

AMINO ACIDS

Tryptophan and its functionality in laying hens

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Abstract

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Tryptophan is one of the essential amino acid. In relation to other amino acids, tryptophan is found in lower concentration in animals; therefore, it is quite likely to become the limiting factor for protein anabolism. Tryptophan is metabolized in the kynurenine and serotonin pathways into other molecules responsible for different functions in the animal. Tryptophan is an amino acid of relevant significance in the feed formulation for laying hens, especially in diets with reduced protein level as well as in complex diets formulated with animal by-products, as it becomes limiting. In the animal, the main function of tryptophan is protein synthesis; in addition, it is metabolized in the kynurenine and serotonin pathways, generating different substances, which are responsible for positive effects on antioxidant capacity, immune system, food intake, animal behavior, gut health and integrity. The tryptophan recommendation for laying hens between 0.15 and 0.23% depending on the statistical model applied, age and genetic lineage, diet composition among other factors.

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Background

Tryptophan in soy corn-based diets it is the third limiting amino acid for laying hens (Bertechini, 2012). Tryptophan is used primarily for protein synthesis in order to maintain egg production, in addition, it has other functions, among which it stands out as a precursor of serotonin. Serotonin is a neurotransmitter, acts on the nervous system helps to reduce aggression and modulate the stress response through social and environmental adaptability (Martin et al., 2000). In addition, some evaluation results suggest that dietary tryptophan promotes positive effects on antioxidant capacity in fish, laying hens, and rats. (Wen et al., 2014; Dong et al., 2012; Raju et al., 2000).

Corn, an ingredient present in greater proportion in Brazilian feed, is deficient in tryptophan, containing only 0.06% of digestible tryptophan, while soybeans contain 0.59% (Rostagno et al., 2017). Thus, the combination of ingredients used in the formulation of feed for laying hens may lead to the need to include L-tryptophan, to meet the demand for this amino acid by laying hens.

Tryptophan metabolism

Tryptophan metabolism occurs in different pathways (Figure 1). Deamination and decarboxylation of tryptophan occurs to form kynurenine in the liver and brain; whereas, tryptophan also act as a precursor for serotonin synthesis in the brain and gastrointestinal tract, this pathway being dependent on the hydroxylation and decarboxylation of tryptophan from the first pathway and the last pathway of tryptophan metabolism occurs through transamination, forming indole-pyruvate (Sallée et al., 2014).

More than 90% of tryptophan is metabolized through the kynurenine pathway, in which several functional metabolites related to energy and immune metabolism are produced. (Stone et al., 2013).

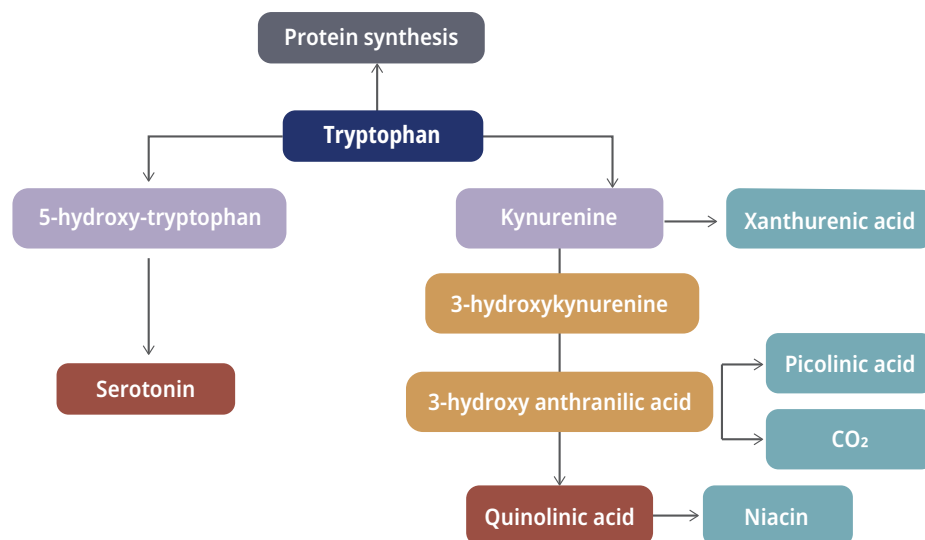


Figure 1. Tryptophan metabolic pathways (Adapted from Le Floo'ch & Seve, 2007)

Nicotinic acid produced from tryptophan in the kynurenine pathway, is essential in the nutrition of monogastric animals and its main function is related to cellular respiration. The energy messengers NADH and NADPH are generated from nicotinic acid

which are essential for the production of ATP in the respiratory chain. Thus, nicotinic acid deficiency can lead to an energy deficit, compromising the physiological homeostasis of the animal organism and causing its death (Santin et al., 2000).

Tryptophan and its functionality

Tryptophan, also known as α -amino- β -indolepropionic acid, it is involved in several physiological functions. (Le Floch et al., 2011). This amino acid is essential for birds, fish and mammals (Wu et al., 2014) as it is not synthesized in these animals therefore tryptophan must be supplied through diet.

Tryptophan is also considered a functional amino acid, simultaneously with other amino acids, such as arginine, cysteine, methionine, which regulate important metabolic pathways to improve health, survival, growth, development and the reproductive process in animals (Wu, 2020). Tryptophan and its metabolites are also involved in immune intestinal homeostasis (Gao et al., 2018). In addition to improve feed intake in poultry, it contributes to improve production performance and helps in the synthesis of hormone-binding substances (Sarsour et al., 2021; Khattak & Helmbrecht, 2019; Ducey & Karsenty, 2010; Le Floch et al., 2008).

Tryptophan is also crucial for different metabolic and physiological activities in the animals as synthesis of neurotransmitters and vitamin B3. It shows positive effects on the balance of the intestinal microbiota,

on enzymatic and non-enzymatic antioxidant capacities. Tryptophan has a positive effect on behavior animal by stimulating serotonin secretion; on immune modulation (Khattak et al., 2019; Bello et al. 2018; Bai et al., 2014; Wen et al., 2014; De Ponti et al., 2007; Tirapegui, 2004).



Effect of tryptophan on animal behavior

Tryptophan is able to modulate behavioral aspects of laying hens, especially by reducing feather pecking, which is considered an initial factor to cause injuries and, consequently, cannibalism and death of the birds. (Birkl et al., 2019; Van Krimpen et al., 2005; Van Hierden et al., 2004).

The behavioral modulation exerted by tryptophan reflects positively on animal welfare. This effect occurs through serotonin which is involved in several

physiological functions such as, regulation of body temperature, food intake, sexual behavior, response to stimuli that cause fear, fight and stress behavior (Lucki, 1998). From 1 to 2% of the body's serotonin is produced in the serotonergic pathway in neurons in the brain, while approximately 95% of serotonin is produced, stored and released by cells in the intestinal mucosa known as enterochromaffin cells (Gershon & Tack, 2007).

Tryptophan and productive performance of laying hens

The requirement of tryptophan in laying hens may vary depending on several factors: age of the birds, nutritional composition of the diet, especially in relation to the concentration of long-chain neutral amino acids; mathematical models adopted in the studies; feed consumption; genetic lineage and others, so tryptophan recommendations for laying hens ranging from 0.15 to 0.23% are observed (Khattak et al., 2019; Mousavi et al., 2018; Dong et al., 2017; Rostagno et al., 2017; Peganova et al., 2003; Harms & Russel 2000; Coon & Zhang, 1999;

NRC, 1994). Studies carried out by Kattak & Helmbrecht (2019) and Peganova et al. (2003) demonstrate high laying rates, 97% and 84%, respectively, through supplementation of L-tryptophan in the diet. Wen et al. (2019) and Cardoso et al. (2014), evaluating different genetic strains and older hens, they observed a high laying rate, 81 and 94%, respectively, by adding L-Tryptophan in the diet. The highest egg production rate found by Cardoso et al. (2014) can be explained by the high level of tryptophan in the diet (Table 1).

Table 1. Tryptophan levels and effects on production parameters in laying hens

Genetic strain	Age, weeks	TRP dig.	Mathematical model	Effect	Reference
Lohmann Brown	31-37	0.15%	Broken-Line	Improved egg mass	Peganova et al. (2003)
Lohmann Brown	31-37	0.18%	Exponential	Improved egg mass	Peganova et al. (2003)
White laying hens	29-49	0.19%	Quadratic	Improved egg mass	Lima et al. (2012)
Dekalb White	60-76	0.19%	Quadratic	Improved egg mass	Cardoso et al. (2014)
Dekalb Brown	44-68	0.20%	Quadratic	Improved egg shell thickness	Moreira et al. (2018)
Lohmann Brown	22-28	0.25%	Linear	Improved egg production	Khattak & Helmbrecht (2019)
Hy-Line W36	41-46	182.3 mg/day	Quadratic Broken-Line	Improved egg production	Wen et al. (2018)
Hy-Line W36	22-34	183 mg/day	Quadratic Broken-line	Improved egg production	Sarsour et al. (2021)

Cardoso et al. (2014) determined the digestible tryptophan:lysine ratio (Trp: Lys) equal to 25.44% for maximum egg production in white Dekalb laying hens from 60 to 76 weeks of age, using a quadratic polynomial model. Meanwhile, the Hendrix Manual (2020) recommends Trp: Lys ratio equal to 22% in the same period. Rostagno et al. (2017) recommend a ratio of 23% for white and brown laying hens in the egg production phase.

Kattak & Helmbrecht (2019) evaluated increasing levels of digestible tryptophan (0.10 to 0.31%) in a corn and wheat based diet for brown laying hens in

the period of peak laying and observed an increase of 2.7% in the feed intake in hens fed diets containing 0.25% digestible tryptophan compared to hens fed diets formulated with 0.10% digestible tryptophan. In addition, these authors observed the effect of tryptophan on egg quality and the regression analysis revealed that the level of digestible tryptophan of 0.22% in the diet promotes better shell quality; thickness and density, corresponding to the digestible tryptophan: digestible lysine ratio equal to 27.5%.

The increase in egg production through L-tryptophan supplementation in the diet of laying hens must be related to the effect of tryptophan in improving gonadotropin release as well as improving protein availability (Dong et al., 2010).

According to Russell & Harms (1999) tryptophan levels below 0.13% cause a reduction in egg production and body weight in laying hens. In addition, special attention must be given to diets formulated with ingredients that have a high concentration of

long-chain neutral amino acids, such as isoleucine, valine and leucine, phenylalanine, tyrosine, since these amino acids influence the requirement of tryptophan (Peganova & Eder, 2002). According to Boa Ventura (2013), tryptophan is the second limiting amino acid, followed by threonine, in complex diets for commercial laying hens, formulated with animal products, which are rich in long-chain neutral amino acids.

Conclusions

L-tryptophan supplementation in the diet of laying hens is positively related to behavior, reducing aggression, feather pecking and cannibalism; performance parameters, improving feed consumption, egg production and quality; the antioxidant capacity, maximizing antioxidant activity at the cellular and enzymatic level; the balance of the intestinal microbiota, reducing the presence of pathogenic microorganisms.

Digestible tryptophan levels up to 0.22% and digestible Trp: Lys ratio up to 27.5% have been effective in increasing egg production and improving egg quality, based on the results obtained by Katthak & Helmbrecht (2019).

Reference

1. Mallin, M.A.; Cahoon, L.B. Industrialized animal production—a major source of nutrient and microbial pollution to aquatic ecosystems. *Popul. Environ.* 2003, 24, 369–385, doi:10.1023/A:1023690824045.
2. Bai M, Liu H, Xu K et al. A review of the immunomodulatory role of dietary tryptophan in livestock and poultry. 2017; 49: 67-74.
3. Bello AU, Idrus Z, Meng GY et al. Gut microbiota and transportation stress response affected by tryptophan supplementation in broiler chickens. *Ita. J. of Ani. Sci.* 2018; 17: 107-113.
4. Bertechini AB. *Nutrição de Monogástricos*. Lavras: Editora UFLA, 2012. 373p.
5. Birkel P, Chow J, McBride P et al. Effects of acute tryptophan depletion on repetitive behavior in laying hens. *Fron. Vet. Sci.* 2019; 6: 230-7.
6. Boa Ventura PV. *Determinação de aminoácidos limitantes em dietas complexas para poedeiras comerciais*. Dissertação (Mestrado em Zootecnia). 2013. 65f. Universidade Estadual Vale do Aracajú, Sobral-CE.
7. Calderano AA, Gomes PC, Lelis GR et al. Digestible tryptophan-to-digestible lysine ratio in diets for laying hens of 42 to 58 weeks of age. *Revi. Bras. Saud Prod. Ani.* 2016; 17: 139-148.
8. Coon G, Zhang B. Ideal amino acid profile for layers examined. *Feed.* 1999; 71: 13-15.
9. De Hass EM, Van Der Eijk JAJ. Where in serotonergic system does it go wrong? Unravelling the route by which the serotonergic system affects feather pecking in chickens. *Neur. Bio. Rev.* 2018; 95: 170-88.
10. Dong X, Azzam M, Rao W et al. Evaluating the impact of excess dietary tryptophan on laying performance and immune function of laying hens reared under hot and humid summer condition. *Brit. Poult. Sci.* 2012; 53: 491-6.
11. Dong X, Zou X. Effects of excess dietary tryptophan on laying performance, antioxidant capacity and immune functions of laying hens. In: *Amino Acid – New Insights and Roles in Plant and Animal*, Toshiki Asao, ed. InTech, ISBN: 978-953-51-3242-4. 2017. DOI: 10.5772/intechopen.68546
12. Dong Y, Bin Z, Wei R et al. Effects of dietary tryptophan levels on performance and egg quality of laying hens. *J. Ani. Nut.* 2010; 22: 1265-70.
13. Ducey P, Karsenty G. 2010. The two faces of serotonin in bone biology. *J. Cell Biol.* 2010; 19:7-13.
14. Gao J, Xu K, Liu H et al. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. *Front. Cell. Infect. Mic.* 2018; 8:13. DOI: 10.3389/fcimb.2018.00013
15. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gast.* 2007; 132: 397-414.
16. Harms RH, Russel GB. Evaluation of tryptophan requirement of the commercial layer by using a corn-soybean meal basal diet. *Poult. Sci.* 2000; 79: 740-2.
17. Jiang SQ, Gou ZY, Lin XJ et al. Effects of dietary tryptophan levels on performance and biochemical variables of plasma and intestinal mucosa in yellow-feathered broiler breeders. *J. Ani. Phy. Ani. Nut.* 2018; 102: e387-e394.
18. Khattak F, Helmbrecht A. Effect of different of tryptophan on productive performance, egg quality, blood biochemistry, and cecal microbiota of hens housed in enriched colony cages under commercial stocking density. *Pou. Sci.* 2019; 98: 2094-2104.
19. Le Floch'h N, Melchior D, Seve B. Dietary tryptophan helps to preserve tryptophan homeostasis in pigs suffering from lung inflammation. *J. Ani. Sci.* 2008; 86: 3473-79.
20. Le Floch'h N, Seve B. Biological roles of tryptophan and its metabolism: potential implications for pig feeding. *Liv. Sci.* 2007; 112: 23-32.
21. Lima MR, Costa FGP, Guerra RR et al. Digestible tryptophan: lysine ratio for laying hens. *Revi. Bra. Zoo.* 2012; 41: 2203-10.
22. Lucki I. The spectrum of behaviors influenced by serotonin. *Biol. Psy.* 1998; 44: 151-162.
23. Martin CL, Duclos M, Aguerre S et al. Corticotropin and serotonergic responses to acute stress with/without prior exercise training in different rat strains. *Acta Physio. Scand.* 2000; 168:421-30. DOI: 10.1046/j.365-201x.2000.00683.x

Reference

24. Moreira TRS, Oliveira LFA, Cruz GFL et al. Relação de triptofano com aminoácidos ramificados para poedeiras semipesadas. In: 55ª Reunião Anual da Sociedade Brasileira de Zootecnia e 28º Congresso Brasileiro de Zootecnia. Goiânia-GO, Brasil. 2018.
25. Mousavi SN, Afsar A, Khalaji S et al. Estimation of digestible tryptophan: lysine ratio for maximum performance, egg quality and welfare of white-egg-laying hens by fitting the different non-linear models. *J. Appl. Anim. Res.* 2018; 46:411-6.
26. NRC. 1994. National Research Council. Nutrient Requirements of Poultry: Ninth Revised Edition, Natl. Acad. Press, Washington, DC. <https://doi.org/10.17226/2114>.
27. Pan X, Wei Z, Wang H et al. Effects of dietary tryptophan on protein metabolism and related gene expression in Yangzhou goslings under different feeding regimens. *Pou. Sci.* 2013; 92: 3196-3204.
28. Peganova S, Eder K. Studies on requirement and excess of isoleucine in laying hens diets. *Pou. Sci.* 2002; 81: 1714-21.
29. Peganova S, Hirche F, Eder K. Requirements of tryptophan in relation to the supply of large neutral amino acids in laying hens. *Poult. Sci.* 2003; 82: 815-22.
30. Raju T, Kanth VR, Reddy PUM et al. Influence of kynurenines in pathogenesis of cataract formation in tryptophan-deficient regimen in Wistar rats. *Indian J. Expe. Biol.* 2007; 45: 543-8.
31. Rostagno HS; Albino LFT, Hannas MI, et al. Tabelas Brasileiras para Aves e Suínos. 4rd. UFV. 2017.
32. Sallée M, Dou L, Cerini C et al. The aryl hydrocarbon receptor-activating effect of uremic toxins from tryptophan metabolism: a new concept to understand cardiovascular complications of chronic kidney disease. *Tox.* 2014; 6:934-49.
33. Santin E, Ahrens NL, Zanella I. Diferentes níveis de ácido fólico e nicotínico em dietas para frangos de corte. *Ciê. Rur.* 2000; 30: 681-5.
34. Sarsour AH, Lee JT, Haydon K et al. Tryptophan requirement of first-cycle commercial laying hens peak egg production. *Poult. Sci.* 2021; 100: 100896. DOI:10.1016/j.psj.2020.11.065
35. Sève B. Physiological roles of tryptophan in pig nutrition. In: Huether G et al. Tryptophan, serotonin, and melatonin: basic aspects and applications. New York: Kluwer Academy. 1999: 729-41.
36. Silva AL, Saraiva EP, Gomes DLS et al. Efeito de diferentes relações triptofano digestível: lisina digestível sobre o peso de órgão e morfometria intestinal de poedeiras leves. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 47, 2010. Salvador. Anais... Salvador: Sociedade Brasileira de Zootecnia, 2010. (CD-ROM).
37. Stone T, Stoy N, Darlington LG. An expanding range of targets for kynurenine metabolites of tryptophan. *Cel. Pre.* 2013; 34: 136-143.
38. Van Hierden YM, Koolhaas JM, Korte SM. Chronic increase of dietary L-Tryptophan decreases gentle feather pecking behavior. *App. Ani. Beh. Sci.* 2004; 89: 71-84.
39. Van Krimpen MM, Kwakkel RP, Reuvekamp BFJ et al. Impact of feeding management on feather pecking in laying hens. *Wor. Pou. Sci. J.* 2005; 61: 663-86.
40. Wen H, Feng L, Jiang W et al. Dietary tryptophan modulates intestinal immune response, barrier function, antioxidant status and gene expression. Of TOR and Nrf2 in young grass carp (*Ctenopharyngodonidella*). *Fish & Shel. Imm.* 2014; 40: 275-87.
41. Wen J, Helmbrecht A, Elliot MA. Evaluation of the tryptophan requirement of small-framed first cycle laying hens. *Pou. Sci.* 2018; 0: 1-9.
42. Wu G, Bazer FW, Dai Z et al. Amino acid nutrition in animals: Protein synthesis and beyond. *Ann. Ver. Ani. Bio.* 2014; 2: 387-417.
43. Wu, G. Functional amino acids in growth, reproduction, and health. *Adv. Nut.* 2010; 1: 31-7.