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In ovo nutrition: Effect of fatty acids on the development of the immune system in poultry

Nutrição in ovo: Efeito dos ácidos graxos no desenvolvimento do sistema imune das aves

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ABSTRACT

This review elucidated the main effects of in ovo nutrition with fatty acids and their relationship with the immune system of producing birds. We searched the main research databases Web of Science and Google Scholar for articles published in the last 20 years, and found 75 files that reported this approach. Ovo nutrition is a technique used in poultry farming, and its efficiency in nutritional management, which combines preventive and health management, has been discussed. This technique's main objective is to provide nutrients even in the embryonic phase of the animal, instigating the functioning of specific intestinal cells, resulting in benefits that can be reflected after birth, as recommended by immune nutrition, in the case of nutrient supplementation that stimulates defense cells of the organism. In the pursuit of precision animal husbandry, advanced and easily applicable techniques are being increasingly explored. Specific nutrients, such as essential fatty acids, enhance the gastrointestinal microbiota, maintain intestinal integrity, and act as necessary substrates to ensure a satisfactory immune response in poultry. This has proven to be highly beneficial for animal production. Studies have demonstrated that the use of these nutrients can effectively improve poultry production in a sustainable and economically viable manner. In conclusion, incorporating essential fatty acids into poultry feed represents a promising and practical strategy within precision animal husbandry. In addition to promoting gut and immune health in birds, this practice contributes to more efficient, sustainable, and economically advantageous production, which aligns with current demands in the poultry industry.

KEYWORDS: fatty acids; immune nutrition; immune system; poultry production.

RESUMO

O objetivo desta revisão é elucidar os principais efeitos da nutrição *in* ovo com ácidos graxos e sua relação com o sistema imune das aves de produção. Buscou-se nas principais bases de pesquisa Web of Science e Google Scholar, artigos dos últimos 20 anos que relatavam este enfoque, totalizando 75 arquivos. A nutrição *in* ovo é uma técnica utilizada na avicultura e tem sido discutida acerca da eficiência em relação ao manejo nutricional visando unir o manejo preventivo e o sanitário. Esta técnica tem como principal objetivo fornecer nutrientes ainda na fase embrionária do animal, instigando o funcionamento de células específicas intestinais em benefícios que poderão ser refletidos após o nascimento, como preconizado pela nutrição imune, no caso da suplementação de nutrientes que estimulam as células de defesa do organismo. Na busca pela Zootecnia de precisão, técnicas avançadas e de fácil aplicação estão sendo cada vez mais exploradas. Nutrientes específicos, como os ácidos graxos essenciais, podem favorecer a microbiota intestinal, manter a integridade intestinal e atuar como substratos necessários para garantir uma resposta imunológica satisfatória nas aves. Isso se mostra altamente benéfico para a produção animal. Estudos na literatura demonstram que o uso desses nutrientes é justificado e comprovadamente eficaz na melhoria da produção avícola, de maneira sustentável e economicamente viável. Concluindo, a incorporação de ácidos graxos essenciais na alimentação das aves representa uma estratégia promissora e prática dentro da zootecnia de precisão. Além de promover a saúde intestinal e imunológica das aves, essa prática contribui para uma produção mais eficiente, sustentável e economicamente vantajosa, alinhando-se às demandas atuais do setor avícola.

PALAVRAS-CHAVE: ácidos graxos; nutrição imune; produção avícola; sistema imunológico.

INTRODUCTION

The immunodeficiency of birds and the constant health challenges found in production systems are daily concerns in poultry production. The precise feeding is, in turn, an essential tool in this process, with the provision of balanced diets being one of the most relevant pillars in the physical condition of the bird to achieve normal growth and optimize egg and/or meat production (AL-DARAJI et al. 2012).

Recent studies have investigated the interaction of nutrients with specific and highly important activities in organisms, such as the action of immune cells. Due to its complexity, the immune system requires several essential nutrients to ensure its effective functioning. Nutritional supplementation has emerged as a promising field to explore its nutritional potential, especially in consideration of the particularities of immune actions in animals (BAKYARAJ et al. 2012).

Among the nutrients necessary for the development of birds, lipids are important sources of fatty acids, which are involved in various metabolic functions of the organism (LIU et al. 2012, MOGHADDAM et al. 2014). Nutrient adjustments, such as fatty acids for birds, can traditionally occur through diets formulated according to the recommendations for each animal phase and by the addition of sources rich in lipid fatty acids derived from vegetable oils and/or animal fat (BHATTACHARYYA et al. 2018).

However, a relatively recent management tool called overnutrition works on the insertion and/or supplementation of nutrients in the feeding management of birds of zootechnical interest, aiming to unite sanitary and nutritional management from the beginning (KERMANSHAHI et al. 2017). The main objective of nutrition *in ovo* is to provide extra nutrients during embryonic development to correct possible nutritional deficiencies and thus provide benefits to the birds at birth. Therefore, this review aims to conduct a literature review on the current use of the *in ovo* nutrition technique with fatty acids and its relationship with the immune system of birds.

METHODOLOGY

A study was conducted at the Department of Animal Science of the Federal University of the Vales of Jequitinhonha and Mucuri, Diamantina, MG, from August 23 to December 15, 2023. The articles were researched in the main electronic databases of literary research, Web of Science and Google Scholar, using search terms that were either associated or not associated, in plural or singular, in English and Portuguese, such as "In vo nutrition", "Fatty acid", "Supplementation" and "Immune system". The years 1999 to 2023 were evaluated to ensure the most recent data (Table 1).

Table 1. Bibliometric inventory of systematic research.

	Files in the database	
Keywords	Web of Science	Google Scholar
In ovo nutrition	1250	988
"In ovo nutrition" and "supplementation”	344	259
"In ovo nutrition" and "supplementation" and "fatty acids"	177	155
"In ovo nutrition" and "supplementation" and "fatty acids" and “immune system"	87	47
Deleted files	44	20
Total number of files	75	

After selecting the studies, a data relevance test was applied, with defined and applied acceptance and exclusion criteria for non-relevant articles, following the methodology proposed by MUNOZ et al. (2002) using a questionnaire composed of questions that generate affirmative or negative responses related to the study's objectives. The responses to the questionnaire were obtained by reading the title, abstract, and part of the results of the articles resulting from the bibliometric search. The two evaluators answered "yes" or "no" to the following questions:

Does the publication date range from 1999 to 2023?

Are keywords included in the title and abstract?

Does the study include nutritional supplementation in ovo animals?

Articles that received 100% YES responses to all questions from both evaluators were included. The inclusion criteria, primary studies in Portuguese, English, and Spanish that were available online in full text or accessible were selected. Studies without abstracts, conference proceedings, or experimental animal models of species other than production birds were excluded. Seventy files were selected and used to support the bibliometric review.

Two files with publication dates before 1999 were used due to the significant relevance of the topic. After the relevance test, the articles selected for use in the study were tabulated in an Excel® spreadsheet with relevant information, such as species, substance, and application location, as exemplified in Table 2.

Table 2. Main information taken from articles on *in* ovo nutrition.

Author(s) *	Species	Substance(s) inoculated	Location(s) of the application
COLES et al. (1999)	Chicken	Peptides	Internal tube
MCREYNOLDS et al. (2000)	Chicken	Amino acids	Amniotic fluid
OHTA & KIDD (2001)	Chicken	Amino acids	Yolk
OHTA et al. (2001)	Chicken	Amino acids	Yolk
TAKO et al. (2005)	Chicken	CHO's, chelated zinc	Amniotic fluid
BHANJA et al. (2004)	Chicken	Amino acids	Yolk
UNI et al. (2005)	Chicken	CHO's	Amniotic fluid
FOYE et al. (2006)	Peru	Egg white, CHO's	Amniotic fluid
BHANJA et al. (2008)	Chicken	Vitamins linoleic acid	Yolk
ZHAI et al. (2008)	Layer	L-carnitine	Amniotic fluid
KADAM et al. (2008)	Chicken	L-threonine	Yolk
BHANJA et al. (2008)	Chicken	Glucose	Yolk
SANTOS et al. (2010)	Chicken	Maltose, multivitamin, glycine, zinc, glutamine	Amniotic fluid
CAMPOS et al. (2011)	Chicken	CHO's, vitamins, and Minerals	Amniotic fluid
BAKYARAJ et al. (2012)	Chicken	Amino acids, AG, Minerals and vitamins	Internal tube
AL-DARAJI et al. (2012)	Quail	Arginine	Internal tube
LIU et al. (2012)	Duck	IGF-1	Albumen
NOWACZEWSKI et al. (2012)	Hen Duck	Vitamins	Internal tube
MOGHADDAM et al. (2014)	Chicken	Royal jelly	Internal tube and Yolk bag
KERMANSHAHI et al. (2017)	Quail	Threonine	Inside and outside the inner tube
GOEL et al. (2017)	Chicken	Nanoparticles	Internal tube and Yolk bag
BHATTACHARYYA et al. (2018)	Peru	Amino acids, AG	Albumen

In ovo nutrition

The growth process of the avian embryo occurs in response to the availability of nutrients found in the egg, where the accelerated development of current strains in the poultry market implies greater metabolic needs, making the period after hatching a critical moment in the pursuit of productive efficiency (TAKO et al. 2004). Certain metabolic substrates are necessary for embryonic growth, with the egg taking on the role of a physical structure that allows for embryonic progress (GONÇALVES et al. 2013). However, eggs contain a finite number of nutrients for development, embryonic growth, and hatching (KOCAMIS et al. 1999).

Although the egg is considered nutritionally complete, the percentages of amino acids, carbohydrates, vitamins, minerals, and lipids may be below desirable levels in the final third and during hatching, especially in birds with high-performance potential (GONÇALVES et al. 2013). These nutrients can generally be almost depleted during hatching; therefore, in ovo nutrition serves as a tool to overcome the initial limitations of post-harvest growth and development in broiler chickens (FOYE et al. 2006). The first signs of interference from exogenous material inside eggs were reported around the 1980s, when SHARMA and BURMESTER (1982) were responsible for research into expanding vaccination techniques for Marek's disease.

The authors also conducted initial tests for the development of the technique, addressing the location and day of inoculation according to embryonic development, without nutritional purpose. AL-MURRANI (1982) was the first to study the improvement of body weight in birds through the introduction of amino acids into the yolk sac of chicken embryos on the seventh day of incubation. In the same year, research involving the administration of nutrients to eggs during the embryonic development phase was reported in the literature on their application in broiler chickens.

Since then, several studies have been conducted with the aim of administering various nutrients, including amino acids, carbohydrates, fatty acids, minerals, and vitamins, mainly to improve hatchability and productive gains. The concept of "in ovo nutrition", also associated with "in ovo inoculation", originated from the translation of the term in English "in egg feeding", which was established more effectively as an auxiliary tool in the nutritional management of birds only in 2004 by Uni & Ferket.

The authors addressed the introduction of nutrients into the egg for the early nutrition of commercial chicken and turkey embryos during embryonic development, resulting in a patent registered in the USA in the same year. Since then, the concept has gained prominence and has been the subject of studies exploring the effects of in ovo supplemental nutrition on various positive aspects of the post-hatching life of birds (FOYE et al. 2007, CHEN et al. 2010).

The practice allows for better nutritional conditions at birth, bringing extra advantages in the early periods after hatching, a phase characterized by the high level of stress to which the bird is subjected, attributed to environmental changes and frequent handling (BHANJA et al. 2004). Among the positive points listed are the more efficient use of nutrients in feed, reduced mortality rates of chicks, stimulation of an effective and early immune response, and gains in muscle development and breast production, reducing production costs per kilogram of meat produced (UNI & FERKET 2004).

Even with the patented technology for over 15 years, there are still inconsistencies regarding the best application site, day of inoculation, and nutritional solution to be injected, which hinders the industrial adoption of the technique by the poultry market but also contributes to new scientific efforts being employed. Positive responses also depend on the osmolarity of the solution injected into the amniotic fluid (UNI et al. 2005).

Unbalanced concentrations and different types of nutrients in the solutions to be inoculated can cause an osmotic imbalance within the egg, leading to the cessation of embryonic development and, consequently, death (DAMASCENO et al. 2017). Many studies have been conducted on the effect of *in ovo* nutrition *on* hatchability and initial growth performance. OHTA & KIDD (2001) demonstrated that different injection sites and dates of *in ovo* inoculation differentially affect hatching; therefore, to recommend the best procedure for embryonic supplementation, it is necessary to understand the different physiological characteristics associated with each phase of embryonic development.

OHTA et al. (1999), applying the inoculation of amino acids in the yolk sac of fertile broiler chicken eggs on the seventh day of incubation, concluded that the hatching rate was not affected and observed an increase in the body weight of the chicks at birth compared with the weight of the eggs. However, embryos that received the same treatment but were inoculated on the 14th day of incubation showed a higher hatching rate and better growth rate after hatching up to 28 days of age (COLES et al. 1999).

With the predominance of *in ovo* nutrition application for chickens, which requires an incubation period of 21 days, inoculation was observed more frequently at the time of transfer to the incubator, approximately from the 17th to the 18th day of incubation, when the embryo begins to ingest the amniotic fluid orally; consequently, the inoculated substances are ingested (PESSÔA et al. 2012). Furthermore, it has been demonstrated that the embryo has digestive enzymes that enable feeding during the pre-hatching phase.

Despite these data, this technique is recent, and little is known about the levels and types of nutrients that can be used in embryo nutrition. SKLAN & NOY (2000) reported that the transfer of yolk content to the intestinal tract is greater when a bird is fed than when it is fasting during the first hours of post-hatching life. *In* ovo nutrition can precede nutritional management during the early life stages of birds (FELLAH et al. 2014).

To meet market needs, research has focused on identifying foods and nutrients that, when supplemented with diets, improve product quality without affecting feed efficiency (NUNES et al. 2010). Considering that some may alter genetic events, influencing animal health and development (GONÇALVES et al. 2009), the supplementation of these nutrients in the embryonic phase would allow for better metabolic responses in a developing organism, favoring the expression of genes of interest at a later stage (BHANJA et al. 2008).

Role of nutrients in embryonic and post-hatching development

The activities of digestive enzymes are present from the embryonic stage in birds, as well as mechanisms that involve the absorptive action of nutrients, mainly in the intestinal medium. The endoderm of the yolk sac secretes extracellular enzymes that act on the substrate, allowing the absorption of the products of digestion, even larger molecules (SKLAN & NOY 2000).

During the incubation period, these digestible products provide energy and nutrients and originate from the yolk, which mainly contains lipids and lower concentrations of proteins and carbohydrates. The lipids of the yolk are transported directly to the embryo's blood by endocytosis, but after hatching, the contents of the yolk are absorbed by the membrane of the yolk sac and by Meckel's diverticulum and are digested and absorbed by the intestinal tract (MORAN JR. 2007).

Among the macromolecules found in the yolk sac, proteins and lipids are among those that most contribute to cellular synthesis and provide support for the construction of passive immunity compared with the energy supply; therefore, when there is no extra energy supply, these nutrients will naturally be directed toward this purpose (OTHA et al. 2004). There are a variety of nutrients, including fatty acids, amino acids, carbohydrates, minerals, and vitamins, with potential use *in sheep nutrition*. The ideal supplementation solution should consider the characteristics of the substance to be inoculated, such as volume, osmolarity, and viscosity (UNI et al. 2005).

Many of the studies conducted with inoculation *in ovo* used vitamins, amino acids, and carbohydrates to stimulate the growth and weight gain of chicks from the beginning (UNI et al. 2005). Nutrient management *in ovo* has direct effects on hatchability, digestive system development, live weight, and post-harvest nutritional status, as access to food is crucial for the early development of chicks in the first days of life (UNI et al. 2005).

SANTOS et al. (2010) reaffirmed the potential of pre-hatching nutrition by highlighting digestive enzymes in embryos during the final stage of incubation. In broiler chicks, starting from the 15th day of incubation, there is the ingestion of amniotic fluid, and by enriching it with nutritious substances, there may be an improvement in the performance of the birds in the first days, as they would have an extra supply of nutrients.

The influence of nutrition on immune system development

Advances in poultry genetics represent a significant increase in productivity efficiency. However, concurrently, there is a notable inadequacy in the immune response of birds in relation to their production potential, which is also observed in sanitary aspects. The techniques applied in production, including management, genetics, and nutrition, exert a natural influence on the health of these animals (RIBEIRO et al. 2008).

The convergence between nutrition and the immune system has been the subject of extensive research, resulting in a considerable volume of studies in the field of poultry production. Furthermore, this research extended to the standardization of chemotherapeutic use in the poultry market (VIEIRA et al. 2015). Advances in this field have provided a deeper understanding of the immune system of birds, allowing valuable insights into the complexity of the interactions between nutritional factors and immune responses. However, gaps remain, and clarifications on this subject are necessary (MORAIS & LIMA 2020).

The immune system of birds operates according to the same principles that govern the immune system of mammals (SHARMA & BURMASTER 1982). VIEIRA et al.” The review (2015) highlights the crucial role of nutrition in immune development. The activities of the immune system, like those of other bodily systems, directly require energy and various specific nutrients (KADAM et al. 2008). Notably, when the immune system is activated, proteins, vitamins, energy, and minerals are diverted to the site of the inflammatory reaction, reducing their availability for the production of meat and eggs.

The main organs of the avian immune system are the primary and secondary lymphoid organs. The primary organs, such as the bursa of Fabricius and the thymus, play a crucial role in the mechanisms of acquired immunity and are responsible for the formation of lymphocytes (O'MALLEY 2005). Secondary organs, such as the spleen, bone marrow, and intestinal lymphoid tissue, complement this function (OLÁH & VERVELDE 2008).

During the embryonic development process, immature embryonic hematopoietic cells migrate from the embryonic sac to the bloodstream and, subsequently, to the spleen, where they form red and white blood cells. These cells colonize primary organs, such as the bursa and thymus, around the sixth day of incubation of the thymus and bursa, and in the third week after hatching, the maturation of these primary and secondary organs occurs (OLÁH & VERVELDE 2008, JUUL-MADSEN et al. 2008).

The early maturation of the immune system differs between birds and other species. This developmental process results in an adaptive response from primary organs such as the thymus and bursa. In broiler chickens, the receptivity of the embryonic thymus at around the sixth day of incubation and the bursa at around the tenth day allows for the formation of lymphocytes. These lymphocytes although morphologically similar to those of post-hatching birds, have limited functionality during the embryonic phase (CAMPOS et al. 2011).

Considering the influence of nutrition on the immune system, some authors, such as SILVA et al. (2013), have discussed the concept of immunonutrition. They emphasized that supplementation with specific nutrients can influence organic activities, including immune system functions, reproduction, and cardiac functionality, among other organs. Research, such as that of CAMARGO et al. (2019), emphasizes that immune system dysfunctions may be associated with malnutrition, deficiencies, or nutritional inadequacies.

These effects manifest as a decrease in various factors, such as antibody secretion and antigen affinity, cell-mediated immunity, lymphocyte production, synthesis of complement system proteins, and phagocytic function. The bioactive components present in diets can interact with the immune response, potentially reducing the susceptibility of birds to infectious diseases (KOGUT & KLASING 2009). Notably, adequate levels of most nutrients, aimed at maximizing production, generally provide a suitable substrate for the immune system to function satisfactorily.

Improving immunity through nutrition can reduce the need for chemical substances, such as antibiotics, which act as growth promoters. Although increased immunity is a desirable aspect of poultry production, it is crucial to consider the potential pathological effects of overstimulation of the immune system. The balance between immune enhancement and the prevention of adverse effects must be carefully considered to promote effective health in modern poultry production systems (QURESHI 2002).

Fatty acids in poultry nutrition

The fatty acids present in the carcasses of monogastric animals reflect what is offered in the diet; when ingested, they pass through the digestive system with almost no change in composition (DALLA COSTA et al. 2016). Thus, enriching diets with fatty acids of interest can result in changes in the lipid profiles of these animals. The vitelline sac, which is present in bird embryos, is of fundamental importance because it contains the nutritional material that allows for embryonic development and is a source of vitamins and fat-soluble fatty acids. According to POWELL et al. (2004), lipids are the main source of energy for bird embryos, representing approximately 35% of the initial volume of energy consumed before birth.

In the last week of the incubation period, a large relative amount of lipid material is used for the development of chicken embryos (TAKO et al. 2005), but another considerable proportion remains at birth and is used until the fifth day after hatching. During these five days, chicks obtain lipids from the yolk through lipoproteins. Linoleic acid must be present in the fertile yolk of the egg to produce high-quality chicks. PEDROSO et al. (2006) observed that the yolk sac surrounding the embryo can contain approximately 14% linoleic acid, which, in a young matrix, represents 160 mg of the egg's content.

Specific nutrients can favor the microbiota, maintain intestinal integrity, and act as necessary substrates to ensure a satisfactory immune response in birds. Among these essential nutrients for the proper development of birds, fatty acids stand out because they are involved in the cellular structure and have energy potential. However, birds are not able to synthesize all the fatty acids they need, and for this reason, some fatty acids, such as linoleic acid, are considered essential (BAIÃO & LARA 2005). Fatty acids are potential food additives for appetite control in poultry production (ZHAI et al. 2008).

Several studies have examined the effectiveness of different saturated short-chain fatty acids and their mixtures on the performance, carcass characteristics, and mortality of broiler chickens, as well as their potential to trigger specific responses. Short-chain fatty acids, such as lauric acid, have specific antimicrobial activity in the intestine and have several additional beneficial effects, such as reducing the pH of the digesta, increasing pancreatic secretion, and exerting trophic effects on the gastrointestinal mucosa (DIBNER & BUTTIN 2002).

In Japanese quails, supplementation with 4% fish oil and 4% soybean oil significantly increased omega-3 concentrations in the yolk sac, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), compared with supplementation at similar levels of other lipid sources, such as sunflower oil and olive oil (GÜÇLÜ et al. 2008).

Conjugated linoleic acid (CLA)

Conjugated linoleic acid (CLA) is an isomeric compound derived from linoleic acid that is present in large quantities in the lipids of milk and in the muscle tissue of ruminants (PREUSS et al. 2013). This compound stimulates the synthesis of immunoglobulins and reduces the production of pro-inflammatory cytokines, playing a role in modulating the inflammatory response (DILZER & PARK 2012).

On the other hand, lauric acid, which is abundant in coconut oil, demonstrates immunological activities that have been described in several studies, including the involution and suppression of humoral and cellular immunity (VERMA et al. 2017). Low concentrations of CLA can be found in human tissues, but the origin of this fatty acid is not well defined in the literature, with the belief that in humans, the formation of CLA occurs through the autoxidation of unsaturated fatty acids from the diet (BOTELHO et al. 2005).

The first evidence that CLA could affect the immune system involved increased immune responses and reduced immune-mediated catabolism in chicks and rats (MOURÃO et al. 2005). Among its various functionalities, CLA has been classified as an anticarcinogenic compound, with potential action on tumors in animal models, associated with a reduction in incidence, number, and size, helping to reduce animal susceptibility to pathogens and, consequently, diverting less energy from production to maintaining immune defense (GATTÁS & BRUMANO 2005). DILZER & PARK (2012) stated that the specific use of CLA has the potential to stimulate the development of lymphoid organs, such as the bursa and the thymus, as this fatty acid influences lymphocyte proliferation and consequently increases antibody productivity.

YAMASAKI et al. (2000), studying the effect of CLA on lipid peroxidation and histological changes in the liver tissues of rats, reported that the action of linolenic and linoleic fatty acids can reverse relapses and the actions of free radicals, as they have antimetastatic and anti-inflammatory properties due to the inhibition of angiogenesis and stimulation of various components of the immune system. Studies using different animal models have linked CLA to several other positive effects that could benefit human health, including the reduction of atherosclerosis and the prevention and treatment of noninsulin-dependent diabetes mellitus (SÉBÉDIO et al. 1999).

The use of CLA in animal diets can increase the efficiency of animal production in a multifactorial manner, whether by increasing animal resistance to diseases or improving the final quality of the carcass. This fatty acid in animal feed is a prominent nutrient that activates lipolysis and minimizes lipogenesis, improving carcass quality by reducing the percentage of fat and consequently increasing the proportion of meat in the carcass, as well as influencing the efficiency of the immune system (GATTÁS & BRUMANO 2005).

The supplementation of CLA in bird diets substantially reduces accumulated liver fat and promotes the incorporation of CLA into liver lipids (BADINGA et al. 2003). There are also reports of negative effects when CLA is used as a supplement, leading to productive damage in specific situations. Some studies have shown that CLA may have a detrimental effect on eggs, such as increasing embryonic mortality during incubation and interfering with growth when offered in inadequate proportions (DALLA COSTA et al. 2016).

HASSANABADI et al. (2014) found an increase in the relative weight of Fabricius bursa and thymus in broiler chickens at 21 and 42 days of age when studying the inoculation of CLA in eggs at levels of 150 and 300 mg, suggesting that CLA could influence the mediation of immune cells in broiler chickens. There was no difference in the number of lymphoid organs between the tested treatment groups; however, TAVES et al. (2017) reported that chronic stress can lead to increased serum corticosteroid concentrations, which can cause atrophy of the thymus and bursa through a mechanism known as apoptosis or programed cell death of lymphoid cells.

Regarding the effects of conjugated linoleic acid (CLA), a study conducted by PAULA et al. (2021) addressed the inoculation of this component and reported a significant reduction in the total cholesterol content in the blood. On the other hand, the level of lauric acid (LA) was markedly increased, regardless of the inoculation level. These results indicate the complexity of the metabolic responses to the application of CLA and LA, highlighting the importance of understanding the implications of these substances in the regulation of the lipid profiles of birds.

Moreover, conjugated linoleic acid derived from the diet can affect the fluidity, permeability, receptor activity, and enzymatic function of biomembrane, altering the composition of fatty acids, mainly at the intestinal level, resulting in disorders and decreasing the potential activity of these damaged enzymes (LIMA et al. 2018). AYDIN & COOK (2004), when evaluating the effects of dietary inclusion of CLA in diets for broiler quails containing canola oil (0.25, 0.5, 1, 2, and 3%), concluded that CLA has the potential to induce embryonic mortality and alter egg quality depending on the dose and the day of inoculation, with higher levels resulting in greater embryonic mortality.

Discussions regarding the CLA are ongoing given the results obtained in many studies. Although the physiological effects of CLA have been studied, its mechanisms of action remain controversial and seem to depend on the animal species, dose, and duration of the experiments.

Laureic acid

Lauric acid (dodecanoic acid) is a saturated fatty acid (C 12:0) that occurs extensively in the fats of the seeds of the Lauraceae plant family (MERÇON 2010). It is the dominant fatty acid in cinnamon oil (80-90%), coconut oil (41-56%), and palm oil (41-55%) (BASTOS-LEITE et al. 2016). Lauric acid-rich glycerides are commonly used in the food industry as flavor enhancers or in their natural state, for example, in the general industrial sector and in surfactant production. Palm kernel oil and coconut oil are the main sources of lauric acid, but uncertainty about the price of coconut oil has led to increased interest in other sources of lauric acid (NOWACZEWSKI et al. 2012).

According to a survey, the saturated fatty acids present in coconut oil are caproic, caprylic, capric, lauric, myristic, palmitic, and stearic acids, and the unsaturated fatty acids are oleic and linoleic acids. Coconut oil is rich in lauric acid, with a concentration above 40% (BARRETO et al. 2006). Due to its high lauric acid content, coconut oil has gained considerable attention in the market in recent years due to its health benefits. It is associated with antibacterial, antiviral, and antifungal effects and contributes to the fight against various pathogenic microorganisms (GOEL et al. 2017).

Some studies have demonstrated the antibacterial activity of lauric acid, especially when impregnated with antimicrobial films (HOFFMANN et al. 2001, DAWSON et al. 2002). Lauric acid also demonstrates antifungal activity. According to OUATTARA et al. (2000), the antifungal effect of lauric acid derivatives was evaluated and their activity against Aspergillus niger. Similarly, BERGSSON et al. (2001) tested the susceptibility of *Candida albicans* to several fatty acids and their monoglycerides, finding that lauric acid was the most active at low concentrations and after a long period of in vitro incubation against the studied fungus, which is a mammalian pathogen. Lauric acid is a precursor of monolaurin (PEREIRA et al. 2004), and it has a modulatory effect on the proliferation of immune cells and antimicrobial activity (BERGSSON et al. 2001).

Another favorable aspect of lauric acid is the evidence that it suppresses the cytokines responsible for tissue inflammation, as reported by SADEGHI et al. (1999), who evaluated the effect of dietary lipids with different fatty acid compositions on the in vivo response of cytokines to bacterial lipopolysaccharide (LPS) in mice and reported that in cases of arterial wall inflammation leading to the development of atheroma, lauric acid also has a suppressive effect on inflammation. RAMALHO & JORGE (2006) stated that the addition of coconut oil modifies the composition of fatty acids in the lipids of the egg yolk, and the incorporation of medium-chain fatty acids derived from coconut oil into the lipids of the egg yolk is limited, but what is incorporated is readily utilized by the embryo.

CONCLUSION

In summary, *in ovo* nutrition has emerged as a promising strategy for poultry farming because of its ability to increase chick hatching and, consequently, strengthen the immune response of the birds. Although it does not replace the importance of early feeding, the combination of these practices minimizes possible adverse effects after hatching.

Given the concerns regarding immune deficiencies, especially in light of genetic and productive advances in poultry strains, the incorporation of specific nutrients, such as fatty acids, has emerged as an effective strategy for supporting the microbiota, maintaining intestinal integrity, and promoting a satisfactory immune response.

In addition to contributing to bird health, *in ovo* nutrition has a positive impact on meat quality by reducing the percentage of fat in the carcass and consequently increasing the proportion of meat. In this context, the literature review supports the effectiveness of this technique and justifies its use as a sustainable and economically viable approach to improving poultry production.

However, for the effective implementation of this practice in the field, it is imperative to carefully evaluate several aspects to fully understand its potential and ensure its success in poultry operations.

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