Arginine and branched chain amino acids in the nutrition of laying hens

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Background

Global egg production has been constantly expanding. World egg production grew by 119%, from 35.5 in 1990 to 76.8 million tons in 2018 (FAO, 2018). In order to meet this growing demand, laying hens have undergone genetic selection for decades and as a result, these birds remain in production longer, between 100 and 110 weeks of age. In addition to presenting higher egg laying rates, its contributing also to better feed efficiency. Obviously, as a result of these changes, nutritional requirements have been altered to meet the demands for high productivity. Thus, it is necessary to continuously evaluate the laying hens response to amino acids levels, key nutrients to optimize the expression of genetic potential.

Fluctuations in the prices of raw materials used in the feed and environmental conditions are additional reasons to understand and properly apply the concept of ideal protein in the laying hens feed, in order to safely reduce the crude protein content. The progressive reduction of dietary protein content leads to a situation in which the amino acids like threonine, isoleucine, valine and arginine become limiting to maintain proper productive performance. The availability of industrial amino acids makes it possible to correct the imbalances in amino acids resulting from the reduction of dietray crude protein.

In addition to the high cost of protein ingredients, commonly used in feed, the high crude protein content in the feed overloads liver and kidney functions, due to the need to excrete excess nitrogen (Andriguetto et al., 2002). It is common to observe hemorrhagic liver syndrome in laying hens, a metabolic disease characterized by excessive accumulation of fat in the liver to the detriment of the health and productive performance of the bird. Bunchasak & Silapsom (2005) observed a 38% reduction in liver fat deposition in laying hens when they reduced crude protein in the feed from 16 to 14%.

Genetic evolution, availability of scientific information and definition of specific requirements for each production parameter have allowed the expansion of the use of amino acids in diets for laying hens. In practical situations, methionine and cystine, lysine, arignine, isoleucine, valine and tryptophan are the most important for poultry. There are studies in the literature reporting the beneficial effects of arginine, isoleucine and valine supplementation in the laying hens diet on productive performance (Lima & Silva, 2007; Souza, 2009; Almeida, 2013; Lieboldt et al., 2016; Parenteau, 2019). Thus, the objective of this review is to discuss the functions and effect of these amino acids in the laying hens feed on egg production and quality.

Arginine

Arginine is an essential amino acid in poultry, other than protein synthesis it has been associated with other important metabolic molecules such as creatine, nitric oxide, glutamate, polyamines, proline, glutamine and collagen (Chevalley et al., 1998; Ball et al., 2007; khajali & Wideman, 2010). The only metabolic pathway for the synthesis of nitric oxide is through the conversion of arginine into citrulline through the enzyme nitric oxide synthase, thus producing all the isoforms of the nitric oxide molecule (Wu & Morris, 1998). Therefore, arginine is crucial for the synthesis of nitric oxide, especially in poultry, as this species is unable to endogenously produce arginine. Nitric oxide has several important functions in the body, such as an immune modulator, it acts in the pathways of energy metabolism, gene expression, blood circulation and the nervous system (Farr et al., 2005; Jobjen et al., 2006).

In laying hens, nitric oxide is essential to maintain the persistence of egg production. In addition to being a highly reactive molecule, nitric oxide is related to the regulation of productive functions in birds (Kumar & Chaturvedi, 2008). Manwar et al. (2006) reported that the serum nitric oxide level is directly related to the laying rate in Japanese quails, in addition, they observed that a 5% L-arginine supplementation in the diet significantly increased egg production. Basiouni (2009) demonstrated that the inclusion of L-arginine in the diet of laying hens increased the level of luteinizing hormone. Luteinizing (LH) and follicle stimulating (FSH) hormones control follicular development, ovulation and egg production through ovarian steroids (Hartree & Cunningham, 1969).

Duan et al. (2016) observed a 10% increase in the laying rate of broiler breeders in the group of hens fed a diet containing 1.36% digestible arginine compared to the group fed a diet formulated with 0.96% digestible arginine. According to the authors, arginine acts directly on the ovaries and ovarian follicles, this amino acid stimulates the release of LH and, consequently, the persistence of egg laying increases.

It is important to mention one of the important imbalances between amino acids that is often not considered in the formulation of feed for laying hens, the antagonism between lysine and arginine. Excess lysine stimulates renal arginase, increasing arginine catabolism, causing a deficiency of the latter amino acid. Increasing the arginine level in a feed with a high lysine content alleviates the negative effect caused by the antagonism from excess lysine (Gadelha et al., 2003). Similarly, arginine overdose can cause an imbalance in the arginine: lysine ratio, increase the activity of renal arginase and, consequently, increase the oxidation of arginine to uric acid (Carvalho et al., 2015). The latest authors (Carvalho et al., 2015) also claim that the proper level of arginine in the diet can increase protein anabolism. L-arginine supplementation in poultry diets may reduce body fat deposition, especially abdominal fat. The low activity of malate dehydrogenase, glucose-6-phosphate dehydrogenase and fatty acid synthase enzymes is observed from L-arginine supplementation in poultry diet (Wu et al., 2011). Meanwhile, stimulation in the expression of the enzyme palmitoyl carnitine transferase-I was observed in broilers fed diets supplemented with L-arginine (Fouad et al., 2013). These enzymes participate indirectly in the lipogenesis process whereas, some of these enzymes are involved in lipid β-oxidation. This is a desired effect, especially in laying hens in the egg production phase, when they are exposed to certain situations that contribute to the occurrence of fatty liver. According to Bertechini (2012), the deficient transport of lipids in the blood and high energy diets contribute to the exaggerated deposition of fat in the liver tissue. Arginine supplementation in the feed allows the reduction of the level of crude protein to be carried out more safely and contributes to the reduction of fat deposition in the liver. Bunchasak & Silapasom (2005) observed a 38% reduction in fat deposition in liver tissue by reducing the crude protein level in the diet from 16 to 14%.

Additionally, as a functional amino acid, arginine supplementation has been shown to relieve oxidative stress and improve antioxidant capacity by reducing superoxide release, relieving lipid peroxidation (Galli, 2009; Petrovic et al., 2008; Atakisi et al., 2009), immunity (Munir et al., 2009; Tayde et al., 2006) and muscle and bone development (Corzo et al., 2009; Fernandes et al., 2009; Castro et al., 2019).

Increasing the inclusion of L-Arginine in feed broiler breeders from 60 to 69 weeks of age promotes an increase in the total antioxidant capacity in the serum and a reduction in the malondialdehydeconcentration in serum and egg yolk (Duan et al., 2016). However, the authors reported that excessive levels of arginine could be harmful by reducing total antioxidant capacity, indicating that a balance in dietary arginine is needed to improve antioxidant capacity. Malondialdehyde, a soluble product of lipid degradation is used to monitor the extent of lipid peroxidation (Wang et al., 2006). Therefore, the reduction in the concentration of this substance in the yolk may contribute to increase the shelf life of eggs.

Studies have shown effects of arginine on the immune system in poultry. For example, Deng et al. (2005) showed that arginine supplementation increases immunoglobulins levels as IgM in laying hens. Yang et al. (2016) evaluated L-arginine supplementation at increasing levels (0.0; 8.5 and 17.0 mg/kg) in the laying hens feed from 25 to 31 weeks of age and they observed a significant increase in the immunoglobulins concentration, improving the poultry immune responsiveness.

In addition to the secretagogue action for reproductive hormones, arginine also acts on insulin and growth hormone (GH) (Collier et al., 2005). These two hormones have a stimulatory action on the production of insulin-like growth factor hormone (IGF-I) in poultry. IGF-I has an effect on feed efficiency due to its ability to modulate protein catabolism, stimulate protein synthesis and reduce protein degradation. (Conlon et al., 2002). Arginine may act on bone development through the action of GH. Lieboldt et al. (2016) when evaluating increasing levels of arginine, in order to meet 70, 100 and 200% of the NRC recommendation (1994) in the feed of different laying hens genetic lines, they observed that the insufficient dietary level of arginine induced growth retardation in the growth phase. The authors attributed the delay in the growth of pullets, regardless of genetic factor, to adverse effects from dietary imbalances between arginine and lysine or between arginine and methionine, as well as the low plasma concentration of arginine and ornithine, caused by an insufficient supply of arginine, inducing the deficiency of these metabolizable amino acids. For laying hens in the egg production phase, arginine supplementation may be an ally in the intestinal calcium absorption and assimilation of this mineral in the hens's body, especially under heat stress conditions, when feed intake is reduced, compromising the availability of nutrients for adequate egg production and quality. Fiore et al. (2000) and Clementi et al. (2001) indicate that arginine is clinically recommended for metabolic disturbances in calcium absorption and calcification. However, when the reduction in crude protein is drastic, arginine supplementation may not be sufficient to maintain satisfactory egg quality. For example, Dao et al. (2021) reduced crude protein from 17 to 13% in the feed of brown laying hens and added 3.5 kg of L-arginine per ton of feed, in order to obtain the level of 0.89% of digestible arginine and a ratio between arginine and digestible lysine equal to 117%. The researchers observed that the egg shell thickness in the control treatment, free of L-arginine was 0.413 mm, while the group fed with diet reduced in crude protein and added with L-arginine the thickness was 0.403 mm.

Arginine supplementation in laying hens feed promotes intestinal growth (He et al., 2011). The stimulation of intestinal growth may contribute to better mucosal integrity and consequently, better feed conversion. Fascina et al. (2017) recommend the supplementation of 968 mg of arginine per kilogram of diet to obtain better feed conversion in brown laying hens and an digestible arginine: lysine ratio of 110%. Liebdolt et al. (2016) demonstrated that deficient arginine levels in the laying hens feed in the early laying phase significantly affect weight gain and feed intake, affecting egg production and quality. Dao et al. (2021) concluded that the reduction of four percentage points in the crude protein level, from 17 to 13%, in a diet based on wheat and sorghum for Hy-Line brown laying hens from 20 to 39 weeks of age was excessive, which may explain the lack of effect on egg production and quality when supplementing 0.35% L-arginine in the reduced crude protein feed. Excessive reduction in crude protein may corroborate a deficiency in non-essential amino acids and other compounds present in soy.

The recommendation of the ideal ratio between digestible arginine and lysine for laying hens in the egg production phase may vary depending on several factors, including methods applied to estimate the nutritional requirement. For example, Rostagno et al. (2017) recommend ideal digestible arginine: lysine ratio equal to 100% for laying hens based on dose-response models. While, Soares et al. (2018) recommend values equal to 109 and 104% of digestible arginine: lysine ratio based on mathematical estimates following Goettingen and Louvain deletion models, respectively.

Branched Chain Amino Acids - Isoleucine, Leucine and Valine

Isoleucine, Valine and Leucine are classified as branched chain amino acids (BCAAs). The availability of feed grade isoleucine and valine offers a new opportunity to formulate diets more efficiently by optimizing the optimal amino acid profile. In addition to protein synthesis, an adequate supply of isoleucine and valine is important to maintain intestinal immunity, gut barrier function and antioxidant capacity. (Azzam et al., 2015; Dong et al., 2016; Wen et al., 2019a). Another important function of this BCAA is the regulation of fatty acid metabolism in the liver. (Bai et al., 2015). Isoleucine positively influences egg production through better liver and body composition. The inclusion of L-Isoleucine in the feed allows to safely reduce crude protein. Reducing the level of crude protein promotes a reduction in hepatic intramuscular fat deposition, which contributes to the health of the laying hens and as a consequence, better egg production (Parenteau, 2019). Leucine's main function is to serve as a substrate for protein synthesis, however, it also acts as a signaling nutrient that regulates protein synthesis and inhibits protein degradation in various body tissues (Escobar et al., 2005). Furthermore, leucine increases the activity of proteins involved in mRNA translation (Wu et al. 2010).

Table 1. Effects of branched chain amino acids

Amino Acid	Effect	Specie	Reference
Leucine	Stimulate protein synthesis	Swine	Escobar et al. (2005)
Leucine	Improve glucose metabolism	Mice	Zhang et al. (2007)
Valine	Improve performance	Broilers	Tavernari et al. (2013)
Isoleucine	Improve antioxidant enzyme activity	Carp	Zhao et al. (2013)
Isoleucine/Valine	Modulate the expression of hepatic genes related to lipid metabolism	Broilers	Bai et al. (2015)
Valine	Increase serum glicose and feed consumption	Laying hens	Azzam et al. (2015)
Valine	Increase cellular antioxidant capacity Improve immune response Increase intestinal digestive enzymes secretion Increase jejunal amino acid transport channels Increase egg production	Laying hens	Wen et al. (2019)
Isoleucine	Improve egg quality and production	Laying hens	Parenteau et al. (2020)

One of the main objectives of laying poultry farming is to improve productivity, persistence of laying and egg mass produced, therefore, considering BCAAs in laying hens nutrition is particularly important, especially leucine, due to its role in regulation of protein synthesis. The ideal ratio of digestible leucine to lysine for white and brown laying hens recommended by Rostagno et al. (2017) is 122%. In addition, isoleucine should be considered in the feed formulation based on corn and soy with a reduction in the crude protein content for laying hens, as under these conditions this amino acid becomes limiting (Shivazad et al., 2002). In a diet based on corn and soybean meal and a reduction of 2.0 percentage points in the crude protein level, meeting the requirements in amino acids, except for isoleucine, which makes it limiting for the maximum egg production and egg weight, possibly indicating a change in dietary amino acid utilization. (Parenteau et al., 2020). Studies have shown that L-Isoleucine supplementation in the laying hens feed increases egg production (Shivazad et al., 2002; Sohail et al., 2002; Ospina Rojas, et al., 2015).

The effect of dietary isoleucine on the intestinal barrier function may be explained by the fact that this amino acid is a substrate for the synthesis of glutamine (Wu, 2009). Oxford & Selvaraj (2019) observed that glutamine supplementation reduced the severity of infection in broiler chickens by reducing intestinal damage as the mRNA content of tight junction proteins increased. These authors verified an increase in the expression of tight junctions, claudin-1, claudin-2 and occluding zonule-1 in broilers challenged with Eimeria, fed diets supplemented with increasing levels of glutamine. The intestinal barrier function is regulated by tight junctions. (Bruewer et al., 2003). These junctions seal the parcellular space between epithelial cells and are necessary to maintain the mucosal barrier. (Tang et al., 2010). An intact intestinal epithelium serves as a vital barrier to prevent the entry of potential pathogens and result in the proper absorption and utilization of nutrients, optimizing the poultry's health and performance (Ritzi et al., 2014). Although, Dong et al. (2016) did not observe alterations in the mRNA expression of the tight junction proteins, claudin-1 and occludin, in the ileum, when they evaluated excessive isoleucine levels in the brown laying hens feed from 28 to 40 weeks of age. This fact could be explained by the lack of production and excessive release of pro-inflammatory cytokines in the intestine, observed by the authors. Excessive production of pro-inflammatory cytokines, especially tumor necrosis factor (TNF-α), implies some pathological responses that occur in inflammatory conditions (Calder, 2001), such as affecting the tight junctions structure through suppression of adenosine monophosphate activated protein kinase (AMPK) expression (Aznar et al., 2016; Sun et al., 2017).

An interaction in response to unbalanced intake of BCAAs has been reported in humans and animals, as excessive leucine levels depress growth and food intake (Harper et al., 1984). Researchers show that excess leucine stimulates the catabolism of isoleucine and valine by increasing the activity of α-ketoglutarate dehydrogenase, a key enzyme involved in the degradation of these two amino acids (Harris et al., 2001; Wiltafsky et al., 2010). This depressive effect from excess leucine in the diet may be aggravated in the presence of valine-deficient diets. Gloaguen et al. (2011) found a reduction in the feed intake of pigs fed diets deficient in valine and a consequent reduction in growth. Wen et al. (2019a) reported that the 1.024% leucine level in the feed was lower than levels that cause antagonism and impair the valine requirement in white laying hens, as they did not observe depression in egg production, feed consumption or egg mass. According to the ratios recommended for laying hens by Rostagno et al. (2017) for digestible BCAAs and lysine, it is observed that there is an adequate ratio between isoleucine: leucine: valine equal to 1.0: 1.56: 1.19; respectively, which must be maintained in order to avoid imbalance between these three amino acids and possible negative effect on egg performance and quality.

In laying hens, some studies have demonstrated the antagonism between BCAAs, in which valine and isoleucine are the most commonly evaluated, since leucine requirements are usually met by protein sources present in conventional diets for laying hens. Peganova & Eder (2002a) reported that a dietary valine concentration of 1.36% reduces feed intake and daily egg mass by 5 and 10%, respectively. Azzam et al. studied valine levels in brown laying hens feed and these authors found no effect on egg mass, even at the maximum level evaluated (1.19%), corresponding to the inclusion of 4 g of L-valine per kg of feed, the egg mass remained unchanged. These authors concluded that high concentrations of L-valine in the laying hens feed do not harm the productive performance or immunity. Peganova & Eder (2002b) studying the response of laying hens to excessive isoleucine levels reported that feed intake was significantly reduced at high L-isoleucine inclusion levels. Peganova & Eder (2003) concluded that diets with high protein concentrations contribute more significantly to increase the isoleucine, valine and leucine levels than diets with low protein content, further enhancing the antagonism between these amino acids. Dong et al. (2016) studing L-isoleucine supplementation (0.0; 1.0; 2.0; 3.0 and 4.0 kg/ton) corresponding to levels of 0.54; 0.64; 0.74; 0.84 and 0.94% of digestible isoleucine in the brown laying hens feed from 22 to 34 weeks of age found no negative effects on egg production and quality. According to the authors, the non-occurrence of the antagonism between the BCAAs and the possible negative effect on performance was due to the low level of crude protein applied in the experimental feed, equal to 14%.

Valine may be considered one of the potential limiting amino acids for laying hens, after methionine, lysine, tryptophan and threonine. According to Kidd & Hackenhaar (2006), in diets composed of corn and soybean meal, valine is the fourth limiting amino acid.

It is necessary to maintain the valine level in laying hens properly in accordance with nutritional recommendations, as well as properly adjusted to feed intake, as there are reports in the literature that unbalanced valine levels may negatively impact laying hens feed intake and reduction in feed intake implies a consequent drop in productive performance. However, Almeida (2013) when evaluating increasing levels of digestible valine from 6.15 to 8.05 g/kg in the laying hens feed found a linear increase in the feed intake, without any effect on the efficiency of lysine utilization. While valine deficiency significantly reduces feed intake, especially in diets formulated with levels below 7.3 g/kg of digestible valine (Wen et al., 2019a). Macelline et al. (2021) concluded that the dietary valine is directly correlated with feed intake.

Lesson & Summers (2005) studying valine levels for laying hens from 32 to 45 weeks of age recommended a total valine: lysine ratio equal 89%. Using egg mass as a parameter, Breghendahl et al. (2008) determined a daily intake of necessary digestible valine equal to 501 mg/hen/day and a digestible valine: lysine ratio of 93%. According to Almeida (2013), egg production in white laying hens may be maximized through the digestible valine: lysine ratio estimated at 97.6% from a quadratic function. The optimal valine requirement estimated by the broken line linear model for maximum egg production was 591 mg per hen per day in a vegetable feed for white laying hens (Wen et al., 2019a). These last authors concluded that white laying hens in the period from 41 to 60 weeks of age have a requirement of 12.2; 14.5; 13.6 and 14.0 mg of valine per day to produce 1 g of egg mass, using linear broken line, quadratic broken line, polynomial quadratic and exponential models, respectively.
Rostagno et al. (2017) recommend ratios of isoleucine and valine with digestible lysine for laying hens equal to 78 and 93% and daily intake for white hens of 648 and 773 mg per hen, respectively. While Oliveira (2018) through the linear response plateau model estimated optimal levels of isoleucine and valine equal to 376 and 679 mg/day/hen, respectively for white laying hens and when applying the quadratic polynomial model, the estimated optimal levels for isoleucine and valine were higher and equal to 633 and 931 mg/hen/day. Marcillene et al. (2021) estimated mean digestible isoleucine and digestible valine requirements equal to 648 and 532 mg/hen/day, respectively, for laying hens.

Final considerations

The availability not only of L-arginine and L-valine, but also of L-isoleucine offers a new opportunity to formulate diets more efficiently and accurately for laying hens, allowing the poultry to express its maximum productive potential throughout its productive life.
The reduction of the crude protein level in the laying hens feed may be safely applied without compromising production performance, through a more comprehensive and balanced industrial amino acids supplementation. According to Parenteau et al. (2020), it is possible to reduce the crude protein level by up to two percentage points, 18 to16% and 16 to 14% in the periods from 20 to 27 and from 28 to 46 weeks of age of white laying hens, respectively, from the valine, isoleucine, arginine in addition to methionine, lysine, threonine and tryptophan supplementation in the laying hens feed. Rostagno et al. (2017) recommended ratios between digestible valine, isoleucine and arginine with lysine for laying hens in the laying phase are: 93; 78 and 100%. In order to improve performance and eeg quality, the recommended rations between valine, isoleucine and arginine with lysine for laying hens are: 90; 82 and 110%.

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