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EXCELÊNCIA
NA PRODUÇÃO
DE ALIMENTOS
SEGUROS.



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PRESENTATION

Dear colleagues and friends of the Brazilian animal production sector,

It is with pleasure that, as President of the Foundation for the Support of Animal Science and Technology– FACTA, I extend the warmest welcome to all who join us for the 41st FACTA Conference 2025, and to all readers now reading the Proceedings of this important event. This compendium of knowledge represents the culmination of months of planning, dedication, and, above all, the spirit of collaboration that permeates our scientific community.

Throughout its trajectory, FACTA has established itself as a fundamental pillar for the development of animal production in Brazil, with a particular and historical focus on poultry farming. In a country of continental dimensions and an undeniable vocation for agriculture, Brazilian poultry farming stands out not only for its impressive productive capacity but also for its resilience and constant pursuit of innovation. The responsibility of feeding millions of people, both domestically and abroad, demands from us a continuous commitment to excellence and sustainability. Since its creation in 1989, the Foundation has played an important role in disseminating knowledge and technologies applicable to the Brazilian poultry sector, in line with our institutional commitment. Over the years, our annual Poultry Science and Technology conferences have brought together more than 25,000 participants, a testament to the impact and relevance of our work. In addition to the conferences, we have held various seminars, refresher courses, and symposia addressing topics such as health, nutrition, management, environment, and animal welfare, always seeking the frontiers of knowledge for the benefit of our sector.

In this dynamic and challenging scenario, the 41st FACTA Conference 2025 emerges as a beacon of knowledge and a catalyst for progress. More than a mere gathering, it establishes itself as a privileged forum for updating cutting-edge technical-scientific knowledge. Here, researchers, industry professionals, students, and policymakers converge to share the latest discoveries, best practices, and innovations that will shape the future of our sector. The exchange of experiences and the discussion of ideas are important elements for us to face contemporary challenges and seize the opportunities that arise.

Beyond the invaluable academic and professional dimension, the FACTA Conference has always been and continues to be a conducive environment for personal interaction and fellowship. It is in the hallways, during breaks between lectures, and in moments of relaxation that bonds are strengthened, partnerships are conceived, and the community unites in a spirit of harmony. We firmly believe that the success of an event like this is not measured solely by the quality of the scientific content but also by the richness of the human connections it fosters. It is the mix of intellect with passion, scientific rigor with human warmth, that drives us to go further.



The 2025 edition of the FACTA Conference, in particular, was conceived with a new format, reflecting our commitment to constant evolution and the pursuit of even more enriching experiences. Every detail was designed to optimize learning, interaction, and comfort for our participants. We highlight the implementation of “Multispecies” and “Multitheme” arenas, which will cover sectors such as swine and fish, expanding the scope of discussions and promoting a more integrated approach to animal production. This innovation aims to ensure that our conference continues to be a relevant space for an even broader audience in the food production chain.

The chosen venue for this conference was carefully selected to provide an inspiring and welcoming environment, suitable to the importance of the topics addressed and the debates that will unfold in it. We believe that a conducive environment is fundamental for ideas to flourish and knowledge to be fully absorbed, and Campinas, a hub of knowledge and innovation, offers the perfect setting for this.

The central theme guiding us on this journey in 2025 – “Management, Innovation, and Excellence in Safe Food Production” – is a direct reflection of the most urgent priorities and challenges that poultry farming and animal production as a whole face right now. In a world that is increasingly aware of the importance of food safety and sustainability, the ability to manage processes efficiently, innovate constantly, and seek excellence at every stage of the production chain is not just a competitive advantage but a non-negotiable responsibility. We will discuss how technology, biosecurity, animal nutrition, welfare, and management practices integrate to ensure that the food reaching the tables of millions of consumers is safe, nutritious, and produced ethically and responsibly.

In a global context marked by economic uncertainties, health challenges, and climate change, the relevance of our unity and purpose becomes even more evident. Difficult times like the one we are going through teach us that the key to overcoming challenges lies in the union of efforts from all sectors. Producers, researchers, industry, government, and consumers: we are all fundamental pieces in this complex ecosystem. It is only through collaboration, open dialogue, and the joint pursuit of innovative solutions that we can build a more prosperous and secure future for Brazilian poultry farming and animal production as a whole.

May the Proceedings of the Lamas Award 2025 serve as a valuable record of all the scientific knowledge shared, the ideas debated, and the connections established. May this material inspire new research, stimulate innovation, and further strengthen the bonds that unite us in favor of an increasingly strong, resilient, and sustainable sector.

I thank each of you for being a part of this story.

Sincerely,
Ariel Antônio Mendes
President of FACTA



LAMAS AWARD

Dear authors,

It is with great satisfaction that we present the Proceedings of the Lamas Award 2025, a publication that symbolizes the tireless dedication and cutting edge knowledge produced by our scientific community. For FACTA, it is an honor to witness the richness and diversity of the research gathered here, which reflect the dynamism and excellence of animal science in Brazil, with a particular emphasis on poultry farming, an important pillar of our food production.

The Lamas Award has established itself as a vital platform for the unification and presentation of the most recent scientific knowledge generated in recent years. In a global scenario that demands increasingly efficient and sustainable solutions for food production, the ability to consolidate and disseminate information is very important. Our efforts aim to create an environment where advances, discoveries, and innovations can be shared transparently and accessibly, driving the progress of the entire sector.

These Proceedings are, therefore, much more than a compilation of abstracts; they serve as a source of inspiration and an initial guide for readers to delve deeper into topics of their interest. By accessing the content of these proceedings, readers will find the results of research conducted with extreme ethical-scientific rigor and innovative methodologies. It is our expectation that the content presented here will stimulate curiosity and encourage the search for full papers published in reputable scientific journals. Only in this way can we truly promote the continuous advancement of animal science, ensuring that the knowledge generated in our laboratories and universities translates into practical applications and benefits for the production chain.

The quality and rigor of the works in these Proceedings are the result of a highly demanding evaluation process. Each abstract submitted to the Lamas Award 2025 was carefully evaluated by an excellent scientific committee, composed of dozens of highly qualified researchers from diverse and respected Brazilian teaching and research institutions. The dedication and professionalism of these ad hoc reviewers are fundamental to ensuring the integrity and scientific relevance of the content presented to you. To them, we express our sincerest gratitude.

One of the central and most rewarding pillars of the Lamas Award is its commitment to providing opportunity and visibility, especially to young researchers. We recognize the immense talent and transformative potential of new generations of scientists. Therefore, the Lamas Award was conceived as a privileged space for these emerging researchers to show the community the results of their research, share their innovative perspectives and, most importantly, receive due recognition through the publication of their works and the awarding of distinctions. We believe that investing in these talents is investing in the future of animal science and Brazilian poultry farming.



The realization of an event of the magnitude of the FACTA Conference 2025 and the publication of the Lamas Award Proceedings are the result of a joint and tireless effort. I would like to express my gratitude to everyone involved, especially thanking the coordinators of the four areas of the Lamas Award: Nutrition (Neyre Shiroma), Health (Marcelo F. Zuanaze), Production (Rodrigo G. Garcia), and Other Areas (Ibiara C. L. A. Paz), whose expertise and leadership were indispensable for the success of the evaluation and selection process of the works.

I extend my gratitude to the ad hoc reviewers, the true pillars of the scientific integrity of this award, who dedicated their time and knowledge to rigorously and impartially evaluate each submission. Without your commitment, the excellence we seek would not be achieved. I also thank the entire FACTA team, whose professionalism and dedication behind the scenes were essential for the impeccable organization of all stages of the Award.

Finally, and most significantly, my special thanks go to all the authors who submitted their works to the Lamas Award 2025. The courage to present your ideas, the passion for research, and the desire to contribute to the advancement of science are the driving force behind this event. You are the protagonists of knowledge and the inspiration for future generations.

May these Proceedings serve as a celebration of intellect, a catalyst for new discoveries, and a testament to the power of collaboration.

Sincerely,

Rodrigo Garófallo Garcia

General Coordinator of the Lamas Award 2025 Scientific Committee



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Effects of Dietary Energy and Protein Density on White Striping Occurrence and Growth Performance in Broiler Chickens

(NU-30 - Winner)

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WINNER



NUTRITION

Abstract:

White Striping (WS), a muscle abnormality characterized by white striations aligned parallel to the fibers of the pectoralis major muscle, has emerged as a significant concern in the poultry industry due to its detrimental impact on meat quality and consumer acceptance. This study aimed to evaluate the effects of dietary energy and protein density on the incidence of WS and production performance in broiler chickens. Male Cobb × Cobb 500 broilers were assigned to either a high-energy (HE; 3,000 to 3,300 kcal ME/kg and 24.19 to 19.56% CP) or low-energy (LE; 2,900 to 3,050 kcal ME/kg and 20.44 to 18.37% CP) diet across four feeding phases (pre-starter: d 1-7; starter: d 8-21; grower: d 22-35; and finisher: d 36-50). Both diets maintained similar levels of minerals, vitamins, and additives, including monensin (100 ppm) and flavomycin (25 ppm), across all feeding phases. At 20, 30, and 50 days of age, birds were weighed and slaughtered in accordance with the requirements of current legislation. All procedures throughout the current study were approved by the Ethics Committee on Animal Use of the Federal University of Rio Grande do Sul. A total of 44 broilers per age (n=132) were randomly selected for WS evaluation (classified as normal, moderate, or severe). Data were analyzed using one-way ANOVA (SAS 9.3), and significant differences were determined using the Tukey test ($p < 0.05$). At 20 days, the LE diet was associated with a higher proportion of normal breast fillets (86.36%) compared to the HE group (40.90%). No severe WS cases were observed in the LE group at this age, whereas 18.18% of birds in the HE group exhibited severe lesions. By 50 days, the incidence of severe WS increased in both groups (HE: 68.18%; LE: 54.54%), suggesting a strong age-related progression of the myopathy, independent of dietary treatment. Despite the higher nutritional density, the HE diet did not confer improvements in productive performance. At 50 days, average carcass and breast weights were comparable ($p > 0.05$) between dietary treatments (4.1 kg carcass weight and approximately 759 g breast weight). Based on the results, it can be suggested that the use of low-energy diets could be a strategy to reduce the incidence of WS in chickens slaughtered at younger ages (griller). Nutritional strategies focused on moderating growth rates through balanced energy and protein levels may be more effective in maintaining muscle integrity and ensuring superior meat quality.

Keywords: Muscle Abnormality, Nutrition, Poultry, Myopathy

Can the Animal Feed Industry Overcome the Challenges of Poor Soybean Meal Pelletizability? (NU-56 - Honorable Mention)

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HONORABLE MENTION



NUTRITION

Abstract:

The composition of soybean meal varies according to the supplier, and some production processes remove lecithin, which has a significant impact on the production of pelleted diets and may result in losses in production capacity. This is all aggravated by the tendency to reduce the metabolizable energy content. The objective of this research was to evaluate, through an alternative specification of pelleting die, compensating the negative effects of these variables while maintaining production capacity and without compromising the quality of the pellets. A mixing and pelletizing line from Embrapa Swine and Poultry was used. The pelletizer used was a Kopper Junior C40/50 HP/37 kW/380 V/71.4 A/1,180 rpm. Two types of soybean meal x two AME levels x two different pelleting die specifications were evaluated, comprising eight treatments. The two types of soybean meal (SM) differed in ether extract (EE), with SM "A" = 2.9% EE and SM "B" = 2.2% EE; the AME levels were according to the nutritional requirements of the birds in the pre-starter phase (standard 2850 kcal/kg) and a decrease of 50 kcal. The diets were composed of diet 1 = Diet with less 50 kcal + SM "B"; diet 2 = Standard feed + SM "B"; diet 3 = Feed with less than 50 kcal + FS "A"; diet 4 = Standard Feed + FS "A". The tested dies were: standard die with hole diameter 4.76 mm x thickness 50 mm, relief only in the two outer rows and the relieved matrix with hole diameter 4.76 mm x 50 mm, with relief in all holes of 8 mm. During pelleting, 10 samples of each treatment were collected and pellet quality parameters were evaluated: Percentage of fines in the sample, Pellet Durability Index (PDI), Pellet Hardness. A loss of production capacity (approx. 25%) was observed when the soybean meal used had low EE content and a conventional die was used ($p < 0.05$), a condition that was overcome by using a alleviated dies, which is desired by the industry. Regarding the pellet quality results, in all treatments the quality was considered good ($> 90\%$). It was observed that the use of the alleviated die in combination with soybean meal "B" with lower ether extract, presented a higher PDI in relation to the diets that used FS "A", thus demonstrating a lower % of fines. A high PDI means that the feed maintains its integrity, minimizing the production of fines (smaller particles). The formulation containing soybean meal with low ether extract and a 50 kcal reduction in the diet had a significant impact on the decrease of pelleting capacity, approximately 25%. The change in the die specification proved to be effective in maintaining production and with a small reduction in quality measured by the PDI from 96% to 94%, when using soybean meal with lower ether extract and diets formulated with less than 50 kcal.

Keywords: Pellet, Processing, Lecithin, Soybean

In Ovolipase Injection on Early Post-Hatch Hepatic Glycogen in Broiler Chicks (NU-01)

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Abstract:

The yolk sac provides essential nutrients for avian embryos, with glucose serving as the primary energy source during the first week of development, followed by increased lipid mobilization. Lipase activity in the vitelline membrane hydrolyzes triglycerides and phospholipids, which is crucial for embryo growth and successful hatching. After hatching, hepatic glycogen acts as the immediate energy reserve, supporting the chick's early metabolic adaptation. This study evaluated the effects of different *in ovo* lipase doses (0, 500, and 1000 U/mL), diluted in a vaccine diluent and injected on day 18 of incubation into 3,744 Cobb500 eggs. All eggs were vaccinated (Marek's, Gumboro, and Newcastle) using an Embritech automatic machine. A total of 396 first-grade male chicks (132 per treatment) were selected and distributed across six cages per treatment (18 experimental units). Chicks were reared under controlled conditions and fed according to Cobb (2018) recommendations. On days 1, 3, 5, and 7 post-hatch, one chick per cage was randomly selected for euthanasia and liver collection. The hepatic glycogen analyses were performed in two steps: the first involved sample digestion, and the second consisted of the colorimetric determination of reducing sugars using the DNS method. Glycogen levels were analyzed using ANOVA (R Software, 2023) followed by Tukey's test ($p \leq 0.05$). Lipase supplementation had no significant effect on hepatic glycogen at hatch ($p > 0.05$), with average values of 6.81, 6.96, and 8.13 mg/g for 0, 500, and 1000 U/mL treatments, respectively. However, at 24 hours post-hatch, chicks that received 1000 U/mL lipase had significantly higher glycogen levels (6.47 mg/g) compared to control group (4.80 mg/g; $p < 0.05$). By day 3, no significant differences were observed ($p > 0.05$), and glycogen content gradually declined until day 7. In conclusion, *in ovo* injection of a higher lipase dose (1000 U/mL) helps preserve hepatic glycogen during the critical first 24 hours post-hatch, potentially supporting early metabolic transitions. However, this effect diminishes as chicks begin exogenous feeding.

Keywords: Glycogen, Chick Quality, Energy Metabolism

The Impact of Bio-Available Silicium Supplementation on Egg Quality Parameters in Broiler Breeder Hens (NU-03)

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Abstract:

Silicium is essential for connective tissue metabolism, mainly through its role in collagen synthesis, which ensures the strength and elasticity of tissues. In birds, collagen is a key component of the eggshell membrane, supporting calcium carbonate deposition and the formation of strong shells. Eggshell quality directly affects embryo protection, fertility, and hatchability in broiler breeders. However, aging reduces silicon absorption and tissue levels, contributing to weaker connective tissues and increased incidence of cracked eggs, as larger eggs are produced with relatively thinner shells. This decline in silicium has been proposed as a marker of tissue degeneration, highlighting the potential need for dietary supplementation in aging flocks. This study aims to evaluate the effect of bio-available silicium on the egg quality of broiler breeder hens. The experiment involved 40,000 Cobb 500 broiler breeder hens, divided into two houses with 20,000 hens each, all aged 20 weeks at the start of the trial in Belgium. From weeks 22 to 30, one house (treatment group) received bio-available Silicium (Poulsil®) at a concentration of 150 g/ton of feed, while the other house (control group) received no supplementation. At 30 weeks of age, 500 eggs were randomly collected from each house for egg quality assessment. The analysis of egg quality parameters revealed statistically significant differences between the control and the group supplemented with bioavailable silicium. Egg weight was significantly higher in the silicium group (58.7 ± 3.3 g) compared to the control (57.8 ± 2.9 g) ($p < 0.001$). Static stiffness also showed a significant increase in the bioavailable silicium group (137.7 ± 19.4 N/mm) relative to the control (134.9 ± 18.9 N/mm) ($p = 0.025$). Similarly, the shell index was significantly higher in the treated group (7.36 ± 0.51 g/cm²) compared to the control (7.29 ± 0.49 g/cm²) ($p = 0.031$). Shell thickness was also improved, with values of 0.313 ± 0.022 mm in the silicium group and 0.310 ± 0.021 mm in the control ($p = 0.031$). In contrast, vitelline membrane rigidity did not differ significantly between the groups, with values of 7.2 ± 0.9 N/mm for the treated group and 7.1 ± 0.9 N/mm for the control ($p = 0.188$). Furthermore, the Shapiro-Wilk normality test indicated that the data did not follow a normal distribution for most parameters, with p -values below 0.05 (egg weight $p = 0.008$, and $p < 0.001$ for the remaining parameters). Conclusion: In summary, adding bio-available Silicium (Poulsil®) to broiler breeder diets at 150 g/ton for 8 weeks significantly improves egg weight, eggshell thickness, eggshell strength (static stiffness and shell index), and numerically increases vitelline membrane strength.

Keywords: Eggshell Strength, Eggshell Thickness, Egg Weight, Membrane Rigidity

In Ovolipase Injection on the Performance of Broiler Chickens (NU-04)

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Abstract:

Lipase acts on the hydrolysis of lipids, such as triglycerides, present in the egg yolk, facilitating the release of fatty acids and glycerol. This action contributes to a more efficient utilization of energy reserves during the hatching process and the post-hatching period. Based on this information, it can be speculated that early stimulation of lipid metabolism may enhance the initial performance of broiler chickens. The objective of this study was to evaluate the effects of different doses of *in ovo* lipase (0, 500, and 1000 U/mL), diluted in vaccine diluent and injected on the 18th day of incubation into 3,744 Cobb500 eggs. All eggs were vaccinated against Marek's disease, Gumboro, and Newcastle using an Embritech automatic vaccinator. After hatching, 396 first-quality chicks (198 males and 198 females) were selected (132 birds/treatment), and distributed into six cages per treatment (18 experimental units), with 3 replicates of males and 3 of females per treatment, simulating a mixed-sex batch. The chicks were raised under controlled conditions and fed according to Cobb (2018) recommendations. On days 1, 7, 14, 21, 28, and 35, the birds and the leftover feed from each replicate were weighed. Performance parameters (feed intake, weight gain, feed conversion ratio, and mortality) were analyzed using ANOVA (R Software, 2023), followed by Tukey's test ($p \leq 0.05$). The use of lipase (500 and 1,000 U/mL) negatively affected feed intake and weight gain up to 14 days of age ($p \leq 0.05$). The average feed intake values were 463.33, 423.42, and 409.15 g, and the weight gain values were 365.75, 338.02, and 330.75 g, for the treatments with 0, 500, and 1000 U/mL, respectively. Moreover, lipase supplementation had no significant effect on feed conversion ratio and mortality ($p \geq 0.05$) at any stage. During the subsequent periods (21, 28, and 35 days), lipase supplementation did not affect feed intake or weight gain ($p \geq 0.05$). It is concluded that *in ovo* injection of lipase negatively affected the early performance of the birds, although this effect was offset over time. The use of high levels of lipase should be carefully evaluated in order to avoid negative impacts on bird performance.

Keywords: Energy Metabolism, Enzyme Supplementation, *In Ovo* Feeding

Effects of Different Fiber Sources on the Performance of Laying Hens (NU-06)

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Abstract:

The inclusion of fiber in the diet of laying hens has gained prominence for its potential positive effects on gut health, animal welfare, and productive performance. This study evaluated the effects of different fiber sources on performance and egg quality in Dekalb White laying hens. A total of 520 hens, 63 weeks old with an average initial body weight of 1572 g, were randomly selected for the study. The diets were formulated to be isoproteic and isoenergetic. The treatments included a control diet (based on corn and soybean meal, with no additional fiber source, 16.5% crude protein, 2,700 kcal/kg metabolizable energy, and 8.1% neutral detergent fiber [NDF]); a grass fiber diet (control + 2.0% elephant grass [Pennisetum purpureum; 9.3% NDF]); a soybean hull diet (control + 20% crude protein + 2.0% soybean hulls [9.3% NDF]); and a commercial insoluble fiber diet (control + 1.5% commercial insoluble fiber [8.1% NDF]). There was a variation in crude protein content in the diet containing soybean hulls. Prior to the experimental period (from 63 to 71 weeks of age), the hens underwent a 15-day adaptation to the diets. The experimental design was completely randomized with four treatments and five replicates, each containing 26 birds. The following variables were evaluated: egg production, egg/hen housed, feed intake, egg weight, feed conversion ratio, egg mass, and viability. Data were analyzed using ANOVA with Tukey's test ($p < 0.05$); non-normal data were analyzed using the Kruskal-Wallis test. No effects of treatments were observed on the main performance variables (egg production, egg/hen housed, feed intake, egg weight, egg mass, and viability) ($p > 0.05$). However, birds fed the soybean hull diet showed better feed conversion compared to the other treatments ($p < 0.05$). Nevertheless, this improved feed conversion may have also been influenced by the variation in crude protein content observed. In conclusion, the evaluated fiber sources can be used in the diet of laying hens.

Keywords: Insoluble Fiber, Pennisetum Purpureum, Shell Thickness

Evaluation of Lithothamnium Calcareum Inclusion in Diets For Laying Hens: Impacts on Performance and Egg Quality (NU-07)

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Abstract:

Limestone is the most commonly used element to supply calcium in animal nutrition. However, due to its inorganic nature, it tends to deplete its resources over time. The emergence of organic sources of calcium may therefore represent a more sustainable and long-lasting supplementation strategy. Among these organic sources, marine algae—particularly *Lithothamnium calcareum*—stand out due to their high calcium content. The exploitation of these bioclastic granules for economic purposes typically involves the harvesting of free-living algal forms, which facilitates collection. *Lithothamnium* is predominantly composed of calcium carbonate and magnesium, in addition to approximately 20 trace elements adsorbed onto its cell walls, which are readily assimilated by both animals and plants. Moreover, this alga exhibits a high degree of porosity, enhancing its efficacy in animal production. It has also been documented for use in poultry litter to retain ammonia and as a soil amendment for pH correction. So, this study aimed to evaluate the effects of two dietary calcium sources on the productive performance and egg quality of commercial laying hens: one organic source derived from the algae *Lithothamnium calcareum*, and one inorganic source, calcitic limestone. The experiment was conducted at the experimental poultry facility of the Federal University of Fronteira Sul (UFFS), using 24 cages of 750 cm² each, equipped with adapted feeders and drinkers, housing three birds per cage, resulting in a total of 72 laying hens. Four treatments were tested (T1 – control; T2, T3, and T4 – with 2.5%, 5%, and 10% inclusion of *L. calcareum*, respectively), distributed in six replicates, in a randomized block design over two consecutive 28-day production cycles. The control diet used 100% calcitic limestone as the calcium source, while the experimental treatments involved partial replacement with the marine algae at the specified inclusion levels. The evaluated parameters included daily feed intake (g/bird/day), egg production (eggs/bird/day), and both internal and external egg quality traits, including calcium content in the eggshell. A significant linear effect ($p < 0.05$) was observed for egg production (eggs/bird/day), and a quadratic effect ($p < 0.05$) was found for feed intake per dozen eggs. Regarding egg quality, no statistically significant differences ($p > 0.05$) were found among treatments, except for yolk color ($p < 0.05$). It was concluded that the organic calcium source *L. calcareum* maintained similar levels of productivity and egg quality as the conventional inorganic source. Among the tested inclusion levels, 2.5% of the algae proved to be the most efficient.

Keywords: Calcareous Algae, Calcium And Eggs, Calcium

Influence of Pre-Placement Feeding During Transport on Organ Development and Performance in Broiler Chickens (NU-08)

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Abstract:

One of the key factors influencing the zootechnical performance of broiler chickens is weight gain during the first week of life, a period in which birds can increase their body weight up to 4.5 times their hatch weight. This early growth rate positively affects subsequent performance indicators such as feed conversion ratio (FCR), daily weight gain (DWG), and mortality rates. A crucial factor for early development is the duration of post-hatch fasting, making the time between hatch and first feed access highly important. The objective of this study was to evaluate whether feed consumption before chick placement, during transportation from the hatchery to the farm, influences organ development, zootechnical performance, and mortality in broiler chickens. Chicks received commercial feed at the hatchery, and placement occurred between March and May 2021 and from November 2021 to January 2022. Assessments included body weight and feed intake measurements, necropsies at 7 days of age for organ biometrics and weights, ileum sampling for histomorphometric analysis, and final evaluation of zootechnical performance parameters at slaughter, including feed conversion ratio (FCR), daily weight gain (DWG), and production efficiency index (PEI), as well as mortality rates at 7 days and at the end of the production cycle. Regarding farm distance from the hatchery, properties were selected within three distance ranges (short, medium, and long). Each farm received one treatment group without pre-placement feeding and one with feed provision, under the same housing and management conditions. For FCR, PEI, and DWG, data from 130 slaughtered Ross broiler flocks, totaling 2,509,500 placed birds, were analyzed. For organ biometrics and histological parameters, 42 and 66 flocks were evaluated, respectively. The experimental design was a completely randomized 2 × 3 factorial scheme, with Tukey's test for means comparison using the SAS statistical software package. Statistically significant differences were found in chick weight at placement, crop, liver, and residual yolk weights in the fed groups, as well as in histomorphometric measurements of villi and crypts. However, no significant differences were observed in overall zootechnical performance. Further studies exploring other aspects of early nutrition are necessary to advance knowledge in this field, which has both economic and animal welfare implications.

Keywords: Early Nutrition, Post-Hatch, Broiler Transport



In Ovo Lipase Injection on Glucose and Serum Lipase Levels in Broiler Chickens (NU-09)

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Abstract:

Lipase is an essential enzyme for lipid digestion, acting in the breakdown of triglycerides into free fatty acids and monoglycerides, thereby facilitating their intestinal absorption. In addition to its digestive function, dietary lipase supplementation in broiler chickens may influence metabolic parameters, such as blood glucose levels and serum lipase activity. However, there are no previous reports of lipase being administered via *in ovo* in broiler embryos. Thus, this study aimed to evaluate the effects of different doses of lipase administered *in ovo* (0, 500, and 1,000 U/mL), diluted in vaccine diluent and injected on day 18 of incubation into 3,744 fertile Cobb500 eggs. All eggs were vaccinated against Marek, Gumboro, and Newcastle using an automatic vaccinator (Embritech), together with the different lipase doses. After hatching, 396 first-quality chicks (198 males and 198 females) were selected and equally distributed among treatments (132 birds/treatment), allocated in six cages per treatment, with three replicates per sex, simulating a mixed flock. Birds were kept under controlled environmental conditions and fed according to Cobb (2018) nutritional recommendations. On days 0, 1, 2, 3, 4, and 7 of age, one bird per replicate was euthanized by decapitation for blood collection (2 mL) on days. The blood was stored in clot activator tubes, centrifuged, and the obtained serum was transferred to 200 µL microtubes. Biochemical analyses were performed using an automatic analyzer (Smart 200 Plus) with specific kits for each parameter, and results were expressed in mg/dL for glucose (Glucose Plus Vet) and U/L for lipase (Lipase Vet). Glucose and plasma lipase data were analyzed by ANOVA (R Software, 2023), followed by Tukey's test ($p \leq 0.05$). Significant differences in glucose levels ($P \leq 0.05$) were observed only on day 0, with the highest value observed in the control group (199.58 mg/dL), the lowest in the 1000 U/mL group (177.31 mg/dL), and no difference in the 500 U/mL group (191.48 mg/dL). No differences were observed on the following days ($p > 0.05$). Regarding plasma lipase, levels were significantly higher ($p \leq 0.05$) on day 3 in chicks that received 500 and 1000 U/mL, with values of 2.81 and 2.62 U/L, respectively, compared to the control (1.32 U/L). No differences were found on days 4 and 7 ($p > 0.05$). It is concluded that *in ovo* lipase injection shows potential to modulate early energy metabolism in newly hatched chicks; however, its effects seem limited to the immediate post-hatch period, highlighting the need for further studies to better understand the duration and mechanisms of these physiological responses.

Keywords: Hematological Parameters, Energy Metabolism, Enzyme Supplementation

***In Ovo* Lipase Injection At Day 18 of Incubation on Hatching Results in Broiler Chickens (NU-10)**

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Abstract:

The *in ovo* injection of nutrients, vitamins, and amino acids to improve the growth performance of broiler chickens has been explored. Among the possible alternatives, the use of the enzyme lipase stands out, considering the large amount of lipids present in the egg yolk. Therefore, the objective was to evaluate the effects of different levels of lipase inoculated *in ovo* on hatchability and chick quality at pulling. A total of 3,744 eggs from a 35-wk-old Cobb500 breeder flock were incubated in a commercial hatchery. On the 18 day of incubation (ED18), candling was performed to select eggs with embryonic development, which were then allocated into three treatments with 13 replicates (incubation tray with 96 eggs) in a completely randomized design. Three levels of lipase were used: 0, 500 and 1000 U lipase, which were inoculated into the amniotic cavity on day ED18, along with Marek's, Gumboro, and Newcastle vaccines using an automatic injector from Embritech. Incubation lasted a total of 507 hours, and the unhatched eggs were counted to calculate hatchability. Subsequently, twenty chicks per treatment, totaling 60 birds, were selected for evaluation of chick weight at pulling. The same chicks were euthanized by cervical dislocation to collect data on yolk sac weight and chick weight without the yolk sac. The data were subjected to ANOVA, followed by Tukey test ($p < 0.05$). Treatments did not affect hatchability, whose average values were 90.63, 91.43, 91.35% for the treatments with 0, 500, and 1,000 U lipase respectively. The analyses of chick weight at pulling (37.65, 36.00, 36.20g), yolk sac weight in relation to chick weight (10.59, 10.03, 10.85%) and chick weight without the yolk sac (33.55, 32.08, 32.09g) also did not present significant results ($p > 0.05$). The *in ovo* lipase injection was not able to influence hatchability or chick weight, refuting the hypothesis that it could have released more energy to assist in the hatching process.

Keywords: Enzyme, *In Ovo* Nutrition, Lipids

Diets With Different Energy Levels For Commercial Laying Hens During the Growing Phase and their Effects on Zootechnical Performance (NU-11)

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Abstract:

Dietary energy is a key component in poultry production, as it directly impacts the performance of the birds. Therefore, evaluating energy levels in diets, especially for hens at different ages, is essential to ensure optimal productive outcomes. Considering that birds regulate their intake based on the energy density of the diet, understanding this relationship is crucial. This study evaluated the effects of different energy levels on the performance of laying hens during the growing phase. A total of 960 Lohmann LSL-LITE® pullets, 10 weeks old with an average body weight of 800 g, were randomly assigned in a completely randomized design with two dietary treatments (2780 and 3080 kcal/kg of AMEn) and eight replicates of 60 birds per experimental unit. The birds were housed in littered floor pens (10 cm wood shavings) with tubular feeders and bell drinkers. The diets were provided from 10 to 15 weeks of age. Performance parameters (feed intake, body weight, and feed conversion ratio) were recorded weekly for each replicate. Data were analyzed using the T-test to assess statistical differences between treatments ($p \leq 0.05$). From the 10th to the 16th week, birds fed the 2780 kcal/kg diet consumed more feed (3054 g/bird) compared to those receiving the 3080 kcal/kg diet (2791 g/bird) ($p \leq 0.001$). No significant differences were observed in initial or final body weight ($p \geq 0.05$), with averages of 797.0 and 802.0 g (initial) and 1196.0 and 1198.0 g (final) for the 2780 and 3080 kcal/kg treatments, respectively. However, dietary energy level significantly affected feed conversion: birds fed the lower-energy diet showed poorer feed conversion (2.53) compared to those on the higher-energy diet (2.34) ($p \leq 0.001$). It is concluded that dietary energy density significantly influences feed intake and feed conversion during the growing phase, and is therefore a determining factor in the zootechnical performance of commercial laying hens.

Keywords: Albumen, Poultry, Haugh Unit, Poultry, Feed Conversion, Soybean Oil

Energy Levels For Commercial Pullets From 10 to 15 Weeks of Age and the Effects on Egg Quality (NU-12)

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Abstract:

Energy is the most expensive component in poultry feed formulation, making it essential to establish appropriate levels in layer diets, as it directly influences productivity and can also affect egg characteristics. This study evaluated the effects of two dietary energy levels on egg quality at the onset of the laying period. During the rearing phase (10 to 15 weeks of age), 960 Lohmann LSL-LITE pullets with an average body weight of 800 g at 10 weeks were used, distributed in a completely randomized design. The birds were fed two experimental diets containing 2780 or 3080 kcal/kg of AMEn, with eight replicates of 60 birds each, throughout the rearing period. In the production phase (17 to 30 weeks), 640 birds were randomly selected from the previous replicates, maintaining the same experimental design with eight replicates of 40 birds each, randomly chosen from each rearing replicate. At 16 weeks of age, the pullets were transferred to California-type cages equipped with trough feeders and nipple drinkers, and began receiving a single standard diet. In the 20th week, eight egg samples per replicate were randomly collected to assess quality parameters: shell thickness and strength, Haugh unit, albumen weight, and albumen diameter. Data were analyzed using the t-test, adopting a 5% significance level ($p \leq 0.05$). The results indicated that birds fed higher-energy diets during the rearing phase showed greater albumen diameter (6.02 cm) and higher Haugh units (99.95 HU) compared to those fed lower-energy diets (5.82 cm and 98.34 HU, respectively). No significant differences were observed for the other evaluated parameters ($p \geq 0.05$). It is concluded that the energy level of the diet provided during the rearing phase positively influences internal egg quality at the beginning of the laying period.

Keywords: Albumen, Poultry, Haugh Unit

Calcium Butyrate Supplementation Improves Performance in Commercial Laying Hens After 46 Weeks of Age (NU-13)

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Abstract:

Modern egg production focuses on extending laying cycles while maintaining high productivity and egg quality. Gut health plays a vital role in nutrient absorption. Encapsulated calcium butyrate is widely used to support intestinal barrier function. This study aimed to evaluate its effects on egg production and intestinal histomorphometry in commercial laying hens in Northeastern Brazil. The study was conducted on a commercial farm in Pernambuco, Brazil, using two flocks of 20,000 Lohmann White hens (totaling 40,000), housed and fed under identical conditions. The study was divided into two phases over time: Phase 1 (1 to 45 weeks, without supplementation) and Phase 2 (46 to 63 weeks), with calcium butyrate (300 g/ton) added to the diet from week 46 onward, only in Phase 2. Therefore, only a before-and-after comparison was performed and no statistical analysis was possible. Egg production and intestinal histomorphometry (duodenum, jejunum, and ileum) were evaluated at week 45 (pre-supplementation) and at week 53 (after 8 weeks of supplementation). Ten birds were euthanized for collection of intestinal samples, with 4 cm segments collected according to Alvarenga *et al.* At 30 weeks, average egg production was 96.0% (flock A) and 95.63% (flock B). After the peak, egg production declined 4% weekly until week 38 above the 0.2% expected by breed standards. From week 38 onward, average production was 92.20% (flock A) and 91.65% (flock B). After calcium butyrate supplementation at week 46, egg production increased weekly by 0.52% (flock A) and 0.44% (flock B), reaching 96.73% and 93.90%, respectively, at week 53. From the perspective of additive supplementation, the added variable was calcium butyrate, and based on the farm's annual history, we could think the improvement maybe by to calcium butyrate. Supplementation continued until week 63, with production remaining above breed standards. To explore physiological changes, intestinal histomorphometry was analyzed in 10 hens from batch A. After supplementation, an increase in villus height and crypt depth was observed in all intestinal segments: Duodenum: +11% villus height; Jejunum: +7% villus height; Ileum: +11% crypt height. Crypt depth also increased in all regions. Hens at 53 weeks of age had greater villus height than those at 45 weeks, suggesting better mucosal health due to calcium butyrate.

Keywords: Butiric Acid, Egg Production, Intestinal Histomorphometry, Supplementation

Impact of Dietary Fiber Sources on Cecal Microbiota Composition and Diversity in White Laying Hens (NU-14)

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Abstract:

This study evaluated the effects of various dietary fiber sources on the cecal microbiota of Lohmann LSL-Lite laying hens. Ninety-six health hens, 55 weeks old at the start of the experiment, were assigned to one of four dietary treatments for 17 weeks: a control diet without added fiber (corn-soybean meal base with 16.0% crude protein, 2,690 kcal/kg metabolizable energy, 2.0% total fiber, 4.3% calcium, 0.25% available phosphorus, 0.74% digestible lysine, and 0.36% digestible methionine); a mixed fiber diet (control + 2.2 g/kg wheat bran); an insoluble fiber diet (control + 1.0 g/kg cellulose); and a soluble fiber diet (control + 1.0 g/kg pectin). Each treatment had six replicates, with four hens per replicate. At 72 weeks of age, cecal content samples were collected from six birds per treatment, selected based on body weight within $\pm 5\%$ of the treatment group average, resulting in 24 samples (one per cage). Hens were euthanized by cervical dislocation for cecal content collection. Microbial analysis was conducted using 16S rRNA sequencing and bioinformatics pipelines, including QIIME 2 (v2020.6) and the phyloseq R package. Sequencing data were processed with DADA2. Alpha diversity analysis showed that microbial richness and diversity varied significantly across treatments. Hens fed diets containing wheat bran and cellulose exhibited higher species richness, reflected in elevated Shannon diversity indices. Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances revealed distinct microbiota compositions between the control and fiber-supplemented groups, highlighting the modulatory effects of dietary fiber on microbial communities. The dominant phyla across all treatments included Bacteroidetes, Campilobacterota, Firmicutes, and Spirochaetota, with a marked increase in Bacteroidetes in fiber-supplemented groups. At the genus level, fiber supplementation enhanced the abundance of key genera such as Bacteroides, Alloprevotella, Faecalibacterium, and Rikenellaceae RC9, compared to the control group ($p < 0.05$). In conclusion, all the groups that received fiber-supplemented diets showed greater microbial diversity and an abundance of potentially beneficial bacteria, indicating positive effects on gut health.

Keywords: Bacteroides, Cellulose, Wheat Bran

***In Ovo* Lipase Injection At Day 18 of Incubation on Post-Hatch Metabolizability in Broiler Chickens (NU-15)**

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Abstract:

Lipase is an enzyme that acts on the metabolism of lipids, breaking them down into fatty acids and glycerol. Since egg yolk is rich in lipids, *in ovo* inoculation of lipase may enhance the utilization of these nutrients during embryonic development and in the early days of life. Therefore, the aim of this study was to evaluate the effects of different levels of lipase injected *in ovo* on metabolizability parameters. A total of 396 chicks, 198 males and 198 females, were used in a completely randomized design with three treatments, six replicates, and 22 chicks per replicate. Three lipase concentrations (0, 500, and 1000 U/mL) were tested and applied to the amniotic cavity on the 18 day of embryonic development (ED18), along with vaccines against Marek's disease, Gumboro, and Newcastle, using an automatic injector from the company Embritech. All chicks received the same pre-starter feed formulated with 2,974 kcal/kg of metabolizable energy and 22.05% crude protein (Cobb, 2018). The total excreta collection method was used from days 4 to 7 of age, with excreta collected directly from the trays of each cage once a day. The feed offered was weighed at the beginning and end of the collection period to determine feed intake. The collected material was ground in a knife mill with a 1 mm screen, and analyses were performed to determine dry matter, crude protein, and ether extract content. Based on feed intake, excreta output, and laboratory analyses, the apparent metabolizability coefficients of dry matter (ADCDM), crude protein (ADCCP), and ether extract (ADCCE) were calculated using the formula: nutrient metabolizability (%) = (nutrient ingested (g) - nutrient excreted (g)) * 100. The data were subjected to ANOVA, followed by Tukey test ($p < 0.05$). There were no differences ($p > 0.05$) between the treatments with 0, 500, and 1000 U/mL lipase for ADCDM (74.20, 73.99, 74.05%, respectively) and ADCCP (86.75, 85.70, 85.03%, respectively). However, there was a difference ($p < 0.05$) for ADCCE, with the treatment of 500 U/mL lipase (92.20%) being more efficient than the 0 and 1000 U/mL lipase treatments (89.05 and 89.52%, respectively). These results suggest that the administration of 500 U/mL lipase *in ovo* may be a way to optimize lipid metabolism in the early days of a chick's life, although further studies are needed to assess the effectiveness of this strategy in later developmental stages.

Keywords: Enzyme, Ether Extract



Increasing Phosphorus Levels With A Fixed Calcium-To-Phosphorus Ratio of 1.65 in the Pre-Starter Diet of Broiler Chickens (NU-16)

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Abstract:

Establishing appropriate levels of available phosphorus (aP) and an optimal calcium (Ca) to phosphorus (P) ratio is essential for maximizing production efficiency and reducing costs in broiler chicken production. This study aimed to evaluate the effects of increasing aP levels, maintaining a fixed Ca:P ratio of 1.65, in the pre-starter diet of broilers. The experiment was conducted at the Animal Metabolism Laboratory (LAMA/UFMG), using 400 1-day-old male Cobb500 chicks. Birds were randomly assigned to four dietary treatments with five replicates per treatment and 20 birds per replicate. The treatments were: T1 (0.30% aP and 0.50% Ca), T2 (0.40% aP and 0.66% Ca), T3 (0.50% aP and 0.83% Ca), and T4 (0.60% aP and 0.97% Ca). Diets were fed from day 1 to day 7, after which all birds received standard diets formulated to meet the nutritional requirements (Cobb, 2024). Performance parameters (feed intake, body weight, and feed conversion ratio) were measured at 7, 14, 21, 28, and 35 days of age. Apparent digestibility of Ca and aP was assessed at 7 days using total excreta collection. Tibia weight and length were measured at 7, 14, and 35 days, and femur bone strength at 14 and 35 days. Data were analyzed using ANOVA (R software, version 2023), and means were compared with Tukey's test ($p \leq 0.05$). At 7 days of age, T3 birds exhibited higher feed intake than T4 birds ($p < 0.05$), with intermediate values for T1 and T2 ($p > 0.05$). Body weight at 7 days was higher in T3 compared to T1 ($p < 0.05$), while T2 and T4 no differ ($p > 0.05$). Feed conversion ratio was improved in T2, T3, and T4 compared to T1 ($p < 0.05$). No significant differences in performance were observed at later ages ($p > 0.05$). Apparent digestibility of P and Ca was significantly lower in T4 compared to the other treatments ($p < 0.05$). No significant treatment effects were found on bone variables ($p > 0.05$). Under a fixed Ca:P ratio of 1.65 in the pre-starter diet, the inclusion of 0.50% available phosphorus is recommended to optimize 7-day performance without compromising nutrient digestibility or bone health.

Keywords: Bone Quality, Minerals, Nutrition



Effects of Increasing Phosphorus Levels With A Fixed Calcium-To-Phosphorus Ratio of 2.09 in the Pre-Starter Diet of Broiler Chickens (NU-17)

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Abstract:

Phosphorus (P) is an essential mineral for bone health and metabolism in poultry, strongly interacting with calcium (Ca), with the Ca:P ratio being a key determinant of mineral bioavailability and utilization efficiency. This study aimed to evaluate the effects of increasing levels of available phosphorus (Pd), under a fixed Ca:P ratio of 2.09, in the pre-starter diet of broilers. The experiment was conducted at the Animal Metabolism Laboratory (LAMA), School of Veterinary Medicine, Federal University of Minas Gerais (UFMG), using 400 one-day-old male Cobb500® chicks. Birds were randomly assigned to four dietary treatments with five replicates per treatment and 20 birds per replicate. The treatments were: T1 (0.30% Pd and 0.63% Ca), T2 (0.40% Pd and 0.83% Ca), T3 (0.50% Pd and 1.05% Ca), and T4 (0.60% Pd and 1.25% Ca). Diets were fed from day 1 to day 7, after which all birds received standard diets formulated to meet the nutritional requirements of the strain. Performance parameters (feed intake, body weight, and feed conversion ratio) were measured at 7, 14, 21, 28, and 35 days of age. Apparent digestibility of Ca and Pd was assessed at 7 days via total excreta collection. Tibia weight and length were measured at 7, 14, and 35 days, and femur bone strength at 14 and 35 days. Data were analyzed using ANOVA (R software, version 2023), with means compared using Tukey's test ($p \leq 0.05$). No significant effects of treatments were observed on performance at any evaluated age ($p > 0.05$). Apparent phosphorus digestibility was lower in birds receiving T4 (0.60% Pd) compared to those on lower phosphorus levels (T1, T2, and T3; $p < 0.05$). A similar trend was observed for calcium digestibility, which decreased as dietary levels increased. No significant differences were observed among treatments for bone variables ($p > 0.05$). Thus, under a fixed Ca:P ratio of 2.09, any level of available phosphorus between 0.30% and 0.60% can be used in pre-starter diets for broilers without compromising performance, digestibility, or bone quality. However, higher phosphorus levels may reduce digestibility and increase environmental excretion without providing additional productive benefits.

Keywords: Bone Development, Minerals, Nutrition, Poultry

Influence of Ph on Phytase Activity on Bone Development and Mineralization in Chicks During the First Week of Life (NU-18)

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Abstract:

The newly hatched chick's digestive tract is immature and must undergo dramatic changes. The pH of the proventriculus and gizzard is still not low enough for complete digestion, and there is still no additional mechanical processing in the gizzard. A reduction in foregut pH has been reported to increase phytase activity on phytate hydrolysis and enhance the release of calcium (Ca) and phosphorus (P). A broiler chick study was conducted over 10 days to evaluate the effect of pH on phytase activity and bone mineralization. A total of 1,920 male Cobb chicks were distributed in a randomized trial (16 replications of 40 birds). Treatments included a negative control (NC): phytase-free with reduced Ca (1.5g/kg) and P (1.4g/kg), positive control (PC): with phytase (100g/ton) and reduced Ca (1.5g/kg) and P (1.4g/kg), a positive control on top (PC on top): with phytase (100g/ton) and without Ca and P reduction. The values of AP were: 0.56, 0.30 and 0.44% and for to Ca: 1.02, 0.75 and 0.90%. At 1, 3, 5, 7, and 9 days of age, 16 birds/ treatment were sacrificed for gizzard pH evaluation and analysis of bone development and mineralization parameters. Results were analyzed using polynomial regression. Gizzard pH evaluation as a function of age showed a decreasing linear trend for the NC and PC on top diets, and a quadratic effect for the PC diet, with a maximum point at 2.97 days. Proventriculus pH showed a quadratic effect for all three diets, with peak values of 4.62, 4.79, and 4.78 for NC, PC, and PC on top, respectively. Ca levels were not altered by the NC diet, while the PC diet showed a decreasing linear trend and the PC on top diet a quadratic effect. For serum P, a quadratic effect was observed in the NC diet, and a linear increasing trend in response to the PC on top diet. The PC diet did not affect P levels with advancing age. Alkaline phosphatase activity showed a quadratic effect for the NC and PC on top diets, with no effect observed for the PC diet. Bone ash analysis and radiographic densitometry of the cortical and trabecular regions showed a linear increasing trend for all three diets. Bone resistance presented a quadratic effect, with minimum points at 2.60, 1.91, and 1.82 days for the NC, PC, and PC on top diets, respectively. Live weight followed the same trend observed in bone breakage resistance, indicating a correlation between these two variables. The use of phytase assuming the availability of 1.5 g/kg of Ca and 1.4 g/kg of P in the pre-starter broiler diet may be overestimating the chicks' digestive capacity during the post-hatching phase. Meeting Ca and P requirements without accounting for the effect of phytase on Ca and P release during the first 10 days of life may be a strategy to reduce future locomotor problems in broilers.

Keywords: Gizzard, Phytate Hydrolysis, Phosphatase Alkaline, Densitometry

Isoleucine Is the Most Effective Branched-Chain Amino Acid For Improving Broiler Breast Yield ($p<0.001$): Systematic Review and Meta-Analysis (NU-19)

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Abstract:

Amino acid supplementation supports feed formulation strategies based on the ideal protein concept. Among the available feed-grade products, branched-chain amino acids (BCAA), particularly valine (VAL), have been increasingly used in corn-soybean meal diets, often representing the fourth or fifth limiting amino acid. All three BCAA, collectively, account for over one-third of total broiler muscle protein and play key roles in preventing proteolysis and promoting muscle accretion. Therefore, supplementing not only VAL but also LEU and ILE may enhance muscle development in broilers. This meta-analysis aimed to compare the individual effects of VAL, LEU, and ILE supplementation on broiler breast yield (BBY). A systematic review was conducted using the search algorithm (chickens OR broiler OR Gallus gallus) AND (valine OR leucine OR isoleucine OR branched-chain amino acids OR BCAA) across Pubmed, Science Direct, Scielo, Scopus and Web of Science, on September 23, 2024. Eligible studies included trials with Cobb, Ross or Hubbard broilers, aged 21 days or older, comparing BCAA-supplemented versus non-supplemented diets. Qualitative and quantitative data were extracted from the included studies and organized by study origin, publication year, broiler sex, genetic line, age, diet composition, BCAA type, source, and inclusion in the feed. Effect sizes were calculated as raw mean differences (RMD) between BCAA-supplemented and not-supplemented groups. Meta-analyses were performed separately for each BCAA, using a REML model. Heterogeneity was explored through meta-regression and subgroup analyses. A total of 11 studies met all the eligibility criteria for VAL, 5 for LEU and 6 for ILE. Each study provided a different number of comparison lines (k) and birds (n) to the dataset, totalizing: VAL (k=61; n=15404), LEU (k=26; n=6524), ILE (k=22; n=7366). Meta-analysis showed that all BCAA increased BBY, but in different intensities: VAL (RMD=0.26%; $p=0.002$); LEU (RMD=0.25%; $p=0.003$), ILE (RMD=1.33%; $p<0.001$). Although this effect of LEU occurred independently of other factors ($p=0.664$), meta-regression demonstrated that the effect of VAL was moderated mainly by the broiler sex ($R^2=31.16\%$) and publication year ($R^2=17.67\%$), while ILE effect by broiler genetic line ($R^2=75.54\%$) and ILE inclusion in the feed ($R^2=27.49\%$). In summary, significant effects of VAL on BBY were detected only until 2020 and, exclusively, in females. Additionally, ILE effects was assessed only in Cobb and their benefits on BBY were higher at inclusion rates between 1.0 and 1.50 g/kg. In conclusion, there is evidence that all BCAA improves BBY, but ILE is the most effective one. More intense responses are obtained in females receiving diets with 1.0 to 1.5 g/kg of ILE supplementation.

Keywords: Bcaa, Carcass Quality, Chickens, Ideal Protein, Muscle Development

Systematic Review and Meta-Analysis on the Effects of Leucine Supplementation on Broilers Growth Performance and Carcass Traits (NU-20)

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Abstract:

Branched-chain amino acids, especially leucine (LEU), have been identified as important signaling molecules for tissue deposition and protein breakdown. However, literature results are discrepant regarding the actual benefits of LEU supplementation on broilers growth performance and carcass quality. This meta-analysis aimed to quantify, with greater precision and accuracy, the effect of LEU supplementation on broilers daily weight gain (DWG), feed conversion ratio (FCR), carcass yield (CYD) and abdominal fat deposition (FAT). For the systematic review, the search strategy (chickens OR broiler OR Gallus gallus) AND (valine OR leucine OR isoleucine OR branched chain amino acids OR BCAA) was applied to Pubmed, Science Direct, Scielo, Scopus and Web of Science, on September 2024. Primary studies using Cobb, Ross, or Hubbard broilers aged 21 days or older, comparing birds fed LEU-supplemented vs. LEU-not-supplemented diets, were selected. Data on the outcomes of interest were extracted and set in an electronic spreadsheet according to study origin, publication year, broilers sex, genetic line and age, experimental diets composition, LEU source and inclusion rate. Data were submitted to meta-analysis using REML model and the inverse of variance method to weight studies according to the precision of their estimates. Leucine effect size was calculated using the raw mean difference (RMD) between LEU-supplemented and LEU-not-supplemented birds. Significant heterogeneity between studies were explained by meta-regression and subgroup analysis. A total of 6 studies, from 2957 retrieved, were included in this meta-analysis. They comprised a dataset of k=31 lines of comparison and a total population of n=7424 broilers. Overall, LEU supplementation increased DWG (RMD=0.67 g/day; $p<0.001$; k=31) and decreased FAT (RMD=-0.23%; $p=0.017$; k=19), without affecting FCR (RMD=0.002; $p=0.761$; k=31). However, LEU supplementation significantly reduced CYD (RMD=-0.95%; $p=0.002$; k=26). No heterogeneity ($p=0.07$) was detected between studies for DWG, meaning its overall result can be applied universally. However, significant heterogeneity ($p<0.001$) was detected for all other variables. Meta-regression identified that the supplementation of other BCAA together with LEU and LEU:LYS ratio in the feed were moderators that significantly affected LEU effect on FCR, CYD and FAT. In short, better responses were observed when LEU was supplemented alone, at LEU:LYS ratios between 0.91 and 1.23. In conclusion, although LEU supplementation improves broiler DWG independently of any other factor, it reduces CYD by nearly 1%, which should be further investigated to optimize supplementation protocols.

Keywords: Branched-Chain Amino Acids, Carcass Yield, Chickens, Ideal Protein, Weight Gain



Symbiotic As A Tool to Reduce Intestinal Permeability in Broiler Breeders (NU-21)

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Abstract:

The use of symbiotics in poultry farming has been highlighted as a strategy for promoting intestinal health and improving performance. These additives, composed of a combination of probiotics — live microorganisms that, when administered in adequate quantities, confer benefits to the host — and prebiotics, such as mannan oligosaccharides (MOS) and beta-glucans, which are substrates selectively used by beneficial microorganisms, have positive effects on modulating the microbiota, strengthening the intestinal barrier and stimulating the immune system. In broiler breeders, the post-transfer period up to the peak phase is critical for enteric health, with intestinal inflammation and dysbiosis being common, compromising nutrient absorption, increasing eggshell contamination and negatively impacting progeny quality. Inflammation of the intestinal environment causes dysfunction of the tight junctions between enterocytes, which results in increased intestinal permeability, allowing the translocation of antigens, toxins and pathogens, and triggering systemic inflammatory responses. This study evaluated the efficacy of a multistrain microencapsulated symbiotic (*Saccharomyces cerevisiae* (CCT 7948), *Bacillus spizizenii* (CCT 8193), *Bacillus amyloliquefaciens* (CCT 8194), *Bifidobacterium bifidum* (ATCC 29521), *Lactobacillus acidophilus* (CCT 7947), mannan oligosaccharides, beta-glucans and inulin) in maintaining the integrity of the intestinal barrier of broiler breeders during the production phase. The study was conducted in a nucleus with two poultry houses, one control group (G1), fed a standard diet, and the other (G2), supplemented with a symbiotic at a dosage of 200 g/ton. Supplementation began at 34 weeks of age and was maintained for 90 days. At the 46th week, oral inoculation of FITC-dextran (0.5 ml/bird) was performed, followed by blood collection from five birds per group for evaluation of serum fluorescence in a spectrophotometer. The comparison between groups was performed using Student's t-test, adopting a significance level of $p < 0.05$. The results demonstrated that the birds in the control group (G1) presented higher detection of FITC-dextran in the serum (0.248 $\mu\text{g/ml}$), compared to the treated group (G2) (0.190 $\mu\text{g/ml}$) ($p < 0.05$), indicating greater intestinal permeability and loss of barrier function in the non-supplemented group. Supplementation with symbiotic exerted a protective effect, preserving intestinal integrity, reducing permeability and, consequently, contributing to the maintenance of enteric health. These findings reinforce the importance of symbiotics as a nutritional tool in supporting intestinal health of broiler breeders.

Keywords: Probiotic, Intestinal Health, Leaky Gut, Intestinal Permeability, Broiler Breeder, Prebiotic

Response of Broiler Chickens to Phytase Supplementation in Starter Diets: Performance, Development and Bone Mineralization Until the Slaughter Age (NU-22)

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Abstract:

During the first few weeks of the chicks' lives, their gastrointestinal tract is still immature, with low hydrochloric acid production and poorly developed gizzard musculature, which can impair the action of phytase in hydrolyzing the ester bonds of phytic acid and consequently release phosphorus (P). As a result, the absorption of P and calcium (Ca) by chicks can be decreased, which can harm bone development and mineralization in the early stages of life and contribute to the occurrence of locomotor problems at slaughter age. A broiler chicken study was conducted from 1 to 42 days of life to evaluate performance, bone development, and mineralization in response to the addition of phytase supplementation to starter diets. 1920 male Cobb chicks were distributed in a completely randomized trial (16 replications of 40 birds). Treatments included a negative control (NC): phytase-free and reduced Ca (1.5g/kg) and P (1.4g/kg); a positive control (PC): with phytase (100g/ton) and reduced Ca (1.5g/kg) and P (1.4g/kg); a positive control on top (PC on top): with phytase (100g/ton) and without Ca and P reduction. From the 11th day onward, all birds received the PC diet, meeting the nutritional requirements for the growth and slaughter phases. Productive performance was evaluated weekly, and 16 birds/treatment were sacrificed for bone assessment at 10, 21, and 42 days of age. Statistical data were analyzed by ANOVA and means were compared by Tukey test. In the period from 1 to 10 days, the PC and PC on top diets showed similar results ($p>0.05$) for feed consumption, weight gain, and feed conversion, but were superior ($p<0.05$) compared to NC. At 21, 28, 35, and 42 days of age, the result was similar to that observed at 10 days of age. Regarding bone development and mineralization analyses at 10 days of age, the evaluations of bone ash, Seedor index, diameter, and cortical bone radiographic density showed similar results between the PC and PC on top diets and superior to the NC diet. The values obtained for trabecular bone radiographic density and cortical thickness were intermediate for the PC on top diet and higher for PC compared to the NC diet. From 10 to 21 days, when all birds were fed the same diet, the bone ash content of birds fed the PC on top diet was equal to that obtained from birds fed the PC diet; however, the bone ash content was higher for the PC diet than for the NC diet. The Seedor index maintained the same behavior observed at 10 days, with superior results for the PC and PC on top diets. The other parameters were not influenced by the diets. No significant differences were observed in bone resistance to breakage between the three diets at any age. At 42 days of age, the diets did not affect any of the bone parameters evaluated.

Keywords: Phosphorus, Phosphatidylinositol, Weight Gain, Gizzard, Trabecular Bone Radiographic Density

Evaluation of High Protein Distiller Dried Grain Inclusion in Diets For Broilers (NU-23)

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Abstract:

Efforts have been made to identify ingredients that meet broilers' nutritional requirements and reduce feed costs. Dried distiller grains (DDG) are a coproduct of the ethanol industry. They have been included in poultry diets due to their potential to partially replace high-protein ingredients, such as soybean meal. One of the new corn co-products being produced is high protein distiller dried grains (HP-DDG), which contain $\geq 40\%$ of CP (Crude Protein), approximately 85% of the energy value of corn, and low fiber levels minimizing the impact of insoluble fiber on nutrient absorption and animal performance. Processing consists of removing fibers before starch fermentation and it controls the quality and quantity of separated materials. The goal of this study was to determine the optimal level of dietary HP-DDG inclusion of this new source in broiler diets on productive performance and carcass yield. 3010 male broiler Ross AP95 chicks were distributed in a completely randomized controlled trial with 5 treatments, and 14 replications with 43 birds each: totaling 602 birds per treatment. The trial period was 43 days and three nutritional programs were used with the following percentages of HP-DDG inclusions per treatment: starter mash feed (1 to 21 days - T1 0%, T2 3%, T3 5%, T4 7% and T5 9%), grower mash feed (22 to 33 days - T1 0%, T2 6%, T3 8%, T4 10% and T5 12%), and finisher mash feed (34 to 43 days of age - T1 0%, T2 9%, T3 11%, T4 13% and T5 15%). Productive performance was evaluated weekly, and 56 birds/treatment were sacrificed for carcass yield and commercial meat cuts at 43 days of age. Statistical data were analyzed by ANOVA and means compared by Tukey test at 5% probability, using the SAS program. The birds that received diets with a 9% HP-DDG inclusion showed higher ($p < 0.05$) feed consumption for the initial period (1 to 7 days of age) compared to the birds fed with a 7% HP-DDG inclusion. However, the other inclusions showed no significant differences ($p > 0.05$). For the weekly performance results (8 to 14, 15 to 21, 22 to 28, 29 to 35, and 36 to 42 days) and for the periods per phase (1 to 14, 1 to 21, 22 to 35, 1 to 42 days) the birds' performance was similar between the treatments. The groups that received diets with HP-DDG up to the inclusion level of 7, 10, and 13%, in the initial, growth and slaughter phases, respectively, presented carcass yield, breast cut yield, and absolute and relative weight of abdominal fat similar to the group of animals that received the reference diet ($p < 0.05$). The result of this study indicated that HP-DDG can be included at levels of up to 7% of the diet in the starter phase, 12% in the growth phase, and 13% in the end phase, without any negative impact on performance and carcass yield.

Keywords: Ethanol Industry, Productive Performance, Carcass Yield, Co-Product.



Does Genetic Line Influence the Effect of Isoleucine Supplementation on Broilers Growth Performance and Carcass Traits? A Systematic Review and Meta-Analysis (NU-24)

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Abstract:

Competition between branched-chain amino acids for intestinal absorption might compromise the fulfillment of broilers requirements. Therefore, using proper supplementation protocols is essential to maximize broilers productivity. This meta-analysis aimed to quantify, based on published results, the effect size of isoleucine (ILE) on broilers daily weight gain (DWG), feed conversion ratio (FCR), carcass yield (CYD) and abdominal fat deposition (FAT). For this, the algorithm (chickens OR broiler OR Gallus gallus) AND (valine OR leucine OR isoleucine OR branched chain amino acids OR BCAA) was applied to Pubmed, Science Direct, Scielo, Scopus, and Web of Science, on 09/23/2024. Primary studies, using Cobb, Ross or Hubbard broilers, aged 21 days or older, that compared birds fed ILE-supplemented and not-supplemented diets were selected. Qualitative and quantitative data were extracted and separated by study origin, publication year, broilers sex, genetic line and age, experimental diets composition, ILE source and inclusion rate. Isoleucine effect size was calculated using the raw mean difference between supplemented and not-supplemented groups. Meta-analysis was performed using REML model and significant heterogeneity between studies were explored by meta-regression and subgroup analysis. Of the 2957 retrieved studies, 8 met all the eligibility criteria and were included in this meta-analysis. They were published between 2004 and 2022, totalizing n=8786 broilers and generating a dataset with k=37 lines of comparison. Overall results revealed that ILE supplementation increased ($p<0.001$) DWG by 5.56 g/day, enhanced ($p<0.001$) FCR by 0.064 points, improved ($p<0.001$) CYD by 1.03% and decreased ($p=0.009$) FAT by 0.11%. Individually, only 4 primary studies had statistical power to detect ILE effects on DWG and FCR. None of the studies had sufficient statistical power to detect the effects of ILE on carcass traits, proving the strength of the meta-analytical model. Heterogeneity was not significant ($p=0.957$) for FAT, only. Meta-regression showed that broilers genetic line was the only moderator that influenced ($p<0.001$) ILE effect on both DWG ($R^2=59.35\%$), FCR ($R^2=34.43\%$) and CYD ($R^2=100\%$). Although Ross broilers increased more intensively DWG than Cobb when supplemented with ILE ($p<0.001$), Cobb broilers responded better than Ross to ILE supplementation on FCR ($p=0.001$). No Hubbard broilers were assessed in primary studies. Only one study used other genetic line than Cobb for CYD, preventing this subgroup analysis for this variable. Therefore, there is evidence in the literature that ILE supplementation improves DWG, FCR and CYD, also reducing FAT in commercial broilers. Genetic line is the moderator most involved in modulating birds responses to ILE.

Keywords: Abdominal Fat, Branched-Chain Amino Acids, Carcass Yield, Chickens, Ideal Protein

Effect of Lignocellulose Inclusion and Corn Grain on Broiler Productivity and Organ Biometry (NU-25)

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Abstract:

Feed characteristics, such as fiber content and particle, directly influence gastrointestinal development and productive performance in broilers. The degree of grain grinding affects not only feed processing quality but also influences its nutritional value, growth performance and gastrointestinal function. The inclusion of dietary fiber improves gizzard development, stimulates peristalsis, intestinal reflexes and digestive enzyme production, promoting digestion and nutrient absorption. The goal of this study was to evaluate the effect of lignocellulose supplementation and corn grind on zootechnical performance and organ biometry in broilers. 2064 one day old male broiler chicks were distributed in a completely randomized controlled trial in a 3 x 2 factorial design (3 levels of lignocellulose: 0%, 0,35% e 0,70%; and two corn grind s: 400 μ m and 980 μ m), totaling 6 treatments with 8 repetitions of 43 birds each. Productive performance was evaluated weekly through measurements of live weight, feed intake and feed conversion. For organ biometry, one bird per replicate was euthanized at 7, 21, and 42 days of age for the evaluation of yolk sac residue, proventriculus, gizzard, and intestinal weights, as well as intestinal length. Data collected were subjected to two-way analysis of variance with 5% significant level. At 7 days of age, birds fed 400 μ m corn exhibited a higher mean weight, weight gain and better feed conversion compared to those fed 980 μ m corn ($p < 0,05$). At 14 days, based on simple effects analysis, the combination of 0.35% lignocellulose and 400 μ m corn resulted in improved ($p < 0.05$) feed conversion, as well as higher ($p < 0.05$) mean weight and weight gain compared to the other diets. At 21 days, birds receiving the finer corn particle presented greater ($p < 0.05$) weight gain than those receiving the coarser grind. At 42 days, better ($p < 0.05$) feed conversion rates and mean weight were noted using a 400 μ m corn and 0,35% lignocellulose combination. Organ biometry at 7 days indicated that a 0,70% lignocellulose inclusion and 980 μ m corn resulted in increased intestinal length. At 21 days, proventriculus weight was greater in chickens that consumed 400 μ m corn, and at 42 days, a significant increase in gizzard weight was noted in birds fed 980 μ m corn. Corn particle has proven to be an influential factor in broiler growth performance and gastrointestinal development. Insoluble fiber inclusion stands out as a promising strategy, particularly when combined with finely ground diets. Therefore, nutritional programs that integrate fine particle s with adequate fiber levels emerge as effective tools to enhance intestinal health and optimize broiler productive performance.

Keywords: Insoluble Fiber, Particle Size, Nutrient Digestibility, Gizzard, Growth Performance



Coarse Particle Inclusion and Lignocellulose-Rich Fiber Addition in Feed Benefit Gut Health of Broiler Chickens (NU-26)

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Abstract:

Physical feed structure and dietary fibers, such as lignocellulose, play key roles in promoting intestinal development and integrity due to a better gizzard development, and potential physiological influence on gut health, epithelial integrity, microflora balance and litter quality. From a nutritional perspective, it is generally believed that smaller particles allow digestive enzymes to better access their substrates, thereby increasing nutrient digestibility and growth performance efficiency. However, broilers may require a certain level of dietary physical structure to enhance gut development and health. When broilers are fed finely ground feed, the gizzard becomes relatively underdeveloped, and this can negatively influence gut motility and gastric digestion. The resultant increase in undigested nutrients passing beyond the ileum may lead to increased growth and multiplication of pathogenic bacteria in the hindgut. The goal of this study was to evaluate the effects of lignocellulose supplementation in mash diets and corn grind on broiler gut health. 2064 one day old male broiler chicks were distributed in a completely randomized trial in a 3 x 2 factorial design (3 levels of lignocellulose: 0%, 0,35% e 0,70%; and two corn grind s: 400 µm and 980 µm), totaling 6 treatments with 8 repetitions of 43 birds each. At 7, 21, and 42 days, 8 birds per treatment were sacrificed and segments of duodenum, jejunum and ileum were collected for morphometric analysis. At 42 days, cecal content was collected to evaluate short-chain fatty acids (SCFA). Data collected were subjected to two-way analysis of variance with 5% significant level. At 7 days, a significant effect ($p < 0,05$) was observed on jejunal villus height, with birds receiving 0% and 0,35% of lignocellulose presenting better results compared to birds fed 0,70%. No significant differences ($p > 0,05$) were noted for any of the variables evaluated at 21 days of age. At 42 days, there was a significant effect concerning the villus: crypt correlation and duodenum absorption area, with birds fed the 980 µm corn presenting a better ($p < 0.05$) correlation and bigger absorption area when compared to the birds that were fed a 400 µm corn diet. SCFA concentration in the ceca was not influenced ($p > 0.05$) by the diets. Dietary particle demonstrated an impact on the intestinal health of broilers during the final growth phases, with larger particles favoring intestinal development and absorptive capacity. The inclusion of insoluble fiber showed potential to modulate intestinal integrity, particularly during the early stages. Therefore, nutritional strategies that combine coarser particles with adequate fiber levels may be effective in promoting gut health and maximizing nutrient digestibility and utilization.

Keywords: Dietary Fiber, Corn Grind Size, Absorption Area, Scfa, Villi Height

Effect of Diets Containing Soluble and Insoluble Fibers on the Cecal Microbial Diversity of Commercial Laying Hens (NU-27)

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Abstract:

This study evaluated the impact of different dietary fiber sources on cecal microbiota composition in Dekalb White laying hens. A total of 520 hens, 63 weeks old at the beginning of the trial, were equally assigned to four dietary treatments for eight weeks: a control diet (corn-soybean meal base with 18.37% crude protein, 2699.1 kcal/kg metabolizable energy, 3.61% calcium, 0.29% available phosphorus, 0.75% digestible lysine, and 0.41% digestible methionine) with no additional fiber; a diet supplemented with elephant grass (control + 20 g/kg elephant grass - *Pennisetum purpureum* Schum); a diet supplemented with commercial insoluble fiber source (control + 15 g/kg insoluble fiber) and a diet including soybean hulls (control + 20 g/kg soybean hulls). Each treatment included five replicates of 26 birds. At 71 weeks of age, five birds per treatment were euthanized by cervical dislocation and cecal content was collected for microbial analysis. Hens were selected based on body weight within $\pm 10\%$ of the treatment group average, resulting in 20 samples (one per cage). Microbial profiling was performed using 16S rRNA sequencing and analyzed via bioinformatic pipelines as QIIME2 and R, with ASVs classified by GTDB (2021) and group differences assessed through Kruskal-Wallis and PERMANOVA tests. No significant differences were observed in alpha diversity across treatments, indicating stability in richness and evenness indices ($p > 0.05$). However, beta diversity analysis based on Bray-Curtis and weighted UniFrac metrics revealed significant shifts in microbial community structure in hens fed with elephant grass, compared to both the control and soybean hull groups ($p \leq 0.05$). The elephant grass treatment differed significantly from both the control and soybean hull groups based on Bray-Curtis distances ($p \leq 0.05$), and from the soybean hull group as assessed by weighted UniFrac ($p \leq 0.05$). Elephant grass supplementation increased the relative abundance of genera *Bacteroides* and *Megamonas* compared to soybean hull and commercial insoluble fiber diets ($p \leq 0.05$), but not from the control ($p > 0.05$). Similarly, the specie *F. prausnitzii* was higher in Elephant grass compared to soybean hull ($p \leq 0.05$), but not to other groups ($p > 0.05$). Another microbiome specie, *Megamonas funiformis* was also more abundant in the elephant grass group versus soybean hull and insoluble fiber groups ($p \leq 0.05$), with no difference observed relative to the control ($p > 0.05$). In conclusion, beta diversity indicated an altered microbial community structure in the elephant grass treatment, with selectively enriched taxa associated with gut health, supporting its potential as a functional fiber source for modulating the microbiota in laying hens.

Keywords: Gut Health, Soybean Hulls, Elephant Grass

Valine Supplementation As An Enhancer of Growth Performance and Carcass Quality in Broilers: A Systematic Review and Meta-Analysis (NU-28)

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Abstract:

A meta-analytical study was carried out to quantify the effect of valine (VAL) supplementation on broilers growth performance and carcass quality. For that, a systematic review was conducted, in the reference date of 2024/09/23, applying the following search strategy in the databases Pubmed, Science Direct, Scielo, Scopus and Web of Science: (chickens OR broiler OR gallus gallus) AND (valine OR leucine OR isoleucine OR "branched chain amino acids" OR BCAA). Retrieved results were screened by title, abstract and full-text and the ones that met all these eligibility criteria were included in this meta-analysis: i) primary study using Cobb, Ross or Hubbard broilers aged 21 days or older; ii) that compared birds fed with VAL-supplemented and VAL-not-supplemented diets; iii) that presented comprehensive composition of experimental diets (ingredients and nutrients); iv) that compared iso-ingredients and iso-nutrients diets, except VAL which had to be included in replacement of filler. Qualitative and quantitative data from the included studies were extracted and analyzed using a random-effect model (REML) to generate and weight raw mean differences between supplemented and non-supplemented groups as the measure of VAL effect. Significant heterogeneity between studies were explained by meta-regression and subgroup analysis. From a total of 2957 retrieved studies, 14 met all the eligibility criteria, generating a dataset with k=92 lines of comparison and n=20564 birds, mostly Cobb (n=14708), males (n=17824), with more than 35 days (n=10568) and receiving VAL from different sources, especially Ajinomoto (n=3900) and Evonik (n=2600). Feed VAL:LYS ratio for most birds (n=17486) ranged from 0.66 to 0.86. Feed crude protein for most birds (n=9916) ranged from 175 to 190 g/kg. Meta-analysis showed that, overall, broilers supplemented with VAL improved ($p<0.001$) daily weight gain by 6.82 g/day, feed conversion by 0.09 points and carcass yield by 0.32% when compared to non-supplemented birds. Also, VAL-supplemented broilers decreased abdominal fat deposition by 0.05% ($p=0.002$). Meta-regression showed that moderators that most influenced VAL effects were broiler genetic line ($R^2=43.91\%$), sex ($R^2=31.16\%$) and feed crude protein content ($R^2=26.34\%$). For those moderators, VAL effects were greater when birds were Ross ($p<0.001$), males ($p=0.001$) and received up to 175 g/kg of crude protein in the diet ($p<0.001$). Valine inclusion level did not influence ($p>0.060$) VAL effect in any of the assessed variables. In conclusion, there is evidence in the literature to support that VAL supplementation improves, regardless of the inclusion level, broilers weight gain, feed conversion and carcass yield, while reducing abdominal fat deposition.

Keywords: Abdominal Fat, Branched Chain Amino Acids, Carcass Yield, Ideal Protein, Weight Gain

Collagen Deposition and Skin Integrity of Broilers Supplemented With Organic Minerals and Raised on Poorly Managed Litter (NU-29)

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Abstract:

The combination of moisture and chemical irritants like ammonia in litter material in intensive breeding systems designed to maximize productivity, can contribute to health issues in chickens, including skin conditions. Although nutritional additives alone will not solve all these issues, their contribution should be considered in improvement strategies. Trace minerals are essentially in the synthesis of structural proteins critical for skin integrity, such as collagen, elastin, and keratin. Arginine (Arg) affects collagen metabolism via proline biosynthesis. The goal of this study was to evaluate the effects of including Arg and the organic minerals to diets of broilers raised in well or poorly managed litters on skin quality. A total of 4032 male chicks were distributed in a trial, in a 4 x 2 factorial design (4 diets: control, control + Arg, control + OM, and control + Arg + OM vs two litter conditions (well and poorly managed) with 12 replications of 42 birds each. Arg was included at 0.30% and an OM was also added, consisting of 40ppm Zn, 30ppm Cu, and 40ppm Mn. For the treatment of poorly managed litter, weekly turning was not performed, the pressure and flow rate of the nipples were adjusted to moisten the litter, and the feeders were kept lower. At 21 and 42 days, 24 birds/treatment were sacrificed, and samples were collected from the right lateral pelvic region. Skin breaking strength (SBS) was measured using a texturometer. Additional samples were processed using standard histological processing techniques and Masson's Trichrome staining. Collagen area obtained from each captured image was calculated and expressed as a percentage of the total area. Epidermal and dermal thickness were measured in 20 different places in each sample. Statistical data were analyzed by ANOVA and means were compared by Tukey test. There was no interaction between the factors for any variable analyzed. SBS were inconsistent in response to nutritional treatments. However, regarding litter conditions, SBS was greater ($p < 0.05$) for birds kept on poorly managed litter. This result may be attributed to lower body weight of birds in these conditions compared to birds kept on well managed litter. The increases in SBS are accompanied by increases in skin fat and decreases in moisture and protein concentrations. At 42 days, collagen deposition was higher ($p < 0.05$) in birds raised on poorly managed litter and that were fed the Arg diet (90.54%) compared to the control diet (88.71%), but similar to the other diets. The same trend was noted for epidermal thickness (57.77 and 53.08 μ m). For birds that were maintained on well managed litter, there was no significant effect ($p > 0.05$) of the diets at 21 or 42 days of age. It was concluded that litter management is fundamental for broiler skin integrity and quality and nutritional strategies can contribute to mitigate negative effects of poorly managed litter.

Keywords: Poultry Litter Moisture, Management, Elastin, Breaking Strength Of Skin

Litter Poultry Quality of Broilers Fed With Arginine and Organic Minerals: Effects on Footpad Dermatitis and Growth Performance (NU-31)

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Abstract:

Footpad dermatitis (FPD) causes downgrades and condemnations of sellable chicken paws but is also an animal welfare concern. FDP is often associated with wet litter or poor litter quality. The possibility of reducing FPD incidence in broiler chickens using dietary supplementation with a more highly available organic mineral (OM) source associated with arginine (Arg) was studied. OM is involved in tissue integrity maintenance and is required for the synthesis of structural proteins, such as collagen and keratin. Arg affects local cell proliferation and possibly collagen metabolism. A total of 4032 male chicks were distributed in a completely randomized trial, in a 4 x 2 factorial design (4 diets: control, control + Arg, control + OM, and control + Arg + OM) VS two litter conditions (well and poorly managed) with 12 replications of 42 birds each. Arg was included at 0.30% and an OM was also added, consisting of 40ppm Zn, 30ppm Cu, and 40ppm Mn. For the treatment of poorly managed litter, weekly turning was not performed, the pressure and flow rate of the nipples were adjusted to moisten the litter, and the feeders were kept lower. Zootechnical parameters were monitored weekly, and pododermatitis was evaluated at 21, 28, 35, and 42 days of life in 5 birds/replication. The means were compared by the Tukey test and the FPD score data were analyzed by the nonparametric Kruskal-Wallis method. Regarding productive performance, in the 1 to 10 days of age period of birds on well-managed litter, the greatest weight gain was obtained in animals that consumed the Arg and Arg + OM diets compared to OM and control, and better feed conversion for birds that received the Arg diet compared to OM, with no differences to the other diets. In the same period, birds on poorly managed litter that consumed the Arg + OM diet presented the best feed conversion when compared to control and OM, but similar to Arg. During the period from 1 to 42 days, no significant differences were observed in feed consumption, weight gain, and feed conversion between the diets evaluated, either for birds raised on well-managed or poorly managed litter. In the evaluation of FPD for birds on well-managed litter, a significant difference was observed at 35 days of age, with higher scores in animals that received the control diet compared to Arg and OM. For birds raised on poorly managed litter, a difference was observed at 28 days, with higher lesion scores in animals fed the control and Arg diets compared to OM and Arg + OM. In conclusion, the addition of Arg and OM to broiler diets improves feed conversion in the first 10 days of life, both in birds raised on well or poorly managed litter, and also reduces the incidence of FDP throughout the cycle.

Keywords: Wet Litter, Footpad Dermatitis Score, Amino Acid, Minerals

Performance of Broiler Chickens Supplemented With Phytogetic Additives (NU-32)

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Abstract:

The search for alternatives to conventional growth promoters has driven the use of phytogetic additives in poultry production. Garlic and cinnamon extracts stand out for their positive effects on bird performance. Evaluating their productive impact is essential for more efficient and sustainable systems. Therefore, this study aimed to evaluate the effect of including garlic and cinnamon extracts in the diet of broiler chickens as a replacement for growth promoters on their performance. The research was conducted at UFGD in an experimental poultry house using broiler chickens. A total of 1,200 one-day-old male Ross TM4® chicks were housed, and the experimental period lasted from 1 to 42 days of age. Birds were distributed in a completely randomized design into five treatments with different inclusion levels of garlic and cinnamon extract: T1 – Positive control (with antimicrobial growth promoter – Bacitracin); T2 – Negative control; T3 – 0.250 kg/ton of garlic and cinnamon extract; T4 – 0.500 kg/ton of garlic and cinnamon extract; and T5 – 0.750 kg/ton of garlic and cinnamon extract. Six replicates per treatment were used, each with 40 birds, totaling 30 experimental pens. Performance was evaluated cumulatively by analyzing feed intake, weight gain, and feed conversion ratio. Data were analyzed for the cumulative periods of 1–7, 1–21, 1–35, and 1–42 days. Statistical analysis was performed using the MIXED procedure of SAS® software. Significant differences were observed in weight gain for the 1–7 and 1–21 day periods, as well as in feed conversion for the 1–7 day period. During this initial period, the 750 g/ton inclusion level of garlic and cinnamon extract resulted in lower weight gain and higher feed conversion, performing similarly to the negative control treatment. Conversely, the 250 g/ton dosage showed the best feed conversion. By 1–21 days, the 750 g/ton level continued to show reduced weight gain. No significant differences were observed for the remaining periods. The use of garlic and cinnamon extracts in broiler diets highlights the importance of proper dosage adjustment to optimize performance, as the highest level evaluated negatively affected some variables. These phytogetic additives demonstrate potential as alternatives to antimicrobials, promoting more sustainable poultry production aligned with consumer demand for safer and more natural food products.

Keywords: Performance Enhancer, Natural Additives, Sustainable Production, Antimicrobials, Phytogetic Additives

Meat Quality of Broiler Chickens Supplemented With Garlic and Cinnamon Extracts (NU-33)

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Abstract:

Garlic (*Allium sativum*) and cinnamon (*Cinnamomum zeylanicum*) extracts have shown potential for improving the meat quality of broiler chickens. Their bioactive compounds can influence characteristics such as color, texture, and water retention. This approach meets the growing demand for more natural and healthier products. Therefore, this study aimed to evaluate the effect of including powdered garlic and cinnamon extracts in the diet of broiler chickens, replacing performance enhancers, and their effects on meat quality. The research was conducted at UFGD, in an experimental broiler facility. A total of 1,200 one-day-old male chicks of the Ross TM4® strain were housed, and the experimental period lasted from 1 to 42 days of age. The birds were distributed in a completely randomized design with five treatments: T1 – Positive control (with antimicrobial performance enhancer – Bacitracin); T2 – Negative control (without antimicrobial or additive); T3 – Inclusion of 0.250 kg/ton of garlic and cinnamon extract; T4 – Inclusion of 0.500 kg/ton of garlic and cinnamon extract; T5 – Inclusion of 0.750 kg/ton of garlic and cinnamon extract. Six replicates were used per treatment, with 40 birds per replicate, totaling 30 experimental pens. At 21 and 28 days of age, two birds per replicate were weighed and slaughtered for meat quality analysis. The parameters evaluated included: fillet weight (kg), length (cm), width (cm), thickness (cm), pH, temperature, lightness (L^*), redness (a^*), yellowness (b^*), water holding capacity, drip loss, thawing loss, cooking loss, and shear force. Data were analyzed using analysis of variance with the MIXED procedure of SAS. At 21 days of age, no significant differences were observed among the treatments. However, at 28 days, a significant difference was found for fillet weight ($p=0.0376$), with the 250 g/ton inclusion level of garlic and cinnamon extract showing the best result. This increase in fillet weight observed with the addition of the extracts to the broilers' diet may be related to the antimicrobial action of these compounds, which improves intestinal microbiota balance and enhances nutrient absorption, in addition to stimulating digestive enzymes, exerting antioxidant effects, and possibly modulating growth-related hormones. Therefore, these results demonstrate the potential of natural extracts to improve productive traits of commercial interest, contributing to a more efficient, healthier, and sustainable poultry production aligned with consumer demands for better quality and more natural food products.

Keywords: Phytogetic Additives, Sustainable Alternative, Physicochemical Characterization

Potential of Probiotic *Bacillus* Strains to Acquire Resistance to Aminoglycosides and Quinolones Under Selective Conditions (NU-34)

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Abstract:

Probiotics are effective alternatives to support gut health and productivity, but must be safe and free of antibiotic resistance genes. Although this is required by entities such as the European Union, few studies have assessed the ability of strains to acquire resistance through selective pressure. This study aimed to evaluate whether selected probiotic strains (*Bacillus velezensis* BV01UNICLON and *Bacillus clausii* ENTPro) could develop resistance after exposure to subdoses of aminoglycosides and fluoroquinolones (amikacin and norfloxacin). Prior to the experiment, the minimum inhibitory concentration (MIC) was determined, and both strains were sensitive to these antibiotics as well as to those required by the EU for probiotic safety: vancomycin, chloramphenicol, tetracycline, and erythromycin. Resistance induction was performed through continuous cultivation over 240 generations in BHI broth. Every 50 generations, antibiotics were added in gradually increasing concentrations. To distinguish the effects of antibiotic pressure from spontaneous mutation, a control group was cultured identically but without antibiotics. MIC was measured every 50 generations, showing increased resistance in both treated and control groups. To evaluate resistance evolution, linear mixed models with random intercepts by treatment were fitted. Generation and antibiotic exposure were included as fixed effects in the first model. A significant effect of generation on MIC was observed ($F_{1,21}=7.74$; $p=0.011$), suggesting increased resistance over generations, while the presence of antibiotics had no significant effect ($F_{1,21}<0.01$; $p=0.990$). In a second model, the aim was to evaluate whether the rate of MIC increase differed among groups. The interaction between generation and treatment was not significant ($F_{1,33}<0.01$; $p=1.000$), indicating that resistance increased similarly across both groups. Based on our data suggesting that *Bacillus* resistance likely resulted from natural mutations over generations rather than antibiotic exposure, then, we hypothesized that sporulation may have occurred in antibiotic-treated groups. To test this hypothesis, cultures underwent heat treatment (90°C for 10 min), acidification (pH 5.5), and incubation at 37°C for 24h to eliminate the vegetative forms and we stained with malachite green staining. Our results showed the sporulated forms indicating a possible link between resistance and the spore form. These promising results indicate that, in addition to improving gut health and withstanding harsh conditions like pelleting temperatures, *Bacillus* does not develop resistance to quinolones or aminoglycosides, making it a safe and advantageous choice for poultry production.

Keywords: Poultry, Antibiotic, Mic, Mutation

Influence of Dietary Components on Laying Hens' Ingestive Behavior: A Systematic Review and Meta-Analysis (NU-35)

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Abstract:

Feeding behavior in laying hens is directly influenced by dietary levels of metabolizable energy (ME), essential amino acids, crude protein (CP) and sodium. The knowledge extraction in metanalytical studies can support information for the development of decision support systems for applications in automated feeders. The objective of this study was to investigate, through meta-analysis and systematic review, the effects of these nutrients on the ingestive behavior of laying hens. The literature search was conducted between August 2024 and June 2025, across the Web of Science, ScienceDirect, Taylor & Francis, Springer Link, and Wiley databases, using the terms and Boolean operators: ("laying hens" AND (feed intake OR body weight) AND (metabolizable energy OR protein OR amino acid)), for publications in english, between the periods of 2013 and 2025. Inclusion criteria were: randomized trials, peer-reviewed articles, corn/soybean meal-based diets, and healthy laying hens in production (20-50 weeks, light-weight category). Research involving other species, challenged birds, additive supplementations, or lacking variability measures were excluded. Screening and data extraction were performed by two independent researchers, and statistical analysis used the standardized mean difference (SMD) with a 95% CI, assessing heterogeneity using the Q and I² indices. The search identified 2,310 studies, of which 68 were fully evaluated and 7 articles met the inclusion criteria for data extraction (n=69). The results demonstrated significant relationships between nutritional elements and ingestive behavior. ME is the primary regulatory factor, with a negative correlation with feed intake (SMD=-0.67; I²=43%). Levels between 2,600 and 2,700 kcal/kg were ideal and did not alter consumption, while concentrations above 3,000 kcal/kg reduced intake, highlighting its influence on satiety. CP showed complex interactions with energy density, with optimal levels between 16% and 18% during peak production. Studies observed that birds adjust consumption based on protein quality and quantity. Regarding amino acids, adequate supplementation of methionine (0.19% to 0.29%) showed a positive effect on consumption regulation when associated with ideal CP levels. Sodium content between 0.15% and 0.18% optimized consumption, but diets with deficient levels (<0.10%) induced higher feed intake, and excesses (>0.25%, >0.4%) decreased consumption and increased water intake. The meta-analysis identified patterns regarding nutrient levels that modulate ingestive behavior, providing insights for precision feeding strategies that consider complex nutritional interactions in the feeding of commercial laying hens.

Keywords: Feed Intake, Feeding Behavior, Metabolizable Energy, Protein, Sodium

Use of An Energy Concentrate Based on Oil Powder For the Total Replacement of Degummed Soybean Oil in Broiler Chicken Diets (NU-37)

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Abstract:

The inclusion of oils in poultry feed formulations plays a crucial role in bird performance and production efficiency, as it increases the energy density of the diet (Dos Santos *et al.*, 2022). The use of oil in powdered form can further enhance the energy value of poultry diets, offering improved oxidative stability, easier handling, and reduced spoilage risks compared to liquid oils. This study aimed to evaluate the effects of total replacement of degummed soybean oil with a powdered energy concentrate, and to determine the ideal inclusion level, on growth performance and carcass characteristics of broiler chickens from 1 to 42 days of age. A total of 640 one-day-old Ross 308 broiler chicks were randomly assigned to five dietary treatments. The control diet (T1) included soybean oil as the lipid source, with a metabolizable energy (ME) value of 8,800 kcal/kg. The energy concentrate (EC) completely replaced soybean oil in diets T2 to T4 at increasing inclusion levels, with ME matrix values of 29,333, 14,667, and 8,800 kcal/kg, respectively, allowing evaluation of EC performance under different energy assumptions. This strategy enabled the assessment of EC performance under varying energy assumptions. Treatments were tested in 8 replicates of 20 birds each. All diets were isoproteic and isophosphoric, formulated to meet the nutritional requirements for the strain. The energy concentrate used (Bio Energia®, Vaccinar Nutrição Animal) was developed to enhance energy density and broiler performance. Performance variables evaluated included feed intake (FI), weight gain (WG), and feed conversion ratio (FCR). At the end of the experiment, three birds per replicate with body weights close to the pen average were selected for carcass evaluation. Data met normality (Shapiro–Wilk) and homogeneity (Levene) criteria and were subjected to ANOVA (SAS v.9.4), with Tukey's test ($p < 0.05$). No significant differences ($p > 0.05$) were observed among treatments for performance parameters. Energy equivalent 14,667, breast yield rose by 3.2 percentage points versus control ($p < 0.05$), whereas lower levels showed no change. Abdominal fat decreased by 12–18% across all EC treatments ($p < 0.05$). All levels of energy concentrate significantly reduced abdominal fat ($p < 0.05$) compared to the soybean oil control. With the equivalent of 29.333 there was no difference in relation to soybean oil. These results support the feasibility of fully replacing soybean oil with a powdered oil energy concentrate in broiler diets, without compromising performance and with potential benefits for carcass traits.

Keywords: Broiler Chickens, Powdered Oil, Growth Performance, Soybean Oil Replacement, Carcass Traits



Milk Replacers on Growth Performance and Litter Uniformity in Suckling Piglets (NU-38)

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Abstract:

Genetic selection for hyper-prolific sows has increased litter but also led to a higher number of low-viability piglets, compromising litter uniformity. This has made it more difficult to meet the nutritional and immunological needs of all piglets, as the number of functional teats and the sow's colostrum and milk production have not increased proportionally. As a result, low-viability piglets often consume less colostrum and milk, impairing immune development and gut maturation, and leaving them less prepared for post-weaning stress. Milk replacer supplementation during lactation may help address these challenges by improving growth and intestinal health. This study evaluated the effects of two commercial milk replacers on piglet growth performance and litter uniformity. The trial was conducted in a commercial farm using 30 litters from third-parity TN70 sows (Topigs Norsvin), randomly assigned to one of three treatments (n=10 per group). Six hours post-farrowing, litters were standardized to 14 piglets per sow. Treatments included: (1) Negative Control (NC; no supplementation), (2) Powder Milk Replacer (PMR; Leitone, Vaccinar Nutrição Animal; 6:1 water dilution), and (3) Liquid Milk Replacer (LMR; 37,5% DM). From day 3 to 19, piglets received 20g of supplement/animal/day; NC piglets received water to control for handling effects. Piglets were individually weighed at birth, day 3, day 19, and weaning (day 25) to assess weight gain and within-litter uniformity. Data were analyzed using ExpDes.pt (R Core Team, 2020, v4.1.0), with litter as the experimental unit and birth weight as a random effect. Significance was set at $p < 0.05$, with trends noted at $0.05 \leq p < 0.10$. Tukey's test was used for post-hoc comparisons. At day 3, body weight (BW) and coefficient of variation (CV) were similar across treatments ($p > 0.05$), as was overall daily weight gain. By day 19, piglets fed PMR tended to have higher BW ($p = 0.062$) and lower CV ($p = 0.071$). At weaning, BW was unaffected, but CV was significantly lower in the PMR group ($p = 0.024$). These findings suggest that milk replacers can enhance growth performance and reduce weight variability, particularly benefiting low-viability piglets. Among the two replacers, the powder formulation was more effective in improving litter uniformity by day 18 and at weaning.

Keywords: Milk Replacers, Suckling Piglets, Growth Performance

Use of A Multifunctional Phosphorus-Releasing Additive For the Total Replacement of Conventional Meat and Bone Meal As A Phosphorus Source in Broiler Chicken Diets (NU-39)

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Abstract:

A major challenge faced by poultry producers is the microbiological quality of meat and bone meal (MBM), as these by-products can harbor pathogens that negatively impact poultry production (Vilela *et al.*, 2018). This study evaluated the effects of supplementing a multifunctional phosphorus-releasing additive on broiler performance, carcass characteristics, and bone mineralization, considering full MBM replacement either from days 21 to 42 (T2) or only during the finisher phase, days 35 to 42 (T3). A total of 480 one-day-old Ross® chicks were randomly assigned to three treatments with eight replicates of 20 birds each: T1 (control – MBM-based diet), T2 (MBM replaced with additive at 1.0 kg/ton from days 21–42), and T3 (MBM replaced from days 35–42). All diets were isoproteic, isoenergetic, and isophosphoric, formulated according to strain requirements. The additive used (Bio Phósforo®, Vaccinar Nutrição Animal) was designed to enhance nutrient use and phosphorus bioavailability, containing citric acid (14.0 g/kg), phosphoric acid (19.0 g/kg), formic acid (35.0 g/kg), pantothenic acid (267.0 mg/kg), glucanase (13.0 U/g), xylanase (382.0 U/g), phytase (2000 FTU/g), pectinase (3.0 U/g), -galactosidase (0.2 U/g), phosphatidylcholine (318.0 mg/kg), total phosphorus (39.0 g/kg), protease (750.0 U/g), niacin (6,400.0 mg/kg), vitamins B2, B6, and E, calcium (50.0 g/kg), zinc and manganese (477.0 mg/kg each). Performance and carcass traits included feed intake (FI), weight gain (WG), feed conversion ratio (FCR), and yields of breast (BI), thigh (TI), drumstick (DI), and wing (WI). Bone traits included femur and tibia breaking strength (BS) and mineral content. At the end, three birds per replicate (close to the average weight) were selected for carcass evaluation; eight birds per treatment were selected for bone analyses. Data normality and variance homogeneity were verified using Shapiro-Wilk and Levene tests. ANOVA was performed in SAS (SAS Institute Inc., 2011), and means were compared using Tukey's test ($p < 0.05$). No significant differences ($p > 0.05$) were found among treatments for performance and carcass traits. However, bone mineralization (BS and mineral content) was significantly higher in birds fed the additive ($p < 0.05$), supporting the feasibility of fully replacing MBM with this additive in broiler diets without compromising performance and suggesting bone health benefits, especially during the 21–42-day period.

Keywords: Broiler Chickens, Meat And Bone Meal, Phosphorus Bioavailability, Enzymatic Blend, Growth Performance

Evaluation of Exogenous Protease Efficacy in Broiler Chickens Under Different Nutritional Plans (NU-40)

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Abstract:

Protein sources are among the most costly components in poultry diets (Beski *et al.*, 2015). When crude protein (CP) levels exceed optimal requirements, nutrient utilization becomes inefficient and environmentally detrimental. Studies have shown that a significant portion of dietary protein remains undigested under such conditions, particularly during rapid growth phases with high feed intake (Ndazigaruye *et al.*, 2019). Moreover, excess protein not only reduces digestibility but also serves as a substrate for harmful gut microorganisms, increasing the risk of disease (Kidd *et al.*, 2001). Supplementing exogenous protease has emerged as a promising strategy to enhance protein digestibility and improve broiler performance by optimizing the utilization of dietary nitrogen and energy (Cowieson *et al.*, 2016). This study aimed to investigate the effects of exogenous protease supplementation on the performance of broiler chickens fed diets based on corn, sorghum, soybean meal, and meat and bone meal. We calculated the basal diet's ileal digestible amino acid (IDA) profile (PC) to create two reduced-IDA matrices—50% (NC1) and 25% (NC2)—for testing protease efficacy. These matrices were then used to formulate two negative control (NC) diets: NC1 and NC2. A total of 3,600 one-day-old male Ross AP95 broiler chicks were randomly assigned to six treatments: T1 – Positive Control (PC) diet without protease; T2 – NC diet with a 50% IDA matrix; T3 – NC diet with a 25% IDA matrix; T4 – PC diet + Protease; T5 – T2 diet + Protease and T6 – T3 diet + Protease. Each treatment included 12 replicates with 50 birds per replicate. Each treatment was tested in 12 pens of 50 birds. Performance parameters evaluated included average body weight, weight gain, mortality, feed intake, and feed conversion ratio at 7, 21, and 42 days of age. Results showed a negative impact on performance when using the 50% IAA matrix, and a milder reduction with the 25% matrix. However, the addition of exogenous proteases effectively restored performance. Notably, the “on top” inclusion of protease in the PC diet resulted in numerical improvements compared to the unsupplemented control. These findings support the potential of exogenous proteases to enhance nutrient utilization and mitigate the adverse effects of reduced digestible amino acid levels. Further research is warranted to refine the nutritional matrix of protease under different protein sources, nutrient levels, or in combination with other exogenous enzymes or additives that may improve nutrient digestibility.

Keywords: Exogenous Enzymes, Protease, Crude Protein Reduction, Broiler Performance

Nutrient Digestibility of Broiler Fed With Diets Containing Different Levels of Emulsifier (NU-41)

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Abstract:

Emulsifiers can increase the digestion and absorption of lipids, reducing the surface tension of the lipid, allowing physical agitation of the gastrointestinal tract to break the droplets into smaller particles and promoting the formation of micelles. The present study aimed to evaluate the nutrient digestibility of the diet of chickens containing different levels of emulsifiers (LipoEnergy®). Total of 300 male Cobb500® broiler chickens were distributed in a completely randomized design, with five treatments and six replicates of 10 broilers each for the metabolism assay. One-day-old chicks were purchased from a commercial hatchery and for the first fourteen days of age, they were group-housed on a floor, in a poultry housing. On the 14th day, all birds were weighed (average weight of 0.800 kg/bird) and homogeneously distributed in metabolic cages, using the traditional total excreta collection method. The treatments used were: 1) basal diet without inclusion of emulsifier (control); 2) basal diet with inclusion of 250g of emulsifier; 3) basal diet with inclusion of 500g of emulsifier; 4) basal diet with inclusion of 750g of emulsifier and 5) basal diet with inclusion of 1,000g of emulsifier/t. Ten chickens were used per experimental unit, from 17 to 24 days, with three adaptation days. Excreta and diets were collected and analyzed to determine dry matter (DM), mineral matter (MM), Crude Protein (CP), and Ether Extract (EE). The apparent dry matter digestibility coefficient, Crude Protein digestibility coefficient, and EE digestibility coefficient were calculated by: $\text{nutrient metabolism (\%)} = ((\text{ingested nutrient (g)} - \text{excreted nutrient (g)}) / \text{nutrient ingested (g)}) \times 100$. The crude energy was determined using adiabatic bomb calorimetry. The data was subjected to regression analysis. The results showed positive quadratic effect for digestibility CP with an increasing emulsifier inclusion level in diet ($\text{CDCP} = 66.77 + 0.000744x - 0.0000103(x - 482.75)^2$), with a maximum point at 540.9 g/t ($r^2 = 0.39$). Metabolizable energy values increased with the addition of emulsifier, the linear equation was positive: $\text{EMAN} = 3051.035 + 0.1608x$ ($r^2 = 0.70$). It is concluded that emulsifiers are additives that improve CP and EE nutrients digestion and absorption; and increase the metabolizable energy of the diet.

Keywords: Additives, Metabolizable Energy, Poultry

Productive Performance of Broiler Chickens Fed Diets With Mycotoxins (NU-42)

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Abstract:

The presence of mycotoxins in feed ingredients used in poultry nutrition poses a significant challenge to the poultry industry, potentially impairing productive performance and, consequently, the profitability of production systems. Aflatoxins, fumonisins, and deoxynivalenol (DON) are among the most common and harmful mycotoxins found in poultry diets. Levels as low as 20–100 ppb of aflatoxins, 1–5 ppm of fumonisins, and 1 ppm of DON can already induce toxic effects in broilers, including reduced growth, poorer feed conversion, and immunosuppression. Therefore, evaluating their effects and seeking effective mitigation strategies are essential for maintaining bird performance and health. A total of 450 male Cobb500 broilers were used, distributed in a completely randomized design with two treatments and nine replicates, each consisting of 25 birds. The treatments were as follows: T1 – basal diet (control); T2 – basal diet with 100 ppb of aflatoxin (AFL), 5 ppm of fumonisin (FUM), and 1 ppm of deoxynivalenol (DON). The experimental period lasted from 1 to 42 days of age. The parameters evaluated were body weight (BW) at 1, 21, 35, and 42 days; average daily gain (ADG) from 1–21, 1–35, and 1–42 days; feed conversion ratio (FCR) in the same periods; and productive efficiency index (PEI). Data were subjected to analysis of variance. The inclusion of mycotoxins in the diet (T2) significantly affected all performance parameters from 21 days of age onward. Birds in T2 showed lower body weights at 21, 35, and 42 days (923 g; 2219 g; 3008 g, respectively) compared to the control group (978 g; 2421 g; 3200 g) ($p < 0.05$). Consequently, ADG was also reduced in T2 during the 1–21, 1–35, and 1–42 day periods (42 g; 62 g; 70 g) relative to T1 (44 g; 68 g; 75 g) ($p < 0.05$). Feed conversion ratio was negatively affected in all phases in T2 (1.41; 1.58; 1.64) compared to T1 (1.32; 1.47; 1.55) ($p < 0.05$). The negative impact of mycotoxins was reflected in the productive efficiency index, with a marked reduction in T2 (400.86) versus the control group (458.54) ($p < 0.0001$). In conclusion, the presence of 100 ppb of AFL, 5 ppm of FUM, and 1 ppm of DON in the diet significantly reduced the performance of broiler chickens from 1 to 42 days of age.

Keywords: Poultry Production, Zootechnical Parameters, Growth Performance, Feed Contamination

Phytase Supplementation in Shrimp Diets (NU-43)

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Abstract:

Phytase supplementation in livestock diets has been widely studied due to its ability to increase phosphorus bioavailability. However, the improper application of this enzyme in shrimp diets may result in adverse effects, including oxidative stress and increased mortality. In this context, the present study aimed to establish a safe and effective protocol for phytase inclusion at different levels in the diet of *Penaeus vannamei*. Post-larvae (PL7) of the Speedline strain were used, previously acclimated and reared for 50 days in ponds. After this period, 11 shrimp with an initial average weight of 4.65 ± 0.010 g were distributed into 100-L tanks under controlled environmental conditions (pH: 8.5–9.1; alkalinity: 180 mg CaCO_3/L ; nitrite, nitrate, and ammonia < 0.1 mg/L; total hardness: 400 mg/L; calcium hardness: 112 mg/L; magnesium hardness: 288 mg/L; salinity: 1.4 ppt). The experimental design was completely randomized, with eight replicates per treatment. Treatments included: a positive control (diet with 1.31% available phosphorus – PC), a negative control (0.59% - NC), and four increasing levels of phytase (1000, 2000, 3000, and 4000 FTU/kg). Data were analyzed using ANOVA, and means were compared by Tukey's test at a 5% significance level. The estimated optimal level of phytase supplementation was determined using a quadratic polynomial (QP) model. Weight gain, feed conversion, and shrimp mortality were evaluated. Seventeen days after the beginning of the trial, shrimp from the positive control showed an average weight of 7.13 g, significantly higher than the negative control (6.05 g; $p < 0.05$). On 28 days, the groups supplemented with 1000, 2000, and 3000 FTU/kg exhibited average weight gains of 7.63 g, 7.44 g, and 7.61 g, respectively, all superior to the negative control and the 4000 FTU/kg group ($p < 0.05$). In addition, at 38 days, all phytase-supplemented groups had significantly better performance than the negative control ($p < 0.05$), with the 1000 and 2000 FTU/kg treatments reaching average weights of 10.60 g and 10.58 g, statistically equivalent to the positive control (10.67 g). Considering weight gain, the quadratic regression analysis ($Y = 9.749 + 0.788x - 0.178x^2$; $R^2 = 0.66$) indicated an optimal supplementation level of 2210 FTU/kg. The treatment with 3000 FTU/kg showed the lowest mortality rate ($p < 0.05$). Feed conversion was significantly improved ($p < 0.05$) in the 1000 FTU group (1.30) compared to the PC, NC, and 4000 FTU/kg groups (1.48, 1.83, and 1.50, respectively), and similar to the 2000 and 3000 FTU/kg groups (1.31 and 1.45). In conclusion, the phytase supplementation positively influenced growth performance, feed conversion, and survival rate in *P. vannamei*, with the recommended level being 2210 FTU/kg during the grow-out phase.

Keywords: Aquaculture, Nutrition, Supplementation, White Shrimp, Enzyme

Effect of Black Wattle Tannin on Incubation Parameters of Brown Egg-Laying Breeder Hens From 53 to 56 Weeks of Age (NU-44)

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Abstract:

The use of phytogetic additives has gained prominence as an alternative to antibiotics in poultry production, aiming not only at gut health but also at improving reproductive and hatchability indices. Among these additives, condensed tannins from black wattle (*Acacia mearnsii*) exhibit antioxidant, anti-inflammatory, and antimicrobial properties, which may positively influence embryonic viability. This study aimed to evaluate the effects of dietary supplementation with Tanfeed (TAN), a black wattle tannin-based product, on incubation parameters of brown egg-laying breeder hens. Four treatments were evaluated: T1 – basal diet (control); T2 – basal diet + 50ppm of Zinc Bacitracin; T3 – basal diet + 750g of TAN/ton of feed; and T4 – basal diet + 1000g of TAN/ton of feed. The following parameters were analyzed: embryonic mortality at stages M1 (0–7 days), M2 (8–14 days), M3 (15–18 days), and M4 (19–21 days); percentage of infertile eggs and total hatchability. A completely randomized design was used with four treatments and nine replicates per treatment, each replicate consisting of six hens. Data were subjected to analysis of variance (ANOVA), and when significant differences were found, means were compared by Tukey's test at a 5% significance level. Treatments with TAN showed a significant reduction in early embryonic mortality (M1), with 9.51% in T3 and 11.03% in T4 compared to 25.06% in the control group (T1) ($p=0.0124$). This reduction may be attributed to the antioxidant action of tannins, which neutralize reactive oxygen species (ROS), minimizing oxidative damage to embryonic cells during the critical early days of incubation. Additionally, a significant decrease in the rate of infertile eggs was observed in groups T3 (1.26%) and T4 (0.75%) compared to T1 (5.22%) and T2 (4.61%) ($p=0.0038$). The inclusion of Tanfeed in the diet of Brown Breeder hens reduced infertility and early embryonic mortality rates. Zinc Bacitracin did not improve incubation parameters and was associated with the highest infertility rate.

Keywords: Antioxidant Activity, Incubation Performance, Phytogetic Feed Additives, Infertility, Hatchability

Use of Marine Algae in Broiler Diets: Impacts on Bone Health and Nutritional Sustainability (NU-45)

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Abstract:

Brazil is a global leader in poultry exports and the second-largest producer of chicken meat. Broiler production is highly profitable but demands efficient nutritional management, with calcium being an essential nutrient for bone health and bird performance. Calcium deficiency can lead to productive losses and locomotor problems. In this context, the marine algae *Lithothamnium calcareum* stands out as a sustainable alternative to limestone, offering high bioavailability and the ability to maintain productive performance. The objective of this study was to evaluate performance, bone health, and potential locomotor issues in broiler chickens fed diets with different levels of limestone replacement by marine algae (*Lithothamnium calcareum*) as a calcium source. The experiment was conducted at the Experimental Poultry facility of the Federal University of Goiás, with approval from the Animal Use Ethics Committee. A completely randomized design was used, with four treatments: 1): 100% limestone; 2): 50% limestone + 50% marine algae; 3): 25% limestone + 75% marine algae; 4): 100% marine algae. Each treatment had ten replicates of 20 birds, totaling 800 birds. Water and feed were provided *ad libitum*. The feed was based on corn, soybean meal, and meat and bone meal. The performance variables analyzed were body weight, body weight gain, feed intake, feed conversion, and uniformity. For bone health evaluation, the following procedures were conducted: Gait Score: assessing bird locomotion; Valgus and varus deformity assessments; Footpad dermatitis and hock burn scoring (scored from 0 to 3 according to severity). After slaughter, the tibias of six birds per treatment were collected. The right tibia was used to calculate the Seedor index and determine bone ash, calcium and phosphorus content levels, while the left tibia was used to measure bone strength through a mechanical force test. Statistical analyses were performed using R software, with analysis of variance (ANOVA) and Tukey's test (5%), along with Shapiro-Wilk tests for normality and homoscedasticity. There were no significant differences ($p>0.05$) among treatments for any performance variables. However, results showed a significant difference ($p<0.05$) in bone strength and bone ash content, with treatment T4 (100% marine algae) presenting the highest values for both. No other significant differences were observed among the evaluated parameters. It is concluded that the inclusion of *Lithothamnium calcareum* marine algae as a calcium source in broiler diets can be implemented without compromising performance, bone health, or causing locomotor problems and contribute positively to bone strength and mineral deposition, presenting itself as a sustainable alternative in broiler nutrition.

Keywords: Alternative Calcium Sources, Animal Welfare, Bone Mineralization, Broiler Nutrition, Gait Evaluation

Broiler Performance When Fed Different Choline Sources From 1 to 21 Days of Age (NU-46)

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Abstract:

Choline plays a crucial role in animal production, performing various metabolic functions such as cell constituent, lipid metabolism, and cell signaling. Therefore, this study aimed to evaluate the use of natural choline in the diet of broiler chickens. The experiment was conducted at the experimental poultry house of the Federal University of Grande Dourados – MS – Brazil. A total of 960 one-day-old Cobb 500 chicks were housed. The basal diet consisted of corn, soybean meal, soybean oil, dicalcium phosphate, limestone, common salt, and other ingredients, formulated according to the specific nutritional requirements for each growth phase of the birds. The diets were distributed in a completely randomized design, comprising three treatments: T1- Synthetic choline (800 g/ton); T2- Negative Control (no choline added); T3-Natural Choline (225 g/ton). There were eight replicates per treatment, with each pen housing 40 birds. Evaluations were performed on live weight, feed intake, weight gain, and feed conversion ratio during the 1 to 21-day period. The obtained data underwent statistical assumption analyses for normality of residuals and homogeneity of variances, using the Shapiro-Wilk and Levene tests, respectively. Analysis of variance was conducted using the MIXED procedure of SAS 9.3 (SAS, 2012). Mean comparisons for each treatment's performance variables were performed using Tukey's test, also adjusted by the MIXED procedure. A significance level of 5% was used for all analyses. Statistical differences were found for feed intake, with the lowest index observed for natural choline at 225 g/ton (1323b), followed by synthetic choline (1378a) and no choline (1379a) ($p=0.0111$). Differences were also found for live weight, where treatments with synthetic choline (1.169 kg b) and 250 g/ton of natural choline (1.169 kg b) showed similar values, unlike the treatment without choline addition (1.208 kg a) ($p=0.0153$). The findings of this study indicate that when the dietary formulation adequately meets the choline requirements of broiler chickens, additional supplementation (whether from natural or synthetic sources) does not confer further benefits in terms of body weight gain. Consequently, the decision to include such supplementation should be made judiciously, considering factors such as cost-effectiveness, dietary composition, and specific management and production conditions.

Keywords: Biocholine, Natural Additives, Choline Sources

Densitometric Analysis of Broiler Chickens Fed With Natural Choline (NU-47)

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Abstract:

Choline is a key nutrient in broiler diets, playing crucial roles in lipid metabolism, cellular signaling, and acting as a methyl group donor in essential metabolic pathways. Natural sources of choline have lower hygroscopicity and higher biological value than synthetic choline chloride, potentially improving feed stability and reducing costs. This study aimed to evaluate the influence of increasing levels of natural choline replacing synthetic choline chloride in the diets of Cobb 500 broilers from 1 to 42 days of age. The trial was conducted in an experimental poultry facility at the Federal University of Grande Dourados. A total of 2,400 male chicks were housed in a completely randomized design with eight replicates and 40 birds per replicate. Treatments consisted of six diets: a positive control (synthetic choline chloride at 800 g/ton), a negative control (no added choline), and four diets with increasing levels of natural choline (75, 150, 225, and 300 g/ton). All diets were isoproteic and isoenergetic, formulated to meet the nutritional requirements of the strain. Body composition variables—such as body weight, fat mass, lean mass, fat percentage, and lean tissue percentage—were assessed by dual-energy X-ray absorptiometry (DXA). Data were tested for normality and homogeneity using Shapiro-Wilk and Levene's tests. Analysis of variance was performed using the MIXED procedure of SAS 9.4, and means were compared by Tukey's test at a 5% significance level. The positive control group showed the lowest fat percentage (4.11%) and highest lean mass proportion (94.51%). Natural choline levels of 150 and 300 g/ton yielded statistically similar results to the positive control, with fat percentages of 4.45% and 4.65%, and lean mass percentages of 94.14% and 93.99%, respectively. Conversely, the 75 and 225 g/ton levels resulted in higher body fat (5.37% and 5.31%) and lower lean mass (92.96% and 93.31%). Although lean mass (kg) and lean tissue (%) did not show statistical significance ($p=0.0727$ and 0.0876 , respectively), trends suggested a favorable response to intermediate and high levels of natural choline. Other variables such as tissue mass (kg) and fat-free mass (kg) were not affected by the treatments, indicating structural stability across diets. Replacing synthetic choline chloride with natural choline at levels of 150 and 300 g/ton proved effective in maintaining broiler body composition, showing performance comparable to the positive control. The results indicate that natural choline is a nutritionally viable alternative and may reduce technological risks associated with the synthetic form.

Keywords: Alternative Additives, Carcass Composition, Poultry Production

Innovative Alternative to Antibiotics: Effects of Rare Earth Elements on Broiler Performance (NU-48)

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Abstract:

With increasing restrictions on the use of antibiotic growth promoters due to antimicrobial resistance, the search for safe and effective additives in animal feed has become essential. Hito Ceres® (Kioel Importação e Comércio LTDA) is a mineral additive derived from rock from south Korea, composed of 17 chemical elements (Rare Earth Elements – REE), including 15 lanthanide-based elements and two with similar properties (yttrium and scandium). Lanthanum, due to its ionic radius being similar to that of calcium, shares properties that enhance its biological activity. Rare Earth Elements (REE) have emerged as promising alternatives as potential growth promoters. These elements are poorly absorbed orally, acting mainly in the gastrointestinal tract, where they promote positive effects through microbiota modulation, enzymatic stimulation, anti-inflammatory, antioxidant, and immunomodulatory activities. Thus, REEs represent a promising tool for sustainable and antibiotic-free production systems. This study aimed to evaluate the effects of the Hito Ceres additive on the zootechnical performance of broiler chickens. The experiment was conducted at the Experimental Poultry House of the Federal University of Goiás, with approval from the Animal Use Ethics Committee. A total of 384 male chicks of the Cobb-500 line were used, distributed in a completely randomized design with three treatments and eight replicates of 16 birds each. The treatments were: 1) Basal diet (control); 2) Basal diet + antibiotic growth promoter (enramycin); 3) Basal diet + Hito Ceres additive (5 kg/ton). Variables analyzed at 21, 35 and 42 days included feed intake, body weight, body weight gain, feed conversion, viability, and flock uniformity. At 41 days, evaluations of carcass yield, commercial cuts, abdominal fat, and liver weight were also performed. Statistical analyses were carried out using R software, with analysis of variance (ANOVA) and Tukey's test (5%), along with Shapiro-Wilk tests for normality and homoscedasticity. The results showed a significant effect ($p < 0.05$) of Hito Ceres on body weight, feed intake, body weight gain, and viability from day 1 to 21. At 42 days, the additive also positively influenced body weight and body weight gain. No significant differences ($p > 0.05$) were observed for carcass yield, commercial cuts, abdominal fat, or liver weight. It is concluded that Hito Ceres can be safely used in broiler feeding, promoting significant improvements in zootechnical performance representing an effective alternative to antibiotic growth promoters.

Keywords: Animal Nutrition, Feed Conversion, Gut Microbiota, Immunomodulation, Mineral Supplementation



Probiotics in the Diet of Laying Hens: Effects of Different Strains on Performance and Egg Quality (NU-49)

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Abstract:

The use of probiotics in poultry farming has proven to be an effective alternative to improve gut health, enhance nutrient digestion, and reduce the use of antibiotics. Among the main types are: NAGF (Normal Avian Gut Flora) probiotics, which contain a wide diversity of bacteria capable of colonizing the entire intestinal tract; non-colonizing strain probiotics, such as *Bacillus subtilis*, which act as transient bacteria without adhering to the intestinal mucosa; and multi-strain colonizing probiotics, composed of microorganisms with adhesion capacity that promote microbiota balance and pathogen control. The aim of this study was to evaluate the effect of different probiotic strains in the diet of commercial laying hens during the production phase. The experiment was conducted in the Poultry Experimental Facility of the School of Veterinary and Animal Science at UFG. The project was approved by the Animal Use Ethics Committee (CEUA/UFG). A total of 256 Lohmann Lite hens at 34 weeks of age were used, distributed in a completely randomized design with four treatments: 1) NAGF probiotic - Normal Avian Gut Flora (Total Anaerobic Bacteria and Lactic Bacteria), 2) non-colonizing strain probiotic (*Bacillus subtilis*), 3) multi-strain colonizing probiotic (*Bacillus subtilis*, *enterobacillus faecius*, *lactobacillus*, *acidophilus*, *delbrueckii*, *plantarum*, *reuteri*, *salivarius*, *acidilactici*) and 4) control, without additives, with eight replicates and eight birds per experimental unit. The experimental phase lasted from 34 to 48 weeks of age, divided into four 28-day cycles. The birds were housed in California cage systems, equipped with nipple drinkers and linear feeders. Water and balanced feed were provided *ad libitum*. The variables analyzed included feed intake, egg production and weight, feed conversion, and egg quality. Egg quality was measured using the Egg Tester Ultimate™ machine, which evaluates egg weight, Haugh unit, shell strength, and shell thickness. Data were analyzed using R software, with ANOVA, and means compared by Tukey's test (5%). When necessary, the Kruskal-Wallis test (5%) was also applied. No significant effects ($p>0.05$) of the treatments were observed on productive performance over the four cycles. The NAGF probiotic treatment resulted in lower average egg weight and greater shell thickness in cycles 3 and 4. For the other parameters, no significant differences were found between treatments. It is concluded that the evaluated probiotics can be used in the diet of commercial laying hens during the production phase.

Keywords: Food Safety, Laying Hens, Microbiota, Nutritional Additives, Zootechnical Parameters



Use of Residue Product From Fish Production in Southern Goiás in Diets of Laying Hens (NU-50)

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Abstract:

The golden mussel (*Limnoperna fortunei*) is an invasive species that causes environmental damage and losses to fish farming, especially in net-cage systems, where its incrustations compromise water quality and fish oxygenation. Due to its high calcium content, the partial or total replacement of calcitic limestone with whole golden mussel meal in the diet of laying hens is being studied. Calcium is essential for bone formation and eggshell quality, making it a fundamental component of poultry feed. Organic sources of minerals, such as this one, may improve bioavailability and, consequently, productive performance. The aim of this study was to determine the effect and the optimal inclusion level of golden mussel meal in the diet of commercial laying hens on productive performance parameters. The experiment was conducted at the Experimental Poultry House of the Federal University of Goiás, with approval from the Animal Use Ethics Committee. A total of 180 Bovans laying hens, 79 weeks of age, were used in a completely randomized design with five treatments and six replicates, with six birds per experimental unit. The treatments consisted of replacing the calcium provided by fine calcitic limestone at 0, 25, 50, 75, and 100% with calcium derived from golden mussel meal in the hens' diets. The birds were housed in California cage systems equipped with nipple drinkers and linear feeders. Water and balanced feed were provided *ad libitum*. The experimental phase lasted from 79 to 90 weeks of age, divided into three 28-day cycles. At the end of each cycle, the following variables were evaluated: feed intake (g/bird/day), feed conversion ratio (kg of feed/dozen eggs and kg of feed/kg of eggs), egg weight, egg mass, and egg production. The collected data were analyzed using R software, subjected to analysis of variance, and means were compared using Tukey's test (5%). When significant, regression analysis was performed. Results showed a significant difference ($p < 0.05$) only for the variable egg weight in the total cycle analysis. The control treatment showed the highest average egg weight (64.54 g), while the others were statistically similar. For the other variables, no significant differences ($p > 0.05$) were found across the three cycles or in the total cycle analysis. It was concluded that golden mussel meal at levels up to 100% in the diet of commercial laying hens did not impair productive performance. However, it reduced egg weight with increasing inclusion of mussel meal. Thus, in addition to adding value to a biological residue, the use of this by-product in laying hen nutrition represents a sustainable strategy with potential to reduce environmental impacts and promote circular economy in animal production.

Keywords: Calcium, Fish Farming, Golden Mussel, Poultry Farming, Productive Performance

Performance and Carcass and Cut Yield of Chickens Fed Diets Containing Butyric Acid (NU-51)

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Abstract:

The objective of this study was to evaluate the performance and carcass yield of broilers fed with butyric acid in the diet. Eight hundred male Cobb chicks were obtained from a commercial hatchery and housed in a 1,500 m² industrial housing divided into 40 1.44 m² boxes. Reused rice straw (3rd cycle) was used as bedding. The experimental design used was completely randomized, with 4 treatments, 10 replicates, and 20 birds per experimental unit. The treatments were: 1) basal diet (control without additives); 2) basal diet with performance-enhancing antibiotic (Enramycin); 3) basal diet with butyric acid, and 4) basal diet with butyric acid plus performance-enhancing antibiotic. The diets were formulated using corn and soybean meal, following commercial levels for broilers. During the experiment, the live weight of the birds (kg), feed intake (kg), weight gain (kg), feed conversion (kg.kg⁻¹), and mortality (%) were evaluated at each stage of rearing (pre-initial, initial, growth and final), and the Productive Efficiency Index was calculated at 42 days of age. At 42 days of age, six birds per treatment were slaughtered to evaluate carcass yield, abdominal fat, and commercial cuts. The data obtained were subjected to analysis of variance, and the averages compared by Tukey's test ($p \leq 0.05$). The results from the period 1 to 21 days of age showed better live weight and weight gain of the birds fed with butyric acid, in relation to the group fed with a basal diet containing a performance-enhancing antibiotic. However, there was no difference from the treatment without the additive (control). For the other variables studied, there was no difference between the treatments in the total period (1 to 42 days of age). The treatments did not differ statistically for the calculated Productive Efficiency Index or the carcass and cut yields obtained at 42 days of age. It was concluded that the butyric acid included in the diet brought benefits to the performance in the initial phase of rearing (1 to 21 days of age).

Keywords: Additives, Antibiotic Substitutes, Organic Acid, Poultry

The Use of Drinking Water Additives in Broiler Chickens: Effects on Zootechnical Performance and Intestinal Health (NU-52)

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Abstract:

The drinking water quality supplied to broiler chickens directly affects their performance and intestinal health, impacting productivity and biosecurity within the poultry production chain. This study aimed to evaluate the effects of different chlorination and acidification treatments of drinking water on the productive performance and intestinal integrity of broilers. The experiment was conducted in four dark house poultry barns on a farm located in northern Rio Grande do Sul, Brazil, using 124,500 ROSS AP95 TM4 birds, monitored from 1 to 37 day of age. Four water treatments were applied: T1 (chlorine dioxide 5 ppm, pH 4), T2 (chlorine 5 ppm, pH 4), T3 (chlorine 1 ppm, untreated water – control), and T4 (chlorine dioxide 1 ppm, pH 4), all with acidifying additives and water hardness treatment when applicable. Zootechnical monitoring and intestinal evaluations were carried out on days 7 and 35, including morphometric parameters, mucus presence, mucosal integrity, and *Eimeria* occurrence. Physicochemical analyses of the water, as well as histological and statistical evaluations (ANOVA, Tukey's test, and Fisher's exact test, $p < 0.05$), were performed to assess treatment effects. On 7 day, treatments with chlorine dioxide (T1 and T4) showed greater average villus height and lower crypt depth in the jejunum, with the highest villus-to-crypt ratio observed in T4. By 35 day, ileum morphometry revealed greater mucosal thickness in T1, indicating better absorption capacity. Regarding intestinal integrity, T1 and T2 had lower lesion scores, whereas T3 (control) showed the highest incidence of lesions and mucus. The birds in treatment 4 showed lowest feed conversion ratio (1.498). Additional, cumulative mortality was lowest in T4 (2.76%), followed by T1 (3.08%), while T2 and T3 presented higher rates (4.00% and 4.36%, respectively). Water physicochemical parameters also remained more stable in T1 and T4 throughout the rearing period. The overall results showed that the chlorine dioxide proved to be effective strategies for enhancing intestinal health and productive efficiency in modern broiler farming.

Keywords: Poultry Drinking Water Quality Parameters, Performance, Water Quality, Chlorine Dioxide, Water Acidification

Intestinal Biometry of Broiler Chickens Supplemented With Natural Extracts During the Pre-Starter Phase (NU-53)

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Abstract:

The increasing demand for more sustainable practices in poultry farming has driven the adoption of natural additives as alternatives to antimicrobial growth promoters. Among these, natural extracts are particularly promising for improving intestinal health in birds, thus contributing to a more efficient, safer, and environmentally friendly production system. This study evaluated the use of garlic and cinnamon extracts in the diets of broiler chickens during the pre-starter phase, and their effects on intestinal biometry and morphometry. The research was conducted at the Faculty of Agricultural Sciences of the Federal University of Grande Dourados (UFGD) in an experimental poultry facility, with 1,200 male Ross-lineage chicks raised from day one to day 42. The birds were assigned to a completely randomized design with five treatments: T1 – Positive control (with antimicrobial growth promoter – Zinc Bacitracin); T2 – Negative control; T3 – Inclusion of 0.250 kg/ton of garlic and cinnamon extract; T4 – Inclusion of 0.500 kg/ton of garlic and cinnamon extract; and T5 – Inclusion of 0.750 kg/ton of garlic and cinnamon extract. Each treatment had six replicates, with 40 birds per replicate, totaling 30 experimental pens. Intestinal biometry and morphometry data were collected at day 7 of age. One bird per replicate was selected for necropsy, followed by the collection and weighing of the liver and intestinal segments (duodenum, jejunum, ileum, and ceca), and measurement of their lengths using a measuring tape. The data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The results showed no significant differences among the treatments for the evaluated biometry variable, indicating that the inclusion of garlic and cinnamon extract did not affect the development of these parameters during the starter phase. It can be concluded that garlic and cinnamon extracts may serve as viable and sustainable alternatives to antimicrobials, as they did not compromise intestinal development, thereby contributing to a more responsible poultry production system that aligns with modern consumer demands.

Keywords: Antimicrobial Growth, Garlic And Cinnamon Extracts, Natural Additives

Carcass and Organ Yield of European Quails Fed With Unconventional Lipid Sources. (NU-54)

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Abstract:

This study aimed to evaluate the carcass yield and internal organ characteristics of European quails fed with alternative lipid sources. For this purpose, 600 birds of the Fujikura commercial strain were used, distributed into five treatments with different lipid sources: soybean oil, conventional corn oil, distilled corn oil, poultry fat, and bovine tallow. The birds were fed until 35 days of age, at which point they were slaughtered for carcass and organ yield analysis. The birds underwent an 8-hour fasting process, followed by manual evisceration and chilling in chillers. Carcass yield was calculated based on the weight of main cuts such as breast, thighs, wings, and back, as well as edible viscera (heart, liver, and gizzard) and non-edible viscera (intestine, spleen, and female reproductive organs). Distilled corn oil provided a higher carcass yield (81.99%) when compared to poultry fat (77.15%), with a significant difference ($p < 0.05$). However, there were no significant differences for the weight of individual cuts (breast, thighs, wings, and back), suggesting that the alternative lipid sources did not directly affect the weight composition of these cuts. Regarding the weight of viscera, such as liver, heart, gizzard, and intestine, there was no significant difference ($p > 0.05$) among treatments. This indicates that the replacement of conventional fat with alternative lipid sources did not significantly interfere with the development and weight of the birds' internal organs. Furthermore, intestinal length and the weight of reproductive organs also showed no significant variation among treatments. These results suggest that the inclusion of alternative lipid sources, such as distilled corn oil, can improve carcass yield without harming the health or development of the quails' internal organs. In comparison to animal-derived lipid sources, such as poultry fat, distilled corn oil demonstrated better results, which can be attributed to the higher digestibility of vegetable fats, which have a more favorable fatty acid profile for animal digestion.

Keywords: Carcass Yield, Internal Organs, Meat Quail, Alternative Lipid Sources, Distilled Corn Oil

Effects of A Symbiotic on the Performance and Intestinal Microbiota of Broilers (NU-55)

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Abstract:

There is great concern worldwide about using alternatives to antibiotics growth promoter (AGP) in animal production. Therefore, an experiment with 1.200 COBB 500® - 1-day-old male chicks was approved by Ethic Committee on Animal Use of FZEA/USP (CEUA 6768100524), and carried out aiming to evaluate the effects of the inclusion of a symbiotic (Pollax® - Ekoa Life Sciences) versus the AGP Zinc bacitracin (ZB) and the anticoccidials (ANC) Salinomycin (SAL) and Nicarbazin (NC). The nutritional plan consisted of a pre-starter diet – 0 to 12 days, growth 1 - 13 to 28 days, growth 2 – 29 to 39 days and finisher – 40 to 43 days, with *ad libitum* provided feed and water. The experimental diets were based on corn and soybean meal in mash feed. The level of inclusion of ZB, SAL and NC were 55 ppm, 60 ppm and 125 ppm, respectively. The weekly measured parameters were feed intake (FI), body weight (BW), body weight gain (BWG), feed conversion (FCR) and mortality (M), with BWG and FCR being corrected with the weight of dead birds. The density was 10 birds/m². The experimental design was completely randomized (CRD), in a 3x2+1 factorial scheme: 3 inclusion levels of Pollax® (0.05%, 0.10% and 0.15%), 2 absence or presence of ANC and AGP plus the Negative Control (NC), totaling 7 treatments, 6 treatments (3x2) with 30 birds with 6 replicates and the NC with 15 birds with 8 replicates. Normality was verified by the Shapiro-Wilk test (1%) and homogeneity of variances by the Bartlett test (1%). Analysis of variance (ANOVA) was performed in CRD, using RStudio and ExpDes.pt package. The effect of Pollax® was analyzed by polynomial regression, while the effect of ANC and AGP and the comparison of the control with the other treatments were evaluated by the F test. Microbiome analysis used amplicon sequencing of the 16S rRNA gene. To prepare metagenomic sequencing libraries of 16S ribosomal RNA - focusing on the variable V4 region - the HiSeq next-generation sequencing platform (Illumina, San Diego, CA, USA) was applied. The total number of species was calculated using Shannon (4.0171 ± 0.5907) and Simpson (0.9289 ± 0.0270) indices, whose results bring high diversity. The distribution of abundance of the species, determined by Chao index (715.1425 ± 165.6171), demonstrated a good amount. Both tests around microbiome were based in alpha diversity. A trend effect was observed for Pollax® use BW ($p=0.05946$), BWG ($p=0.05800$), corrected BWG ($p=0.06465$) and FCR ($p=0.08953$), at 42 days. The 0.15% Pollax® inclusion brought the most interesting values for the indices tested. In conclusion, the supplementation whit Pollax® enhanced the abundance and diversity of microbiota, such as broiler's performance.

Keywords: Benefical Microorganisms, Nutrition, Performance, Poultry



Radiographic Density Correlation Between Trabecular and Cortical Bones of Broiler Chicken Lines From 1 to 42 Days of Age

(PR-11 - Winner)

Paula Vitoria Black, Lays Priscilla Da Silva Melo, Camille Maróstica,
Amanda Luiza Dieckmann, Ingrid Vitória Scheffmacher Josefi e
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WINNER



PRODUCTION

Abstract:

During their growth, the skeleton of broilers must adapt its morphology and material properties to successfully withstand the mechanical effects of rapidly increasing body weight. The proximal metaphysis contains a large volume of trabecular bone, which plays a crucial role in distributing impact loads applied to the cortex, thus contributing to the mechanical strength of long bones, leading to microfractures, necrosis, and loss of bone mineral content. Cortical and trabecular bones are distinguished based on their degree of porosity. Cortical bones have porosity between 0–30%, while trabecular bones range from 30–90%. Therefore, cortical bone density can, to some extent, be represented by radiographic opacity alone; however, since trabecular bone consists of a mix of plate-like and columnar structures, its radiographic density is more difficult to quantify accurately. The goal of this study was to evaluate the pattern of radiographic bone density of the trabecular (RBDT) and cortical (RBDC) regions and assess the correlation between these values in broiler tibiae from 1 to 42 days of age. 1584 male Cobb, Ross and Embrapa line broiler chicks were distributed in a randomized trial with 12 repetitions of 44 birds per pen. Twelve birds per treatment were slaughtered weekly (from 1 to 42 days of age), and their tibiae were collected for radiographic densitometry of the proximal metaphysis and cortical region. A 10-step aluminum ladder, with 1 mm thickness between steps, was used as the radiographic reference. Images were processed, and grayscale values were converted to aluminum-equivalent thickness (mmAL). Data were analyzed using a polynomial regression in function of bird age. Pearson's correlation test was also applied to assess the correlation between RBDT and RBDC. Radiographic assessments of RBDT and RBDC revealed significant quadratic pattern ($p < 0.05$) over time for all three genetic lines. Based on the derivative of the regression equations, the peak RBDT and RBDC values for Cobb occurred at 27 ($R^2 = 0.53$) and 24.4 ($R^2 = 0.60$) days of age, respectively; for Ross, at 25.22 ($R^2 = 0.81$) and 25.26 ($R^2 = 0.64$) days; and for the Embrapa, at 30 ($R^2 = 0.60$) and 26.98 ($R^2 = 0.85$) days. These findings indicate that the Embrapa line, which exhibits a lower growth rate, also demonstrates a delayed mineralization in both trabecular and cortical bone regions. The Cobb and Ross lines followed similar patterns. Pearson's correlation coefficients between RBDT and RBDC were positive and significant ($p < 0.001$) for all lines: Cobb ($r = 0.46$), Ross ($r = 0.47$), and Embrapa ($r = 0.60$). The assessment of RBDT can estimate the RBDC and can be measured in association with the diagnosis of dyschondroplasia and chondronecrosis, which represent major causes of lameness in broiler chickens.

Keywords: Fast-Growing, Lameness, Proximal Metaphysis, Porosity



Effect of Embryonic Thermal Manipulation Between Days 7 and 16 of Incubation on Hsp70 and Myod Mrna Expression

(PR-20 - Honorable Mention)

HONORABLE MENTION



PRODUCTION

Marcos Henrique Soares, Henrique Carneiro Lobato,
Cristhian David Andrade Alfaro, Kirsty Ximena Noboa Carrasco,
Mariana Cristina Vieira, Julia Valadares Pereira,
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Abstract:

Thermal manipulation (TM) during the embryonic development is an epigenetic strategy used to enhance physiological responses. When properly adjusted to stimulate myogenic precursors and regenerative satellite cells, taking advantage of the third wave of myoblast proliferation (embryonic days 14 and 18), this technique has the potential to improve broiler performance under heat stress. This research aimed to assess TM protocols based on eggshell temperature (EST) and their influence on expression of heat shock protein 70 (Hsp70) and myogenic differentiation 1 (MyoD) genes during the rearing phase. A total of 4,300 fertile eggs from 39-wk-old Ross 308 breeders were incubated across five setters (860 eggs each). Each setter contained 10 trays with 86 eggs per tray. Group 1 (control) was set at 37.5°C and 60% relative humidity (RH) from day 1 to 19 day of incubation. TM was applied in Groups 2 to 5 covered days 7 to 16 of incubation, employing different EST and 65% RH (group 2: 39.5°C/6h/day; group 3: 39.5°C/12h/day; group 4: 40.5°C/6h/day and group 5: 40.5°C/12h/day). From days 17 to 19, all groups were incubated at 37.5°C and 60% RH, and from days 19 to 21, at 36.5°C and 65% RH. After hatch, 540 male chicks (108 per treatment) were reared with six cages of 18 chicks each. Chicks were reared under standard conditions until day 21 (Aviagen, 2018). From day 21 to 35, birds were subjected to daily heat stress (32°C for 8 h/day). After hatching, pectoral muscle samples were collected from 30 chicks (6 from each treatment group per day) at post-hatch days 0, 6, 21, 35 to quantification of muscle mRNA levels of Hsp70 and MyoD by real time PCR. The data were subjected to ANOVA (R Software, 2023), means were compared using Tukey's test ($p < 0.05$). At day 0, the group 5 exhibited the highest Hsp70 gene expression level (fold change=380.38) in comparison to all the other groups: fold change 1.0; 1.33; 1.13 and 0.7 to groups 1, 2, 3 and 4, respectively ($p < 0.05$). On the day 6, there was a decrease in Hsp70 expression in the TM treatments: fold change 0.0; 0.0; 0.05 and 0.04 to groups 2, 3, 4 and 5, respectively, compared to control group (fold change=1.00); however, group 5 showed higher expression than groups 2 and 3 ($p < 0.05$). On day 21 after thermal stress, an increase in Hsp70 expression was observed in group 5 (fold change= 1.52) followed by group 1 (fold change= 1.00) and then by the others ($p < 0.05$). At day 35, Hsp70 gene expression was reduced in all experimental treatments: fold change 0.01, 0.46, 0.12, and 0.16 to groups 2, 3, 4, and 5, respectively compared to the control group (fold change=1.00; $p < 0.05$). It can be observed that on day 0 of rearing the MyoD gene is expressed in group 1 (fold

change=1.00) and inhibited in all TM treatments: 0.03; 0.02; 0.03 and 0.03 to groups 2, 3, 4 and 5, respectively ($p<0.05$). At day 6, gene expression was higher in the control group and suppressed in the treated groups: 0.00, 0.02, 0.01, and 0.02 for groups 2, 3, 4, and 5, respectively ($p<0.05$). At day 21, group 3 exhibited an upregulation of Hsp70 expression (fold change=2.05), higher than other TM groups: 0.05, 0.19, and 0.17, groups 2, 4, and 5 respectively ($p<0.05$), but not significantly different from the control group (fold change=1.00). At 35 days of age, group 2 increase in MyoD gene expression (fold change=1.28) compared to all other TM treatments ($p<0.05$), although it did not differ from group 1 (fold change= 1.00; $p>0.05$). In conclusion, TM at 39.5°C/6 h/day improved MyoD gene expression patterns comparable to the control, which translates into a greater ability to mobilize satellite cells for fusion after the onset of heat stress. Prolonged exposure to higher temperatures, as 40°C/12h/d enhance thermotolerance but effects on hatchability and performance results needs be considerate.

Keywords: Satellite Cells, Myogenesis, Heat Stress

Biological Compounds in Mealworm Control (*Alphitobius Diaperinus*) in Poultry Production Systems (PR-01)

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Abstract:

Mealworm control has been a challenge for the broiler production's system. The technical, economic, environmental and public health impacts are difficult to quantify, but economic losses are certain, considering that the adult insect and larvae, causes damage to the structure of the poultry house. In addition, the Mealworm contributes to the transmission of several diseases (viruses, fungi and bacteria), especially Salmonellosis, which causes illness in birds and humans, reducing zootechnical performance, such as weight gain and feed efficiency, and impacts to the producer health and the products quality, when the chemical insecticides are used for its control. Conventional control has been carried out using chemical insecticides, which have been facing a increasing challenges, such as resistance developed by insects and concerns about the sustainability of the production system. The present study aims to evaluate the degree lethality (DL) of the microorganisms *Bacillus Thuringiensis* (Bt), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* (Ma), in isolated and associated forms (Bt+Bb, Bt+Ma, Bb+Ma and, Bt+Bb+Ma), as well as a possibility of additive effects and synergism. Adult insects and larvae, 30 per development stage, were placed in petri dishes lined with filter paper and sprayed with compounds diluted at 3% in distilled water. In addition to the treatments, distilled water and a chemical/organic insecticide in development (QUIEMB-C), were used as a negative and positive control, respectively. A completely randomized design was used, consisting of 9 treatments, including isolated compounds (3), associated compounds (4) and controls (2), with 10 replicates/treatment. Statistical analysis demonstrated a significant effect between treatments for both stages of insect development. Regarding the larvicidal potential, the best results were obtained for Bt+Bb (73.00%), Bt (71.64%) and Bt+Ma (62.67%), while for adult insects the best results were for Bt+Bb (77.61%), Bb (75.34%) and Bb+Ma (62.31%). The Bt+Bb compound showed a significant additive effect, enabling high (DL) for both larvae and adult insects. The chemical/organic compound under development showed high DL for both adult insects (98.09%) and larvae (98.47%), indicating low resistance of the insects.

Keywords: Poultry Farming, Biological Control, Pests, Bioassay

Natural Plants Extracts As Means to Control Mealworm (*Alphitobius Diaperinus*) in Poultry Production Systems (PR-02)

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Abstract:

Mealworms control using chemical insecticides have been faces challenges, as a developed resistance and concerns about the sustainability of the poultry production system. The experiment objective was to evaluate the insecticidal potential of plants extracts, as an alternative to chemical compounds, through the degree of lethality (LD), for adult insects. Plants extracts (5) were tested: Timbó (Derris urucu), Neem oil (Azadirachta indica), Andiroba (Carapa guianensis), Cinnamon (Cinnamomum verum) and Rotenone. A completely randomized design was used, with 8 treatments, with 10 replications/treatment, totaling 80 experimental units. Two bioassay methodologies were used, Petri plates, lined with filter paper and, 500 ml pots, containing a substrate composed of reused bedding and ration. The compounds were diluted in water (3%) while the positive controls (Pankada and Nokaut) followed the dilution indicated by the manufacturer (0.3%). The results on Petri plates showed 3 distinct groups, in terms of efficiency in controlling the adult insect. Rotenone (95.63%) had the highest insecticidal potential (DL), followed by Neem (83.73%), Andiroba (81.95%), Canela (81.17%) and Timbó (47.26%). In the bioassay conducted on poultry litter, the efficiency of all products was low (DL<50), with Rotenone (40.57%) and Neem (25.44%) showing the best results and Andiroba (10 .75%), Canela (4.06%) and Timbó (3.70%) showing DL<10%. In summary, Rotenone stood out as the most promising compound, suggesting a viable path for the development of new bioinsecticides. The disparity in efficacy between the two methodologies used indicates, however, that the plant extracts showed limitations in field environments. This limitation highlights the importance of developing laboratory methodologies that simulate better the poultry house conditions, besides the need to investigate possible improvements in the formulation and application of botanical compounds.

Keywords: Broiler Chicken; Mealworm; Bioinsecticide; Compounds; Plant Extracts

Effect of Embryonic Thermal Manipulation Between Days 7 and 16 of Incubation on Hatchability and Chick Quality (PR-03)

Thales Meireles de Melo Diniz, Victor Fernandes de Araújo, Henrique Carneiro Lobato, Marcos Henrique Soares, Julia Valadares Pereira, Luiza de Araújo Nascimento e Itallo Conrado Sousa de Araújo

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Abstract:

Embryonic thermal manipulation (TM) is a strategy used to induce physiological adaptations in broilers, such as thermotolerance, which can enhance performance under suboptimal environmental conditions. However, increasing incubation temperature may also induce physiological stress in embryos, potentially compromising hatchability and chick quality. This study aimed to evaluate TM protocols based on eggshell temperature and their effects on hatchability, chick quality, and day-old chick relative yolk sac weight. A total of 4,300 fertile eggs from 39-week-old Ross 308 breeders were incubated across five setters (860 eggs each). Each setter contained 10 trays with 86 eggs per tray, and the tray was considered the experimental unit (replicate). Group 1 (control) was incubated at 37.5°C and 60% relative humidity (RH) from day 1 to 19. Groups 2 to 5 involved TM from day 7 to 16 at 65% RH with the following protocols: Group 2: 39.5°C/6h/day; Group 3: 39.5°C/12h/day; Group 4: 40.5°C/6h/day and Group 5: 40.5°C/12h/day. From days 17 to 19, all groups were incubated at 37.5°C and 60% RH, and from days 19 to 21, at 36.5°C and 65% RH. Temperature was monitored daily using a pediatric ear thermometer (Braun Healthcare, El Paso, USA) on one randomly selected egg located at the center of each tray. Hatchability was calculated based on the number of fertile eggs. After pulling, chick physical quality was assessed in 15 chicks per tray (Tona *et al.*, 2003), and the relative yolk sac weight (as a percentage of chick weight) was evaluated in 5 chicks per tray. The data were subjected ANOVA, means were compared using Tukey's test ($p < 0.05$). Hatchability was significantly lower in group 5 (40.5°C/12h), at 31.55%, followed by group 4 (35.3%). In contrast, groups 1, 2, and 3 showed higher hatchability rates of 86.0%, 84.4%, and 84.6%, respectively ($p < 0.05$). Chick quality scores were similar among groups 1 to 4 (96.5, 97.0, 93.4, and 95.0, respectively), but were significantly higher than in group 5 (73.5) ($p < 0.05$). Relative yolk sac weight was 15.8% in group 5 which did not differ from groups 3 (13.9%) and 4 (14.6%) but was significantly higher than in groups 1 (12.8%) and 2 (12.0%) ($p < 0.05$). In conclusion, TM protocols of 39.5°C for 6h or 12h/day between days 7 and 16 of incubation resulted in hatchability, quality score and relative yolk sac weight comparable to the control group. Prolonged exposure to higher temperatures, as in group 5, severely impaired hatch outcomes and is not recommended.

Keywords: Embryo Mortality, Embryonic Development, Epigenetics, Heat Stress, Yolk Sac Utilization

Effect of Navel Quality in Day-Old Chicks on Pathological Examination of Internal Organs and Microbiological Evaluation of the Yolk Sac (PR-04)

Mariana Cristina Vieira, Christopher Oliveira Napier, Hemille Antunes Ferreira Miranda, Artur Cavalcanti de Souza, Thales Meireles de Melo Diniz, Victor Fernandes de Araújo e Itallo Conrado Sousa de Araújo

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Abstract:

The physical quality assessment of day-old chicks is a practice used to monitor the incubation process and can be performed using quantitative or qualitative methods. Among these, navel healing scoring is a widely used qualitative method in commercial hatcheries. This study aimed to evaluate macroscopic alterations in organs and investigate the microbiology of the yolk sac in chicks of different physical quality. A total of 372 male Ross 308 chicks, from 39-week-old breeder hens, were used. They were divided into two treatments: chicks with completely healed navels, without black buttons or vitelline membrane (first quality – Q1), and chicks with the presence of a black button around 2 mm (second quality – Q2). The chicks were housed in 12 cages in a climate-controlled room (6 per treatment, 31 chicks per cage). At placement, on day three, and on day five, six chicks per treatment (one per cage) were randomly selected, euthanized by cervical dislocation for necropsy. The following organs were examined: yolk sac, heart, air sacs and lungs, liver and gallbladder, proventriculus, gizzard, small intestine, pancreas, and ceca. Yolk sac contents were also collected for bacterial isolation and subsequent identification using MALDI-TOF technique. Q2 chicks showed a higher incidence of caseous material in the yolk sac (16.7%), airsacculitis (50%), hydropericardium (16.7%), and liver enlargement (33.3%). At placement, only Q2 chick samples showed bacterial growth, with the identification of *Enterococcus faecalis*. At three days of age, no bacterial growth was observed. However, at five days, *E. faecalis*, *E. faecium*, and *Escherichia coli* were identified in Q2 samples, while Q1 samples showed *Enterococcus hirae* and *E. coli*. These findings suggest that bacterial infections are present in chicks regardless of initial navel score. It can be concluded that physical quality at hatch, while useful as an initial screening tool and for evaluating incubation processes, was not effective in predicting bacterial infections in chicks. Furthermore, initial navel quality was not a determining factor in the birds' susceptibility to infections, as both groups presented macroscopic changes and bacterial growth in the yolk sac content, consistent with infectious conditions.

Keywords: Colibacillosis, Enterococcosis, Incomplete Navel Healing, Omphalitis

Effect of One-Day-Old Chick Physical Quality on Cecal Microbiota At 7 and 14 Days (PR-05)

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Abstract:

Poor navel healing and the presence of a black button in day-old chicks may indicate early microbiological alterations that can impair digestion, nutrient absorption, and immune system development. Given the limited information on how chick physical quality influences early cecal microbiota, this study aimed to assess the cecal microbial composition of broiler chicks with different physical quality scores at 7 and 14 days of age. Additionally, comparisons were made between the two ages within each quality group. A total of 360 male Ross308 chicks were assigned to two treatment groups based in chick Pasgar Score 5 to 10, according to Boerjan (2002): first-class chicks (Q1), chicks with fully healed navels, no black button and/or vitelline membrane, and a Pasgar score of 10 (optimal). Second-class chicks (Q2): chicks with a 2 mm black button and/or vitelline membrane (Pasgar score of 8). Chicks were housed in 12 climate-controlled cages (6 per treatment, 30 chicks per cage). At 7 and 14 days of age, one bird per replicate was euthanized via cervical dislocation, and cecal contents were aseptically collected for analysis. DNA was extracted from the samples, and the V3-V4 hypervariable region of the 16S rRNA gene was amplified. Amplicons were purified, quantified, and used to construct libraries for sequencing on the Illumina platform. The resulting sequences were processed using bioinformatics tools, and taxonomic data were subjected to multivariate statistical analyses ($p < 0.05$). At 7 days, chicks in the Q1 group showed a higher abundance of the genus *Lhubacter* compared to Q2 ($p = 0.04$), and *Dysosmobacter* abundance was greater in Q1 at 7 days than at 14 days ($p = 0.027$). At 14 days, Q1 chicks exhibited a higher proportion of unclassified microorganisms compared to Q2 ($p = 0.024$). In contrast, Q2 chicks showed increased abundances of *Fournierella*, *Oscillibacter*, *Flavonifractor*, *Anaerotignum*, and *Erysipelatoclostridium* at 14 days compared to 7 days ($p < 0.05$). Principal Component Analysis (PCA) revealed a clear separation between treatment groups at both sampling points, indicating that initial chick quality influences the development and structure of the cecal microbiota. In conclusion, chicks with poor navel healing (Pasgar score 8) developed a distinct cecal microbiota characterized by a higher abundance of bacterial genera such as *Fournierella*, *Oscillibacter*, *Flavonifractor*, *Anaerotignum*, and *Erysipelatoclostridium* at 14 days of age. Several of these genera have been associated in previous studies with intestinal dysbiosis, inflammation, or metabolic disturbances. These findings suggest that poor physical quality at hatch may compromise early gut microbial development, potentially impacting intestinal health and immune maturation.

Keywords: Navel Healing, Microbial Composition, Bacterial Genera

Effect of Navel Quality in Day-Old Chicks on Organ Development and Yolk Sac Utilization (PR-06)

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Abstract:

The absorption of the yolk sac and the development of the gastrointestinal tract in chicks are critical for early nutrient utilization. However, limited information is available on how the physical quality of the navel in day-old chicks affects the mobilization of energy reserves stored in the yolk sac. This study aimed to evaluate the impact of navel quality on yolk sac absorption and digestive organ development in broiler chicks. A total of 372 one-day-old male Ross 308 chicks from a commercial hatchery were used. Birds were assigned to two treatments: Q1, with completely healed navels (no black button or vitelline membrane), and Q2, with the presence of a 2 mm black button. Chicks were housed in 12 climate-controlled cages (6 per treatment, 31 birds per cage). From placement until day 5, one chick per cage (6 per treatment/day) was randomly selected daily for organ and yolk sac collection and weighing. The evaluated organs included the yolk sac, heart, liver (gallbladder removed), proventriculus, gizzard, small intestine, pancreas, and ceca. Relative organ weights were calculated based on yolk-free body weight. During the first 24 hours (at pulling and day 1), chicks in the Q1 group had significantly lower yolk sac weights compared to Q2 ($p < 0.05$), indicating more efficient nutrient absorption. At pulling, the yolk sac represented 6.94% of body weight in Q1 chicks, while in Q2 chicks it accounted for 11.33%. On day 1, these values were 2.11% and 4.36%, respectively. No significant differences were observed between groups on subsequent days ($p > 0.05$). No differences were found in the weights of the proventriculus, gizzard, ceca, or heart throughout the evaluation period ($p > 0.05$). However, on day 1, Q1 chicks showed a higher relative weight of the small intestine (7.27%) compared to Q2 chicks (6.34%) ($p < 0.05$). No further differences were observed between groups in the following days ($p > 0.05$), indicating a compensatory development in Q2 chicks over time. In summary, chicks with better navel quality exhibited faster yolk sac absorption and more advanced gastrointestinal development during the first day's post-hatch. However, this advantage did not persist until the end of the first week, demonstrating that chicks with unhealed navels are capable of compensating when raised under appropriate management conditions.

Keywords: Nutrient Absorption, Post-Hatch Metabolism, Navel Healing

Effect of Embryonic Thermal Manipulation Between Days 7 and 16 of Incubation on Serum Levels of Triiodothyronine, Thyroxine and Cortisol (PR-07)

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Abstract:

Thermal manipulation (TM) during embryogenesis may induces epigenetic modifications that accelerate hypothalamic-pituitary-thyroid/adrenal axis maturation, leading to reduce post-hatch thyroid hormone levels. This hormonal modulation lowers metabolic heat production during growth, enhancing performance under heat stress. This study aimed to evaluate the effects of thermal manipulation (TM) on the levels of triiodothyronine (T3), thyroxine (T4), and cortisol in broilers at pulling and under heat stress conditions at 21 and 35 days of age. A total of 4,300 fertile eggs from 39-wk-old Ross 308 breeders were incubated across five setters (860 eggs each). Each setter contained 10 trays with 86 eggs per tray. Group 1 (control) was set at 37.5 °C and 60% relative humidity (RH) from day 1 to 19 day of incubation. TM was applied in groups 2 to 5 covered days 7 to 16 of incubation, employing different EST and 65% RH (group 2: 39.5 °C/6h/day; group 3: 39.5 °C/12h/day; group 4: 40.5 °C/6h/day and group 5: 40.5 °C/12h/day). From days 17 to 19, all groups were incubated at 37.5 °C and 60% RH, and from days 19 to 21, at 36.5 °C and 65% RH. After hatch, 540 male chicks (108 per treatment) were reared with six cages of 18 chicks each. Chicks were reared under standard conditions until day 21 (Aviagen, 2018). From day 21 to 35, birds were subjected to daily heat stress (32 °C/8h/day). On days 0, 21, and 35 of life, six chicks from each treatment were selected for blood sample collection. On the days when the thermal heat cycle was applied, samples were collected 4 hours after the beginning of heat exposure. The blood samples were centrifuged at 5,000 RPM for 5 minutes, and the serum was analyzed using the Immulite 2000 analyzer (Siemens Healthcare Diagnostics, Berlin, Germany). The data were subjected ANOVA, followed by Tukey's test ($p < 0.05$). At pulling, serum cortisol levels were lower in control group (1.77 ng/dL) compared to the other groups ($p < 0.05$), which did not differ significantly from each other: group 2: 2.97 ng/dL; group 3: 3.01 ng/dL; group 4: 2.84 ng/dL; group 5: 2.84 ng/dL ($p > 0.05$). On subsequent days, cortisol levels in TM groups did not differ from those in the control group ($p > 0.05$). Regarding T3, differences were observed only on day 21, chicks from group 5 (176.20 ng/dL) and control group (142.50 ng/dL) showed higher levels ($p < 0.05$) compared to groups 2 (128.50 ng/dL), 3 (126.43 ng/dL), and 4 (140.16 ng/dL), which did not differ among themselves ($p > 0.05$). As for T4, at pulling, the TM groups did not differ among themselves: group 2 (2.40 ng/dL), group 3 (2.42 ng/dL), group 4 (2.30 ng/dL), and group 5 (2.34 ng/dL), but all showed higher serum concentrations than control group (1.48 ng/dL; $p < 0.05$). No significant differences in T4 levels were observed on the subsequent days ($p > 0.05$). In conclusion, TM protocols increased embryonic stress and post-hatch metabolism. TM at 39.5 °C (6 or 12 h/day) and 40.5 °C/6 h/day from days 7-16 reduced T3 levels by day 21, indicating improved thermotolerance. In contrast, prolonged exposure (40°C/12h/day) failed to optimize metabolism, likely due excessive thermal stress.

Keywords: Epigenetics, Heat Stress, Thermotolerance

Effect of Day-Old Chick Physical Quality on Organs Development, Yolk Sac Consumption, and Hepatic Glycogen Quantification (PR-08)

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Abstract:

The physical and behavioral characteristics of day-old chicks such as navel healing and activity level are commonly used by hatchery to assess their quality. These features indicate the chick's early vigor, but their direct effects on development and performance are not fully understood. This study aimed to evaluate how physical chick quality influences the development of digestive organs, yolk sac utilization, and hepatic glycogen. A total of 540 one-day-old male Ross 308 chicks were allocated to three treatment groups: Q1: first-class chicks with a Pasgar score of 10, Q2: second-class chicks with unhealed navels and a Pasgar score of 8, and Q1+Q2: a mixed half/half group of both chick types. The birds were housed in 18 battery cages (6 replicates per treatment, 30 chicks each) for 35 days under climate-controlled conditions, with free access to feed and water and a 16L:8D cycle. Measurements included the relative weight (to body weight) of organs (liver without gallbladder, heart, spleen, proventriculus, gizzard, small intestine, pancreas, and ceca), yolk sac absorption, and hepatic glycogen concentration (mg/g). Daily during the first week post-hatch, one chick per cage was randomly selected for euthanasia and organs collection assessed. The hepatic glycogen analyses were performed in two steps: the first involved sample digestion, and the second consisted of the colorimetric determination of reducing sugars using the DNS method. All the data were analyzed using ANOVA (R Software, 2023) followed by Tukey's test ($p < 0.05$). Q2 chicks exhibited significantly higher relative yolk sac weights on days 0, 1, 4, and 7 (10.11%, 3.58%, 0.44%, and 0.20%, respectively) compared to Q1 (6.23%, 1.79%, 0.22%, and 0.05%) and Q1+Q2 (9.58%, 3.00%, 0.23%, and 0.05%) ($p < 0.05$), indicating reduced energy reserve utilization. No significant differences were observed among treatments in the relative weights of the heart, proventriculus, gizzard, small intestine, pancreas, and ceca, or in hepatic glycogen concentration during the evaluation period ($p > 0.05$). However, Q1 chicks had significantly higher liver weights on days 1 (4.62%) and 5 (4.65%) compared to Q2 (3.86% and 4.03%) and Q1+Q2 (3.74% and 4.19%) ($p < 0.05$). Additionally, Q1 chicks had lower spleen weights on day 1 (0.03%) compared to Q2 (0.06%) and Q1+Q2 (0.08%). In conclusion, chicks with a lower Pasgar score (Q2) showed reduced yolk sac absorption during the first week of life compared to high-quality chicks (Q1). However, no lasting effects were observed on organ development or hepatic glycogen content during this period, suggesting that early developmental differences may be transient and potentially compensated for as chicks grow.

Keywords: Pasgar Score, Energy Reserves, Relative Organ Weight, Post-Hatch Development, Glycogen Metabolism

Bone Development and Biomechanical Characteristics in Broiler Lines (PR-09)

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Abstract:

The productivity of broiler chickens is directly linked to bone health, which must support rapid growth and ensure mobility and welfare. The accelerated growth rate of broilers can be a contributing factor to various disorders metabolic and bone problems. Genetic improvement can impact bone structure and growth, influencing both growth curves and bone quality traits. These conditions often result in locomotor abnormalities, negatively affecting both welfare and performance. The goal of this study was to evaluate bone development and biomechanical characteristics in broilers of different strains from 1 to 42 days of age. 1584 male Cobb, Ross and Embrapa line broiler chicks were distributed in a completely randomized controlled trial with 12 repetitions of 44 birds per pen. Twelve birds per treatment were slaughtered weekly (from 1 to 42 days of age), and their humerus, tibiotarsus and femur were collected for bone strength (BS), Seedor index (SI - correlation between bone length and weight) and cortical bone thickness (CBT) evaluations. The results were analyzed using a polynomial regression in function of the birds' ages. BS analysis presented quadratic pattern ($p < 0.05$) over time for all three genetic lines. According to the adjusted equations, the peak values for the Ross line occurred at 33.14, 28.95, and 31.52 days of age for the tibiotarsus, femur and humerus, respectively. For Cobb, these were observed at 37.14, 31.70, and 35.66 days for tibiotarsus, femur and humerus, respectively. For the Embrapa line, the values occurred at 30.23 and 33.79 days for the femur and humerus, while the tibia showed a cubical pattern. The mechanical strength of a long bone depends on the amount and spatial distribution of bone mass and tissue properties, such as the material density and composition of the organic matrix. Due to the slower growth rate of the Embrapa line compared to the others, tibial resistance to breakage may suffer variations over time. SI increased linearly ($p < 0.05$) with age for all genetic lines across all bone types evaluated. CBT presented significant quadratic pattern ($p < 0.05$) over time for all three lines. Based on the first derivative of the regression equations, the highest CBT values for Ross occurred at 31.39, 29.45, and 32.09 days of age for the tibiotarsus, femur and humerus, respectively. For Cobb, at 29.00, 29.26, and 37.02 days for the tibiotarsus, femur and humerus, respectively and for Embrapa at 37.20, 32.91, and 37.32 days for the tibiotarsus, femur and humerus, respectively. The study showed that the lines evaluated present different bone development and biomechanical characteristic pattern across growth, which should be considered when implementing nutritional and management strategies.

Keywords: Genetic Improvement, Bone Strength, Seedor Index, Cortical Bone Thickness, Welfare

Impact of Litter Management on the Occurrence of Locomotor Problems and Arthritis in Broiler Chickens (PR-10)

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Abstract:

The presence of litter clumps and uneven surfaces in poorly managed litter contributes to joint instability in long bones, which can lead to microfractures, cleft, degeneration and necrosis of the metaphyseal area, accompanied by loss of bone trabecular. On the other hand, wet litter can predispose birds to plantar pad injuries, which cause pain and restrict access to water and feed. These lesions increase the rate of carcass condemnation at slaughter due to bone fragility, dermatitis/cellulitis, airsacculitis, and serve as entry points for litter-borne bacteria and viruses. Lameness and impaired gait are major welfare issues in broiler chickens. A total of 4032 male chicks were distributed in a completely randomized controlled trial with two litter conditions (well and poorly managed) with 24 replications of 42 birds each. For the treatment of poorly managed litter, weekly turning was not performed, the pressure and flow rate of the nipples were adjusted to moisten the litter, and the feeders were kept lower. At 21 and 42 days of age, 2 birds/replication were sacrificed for sample collection. The arthritis index was calculated using the formula: $(a+c)/b$ (diameters of the distal tibiotarsus (a), intertarsal joint (b) and proximal tarsometarsus (c)). The following parameters were also assessed: radiographic bone density of the trabecular (RBDT), radiographic bone density of the cortical (RBDC), bone strength (BS), Seedor index (SI - correlation between bone length and weight) and cortical bone thickness (CBT). Results were analyzed using the SAS statistical program and the means were compared by the Tukey test. At 21 days of age, birds raised on poorly managed litter presented lower ($p<0.05$) arthritis index, RBDT, RBDC, and SI values, and higher BS values ($p<0.05$). Traumatic arthritis caused by poorly managed litter can predispose to infectious arthritis caused by viruses or bacteria. No significant effect ($p>0.05$) of litter condition was observed on CBT. At 42 days of age, no litter management effect ($p>0.05$) was observed on the arthritis index, CBT, and RBDC. For BS and SI evaluations, the lowest values ($p<0.05$) were observed in tibias of birds raised on poorly managed litter. However, for RBDT, higher values ($p<0.05$) of RBDT were observed for birds raised on poorly managed litter. Heavier body weight is a factor that predisposes to lesions in the proximal metaphysis, which may explain increased trabecular bone porosity due to higher mechanical load observed in heavier birds from the well-managed litter group. Improper litter management is a significant factor that contributes to locomotor problems in broilers, impacting bird health, welfare and productivity due to excessive moisture, compaction, ammonia emission and high pathogen load.

Keywords: Wet Litter, Litter Clumps, Litter Management, Mineralization, Bone Loss

Application of Microbiological Solutions in Egg Incubation: Preliminary Results and Industrial Perspectives (PR-12)

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Abstract:

Eggshell sanitization is a routine practice in commercial hatcheries aimed at reducing contamination by pathogenic microorganisms. However, this process also removes nearly all of the natural microbiota present on the eggshell surface, including commensal or potentially beneficial microorganisms that may contribute to the early health of chicks. On the other hand, studies involving the *in ovo* injection of probiotics have shown positive effects on gut health and performance in broilers, but this technique is invasive and not easily applicable on a commercial scale. As an alternative, using microbiological solutions sprayed during incubation is a practical and new approach that fits well with current hatchery practices. Nevertheless, despite its potential, important challenges remain – such as limited knowledge about the natural eggshell microbiota – which hinder the safe and effective implementation of this technology. Therefore, this preliminary study aimed to (1) characterize the cultivable microbiota present on the eggshells of eggs from different production systems (free-range and commercial hatchery) and (2) evaluate the effects of the non-invasive application of selected beneficial microorganisms during incubation on hatchability and early chick performance. Eggshell samples from both sources were collected for microbiological isolation using various culture media, followed by morphological and MALDI-TOF MS identification. Next, 63 fertile Cobb eggs were divided into three groups: one control group that was only sprayed with a sterile solution, and two treatment groups that received different microorganisms sprayed on days 0, 3, 7, 10, 14, and 18 of incubation. Hatchability was assessed on day 21, and the chicks were monitored until 15 days of age for body weight evaluation. The isolated microorganisms showed considerable morphotypic diversity and a low incidence of hemolytic strains. Among the treatments, one of the tested microorganisms increased hatchability compared to the control group and showed a trend toward improved early performance. The spray application method proved to be simple, safe, and compatible with current hatchery practices. In conclusion, the non-invasive application of microbiological solutions during incubation is a practical and new approach to enhance bird health and performance, which could lead to new products. Acknowledgment: The authors thank CNPq and FAPEMIG for their support of this research.

Keywords: Probiotics, Eggshell Microbiota, Egg Sanitization, Early Chick Performance, Incubation Technology

Serological Profile of Broilers Chickens With Different Degrees of White Striping Fed Diets of Varying Density (PR-13)

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Abstract:

White striping (WS) is a common muscle abnormality in modern poultry production, associated with reduced meat quality and economic losses throughout the production chain. This study aimed to evaluate the relationship between different degrees of WS severity and the serological profile of broilers fed diets of varying density. Broilers (Cobb × Cobb 500) were fed either high-energy (HE; 3,000 to 3,300 kcal ME/kg and 24.19 to 19.56% CP) or low-energy (LE; 2,900 to 3,050 kcal ME/kg and 20.44 to 18.37% CP) diets over four phases (pre-starter: d 1–7; starter: d 8–21; grower: d 22–35; and finisher: d 36–50). A total of 132 male birds were analyzed, slaughtered at 20, 30, and 50 days of age. Breast fillets were classified by WS severity (normal, moderate, or severe). Serum analysis of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), uric acid, and albumin levels was performed using a spectrophotometer with automatic calibration and high-performance reading. Data were analyzed using one-way ANOVA (SAS 9.3), and mean comparisons were conducted using the Tukey test at a 5% significance level. Broilers with moderate and severe WS showed statistically elevated ($p < 0.05$) CK levels (up to 21,725 IU/L in the severe group at 50 days on the LE diet), AST (up to 556 IU/L), and LDH (up to 4,096 IU/L), along with increased albumin concentrations (up to 0.8 g/dL), indicating muscle damage and impaired cellular integrity. The HE diet resulted in earlier enzymatic peaks: at 30 days in the moderate group, CK levels reached 10,505 IU/L in the HE group and 6,823 IU/L in the LE group. However, at 50 days, the highest CK and AST values were observed in birds fed the LE diet, suggesting cumulative effects of prolonged growth under lower energy availability. CK was the most responsive biomarker to WS severity, consistently exceeding 14,000 IU/L in severe cases. In contrast, ALT and uric acid levels remained statistically unchanged, suggesting a closer association with hepatic and renal functions than with muscular damage. Although uric acid approached critical thresholds (>15 mg/dL), it was not significantly influenced by diet or age. In conclusion, WS exerts systemic effects on muscle integrity, and the enzymatic profile—particularly CK—represents a promising tool for early detection of muscle lesions in broilers. Understanding the interplay between age-specific nutrition and muscle abnormalities may support the development of more balanced diets that limit excessive growth while preserving meat quality.

Keywords: Biomarkers, Muscle Abnormality, Nutrition, Poultry



Effect of Day-Old Chick Physical Quality on the Performance of Broilers Up to 35 Days of Age (PR-15)

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Abstract:

The physical quality of chicks has been used in hatcheries as a tool to assess incubation efficiency. Despite the frequent use of subjective evaluation methods, few studies have linked chick quality to their adaptation throughout the rearing period and its effects on performance. This study aimed to evaluate the performance of chicks with different physical qualities from day 1 to day 35. A total of 540 Ross 308 chicks were used, divided into three groups: Q1 (navel completely healed, no black button or vitelline membrane, Pasgar score 10), Q2 (2 mm black button, Pasgar score 8), and Q1+Q2 (50% of each). Chicks were housed in 18 cages (6 replicates per group with 30 chicks each one) in a climate-controlled room with *ad libitum* access to water and feed, formulated according to Aviagen (2018) guidelines. Performance parameters were recorded weekly, and data were analyzed by ANOVA (R Software, 2023), followed by Tukey's test ($p \leq 0.05$). Q1 showed higher weight gain ($p \leq 0.05$) from day 1 to 7 (177.5 g), 1 to 14 (547.6 g), and 1 to 21 (986.2 g), compared to Q2 (160.3 g, 503.8 g, 924.7 g) and Q1+Q2 (165.8 g, 521.2 g, 940.7 g). At 14 days, feed intake was 643.8 g (Q1), 652.1 g (Q2), and 652.3 g (Q1+Q2), with feed conversion ratios of 1.18, 1.29, and 1.25, and mortality rates of 2.22%, 2.78%, and 2.22%, respectively ($p > 0.05$). Chick quality score did not affect performance from 1-21; 1-28 and 1-35 days ($p > 0.05$). At 21 days, intake was 1,178.9 g (Q1), 1,195.4 g (Q2), and 1,193.2 g (Q1+Q2); feed conversion ratios were 1.19, 1.29, and 1.27; mortality remained 2.22%, 2.78%, and 2.22%. At 28 days, body weights were 1,656.7 g (Q1), 1,626.3 g (Q2), and 1,647.6 g (Q1+Q2); feed intake was 2,643.7 g, 2,656.5 g, and 2,651.2 g; feed conversion ratios were 1.26, 1.30, and 1.29; mortality remained unchanged. At 35 days, body weights were 2,257.3 g (Q1), 2,228.9 g (Q2), and 2,245.6 g (Q1+Q2); total feed intake was 4,463.2 g, 4,484.6 g, and 4,474.2 g; feed conversion ratios were 1.33, 1.36, and 1.34. It is concluded that physical chick quality influences only early performance up to 14 days post-placement, with no observed effect on chick mortality.

Keywords: Feed Conversion Ratio, Omphalitis, Pasgar Score

Estimating the Carbon Footprint of Commercial Egg Production in the Amazonian Savanna (PR-16)

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Abstract:

The global poultry industry is facing increasing pressure to align with a low-carbon economy; however, a significant information gap exists regarding greenhouse gas (GHG) emissions from egg production, particularly in unique ecosystems such as the Amazonian savanna. This study aimed to estimate the carbon footprint of a commercial brown egg production system in this previously unquantified region of Roraima, Brazil. Data on key inputs, including feed provision, energy consumption, and manure management, were collected over one year (2022) from a farm with 10 conventional poultry houses. Using 1 kg of eggs as the functional unit, emissions were modeled with the Carbon Footprint Estimation Toolkit and 2006 IPCC emission factors. The egg production system comprised ten aviary units, housing a total of 240,000 laying hens from the Hisex Brown and Hy-Line Brown strains. The egg production system included 10 aviaries, a total of 240,000 birds of the Hisex Brown and Hy-Line Brown lines, between 2 and 490 days of age, distributed in 3 breeding phases (breeding, rearing, and egg production) and with a total volume of 39,420,000 eggs produced. The total annual GHG emissions for the farm were estimated at 373.42 metric tonnes of CO₂ equivalent (t CO₂e). The primary source was off-farm electricity consumption, accounting for 224.90 t CO₂e/year (60.2% of the total). On-farm activities constituted the second-largest source at 148.52 t CO₂e/year (39.8%), with manure management being the predominant component, releasing 71.09 t/y of methane (CH₄) and 67.80 t/y of nitrous oxide (N₂O). When normalized by the functional unit, the carbon footprint was calculated to be 0.158 kg of CO₂e per kilogram of eggs. The study establishes that the emissions profile is dominated by the consumption of electrical energy from external sources, and secondarily, by manure management. For this specific system, electrical energy is the primary factor to consider for mitigating the carbon footprint. Therefore, mitigation strategies must be primarily directed towards improving energy efficiency and implementing advanced manure treatment technologies to reduce the environmental impact of egg production in tropical systems.

Keywords: Laying Hen Production, Greenhouse Gases Emissions, Environmental Impact

Egg Yolk Metabolites As Potential Indicators of Quality (PR-17)

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Abstract:

Egg quality is crucial for hatchability, and although several factors can interfere with this quality, the influence of yolk compounds is poorly studied. This study aimed to evaluate the association of yolk compounds on eggshell quality. Hatchable eggs from five flocks of Lohmann LSL Lite light breeders, aged 52 to 64 weeks, were used. A total of 99 eggs underwent the specific gravity test based on Archimedes' principle. Eggs were classified into low (between 1064 and 1069 g/L) and high specific gravity groups (between 1079 and 1090 g/L), with 44 eggs in the low and 55 eggs in the high specific gravity group. Metabolite extraction was followed by analysis using high-performance liquid chromatography coupled with a high-resolution mass spectrometer with electrospray ionization. After data alignment, normalization, and filtering, multivariate analysis was performed using Hotelling's T^2 test. Subsequently, Principal Component Analysis (PCA) and logistic regression were performed. The results revealed that Melatonin Glucuronide was more abundant in eggs with low specific gravity, with a mean intensity of 14.96 compared to 10.68 in eggs with high specific gravity ($p < 0.05$). Conversely, Dihydroxytetradecanoic Acid was more abundant in eggs with high specific gravity, with a mean intensity of 18.90 versus 15.42 in the low specific gravity group ($p < 0.05$). Although differences in mean abundance were observed, the PCA did not show a clear separation between groups. Moreover, logistic regression analysis resulted in a low area under the curve (AUC), indicating that these two metabolites alone are insufficient to accurately discriminate between eggshell quality groups, likely due to the influence of additional variables. Melatonin Glucuronide, a powerful antioxidant metabolite formed during melatonin metabolism, is found in the yolk. Previous studies suggest that melatonin supplementation in laying hens increases bone strength but may reduce eggshell thickness. The higher presence of this metabolite in yolk of eggs with lower specific gravity could suggest a physiological trade-off in calcium allocation, favoring bone development over eggshell deposition. Dihydroxytetradecanoic Acid, a long-chain fatty acid produced by beneficial gut bacteria, has recognized anti-inflammatory and antimicrobial properties. Its greater abundance in eggs with higher specific gravity may indicate a link between microbiota composition and eggshell quality. This study highlights the possible role of melatonin metabolism in modulating calcium utilization and egg quality, as well as the potential association between intestinal bacteria and egg quality, being the first to demonstrate the relevance of the reproductive-intestinal axis.

Keywords: Egg Quality, Metabolomics, Melatonin Glucuronide, Dihydroxytetradecanoic Acid

Behavior of Broiler Chickens: Effects of Supplementation With Garlic and Cinnamon Extracts As A Sustainable Alternative to Growth Promoters (PR-18)

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Abstract:

The use of natural additives such as garlic and cinnamon extracts represents a sustainable alternative to growth promoters in poultry production. These compounds may positively influence bird behavior and welfare. Therefore, this study aimed to evaluate the effects of including garlic and cinnamon extracts in the diet of broiler chickens on animal behavior, as a replacement for conventional performance enhancers. The research was conducted at UFGD, in an experimental broiler facility, using 1,200 one-day-old male chicks of the Ross® TM4 strain. The experimental period lasted 42 days. Birds were distributed in a completely randomized design with five treatments and six replicates of 40 birds each, totaling 30 experimental pens. Treatments were as follows: T1 – positive control (with the antimicrobial performance enhancer zinc bacitracin); T2 – negative control (without zinc bacitracin or additives); T3 – inclusion of 0.250 kg/ton of garlic and cinnamon extract; T4 – inclusion of 0.500 kg/ton; and T5 – inclusion of 0.750 kg/ton. At 42 days of age, bird behavior was evaluated through 1-hour video recordings conducted at 8 a.m., 12 p.m., and 4 p.m. A single observer analyzed the videos every 10 minutes, totaling six observations per period. Behaviors were recorded based on a pre-established ethogram. Statistical analysis was performed using SAS software (version 9.4) with the GLIMMIX procedure. The inclusion levels were compared to the positive control using Dunnett's test, and results were expressed as percentages representing the frequency of observed behaviors. Significant differences were found in inactive behaviors ("standing" and "sitting"). Birds supplemented with 750 g/ton of garlic and cinnamon extract showed the lowest percentage of standing behavior. Moreover, a decrease in the percentage of sitting behavior was observed as the inclusion level increased. This reduction in inactivity was associated with a proportional increase in active behaviors such as walking, feeding, and environmental exploration, suggesting greater overall activity. Therefore, the results of this study indicate that the inclusion of garlic and cinnamon extracts can promote increased activity and a potential improvement in the behavioral welfare of broiler chickens, as reflected by the reduction in inactive behaviors and increased engagement in feeding and exploratory activities. These findings suggest that such additives hold potential as tools for improving animal welfare and quality of life in poultry farming.

Keywords: Natural Additives, Behavioral Evaluation, Animal Welfare



Effects of Thermal Manipulation During Embryonic Development on Gene Expression and Performance of Broiler Chickens (PR-19)

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Abstract:

Poultry farming is one of the main sectors of Brazilian agribusiness, placing the country among the world's largest producers and exporters of chicken meat. However, frequent heat waves affecting various regions have posed a serious challenge to the poultry production chain, causing heat stress in birds and resulting in significant losses in performance and increased mortality. In this situation, the development and adoption of technologies to mitigate the negative effects of excessive heat are essential to ensure the sector's sustainability and competitiveness. Within this context, thermal manipulation (TM) during the incubation of fertile eggs has been investigated as a strategy to promote thermotolerance in broiler chickens, although its effects on performance and physiological parameters remain not fully understood. Therefore, the objective of this study was to evaluate the effects of late-incubation TM on hepatic HSP70 gene expression and the productive performance of broiler chickens. A total of 864 fertile Ross® eggs were distributed across four treatments: control (37.5 °C throughout incubation), T3h (39 °C for 3 h on days 16, 17, and 18 of incubation), T12h (39 °C for 12 h on the same days), and T24h (39 °C for 24 h on those days). After hatching, chicks were reared until 42 days of age, and performance parameters and hepatic HSP70 gene expression were evaluated. Birds from the T24h group showed reduced ($p<0.05$) feed intake throughout the entire rearing period and lower weight gain at 42 days of age ($p<0.05$). Feed conversion ratio was not affected ($p>0.05$). Regarding gene expression, higher hepatic HSP70 expression was observed in birds from the T3h group compared to the other treatments. It is concluded that TM for 3 h per day during the final days of incubation may promote physiological responses associated with thermotolerance without compromising performance. In contrast, continuous exposure to 39 °C for 24 h resulted in initially favorable responses up to 33 days of age but negatively impacted final performance at 42 days. Acknowledgment: The authors thank CNPq and FAPEMIG for their support of this research.

Keywords: Heat Stress, Hsp70, Incubation Temperature, Poultry Production, Thermotolerance



Could Incubation Temperature Affect the Locomotor System and Performance of Broilers During Rearing? (PR-21)

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Abstract:

Bone formation and growth begin during embryogenesis and can be influenced by incubation temperature, which regulates the development of organs and body structures. However, few studies have evaluated how incubation management practices affect broiler chickens during the rearing period. This study aimed to evaluate whether deviations from the standard eggshell temperature (EST), either above or below the recommended range during the mid-incubation phase, influence physiological, skeletal, locomotor and performance traits of broilers at slaughter age. A total of 2,408 fertile eggs from 45-week-old Cobb 500® breeder hens were incubated from day 8 to 18.5 under four constant EST treatments: (1) Control (37.8 °C); (2) Low EST (36.7 °C); (3) High EST (38.9 °C); and (4) Very High EST (39.4 °C). 800 male chicks were selected and reared under standard commercial conditions until 42 days of age. The following parameters were evaluated at the end of the rearing period: blood variables (calcium [Ca], phosphorus [P], alkaline phosphatase [ALP], growth hormone [GH], thyroid hormones [T3,T4], parathyroid hormone [PTH], and vitamin D3); rectal temperature (RT); tibial parameters (weight, length, width, Ca, P, ash content, and bone strength); incidence of skeletal and locomotor disorders (tibial dyschondroplasia [TD], valgus-varus deformity [VV], footpad dermatitis [FPD], gait score [GS], and latency-to-lie [LTL]); productive performance (feed intake, weight gain, body weight, feed conversion ratio, and viability); carcass and cut yields (breast, wing, thigh, and drumstick). Data were analyzed using a completely randomized design and Tukey's test ($p \leq 0.05$) with SAS® OnDemand. Incubation temperature deviations significantly affected most evaluated variables. Low, high, and very high ESTs impaired ($p \leq 0.05$) broiler performance, carcass and cut yields, serum PTH and ALP levels, tibial Ca, P, ash content, weight, length, and width. VV and LTL disorders were also more prevalent ($p \leq 0.05$) in these groups. The very high EST group showed the most detrimental results across all assessments. For serum Ca, P, vitamin D3, bone strength, wing yield, TD and GS incidence, no differences ($p > 0.05$) were observed between the low and control EST groups, with significant impacts only under high and very high EST ($p \leq 0.05$). GH levels and FPD incidence were negatively affected exclusively by very high EST ($p \leq 0.05$). RT was influenced by both low and high ESTs ($p \leq 0.05$). In conclusion, constant deviations in EST (either above or below the standard) during the mid-embryonic phase are not recommended, as they negatively affect physiological, skeletal, locomotor, performance, and carcass yield traits of 42-day-old broilers, with high temperatures causing the most severe impairments.

Keywords: Carcass Yield, Eggshell Temperature, Embryonic Development, Grow-Out Period, Osteogenesis



Intermittent Prenatal Thermal Manipulation and Its Effects on Bone Development and Performance of Broilers At Slaughter Age (PR-22)

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Abstract:

Intermittent prenatal thermal manipulation (IPTM) has been proposed as a tool to induce epigenetic adaptation, potentially improving thermotolerance in broilers. Additionally, as skeletal disorders can originate during embryogenesis, IPTM may also influence bone characteristics and overall performance post-hatch. This study evaluated the effects of different eggshell temperatures (EST) applied intermittently during incubation on physiological, skeletal, locomotor, performance, and carcass yield parameters in broilers at slaughter age. A total of 2,408 fertile eggs from 52-week-old Cobb 500® breeders were distributed across four incubators. The control group (C) was maintained at a constant EST of 37.8 °C throughout incubation. In the other three incubators, from day 8 to 18.5, for 6 hours daily, ESTs were set to 36.7 °C (Low-IPTM), 38.9 °C (High-IPTM), and 39.4 °C (Very High-IPTM). 800 male chicks were reared under standard management conditions until 42 days of age. At slaughter age, the following parameters were assessed: blood mineral, biochemical and hormonal profiles (Ca, P, alkaline phosphatase [ALP], growth hormone [GH], thyroid hormones [T3, T4], parathyroid hormone [PTH], vitamin D3), and rectal temperature [RT]. In addition to physiological parameters, bone characteristics were tested: tibial morphometry, chemical composition, and mechanical properties (weight, length, width, calcium [bone C], phosphorus [bone P], ash content, and strength); incidence of skeletal and locomotor disorders (tibial dyschondroplasia [TD], valgus-varus [VV], pododermatitis [PD], gait score [GS], and latency-to-lie [LTL]). Growth performance (feed intake, weight gain, body weight, feed conversion ratio, and viability); and carcass and cut yields (breast, wing, thigh, and drumstick) were also evaluated. Data were analyzed using a completely randomized design and Tukey's test ($p \leq 0.05$) in SAS® OnDemand. The Low-IPTM treatment negatively affected most evaluated parameters ($p \leq 0.05$), including performance, Ca, P, ALP, PTH, vitamin D3, RT, bone Ca, P, ash content, tibial morphometry and strength. However, carcass yield and GS were not significantly affected by this treatment. In contrast, High-IPTM and Very High-IPTM groups showed improved outcomes ($p \leq 0.05$) in performance, carcass yield, serum Ca, P, ALP, T3, T4, PTH, vitamin D3, RT, bone C and P, ash content, morphometry and tibial strength, as well as GS. All IPTM-treated groups showed reduced incidence of VV and improved LTL compared to the control ($p \leq 0.05$). Based on the presented data, the use of IPTM at 38.9 °C or 39.4 °C for 6 hours daily between embryonic days 8 and 18.5, may be a technique applied to improve bone development and performance in broiler production.

Keywords: Eggshell Temperature, Embryogenesis, Grow-Out Period, Locomotor Disorders, Physiological Parameters

Egg Grading Method and Age of Broiler Breeders and their Impact on Incubation Efficiency (PR-23)

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Abstract:

Grading of fertile eggs from heavy breeder flocks is a critical step that directly influences incubation efficiency and chick quality. Defects such as cracks, inversions, deformities, dirt, and rejects can significantly compromise hatchability and progeny performance (Boleli *et al.*, 2016; Demirel & Demirel, 2020; Vekic *et al.*, 2021; Vekić *et al.*, 2022). Proper identification and management of these nonconformities are essential to optimize incubation outcomes (Reijrink *et al.*, 2009; Boleli *et al.*, 2016; Silva *et al.*, 2017). The grading method directly affects egg uniformity and, consequently, incubation conditions. Weight-based grading, for instance, promotes homogeneous thermal distribution and supports improved embryonic development (Ab Nasir *et al.*, 2018; Nyalala *et al.*, 2021). This study aimed to evaluate the effects of grading methods (manual vs. automated) and breeder age groups (28–42, 43–56, and 57–70 weeks) on hatchability, chick quality, and embryonic mortality in Cobb heavy breeders. The experiment was conducted in a commercial hatchery using 54,432 fertile eggs distributed in a 2×3 factorial design (two grading methods × three age groups). Eggs were examined for defects such as cracks, inversions, deformities, and discards. Response variables included hatchability rate, chick quality (first- and second-grade), cull percentage, and embryonic mortality (classified by developmental stage, including live/dead pipped eggs). Hatchability was significantly influenced by the proportions of first- and second-grade chicks and culls ($p < 0.0001$), highlighting their role as critical quality indicators. A significant interaction was observed between grading method and the presence of cracked eggs ($p = 0.0070$), with manual grading proving more effective in detecting and removing these defects, particularly in older flocks where eggshell fragility is more prevalent. Breeder age also had a significant impact, with lower hatchability rates recorded in the 57–70-week group compared to the 28–42-week group ($p = 0.0205$). These findings underscore the importance of adjusting egg grading strategies based on breeder age, integrating the precision of automation with the tactile sensitivity of manual inspection when necessary. However, manual grading performed slightly better in older flocks due to its ability to detect more fragile eggs. In such cases, the operator's tactile sensitivity remains a valuable asset for effective egg selection.

Keywords: Chick Quality, Commercial Incubation, Embryodiagnosis, Egg Grading, Fertile, Proctive Efficiency

Economic Viability of Using Plastic Flooring As Bedding Substrate in Broiler Production (PR-25)

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Abstract:

This study aimed to evaluate the economic viability of replacing traditional litter materials such as sawdust and rice husks with plastic flooring in broiler chicken production. This technological alternative has gained attention due to its potential to enhance sanitary conditions and reduce labor demands related to litter management. The financial indicators used to assess feasibility included Net Present Value (NPV), Internal Rate of Return (IRR), Modified Internal Rate of Return (MIRR), Profitability Index (PI), Discounted Payback Period (DPP), and Equivalent Uniform Annual Value (EUAV). To complement the financial analysis, scenario analysis, sensitivity analysis, and Monte Carlo simulation were conducted to assess potential risks and uncertainties. The initial investment required for the installation of plastic flooring was R\$ 350,000.00, corresponding to a single poultry house with an area of 2,970 m² and a housing capacity of approximately 41,000 broilers per production cycle. Additionally, the average price received for manure was R\$ 250.00 per ton, contributing to the project's revenue stream. The financial outcomes were positive, with an NPV of R\$ 64,786.23 over the analysis period. The IRR reached 18.5% annually, while the MIRR was 16.7%, both confirming economic feasibility under prevailing market conditions. The PI was calculated at 1.35, and the DPP was 4.5 years, indicating a reasonable time frame for return on investment. Results from the Monte Carlo simulation showed a low probability of negative NPV, reinforcing the project's economic robustness. In conclusion, the replacement of conventional litter with plastic flooring is economically viable for broiler producers and represents a promising investment strategy. Although additional benefits such as improved hygiene, bird welfare, and environmental sustainability are often discussed in the literature, they were not within the scope of this financial assessment.

Keywords: Economic Viability, Indicators, Poultry, Production, Bedding Substrate

Acetylsalicylic Acid As A Strategy to Support Thermoregulation in Broiler Chickens (PR-28)

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Abstract:

The main problems faced in poultry production, especially in tropical and subtropical climate regions, is heat stress, which negatively impacts the productive performance and welfare of broilers. Therefore, several strategies have been investigated as auxiliary tools to mitigate the deleterious effects of heat. Among these strategies, the use of acetylsalicylic acid (ASA) has been studied. The ASA is a non-steroidal anti-inflammatory drug, with low cost, widely used in human medicine, the drug has antipyretic and prostaglandin synthesis modulating properties, which can act directly on the thermal response of birds. The experiment was conducted with the objective of evaluating the effects of ASA on the physiological and behavioral parameters of broilers exposed to heat stress. For this purpose, 120 male Ross® chicks were distributed in a completely randomized design with four treatments: T1 – control (without ASA), T2 – 250mg/L of ASA, T3 – 500mg/L of ASA and T4 – 1000mg/L of ASA diluted in drinking water, for five consecutive days (40 to 45 days of rearing). Thermal stress was induced by turning off the ventilation system until the critical room temperature was reached. Heart rate (HR), respiratory rate (RR), cloacal temperature, crest temperature (infrared thermography) and the latency to lie (LTL) test were evaluated. The data were subjected to analysis of variance (ANOVA), considering a significant level of 5%. The results demonstrated that RR was significantly reduced with the increase in ASA doses ($p=0.0049$), indicating less respiratory effort of the birds and a possible antipyretic effect of ASA. The temperature of the comb increased significantly ($p=0.0014$), suggesting greater peripheral vasodilation and greater efficiency in heat dissipation. The cloacal temperature also showed a statistical difference ($p=0.0125$), with a slight increase at higher doses. On the other hand, HR ($p=0.394$) and the latency to lie test ($p=0.3407$) did not show significant differences between treatments, demonstrating that ASA did not alter behavior related to thermal discomfort or cardiac function under the conditions evaluated. The absence of variation in these two points evaluated may indicate that the dosage protocol or time of exposure to ASA was not sufficient to cause noticeable changes in the birds. It is concluded that ASA has potential as a physiological modulator of the response to heat stress in broilers, acting especially on peripheral thermoregulation. However, no behavioral changes were observed, suggesting the need for further studies.

Keywords: Animal Welfare, Antipyretic, Latency To Lie, Poultry Farming, Thermography

Bioacoustics As A Tool For Monitoring the Well-Being of Broiler Chickens (PR-29)

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Abstract:

The improvement of poultry production systems has intensified the need for more sensitive and non-invasive methods for assessing animal welfare. In this scenario, bioacoustics stands out as a promising tool, capable of identifying changes in the emotional states of broilers by characterizing vocal patterns. This study aimed to verify whether basic acoustic characteristics allow the differentiation of vocalizations associated with heat stress and fear in broiler chickens. The study was conducted in a positive pressure experimental aviary located at the Edgárda Experimental Farm (UNESP – Botucatu/SP). Ross® male broilers were used, housed in 1.50 m x 2.50 m boxes, with *ad libitum* feeding and balanced feed according to the production phase (initial, growth, and final). Three broilers per box were marked with different colors to standardize vocalization recordings throughout the production cycle (45 days). The recordings were made in weeks 2, 3, 4, and 6, under intermittent stress stimuli, including: thermal stress (heating bell) and fear stress (unknown object (colored ball); predator simulation (snake replica) and direct human interaction (manual catching). Vocalizations were captured with a directional microphone (shotgun) and analyzed using Raven Pro 1.6 software. The data were submitted to PROC GLIMMIX statistical analysis (SAS®), with Tukey-Kramer tests ($p \leq 0.05$). The results indicated that the type of stress influenced the vocal parameters ($p < 0.0001$). The number of vocalizations was higher under fear stress, peaking in the third week (56.9), while heat stress caused a progressive reduction reaching the lowest average in the sixth week (20.8). The duration of vocalizations was also significantly shorter under heat stress in the sixth week (0.089 s) and longer under fear stress in the second and sixth weeks (0.147 and 0.146 s). The low peak frequency decreased over time ($p < 0.0001$), accompanying the growth of the broilers, with the high peak frequency being higher in fear vocalizations (3,950 Hz) than in heat vocalizations (3,312 Hz, $p = 0.0018$), evidencing an alarm and escape vocalization pattern. It is concluded that broiler chickens emit distinct acoustic signatures in response to different stressors, with fear associated with more frequent, longer, and higher-pitched vocalizations, while heat stress suppresses vocal emission. The physiological prioritization of thermoregulation through panting may justify this behavior. Basic acoustic analysis shows promise for differentiating emotional states and highlights its potential for composing automated animal welfare monitoring systems in precision poultry farming.

Keywords: Animal Welfare, Precision Poultry Farming, Stress, Vocalization

Interaction Between Probiotics and Environmental Enrichment on the Performance of Broiler Chickens (PR-30)

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Abstract:

The restriction on the use of antimicrobials in poultry farming, due to the risk of increased microbial resistance, has driven the search for alternatives that combine zootechnical performance with sanitary safety. Among these, probiotics stand out for positively modulating the intestinal microbiota; and environmental enrichment, for promoting animal welfare, reducing stress in birds, enhancing broiler health and consequently their productivity. Thus, this study aimed to evaluate the effects of the interaction between probiotics and environmental enrichment on the performance of broiler chickens. The experiment was conducted at the Faculty of Agricultural Sciences of the Federal University of Grande Dourados (UFGD), with 1,440 male Ross® 308 chicks (from 1 to 35 days old), in a completely randomized design, in a 3 x 2 factorial scheme (additives x enrichment), totaling six treatments with eight replications: T1: Negative control with enrichment; T2: Negative control without enrichment; T3: Positive control (antibiotic) with enrichment; T4: Positive control without enrichment; T5: Probiotic with enrichment; T6: Probiotic without enrichment. The environmental enrichment consisted of a permanent platform to simulate the roosting behavior, associated with different enrichments like colored balls, hay bales, among others, which were replaced periodically to maintain the novelty stimulus. Weight gain, body weight, feed conversion, and mortality were evaluated at 7, 21, and 35 days. Data were tabulated and subjected to analysis of variance (ANOVA, $p < 0.05$) using Minitab® software. No significant differences ($p < 0.05$) were observed between treatments for the evaluated variables. Therefore, it is inferred that under conditions without sanitary challenge, as in the present study, the replacement of growth-promoting antimicrobials with probiotics combined with the use of environmental enrichment does not compromise productive performance, indicating the potential of integrating these strategies in systems focused on food safety, broilers health and productivity.

Keywords: Gut Health, Welfare-Based Farming, Antibiotic-Free Production, Alternative Growth Promoters, Microbiota Modulation

***Bacillus Velezensis*: An Underexplored Probiotic Bacterium With the Potential to Improve Egg Production and Quality in Advanced Age Light Strain (PR-31)**

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Abstract:

Light strains at an advanced age present a reduction in egg production linked to age and oxidative stress in the ovary. Plant extracts can have significant antioxidant effects, as we demonstrated in another study with *Libidibia ferrea* (LF). Working with omics techniques, our research group has observed the association between eggshell quality and metabolites from bacteria of the genus *Bacillus* in the yolk. Therefore, the present study evaluated the effects of supplementation with *Libidibia ferrea* (LF) extract derived from the pod of the plant and *Bacillus velezensis* BV01 UNICLON (BV01), either alone or in combination, on the production and quality of eggs from White Plymouth Rock light strain hens, aged 80 to 92 weeks. The experiment was carried out on the light strain farm of Layer Genetics Brasil in partnership with the Laboratory of Infectious Diseases (LADOC) of the Federal University of Uberlândia (UFU), using 100 birds, distributed into four groups (n=25): NC (negative control), BV (supplemented with BV01), LF (with LF) and LF+BV (association of LF and BV). The treatments were administered individually at 80-week-old birds, directly into the beak of each bird (100 µL/bird), with *L. ferrea* at 62.5 mg/mL and *B. velezensis* at 107 CFU/mL. After 12 weeks of treatment, we evaluated daily and weekly egg production, bird weight, egg weight, and shell strength using ANOVA ($p < 0.05$). The results showed that the BV group had a significant increase in daily ($p = 0.0024$) and weekly ($p = 0.0176$) production, with 5.55% and 5.61% more production, respectively, compared to the NC. The LF group showed no significant differences in daily or weekly production. The LF+BV group had a significant increase in daily ($P < 0.0001$) and weekly ($P = 0.0002$) production, increasing egg production by 8.59% and 8.44%, respectively, compared to the NC. Regarding bird weight, LF promoted a significant decrease compared to NC ($P = 0.0157$); however, it was not possible to correlate which component of the extract caused this effect on bird weight, while BV and LF+BV did not differ from NC. Egg weight was not influenced by the treatments. In the eggshell strength, only NC showed a significant increase in fracture susceptibility between the pre- and post-experiment phases ($p = 0.0058$), while the other treatments did not influence the susceptibility to fracture. Therefore, BV01, isolated or in association with LF, promoted a significant increase in the production and quality of eggs from pure lines of advanced age, without compromising eggshell quality, making it an alternative for improving performance at this stage.

Keywords: Poultry Performance, Supplementation, Pure Lines, Antioxidants, Microbial Bioactives

***Bacillus Velezensis* and Phytogenics: A New Approach to Improve Egg Production and Quality in Light Layer Lines Over 80 Weeks of Age (PR-32)**

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Abstract:

Studies show that plant extracts may be potential alternatives to combat ovarian oxidative stress in aged hens, which is one of the causes of decreased egg production. In parallel, our research group has observed an increase in metabolites with antioxidant activity in broilers treated with *Bacillus velezensis* BV01 UNICLON (BV01), as well as a positive association between egg quality and *bacillus*-derived metabolites in the yolk. This study aims to evaluate the effect of a commercial phytogenic additive (based on carvacrol, thymol, eugenol, and diterpenic acids derived from pine resin) and BV01 on egg production and quality in aged White Plymouth Rock light pure lines. The trial was conducted with 100 hens at 80 weeks of age, divided into four treatment groups: a negative control (NC), which received 100 µL of saline; a BV01 group, which received 10⁶ CFU/hen in 100 µL between 80 and 92 weeks of age; a commercial phytogenic blend (PB) group, which received 100 µL (20 mg/hen of PB); and a combination group (BV+PB), which received both treatments simultaneously. Statistical analysis was performed using ANOVA, followed by Tukey's test and two-way ANOVA ($p < 0.05$). There was no significant difference in body weight gain; however, egg production increased across all treated groups compared to the NC ($p < 0.05$). The weekly increase was 5.97%, 6.65%, and 6.96% compared to the control group, with no decrease in egg weight. Both albumen height and Haugh unit increased with age in the NC and BV-treated groups, whereas the groups treated with PB and BV+PB showed a decrease in these two variables. All treatments helped preserve eggshell quality, as shell-breaking strength did not decline over time, suggesting a protective effect regardless of the additive used. These results indicate that supplementation with *Bacillus velezensis* and PB significantly improved egg production and eggshell quality. Additionally, BV showed a positive effect on internal egg quality by increasing the Haugh unit. This approach may provide new support for the production of light lines in advanced age.

Keywords: *Bacillus Velezensis*, Phytogenic Additive, Egg Production, Laying Hens, Egg Quality

Safety and Colonization of *Lactococcus Lactis* and *Bacillus Velesensis* Chicken Embryos (PR-33)

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Abstract:

Probiotics supplied to embryos are a fascinating approach since they can protect newborn chickens. *Lactococcus lactis* and *Bacillus velezensis* are promising probiotics but have not been tested or used in poultry yet. This study aimed to evaluate the safety and colonization of *L. lactis* sub *lactis* (LLL01) and *Bacillus velezensis* (BV01) in newborn broiler chickens inoculated in chicken embryos (CE) at 19 days of incubation (DI). The probiotic bacteria were grown in Mueller Hinton broth with added minerals and dried in maltodextrin using a spray dryer. We inoculated 3 log CFU/CE of BV01 or LLL01, or a mix of them (BVLLL) into the amniotic fluid of the CE at 19 DI. A total of 40 CEs were tested and divided into the following groups: (i) negative control (NC) (inoculated with water), (ii) group inoculated with LLL01, (iii) group inoculated with BV01, and (iv) group inoculated with BVLLL. After hatching, the CEs were euthanized, and the entire intestines were collected and placed in 10 ml of saline solution. The intestines were macerated and homogenized, followed by serial dilution. The LLL01, BVLLL, and NC groups were incubated in triplicate for colony counting on M-17 agar in a microaerophilic atmosphere at 35°C. For the BV01, BVLLL, and NC groups, we incubated them in nutrient agar (NA) under aerobic conditions at 35°C. After hatching, we observed that the CEs were dry and alert, with no signs of navel or joint injuries, dehydration, or other damage. On M-17 agar, all CE intestines of the groups inoculated with LLL01 and BVLLL showed growth. The Gram test revealed only gram-positive cocci, confirming that only LLL01 grew. In NA, colonies grew in only one sample (3.39 log CFU/mL) from the group inoculated with BV01, while all samples from the combination of LLL01 and BV01 groups exhibited growth. The pattern of colonies differed between samples from the group inoculated with BV01 and BVLLL. With the Gram test, the group inoculated with BV01 demonstrated the presence of gram-positive bacilli, while the group inoculated with BV01 and LLL01 showed only the growth of gram-positive cocci. There was no bacterial growth in the NC group. This work proves that LLL, a probiotic with features of intestinal protection, due to its ability to modulate the gut barrier, increase the mucus production, and modulate the intestinal inflammation, can colonize CE and improve broiler health in the first days of life.

Keywords: Probiotic Colonization, Intestinal Health, Broiler Development, Embryonic Microbiome



First Report on the Identification of *Eimeria nagambie* in a Commercial Free-Range Laying Hens Farm (SA-02 - Winner)

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Abstract:

Coccidiosis is associated with enteritis, reduced feed efficiency, and mortality in commercial poultry. Prophylactic strategies include live vaccines, which provide species-specific immunity, and in-feed anticoccidial drugs. Until recently, seven *Eimeria* species (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*) were recognized as specific to domestic chickens. Three new species, named *Eimeria lata*, *Eimeria nagambie* and *Eimeria zaria*, have been identified in several countries, including in alternative chicken production systems in Brazil. The objective of this study was to screen for *Eimeria* spp. in a commercial farm of free-range certified laying hens located in the municipality of Jambeiro, São Paulo, Brazil. The farm facilities consist of four barns and seven flocks, each containing 1000-1500 birds. Due to avian influenza prevention efforts, hens are kept indoors and raised as cage-free, lacking access to areas outside the barn. Samples were collected from a 20-week-old flock of 1000 NOVOgen Tinted layer hens that were vaccinated at the hatchery with a vaccine containing precocious lines of the seven *Eimeria* species mentioned above. Four pools of ten fecal samples were collected from different spots in the barn and processed following standard procedures for oocyst purification and sporulation. Fecal pools were then combined for DNA extraction. Species-specific PCRs targeting SCARs markers were used to detect the seven well-recognized *Eimeria* species. In addition, three nested PCR protocols targeting the IMP-1, rRNA (ITS2), and TBP genes were employed to detect *E. lata*, *E. nagambie* and *E. zaria*, respectively. PCR protocols identified *E. acervulina*, *E. brunetti*, *E. nagambie* and *E. necatrix*. PCR-positive amplifications are expected for the species included in the vaccine, even in the presence of specific immunity. However, PCR does not allow for differentiation between vaccine and field strains of *Eimeria* spp. The identification of *E. nagambie* was expected, since it has been detected previously at high prevalence in chickens from alternative production systems in the state of São Paulo. The finding of *E. nagambie* may pose a threat to commercial poultry systems, since there is no cross-immunity among *Eimeria* spp. from domestic chickens, and *E. nagambie* is not included in commercial vaccines. This study represents the first identification of *E. nagambie* in layer hens housed in a cage-free commercial production system.

Keywords: Coccidiosis, Laying Hens, Free-range



Gastrocnemius Tendon From Broilers With Arthritis/Tenosynovitis at Slaughter Age Provides More Consistent and Sensitive Detection of Avian Reovirus Than Synovial Fluid After Blind Passage in Spf Embryonated Eggs (SA-30 - Honorable Mention)

HONORABLE MENTION

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ANIMAL HEALTH

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Abstract:

Arthritis and Tenosynovitis in broiler chickens causes significant economic losses in the poultry industry and one of the main pathogens involved in these lesions is the Avian Reovirus (ARV). In order to improve the diagnostic accuracy, this study compared the efficacy of ARV recovery from the gastrocnemius tendon (GT) and swabs of synovial fluid (SF) as biological samples, as well as evaluated the effect of a double freeze-thaw technique in both samples. At the slaughterhouse, 25 legs of broilers with joint gross lesions classified from grades I to V (five legs per grade), according to a previously study, were harvested from a single poultry farm. GT were cut lengthwise and macerated before elution. GT and SF were eluted with PBS and each sample was equally divided into two microtubes, which were either subjected or not to a -80 °C double freeze-thaw process, followed by blind passage in Specific Pathogen-Free (SPF) embryonated eggs for further RT-qPCR. Data were submitted to Bland-Altman analysis and Chi-square test for statistical analysis using GraphPad Prism v.10.4.0. Significant difference was considered when *p*-value was lower than 0.05. Results showed that all GT samples from grades I to IV (20/20) were ARV-positive with higher viral loads compared to SFs. In grade V, three GT samples tested positive (3/10), while none of the SF samples were positive. As previously described in literature, this advanced gross lesion grade is less likely to have detectable ARV. SFs had false-negative results, especially in grades I, II, and IV. The double freeze-thaw step had no significant impact on diagnostic efficacy in both GT and SF samples. Although not statistically significant, some SF samples tested positive only after thermal treatment. Moreover, GT-isolates had lower variability in Quantification Cycle (Cq) values. On the other hand, SF-isolates exhibited a high dispersion of data. Bland-Altman analysis revealed that GT samples had a lower Cq that ranged from -4.1 to -4.9 in comparison to SF samples, indicating a higher concentration of viral RNA in this matrix. Limitations of the study regards on sampling from a unique poultry farm and untested efficacy of nitrogen-based freezing. Nevertheless, it shed lights on the importance of GT samples for ARV diagnosis. Considering both feasibility and ARV detection efficiency, the GT without the double freeze-thaw step is the ideal matrix for ARV diagnosis in arthritis/tenosynovitis cases of broilers at slaughter age after blind-passage in SPF eggs, providing superior sensitivity and consistent results. Although easier to harvest, SFs had lower sensitivity and a higher likelihood of false-negative outcomes, making it less suitable as a standard diagnostic matrix.

Keywords: Rt-Qpcr, Viral Diagnosis, Avian Arthritis, Tendinous Tissue, Freeze-Thaw Processing

***Alphitobius Diaperinus*: Is There Interference in the Zootechnical Performance of Broilers? (SA-01)**

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Abstract:

Alphitobius diaperinus (common name: darkling beetle or mealworm) is a relevant pest in poultry farming, acting as a vector of important pathogens and damaging facilities and equipment. Scientific articles also report interference in broiler performance. This study evaluated the zootechnical performance of chicks fed with *A. diaperinus*. The study was conducted in an experimental poultry shed containing twelve 1.5 m² boxes and wood shavings as bedding. The experimental design was completely randomized, consisting of two homogeneous groups, with five replicates of 29 one-day-old (DO) Ross broiler chicks. The experimental period covered the 1st to the 42nd DO, providing water and feed formulated according to different stages of broiler development. From the 3rd to the 7th DO, the birds in Group I (GI) were fed individually with 10 adults and 10 larvae of *A. diaperinus* collected from infested poultry sheds. The diet of GII did not include the darkling beetle. On the 2nd, 3rd, 5th, 6th, 14th, 21st, 28th, 35th and 42nd DO, birds and feed were weighed to calculate weight gain, feed intake and feed conversion, and the mean values were compared by the F test with a 95% confidence interval. Bird mortality was compared by Fisher's exact test ($p \geq 0.05$). From the 4th to the 8th, 10th and 14th DO, two birds per replicate were necropsied to evaluate lesions in the digestive system. The findings indicated the absence of any significant difference ($p > 0.05$) between GI and GII, in all the evaluations. The weight of birds in GI was 72.92 g on the 2nd DO and 3146.87 g on the 42nd DO. Birds in GII weighed 72.98 g and 3091.95 g, respectively, on the 2nd and 42nd DO. Feed consumption during the experimental period was 4907.04 g (GI) and 4814.28 g (GII). The feed conversion rates of the birds were 0.30 (GI) and 0.35 (GII) in the first week and 1.73 (GI) and 1.77 (GII) in the last week. Necropsy revealed traces of insects in the crop, gizzard and intestine of birds fed with darkling beetles (GI), but no noteworthy lesions were identified in birds belonging to the two experimental groups (GI and GII) on the dates evaluated. It was therefore concluded that, under the conditions of this study, broiler zootechnical performance was not affected by the ingestion of *A. diaperinus*.

Keywords: Darkling Beetle, Zootechnical Performance, Broiler

Microencapsulated Essential Oils Can Control *Salmonella Enteritidis* Infection in Broilers (SA-03)

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Abstract:

Salmonella Enteritidis (SE) is responsible for one of the leading foodborne infections in humans. Although infection in poultry is often asymptomatic, there is the excretion and dissemination of the SE within and beyond the poultry environment. Therefore, controlling that infection in poultry is a key strategy to reduce the dissemination of this pathogen to other animals and humans. A proposed natural alternative involves the use of essential oils (EOs), which exhibit antimicrobial activity against different bacteria. In this context, the present study aimed to evaluate the antimicrobial efficacy of three microencapsulated EOs against SE in experimentally infected broilers. To this end, cinnamon (1.10 g/kg of feed), oregano (1.25 g/kg), and clove (1.75 g/kg) EOs were microencapsulated in a lipid matrix (36.9 g/kg) as a blend, which was then incorporated into the feed of treated broilers throughout the experimental period. A total of 135 one-day-old chicks were randomly assigned to three groups, with 45 chicks per group. All groups were housed in metal cages with *ad libitum* access to feed and water. Group A received a half dose of the EO blend (20.5 g/kg of feed), group B received the full dose (41 g/kg of feed), and group C received no treatment. On the second day of age, all chicks were orally challenged via gavage with SE. To assess fecal shedding of SE, cloacal swabs were collected twice per week. At 2, 5-, 7-, 14-, and 21-days post-infection (dpi), cecal content samples were collected from five broilers per group for bacterial enumeration. Regarding fecal shedding, 54.44 % (49/90) of cloacal swabs were positive for SE in group A, 77.77 % (70/90) in group B, and 90 % (81/90) in group C. In this study, EO blend supplementation in group A reduced fecal shedding of SE by approximately 36 % compared to group C. As for cecal colonization, SE counts were significantly reduced (by 4 log₁₀) in group A compared to group C at 7, 14, and 21 dpi. A modest reduction (1–2 log₁₀) was observed in group B at 7 and 14 dpi, but not statistically significant compared to group C. Interestingly, group A, which received half the dose of the EO blend, demonstrated the greatest antimicrobial effectiveness in controlling cecal colonization by SE in broilers. This paradox may be explained by the non-selective antimicrobial properties of EOs, which at higher concentrations could disrupt the intestinal microbiota and damage the mucosal barrier, including reductions in beneficial genera such as *Bifidobacterium* and *Lactobacillus*. Therefore, the results indicate that microencapsulated EOs represent an effective alternative to reduce SE and, consequently, minimize horizontal dissemination in poultry production.

Keywords: Phytochemicals, Paratyphoid Salmonellosis, Cinnamon

Avian Reovirus Clusters Isolated in Broilers With Suggestive Clinical Symptoms in Brazil in 2024 (SA-04)

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Abstract:

The Avian Reovirus (ARV) is of significant relevance in poultry production, as it is the primary causative agent of avian viral arthritis and tenosynovitis. This study aimed to investigate the occurrence of ARV in broilers exhibiting clinical symptoms and analyze the relationship between the identified cluster, age, location, and its distribution across Brazil. A total of 960 broiler flocks, presenting clinical signs suggestive of ARV (leg problems and increased passage rate), from various Brazilian states were analyzed. Isolation was performed on pooled samples of the small intestine (duodenum and pancreas regions), heart, or joints, with each pool consisting of samples from five birds. The samples were inoculated into 6-day-old SPF (Specific Pathogen-Free) embryonated eggs and passaged in chicken embryo kidney. After isolation, RT-qPCR was conducted to confirm the presence of the viral kidney Sequencing and phylogenetic analysis were performed by amplifying the sigma C gene and sequencing the nucleotides. Descriptive statistics and Pearson correlation analysis ($p < 0.05$) were applied using GraphPad Prism 10.2. A total of 390 ARV isolates were obtained from 360 flocks, and 190 isolates were sequenced. Cluster 4 was the most prevalent (41.05%), followed by clusters 2 (20.53%), 6 (14.74%), 5 (14.21%), 1 (11.58%), and 3 (8.95%). All isolates originated from flocks younger than 45 days, with no association between the identified cluster and the age of the birds isolated (< 45 days). The highest ARV isolation rate was observed in the intestine (58.61%), followed by tendons (48.01%) and heart (20.20%). In the intestine, the distribution of clusters mirrored the overall results, but a lower isolation rate of cluster 6 (6.67%) was observed in tendons, and a lower isolation of cluster 5 (2.63%) was seen in the heart. Cluster 5 was present in 15% of tendon samples. The distribution of different clusters across regions showed variation; in the Northeast, clusters 2 and 6 were absent, with the highest isolation of clusters 1 and 3 (both 33.33%). In the Southeast and South, all clusters were identified, with clusters 4 and 2 being more prevalent, and cluster 5 occurring at 12.5% and 11.11%, respectively. In the Central-West, cluster 3 was not identified, and clusters 4 (40.74%) and 1 (29.63%) were the most frequent. This study highlights the widespread presence of various ARV clusters in Brazil, including clusters associated with milder lesions (such as cluster 4) and those linked to more severe lesions (clusters 5 and 6).

Keywords: Flocks, Rt-Qpcr And Arv, Flocks, Rt-Qpcr, Arv

Genetic Characterization and Pathogenicity Assessment of *Salmonella Minnesota* Isolates in Broiler Chickens (SA-05)

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Abstract:

Salmonella spp. is a global public health concern, with poultry products being major transmission vehicles. In Brazil, the emergence of *Salmonella Minnesota* (SM) has gained relevance and drawn the attention of health authorities. This study aimed to sequence and analyze SM strains isolated from broilers and evaluate intestinal infection, systemic invasion, and fecal shedding in experimentally infected birds. Whole-genome sequencing and analysis were conducted on 268 genomes to identify virulence and resistance genes, including 263 publicly available genomes from the Enterobase database and five genomes from broiler chicken isolates collected in the southeastern region of Brazil. A total of 191 multidrug-resistant strains were identified as well as virulence genes related to adhesion, iron uptake, and the type III secretion system. Based on genomic data, three strains SM574, SM556 and SM573 were selected. The strains were adapted *in vitro* for antimicrobial resistance to nalidixic acid, spectinomycin, and ampicillin. Birds were divided into four groups, uninfected control, A (SM574), B (SM556), and C (SM573). On the second day of life, birds received 1×10^8 CFU/mL of their respective strain orally. Systemic infection, cecal colonization, and fecal shedding were assessed over 28 days. Bacterial counts were analyzed using two-way ANOVA followed by Bonferroni's test, while shedding was evaluated using the Chi-square test ($p < 0.05$). Bacterial growth in the liver was detected only in birds from group A at 2 dpi ($p < 0.05$). Fecal shedding was significantly higher in birds from group B ($p < 0.05$). At 2 dpi, cecal bacterial counts were lower in birds from group A compared to those from group C ($p < 0.05$). At 7 dpi, birds from group A exhibited the lowest cecal counts ($p < 0.05$). At 14 dpi, birds from group C showed higher counts than those from groups A and B ($p < 0.05$). At 21 dpi, birds in group B had lower bacterial counts compared to those in groups A and C ($p < 0.05$). At 28 dpi, bacterial counts in birds from group B remained lower than those observed in group A ($p < 0.05$). The results indicate that strain SM574 had greater potential for early systemic infection. Strain SM556 showed more intense fecal shedding. With regard to cecal colonization, distinct dynamics were observed among the strains, with SM573 exhibiting the highest bacterial loads at 14 dpi, while SM556 maintained the lowest levels during the final stages of the study. These findings highlight strain-specific differences in terms of invasion, fecal shedding, and intestinal colonization behavior, with strain SM574 appearing to be more pathogenic, as evidenced by the presence of systemic infection.

Keywords: Fowl Paratyphoid, Intestinal Colonization, Poultry Production, Salmonellosis, Systemic Infection

Genes Encoding Anti-Inflammatory Proteins Involved in Persistent *Salmonella Pullorum* Infection in Chickens (SA-07)

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Abstract:

Pullorum disease is a non-zoonotic avian illness caused by *Salmonella Pullorum* (SP), vertically transmitted and causing high mortality. Persistent infection in part of the flock is one of its main characteristics. Although many studies have investigated SP persistence, mechanisms of survival and immune evasion remain unclear. This study evaluated if anti-inflammatory effector proteins linked to NF- κ B inhibition affect SP persistence and pathogenicity. Four mutant strains, SP Δ avrA, SP Δ gtgA, SP Δ pipA, and SP Δ sseL, were constructed using the Lambda Red system, with deletions confirmed by PCR and sequencing. The wild-type *Salmonella Pullorum* ATCC 9120 (wt-SP) and Brazilian isolate *Salmonella Gallinarum* SG287/91 (SG) were used for pathogenicity comparison. Susceptible birds were divided into groups: A (SP Δ avrA), B (SP Δ gtgA), C (SP Δ pipA), D (SP Δ sseL), E (SP), F (SG), and G (non-challenged). At seven days old, birds were orally inoculated and monitored for 10 weeks. Samples were collected at 7, 14, 21, 35, 49, and 63 days post-infection (dpi) to detect *Salmonella* spp. in spleen and liver and assess macroscopic lesions. Clinical signs were monitored throughout. Organ counts (liver and spleen) were tested for normality, analyzed by two-way ANOVA, and means compared by Sidak's test. Macroscopic lesion data and *Salmonella* detection were binarized and analyzed by chi-square. Statistical significance was considered when the *p*-value was lower than 0.05. At 7 dpi, SP was detected in higher amounts than mutants, with similar detection frequency over time. Birds inoculated with SP Δ avrA showed more intense clinical signs than other mutants, but no significant differences in lesions or bacterial counts among challenged groups (*p*>0.05). During the acute phase, SG showed higher spleen and liver counts, while mutants showed lower counts, indicating reduced systemic survival. Deletions of avrA and gtgA caused severe liver and lung lesions, showing their role in inflammatory modulation and pathogenesis. Deletions of pipA and sseL did not affect pathogenicity. Although some differences in pathogenicity were observed, no differences were found in the persistence of the mutant strains compared to the wild-type SP.

Keywords: Mutagenesis, Pullorum Disease, Poultry Farming, Effector Proteins, Pathogenicity

Evaluation of Marek'S Disease Virus Replication in Different Tissues Under Two Vaccination Protocols (SA-08)

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Abstract:

Marek's Disease (MD) is a highly contagious lymphoproliferative disorder in chickens, caused by an oncogenic herpesvirus with a strong affinity for CD4+ T cells. Vaccination remains the primary control strategy, with serotype 1 (CVI988/Rispens) and serotype 3 (vectored HVT) vaccines being the most widely used. This study aimed to quantitatively compare the replication of MD vaccine virus in three tissues (feathers, thymus, and spleen) under two distinct broiler vaccination protocols: one using a reduced dose (¼ of the commercial dose) of CVI988 and the other using a full dose of vectored HVT. The experiment was conducted in collaboration with a poultry company in Paraná, Brazil. Both vaccines were administered *in ovo* on day 19 of incubation. Tissue samples were collected from 20 birds per group at 21 and 28 days of age. Quantification of viral replication was performed using real-time PCR (qPCR), targeting the Meq gene for CVI988 and the sORF1 gene for HVT. Viral loads were expressed as log₁₀ copies per 10,000 somatic cells. Statistical analysis was conducted using the Kruskal-Wallis test due to non-normal data distribution, with significance set at $p < 0.05$. Quantitative results revealed that the CVI988 group consistently exhibited significantly higher viral loads across nearly all tissues and time points. At 21 days, the mean viral load in the spleen was 1.57 log₁₀ for CVI988 versus 0.06 log₁₀ for HVT. In the thymus, the values were 1.16 versus 0.11 log₁₀, respectively. Although the difference in feathers was not statistically significant at this stage, the CVI988 group still showed more than double the viral load. By 28 days, the disparity became more pronounced, particularly in feathers, where CVI988 reached 3.28 log₁₀ compared to 0.83 log₁₀ for HVT. These findings confirm that even at a reduced dose, the CVI988 vaccine demonstrates a higher replicative capacity and broader tissue distribution than the full-dose vectored HVT vaccine. The feather tissue consistently showed the highest viral loads, aligning with its known sensitivity for detecting active MD virus replication. The results underscore the importance of considering viral load and sampling time when evaluating vaccine efficacy. Further studies involving challenge trials and cellular immune response assessments are recommended to support strategic decisions in MD control programs.

Keywords: Cvi988, Rhvt, Qpcr, Feathers Pcr, Quantitative Pcr

Analyses of the Prevalence of *Eimeria* Spp. on Commercial Broiler Chicken Farms in the State of São Paulo Using 18S Rrna Targeted Next-Generation Sequencing (SA-09)

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Abstract:

Coccidiosis is one of the most economically significant diseases for the commercial broiler production industry. Although the commercial broiler chicken sector is economically significant for the state of São Paulo, there is a paucity of studies on the epidemiology of coccidiosis in broiler farms within this state. This study aims to determine the prevalence of the 10 *Eimeria* species of domestic chickens and new *Eimeria* operational taxonomic units (OTUs) in commercial broiler chicken farms (CBCF) located in 20 municipalities within the northwest region of the state of São Paulo. Five fecal pools were collected in each CBCF, each containing fresh droppings from 10 chickens, including cecal droppings. Each fecal pool was processed individually, and the sediments resulting from the process of purification were combined into a single sample. The purification and concentration of oocysts yielded one sample per CBCF, resulting in a total of 88 samples. Samples were examined by microscopy to detect *Eimeria* oocysts. We performed nested PCR targeting the 18S rRNA gene and next-generation sequencing to detect and identify *Eimeria* spp. and potential new *Eimeria* OTUs. The amplicons from nested PCR were processed using the Illumina 16S metagenomic protocol, which included paired-end reads of 150 bp and the MiSeq® Reagent kit v2 (Illumina). Next-generation sequencing was performed at the Epigenomics Laboratory of the Faculty of Veterinary Medicine of Araçatuba, UNESP, on the MiSeq® sequencer (Illumina). Microscopic screening identified 88/88 (100%) samples as positive for *Eimeria* spp. Next-generation sequencing analyses identified the following order of prevalence of *Eimeria* spp.: *Eimeria maxima* (82/88; 93.18%); *Eimeria acervulina* (75/88; 85.23%); *Eimeria necatrix/tenella* (19/88; 21.59%); *Eimeria mitis* (3/88; 3.41%); *Eimeria mivati* (10/88; 11.36%); and *Eimeria praecox* (19/88; 21.59%). This study identified the species prevalent in broiler chicken farms in Brazil and other countries. As expected, we observed the highest prevalence of *E. maxima* and *E. acervulina*, the two species of greatest economic significance to the poultry sector. In contrast with the higher number of studies performed in other Brazilian states, such as Santa Catarina, there is a paucity of data concerning the prevalence of *Eimeria* spp. in CBCFs within the state of São Paulo. Although we didn't find the newly identified *Eimeria* species of domestic chickens (*Eimeria lata*, *Eimeria nagambie*, and *Eimeria zaria*) and new OTUs in this study, their previous detection in alternative farming systems in São Paulo indicates that future studies employing methods with discriminatory power to identify all *Eimeria* species and new OTUs are needed.

Keywords: Coccidiosis, Domestic Chicken, Molecular Diagnosis, Deep Amplicon Sequencing

Molecular Characterization of *Eimeria Zaria* in Domestic Chickens From Alternative Poultry Production Systems in the State of São Paulo (SA-10)

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Abstract:

Coccidiosis represents one of the main sanitary and economic threats to global poultry farming. Domestic chickens are infected by ten species of *Eimeria*, including three recently identified species: *Eimeria lata*, *Eimeria nagambie*, and *Eimeria zaria*, which have already been documented in several countries, including Brazil, where they were identified in alternative poultry production systems (APPS). *Eimeria lata*, *E. nagambie*, and *E. zaria* infect chickens previously vaccinated against coccidiosis, highlighting their relevance in the context of coccidiosis control. This study aims to molecularly characterize *E. zaria* isolates identified in APPS located in the state of São Paulo. Samples consisted of 26 DNA samples extracted from feces of domestic chickens unvaccinated against coccidiosis and previously determined to be positive for *E. zaria* by genus-specific PCR followed by next-generation sequencing. DNA samples were subjected to two one-step *E. zaria*-specific polymerase chain reaction (PCR) protocols to amplify partial fragments of the tubulin-binding protein (TBP) (907 bp) and the microneme 2 protein (MIC2) (592 bp) genes, followed by Sanger genetic sequencing. Two nested PCR protocols were alternatively employed (861 bp, TBP; 594 bp, MIC2), using primers that anneal within the internal region of the amplicon generated by one-step PCRs. The positive samples were subjected to genetic sequencing. All samples yielded negative results in one-step PCRs. Six samples tested positive by nested PCRs. The six sequences of the tubulin-binding protein revealed 99.54% genetic similarity (six nucleotide substitutions: five synonymous and one nonsynonymous) and 99.31% genetic similarity (four nucleotide substitutions: three synonymous and one nonsynonymous) when compared to the Italian (OR645464) and Greek (OR645463) sequences of *E. zaria*, respectively. Moreover, these sequences shared 100% similarity with the *E. zaria* whole genome shotgun sequence (CAJFCY010000041) from Australia. The microneme 2 protein genetic sequencing resulted in four different sequences with nucleotide substitutions and insertions in a noncoding region that shared 99.39% to 99.83% genetic similarity to the *E. zaria* sequences described in Greece (OR645465) and Italy (OR645466). Moreover, these sequences shared 100% similarity with the *E. zaria* whole genome shotgun sequence (CAJFCY010000001) from Australia. In conclusion, this study provides additional data on the gene sequences of *E. zaria* and its first molecular characterization in South America. The analyses reveal the presence of genetic variability among Brazilian, Italian, and Greek isolates of *E. zaria*. The comparison with published sequences from both genes showed 100% genetic similarity with *E. zaria* sequences from Australia.

Keywords: Coccidiosis, Diagnosis, Epidemiology, Backyard Farming

Efficacy of An Attenuated *Salmonella Enteritidis* Vaccine Applied During Laying Period As A Fourth Dose in Commercial Hens (SA-11)

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Abstract:

The vaccination of laying hens with live vaccines against *Salmonella Enteritidis* applied in a 3-dose vaccination program during rearing is one of the measures implemented that have reduced the prevalence of *Salmonella* in poultry and, accordingly, also the risk of transmission of salmonellosis to humans through the consumption of contaminated eggs. The current trend of extending the production period beyond the 90th week of life led Laboratorios Calier to consider the introduction of a fourth dose of the live *Salmonella Enteritidis* vaccine during laying to ensure the protection of hens until the end of a lengthy productive period. 65 commercial hens (Novogen breed) vaccinated in the farm with a live vaccine against *S. Enteritidis* at 1, 6 and 15 weeks of age, were transported to the experimental facilities to be vaccinated during laying (55 weeks of age) with a 4th dose. A safety assessment was performed the month after the vaccination. To evaluate the degree of protection at the end of the laying period (95 weeks of age), a challenge was carried out on 40 vaccinated hens and on 30 control (non-vaccinated) hens, and excretion and colonization of challenge strains was evaluated. The application of a 4th dose of the vaccine at 55 weeks of age demonstrated that hens are protected until the end of an extended laying period. The colonization of challenge strains (tested by culture method) in liver, spleen, cecum, ovary, and oviduct of vaccinated animals (n=20) was reduced in comparison to non-vaccinated animals (n=20): 54% infected organs versus 74% in control the 1st week, and 28% versus 56% the 2nd week after challenge. The rates of *Salmonella Enteritidis* excretion in cloacal swabs were reduced within 2 weeks in vaccinated animals, while hens of control group were still positive in the 3rd week after challenge. The results from this experiment indicate that a 4th dose of *Salmonella* vaccine during laying can be an important and effective tool within a comprehensive biosecurity program designed for successful *Salmonella* control in industrial poultry farms.

Keywords: Layers, Biosecurity, Excretion, Salmonellosis

Evaluation of Viral Recovery Techniques For Avian Reovirus (Arv) and Infectious Bursal Disease Virus (Ibdv) in Poultry Litter (SA-12)

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Abstract:

Avian Reovirus (ARV) and Infectious Bursal Disease Virus (IBDV) are major threats to global poultry, causing significant economic losses. ARV leads to tenosynovitis/arthritis in 3–7-week-old chickens, while IBDV causes severe immunosuppression and high mortality in young chickens (3-6 weeks) by targeting the bursa of Fabricius. Both are highly contagious, environmentally stable dsRNA viruses spreading horizontally, with pathogenesis involving initial gut replication followed by systemic dissemination. While clinical signs and lesions aid in diagnosis, control relies on vaccination and stringent biosecurity measures. However, detecting ARV and IBDV in diverse poultry litter types (e.g., wood shavings, sawdust, and rice husk) presents a unique challenge for surveillance. Therefore, this study aimed to evaluate the recovery of IBDV, ARV, using Murine Norovirus 1 (MNV-1) as an internal control from different poultry litter materials. For viruses recovery evaluation, three different types of poultry litter (sawdust, rice husk, and wood shavings) were collected from poultry houses in the Brazilian states of Minas Gerais (sample 395), Distrito Federal (sample 397), and Ceará (sample 407), respectively during November/December 2024. Later, 100g of each sample was inoculated with 10 mL of MNV-1 at 1010 copies/mL, Poulvac® Reo (Zoetis®) vaccine at 1×101.7 DIOE50, and Poulvac® Bursa F (Zoetis®) vaccine at 1×102.0 DIOE50. Then, one litter sample of each type was selected to be processed using the following viral concentration methods: (i) Polyethylene Glycol 6000 (PEG), using 25.0 g of the litter; (ii) Tris Glycine Beef Extract (TGBE) + PEG 6000, using 12.5 g of sample; and (iii) TGBE + Ultracentrifugation (UC – 100.000×g), using 12.5 g of sample. All samples, along with an aliquot of both vaccines, were subjected to RNA/DNA extraction using the Quick-DNA/RNA Viral MagBead Kit (Zymo Research), followed by reverse transcription using M-MLV Reverse Transcriptase (Merck). For the detection of MNV-1, an RT-qPCR was employed using the QuantiNova Probe RT-PCR Kit (QIAGEN); meanwhile, for detecting ARV and IBDV, a PCR, and qPCR reaction were employed using the Taq DNA Polymerase Kit (Thermo Scientific), and SYBR-Green PCR Master Mix (Thermo Scientific) respectively. The TGBE + PEG method demonstrated the highest viral recovery rates across all litter types (395: 118.72%; 397: 100.03%; 407: 124.99%), followed by PEG (395: 81.16%; 397: 53.31%; 407: 41.40%). Detection of vaccinal IBDV and ARV by PCR occurred mainly in samples concentrated with TGBE + PEG and PEG alone, which is consistent with the MNV-1 recovery. These potential detections still require confirmation through genome sequencing to allow for viral genotyping.

Keywords: Tenosynovitis, Arthritis, Immunosuppression



Surveillance of Avian Reovirus (Arv) in Poultry Litter and Broiler Chickens on Brazilian Commercial Farms (SA-13)

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Abstract:

Avian Reovirus (ARV) is an important viral pathogen in poultry production, associated with various clinical conditions and significant economic losses. Understanding its distribution in both birds and the environment is essential for effective surveillance and control strategies. This study aimed to investigate the presence of ARV in broiler chickens and poultry litter on commercial poultry farms across several Brazilian states. The study was conducted in two phases. In the first phase (August–November 2021), ARV monitoring was performed in 100 broiler flocks through nasotracheal swab sampling of the animal carcasses. Birds aged 4–6 weeks, either clinically healthy or showing mild respiratory signs, were sampled. In the second phase (November 2024), 30 poultry litter samples—including wood shavings, sawdust, and rice husks—were collected using litter pooling and drag swab methods. All samples were stored at 4 °C, processed using organic precipitation and RNA extraction, and analyzed by RT-PCR targeting the S3 gene. ARV was detected in 12% (12/100) of broiler flocks, with the highest detection rate in Paraná (58.3% of the positive flocks). In poultry litter, ARV was detected in 33% (10/30) of samples, with positive farms located in Minas Gerais (3 farms), Ceará (1), Paraná (1), and Santa Catarina (3). The detection of ARV in both birds and environmental samples across multiple regions highlights the widespread presence of the virus in Brazilian poultry farms and underscores the importance of continuous monitoring to inform biosecurity and disease control measures. It is recommended to monitor both poultry and their surrounding environment to better understand viral stability, potential cross-exposure between different flocks, and to support effective vaccination control strategies.

Keywords: Environmental Surveillance, Poultry Health, Broiler Chickens, Rt-Pcr Detection

Establishment of Histomorphological Parameters For Evaluating Metaphyseal and Epiphyseal Regions of the Tibia in Broiler Chickens (SA-14)

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Abstract:

The intense growth rate of modern broiler lines within a short time lead results in skeletal overload due to insufficient bone mineralization. The chickens' center of gravity has shifted forward compared to their ancestors, which affects the way the chicken moves and results in additional pressure on the legs, affecting tibia and femur proximal metaphysis, with losses in mineral density of the bone trabeculae. In these areas, mechanical injuries and microfractures occur, damaging blood vessels. Aspects such as genetics, sanitary challenges, nutrition and embryonic development influence the occurrence of lameness, with possible interactions among these factors. The objective of this study was to establish histomorphological parameters for evaluating metaphyseal and epiphyseal regions of the tibia in broilers fed diets deficient in calcium and phosphorus during the first week of life. A total of 32 tibias of 42-day-old broilers were collected: 16 from birds fed Ca and P-deficient diets, and 16 from birds fed nutritionally adequate control diets. The metaphyseal and epiphyseal regions, were fixed in formalin, decalcified, embedded in paraffin and stained with hematoxylin-eosin. The slides were analyzed by a single evaluator under an Olympus microscope (10x objective lens), using a scoring system comprising seven parameters: Alterations in the growth plate were scored from 0 (normal) to 3 (complete cellular disorganization). Non-mineralized cartilage casts in the calcification and ossification zone, as well as in the bone periphery, were scored as follows: 0 (absent), 1 (<33%), 2 (34–66%) and 3 (67–100%). Bone degeneration was assessed from 0 (normal) to 3 (structural loss and degenerated chondrocytes). Necrosis was assessed as to percentage (0 to 3) and chronicity (0 to 3), considering characteristics such as presence of inflammatory cells, matrix replacement and loss of osteocytes. Score data were analyzed using the non-parametric Kruskal-Wallis test. Results indicated that dietary Ca and P deficiency did not significantly affect ($p>0.05$) the distribution or severity of histological lesions in the metaphyseal and epiphyseal regions of broiler tibias. Nonetheless, high lesion scores across both groups suggest that the evaluated alterations are biologically relevant and may correlate with macroscopic lesions observed during flock-level health monitoring. It is important to consider that although mineral-deficient diets reduced overall body weight and skeletal development, in this study, it did not influence in the biomechanical integrity of the bone. The diagnosis and investigation of microscopic aspects involved in these lesions can help to target the causes and adopt strategies that can mitigate locomotor problems.

Keywords: Mechanical Injuries, Necrosis, Bone Degeneration, Trabecular Bone Loss

Morphological Identification of Large-Sized Oocysts of *Eimeria* Spp. From Domestic Chickens (SA-15)

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Abstract:

Morphological diagnosis is considered unreliable for species-specific differentiation of the 10 *Eimeria* species of domestic chickens and has been neglected for decades. This study aimed to conduct a morphological analysis of large-d oocysts ($\geq 23.9 \mu\text{m}$) of *Eimeria* spp. from domestic chickens, specifically *E. brunetti*, *E. lata*, and *E. maxima*. Oocysts originated from pure isolates of each species stored at FMVA/UNESP. Morphological analyses were conducted using an optical microscope with a 100X objective through measuring and observation of the sporulated oocysts and their internal structures. Oocysts of *E. lata* have a two-layered wall, with the outer layer being smooth; a micropyle in the outer layer, but it may be inconspicuous or barely discernible in some oocysts; a polar granule and, in some oocysts, a second polar granule; ovoid to elliptical sporocysts, with a flattened Stieda body and a rounded sub-Stieda body; a compact sporocyst residuum; and sporozoites with anterior and posterior refractile bodies and one nucleus. Oocysts of *E. maxima* have a two-layered wall, with the outer layer being rough; a micropyle in the outer layer, but it may be inconspicuous or barely discernible in some oocysts; a polar granule and, in some oocysts, a second polar granule; ovoid sporocysts; a knob-like Stieda body and a rounded sub-Stieda body; a compact sporocyst residuum; and sporozoites have anterior and posterior refractile bodies and one nucleus. Oocysts of *E. brunetti* have a two-layered wall, with the outer layer being smooth, and an absence of a micropyle; a polar granule and, in some oocysts, a second polar granule. The sporocysts, ovoidal to ellipsoidal, have a flattened Stieda body and a trapezoidal sub-Stieda body; a compact sporocyst residuum; and sporozoites with anterior and posterior refractile bodies and one nucleus. Despite the consensus in the literature that it is not possible to differentiate *Eimeria* oocysts of domestic chickens through morphological analysis, our results show that this analysis, if conducted meticulously and under a 1,000X magnification, enables the identification of morphological differences among *E. lata*, *E. maxima*, and *E. brunetti* oocysts. The oocysts of *Eimeria lata* and *E. maxima* exhibit differences in their outer layer — smooth in *E. lata* and rough in *E. maxima* — in addition to a subtle difference in the and shape of the Stieda body. The differences in the shape and of the sub-Stieda body, along with the lack of a micropyle in the oocysts of *E. brunetti*, allow accurate differentiation from the oocysts of *E. lata* and *E. maxima*. Nevertheless, further data from oocysts of additional isolates of these three species must be obtained to draw definitive conclusions about their morphological diagnosis.

Keywords: Coccidiosis, Morphology, Apicomplexa, Eimeriosis

The Phylogenetic Analysis of the VP2 Gene Reveals Genetic Diversity and the Presence of Divergent Strains of IBDV in Brazilian Samples (SA-16)

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Abstract:

Within genome segment A and is considered the most informative region for genotypic differentiation. This approach has been widely accepted due to the availability of sequence data in GenBank. In this context, the present study aimed to characterize IBDV strains from clinical samples collected in 2024 and 2025 in commercial poultry flocks in Brazil. The samples were from broilers, breeders and layers aged between 18 and 45 days. Phylogenetic analysis of hvVP2 nucleotide sequences was performed to determine the phylogenetic classification of these isolates in relation to the previously described genogroups and to evaluate the possible occurrence of regionally different variants. Twenty-six IBDV-positive samples previously identified at CDMA by PCR were subjected to phylogenetic analysis based on partial sequencing of the VP2 region. The resulting sequences were aligned with IBDV reference strains from GenBank and a phylogenetic tree was constructed. The analysis revealed two main clades. One well-supported clade included strains classified as MG4 and MG8 (group 4 – G4), which showed genetic similarity to IBDV isolates from Brazil, Uruguay and Japan. The second clade included strains clustered with classical IBDV strains such as STC, Lukert and the F52/70 strain (group 1 – G1). Notably, robust statistical support (bootstrap > 0.9) was observed for a proposed subdivision within G4, referred to here as G4B, as the Brazilian isolates formed a genetically distinct subcluster compared to the classical G4 references, despite being phylogenetically related. Based on the samples analyzed, IBDV strains belonging to the G1 and G4 genogroups are circulating in Brazilian commercial poultry flocks. The literature indicates that G4 strains are particularly associated with immunosuppression due to severe lymphoid depletion, even in the absence of overt clinical signs. Our results confirm this association and emphasize the importance of G4 IBDVs in subclinical immunosuppressive disease. Analysis of the VP2 gene, including the hypervariable region, revealed the prevalence of G1 and G4 IBDV strains in Brazil and supports the proposal of a new subdivision within G4 (designated G4B) based on sequence divergence. Macroscopic lesions and performance indicators (including mortality) alone are not sufficient to predict the specific IBDV genogroups in flocks or their potential impact on avian immunocompetence. Therefore, genotypic characterization should be integrated with histopathological and serological findings for a comprehensive assessment of IBDV infection dynamics.

Keywords: Gumboro; Infectious Bursal Disease Virus; Genogroups; Molecular Surveillance; Vp2

Molecular Characterization of *Eimeria* *Nagambie* in Domestic Chickens From Alternative Poultry Production Systems in the State of São Paulo (SA-17)

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Abstract:

Coccidiosis, a disease caused by the genus *Eimeria* in domestic chickens, is among the most economically significant diseases for the poultry industry, resulting in an estimated yearly global loss of over 3 billion dollars. Recently, novel genetic variants of *Eimeria* from domestic chickens, designated OTU x, y, and z (*Eimeria lata*, *Eimeria nagambie*, and *Eimeria zaria*, respectively), have been identified. Similar to other *Eimeria* species, these variants are associated with a reduction in productive parameters in domestic chickens. This study aims to develop a nested PCR protocol and conduct the molecular characterization of *E. nagambie* in domestic chickens kept in extensive and semi-intensive farming systems in the state of São Paulo. This study employed 32 DNA samples extracted from fecal samples of domestic chickens from alternative farming systems (AFS) in the state of São Paulo, which had previously tested positive for *E. nagambie* through next-generation sequencing of 18S rRNA gene amplicons. The AFS are located in rural regions, each housing between 6 to 250 birds of diverse ages and breeds, all of which have not been vaccinated against coccidiosis. DNA samples were subjected to nested PCR to amplify a partial fragment of 322 to 330 bp of the rRNA gene (ITS-2) for the specific detection of *E. nagambie*, followed by genetic sequencing. Nested PCR detected 65.6% (21/32) of the samples as positive, which were subsequently sent for Sanger sequencing. Nineteen samples with high-quality sequences were obtained. The genetic similarities of sequences from this study in comparison to GenBank sequences from *E. nagambie* are described as follows: One sequence exhibited 99.7% similarity to the sequences OR229151 (Brazil) and AM922253 (Australia); another sequence showed 99.38% similarity to the whole genome shotgun sequence from Australia (CAJFCZ010007291); two sequences demonstrated 100% similarity to the sequence OR229152 (Brazil); and fifteen sequences displayed 100% similarity to both sequences OR229151 (Brazil) and AM922253 (Australia). This study provides additional data on the gene sequences of *E. nagambie* and presents its first molecular characterization in South America. The comparison with published sequences from GenBank revealed genetic similarities with *E. nagambie* sequences originating from Australia.

Keywords: Coccidiosis, Diagnosis, Epidemiology, Backyard Farming

Atr-Ftir Spectroscopy Combined With Artificial Intelligence Can Offer An Alternative Method For Detecting *Campylobacter jejuni* (SA-18)

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Abstract:

Campylobacter jejuni (CJ) is a leading cause of human gastroenteritis globally, and its diagnosis remains challenging due to difficulties in isolating, growing, and identifying the organism. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) measures how molecules in a sample absorb infrared light at various wavelengths, causing vibrational transitions. Each type of chemical bond has a unique vibrational signature, making ATR-FTIR a powerful diagnostic tool. We utilized ATR-FTIR combined with artificial intelligence (AI) to detect CJ. Infrared spectra were recorded from 145 CJ samples (50 reading each sample) of diluted colonies as positive controls. After reading, a total of 29 samples of CJ strain 1997/6 (20%), 37 of CJ strain 35528/8 (26%), 30 of CJ strain 39259/3 (21%), 19 of CJ strain 9591/6 (13%) isolated from chickens, and 30 of CJ strain IAL (21%) isolated from humans. For negative controls, 146 samples with total of 50 reading each sample were tested. Then, total of 29 avian pathogenic *Escherichia coli* (ST131) (20%), 27 *Campylobacter coli* strain 1 (18%), 33 *Campylobacter coli* strain 2 (23%), 28 *Salmonella enteritidis* (19%), and 29 *Staphylococcus aureus* (20%) reading was analyzed. The data were randomly divided into training data (291 samples) and external validation data (200 samples) to construct the CJ database. Multivariate analyses were performed using Orange 3.34.1 software to develop the best algorithm models for classifying the groups as positive and negative. Sensitivity, specificity, and accuracy calculations were done using the MEDCALC online platform. After creating the AI algorithms, the prediction models were trained with 10-fold cross-validation in a Training Confusion Matrix, and the three best models—Random Forest (RF), Gradient Boosting, and Neural Network—were subjected to external validation. RF showed the best results in both cross and external validation. Cross-validation results included sensitivity, specificity, accuracy, and positive likelihood ratio (LR+) of 99.31%, 99.32%, 99.31%, and 144.9, respectively. In external validation, sensitivity, specificity, and accuracy reached 100%, proving the model's success. The area under the curve (AUC) also showed excellent results for the three models in training and external validation data, with RF having the best AUC in both. Combining ATR-FTIR spectroscopy with AI presents a promising method for detecting CJ, leveraging ATR-FTIR's rapid analysis capabilities and AI's sophisticated data processing power. This approach enables accurate and efficient pathogen identification, significantly enhancing diagnostic processes and offering a robust tool for improving food safety and public health monitoring.

Keywords: Bacteria, Human Gastroenteritis, Diagnostic Tool, Biochemical Fingerprint, Random Forest

Macroscopic and Visceral Evaluation of Condemned Broiler Carcasses With Cachexia or Repugnant Appearance (SA-19)

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Abstract:

Market demand has driven improvements in Brazilian poultry production, which, as the third-largest global broiler producer, must maintain high quality standards. Despite high productivity, carcass condemnations still lead to economic losses. Proper identification of lesions, their causes, and the sanitary involvement of affected tissues is essential to guide management strategies during slaughter, thereby ensuring the safe production and high-quality animal protein. This study aimed to evaluate the infectious and inflammatory involvement of broiler carcasses condemned due to repugnant macroscopic characteristics or cachexia. A total of 256 broiler carcasses (64 per group) were necropsied. Sampling occurred across four consecutive slaughter days. Carcasses were stratified into four groups based on external macroscopic characteristics: Group A: well-developed carcasses with skin discoloration (localized or generalized) or other repugnant features; Group B: carcasses with a "triangle"-shaped breast tip, with or without prominent keel, no color changes, and no visible compromise (small carcasses); Group C: carcasses with a "triangle"-shaped breast tip and prominent keel and/or protruding bones and joints on the back, with no discoloration or visible compromise (cachectic carcasses); Group D (control): normal carcasses not subject to total condemnation. Descriptive statistical analysis was performed. In group A, 20% of the carcasses showed macroscopic alterations in more than one internal organ along with external changes, characterizing systemic inflammation. Another 13.84% presented isolated macroscopic findings in internal organs, while 66.15% (43 carcasses) had no macroscopic alterations in internal organs. In group B, small carcasses, 87.09% had no internal organ alterations, and no carcasses presented signs of systemic disease. Isolated macroscopic findings included hydropericardium, air sac opacity, pericarditis, pale liver tissue with hyperplasia, and proventriculitis. In Group C, cachectic carcasses, 15.38% of carcasses showed internal organ alterations. Two carcasses had isolated findings, while 12.30% presented multiple macroscopic lesions in the viscera, such as ascites, air sac opacity, hydropericardium, pericarditis, perihepatitis, presence of caseous material in the lungs and air sacs, proventriculitis, and liver and gizzard changes. In group D, no macroscopic alterations were observed in internal organs. The carcass evaluations showed that visible external alterations are not always associated with infectious or inflammatory internal lesions, highlighting the importance of technical criteria that are more assertive when dealing with systemic problems.

Keywords: Macroscopic Lesions, Poltry, Slaughterhouse



Detection of IgY in Egg Yolks From Broiler Breeder Hens Vaccinated and Non-Vaccinated With Autogenous Vaccines Against Aviadenovirus and Reovirus. (SA-20)

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Abstract:

Even with major technological advances in production, the national poultry industry still faces significant challenges caused by infectious agents, which are responsible for major losses to the production chain. Two of these infectious agents are Aviadenovirus and Reovirus. This study evaluated and quantified the transmission of antibodies (IgY) from breeder hens to chicks in cases where the birds were vaccinated with autogenous vaccines for Aviadenovirus and Reovirus. It also demonstrated the transmission of these antibodies in field infections caused by these agents. Eight flocks of broiler breeders were analyzed, each containing 50,000 birds. These were divided into four groups (G1 to G4) as follows: G1: birds vaccinated with autogenous vaccine against Aviadenovirus, G2: birds vaccinated with autogenous vaccine against Reovirus, G3: birds naturally infected with Aviadenovirus, G4: birds naturally infected with Reovirus. In addition, a control group (G5) composed of Specific Pathogen Free (SPF) birds was used as a negative group. The birds in the vaccinated groups were immunized with two doses of an oil-based autogenous vaccine at 12 and 18 weeks of age. When the breeder flocks reached 30 weeks of age, 30 eggs from each group were collected. The yolks were subjected to IgY extraction and quantification using the ELISA test. In addition to detecting IgY in the different groups, weight gain and total mortality rates were also evaluated in broiler flocks originating from the studied breeder flocks. The study showed that, in breeder flocks with clinical disease problems, the transfer of antibodies via the egg to the progeny, for both Aviadenovirus and Reovirus, occurred in a non-uniform manner (high standard deviation) and with higher antibody titers compared to birds immunized with autogenous vaccines, which showed lower and more uniform antibody titers. Regarding the broilers analyzed, the flocks originating from vaccinated breeder hens showed better weight gain and lower total mortality compared to flocks originating from breeders that were naturally infected with the agents.

Keywords: Circulating Antibodies, Vaccinated

Emergence of GI-23 IBV Lineage in Non-Commercial Birds in Brazil: Virus Characterization and Implications (SA-21)

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Abstract:

Infectious Bronchitis Virus (IBV) variants of the GI-23 lineage, first identified in the Middle East in 1998, have since disseminated globally, reaching commercial poultry in Paraná, southern Brazil, in 2021. Until then, the predominant IBV genotypes circulating in Brazil belonged to the GI-1 and GI-11 lineages. This study investigated the presence of IBV in backyard poultry in São Paulo State, a region with significant biodiversity among wild birds and an important poultry-producing area. Between December 2022 and April 2024, active surveillance was conducted by the São Paulo Agricultural Defense Coordination, involving the sampling of 742 birds (718 chickens, 17 ducks, and 7 geese) from 140 backyard flocks across 15 municipalities. All birds were clinically healthy and unvaccinated. Molecular screening of 304 cloacal and tracheal swabs was performed using RT-qPCR targeting the 5' untranslated region (5'UTR) of the IBV genome, followed by RT-PCR amplification of the S1 gene with a focus on GI-1 and GI-11 lineages. IBV RNA was detected in 29 backyard flocks (20.7%), comprising 42 positive samples (13.81%), all from chickens. Notably, 80% (12/15) of the surveyed municipalities reported at least one positive case, with the highest detection rates observed in Ilha Comprida (40.47%) and São Sebastião (14.28%). Crucially, none of the positive samples belonged to the GI-1 or GI-11 lineages. Seven unclassified strains, all collected in 2023, were subsequently sequenced using GI-23-specific primers, revealing high nucleotide identity (over 95%) with the GI-23 lineage based on partial S1 gene analysis. This represents the first detection of GI-23 IBV in backyard poultry populations in São Paulo state. Two viral isolates were obtained, and whole-genome sequencing via next-generation sequencing (NGS) was performed for one isolate. Phylogenetic analysis classified all seven sequences as GI-23 viruses, and phylodynamic analyses identified at least three independent introductions of GI-23 into Brazil: three sequences clustered with previously reported Brazilian isolates from 2022; one group clustered with European, Mexican, and Bolivian isolates, and three formed a distinct clade within the GI-23 lineage. Recombination analysis using the complete genome sequence further revealed multiple recombination events involving different IBV lineages. These findings highlight the recent emergence and complex molecular dynamics of GI-23 IBV in São Paulo's backyard poultry, underscoring the urgent need for strengthened molecular surveillance and improved biosecurity in commercial flocks to avoid virus dissemination to non-commercial flocks.

Keywords: Infectious Bronchitis Virus, Avian, Backyard, Poultry, Coronavirus

Mapping Avian Influenza Virus Risk Hotspots in São Paulo State: Strategic Insights For Epidemiological Surveillance and Backyard Poultry Protection (SA-22)

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Abstract:

This study aimed to identify high-risk areas for avian influenza virus (AIV) transmission between waterfowl and backyard poultry in São Paulo State, Brazil, to enhance surveillance, protect poultry health and food security, and mitigate socioeconomic impacts. Between December 2022 and April 2024, the Secretary of Agriculture and the State Poultry Health Program collected 404 cloacal/tracheal swabs from 1,212 birds (chickens, ducks, and geese) across 175 backyard poultry flocks in 16 municipalities. Molecular RT-qPCR targeting the matrix gene assays were conducted to assess viral presence. A geodatabase integrating migratory waterfowl occurrence data (CEMAVE/ICMBio) and 54 cases of highly pathogenic avian influenza virus (HPAIV) H5N1 in wild birds was developed. Spatial analysis using kernel density estimation (quartic function, 10 km bandwidth, cross-validated) generated five-class relative density maps highlighting areas of elevated wild bird density. All swab samples tested negative for AIV by RT-qPCR. Spatial risk mapping identified the São Paulo Metropolitan Region and the Paraíba Valley as high-density wild bird mesoregions, with Bertioga, Itanhaém, Santos, and São Sebastião emerging as high-risk hotspots for AIV introduction into backyard poultry. Given the recent HPAIV outbreaks in Brazil's commercial poultry and multiple detections in wild birds, enhancing and prioritizing targeted active surveillance in these areas is critical. This proactive strategy provides actionable data for early detection, optimized resource allocation, and effective mitigation of economic losses and zoonotic transmission risks, thereby protecting backyard poultry, commercial flocks, and public health. Funding: FAPESP (2022/08528-3 and 2023/08501-0) and RedeVirus-MCTI-CNPq (403761/2020-4 and 400172/2022-4).

Keywords: Highly Pathogenic Avian Influenza, Active Surveillance, Exposure Risk, Viruses, Birds

Immune Response of Breeder Hens Vaccinated With Live Vaccine Against Colibacillosis – Field Study (SA-23)

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Abstract:

One of the major challenges in poultry production is the effective control of Avian Pathogenic *Escherichia coli* (APEC). The emergence of antibiotic-resistant strains has reduced the efficacy of traditional treatments, highlighting the need for preventive strategies such as vaccination. This study aimed to evaluate the immune response in breeder hens vaccinated with a live attenuated *Escherichia coli* vaccine under field conditions. The study was conducted on a breeder farm in Paraná, Brazil. Two groups were evaluated: one vaccinated with two oral doses of a live *Escherichia coli* vaccine (at 1 and 15 weeks of age), and a non-vaccinated control group. At 24 weeks, 20 blood samples and tracheal and cloacal swabs were collected from each group. Blood samples were analyzed by flow cytometry to assess mucosal helper T lymphocytes (CD4+ TCRVβ1+), while swabs were tested for anti-*E. coli* IgA levels using ELISA. Additionally, tissue samples (liver, spleen, intestines, bone marrow) from progeny of both groups were tested for APEC presence. Vaccinated birds showed a statistically significant increase in mucosal CD4+ TCRVβ1+ lymphocytes, indicating a strong cellular immune response. IgA levels in tracheal and cloacal swabs were also higher in the vaccinated group, suggesting enhanced mucosal immunity. No APEC was detected in progeny from either group, indicating effective biosecurity and vaccination protocols. These findings align with previous studies showing that live *E. coli* vaccines stimulate mucosal immunity and reduce clinical signs of colibacillosis. The persistence of immune response up to 9 weeks post-vaccination was also demonstrated, reinforcing the long-term efficacy of the vaccine. The live *Escherichia coli* vaccine induced a robust mucosal and cellular immune response in breeder hens, with increased levels of CD4+ TCRVβ1+ lymphocytes and IgA production. These responses are critical for protection against APEC, particularly in the respiratory and gastrointestinal tracts. The study supports the use of live vaccines as part of an integrated strategy to control colibacillosis, especially in the context of reduced antibiotic use. Laboratory tools such as flow cytometry and ELISA proved effective in monitoring immune responses and can guide future vaccination programs.

Keywords: Immunity, Antibodies, IgA

Evaluation of the Pathogenicity of Avian Reovirus Isolates From Commercial Broiler Flocks in Brazil (SA-24)

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Abstract:

Avian reovirus (ARV) is a globally distributed pathogen that affects poultry, particularly broiler chickens, and is associated with several clinical syndromes, including arthritis, runting-stunting syndrome, and most notably, tenosynovitis. This study aimed to evaluate the pathogenicity and transmissibility of eight ARV field isolates collected from commercial broiler flocks in Brazil, each representing distinct clinical presentations and geographic origins. A total of 90 one-day-old specific-pathogen-free (SPF) chicks were randomly assigned to nine experimental groups—eight inoculated groups and one negative control—with 10 birds per group. Viral concentrates were prepared using primary renal cell cultures and standardized to a final titer of 10^3 /bird. Inoculation was performed via the plantar footpad (0.1 mL/bird) to mimic natural exposure, following protocols reported in scientific literature. Birds were monitored clinically and euthanized at 0, 7, 14, and 21 days post-inoculation (dpi) for organ collection (tendons, intestines, heart, and liver), followed by real-time PCR and histopathological evaluation. All eight ARV isolates were detected in tendons as early as 7 dpi, indicating rapid tropism and efficient migration from the inoculation site. By 14 and 21 dpi, ARV was detected in intestinal tissues across all challenged groups, including initially negative cases, suggesting horizontal transmission among birds housed in the same epidemiologic unit. Histopathological analysis confirmed tenosynovitis in 100% of infected birds, with 62% of the isolates inducing progressive lesion severity. Myocarditis and hepatitis appeared from 7 dpi onward, with clinical evolution varying according to the ARV strain used for inoculation. These results demonstrate considerable heterogeneity in pathogenic potential among ARV strains circulating in broiler production and underscore the importance of comprehensive diagnostic surveillance and targeted vaccination strategies to reduce ARV-associated economic losses in poultry systems.

Keywords: Avian Reovirus, Tenosynovitis, Pathogenicity, Broilers, Horizontal Transmission

***Bacillus Velezensis* As A Probiotic Alternative Against *Salmonella Heidelberg* in Broiler Chickens: An Omics-Based Approach (SA-25)**

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Abstract:

Although probiotics are widely recognized for their role in pathogen control and improving gut health, few studies have used omics-based techniques to deepen understanding of host–probiotic interactions. *Bacillus velezensis* (BV) remains underexplored as a probiotic candidate in poultry. This study assessed BV's effectiveness in inhibiting *Salmonella Heidelberg* (SH) using metagenomic and metabolomic approaches to evaluate its impact on poultry health and food safety. The research started with *in vitro* assays to determine inhibition zones, followed by an *in vivo* trial involving 100 broiler chicks divided into four groups: Positive Control, Negative Control, SH-challenged birds treated with BV, and non-challenged birds treated with BV. The study examined BV's effects on cecal microbiota composition, fecal metabolite profiles, and SH colonization in the cecum. Metagenomic and metabolomic analyses were conducted to assess microbial diversity and metabolic changes. SH counts were analyzed using the Shapiro-Wilk test and two-way ANOVA. For metabolomics, metabolites with significant differences by t-test ($p < 0.05$) and a fold change > 2 were further examined with Pearson's correlation analysis. Due to high multicollinearity among metabolites within age groups, principal component analysis (PCA) was performed separately for each group. Additional statistical analyses included multivariate analysis of variance (MANOVA). Alpha and beta diversity metrics were derived from the metagenomic data, and PCA results were used in a k-nearest neighbors (k-NN) classification model. BV supplementation significantly decreased SH counts in cecal content at 28 days of age ($p < 0.05$). Metabolomic analysis identified five major classes affected by treatment, including increased levels of beneficial compounds such as fatty acyl glucosides (surfactant molecules), lignin, artemisinin (antioxidants), and taurodeoxycholic acid (a bile acid), along with a decrease in metabolites associated with SH infection. Treatments also significantly influenced microbial richness and diversity, with BV supplementation and SH challenge producing diverse yet distinct microbial profiles. These findings support the potential of *Bacillus velezensis* as a probiotic alternative to antibiotics in poultry, promoting gut health, reducing SH colonization, and modulating key microbial and metabolic signatures linked to intestinal protection.

Keywords: Poultry Health, Food Safety, Antibiotic Resistance, *In Vitro*, *In Vivo*

Distribution of Avian Infectious Bronchitis Virus Genomic Groups in Commercial Poultry Flocks in Southeastern and Northeastern Brazil (2024–2025) (SA-26)

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Abstract:

Since 2021, Brazilian poultry breeding has been confronted with the spread of another genomic group (G) of infectious bronchitis virus (IBV), G23 (representative Var2). This genomic group has been associated in our flocks with an increase in condemnations due to airsacculitis as well as other lesions due to opportunistic bacterial infections, in addition to increased mortality in the flocks. However, IBVs of G11 (or formerly BRI) have already been detected in the last 40 years, in addition to a smaller proportion of IBVs of G1 (Massachusetts), which are also responsible for numerous clinical diseases in poultry flocks. Therefore, the aim of the present study was to evaluate the distribution of IBVs in different genomic groups (G1, G11 and G23) in samples collected between January 2024 and May 2025 from lots of broilers, breeders and layers from farms in Southeast and Northeast Brazil. Pools of organs were evaluated separately (pools of trachea, lungs and air sacs, kidneys and caecal tonsils) and/or cloacal swabs from batches vaccinated against Mass (G1) in conjunction with the G11 vaccine or not (BR1). All samples were analyzed by RT-PCR, amplifying a portion of the gene encoding the nucleocapsid protein. Those with a positive result were genotyped by real-time PCR for a portion of the gene encoding the spike protein (S1), covering variable and conserved regions. Results: 54% of the infections were mixed infections, and of these, 50% of the detected IBVs were from G11 (field virus), 44% from G23 and 22% from G1 (vaccine strains). Among the mixed infections, G11 (field IBV) and G23 (32%) were the most common, followed by G1 + G11 (field) (10%), mixed G1 + G11 (field) + G23 (8%) and G1 + G23 (4%). 42% of the detected IBVs belonged to only one genomic group/sample evaluated, with the following distribution: 19% G23, 12% G11 (field IBV) and 11% G11 (vaccine strains). There appears to be a coexistence of IBV genome groups, with a higher prevalence of G11 (field) in mixed infections and G23 in single genogroup infections. This high prevalence of G11 (field), even in vaccinated herds, can be explained by the possible genomic variability of G11-IBV and by possible errors in vaccination procedures that result in some herds being susceptible even when vaccinated. Given the increasing genetic variability of IBV in industrial poultry flocks in recent years, it is recommended that surveillance should not be based solely on serological testing. Genotyping of the virus groups involved is essential for effective adaptation of control programs.

Keywords: Ibv, Genogroups, Molecular Surveillance, Genomic Diversity

A Novel Next-Generation Sequencing Method For Mitochondrial Genome-Based Identification of *Eimeria* Spp. From Domestic Chickens (SA-27)

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Abstract:

Coccidiosis poses a significant economic threat to commercial poultry systems. Detecting and differentiating the ten *Eimeria* species of domestic chickens is particularly challenging, especially in samples containing a large number of multiple *Eimeria* species. This study aimed to develop a next-generation sequencing (NGS) method for sequencing amplicons produced via PCR that targets the mitochondrial genome, allowing the detection and identification of *Eimeria* spp. in fecal samples from domestic chickens. A PCR protocol was standardized using genus-specific primers that anneal to putative rRNA (forward primer) and cytochrome B (reverse primer) sequences within the mitochondrial genome of *Eimeria* spp. PCR amplicons (223 bp, excluding primers) were analyzed using NGS on the Illumina MiSeq platform according to the Illumina 16S metagenomic protocol, with 150 bp paired-end reads and a MiSeq Reagent kit v2 (Illumina). Following assay standardization, the NGS method was evaluated by comparing its results with those obtained from 91 DNA samples extracted from fecal samples of domestic chickens raised in commercial broiler chicken farms (CBCF) or alternative poultry production systems (APPS). These samples have been previously examined via NGS of partial fragments of the 18S rRNA gene of *Eimeria* spp. NGS analyses identified the following order of prevalence of *Eimeria* spp.: 1) CBCFs: *Eimeria maxima* (44/45; 98%); *Eimeria acervulina* (39/45; 87%); *Eimeria tenella* (13/45; 29%); *Eimeria mitis* (6/45; 13%); and *Eimeria praecox* (5/45; 11%). 2) APPS: *E. mitis* (27/46; 59%); *E. acervulina* (23/46; 50%); *Eimeria nagambie* (18/46; 39%); *E. maxima* (13/46; 28%); *E. praecox* (11/46; 23%); *Eimeria lata* (10/46; 22%); *E. tenella* (9/46; 20%); *Eimeria brunetti* (8/46; 17.4%); *Eimeria zaria* (7/46; 15%); *Eimeria* sp. (6/46; 13%); and *Eimeria necatrix* (6/46; 13%). The comparison of NGS results through a retrospective analysis of 91 DNA samples demonstrated that the NGS protocol developed in this study identified the same *Eimeria* species, with a similar order of prevalence, in both NCFC and SCA. The exceptions were *E. lata*, which was detected in the SCA in samples that had previously tested negative for this species, and *E. necatrix*/*E. tenella*, which cannot be differentiated by 18S rRNA-targeted NGS. The identification of the ten *Eimeria* species of domestic chickens, along with a novel *Eimeria* operational taxonomic unit (OTU), shows that the NGS protocol from this study successfully detects and differentiates *Eimeria* species and new OTUs from domestic chicken fecal samples with high specificity, which is essential for implementing control measures for coccidiosis in the poultry industry.

Keywords: Coccidiosis, Molecular Diagnosis, Poultry Farming

Distribution of the *Tsh* gene in *Escherichia coli*: Diversity of Serogroups and Association With High-Risk Apec Strains (Brazil, 2017-2025) (SA-28)

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Abstract:

Avian pathogenic *Escherichia coli* (APEC) represents a major threat to global poultry production, causing respiratory diseases and sepsis. Among APEC virulence factors, temperature-sensitive hemagglutinin (*Tsh*) is notable for its prevalence in virulent strains and its relevance in avian respiratory infections. *Tsh* belongs to the SPATE family (Serine Protease Autotransporter of Enterobacteriaceae) and acts via a type V secretion system. It has a dual function: the secreted N-terminal fragment acts as a mucinase, degrading mucus and facilitating access to epithelial cells, while the membrane-bound C-terminal portion promotes hemagglutination and bacterial adhesion to host cells. These mechanisms appear to play an important role in the early stages of colonization of the respiratory tract.

Environmental factors influence the expression of *tsh*, which increases in levels at temperatures similar to those of avian hosts, suggesting adaptive regulation during infection. Its gene is usually located on large virulence plasmids, such as ColV, which suggests the possibility of horizontal gene transfer. Due to its immunogenicity and function at the beginning of infection, *Tsh* is considered a possible vaccine or therapeutic target. This study aimed to evaluate the frequency of the *tsh* gene in APEC strains isolated from cases of avian colibacillosis in Brazil, between 2017 and 2025. A total of 130 strains of APEC from AviBac-Br collection were subjected to the WGS using MiSeq (Illumina). *De novo* assembly was performed using SPAdes v 4.1.0. The assembly's quality was assessed with QUAST v.5.2.0. Serotype, resistance and virulence genes were determined using services available on the website of the Center for Genomic Epidemiology and Galaxy Europe. The results show that the frequency of occurrence of *tsh* gene was 39.2% (51/130). These strains were distributed in 5 Brazilian states (SP, MG, PR, GO and RR). We identified 16 distinct serotypes: O5, O8, O15, O23, O24, O33, O36, O45, O53, O71, O88, O101, O103, O125, O153 and O157. In addition, some of these strains were classified as high-risk lineages: 6/51 were from phylogroup G-ST117; 6/51 were from group D-ST69 and 1/51 was classified in phylogroup C-ST23. The presence of resistance genes ranged from 0 to 15. Most of the strains were classified as susceptible, and 15.49% were considered MDR (multidrug resistant). In this context, highlight three strains from high-risk lineage ST117, that presented 9 to 15 resistance genes, which could codify resistance to 19 until 22 antibiotics. In conclusion, our results show that the *tsh* gene is moderately prevalent in APEC, and despite the diversity of serogroups, it was associated with high-risk lineages of APEC.

Keywords: Spate Proteins, Whole-Genome Sequencing, Virulence Factors, Apec (Avian Pathogenic *Escherichia Coli*)

Intestinal Lesion Score Reduction in Broilers Challenged with *Eimeria Maxima* Using Hydrolyzable Tannin and Arsenical Additives (SA-29)

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Abstract:

Coccidiosis is a major enteric disease in broilers, with documented resistance of *Eimeria* spp. to conventional anticoccidials. Moreover, arsenical combinations have been banned in several countries, reinforcing the need for safer alternatives. This study aimed to assess the efficacy of different additives in broilers challenged with *Eimeria maxima*. The experiment was conducted at the CAPEV Research Center, located in Amparo-SP, under ethical approval nº 003/2024. A total of 400 male Cobb broilers, not vaccinated against coccidiosis, were housed in suspended cages free from coccidial contamination. At 11 days of age, 240 birds with uniform body weight were selected and allocated into five treatments, with eight replicates of six birds each. Treatments included a positive control (unchallenged-PC), a negative control (challenged, untreated-NC), 3-nitro-4-hydroxyphenylarsonic acid + salinomycin (44 ppm + 72,6 ppm– NHS), hydrolyzable tannin + salinomycin (550 ppm + 72 ppm-HTS), and hydrolyzable tannin alone (550 ppm -HT). On day 14, birds from the challenged groups received 1 mL of an oral suspension containing 12,000 *E. maxima* oocysts/mL. At 20 days, birds were euthanized and intestines evaluated for lesion presence and severity using Johnson & Reid (1970) scores (0=no lesion; 1–3=increasing severity). Data were analyzed using the Kruskal-Wallis test, and means were compared by Fisher's post-hoc test, adopting a significance level of 5%. PC group showed 93.75% of birds with a score of 0, while the NC group had 92.86% of birds with lesion scores above 2. Among the challenged groups, the NC presented the highest mean intestinal lesion score (2.10; $p<0.05$), significantly higher than those observed in the NHS, HTS, and HT groups, which had mean scores of 1.67, 1.73, and 1.73, respectively. The tested additives NHS, HTS, and HT were able to reduce lesion scores by 0.43, 0.37, and 0.37, respectively. The observed frequencies for scores 0, 1, 2, and 3 were 93.75%, 6.25%, 0%, and 0% for PC; 0%, 7.08%, 75%, and 17.92% for NC; 0%, 37.5%, 58.33%, and 4.17% for NHS; 0%, 37.5%, 52.08%, and 10.42% for HTS; and 2.08%, 35.42%, 50%, and 12.5% for HT, respectively. The frequency of score 2 lesions was significantly reduced ($p<0.05$) in birds treated with hydrolyzable tannin, either alone or with salinomycin. Regarding score 3, although the overall incidence was lower, treatment with NHS significantly reduced it ($p<0.05$) compared to NC, showing similar performance to HTS and HT treatments. In conclusion, the evaluated additives (NHS, HTS, and HT) proved effective in reducing the severity of intestinal lesions caused by *Eimeria maxima*. Notably, hydrolyzable tannin emerged as a promising alternative for coccidiosis control in broiler production.

Keywords: Anticoccidial Efficacy; Hydrolyzable Tannin; Salinomycin; Poultry Health; Natural Additives

Epidemiological Investigation of *E. Coli* in Commercial Layer Hens (SA-31)

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Abstract:

The Brazilian poultry industry continues to expand, accompanied by growing concern for animal health and welfare. In this context, avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), stands out as one of the main diseases, leading to significant economic losses. APEC can act as a primary or secondary agent, affecting various organs in birds, which complicates its identification. Traditional diagnostic methods, such as serotyping, are limited, as serotype is not always correlated with pathogenicity. Advances in molecular biology have contributed to a better understanding of the characteristics and behavior of these strains. This study aimed to characterize *E. coli* isolates obtained from six flocks of commercial laying hens in southeastern Brazil, at 7, 18, and 30 weeks of age. A total of 63 organ samples were collected, followed by bacterial isolation and conventional PCR detection of five virulence genes: *iss*, *iroN*, *iutA*, *hlyF*, and *ompT*. Samples with four or more genes were subjected to phylogrouping (A, B1, B2, C, D, E, F) via multiplex PCR and tested for susceptibility to 16 antimicrobials using the disk diffusion method. Of the 63 isolates, 52 (82.5%) showed *E. coli* growth, with 104 virulence factors detected (27 *iroN*, 25 *ompT*, 20 *iutA*, 19 *iss*, and 13 *hlyF*). Most samples (34.6%) did not present virulence genes, while 28.8% showed four or five genes. Phylogrouping revealed predominance of groups B1 (8), A (2), D (2), E (2), and C (1). In the antimicrobial susceptibility test, 71.4% of isolates were sensitive, 3.6% intermediate, and 25% resistant. The association between virulence genes and Clermont phylogrouping proved to be an effective tool for the epidemiological assessment of colibacillosis. Of the 15 samples selected for phylogrouping, 10 belonged to commensal groups (A and B1), while only 2 belonged to group E, which is associated with higher pathogenicity. The results reinforce the importance of molecular characterization of APEC as a tool to support control and treatment strategies, especially considering the high sensitivity observed to the tested antimicrobials.

Keywords: Colibacillosis, Diagnosis, Laying Hens/ Layers, Control, Biosecurity

Anticoccidial Sensitivity Test For *E. Maxima* Sampled from a Broiler Company in Minas Gerais, Brazil (SA-32)

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Abstract:

Coccidiosis is a globally widespread disease in poultry production with significant impacts on the intestinal health of broilers as well as economic losses associated with medication and zootechnical performance. Ionophores, nicarbazin-potentiated ionophores and chemical anticoccidials are important tools for coccidiosis control and remain the most used strategy in Brazil to prevent the disease in broilers. This study aimed to evaluate, under experimental conditions, the efficacy of the main in feed anticoccidials used in Brazil for coccidiosis prevention. Approximately five kilograms of feces and intestinal scraping were collected from poultry houses of the same integrated broiler company in the state of Minas Gerais, southeastern region of Brazil. The samples were stored in a potassium dichromate solution and sent to a research center in Amparo/SP, where the oocysts were purified, identified, sporulated, and quantified to perform the Anticoccidial Sensitivity Test - AST. The prevalent specie was *Eimeria maxima* which was tested in the following design: T1: UUC – uninfected untreated control; T2: IUC – infected untreated control; T3: nicarbazin plus semduramicin; T4: nicarbazin plus narasin; T5: semduramicin; T6: monensin; T7: salinomycin; T8: lasalocid and T9: decoquinate, Each treatment had four replicates of six birds each, totaling 216 broiler chickens. Chicks were housed at one day of age and at d12 received medicated feed according to the respective treatments (T3 to T9). At d14, birds were orally inoculated via gavage with approximately 10,000 *E. maxima* sporulated oocysts diluted in 1 mL per bird (T2 to T9). On d20, weight gain (WG), feed conversion ratio and *E. maxima* lesion score were evaluated. Treatments T8 and T9 did not differ statistically from T1 in WG and were considered the best groups in this parameter. Treatments T4, T6 and T7 obtained the lowest WG, not differing statistically from T2. Regarding *E. maxima* lesion score, T1 was the only treatment with statistical difference in relation to the other treatments in the study i.e. anticoccidial treatments were statistically equal to each other. Among the ionophores evaluated, lasalocid resulted in the greatest weight gain. Decoquinate showed the highest efficacy among the chemical anticoccidials. Nicarbazin plus semduramicin combination was the most effective among the nicarbazin-potentiated ionophores.

Keywords: Coccidiosis, Ionophores, Chemical Anticoccidials

The Importance of the Integrated Disease Surveillance Plan for Swine Farming in Brazil (SA-33)

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Abstract:

Brazilian swine farming has a very favorable sanitary status as it is considered free from economically significant diseases, notably african swine fever (ASF) and porcine reproductive and respiratory syndrome (PRRS), and also because it has three zones free of classical swine fever (CSF). Maintaining this sanitary status in Brazil ensures a competitive advantage in accessing international markets. To improve the surveillance system for diseases affecting swine farming, the Animal Health Department published the Integrated Swine Disease Surveillance Plan (PIVDS) in 2020. Developed by the Official Veterinary Service (OVS) in collaboration with private sector stakeholders, it redefined the components of the surveillance system for CSF, ASF and PRRS. The PIVDS is implemented in Brazil's CSF-free zone and consists of five components: risk-based serological surveillance (1), inspections at breeding establishments (2), investigations of suspected cases (3), slaughterhouse inspections (4), and serological surveillance of feral swine (5). For the implementation of components 1 and 2 of the PIVDS, the number of establishments to be sampled was defined based on their category, considering the risk assigned to each. Four types were selected: certified swine breeder farms (GRSC), piglet-producing units, full-cycle farms, and non-technified swine farming establishments for local trade. The implementation of component 1 resulted in 55,369 serum samples tested by ELISA for CSF in 2,087 establishments during the first cycle, and 66,476 samples in 2,058 establishments during the second cycle. The results obtained, following the protocol, confirmed the absence of viral circulation in the three areas covered by the integrated plan. For component 2, 1,424 establishments were inspected in the first cycle and 1,276 in the second, with no probable cases of CSF, ASF, or PRRS detected. Regarding passive surveillance, 146 investigations of suspected hemorrhagic syndrome were conducted in the first cycle and 30 in the second. All investigations were concluded with negative results for the target diseases. In slaughterhouse inspections, 57,590,371 and 59,571,792 swine were sent for slaughter during the periods corresponding to the first and second cycles, with no clinical signs of the target diseases observed. In the serological surveillance of feral swine, 1,787 and 1,595 samples were tested in the first and second cycles, respectively, with negative results at the end of the investigation. The PIVDS fulfills its purpose of strengthening surveillance and emergency response, protecting swine farming and Brazil's economy.

Keywords: Classical Swine Fever (Csf), African Swine Fever (Psa), Porcine Reproductive and Respiratory Syndrome (PrRs)

Immunological Status and Productive Performance of Broiler Chickens in Sentinel Farms in Northeastern Brazil Using a Monitoring Tool for Vaccination Program Evaluation (SA-34)

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Abstract:

Monitoring in poultry production involves systematically tracking and recording data using tools to support better management and decision-making. Health assessment of broiler flocks is essential for controlling processes such as vaccination and contributes to biosecurity strategies. This study aimed to monitor the zootechnical and health performance of broiler chickens raised in sentinel farms of an integrator in Northeastern Brazil, throughout the birds' entire life cycle, using an innovative monitoring tool. The tool enabled the assignment of a final score to each flock based on various parameters aligned with the vaccination program, facilitating interpretation and guiding interventions along the production chain. Data and biological samples were collected at three key time points: placement, approximately four weeks of age, and pre-slaughter. A total of 137,000 birds from different genetic lines and origins were monitored across four flocks, all managed under similar conditions. The vaccination program for Infectious Bursal Disease (IBD) included immune complex vaccines administered *in ovo*, in combination with recombinant and live attenuated vaccines for other diseases. In one of the monitored flocks, the and macroscopic appearance of the bursa of Fabricius were consistent with the expected response to the antigen-antibody type vaccine. Histopathological analysis revealed moderate to average medullary lymphoid depletion, with lesions attributed to the vaccine virus and cytokines from T cells inducing apoptosis. The basal membrane remained intact, indicating tissue recovery. All bursa samples tested positive for IBDV, and the high viral load (low Ct) allowed for subsequent strain typing. ELISA serology results showed antibody titers within expected ranges, supporting the effectiveness of the vaccination program. Ongoing surveillance proved essential for evaluating immune status and flock health. The results confirmed the absence of IBD with sanitary impact and demonstrated that the current vaccination strategy was effective and did not compromise performance. This approach highlights how technology can support continuous improvement in industrial poultry production.

Keywords: Monitoring, Health Performance, Data Collection, Vaccination Program, Immune Status



Evaluation of the Impact on Production Cost and Zootechnical Performance of Broiler Chickens Immunized with Different Recombinant Triple-Action Vaccines (SA-35)

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Abstract:

Field vaccination errors, once frequent, have been significantly reduced due to advances in biological technologies. Vector vaccines, which do not contain live virus, are unaffected by maternal antibodies and rely on the HVT (Marek's disease virus) vector to initiate protection. For Gumboro disease, immunity is induced by the VP2 protein, and through the insertion of the F protein gene from NDV into the HVT genome, triple protection against Gumboro, Newcastle, and Marek's diseases has been achieved. This study aimed to compare zootechnical performance and production cost (R\$/ton) between two vaccination programs in a broiler integration in Northeastern Brazil. In T01, birds received a recombinant vaccine from a competing laboratory, while in T02, birds were immunized with a triple vector vaccine from Zoetis. Both vaccines were administered subcutaneously at the hatchery. The study was conducted from January to March 2025, involving 252,060 birds (T01: 116,060; T02: 136,000), evaluated across two flocks (T01) and three flocks (T02). Statistical analyses were performed using MINITAB® software. One-way ANOVA was used to assess differences among variables, and the Kruskal-Wallis test was applied to evaluate average slaughter weight. When significant differences were found ($p < 0.05$), the t-test was used for mean comparisons. No statistically significant differences ($p > 0.05$) were observed between groups for average weight, age, daily weight gain (DWG), feed conversion ratio (FCR), or productive efficiency index (PEI). However, T02 showed significantly lower mortality ($p = 0.034$) and higher viability, along with a significantly lower production cost per ton ($p = 0.015$), indicating a better return on investment (ROI). These results support the use of the T02 vaccine as a safe, effective, and economically advantageous option for future broiler vaccination programs.

Keywords: Recombinant Vaccine, Triple Vector Vaccine, Return On Investment (Roi), Production Cost (R\$/Ton), Zootechnical Performance

***Campylobacter* spp in Samples From Broiler Chicken Slaughter Plants (SA-36)**

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Abstract:

Campylobacter spp. are important etiological agents of foodborne gastroenteritis in humans, with *C. jejuni*, *C. coli*, and *C. lari* being the most frequently involved species. Broiler chickens are recognized as natural reservoirs and represent the main source of human infection. Contamination may occur throughout the entire production chain, with critical control points during slaughter stages. This study aimed to monitor the presence of *Campylobacter* spp. in poultry slaughterhouses and was conducted in three slaughterhouses under the supervision of the Sao Paulo State Inspection Service (SISP-SP). Five weekly sampling sessions were carried out in each facility, with two flocks sampled per session at seven points along the slaughter process, totaling 210 samples. The sampling points were as follows: cloacal swabs from birds, swabs from poultry transport crates, environmental swabs from mechanical defeathering equipment and evisceration equipment, water samples from chilling tanks and pre-chilling tanks, and carcasses at the pre-packaging stage. Carcasses were subjected to rinsing procedures and all samples were enriched in Bolton broth, and 2 mL aliquots were stored at -80°C . DNA was extracted using the commercial IndiMag Pathogen Kit in conjunction with the automated nucleic acid extraction system Indimag 48 (INDICAL BIOSCIENCE), according to the manufacturer's instructions. Detection of *C. jejuni*, *C. coli*, and *C. lari* was performed using a multiplex real-time PCR assay with the commercial Kylt® *Campylobacter jejuni, coli & lari* kit (KYLT). *Campylobacter* spp. were detected in 76.2% of the samples, corroborating findings from previous studies. Pre-slaughter contamination was confirmed by the simultaneous detection of *C. jejuni* and *C. coli* in cloacal swabs (18.4%) and poultry transport crates (25%), highlighting their role as primary sources of infection. Mixed contamination with *C. jejuni* and *C. coli* was detected on defeathering equipment (40%) and evisceration equipment (46.7%). Only *C. jejuni* was found in water samples from chilling tanks (40%), supporting its ability to survive at low temperatures. A high level of contamination by *Campylobacter* spp. was observed in poultry carcasses at the pre-packaging stage (58.3%), with co-occurrence of *C. jejuni* and *C. coli* in 26.7% of those samples. The results of this study are consistent with previous reports regarding the predominance of *C. jejuni* and the lower prevalence of *C. coli* in poultry slaughter plants. These findings highlight the importance of controlling *Campylobacter* spp. in slaughterhouses and reinforces the need to include routine monitoring of this pathogen in the poultry production chain to ensure the microbiological quality and food safety of chicken meat.

Keywords: Campylobacteriosis, Critical Points, Cross Contamination, Environmental Samples, Carcasses

Characterization and Dissemination of Avian Pathogenic *Escherichia Coli* St117 in Brazilian Poultry Production (SA-37)

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Abstract:

Escherichia coli infection in global poultry production has proven to be a increasingly problem, causing a decline in production indicators, significant economic losses and affecting animal welfare issues. In general, APEC isolates from chickens constitute a genetically diverse population with numerous of different characteristics and sequence types (STs) and the ST117 have been frequently observed (Ronco, T. et al, 2017). The aim of this study was to characterize APEC ST117 strains isolated from birds with colibacillosis in Brazil. A total of 105 strains isolated from organs of birds with colibacillosis, previously classified as APEC by PCR (Johnson *et al.*, 2008), were subjected to the Whole Genome Sequencing using MiSeq (Illumina, San Diego, USA). The assemblies were assessed with QUAST v.5.2.0. Serotypes, ST and virulence genes were determined using services available on the website of the Centre for Genomic Epidemiology (<https://cge.food.dtu.dk/>) and Galaxy Europe (<https://usegalaxy.eu/>). A total of 25/173 (14,4%) strains were classified as ST117, of which 12/25 were isolated from broilers, 8/25 were isolated from layers and 5/25 isolated from broiler breeder. These strains were recovered from 5 Brazilian states (SP, MG, PR, SC and PE). The serotypes identified were: O8:H4, O24:H4, O33:H4, O53:H4, O71:H4, O78:H4, O125ab:H4, O143:H4, O143:H100 and O161:H4. All strains were positive for genes that codified FimA, curli and S fimbriae, iss, ompT and iron uptake systems (iroN, iucD, irp and iutA). In addition, 12/25 strains were positive for Vacuolating autotransporter toxin (Vat) and 10/25 were tsh+ (temperature-sensitive hemagglutinin). The Vat and TSH are member of serine protease autotransporter proteins of Enterobacteriaceae (SPATEs), present in human extraintestinal pathogenic *E. coli* (ExPEC) are associated with the development of cellulitis and colonization of respiratory tract in birds with colibacillosis, respectively (Parreira and Gyles, 2003). The high-risk lineage from phylogroup G and ST-117 was predominant (15/25), but other sequence types were identified. In conclusion, our results shows the lineage ST-117 APEC is disseminated in broiler, breeder broiler and layers production. Further investigations are needed to determine the role of Vat toxin in colibacillosis and the zoonotic risks associated with poultry meat.

Keywords: *Escherichia Coli*, Colibacillosis, St117

Phage Display-Derived Peptides: A Promising Alternative for *Campylobacter jejuni* Control through Theragnostic Approach (SA-38)

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Abstract:

Considering the importance of *Campylobacter jejuni* (CJ) for human health and the need for new theragnostic approaches, this study aimed to select and characterize specific binding peptides from CJ by using phage display, to control this bacterium. We performed peptides selection using the biopanning technique and a total of 23 phages were selected using CJ as the target. These clones were sequenced and tested via screening phage-ELISA, where we considered with significant binding effect those phages with higher absorbance compared to a wild phage (M13). To help choose the best peptide, we performed the molecular docking and based at the binding strength. After molecular docking, we selected five peptides and performed a second phage-ELISA screening, which gave us the two best choices (peptide B6 and C5). Then, we followed a protocol of *in vitro* assay to evaluate the minimal inhibitory concentration (MIC) using CJ IAL 2383 and CJ 1997/6 strains and the peptides alone and mixed. Compared to the positive control, peptide B6 was the one with the most bacteria reduction power. It reduced CJ IAL 2383 to 93.93% at 1000ug/mL while peptide C5 did not reach 90% reduction. To the strain CJ 1997/6 peptide B6 decreased CJ from 125 ug/mL (92.02% reduction) with 96% reduction at 1mg/mL. Peptide C5 reduced bacteria from 250µg/mL (91.43% of reduction). At 1mg/mL peptide C decreased CJ in 95.8%. When using both peptides (mix), the reduction of CJ IAL 2383 was from 500ug/mL with 91.72% reduction reaching 93% reduction at 1000ug/mL. To the mix, the results of reduction to strain 1997/6 started from 125ug/mL (92.71% of reduction) and at peptide at 1mg/mL decreased CJ in 94.73%. Considering the worst result of the C5 compared to B6 we performed another analysis with C5 in triplicate, but with CJ initial inoculum of 4 UFC/mL to evaluate if the initial inoculum changed the results. In this case, the peptide C5 reduced bacteria growth in 100% from concentration of 250µg/mL and 97.62% at 62.5 ug/mL. This shows that the peptide has high power of inhibiting the CJ when the initial dose is low. The use of peptides is also interesting for diagnosis because they can be more specific (once they recognize a specific epitope) and safer, since it is not necessary to handle the pathogen.

Keywords: Binding Peptides, Biopanning, Pathogen Control

First Human Isolation of *Erysipelothrix Piscisarius* in Brazil: Clonal Relatedness to Turkey Strains and Multi-Omic Characterization of a Potentially Underrecognized Pathogen (SA-39)

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Abstract:

The identification of new and emerging zoonoses is crucial for implementing control measures. A multi-omic approach was used to characterize a bacterial isolate from a sick male patient, initially identified as *Erysipelothrix rhusiopathiae* (ER) by MALDI-TOF. However, PCR identified it as *Erysipelothrix piscisarius* (EP) here the strain was named as EP h2B. Next-generation sequencing (NGS) was performed, and the results showed high similarity to EP isolates previously associated with infections in turkeys (first identified by us), fish in the USA, and humans in China, based on phylogenetic and genomic clustering analyses. Visual genome comparison with the Proksee program revealed high genomic conservation among EP isolates, with few specific regions indicating possible insertions or deletions. The biochemical analyses demonstrated similar biochemical profiles between the human isolate EP h2B and (ER), except for the ortho-nitrophenyl- β -galactopyranoside (ONPG) test, which indicated that EP h2B does not produce the β -galactosidase enzyme. Comparative metabolomic analysis distinguished EP h2B, ER, and *Staphylococcus* spp. ATCC 25923 (SA) by six metabolites. EP h2B and ER had more L-arginine and Ile Gly than SA. The metabolites 1,6-anhydro-N-acetyl-beta-muramate and cysteine were found at higher relative abundance in EP h2B compared to ER. The unknown metabolite and Reveromycin A 4'-methyl ester (or Reveromycin I) showed lower levels in EP h2B compared to ER. We report here, for the first time in Brazil, the isolation of EP from a human case. Phylogenetic analysis indicates that the EP h2B strain is related to EP isolates previously obtained from turkeys by our group, suggesting a newly recognized zoonosis that has been little studied. Furthermore, the EP h2B strain also presents a distinct metabolic profile compared to other EP strains, which may suggest species-specific adaptations.

Keywords: Genome, Metabolomic, Biochemical

Serum Amyloid A Can Be Used as a Biomarker of Inflammation in Allantoic Fluid of Chicken Embryos in the Early Stages of Development (SA-40)

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Abstract:

Monitoring flock health during incubation can be an approach that has been little explored for early detection of infections and for implementing effective sanitary measures in hatcheries. Furthermore, chicken embryos have been used as a viable, ethical, and sustainable alternative for pre-clinical tests on inflammation. However, non-invasive biomarkers for detecting inflammation and infection within embryonated eggs are still lacking. The aim of this study was to identify whether reliable inflammatory markers in egg samples could provide valuable tools for quality control of incubation using a less invasive approach, such as allantoic fluid in the early stages of incubation. To address this, serum from chicken embryos (CE) inoculated with 2log UFC/mL of *Salmonella Pullorum* at 7 or 9.5 days of embryonic development (DED) via the allantoic cavity were evaluated after 48 hours to investigate acute inflammatory markers. Serum and allantoic fluid were collected to quantify specific acute phase proteins from birds (Serum Amyloid A, Alpha-1-Acid Glycoprotein, Ovotransferrin) using ELISA for chicken samples. Differences in serum concentrations of the acute-phase proteins serum amyloid A, alpha-1-acid glycoprotein, and ovotransferrin were not statistically significant ($p>0.05$) between embryos inoculated with SP at 7 and 9.5 DED and collected 48 hours post-inoculation, and those inoculated with saline (NC). In the allantoic fluid, however, only serum amyloid A showed increased concentration in embryos inoculated with SP (1,054 μ g/mL) at 9.5 days compared to the NC group (0,1193 μ g/mL). Serum Amyloid A is a highly sensitive acute phase protein produced mainly by hepatocytes during early infection. Its levels can rise over 1000-fold, playing a key role in host defense by opsonizing gram-negative bacteria activating immune cells. In addition to serving as a reservoir for embryonic excreta, the allantoic fluid also receives amino acids, peptides, and proteins resulting from embryonic metabolism including acute-phase proteins produced by the liver. Additionally, the direct transfer of gut-derived proteins to this compartment is likely, particularly considering the extrahepatic production of acute-phase proteins in the intestine. Overall, the allantoic fluid serves as a suitable sample for measuring acute phase proteins and identifying inflammation *in ovo*, highlighting its potential as a practical biomarker source for inflammation monitoring and quality control in poultry production.

Keywords: Acute Phase Protein, Elisa, *Salmonella Pullorum*

Pro-Inflammatory Cytokines in Early-Stage Chicken Embryos (SA-41)

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Abstract:

Despite the broad use of chicken embryos (CE) as a research model, its application in inflammation studies still lacks standardization and validation at earlier developmental stages. Although late-stage embryos offer advantages over post-hatch models, including reduced stress, investigating earlier stages (before 12 days of embryonic development) is essential to enhance the ethical considerations and sustainability, as well as the scientific value of CE in infection and inflammation research. This study aimed to assess the suitability of early-stage embryos as an inflammation model by inoculating *Salmonella Pullorum* (SP) and analyzing cytokine expression up to 17 DED. Embryos were inoculated at 9.5 DED with 100 µL of 2 log CFU/mL SP via the allantoic cavity. Liver and spleen samples were collected at 11.5 and 17 DED for RT-qPCR TaqMan analysis of cytokine gene expression. For statistical analysis, we performed a two-way ANOVA ($p < 0.05$). TGF expression was higher at 11.5 DED compared to 17 DED, suggesting a decrease in TGF levels with advancing embryonic age. However, no differences were observed in response to the challenge with SP at either age. Although TGF-β is widely recognized for its roles in adult physiology, including the regulation of inflammation, it also plays a crucial role during embryonic development by controlling differentiation, morphogenesis, and tissue remodeling. IL-6 expression did not differ significantly between groups or developmental stages, indicating that it was not influenced by either the infection or the embryo's age. In contrast, IL-8 expression increased with embryonic age in both groups. At 11.5 days of development, we observed upregulation in the group challenged with SP compared to the negative control. IL-8 is a chemokine that has been detected early during embryonic development, with its levels increasing in later embryonic stages. Its increase is a response to pro-inflammatory stimuli and plays a key role in the innate immune response by recruiting heterophils. A slight increase at 11.5 DED in the infected group (PC) may reflect a transient inflammatory response. These findings support the use of chicken embryos before 12 DED as a viable model for studying acute inflammation, demonstrating age-dependent modulation of cytokine expression in response to infection.

Keywords: Avian Immunology, Innate Immune Response, Experimental Inflammation Model

Antimicrobial Resistance Profile and Biofilm Formation of *Escherichia coli* Isolated From Finger Rubbers of Poultry Plucking Machines (SA-42)

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Abstract:

The widespread use of antimicrobials in animal production promotes the selection of resistant bacteria, compromising therapeutic efficacy and posing risks to public health, as such microorganisms can be transmitted along the food chain. In poultry slaughtering, steps such as defeathering, evisceration, and immersion chilling represent critical points for bacterial cross-contamination in the processing plant environment. Given this context, the present study aimed to characterize the biofilm-forming ability and phenotypic antimicrobial resistance profile of *Escherichia coli* isolates obtained from rubber finger components of industrial poultry plucking machines. Fourteen pools were prepared, each consisting of three rubber fingers replaced after wear from use. These materials were incubated in BHI broth at 37 °C for 24 hours and subsequently plated on MacConkey agar, followed by incubation under the same conditions. Colonies with characteristic morphology were subjected to biochemical tests for confirmation as *Escherichia coli*. To assess biofilm production, the isolates were first incubated in TSB broth at 37 °C for 18 hours, standardized to 0.5 on the McFarland scale, and distributed in triplicate into polystyrene plates. They were then incubated again in TSB at 37 °C for 24 hours. The *Escherichia coli* EAEC ATCC 042 strain was used as a positive control. After biofilm formation, the samples were washed with PBS, fixed with methanol (99%), stained with crystal violet (0.1%), eluted with methanol (99%), and subjected to absorbance reading at 630 nm. Antimicrobial resistance was evaluated using the disk diffusion method against 17 antimicrobials from different pharmacological classes. It was observed that 64.28% (9/14) of the isolates were classified as strong biofilm producers, while 35.71% (5/14) showed moderate production, with no weak producers detected. Regarding the antimicrobial resistance profile, the highest rates were observed for the tetracycline class (71.43%; 10/14) and penicillins (57.14%; 8/14). Additionally, 21.43% (3/14) of the isolates tested positive for extended-spectrum beta-lactamase (ESBL) production, and 57.14% (8/14) were classified as multidrug-resistant (MDR). It is concluded that the *E. coli* isolates showed a high capacity for biofilm formation and a notable resistance profile to multiple classes of antimicrobials, highlighting the rubber fingers of plucking machines as potential sources of cross-contamination in the slaughterhouse environment.

Keywords: Enterobacteriaceae, Cross-Contamination, Antimicrobial Susceptibility Testing, Slaughterhouse

Metabolomic Insights Into the Synergistic Role of *Bacillus*-Derived Extracellular Vesicles Against *Salmonella Heidelberg* (SA-43)

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Abstract:

The role of extracellular vesicles (EVs) in bacterial communication and pathogenesis has garnered significant attention in recent years, but studies related to EVs of probiotic strains are still scarce. Our preliminary investigations using the methodology of the spot in diffusion in agar revealed that while *Bacillus cereus* (BC), *Bacillus subtilis* (BS), and *Bacillus licheniformis* (BL) individually do not exhibit significant antimicrobial effects against *Salmonella Heidelberg* (SH) *in vitro*, a synergistic effect emerges when these strains are tested together. To assess the action of EVs in this context, this study aimed to evaluate their role in the inhibition and synergism among BC, BS, and BL against SH, as well as to identify the main metabolites present in the EVs. To this end, we cultured these *Bacillus* strains both individually and in combination, isolated their total metabolites (MT), and further separated EVs and low molecular weight metabolites (MB) using ultracentrifugation and ultrafiltration. We then characterized these components, particularly focusing on pH, quantification of protein content, number, and size of particles. Finally, we assessed their antimicrobial activity against SH. Our results demonstrate a strain-dependent antimicrobial activity of EVs, with specific combinations of BL+BS and BL+BC leading to a 99% reduction in different SH strains grown. These findings suggest that EVs may be key contributors to the enhanced antimicrobial effects observed in mixed cultures of these probiotic strains. Complementing these findings, metabolomic analysis of EVs identified the presence of key antimicrobial compounds, including thiouracil, indoleacrylic acid, D-tryptophan, L,L-cyclo (leucylprolyl), and bacitracin, reinforcing the potential of both EVs and associated metabolites in driving the observed synergism. This study not only deepens our understanding of the role of bacterial EVs in synergistic interactions but also highlights their potential application in the development of novel antimicrobial strategies.

Keywords: Probiotic, Antimicrobial, Metabolites, Inhibition, Bacteria

Genotypic and Phenotypic Profile of *Salmonella Infantis* Isolates from a Poultry Slaughterhouse (SA-44)

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Abstract:

Salmonella spp. is a pathogen of One Health importance, whose ability to form biofilm represents one of the main mechanisms of persistence and resistance in the environment, hindering the action of antimicrobials and sanitizers, especially in poultry slaughterhouses. This study aimed to evaluate the phenotypic and genotypic biofilm production, as well as the RDAR (red, dry, and rough) morphology of *Salmonella Infantis* (SI) isolates (n=7) from a broiler slaughterhouse. Seven isolates obtained from cecum and carcasses after the chilling step were analyzed. Genomic DNA was extracted and subjected to PCR for the detection of the genes *agfA* (bacterial adhesion), *csgD* (regulation of curli fimbriae synthesis), *adrA* (extracellular matrix production), and *luxS* (associated with the quorum sensing system), all related to biofilm formation. Phenotypic evaluation of biofilm production was performed in polystyrene plates incubated at 35°C. Morphological characterization was conducted on Luria-Bertani agar (without NaCl) supplemented with Congo red and brilliant blue G, incubated at 28°C and 35°C to identify the RDAR phenotype. All isolates (7/7; 100%) were positive for the *agfA*, *adrA*, and *csgD* genes, while two isolates (2/7; 28.6%) carried the *luxS* gene. Regarding phenotypic profile, five isolates (5/7; 71.43%) were classified as strong biofilm producers, and two (2/7; 28.57%) as moderate producers. RDAR morphology was observed in 57.14% (4/7) of the isolates incubated at 28°C, while no strain expressed this phenotype at 35°C. Notably, two isolates (28.57%; 2/7) were positive for all four genes investigated, showing RDAR morphology and moderate or strong biofilm production, respectively. These results demonstrate that the evaluated *Salmonella Infantis* isolates have genotypic and phenotypic characteristics strongly associated with biofilm formation, favoring their persistence on surfaces in slaughterhouse environments and representing a risk to food safety.

Keywords: Rdar Morphology, Resistance, Virulence, Poultry, Rdar Morphology

Inhibitory Effect of *Lactobacillus* spp. Against *Salmonella* spp. Isolated from Avian Sources (SA-45)

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Abstract:

Salmonella spp. is one of the main agents associated with foodborne disease outbreaks and is a zoonosis of great public health concern. The emergence of antimicrobial-resistant strains worsens this scenario by reducing the effectiveness of conventional treatments and promoting the spread of resistance genes throughout the poultry production chain. In this context, research on probiotics as promising alternatives for microbiological control has intensified. This study aimed to evaluate the antagonistic activity of a commercial pool of *Lactobacillus* spp. (PL), composed of 11 strains, against *Salmonella* spp. serovars isolated from poultry. A total of 20 isolates (10 *Salmonella* Heidelberg – SH and 10 *Salmonella* Typhimurium – ST), stored in the culture collection of the Avian Medicine Laboratory – UEL, were used. The isolates were subjected to the Spot on the Lawn and co-culture techniques. In the Spot on the Lawn assay, *Lactobacillus* spp. was incubated in MRS broth (10^6 CFU/mL) at 37°C for 24 hours. Then, 20 µL of the inoculum was plated onto MRS agar. After a further incubation, *Salmonella* spp. isolates (previously incubated in BHI for 24 h) were added to semi-solid BHI and poured over the surface of the plates. After 24 h, inhibition halos were measured, and diameters greater than 1 mm were considered satisfactory. In the co-culture assay, PL, SH, and ST inocula were prepared at double concentrations and mixed in MRS and MH media. Cultures were incubated at 37°C for 24 h and evaluated at 0, 6, 10, and 24 hours on MacConkey and Rogosa agars, forming the following groups: G1 (recovery of ST or SH), G2 (recovery of *Lactobacillus* spp.), G3 (co-culture – recovery of ST or SH), and G4 (co-culture – recovery of *Lactobacillus* spp.). Data were analyzed using repeated measures ANOVA in Statistica software version 13.5, considering group as the between-subject factor and time as the within-subject factor. When a significant Group × Time interaction was observed, Tukey's test was applied ($p < 0.05$). Mauchly's test was used to assess sphericity, with Greenhouse-Geisser correction applied when necessary. The PL was able to inhibit 100% ($n = 20$) of the isolates in the Spot on the Lawn technique, with average inhibition halo diameters of 5.53 mm for SH and 7.13 mm for ST. In the co-culture assay, a significant reduction in the growth of both serovars was observed, reaching undetectable levels after 10 hours of incubation. It is concluded that the PL demonstrated antagonistic activity against *Salmonella* spp. of avian origin, showing potential as an alternative strategy for controlling this pathogen.

Keywords: Probiotic, Food Safety, Antagonism, Poultry Production

Evaluation of Biofilm Production on Stainless Steel Under Different Temperatures and Detection of Biofilm-Associated Genes in *Salmonella Minnesota* Samples Isolated from a Poultry Slaughterhouse (SA-46)

Larissa Justino, Evelin Lurie Sano, Maria Fernanda Marques Pilli, Ana Carolina Bergamo Benteo, Rebecca Zandonai Gil, Rafael Humberto de Carvalho, Lidia Laiana Nogueira de Paula, Alexandre Oba e Ana Angelita Sampaio Baptista

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Abstract:

Salmonella Minnesota (SM) has been frequently isolated from broiler farms in Brazil in recent years and has also been associated with multidrug-resistant (MDR) antimicrobial profiles. In addition, the presence of virulence factors, such as the ability to form biofilms, contributes to resistance against environmental stressors, especially on inert surfaces relevant to the industry, such as stainless steel. This may result in cross-contamination in slaughterhouses and deterioration of poultry products, representing a public health risk. Given this, the present study aimed to evaluate the biofilm-forming ability of MDR SM strains on stainless steel surfaces under different temperatures, as well as to investigate the presence of biofilm-associated virulence genes. The SM isolates were obtained from chicken carcasses (n=9) and water from the cooling equipment (n=5) within the slaughterhouse environment. Biofilm production was assessed on stainless steel discs. The isolates were incubated in BHI broth at 35°C for 24 hours and diluted to reach a concentration of 10⁸CFU/mL. Then, 600 µL were applied in triplicate to 24-well polystyrene plates containing the metal discs and incubated at 35°C, 16°C, and 12°C for 96 hours. Samples were then washed, stained with 0.1% crystal violet, solubilized in glacial acetic acid, transferred to 96-well plates, and measured using a spectrophotometer at 560 nm. The samples were classified as strong, moderate, weak, or non-biofilm producers. Detection of the *adrA*, *csgD*, *agfA*, and *luxS* genes was performed by PCR, and the amplified products were visualized on 1.5% agarose gel stained with GelRed. For statistical analysis, a 3x2 factorial scheme was adopted, with three different temperatures (12°C, 16°C, 35°C) and two origins (carcass and chiller water). Tukey's post-hoc test with 5% significance was applied, and the data were analyzed using the General Linear Models (GLM) software. Regarding biofilm formation, a significant difference was observed ($p<0.05$), with a higher frequency of moderate biofilm production (35.71% – 5/14) at 16°C, which did not differ significantly from 12 °C (21.43% – 3/14). There was no significant difference ($p>0.05$) in biofilm production on stainless steel at different temperatures between the two origins (carcass and chiller water). All SM isolates (100% – 14/14) were positive for the *adrA* and *csgD* genes. A total of 92.85% (13/14) were positive for the *agfA* gene, while only 21.43% (3/14) tested positive for *luxS*. It is concluded that SM samples were capable of producing biofilm on stainless steel and exhibited genes associated with biofilm production.

Keywords: Resistance, Virulence, Public Health, Poultry

***Lactobacillus acidophilus* Mitigates Embryonic Mortality Induced By Avian Pathogenic *Escherichia Coli* (SA-47)**

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Abstract:

Avian Pathogenic *Escherichia coli* (APEC) is recognized as a major pathogen that causes severe diseases in poultry, affecting not only adult birds but also chicken embryos (CE), ultimately leading to substantial economic losses for the poultry industry. In response to increasing restrictions on the use of antibiotics in animal production, the application of probiotics, such as certain strains of *Lactobacillus acidophilus* (LA), has emerged as a promising strategy to promote bird health and mitigate the impact of bacterial pathogens. The objective of the present study was to assess the effectiveness of LA in reducing embryo mortality caused by APEC infection. For this purpose, a total of 75 Cobb line CE were obtained and, at 16 days of embryonic development (DED), were weighed and identified. These embryos were then randomly allocated into three experimental groups, each consisting of 25 CE: (i) the positive control group (PC), which was inoculated at 16 DED via the eggshell membrane (EM) with 103 log CFU per embryo of APEC isolated from chickens; (ii) a treatment group inoculated with APEC in the same manner as the PC and treated with LA at 106 log CFU/CE via amnion; and (iii) the negative control group (NC), which received a 0.85% saline solution at 16 DED via the eggshell membrane and again at 18 DED via amnion. Following inoculation, all eggs were placed in an automatic incubator maintained at a temperature of 36.5°C and a relative humidity of 55%. The embryos were continuously monitored throughout the incubation period until hatching, which occurred at 21 DED. After hatching, the chicks remained inside the incubator until their feathers were completely dry, at which point embryonic mortality was recorded and analyzed. Statistical analysis of the data was performed using the chi-square test, followed by a binomial test comparing two proportions. The results revealed that mortality in the NC was 0% (0 out of 25), while the PC exhibited a mortality rate of 48% (12 out of 25), and the group challenged with APEC and treated with LA showed a rate of 12% (3 out of 25). The statistical comparison demonstrated significant differences between PC and NC, as well as between the PC and the group treated with LA. These findings indicate that the administration of *Lactobacillus acidophilus* was effective in mitigating the deleterious effects of Avian Pathogenic *Escherichia coli* on chicken embryos, thereby contributing to increased hatchability and offering a potential alternative approach to promote poultry health.

Keywords: Poultry Farming, Colibacillosis, Antibiotics, Probiotics

Analysis of the Immunocompetence of Chicks with Different Body Conditions at Birth (SA-48)

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Abstract:

The intensification of poultry production has brought new challenges to maintaining the health and immunocompetence of birds. The increased demand for chicks, pressure for higher productivity from broiler breeders, and various stressors associated with modern poultry production present ongoing challenges to chick uniformity and quality. In this context, the present study aims to evaluate the effects of different hatch weights on the immunological and enteric development of broiler chicks during the first 21 days of life. A total of 200 one-day-old chicks from 40-week-old broiler breeders were used. The chicks were weighed and divided into two distinct groups: standard-light (45–49g) and heavy (56–59g). Birds were kept under standardized management conditions with *ad libitum* access to water and commercial feed. Blood samples, lymphoid organs (spleen, thymus, and bursa of Fabricius), and intestines were collected on days 7, 14, and 21 for immunological, histological, and morphometric analyses. Evaluations included: body weight and feed conversion ratio; immunophenotyping of CD4⁺ and CD8⁺ lymphocytes in blood and spleen by flow cytometry; phagocytic activity and leukocyte oxidative burst; serological response to vaccination against Infectious Bronchitis, Newcastle Disease, and Gumboro Disease (ELISA); and histomorphometry of the intestine and lymphoid organs, assessing diameter, area, and cell density in the thymus and bursa, as well as villus height, crypt depth, and mucosal thickness in the intestine (ImageJ). The results showed that although the light group had lower initial performance (day 7) in terms of body weight, both groups reached similar weights by around day 21, indicating compensatory growth. From an immunological perspective, the light group showed a higher percentage of CD4⁺ and CD8⁺ lymphocytes during the first two weeks, as well as increased phagocytic activity. However, these differences were compensated over time, with both groups showing similar values by day 21. No significant differences were observed in the morphology of the intestine, bursa, or thymus, nor in vaccine titers, indicating that initial weight did not compromise adaptive immune response or organ morphology. It is concluded that initial body weight temporarily influences immunological and zootechnical parameters, but does not have a lasting effect on immunological development, intestinal morphology, or vaccine response. These findings reinforce the importance of nutritional and health monitoring in early stages and highlight the recovery capacity of low birth weight chicks, allowing for more efficient and individualized management adjustments.

Keywords: Broiler, Immunocompetence, Hatch Weighth, Enteric Development, Animal Performance

Early Responses of Lymphocytes and Antigen-Presenting Cells in Chicken Embryos: An Underexplored Approach. (SA-49)

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Abstract:

The chicken embryo (CE) is a valuable model for studying immune responses, as it represents a more cost-effective, environmentally sustainable, and ethically acceptable alternative compared to traditional animal models. Moreover, in the context of poultry science, understanding the immune response in CE may support future strategies for treatment, nutrition, and vaccine development. This study aimed to quantify leucocytes at different developmental stages and to assess their immune response to an infection challenge during early development. Therefore, CE were inoculated with 1 log UFC of *Salmonella Pullorum* (SP) at 9.5 days of embryonic development (DED), and blood samples were collected at 11.5 and 17 DED for flow cytometric analysis of lymphocyte and antigen-presenting cell populations. Although the frequency of CD3⁺ cells decreased with age in infected embryos, this likely reflects increased total cellularity during development rather than a true decrease in T cells. Specific T cell subsets, such as TCRγδ⁺ cells, increased significantly with age, especially following SP infection. TCRγδ⁺ cells are among the first T cells to develop and mature in the embryonic thymus and are involved in early immune responses to bacterial infection. CD4⁺ cells within the CD3⁺ population remained stable across ages and infection status, indicating preservation of helper T cell proportions. In contrast, CD8⁺ cells decreased markedly at 11.5 DED in infected embryos, suggesting that SP infection may reduce cytotoxic T cell representation at this stage. While some studies report increased CD8⁺ cells after *Salmonella* infection, others describe transient lymphocyte suppression and lymphoid organ atrophy early post-infection. *Salmonella* can also negatively regulate CD8⁺ cytotoxic responses to persist within the host. A higher CD8⁺/CD4⁺ ratio at 12 DED may reflect a relative predominance of CD8⁺ cells during early thymic development. The proportion of MHCII⁺ antigen-presenting cells increased with age in both control and infected groups, indicating maturation and expansion of dendritic cells, B cells, or activated monocytes. MRC1⁺ cell frequency decreased with age in controls but increased at 17 DED in infected embryos, suggesting SP infection triggers recruitment or activation of mononuclear phagocytes. Overall reductions in CD3⁺ and BU⁺ cell frequencies during development may be explained by earlier functional maturation of the thymus (10–11 DED) compared to the bursa of Fabricius (16 DED), allowing T cells to mature and circulate earlier. CD3⁺ cells contribute to immediate cellular immune responses and immune surveillance, while BU⁺ cells and antibody production become more relevant near and after hatching. Beyond cell counts, we observed that 48 hours after inoculation.

Keywords: Mhcii, Monocytes, Experimental Model



Early Incubation Temperature Profiles and their Impact on Mineral Utilization From Egg Components and Broiler Bone Development (OA-08 - Winner)

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Abstract:

Temperature control throughout the incubation (INC) is critical to ensure optimal utilization of egg components, bone mineralization (BM), and proper skeletal development. While the effects of physical factors during the mid- and late-incubation have been extensively studied, the initial stage remains underexplored. This trial investigated the influence of gradually applied thermal profiles during the first 8 days of embryogenesis on mineral utilization from the eggshell and yolk, as well as on mineral deposition, morphometric, biophysical and mechanical properties of the tibia (TB) in embryos and day-old chicks. A total of 2,408 eggs from 45-week-old Cobb 500® breeders were allocated to four incubators (P. Ecológica IP600®). From day 0 to day 8 of INC, treatments consisted of increasing or decreasing eggshell temperatures (EST): TCO-L (low initial temperature – 98 °F), TCO-H (high initial temperature – 103 °F), TCO-VH (very high initial temperature – 104 °F), and TCO-C (control – 100 °F). From day 9 onward, all incubators were set at 100 °F. Calcium (Ca), phosphorus (P), manganese (Mn), and magnesium (Mg) concentrations were analyzed via inductively coupled plasma optical emission spectrometry (ICP-OES), following the methodology of Yair & Uni (2011) for yolks and eggshells (at 0d, 8d, 18.5d, and hatch), and Yair *et al.* (2012) for TB (at 18.5d and hatch). TB were weighed and subjected to fracture force testing according to Shim *et al.* (2012), using a three-point bending test (EMIC®, Instron, DL 3000) and BlueHill® software. Ash content was determined as described by Yair *et al.* (2012). Bone calcification was assessed through differential staining using Alcian Blue and Alizarin Red (Blom & Lilja, 2004); and image analysis by ImageJ®. Hatchability of fertile eggs (HF) were also recorded. Data were analyzed following a completely randomized design, with means compared by Tukey's test at $p \leq 0.05$ (SAS® 9.2). Higher ESTs (TCO-H and TCO-VH) increased the retention of Ca and Mg in the eggshell, and P and Mn in the yolk ($p \leq 0.05$). TB mineral composition analysis revealed a decrease in mineral content with increasing EST, with TCO-L showing the highest levels of BM ($p \leq 0.05$). Moreover, TCO-H and TCO-VH led to underdeveloped TB, reduced fracture resistance and HF, whereas TCO-L produced results that were statistically similar or superior to the control in terms of morphometric, biophysical and mechanical TB characteristics. In conclusion, elevated initial ESTs (above the standard) are not recommended. Conversely, a lower initial EST (98 °F) gradually increased until day 8 of incubation may represent a viable strategy to optimize mineral mobilization and deposition in bone, improve skeletal development and potentially prevent locomotor disorders during rearing.

Keywords: Eggshell Temperature, Embryonic Development, Incubation Management, Mineral Mobilization, Tibia Quality



Semen Quality of Rhode Island Red Roosters From 53 to 56 Weeks of Age Fed Black Wattle Tannin: Evaluation of Physical and Morphological Parameters (OA-07 - Honorable Mention)

Amanda de Moura Dani¹, Julia Schneider¹, Marcela Ziegler Arruda¹, Manuela Castilho Matsdolf de Mello¹, Cassiane Turchetto Ditadi¹, Vitor Silva¹, Alexandre Pires Rosa¹, Bruna Poletti² e Alcides Oliver Sencio Paes²

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HONORABLE MENTION



OTHER AREAS

Abstract:

The search for natural alternatives to antibiotic growth promoters has driven research on the use of phytogetic additives in poultry nutrition, especially in breeders, where semen quality is critical for flock productivity. In this context, the present study aimed to evaluate the effects of dietary supplementation with Tanfeed (TAN), a product based on condensed tannins from black wattle (*Acacia mearnsii*), on the semen quality of Rhode Island Red roosters between 53 and 56 weeks of age.

Four treatments were evaluated: T1 – basal diet (control); T2 – basal diet + 50ppm of Zinc Bacitracin; T3 – basal diet + 750g of TAN/ton of feed; and T4 – basal diet + 1000g of TAN/ton of feed. A completely randomized design was used, with four treatments and ten replicates per treatment, each replicate consisting of one rooster. The parameters analyzed were sperm vigor, sperm motility, semen volume, and percentage of abnormal spermatozoa (PAS). Data were subjected to analysis of variance (ANOVA), and when significant (5%), means were compared using Tukey's test. Results showed that treatments with TAN (T3 and T4) resulted in significant increases in semen volume (0.99 mL and 1.05 mL, respectively), higher than T2 (0.80 mL) ($p < 0.0001$). The highest PAS was observed in the Zinc Bacitracin group (5.72%), whereas T3 and T4 showed significantly lower abnormality rates (3.14% and 3.32%, respectively). These were also lower than the control group (4.12%) ($p < 0.0001$). This reduction may be attributed to the antioxidant effects of Acacia tannins, which can neutralize reactive oxygen species (ROS) in the testicular environment, protecting the structural integrity of sperm and mitigating oxidative damage that leads to cellular abnormalities. No significant differences were found between treatments for sperm vigor ($p = 0.7599$) or motility ($p = 0.8442$), with overall means of 4.26 and 80.4%, respectively. In conclusion, dietary inclusion of Tanfeed improved seminal quality in breeder roosters by increasing ejaculate volume and reducing the percentage of abnormal spermatozoa. Zinc Bacitracin did not improve semen quality and resulted in the highest rate of sperm abnormalities.

Keywords: Reproductive Performance, Sperm Morphology, Oxidative Stress, Phytogetic Additives

Artificial Incubation As A Tool to Strengthen Brazilian Family Poultry Farming (OA-01)

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Abstract:

Family farming accounts for 67% of rural employment and 23% of the gross value of national agricultural production in Brazil (IBGE, 2024). Despite its economic and social importance, many small-scale farmers face challenges in accessing technologies that could enhance their productivity, particularly in free-range poultry farming. Among such technologies, artificial egg incubation stands out as a viable alternative to natural incubation. This method offers greater control over environmental conditions, improves hatchability rates, and reduces sanitary risks. This study was funded by FAPEMIG through Call APQ-04190-22 (Research with Extension Interface) and aimed to characterize family poultry farmers from four regions of Minas Gerais and assess their knowledge of artificial incubation. A total of 87 producers from the municipalities of Bambuí, Igarapé, Ituiutaba, and Lavras were interviewed using structured and semi-structured questionnaires. Descriptive statistics were used to present the results. Most respondents were male (55%), aged 50 or older (64%), married (71%), and had not completed primary education (36%). The predominant monthly income ranged between one and two minimum wages (40%), and 30% reported agriculture as their main source of income, supplemented by retirement benefits. Chicken farming was identified as the main livestock activity on 61% of these small properties. Regarding incubation practices, although 74.7% of producers reported being familiar with the term “artificial incubation”, only 48.3% had ever used incubators, and just 13.8% used them regularly. Natural incubation remained the most common method (49.4%), followed by the purchase of one-day-old chicks (25.3%). Only 39.1% of farmers sanitized the eggs before incubation. Despite these limitations, 71.3% considered incubators the best option, mainly due to their capacity to incubate a larger number of eggs. The findings reveal a profile of farmers with low educational attainment, limited financial resources, and small-scale production, yet open to adopting new technologies. Artificial incubation shows strong potential as a tool to strengthen family poultry farming, as producers believe it can improve chick production. Therefore, implementing educational and technical support initiatives is essential to promote the adoption of artificial incubation and enhance the sustainability of family-based poultry systems.

Keywords: Family Farming, Free-Range Chicken, Natural Incubation

Application of Bioimpedance and Machine Learning in the Estimation of Poultry Body Composition (OA-02)

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Abstract:

The standard method for determining poultry body composition is comparative slaughter. Despite its accuracy, this technique is labor-intensive, costly, and incompatible with longitudinal monitoring of individual animals. Although nutritional programs aim to standardize body weight, appropriate morphological conformation at the onset of photostimulation is more strongly associated with reproductive performance than mere attainment of weight targets. In this context, non-lethal techniques such as bioelectrical impedance analysis (BIA) present advantages, as they are non-invasive, portable, low-cost, and capable of estimating body composition based on electrical conductivity of body fluids and the resistive properties of adipose tissue. Machine learning (ML) encompasses computational methodologies that enable systems to learn from data and make predictions or decisions without being explicitly programmed. Among ML models, the Multi-layer Perceptron (MLP) neural network is particularly suitable for modeling non-linear relationships between input and output variables. The objective of this study was to evaluate the feasibility of using BIA to predict body composition parameters in poultry, employing MLP neural networks for data analysis. The experiment was conducted at the Experimental Farm of the Federal University of Minas Gerais (UFMG), using 640 commercial laying hens allocated in a completely randomized design within a cross-sectional study framework. Birds were subjected to two dietary energy levels (2780 and 3080 kcal/kg). At 10, 15, 18, 22, 26, and 30 weeks of age, one bird per replicate was randomly selected for BIA measurements and chemical composition analyses. BIA assessments were conducted using the Quantum II device (RJL Systems), which provided measurements of reactance (X_c) and resistance (R_e). Impedance parameters, combined with morphometric measurements, were used to estimate body tissue composition. Data from the 2780 kcal/kg treatment group were used to train the MLP model, while data from the 3080 kcal/kg group were used for model validation. The model yielded coefficients of determination (R^2) of 0.90 for carcass protein, 0.76 for organ protein, 0.77 for carcass fat, and 0.59 for abdominal fat (expressed in grams). The results demonstrate the potential of combining BIA with MLP-based machine learning for non-destructive prediction of poultry body composition. This approach may support the development of integrated systems capable of real-time analysis of impedance data (X_c and R_e), contributing to precision management and welfare-friendly phenotyping in poultry production systems.

Keywords: Multi-Layer Perceptron, Predictive Modeling, Laying Hens, Electrical Resistance, Energy Levels

Nanotechnology in Broiler Flooring: Connecting Performance, Health, Welfare and Carcass Quality (OA-03)

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Abstract:

This study investigated the effects of two types of plastic flooring—one with and one without nanotechnological antimicrobial additives, used as complete or partial replacements for traditional wood shavings on broiler chickens throughout their 42-day rearing period. We evaluated zootechnical performance, yield, meat quality, and litter microbiology in 1500 male Ross 408® broiler chicks, randomly assigned to five treatment groups: wood shavings (control), plastic flooring (PF), a 50/50 mix of plastic flooring and wood shavings (PF + WS), plastic flooring with antimicrobial additives (PFA), and a 50/50 mix of PFA and wood shavings (PFA + WS). Analyses included organ biometrics (liver, heart, spleen, and gizzard), severity of *Eimeria* lesions, microbiological profiles, performance indices, and meat quality. The results revealed that the use of plastic flooring, especially when exclusive, presented significant challenges. An increase in intestinal lesions related to coccidiosis and a higher prevalence of *Salmonella* were observed in birds raised on these floors. Additionally, broilers on plastic flooring exhibited worse feed conversion, lower body weights, and inferior carcass yields compared to those on wood shavings. Meat quality was also negatively affected, with plastic flooring resulting in less favorable fillet characteristics. In summary, the implementation of plastic flooring under the conditions of this study compromised broiler chicken performance and health parameters.

Keywords: Antimicrobial, Carcass Yield, Feed Conversion, *Salmonella*, Wood Bedding

Expression Units For Pain and Fear Detection in Pre-Slaughter Beef Cattle (OA-04)

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Abstract:

The objective of this study was to identify and catalog the parameters of facial action units (FAUs) that characterize the expression of pain and fear in beef cattle at the pre-slaughter stage. Pre-slaughter images of beef cattle were acquired in the holding pens at a regional slaughterhouse during 3 days, where animals are kept before slaughter. A total of 139 animals were subsequently evaluated and categorized based on pre-slaughter visual assessments. During this period, the animals had access to water, misting systems, and ventilation while awaiting slaughter. Microexpressions were then cataloged based on the criteria of FAUs, a system that categorizes expressions by deconstructing them into discrete, individual muscle actions in the head region. Subsequently, an ethogram was constructed based on these FAUs to detect patterns indicative of pain and fear at the pre-slaughter stage, including actions in the head region, each accompanied by a detailed description and its corresponding cataloged FAU image. The direct consisted of categorization of the described FAUs, with coding parameters ranging from visible presence and static state to duration in seconds. Head Region: Orbital: a) Partially closed eye: Eyeball not fully visible; the eyelid is visible, partially closed, and not covering the entire eyeball. b) Open eye (partial sclera): Eyeball is fully visible; the eyelid is visible and not drooping. c) Open eye (full sclera): Eyeball is fully visible; the eyelid is visible and not drooping. Tearing is associated with the onset of extreme fear. d) Closed eye: Eyeball is not visible; the eyelid is fully visible and covers the entire eyeball. e) Eye closure (combination): The eyeball is either partially or not visible; the eyelid is partially visible and semi-closed. Tearing. f) Wide open eye: The eyeball is visible; eyelids are retracted, almost invisible, while most of the sclera is exposed. Extreme fear. g) Brow striations: Vertical furrows on the medial part of the brow ridge. h) Brow bulging: Bulging of the brow ridge. Tension above the eye is noted, with the inner brow raised (initial tension) and the outer brow raised (final tension). Head Region: Muzzle and Mouth: a) Immobile muzzle: The muzzle is held still. It is moist, with or without mucous discharge. The mouth is immobile. b) Nostril dilation: The nostrils are dilated in the static state. This research constitutes a cornerstone in applied ethology, operationalizing our understanding of bovine affective states into a systematic and robust assessment framework. By establishing a validated ethogram for FAUs, this study moves beyond subjective observation to provide an objective, non-invasive tool with profound implications for the entire beef production chain.

Keywords: Facial Expression, Behavioral Indicators, Welfare

Decision Support System For Broiler Density Determination: A Fuzzy Modeling Approach (OA-05)

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Abstract:

Determining the ideal stocking density to ensure a thermal environment comfort for different types of broiler chicken house is a major challenge for modern poultry farming due to extreme heat events. Fuzzy decision systems, a type of artificial intelligence, provide an effective way to optimize decisions in challenging environmental conditions. The aim of this study was to develop a fuzzy inference model to predict broiler chicken density while prioritizing the birds' thermal comfort previously. The model was constructed and validated based on two indices: the Relative Air Temperature Reduction Potential of Climate Control Systems (PRT), which considers the constructive typology of the poultry houses and their thermal control capacity, across six levels, ranging from PRT 1 (poor), representing a house with low temperature reduction capacity, to PRT 6 (ideal), a house with high thermal reduction capacity; and the Temperature and Humidity Index (THI), defined in four risk scenarios: comfort, alert, danger, and emergency, based on the progressive increase in ambient temperature, as described by De-Souza *et al.* (2003) and Tao & Xin (2003). The model was built using FisPro® 3.9 software as a multiple-input, single-output (MISO) system. The PRT and THI indices were defined as input variables, with density defined as the output variable. Fuzzy sets were defined using triangular membership functions in three intervals (minimum, medium and maximum) and inference was performed using the Mamdani method with 24 association rules. To validate and evaluate the model, the density data resulting from the fuzzy inference were categorized and compared with 12 scientific articles from which data on density, air temperature, relative air humidity and facility descriptions were extracted (n=67). The model's performance was evaluated using a confusion matrix in RStudio, with the following metrics calculated: precision (78%), sensitivity (76%), and F-score (77%). These results indicate good predictive performance. The estimated mean density was found to be 20.6% lower than that reported in the literature, with a median difference of 39.4%. The variation was ± 17.2 percentage points and the correlation between the data was weak and negative (-0.17). This does not represent model failure, but rather a proposal to avoid conventional practices and suggest conservative adjustments focused on thermal comfort. Although reducing density is recommended in extreme heat conditions, there are few tools to support this decision. The developed fuzzy model can be a decision support system for density determination, especially when integrated with meteorological indices and predictions, aiming to minimize productive losses associated with heat stress.

Keywords: Animal Welfare, Environmental Management, Poultry Housing, Stocking Density, Thermal Comfort



Influence of Inoculation Route on the Toxicity and Pharmacokinetics of Commercial and Nanoformulated Drugs in the Chicken Embryo Model (OA-06)

Fabiana Oliveira Notário, Simone Sommerfeld, Gustavo Martins Felix Silva, Tiara da Costa Silva, Arlene Bispo dos Santos Nossol, Mario Machado Martins, Isabelle Ezequiel Pedrosa, Ana Clara Feitosa Silva, Disney Oliver Sivieri Júnior e Belchiolina Beatriz F

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Abstract:

The chicken embryo has proven to be a practical and valuable model for preclinical pharmacokinetic and toxicity studies due to its ease of handling. However, there is a lack of standardized protocols considering the route of administration. This study investigated whether different inoculation routes affect the toxicity of commercial and nanoformulated drugs (norfloxacin, indomethacin, and lidocaine) and influence the pharmacokinetics of commercial and nanoformulated norfloxacin in serum and allantoic fluid. For the toxicity assessment, embryos at 10 days of development received five concentrations of each formulation via four routes: shell membrane, allantois, chorioallantoic membrane, and amnion. At 16 days, mortality, body weight, and histological alterations in liver and kidney were evaluated. In parallel, pharmacokinetic parameters were assessed after a single dose of norfloxacin (commercial and nanoformulated), with biological samples collected at 30 min, 1 h, 4 h, and 24 h post-inoculation. No LD₅₀ was detected for any compound. Norfloxacin did not affect embryo weight. Commercial lidocaine increased weight, while its nanoformulation did not. Indomethacin reduced weight significantly in both forms. Histopathological alterations occurred only in embryos treated with nanoformulated drugs via the amnion. Norfloxacin caused kidney inflammation and liver degeneration; lidocaine led to kidney damage; indomethacin caused liver lesions. Only the commercial formulation yielded observable pharmacokinetic parameters. Norfloxacin reached maximum serum concentration (C_{max}) at 1 h post-inoculation for all routes, except the amnion, where C_{max} occurred at 24 h. Drug detection in allantoic fluid occurred exclusively in embryos inoculated via the amnion. Results show that the inoculation route alters pharmacokinetic behavior and toxicity. The amniotic route displayed a typical absorption curve with slower systemic distribution and increased toxicity, suggesting possible biotransformation. Other routes led to rapid absorption and elimination but did not allow drug detection in the allantois. Therefore, the chicken embryo is a viable model for pharmacokinetic studies, but findings depend on the route of administration, particularly when using nanoformulated drugs.

Keywords: Drug Delivery, Embryo Model, Histopathology, Nanoformulation, Pharmacokinetics

Effects of Thermal Stress on Broiler Litter Quality: Internal Temperature, Moisture, Ph, and Ammonia Dynamics (OA-09)

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Daniella Moura¹ e Nilsa Duarte da Silva Lima³

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Abstract:

This study investigated how thermal stress affects broiler litter's physical and chemical properties, specifically internal temperature, moisture, pH, and ammonia (NH₃) concentration. The trial took place in an environmental chamber divided into three compartments (1.6x1.4x3.0 m), each housing 25 birds under neutral, cold, or hot conditions. Treatments were randomly assigned at 14 and 21 days for Experiment I, and at 35 and 39 days for Experiment II. In Experiment I, we collected data at 7, 14, 21, and 28 days to analyze early-stage thermal stress impacts on 7- to 28-day-old broilers. For each compartment, we selected five distinct sampling points. At each point, litter internal temperature was measured for three minutes using mercury thermometers, followed by the collection of a litter substrate sample. All samples were taken when compartments were empty, immediately after birds were removed for weighing and processing, and stored in labeled, sealed hermetic bags. Each compartment's litter sample was split into six 10g subsamples for pH and moisture analysis. For pH, three subsamples per compartment were dissolved in 100 mL of distilled water, stirred to dissolve lumps, and rested for 30 minutes before measurement with a digital pH meter. Experiment I results showed no detectable ammonia. However, we found significant interactions between treatment and age for litter moisture and pH ($p < 0.05$). Litter internal temperature consistently decreased over time, especially from the second week onward ($p < 0.05$). Moisture was highest in heat-stressed environments during week four (11.1%) and lowest in the first week under heat stress (4.6%). Litter pH increased with age across all environments, peaking at 6.9 in neutral conditions during week four. Experiment II assessed late-stage effects on older broilers (35 to 39 days) exposed only to hot stress. Here, ammonia concentrations significantly increased post-stress ($p < 0.05$), peaking at 6.7 ppm under neutral conditions but lowest (2.6 ppm) in moderate heat. Internal litter temperature increased with age but showed no significant treatment differences. Moisture content in week six was highest in moderate heat (10.3%) and lowest in neutral conditions (9.3%). Litter pH also rose with age, reaching a maximum of 8.8 in the neutral environment by week six. These findings clearly indicate that both age and environmental temperature profoundly influence litter quality. While early-stage ammonia volatilization was undetectable, it significantly increased after thermal stress in later growth stages. This highlights the critical importance of environmental control in broiler housing to maintain optimal litter conditions and minimize ammonia emissions.

Keywords: Bedding Quality, Ammonia Volatilization, Environmental Control

Influence of Elife® Supplementation on Sperm Morphology in Rhode Island Red Roosters From 67 to 70 Weeks of Age (OA-10)

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Abstract:

Semen quality in poultry breeders is a critical factor for reproductive success, directly affecting fertility and hatchability rates. This quality can be influenced by nutrition, age, and oxidative stress. In this context, dietary supplementation with antioxidant compounds has emerged as a promising strategy to preserve sperm integrity by effects of reactive oxygen species (ROS). This study aimed to evaluate the impact of Elife®—a commercial antioxidant based on grape seed extract—on sperm quality parameters in Rhode Island Red roosters. Thirty roosters were distributed in a completely randomized design, with three treatments and ten replicates (one rooster per replicate). The treatments were: T1 – control diet; T2 – diet supplemented with 0.5 kg of Elife®/ton of feed; and T3 – diet supplemented with 1 kg of Elife®/ton of feed. From 67 to 70 weeks of age, semen samples were collected and analyzed for the following parameters: sperm vigor, sperm motility (%), ejaculate volume (mL), and percentage of abnormal sperm cells (TAS, %). Data were subjected to analysis of variance (ANOVA), and when significant ($p < 0.05$), means were compared using Tukey's test. Elife® supplementation positively influenced sperm motility ($p = 0.0118$), with T3 (96.40%) showing superior results compared to T1 (85.30%). Ejaculate volume was also significantly higher in T2 (0.63mL) compared to T1 and T3 (0.40mL and 0.41mL, respectively) ($p = 0.0369$). The antioxidant reduced the percentage of abnormal sperm cells ($p = 0.0016$), with T3 showing 3.70% and T1 12.50%. These results suggest that the evaluated antioxidant contributed to maintaining sperm cell integrity, likely by neutralizing the harmful effects of oxidative stress. No significant differences were observed for sperm vigor ($p = 0.5818$). It is concluded that supplementing Rhode Island Red roosters from 67 to 70 weeks of age with 1 kg of Elife®/ton of feed improved sperm motility and reduced abnormal sperm rates. Therefore, the product may represent an effective nutritional strategy to enhance semen quality and, consequently, reproductive performance of males.

Keywords: Sperm Integrity, Semen Quality, Dietary Antioxidant, Reproductive Performance

Feeding Mechanics in Motion: How Feed Texture Influences Beak Kinematics in Broiler Chickens (OA-11)

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Abstract:

Understanding how feed structure interacts with broiler development is crucial for optimizing both performance and welfare. This study explored the biomechanical demands of feed ingestion across three growth stages (starter, grower, finisher) and three feed types (fine mash, coarse mash, pellets) in broiler chickens using high-speed kinematic video analysis. Broiler chickens (n=9) for each growth phase were placed individually in a mobile studio: initial (3-4 days old), growth (20-21 days old), and final (34-36 days old). Kinematic measurements of the beak were taken across three feed forms: fine mash, coarse mash, and pellets. For the initial phase, we used a crumbled pellet. Each bird was individually placed in a glass-made box, where different feeds were offered separately and then removed after an 8-second video was captured. After the video footage, their beak was measured using a digital caliper. The video calibration consisted of taking a picture with a ruler located at the feeder. Each video frame was equivalent to 0.0033 seconds (3.3 milliseconds). The assessment of each movement was done by observing the video frame by frame. We started counting at the frame where the movement began and finished when the movement ended. Key mandibular metrics, including beak gape and movement duration, revealed that feed texture significantly modulates oral processing, especially in older birds. Pearson correlation coefficients were calculated to explore potential compensatory relationships among morphometric and biomechanical traits. These correlations were beneficial for identifying mechanical trade-offs, such as gape width vs mandibular movement time. ANOVA was applied to examine the effect of the variables (η^2), which represents the proportion of variance in a dependent variable that is associated with one or more predictors in a statistical model. Pellets consistently reduced mandibular effort, whereas fine mash increased both movement time and head displacement, suggesting higher biomechanical load. Particularly, interactions between bird age and feed form were statistically significant, with effects increasing across developmental stages. These findings offer novel insights into how matching feed physicality to ontogenetic capacity can enhance feeding efficiency while supporting natural behaviors. This mechanistic understanding lays a foundation for precision nutrition strategies that consider both physiological development and animal welfare.

Keywords: Broiler Biomechanics, Beak Kinematics, Welfare-Optimized Feeding

Influence of Blood Collection Sites and Methodologies For Glucose Determination in Hens: Systematic Review (OA-12)

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Abstract:

Glucose is the main source of energy for birds and is essential for metabolic and physiological activities. Observed changes in its concentration are often correlated with nutritional problems and physiological indicators of stress in animals. The study was carried out through a systematic literature review with the aim of identifying whether there are differences in serum glucose concentration among different blood collection points and the methodology applied for its measurement. The search for articles was performed in the ScienceDirect and Scielo databases in June 2025. The search criteria adopted were articles published in English, from 2012 to 2025, with the following search terms and boolean operators (Laying hens AND glucose OR broiler breeder), obtaining a total of 4492 articles in this search, of which 193 studies were selected by title and abstract and imported into Mendeley for filtering. The inclusion criteria were the selection of articles with laying hens or breeders, with data on age, glucose concentration, blood collection points, analysis methods, and sample processing equipment. Exclusion criteria were duplicate articles, articles without glucose concentration data, other species, broilers, with the presence of contamination by microorganisms or pathogens, presence of additive or supplement inclusions. After the inclusion and exclusion criteria, 8 articles were selected to compose the database. Data extracted from the studies (n=47) were organized in a standardized electronic spreadsheet and filtered to include information on collection point, analytical method, equipment used, age of birds, and glucose concentration. Statistical analyses were performed in RStudio. ANOVA results indicated that the blood collection point exerted a significant influence on glycemia levels. The F-statistic value was 27.1, with degrees of freedom of 3 and 39, and a p -value <0.001 , with samples collected from the wing vein showing the lowest concentration (157.8 ± 17.8 mg/dL; $n=8$), which was significantly lower than those obtained from the brachial vein (241.4 ± 1.9 mg/dL; $n=4$; $p<0.001$), jugular veins (210.3 mg/dL; $n=2$; $p<0.001$) and medial metatarsal vein (222.6 ± 15.9 mg/dL; $n=32$; $p<0.001$). There were no significant differences among the other collection points (brachial, jugular, and medial metatarsal). There were no differences in glucose concentration among the analysis method, equipment, and age of the birds. The results demonstrate that the blood sampling site is the main determinant of variations in serum glucose levels in birds, while laboratory methodology, equipment employed, and animal age did not significantly alter the results of avian glycemia.

Keywords: Glucose, Physiological Indicators, Analytical Methods, Serum Biochemical Profile, Analytical Variability



Effects of the Interaction Between Probiotics and Environmental Enrichment on Anxiety in Broiler Chickens (OA-13)

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Abstract:

Anxiety in chickens is a more complex type of fear, characterized by birds with higher reactivity to human presence, restlessness, and vocalizations. Probiotics and environmental enrichments are promising tools to mitigate the effects of this negative affective state. This study aimed to infer anxiety levels in broiler chickens submitted to a combination of probiotics and environmental complexity (enrichment). 1,440 one-day-old male Ross TM4® chicks were used. The experimental design was completely randomized in a 3×2 factorial. Factors used in this study were: additives (negative control vs antibiotic vs probiotic) and environmental complexity (low- vs high-complexity). Each factor combination had 8 replicates of 30 birds. In the low-complexity treatments, birds were raised in an environment similar to commercial settings, and in the high-complexity treatments, birds had access to permanent and temporary enrichments. At 40 and 41 days of age, 6 birds per replicate were arbitrarily selected and submitted to an attention bias test (ABT) to quantify anxiety levels through behavioral responses to a positive and a negative stimuli (n=16 groups of 3 birds/treatment). Birds were tested in groups of three in a test arena contained pine shavings, one feeder with feed and mealworms (positive stimulus), and one bluetooth speaker to play a ground predator conspecific alarm call for 8s (negative stimulus). The behaviors measured were latency to begin feeding(s), latency to resume feeding after the 2nd alarm call(s), latency to first step(s), and latency to first vocalization(s). Data were analyzed in SAS using GLIMMIX followed by Tukey's multiple comparison test ($p < 0.05$). Additives and environmental complexity were used as predictors and replicate and bird ID as random effects. An interaction was found for latency to begin feeding ($F_{2,281}=3.80$, $p=0.024$), with birds in the antibiotic and low complexity treatment showing higher latencies ($238 \pm 31s$) compared to birds in the antibiotic and high complexity treatment ($139 \pm 18s$, $p=0.028$). No differences were found among the other factors ($p > 0.119$). Latency to resume feeding was affected by environmental complexity ($F_{1,74}=5.49$, $p=0.022$). Birds in low-complexity environments resumed feeding later ($25 \pm 5s$) than those in high-complexity environments ($11 \pm 4s$). An interaction was found for latency to first step ($F_{2,168}=3.19$, $p=0.043$). Birds in the antibiotic and low-complexity treatment vocalized faster ($26 \pm 2s$) than those in the control and low-complexity treatment ($40 \pm 4s$, $p=0.023$). No differences were found among the other factors ($p > 0.182$). A tendency was observed for latency to first vocalization for the environmental complexity treatments ($F_{1,39}=3.11$, $p=0.085$). Birds in low-complexity vocalized earlier ($19 \pm 2s$) than those in high-complexity ($23 \pm 2s$). The use of probiotics did not affected the affective states of broiler chickens. The study results suggest that providing a more complex environment in poultry houses through environmental enrichment reduces anxiety levels and bird reactivity to human presence, favoring human-animal relation and potentially reducing economic losses in the poultry industry.

Keywords: Poultry, Animal Welfare, Human-Animal Relation, Affective States

Field Conditions and Its Relationship With Carcass Condemnations in Broilers: A Mixed Data Approach (OA-14)

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Abstract:

The application of new technologies in poultry farming has led to increased domestic production, positioning Brazil prominently in the global market. However, the adaptation of these technologies during the incorporation process may present challenges in the field due to structural and climatic conditions, consequently resulting in higher rates of carcass condemnations. Therefore, understanding the profile of carcass condemnations is crucial for informed decision-making in the field and slaughterhouses operations. This study aims to establish the relationship between field qualitative factors such as sex, hatchery of origin, type of poultry house acclimatization, and lineage, and the condemnations causes of broiler carcasses using factorial analysis of mixed data. The study analyzed 445 batches of griller chickens slaughtered between January 2021 and August 2022 in the state of Mato Grosso do Sul – Brazil. The data related to condemnation were obtained from the evaluation carried out by the SIF (Federal Inspection System), while the structural data were provided by the integrator. Thus, these pieces of information were cross-referenced, and the comparison was made based on the number of carcasses analyzed. The technological level of the evaluated poultry houses emerged as the primary factor explaining the diversity in condemnations, negative pressure poultry houses demonstrating higher rates of carcass condemnations. Batches of male chickens and single origins hatcheries batches exhibited the lowest rates of condemnation during the slaughter process. The Ross95 strain exhibited the least impact on carcass condemnations. Therefore, in the context of this study, housing batches of Ross95 males with a single origin from a hatchery in a positive pressure aviary presents the most favorable scenario for reducing carcass condemnations.

Keywords: Chicken Slaughtering, Clusters, Dark Houses, Principal Components, Rearing Conditions

Development of a Collective Nest for the Recording of Laying Hens Behavioral Data (OA-16)

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Abstract:

Artificial intelligence (AI) has revolutionized poultry farming by enabling the measurement of bird behavior and preferences. However, development in this field requires labeling of behavioral events to improve equipment functionality. Precision livestock farming integrates automation technologies with continuous data collection of sounds and images, using microcontrollers connected to sensors to monitor animal interactions with equipment and allow automatic labeling of images and sounds associated with behaviors for the development of automation tools. The objective of this study was to develop and calibrate a nest with automated data collection for application in behavioral studies of laying hens. A collective-access nest prototype was developed, consisting of three compartments: a slatted perch area (120 cm long x 40 cm wide x 45 cm high); a collective nest (120 cm x 45 cm x 45 cm) with a red curtain and Astroturf® synthetic mat; and an egg collection grate divided into three sections at the back of the nest. The slatted area and nest interior were equipped with Arduino Uno microcontrollers with a datalogger module containing a clock and SD card for data storage. Each compartment included two 50 kg load cells and an HX711 module (24-bit analog-to-digital converter). The egg collection grate had the same processor and datalogger configuration, but with three independent 5 kg load cells, each with its own HX711 converter, capable of recording weight with a ± 2 g tolerance. During calibration, the sensitivity of the load cells was adjusted according to the position of simulated weights. Calibrations of the entrance slatted area and the interior of the nest were analyzed, identifying weight thresholds at the edges to record bird presence and absence. The egg grate was calibrated for precise weight recording, associated with timestamps logged every second in a spreadsheet. A total of 2,906 events were recorded, with 29.4% simulating hens moving from the slatted area to the nest, and 28.9% indicating the reverse, with no registration failures. This pattern simulates the hen entering the nest via the slatted area and moving to and from the egg-laying zone. In 41.7% of readings, the absence of weights was correctly detected by all sensors, with no data recorded, as expected. The methodological standardization demonstrated potential to measure behaviors related to egg-laying and temporal preference for nest access and use. The clock module enabled precise date, hour, and minute recording, allowing synchronization with sound and image capture for AI training.

Keywords: Artificial Intelligence, Microcontrollers, Poultry Farming, Precision Livestock Farming, Technology

Development and Validation of a Nipple Drinker For Poultry to Monitor Water Consumption (OA-17)

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Abstract:

Poultry water consumption contains important information, but recording it is difficult because low individual consumption cannot be recorded by conventional meters. The objective was to develop and validate an automated prototype drinker for recording individual poultry water consumption. The system consisted of an Arduino Uno processor board, a datalogger module with a clock and SD card for data recording, and two sets of HX-711 digital load cell conversion modules (20kg capacity), with an 8-liter PVC compartment (10 cm diameter x 110 cm height) for storing water, coupled with a nipple-type drinker with tray. The weight variation recorded by the datalogger was considered the record of water consumption per bird. The initial calibration of the load cells was performed with increasing volumes of water (total capacity of 8L per unit), measuring the weight indicated on the serial monitor of the processor interface and correlating it with the volume actually added. The experimental validation was divided into four stages and performed in triplicate: (1) Determination of the total capacity of the drinkers by successive additions of 2 L until the maximum volume was reached; (2) Measurement of the flow rate, performing controlled withdrawals of 50 mL under three different configurations: simultaneous, individual, and alternating, totaling 50 repetitions per configuration and timing the withdrawal time with a digital stopwatch and the processor interface; (3) Reading stability test (oscillation), consisting of sequential withdrawal of 50 mL from a single drinking fountain, alternating between the two collection points and 30 repetitions; (4) Cross-interference assessment, in which 30 withdrawals of 50 mL were performed in a single drinking fountain while monitoring the stability of the adjacent sensor, checking for possible reading variations. The data were recorded digitally in spreadsheets. Statistical analyses were conducted by simple linear regression (volume x weight), with the aid of Excel® and validated with R® version 4.3. The system showed high accuracy (mean error <2%), with a strong correlation between volume and recorded weight ($r > 0.99$). Flow measurement showed a maximum variation of $\pm 3\%$ in standardized 50 mL withdrawals. Stability tests showed high repeatability, with variations within the $\pm 5\%$ margin. Interference assessments between the two load cells indicated deviations of less than 0.5%, validating their operational independence. The prototype demonstrated high accuracy, stability, and reliability under controlled laboratory conditions, showing potential application in poultry experimentation as a tool for monitoring individual water consumption.

Keywords: Automation, Precision Poultry Farming, Smart Drinker, Dynamic Monitoring, Sensors

Physiological Status of Newly Hatched Chicks Subjected to Different Eggshell Temperature Profiles During the Early Incubation Phase (OA-18)

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Abstract:

Disruptions in homeostasis can negatively affect chick performance during rearing. The aim of this study was to investigate whether the physiological parameters of newly hatched chicks are influenced by thermal modulations in the early incubation (INC) phase. 2408 eggs (63-66g) from 45-week-old Cobb 500® breeders were distributed into 4 incubators (P.Ecológica IP600®). Between days 0 and 8 of INC, eggs were exposed to increasing or decreasing eggshell temperature (EST) profiles: TCO-L (low initial EST-98 °F); TCO-C (standard EST-100 °F); TCO-H (high initial EST-103 °F); TCO-VH (very high initial EST-104 °F). From day 9 onwards, all incubators were set at 100 °F. At hatch, after measuring the chicks rectal temperature (RT), blood samples were collected from the jugular vein. Hormonal analyses were performed using commercial ELISA kits to measure Triiodothyronine (T3), Thyroxine (T4), Vitamin D3 (D3), Parathyroid hormone (PTH), and Calcitonin (CALC). Hemogasometry was conducted by i-STAT®, and the following parameters were analyzed: pH, partial pressure of CO₂ and O₂, bicarbonate (HCO₃⁻), O₂ saturation, ionized calcium (iCa), Na⁺, K⁺, hematocrit, and hemoglobin. Glucose (GLI), lactate (LAC), uric acid (UA), calcium (CA), phosphorus (P) and alkaline phosphatase (AP) levels were determined using an automatic biochemical analyzer. Hepatic glycogen (HEPG) was quantified in liver samples according Bidinotto *et al.* (1998). All analyses were performed in duplicate for 14 samples per treatment. Differences between means were determined by the CRD and Tukey's test ($p \leq 0.05$) using SAS® 9.2. No significant differences were found for RT, T3, and T4 ($p > 0.05$). The lowest levels of GH, CALC, and D3, as well as the highest levels of PTH, were observed in the TCO-H and TCO-VH ($p \leq 0.05$). Hemogasometric parameters indicated that exposure to high EST profiles (TCO-H and TCO-VH) early in INC, impaired respiratory efficiency and the buffering capacity, compromising tissue oxygenation and calcium homeostasis of chicks. Conversely, embryos incubated at lower EST (TCO-L) exhibited stable acid-base balance and appropriate levels of iCa and HCO₃⁻. TCO-L showed the highest levels of CA, P, and GLI, while TCO-VH had the lowest values. For the LAC, the opposite pattern was observed ($p \leq 0.05$). There was a significant reduction in AP and HEPG, and an increase in UA levels in treatments exposed to high ESTs. It was concluded that elevated ESTs negatively affected the chicks homeostasis. In contrast, the gradual increase in EST during the first week of incubation resulted in similar or superior outcomes to the standard EST, suggesting that the TCO-L thermal profile may optimize the regulation of physiological processes throughout embryonic development.

Keywords: Acid Base Balance, Blood Metabolites, Chick Performance, Homeostasis, Hormonal Profile

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