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Glutamine in ovo - Effects on the development and health of the gastrointestinal tract, antioxidant status and immune response in chickens

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RESUMO
Observamos que nas aves ocorre uma extensa mortalidade embrionária no final do período de incubação, período esse no qual ocorrem eventos relacionados à eclosão tais como a abertura da membrana e da casca do ovo, o início da respiração pulmonar e a emergência, propriamente dita, do ovo. A frequente atividade dos embriões implica em um grande consumo de energia, o que em conjunto com o acelerado desenvolvimento intestinal, observado especialmente ao final da incubação, ocasionam em uma demanda energética muito alta por parte dos mesmos. Nesse momento, as aves contam com o saco vitelino residual como único suprimento de nutrientes para fornecer energia. O que, geralmente, não se constitui em atendimento das altas exigências nutricionais do momento, ocasionando em catabolismo de recursos corporais como músculos e células imunes, ocasionando em diminuição do peso corporal, redução do peitoral e da imunidade, além de declínio do peso dos órgãos. De outro norte, observamos que o desenvolvimento do intestino delgado também pode ser melhorado antes da eclosão através da estimulação nutricional por meio da alimentação in ovo (IOF). A glutamina é um aminoácido condicionalmente essencial

que está fortemente ligada ao status antioxidante de pintos e às suas defesas imune. Possui ainda, efeitos estimulatórios bem documentados na proliferação de células epiteliais do intestino delgado que ocasionam em melhor morfologia do intestino delgado em várias espécies. Esse conjunto de potenciais benefícios da glutamina em adição com a necessidade de se estudar e entender melhor os mecanismos biológicos envolvidos nas relações entre os aditivos nutracêuticos inoculados via in ovo (IOF) e as aves, explicam a escolha desse aminoácido para esse estudo, bem como, descrevem o objetivo do mesmo.

Palavras-chave: Aminoácidos; Avicultura Industrial; Desempenho; Imunocompetência.

1 INTRODUCTION

It has been observed that in birds there is extensive embryonic mortality towards the end of the incubation period, a period in which events related to hatching take place, such as the opening of the egg membrane and shell, the start of pulmonary respiration and the actual emergence of the egg.

The frequent activity of the embryos means that they consume a lot of energy, which, together with the accelerated intestinal development seen especially towards the end of incubation, results in a very high energy demand on their part.

In addition, after hatching, most neonates are often subject to a delay in feed intake, which can take between 24 and 72 hours. This is due to differences in hatch times within the hatch window, as well as transportation in commercial practice.

During this period of delayed feed intake, the birds rely on the residual yolk sac as their only nutrient supply to provide energy. However, the remaining yolk sac appears to make an insufficient contribution to the nutritional needs for both maintenance and growth in today's newborn poultry, which in turn drives the mobilization of critical body resources (mainly muscle) to provide the energy needed for maintenance, causing a decrease in body weight, a reduction in chest size and a decline in organ weight.

For all these reasons, the last days of incubation, as well as the first days after hatching, are a critical period for the survival and development of late embryos and neonates due to the considerable catabolism of energy.

On the other hand, we have observed that the development of the small intestine can also be improved before hatching by nutritional stimulation through in ovo feeding (IOF). In ovo feeding is a method of supplying nutrients to the small intestine of poultry embryos by injecting a nutrient solution into their amniotic fluid, prior to amniotic ingestion, which occurs from the 17th day of incubation until hatching.

In the small intestine of birds, glutamine and fructose-6-phosphate are known to be substrates for the synthesis of glucosamine-6-phosphate and therefore glycoproteins (including mucins and membrane receptors). Glutamine is therefore strongly linked to the antioxidant status of chicks and their immune defenses. It also has well-documented stimulatory effects on small intestinal epithelial cell proliferation via the mitogen-activated protein kinase (MAPK) and mechanistic target of rapamycin (mTOR) signaling pathways, resulting in improved small intestinal morphology in various animals and humans.

This set of potential benefits of glutamine, in addition to the need to better study and understand the biological mechanisms involved in the relationship between nutraceutical additives and poultry, explains the choice of this amino acid for the present study, as well as describing its objective.

2 DEVELOPMENT

2.1 INTESTINAL DEVELOPMENT X IN OVO NUTRITION (IOF)

The mucosal lining of the small intestine is a highly functional epithelium composed of absorptive, secretory and sensory cells, which are constantly renewed by multipotent intestinal stem cells (ISC). ISC reside within the crypts and constantly proliferate for self-renewal and generation of progenitor cells, which differentiate into the functional cells that line the villi facing the lumen1.

Compartmentalization of the multipotent and differentiated regions of the small intestinal epithelium occurs during villus formation, through a polarized mesenchymal bone morphogenetic protein (BMP) signaling gradient that limits all proliferative and Wnt-responsive ISCs to the lower regions of the developing villi. These regions develop into crypts as the small intestine matures, while the villi become exclusively filled with differentiated cells2.

In chicken embryos (Gallus gallus), the formation of villi occurs on the 15th day of embryonic development (embryonic 15 d, E 15), and the crypts develop on the 21st day (day of hatching) 3,4.

After hatching, initial feeding stimulates the completion of small intestinal maturation by expanding the epithelium of the crypts and villi, mediating cell proliferation and differentiation and activating nutrient transporters, digestive enzymes and mucin secretion 4,5.

On the other hand, the development of the small intestine can also be improved before hatching by nutritional stimulation through in ovo feeding (IOF). IOF is a method of providing nutrients to the small intestine of poultry embryos by injecting their amniotic fluid with a formulated nutrient solution on day 17 of incubation, prior to amniotic ingestion, which occurs up until the day of hatch 6.

Several studies have shown that IOF of specific nutrients improved perihatch gut development and functionality, expanding the dimensions of the villi and crypts and increasing the ability to digest and absorb nutrients 7,8.

Reicher et al. (2022)9 confirmed that IOF of leucine and glutamine on day 17 of incubation increased intestinal cell counts by 33% and 40%, respectively, compared to the control. Importantly, they explained that glutamine and leucine supplementation via the in ovo route accelerated the development and maturity of the small intestine throughout the pre-hatching phase, positively regulating the proliferation and differentiation of multipotent cells.

Therefore, the main objective of IOF is to accelerate the initial development of the gastrointestinal tract and stimulate its ability to digest and absorb nutrients in newborn chicks and, consequently, enhance their rapid growth genetics. The literature confirms that IOF of carbohydrates (Slawinska et al. 2020; Asa et al. 2022)10,11, amino acids (Nazem et al. 2019; Gonzales et al. 2022; Reicher et al. 2022)12,13,9 and probiotics (Abdel-Moneim et al. 2020a; El-Moneim at al. 2020)14,15 improves intestinal histomorphology (taller villi, higher villus-to-crypt ratio and larger goblet cells).

2.2 GUT HEALTH X IN OVO NUTRITION (IOF)

The gut microbiota plays an essential role in the function and health of the gastrointestinal tract. Therefore, modulating the microbiota of the gastrointestinal tract has been suggested as an effective strategy to improve the host's intestinal health, nutrient digestion, immunity and productivity 15.

Unlike mammals, chicks hatch with a sterile gastrointestinal tract and, consequently, any contact with pathogenic bacteria represents a risk of infection and disease. Previous colonization of this tract by useful bacteria helps the birds to cope with environmental and pathogenic stressors 14,15.

In ovo nutraceutical injection (IOF) technology is a useful tool for establishing early colonization of beneficial microorganisms in the embryonic gastrointestinal tract. Extensive evidence illustrates that IOF of probiotics (Castaneda et al. 2021)16, prebiotics (Tavaniello et al. 2018; Stadnicka et al. 2020)17,18 and symbiotics (Boguslawska-Tryk et al. 2021; Shehata et al. 2022)19,20 help maintain a healthy microbial balance by strengthening useful microorganisms such as Lactobacillus, Bacillus and Bifidobacterium.

Castaneda et al. (2021)16 observed that B. subtilis IOF (106 CFU/50 µl/egg) on day 18 of incubation reduced pathogenic bacteria, including total bacterial counts, aerobic bacteria and total coliforms in the ileum and cecum on different days of the broiler growing period.

Improving intestinal health improves the general health of the host. This can be confirmed by the work of Tavaniello et al. (2018)17, who elucidated that raffinose IOF stimulated the development of useful bacteria (lactobacilli and Bifidobacteria) and suppressed the colonization of pathogenic bacteria in the gastrointestinal tract. Similarly, Stadnicka et al. (2020)18 found that raffinose IOF improved intestinal health by decreasing intestinal populations of Clostridium, E. coli, as well as coccidia counts and increasing populations of Bifidobacteria and Lactobacilli.

Several recent studies have postulated that amino acid IOF improved the development and health of the GIT 21,22,9. Gonzales et al. (2022)13 revealed that IOF of a combination of sulphur amino acids (5.90 mg of l-methionine and 3.40 mg of l-cysteine) plus 0.15 mg of folic acid increased ileal crypt depth in neonatal chicks.

In terms of gene expression, Gao et al. (2018b)21 elucidated that the IOF of 1% l-arginine on day 17.5 of embryonic development was involved in regulating the functions of the intestinal barrier in post-hatch broiler chickens by increasing the count of proliferating cells positive for villus nuclear antigen and gene expression of claudin-1, mucin-2, zonula occludens-1 and -2 in the jejunal mucosa of 21-day- old broilers.

We should also highlight the work of Shehata el al. (2022)20, who observed that IOF of probiotics, prebiotics and/or symbiotics increased the caecal content of acetic, propionic, butyric, pentanoic, ovaleric and isobutyric acids. These organic acids lowered the intestinal pH and consequently played vital roles in suppressing pathogens in the gastrointestinal tract, supporting structural integrity, improving the barrier function of the intestinal epithelium, physiological function, intestinal immunity and health23.

Therefore, the colonization and early establishment of beneficial bacteria in the gastrointestinal tract of chickens, as well as the use of nutraceuticals in ovo can block the colonization of pathogens, improving intestinal development and health, which translates into better performance.

2.3 GLUTAMINE - CHARACTERIZATION AND POTENTIAL FOR USE IN IN OVO TECHNOLOGIES

Amino acids are beneficial in improving the general physiological state, immunization against infectious diseases and stabilization under non-infectious or management conditions (Saleh et al. 2018)24, thus improving the productive performance of poultry 25.

Enteric infections in broilers can have a major influence on endogenous amino acid losses in the gastrointestinal tract (GIT). (2017)26 stated that supplementing the diet with amino acids (glutamine, arginine and threonine) above the levels recommended for growth may be necessary to improve the immune response against Eimeria and E. coli.

Glutamine is an important substrate for the synthesis of peptides, proteins, lipids, purines, pyrimidines, amino acids, nicotinamide adenine dinucleotide phosphate (NADPH), glucosamine, antioxidants and for many other biosynthetic pathways involved in regulating cellular function. Several enzymes are involved in the metabolism of this amino acid. Glutamine is predominantly synthesized from l-glutamate (Glu) and ammonia (NH 3) by the action of the largely cytosolic enzyme Gln synthetase (GS), while the mitochondrial enzyme glutaminase (GLS) is responsible for the hydrolysis of Gln to Glu and NH3. GS is highly expressed in skeletal muscle, while GLS is found in most cells with the small intestine, kidney, leukocytes and vascular endothelium having the highest activities 27, 28.

Glutamine is also known to provide nitrogen and an energy source for the proliferation of immune cells and intestinal mucosa, and is needed along with cysteine to synthesize antioxidants such as glutathione27,28. Its supplementation has been studied in animal diets due to its effects on intestinal structure and function 29,30.

It serves as an important source of energy for enterocytes, particularly during periods of increased proliferation. It is also a so-called non-essential nutrient, but becomes essential for animals in challenging situations such as stress, infection, injury or high temperature 31.

Among the amino acids with potential for use in in ovo nutrition, glutamine stands out for being a trophic agent, important in the process of cell proliferation, since it induces the enzyme ODC (ornithine decarboxylase), as well as producing vital polyamines for the process 32,33.

Finally, glutamine can also be hydrolyzed into glutamate, acting as an immunomodulatory agent, inhibiting pro-inflammatory cytokines, protecting the mucosa from exacerbated immune response and pH changes.

2.4- GLUTAMINE X ANTIOXIDANT STATUS OF POULTRY

Several enzymes are involved in glutamine metabolism. This amino acid is predominantly synthesized from l-glutamate (Glu) and ammonia (NH 3) by the action of the largely cytosolic enzyme Gln synthetase (GS), while the mitochondrial enzyme glutaminase (GLS) is responsible for the hydrolysis of Gln to Glu and NH3. GS is highly expressed in skeletal muscle, while GLS is found in most cells with the small intestine, kidney, leukocytes and vascular endothelium having the highest activity. Glutamine is also metabolized by glutamine:fructose-6-phosphate aminotransferase (GFAT), which condenses the amino group of glutamine and fructose-6-phosphate into glucosamine-6-phosphate, a precursor for N- and O-linked glycosylation reactions 34.

The glutamate product is used for the synthesis of the antioxidant glutathione: a small three-amino acid peptide (Glu-Cys-Gly) that is an efficient peroxide-based free radical neutralizer. Alternatively, it can be further metabolized by Glu dehydrogenase and/or aminotransferases into α-ketoglutarate, which then enters the Krebs cycle generating ATP and serving as an anaplerotic carbon source for the formation of non-essential amino acids and lipids 35.

In addition, the production of NADPH by malate-pyruvate cycling promotes redox homeostasis, providing the reducing equivalents for glutathione reductase to regenerate glutathione. Finally, in the intestine, enterocytes convert Glu into delta1-pyrroline-5-carboxylate allowing the formation of l-proline, l-ornithine and l-citrulline. By generating l-citrulline, which is subsequently metabolized to the NOS substrate l-arginine by the concerted action of argininosuccinate synthetase and argininosuccinate lyase in the kidney, Gln also functions as a precursor of l-arginine to drive NO synthesis 36.

Confirming this, Hu et al. (2020)37 studying different levels of glutamine supplementation in the diet of chickens under cyclic heat stress, concluded that the inclusion of glutamine increased the activity of antioxidant enzymes and reduced the expression of markers related to lipid peroxidation and muscle oxidation.

In addition, Bai et al. (2019)38 in a study supplementing chicken diets subjected to different periods of heat stress with glutamine observed that Gln supplementation was associated with improvements in antioxidant capacity and lipid peroxidation in heat-stressed broilers.

Surai et al. (2019)39 also state that in the case of the antioxidant system of the developing embryo, it can be said to consist of: antioxidant enzymes (glutathione peroxidase GSH-Px, superoxide dismutase SOD and catalase CAT), water-soluble antioxidants (ascorbic acid, glutathione, taurine, carnitine, etc.), fat-soluble antioxidants (vitamin E, carotenoids, coenzyme

Q) and mineral antioxidants (Se, Mn, Zn, etc.). Poultry embryos are more susceptible to oxidative stress due to their extremely rapid development, high metabolic rates and high content of polyunsaturated fatty acids in their tissues. In ovo inoculation (IOF) using natural antioxidants increases the activity of antioxidant enzymes, which play a fundamental role in the elimination and detoxification of free radicals and also decreases the concentration of malondialdehyde (MDA) as an index of lipid peroxidation 11,40.

Amino acids have a high antioxidant capacity as they maintain a balance between the synthesis and removal of free radicals.

2.5 IN OVO GLUTAMINE (IOF) X BIRDS' IMMUNE RESPONSE

The state of health of poultry is directly related to their immune system; birds with an adequate immune system grow better.

Despite this, modern chickens have become more sensitive to infectious diseases due to their high growth pattern. Therefore, much attention has been paid to improving the immune system and activating the immune response in the early stages of growth. The IOF of nutraceuticals, immune stimulants and bioactive components could be a promising tool for improving the post-hatch immune response in poultry and providing protection against diseases 41,20.

Amino acids are extremely beneficial structures in improving the general physiological state, immunization against infectious diseases and stabilization under non-infectious or management conditions (Saleh et al. 2018), thus improving the productive performance of poultry 25.

In addition, amino acid metabolism is different depending on the state of health in birds (challenge vs. non-challenge conditions) 42. digestibility, absorption and metabolism of amino acids is affected in health and disease. Their deficiency can predispose and their supplementation can prevent or alleviate diseases 42.

Amino acid supplementation resulted in cecal butyric acid production and total short-chain fatty acid production and supported growth, development, feed conversion efficiency and improved immunity 42.

An adequate supply of dietary amino acids is necessary to maintain normal immunocompetence and host protection against some diseases in all species. Dietary supplementation with free methionine attenuates intestinal oxidative stress induced by Eimeria spp. in broiler chickens 43.

In another study, Toghyani et al. (2019)44 reported that IOF of arginine (35 mg) and/or threonine (25 mg) on day 14 of incubation significantly increased antibody titers (SRBCs), while titers against Newcastle disease and avian influenza did not increase significantly. In addition, arginine IOF significantly increased the relative weights of the bursa of Fabricius and spleen compared to the simulated control at 11 days of age in broilers.

Similarly, Gonzales et al. (2022)13 revealed that IOF with a combination of sulphur amino acids (5.90 mg of l-methionine and 3.40 mg of l-cysteine) plus 0.150 mg of folic acid increased the relative weights of the thymus and spleen at 21 days of age. Furthermore, it has been shown that that IOF of 100 μg l-arginine (Subramaniyan et al., 2019)41 or 100 μg of l-arginine conjugated with 1,000 μg of silver nanoparticles (Subramaniyan et al. 2021)45 on day 14 of incubation increased serum IgM concentration in newly hatched chicks.

This amino acid plays an important role in necrotic enteritis, which causes significant economic losses in the broiler industry. L-glutamine compensates for the metabolic losses of this infection, improves intestinal development and intestinal morphology, improves growth performance and serum biochemical indices 46.

The protective effects of Gln may be the result of increased expression of junction cells (TJP) in the small intestine and decreased intestinal permeability during stress. The mechanism for these protective effects may be through Gln activation of occludin, claudin-1, zonula occludens-2 (ZO-2), zonula occludens-3 (ZO-3) and corticotrophin-releasing factor 47.

This demonstrates that Gln supplementation can regulate TJP expression, which has a beneficial effect on mucosal barrier function and health under conditions of heat stress. (2018)48 are consistent with these reports and suggest that Th1 and Th2 responses were inhibited by the presence of glutamine and that it plays a beneficial role in alleviating the intestinal inflammatory response.

In addition, in the trials conducted by Salmanzadeh et al. (2020)49 with glutamine IOF at different levels for 43-week-old layers, an increase was observed in the number of lymphocytes and heterophils, as well as in the heterophil/lymphocyte (H/L) ratios of newly hatched chicks and heterophil/lymphocyte (H/L) and 10-day-old chickens. All the Gln levels used increased the relative spleen and bursa weights of newly hatched chicks and the spleen weight of broilers at 10 days of age. On the 26th day of age, increasing the dose of Gln increased the concentration of immunoglobulins G and M.

These data and reports reinforce the potential of glutamine, especially that supplied via in ovo feeding (IOF), as an amino acid of unparalleled importance for the general health of chickens and, consequently, for broiler performance.

3 FINAL CONSIDERATIONS

After analyzing the reports presented, it can be concluded that glutamine has a huge range of benefits for animals and humans, many of which still need to be better studied and elucidated.

In addition, when combined with in ovo technology (IOF), it can be assumed that these effects can be taken advantage of earlier by the birds, which will have an earlier and more complete intestinal development and a modulation of the intestinal cells in order to improve its health, generating better immune responses which, in the end, will result in better all-round health for these birds.

REFERENCES

1. Chen W, Xu J, Tangara M, Peng J. Effects of in ovo injecting disaccharides and alanyl-glutamine dipeptide on the energy status in duck embryos and neonates. Animal reproduction science. 2010 Oct 1;122(1-2):29-35.

2. Hansi N, Thoua N, Carulli M, Chakravarty K, Lal S, Smyth A, Herrick A, Ogunbiyi O, Shaffer J, Mclaughlin J, Denton C. Consensus best practice pathway of the UK scleroderma study group: gastrointestinal manifestations of systemic sclerosis. Clin Exp Rheumatol. 2014 Nov 1;32(6 Suppl 86):214-.

3. Shyer AE, Huycke TR, Lee C, Mahadevan L, Tabin CJ. Bending gradients: how the intestinal stem cell gets its home. Cell. 2015 Apr 23;161(3):569-80.

4. Uni Z, Tako E, Gal-Garber O, Sklan D. Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poultry Science. 2003 Nov 1;82(11):1747-54.

5. Reicher N, Melkman-Zehavi T, Dayan J, Uni Z. It’s all about timing: Early Feeding promotes intestinal maturation by shifting the ratios of specialized epithelial cells in chicks. Frontiers in Physiology. 2020 Dec 17;11:596457.

6. Uni Z, Ferket PR, Tako E, Kedar O. In ovo feeding improves energy status of late-term chicken embryos. Poultry Science. 2005 May 1;84(5):764-70.

7. Dai D, Wu SG, Zhang HJ, Qi GH, Wang J. Dynamic alterations in early intestinal development, microbiota and metabolome induced by in ovo feeding of L-arginine in a layer chick model. Journal of animal science and biotechnology. 2020 Dec;11:1-6.

8. Wang J, Lin J, Wang J, Wu S, Qi G, Zhang H, Song Z. Effects of in ovo feeding of N-acetyl-L- glutamate on early intestinal development and growth performance in broiler chickens. Poultry science. 2020 Jul 1;99(7):3583-93.

9. Reicher N, Melkman-Zehavi T, Dayan J, Wong EA, Uni Z. Nutritional stimulation by in-ovo feeding modulates cellular proliferation and differentiation in the small intestinal epithelium of chicks. Animal Nutrition. 2022 Mar 1;8:91-101.

10. Slawinska A, Zampiga M, Sirri F, Meluzzi A, Bertocchi M, Tavaniello S, Maiorano G. Impact of galactooligosaccharides delivered in ovo on mitigating negative effects of heat stress on performance and welfare of broilers. Poultry science. 2020 Jan 1;99(1):407-15.

11. Mete O, Asa SL, Gill AJ, Kimura N, de Krijger RR, Tischler A. Overview of the 2022 WHO classification of paragangliomas and pheochromocytomas. Endocrine pathology. 2022 Mar;33(1):90-114.

12. Nazem MN, Amiri N, Tasharrofi S. Effect of in ovo feeding of amino acids and dextrose solutions on hatchability, body weight, intestinal development and liver glycogen reserves in newborn chicks. InVeterinary research forum 2019 (Vol. 10, No. 4, p. 323). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

13. Salgado Pardo JI, Navas González FJ, González Ariza A, Arando Arbulu A, Leon Jurado JM, Delgado Bermejo JV, Camacho Vallejo ME. Traditional sexing methods and external egg characteristics combination allow highly accurate early sex determination in an endangered native turkey breed. Frontiers in Veterinary Science. 2022 Aug 15;9:948502.

14. Abdel-Moneim AM, Elbaz AM, Khidr RE, Badri FB. Effect of in ovo inoculation of Bifidobacterium spp. on growth performance, thyroid activity, ileum histomorphometry, and microbial enumeration of broilers. Probiotics and antimicrobial proteins. 2020 Sep;12:873-82.

15. El-Moneim AE, El-Wardany I, Abu-Taleb AM, Wakwak MM, Ebeid TA, Saleh AA. Assessment of in ovo administration of Bifidobacterium bifidum and Bifidobacterium longum on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. Probiotics and Antimicrobial Proteins. 2020 Jun;12:439-50.

16. Castañeda CD, Gamble JN, Wamsley KG, McDaniel CD, Kiess AS. In ovo administration of Bacillus subtilis serotypes effect hatchability, 21-day performance, and intestinal microflora. Poultry Science. 2021 Jun 1;100(6):101125.

17. Tavaniello S, Slawinska A, Prioriello D, Petrecca V, Bertocchi M, Zampiga M, Salvatori G, Maiorano G. Effect of galactooligosaccharides delivered in ovo on meat quality traits of broiler chickens exposed to heat stress. Poultry science. 2020 Jan 1;99(1):612-9.

18. Stadnicka K, Bogucka J, Stanek M, Graczyk R, Krajewski K, Maiorano G, Bednarczyk M. Injection of raffinose family oligosaccharides at 12 days of egg incubation modulates the gut development and resistance to opportunistic pathogens in broiler chickens. Animals. 2020 Mar 31;10(4):592.

19. Bogusławska-Tryk M, Ziółkowska E, Sławińska A, Siwek M, Bogucka J. Modulation of intestinal histology by probiotics, prebiotics and synbiotics delivered in ovo in distinct chicken genotypes. Animals. 2021 Nov 18;11(11):3293.

20. Shehata AM, Paswan VK, Attia YA, Abougabal MS, Khamis T, Alqosaibi AI, Alnamshan MM, Elmazoudy R, Abaza MA, Salama EA, El-Saadony MT. In ovo inoculation of Bacillus subtilis and raffinose affects growth performance, cecal microbiota, volatile fatty acid, ileal morphology and gene expression, and sustainability of broiler chickens (Gallus gallus). Frontiers in Nutrition. 2022 May 31;9:903847.

21. Gao T, Zhao MM, Li YJ, Zhang L, Li JL, Yu LL, Gao F, Zhou GH. Effects of in ovo feeding of L- arginine on the development of digestive organs, intestinal function and post-hatch performance of broiler embryos and hatchlings. Journal of animal physiology and animal nutrition. 2018 Feb;102(1):e166-75.

22. Elwan HA, Elnesr SS, Xu Q, Xie C, Dong X, Zou X. Effects of in ovo methionine-cysteine injection on embryonic development, antioxidant status, IGF-I and TLR4 gene expression, and jejunum histomorphometry in newly hatched broiler chicks exposed to heat stress during incubation. Animals. 2019 Jan 12;9(1):25.

23. Ebeid TA, Ketta M, Al-Homidan IH, Barakat H, Abdel-Moneim AM. In ovo feeding of nutraceuticals and its role in adjusting the gastrointestinal tract, antioxidative properties,

immunological response, and performance in poultry: An updated review. Czech Journal of Animal Science. 2023 Jan 1;68(1).

24. Saleh AA, Ragab MM, Ahmed EA, Abudabos AM, Ebeid TA. Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature. Journal of applied animal research. 2018 Jan 1;46(1):820-7.

25. Ghoreyshi SM, Omri B, Chalghoumi R, Bouyeh M, Seidavi A, Dadashbeiki M, Lucarini M, Durazzo A, van den Hoven R, Santini A. Effects of dietary supplementation of L-carnitine and excess lysine-methionine on growth performance, carcass characteristics, and immunity markers of broiler chicken. Animals. 2019 Jun 16;9(6):362.

26. Gottardo ET, Burin Junior ÁM, Lemke BV, Silva AM, Busatta Pasa CL, Muller Fernandes JI. Immune response in Eimeria sp. and E. coli challenged broilers supplemented with amino acids. Austral journal of veterinary sciences. 2017 Sep;49(3):175-84.

27. Newsholme P. Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection?. The Journal of nutrition. 2001 Sep 1;131(9):2515S-22S.

28. Le Floc'h N, Melchior D, Obled C. Modifications of protein and amino acid metabolism during inflammation and immune system activation. Livestock Production Science. 2004 Apr 1;87(1):37- 45.

29. Santos RG, Quirino IE, Viana ML, Generoso SV, Nicoli JR, Martins FS, Nogueira-Machado JA, Arantes RM, Correia MI, Cardoso VN. Effects of nitric oxide synthase inhibition on glutamine action in a bacterial translocation model. British journal of nutrition. 2014 Jan;111(1):93-100.

30. Soares AD, Costa KA, Wanner SP, Santos RG, Fernandes SO, Martins FS, Nicoli JR, Coimbra CC, Cardoso VN. Dietary glutamine prevents the loss of intestinal barrier function and attenuates the increase in core body temperature induced by acute heat exposure. British Journal of Nutrition. 2014 Nov;112(10):1601-10.

31. Soltan MA. Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and some blood parameters of broiler chickens. Int. J. Poult. Sci. 2009;8(1):60-8.

32. Maiorka, A. Impacto da saúde intestinal na produtividade avícola. simpósio brasil sul de avicultura, 2004, 5, 119-129.

33. Maneewan, B., & Yamauchi, K. (2005). Recovery of duodenal villi and cells in chickens refed protein, carbohydrate and fat. British poultry science, 46(4), 415-423. https://doi.org/10.1080/00071660500158105

34. Rodas PC, Rooyackers O, Hebert C, Norberg Å, Wernerman J. Glutamine and glutathione at ICU admission in relation to outcome. Clin Sci (Lond). 2012;122(12):591-7.

35. CRUZAT, Vinicius Fernandes; NEWSHOLME, Philip. An Introduction to Glutamine Metabolism. In: Glutamine. CRC Press, 2017.1-18.

36. Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: metabolism and immune function, supplementation and clinical translation. Nutrients. 2018 Oct 23;10(11):1564.

37. Hu H, Dai S, Li J, Wen A, Bai X. Glutamine improves heat stress–induced oxidative damage in the broiler thigh muscle by activating the nuclear factor erythroid 2–related 2/Kelch-like ECH- associated protein 1 signaling pathway. Poultry science. 2020 Mar 1;99(3):1454-61.

38. Bai X, Dai S, Li J, Xiao S, Wen A, Hu H. Glutamine improves the growth performance, serum biochemical profile and antioxidant status in broilers under medium-term chronic heat stress. Journal of Applied Poultry Research. 2019 Dec 1;28(4):1248-54.

39. Surai PF, Kochish II, Romanov MN, Griffin DK. Nutritional modulation of the antioxidant capacities in poultry: the case of vitamin E. Poultry science. 2019 Sep 1;98(9):4030-41.

40. Mousstaaid A, Fatemi SA, Elliott KE, Alqhtani AH, Peebles ED. Effects of the in ovo injection of L-ascorbic acid on broiler hatching performance. Animals. 2022 Apr 14;12(8):1020.

41. Subramaniyan SA, Kang D, Siddiqui SH, Park J, Tian W, Park B, Shim K. Effects of in ovo supplementation with nanonutrition (L-arginine Conjugated with Ag NPs) on muscle growth, immune response and heat shock proteins at different chicken embryonic development stages. Animals. 2020 Mar 27;10(4):564.

42. Hilliar M, Hargreave G, Girish CK, Barekatain R, Wu SB, Swick RA. Using crystalline amino acids to supplement broiler chicken requirements in reduced protein diets. Poultry Science. 2020 Mar 1;99(3):1551-63.

43. Khatlab AD, Del Vesco AP, de Oliveira Neto AR, Fernandes RP, Gasparino E. Dietary supplementation with free methionine or methionine dipeptide mitigates intestinal oxidative stress induced by Eimeria spp. challenge in broiler chickens. Journal of Animal Science and Biotechnology. 2019 Dec;10(1):1-7.

44. Cowieson AJ, Toghyani M, Kheravii SK, Wu SB, Romero LF, Choct M. A mono-component microbial protease improves performance, net energy, and digestibility of amino acids and starch, and upregulates jejunal expression of genes responsible for peptide transport in broilers fed corn/wheat-based diets supplemented with xylanase and phytase. Poultry science. 2019 Mar 1;98(3):1321-32.

45. Subramanian P. Lipid-based nanocarrier system for the effective delivery of nutraceuticals. Molecules. 2021 Sep 10;26(18):5510.

46. Xue GD, Barekatain R, Wu SB, Choct M, Swick RA. Dietary L-glutamine supplementation improves growth performance, gut morphology, and serum biochemical indices of broiler chickens during necrotic enteritis challenge. Poultry science. 2018 Apr 1;97(4):1334-41.

47. Wang B, Wu G, Zhou Z, Dai Z, Sun Y, Ji Y, Li W, Wang W, Liu C, Han F, Wu Z. Glutamine and intestinal barrier function. Amino acids. 2015 Oct;47:2143-54.

48. Wu QJ, Liu N, Wu XH, Wang GY, Lin L. Glutamine alleviates heat stress-induced impairment of intestinal morphology, intestinal inflammatory response, and barrier integrity in broilers. Poultry Science. 2018 Aug 1;97(8):2675-83.

49. Salmanzadeh M, Ebrahimnezhad Y, Aghdam Shahryar H, Ghiasighaleh Kandi J. The effects of in ovo administration of glutamine on hatchability, subsequent performance, digestive enzyme activities, immune response and some of blood parameter in broiler chickens. Iranian Journal of Applied Animal Science. 2020 Sep 1;10(3):535-45.