Males exhibited greater body weight (3741–3794 g) and feather yield (12.33–12.54%) than females (3069–3084 g and 11.09–11.38%, respectively; $P < 0.05$). Females showed greater abdominal fat (1.11–1.13 vs. 0.85–0.88%), visceral fat (0.83–0.89 vs. 0.59–0.60%), total fat deposition (1.96–2.00 vs. 1.43–1.46%), and relative viscera weight (3.85–3.90 vs. 3.50–3.60%) than males ($P < 0.05$). A significant ventilation system $\times$ sex interaction was observed for relative liver weight, with females reared under positive-pressure ventilation exhibiting the highest values (1.57%; $P < 0.05$). In conclusion, sex was the primary determinant of body composition in commercial broilers, with females exhibiting greater adiposity and males exhibiting greater body weight and feather yield. Ventilation system exerted limited effects on productive traits but influenced liver development through a sex-dependent response.

Keywords: body composition; carcass yield; fat deposition; sex effect

317P Evaluation of Shell Translucency in Commercial White Eggs from Floor-Reared Laying Hens Marketed in Supermarkets in the Metropolitan Region of Goiânia, Brazil

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In this study we evaluated commercial white eggs from floor-reared laying hens and purchased from supermarkets in Goiânia, Brazil, to investigate eggshell translucency and shell quality. The experiment was conducted at the Poultry Research Unit EVZ/UFG. 180 eggs were assigned to 2 treatments: group 1 was evaluated immediately after purchase (TA), and the group 2 was stored under refrigeration until the expiration date (TB). Eggs were distributed into 3 batches of 60 eggs each. Individual egg weight (EW) was recorded, and specific gravity (SG) was determined using 8 saline solutions ranging from 1065 to 1100 g/cm³ at 0.005 intervals. Eggs were arranged in trays in the Blue Box® LED imaging system. Images were processed with the Procam® application, and eggs were classified in translucency scores (TS) from 1 to 4. In each treatment, 30 eggs were randomly selected for external shell quality assessment using an Egg Tester®. Shell breaking strength (BS) and shell thickness (ST) were determined. The remaining eggs were refrigerated until the expiration date. After evaluation, shells were washed and dried

in a forced-air oven at 55°C for 48 h. Shell percentage (SP) was calculated as the ratio between dry shell weight and total egg weight. EW, TS, and SG were analyzed using a randomized complete block design, whereas ST, BS, and SP were analyzed using a completely randomized design. EW, SG, BS, ST, and SP were expressed as means, and TS expressed as the modal value. For the 1st batch, TA presented EW, SG, BS, ST, SP and TS, respectively following values: 68.9 g, 1.065 g/cm³, 3.8 kgf, 0.44 mm, 6.5 %, and 1. In TB, the values were: 69.0 g, 1.065 g/cm³, 4.12 kgf, 0.44 mm, 6.9 %, and 1. In the 2nd batch, TA showed: 63.5 g, 1.080 g/cm³, 3.97 kgf, 0.44 mm, 6.0 %, and 1. TB presented: 61.8 g, 1.065 g/cm³, 3.88 kgf, 0.43 mm, 5.85 %, and 2. For the 3rd batch, TA presented: 59.8 g, 1.090 g/cm³, 5.59 kgf, 0.50 mm, 6.32 %, and 1. TB showed: 59.8 g, 1.075 g/cm³, 5.41 kgf, 0.49 mm, 6.25 %, and 2. EW decreased during storage in all batches compared to day 0 with the end of the storage period. Likewise, ST scores increased over time, indicating a deterioration in shell translucency, while SG also declined during refrigerated storage. No significant differences were observed for shell quality traits, except in the 1st batch, in which SP increased after refrigerated storage.

Keywords: Storing; Score; Floor system; Translucency

318P Prototype of a computer vision-based system for internal egg quality assessm

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Egg internal quality progressively declines during storage due to physicochemical changes affecting yolk integrity and thick albumen consistency. This study developed a prototype computer vision system using Python and OpenCV to objectively assess internal egg quality from photographs of broken eggs placed on graph paper. The system automatically segments yolk and albumen, measures yolk area, identifies multiple yolks, classifies yolks as intact or broken, and estimates the proportion of thick albumen relative to the total albumen area. The prototype was applied to photographs of 80 broken eggs, yielding 80 yolk images and 64 analyzable albumen masses. Among the 80 yolks analyzed, 65 (81.2%) were identified as intact, with a median area of 1,572.0 mm² (range: 1,123.3–2,622.8 mm²). Thick albumen was successfully identified in 47 (73.4%) albumen masses, representing a median of 26.5% (range: 12.7–52.3%) of the total albumen area. These preliminary findings demonstrate the technical feasibility of automatically extracting objective image-derived measurements associated with internal egg quality, supporting the development of low-cost tools for egg grading, quality monitoring, and industrial processing. The principal limitation was the sensitivity of color-based segmentation to variations in lighting conditions between image acquisition sessions. Future studies will incorporate color calibration targets, validate image-derived measurements against conventional egg quality traits, and expand the prototype using deep learning-based image segmentation to improve robustness and automation.

Keywords: Computer vision; Egg quality; Dense albumen; yolk

319P "Effect of three storage temperatures and times on the freshness of laying hen eggs for consumption"

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Hypothesis – Objectives: Maintaining good internal egg quality is important for economic and productive purposes and impacts food security. Freshness is measured using Haugh Unit and Yolk Index analyses. Our hypothesis was that different storage temperatures affect egg freshness. The objective was to determine the effect of three storage temperatures and times on egg freshness. **Experimental Design:** 120 samples of freshly laid eggs from laying hens were randomly collected. Forty eggs were placed at room temperature ($22\text{ }^{\circ}\text{C} \pm 2.4$), 40 eggs at high temperature ($29.5\text{ }^{\circ}\text{C} \pm 2.1$), and 40 eggs at refrigeration temperature ($4\text{ }^{\circ}\text{C} \pm 0.34$) for a period of 21 days. Analyses were performed every 3 days. **Materials and methods:** This study was conducted in the Nutrition Research Laboratory R&D – FMVZ – UNICA. One hundred and twenty eggs from 35-week-old HY-LINE Brown hens were randomly selected. An egg analyzer (Digital Egg Tester-6500) and a Mitutoyo micrometer were used. **Statistical analysis:** The general linear model procedure of SAS v 9.4 was used to determine the effect of storage time at three temperatures on egg freshness. The Kruskal-Wallis test was used to determine the significant effect, and the Bonferroni correction test was used for comparison of means. Differences were considered statistically significant when $P < 0.05$. **Results:** A significant reduction ($P < 0.05$) was found for ambient temperature ($22\text{ }^{\circ}\text{C} \pm 2.4$) on Haugh unit and yolk index, which decreased from 91.84 and 0.399 (day 0) to 42.38 and 0.275 (day 21), respectively. High temperature ($29.5\text{ }^{\circ}\text{C} \pm 2.1$) significantly affected ($P < 0.05$) Haugh unit and yolk index, which decreased from 89.43 and 0.413 (day 0) to 27.21 and 0.153 (day 21), respectively. The refrigeration temperature ($4\text{ }^{\circ}\text{C} \pm 0.34$) significantly affected ($p < 0.05$) the Haugh unit and yolk index, which decreased from 93.73 and 0.418 (day 0) to 79.75 and 0.386 (day 21), respectively. **Conclusions:** It is concluded that storing eggs in a high-temperature environment has the most negative impact on egg freshness. Refrigeration temperature best maintains egg freshness. This baseline information is of interest and important to consider for the proper management of internal egg quality under appropriate storage and marketing conditions.

Keywords: Haugh unit; yolk index; Egg Freshness; Laying; Temperature

320P Evaluation of Shell Translucency in Commercial White Eggs from Cage-Reared Laying Hens Marketed in Supermarkets in Goiânia, Brazil

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This study evaluated commercial white eggs produced by cage-reared laying hens and marketed in supermarkets in Goiânia, Brazil, to investigate the relationship between shell translucency and external egg quality. The experiment was conducted at the Poultry Research Unit of EVZ/UFG. A total of 180 white eggs were assigned to two treatments: evaluation immediately after purchase and evaluation after refrigerated storage until the expiration date indicated on the package. Eggs were divided into three groups (G1, G2, and G3) of 60 eggs each. Egg weight (EW) was recorded, and specific gravity (SG) was determined using the saline flotation method with eight saline solutions ranging from 1.065 to 1.100 g/cm³. Shell translucency (TS) was assessed in both treatments using the Blue Box®

imaging system, and images were analyzed with the Procam® application to assign translucency scores from 1 to 4. In each group, 30 eggs were randomly selected to determine shell breaking strength (BS) and shell thickness (ST) using an Egg Tester®. After refrigerated storage, the remaining eggs were evaluated using the same procedures. Eggshells were washed, dried at $55\text{ }^{\circ}\text{C}$ for 48 h, and shell percentage (SP) was calculated as the ratio of dry shell weight to egg weight. EW, TS, and SG were analyzed using a randomized complete block design, whereas BS, ST, and SP were analyzed using a completely randomized design. Descriptive statistics were used to calculate means for EW, SG, BS, ST, and SP, whereas TS was expressed as the mode. Mean values (Treatment 1 vs. Treatment 2) were: G1—EW: 64.30 vs. 63.40 g; SG: 1.080 vs. 1.065 g/cm³; BS: 3.35 vs. 3.90 kgf; ST: 0.42 vs. 0.44 mm; SP: 9.46 vs. 9.53%; TS: 2 vs. 2. G2—EW: 64.37 vs. 63.05 g; SG: 1.083 vs. 1.065 g/cm³; BS: 4.00 vs. 4.14 kgf; ST: 0.43 vs. 0.45 mm; SP: 9.47 vs. 9.82%; TS: 2 vs. 2. G3—EW: 53.56 vs. 52.43 g; SG: 1.078 vs. 1.065 g/cm³; BS: 4.80 vs. 5.17 kgf; ST: 0.47 vs. 0.47 mm; SP: 10.12 vs. 10.57%; TS: 3 vs. 2. Refrigerated storage for up to 30 days reduced egg weight and specific gravity, whereas shell quality traits showed only minor changes. The variability observed among commercial eggs emphasizes the need for improved quality control throughout the commercial distribution chain.

Keywords: Storing; eggshell; score; specific gravity; egg weight

321P Comparative Genomic and Metabolomic Analysis of *Erysipelothrix piscisicarius* Isolates

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Erysipelothrix piscisicarius (EP) is still a newly described species that remains poorly explored. However, it may cause high mortality in poultry, and its zoonotic potential remains incompletely elucidated. Therefore, this study aimed to perform a comparative genomic analysis, through whole-genome sequencing, of EP isolates from humans and turkeys originating from the same geographical region, although recovered during different time periods, in order to investigate their genomic relatedness and possible evidence of zoonotic transmission. In addition, the intracellular and extracellular metabolite profiles of human fibroblasts infected with *E. piscisicarius* were evaluated, integrating the metabolomic findings with the discussion of metabolically relevant pathways identified at the genomic level. For comparative genomic analysis, we used the sequences of 17 isolates obtained from turkeys during an outbreak and one strain isolated from a human patient with clinical disease, along with other sequences deposited in public databases. To evaluate metabolites in cell culture, two EP strains isolated during an outbreak in commercial turkey flocks (EP6 and EP) were used, along with two human strains (AH2 and AH1), *E. rhusiopathiae* (ER), and *Staphylococcus aureus* (SA). Multivariate analysis was performed using PCA and MANOVA, considering $p < 0.05$ as statistically significant. Human and avian isolates were classified as clonal strains, demonstrating a close genomic relationship and reinforcing the zoonotic potential of EP. In the intracellular metabolomic profile, infected cells exhibited distinct patterns compared with non-infected cells, indicating a metabolic reprogramming involving amino acid metabolism and nitrogen compound-associated pathways. However, no relevant differences were observed among the EP, ER and SA isolates, the lat-

ter representing a more phylogenetically distant microorganism. In the cell culture supernatant, a clear metabolic separation between genera was observed, with *Erysipelothrix* spp. distinctly segregating from SA. At the strain level, metabolomic profiles were largely similar, except for one related to nitrogen metabolism, which was detected at higher level of EP compared with ER. Alterations in sulfur assimilation pathways, cysteine biosynthesis, unsaturated amine formation, and tryptophan degradation through indole-derived metabolites associated were different at level strains of ER. These findings highlight EP as a potential emerging zoonotic pathogen, exhibiting metabolomic signatures distinct from SA, but only minor metabolic differences among isolates within the genus *Erysipelothrix*.

Keywords: Poultry ; microbiology; zoonotic; disease; pathogens

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 β -lactam 207STP
 β -mannanase 140
 β -mannans 92

DRAFT