AMINONews®

C. Bortoluzzi*; J.I.M. Fernandes†; K. Doranalli‡; T.J. Applegate*
*Department of Poultry Science, University of Georgia, Athens, GA, USA
†Department of Animal Sciences, Federal University of Parana, Palotina, PR, BR
‡Evonik Nutrition & Care GmbH, Hanau, Germany.

How could dietary amino acids ameliorate the intestinal endogenous losses due to enteric challenges in broiler chickens? A review

KEY INFORMATION

Some of the important roles that amino acids play on maintaining and ameliorating the function of the gastrointestinal tract in broilers were discussed. It is evident that there is a lack of information regarding the losses of endogenous amino acids caused by enteric infections. However, from the available literature, it is possible to formulate some hypothesis which may drive further studies aiming towards a better understanding of the functions of the dietary amino acids and their metabolism within the gastrointestinal tract of broiler chickens submitted to various conditions. Some of the questions that remain unanswered include:

- Does coccidiosis alter the immune and metabolic pathways of enterocytes in order to use dietary amino acids more efficiently to cope with the infection?
- Do enteric infections increase the use of dietary amino acids by the intestinal cells because of:
    - Higher tissue/body need?
    - Compensatory for the lower digestibility?
    - Similar tissue needs but lower feed intake?
- Do broilers have the same intestinal metabolism rate of amino acids, mainly threonine and methionine, as piglets?
- Do amino acids counteract the negative effects of enteric infections by changing the metabolism of enterocytes in order to reduce the endogenous losses of amino acids?
- Is tryptophan metabolized by the intestinal microbiota of chickens leading to the production of metabolites with beneficial actions on the host?

The true cost of enteric diseases in broilers must be determined in order to formulate diets with more precise concentrations of amino acids to maximize growth while meeting the requirements of the immune system and the intestinal microbiota. Clearly, the intestine demands a large amount of energy and amino acids for its maintenance, which may be increased during enteric diseases, because, besides inflammation, the dysbiosis generated in the intestine may also increase the use of nutrients, and their endogenous losses into the gastrointestinal tract.

SUMMARY

Even though little is known about the effects of enteric infections such as coccidiosis and necrotic enteritis on the endogenous amino acids (EAA) losses within the gastrointestinal tract (GIT) of broilers, more information is available on the effects of these diseases on the apparent ileal digestibility (AID) of amino acids. There are many factors that must be considered when attempting to determine the intestinal flow of endogenous amino acids, including age of the birds, strain, presence/absence of a pathogen, intestinal metabolism of amino acids, among others. The GIT and the liver

share the responsibility of releasing amino acids into the peripheral blood which are necessary to support protein synthesis. In general, intestinal amino acid metabolism reflects a very active organ

system that will meet its own requirement for amino acids first, and then will delivery amino acids for the rest of the organism. Higher concentrations of some amino acids from highly available sources may help compensate for malabsorption during periods of intestinal challenge and by minimizing the EAA losses. For instance, threonine (Thr) is a major constituent of the mucins secreted into the intestine, and acts as a barrier to infections and antigen materials presented to the gut; Glutamine (Gln) is regarded as a conditionally essential nutrient under stress conditions; Arginine (Arg) enhances the intestinal barrier functions and vascular development and modulates the intestinal microbiota. Sulfur amino acids (SAA) and their metabolites are also of great interest for maintaining intestinal integrity and function of mainly rapid proliferating cells such as enterocytes. Tryptophan (Trp) has essential roles on the metabolism of the GIT function, mainly through the function of its metabolites including antioxidant, inhibition of pro-inflammatory cytokines, and improvement of the immune function. Therefore, the objectives of this review is to discuss: 1) the factors that influence EAA flow within the intestine with special emphasis on enteric infections; and 2) how the utilization and metabolism of amino acids by intestinal cells is affected by these infections and by dietary amino acids, with a main focus on Thr, Arg, Gln, SAA, and Trp.

INTRODUCTION

The concept of intestinal health is a broad topic. As proposed by Conway (1994), diet, mucosa, and the luminal microbiota are the major components when discussing gut health. Maintenance of the overall health of the intestine relies on a balance between these three factors, including the digestive epithelium and overlying mucus layer (Montagne *et al.*, 2003). As recently reviewed by Bortoluzzi *et al.* (2018) two main components help keep the integrity of the intestinal mucosa. The mucus layer serves as a protection between the luminal contents and the enterocytes, and the epithelial cells layer, which is in constant process of renewal and is the barrier between the “external” and “internal” environments. Enteric pathogens, such as bacteria and parasites, and their metabolites may perturb digestive functions, and lead to diarrhea, poor growth performance, and even death (Montagne *et al.*, 2003).

Endogenous amino acids losses are correlated with the health of the GIT and their estimation is of paramount importance to determine the standardized amino acid digestibility of feed ingredients. Yet, because not all nutrients in feedstuffs for poultry are available for growth and development, it is necessary to separate the total nutrients of a feed ingredient and the nutrients that are bioavailable for digestion and absorption (Adedokun *et al.*, 2011). Furthermore, EAA losses may be separated into two categories: basal and specific (Nyachoti *et al.*, 1997). The basal losses represent the amount of amino acids naturally lost by the intestine of an animal, while the specific losses refer to the amino acids that are lost depending on the dietary ingredient composition (Stein *et al.*, 2007). Therefore, when ingredients with highly digestible protein are used, the EAA losses are lower than when using ingredients rich in antinutritive factors such as fiber and phytate (Stein *et al.*, 2007).

As a consequence of enteric infections, the intestine will respond with increased mucus production and cell proliferation in an attempt to eliminate the pathogen and/or reestablish a homeostatic state. This condition will also exacerbate EAA losses originated from various digestive secretions, mucoproteins, and desquamated epithelial cells from the lining of the gut (Ravindran and Hendriks, 2004). The losses of EAA and their measurement may vary depending on the presence/absence and concentration of the amino acid in the diet, the age of the birds, the strain of the chicken (Adedokun *et al.*, 2011), and the presence/absence of pathogens.

For instance, the dynamic nature of synthesis and degradation of mucin, a protein produced by goblet cells in the intestine and rich in the amino acids Thr, serine (Ser), and proline (Pro; Lien *et al.*, 2001), is dependent upon the presence of feed in the GIT (Smirnov *et al.*, 2004), the ingredients and supplemental enzymes used in the diet (Sharma *et al.*, 1997), and sanitary conditions (Corzo *et al.*, 2007). Therefore, it has been difficult to establish a standardized

method to measure EAA losses among different laboratories (Adedokun *et al.*, 2011) which may impose further challenge on the determination of these losses in challenged chickens and how different nutrients and feed additives may interfere with the flow of amino acids into the intestine.

The GIT and the liver share the responsibility of releasing amino acids into the peripheral blood that is necessary to support protein synthesis. In general, the intestinal amino acid metabolism reflects a very active organ system that will meet its own requirement for amino acids first, and then will uptake amino acids for the rest of the organism (Baracos, 2004). These observations make sense as the appearance of amino acids in the portal system is different in quantity and proportion from the amino acids that are being absorbed by the intestine (Baracos, 2004). Enteric diseases in poultry may not only affect the flow of EAA in the intestine, but also modify their metabolism in the intestinal epithelial cells. Moreover, how these cells utilize the amino acids provided by the diet has further consequences on their distribution among other tissues of the body. Therefore, increasing dietary concentrations of highly digestible amino acids in the diet may compensate for periods of intestinal challenge whereby the digestive and absorption processes may be impaired (Bortoluzzi *et al.*, 2018), even though it has to be considered that the intestine is also regulating the rate of amino acids utilization and their potential toxic effects (Baracos, 2004).

The objective of this review is to discuss the factors that influence EAA flow in the intestine with a special emphasis on enteric infections (coccidiosis and necrotic enteritis, NE), and how the utilization and metabolism of amino acids by the intestinal cells may be affected by these infections. Additionally, due to their functional role in ameliorating the intestinal function, insights regarding the effects of Thr, Arg, Gln, sulfur-amino acids (SAA), and Trp and how their supplementation could diminish the impact of enteric diseases on the EAA losses of amino acids will be provided.

CONSIDERATIONS ON INTESTINAL ENDOGENOUS LOSSES CAUSED BY ENTERIC INFECTIONS

Coccidiosis and NE are considered the most significant health problems in broilers in the southeastern region of the United States (Hofacre *et al.*, 2018), and most likely worldwide. Necrotic enteritis, caused by the bacterium Clostridium perfringens, is often associated with coccidiosis, caused by parasites of the genus Eimeria; thus, the control of coccidiosis may significantly reduce the incidence of clinical cases of NE in broiler flocks (Smith, 2018). Not only subclinical NE but also dysbacteriosis may be an issue when implementing different programs to control coccidiosis, such as vaccination and chemical coccidiostats programs (Smith, 2018). As stated by Hofacre *et al.* (2018) the success in the prevention of both subclinical and clinical NE must rely on the reduction of intestinal damage, overgrowth of pathogenic C. perfringens, and maintenance of a healthy microbiota. Therefore, information must be available to nutritionists who formulate diets without using antimicrobial drugs to help understand the extent of intestinal losses of amino acids caused by these diseases, and how different nutritional approaches can help birds cope with such infections.

Most of the studies determining EAA losses in poultry have been conducted with birds raised in battery cages; however, chickens raised on litter have more contact with bacteria (pathogenic or commensal) that modify mucus secretion, epithelial turnover, and passage rate (Adedokun *et al.*, 2011). Early studies have demonstrated that the simple presence of the microbiota increases the epithelial turnover rate 1.7 times in the duodenum of conventional vs. germ-free chickens (Cook and Bird, 1973). Fernando and McCraw (1973), however, reported that the presence of Eimeria acervulina infection in chickens increased the epithelial turnover in 3.1, 1.9, and 1.9 times in the duodenum, jejunum, and ileum, respectively. Noteworthy, higher turnover rate in the duodenum compared to jejunum and ileum would be expected as E. acervulina infects the duodenum. Other Eimeria species which infect other portions of the GIT, will most likely also lead to higher cell regeneration in the jejunum and ileum, for example.

Even though little is known about the effects of intestinal infections such as coccidiosis and NE and their effects on EAA losses, more information is available on the impact of coccidiosis on the AID of amino acids. Rochell *et al.* (2016)

reported that with the exception of glycine (Gly) and Trp, E. acervulina infection reduced AID of all amino acids in broilers (Table 1).

Table 1: Effect of E. acervulina dosage inoculation in 21-d-old broiler chickens fed a regular diet, and effect of coccidia infection to 21-d-old broiler chickens fed low or high supplemental amino acid diets on the apparent ileal digestibility of amino acids. Adapted from Rochell *et al.* (2016, 2017b).

	E. acervulina oocysts per bird[1]				Unifected		Infected[2]	
	0	2.5 x 10^5	5.0 x 10^5	1.0 x 10^6	Low AA	High AA	Low AA	High AA
Indispensable amino acids								
Arg	90.3	89.7	88.3	88.5	89.5	89.4	85.3	85.2
His	86.3	85.6	83.7	84.4	85.5	85.8	80.7	80.3
Ile	85.2	83.9	81.6	81.9	83.4	83.9	76.1	75.8
Leu	87.1	85.8	83.8	83.8	85.4	85.4	77.9	77.6
Lys	88.0	87.0	84.9	85.2	86.7	88.3	81.2	81.4
Met	93.6	92.7	91.1	91.2	92.6	92.9	87.4	86.2
Phe	86.9	85.7	83.7	83.8	85.1	87.3	78.2	77.7
Thr	79.7	78.3	75.7	76.5	77.0	79.1	70.1	71.7
Trp	88.0	88.9	88.3	89.7	87.1	89.3	87.7	89.6
Val	83.9	82.5	80.0	80.5	82.0	82.6	74.4	74.4
Dispensable amino acids								
Ala	85.6	84.2	81.5	81.6	84.1	84.3	75.8	75.3
Asp	82.3	81.4	79.1	79.7	81.1	81.5	74.9	74.4
Cys	73.2	71.3	67.6	68.1	72.3	71.1	63.0	58.2
Glu	89.2	88.5	86.7	86.8	88.2	88.1	82.6	81.8
Gly	79.3	78.8	76.5	77.4	78.0	78.7	72.5	72.6
Pro	85.0	84.2	82.4	82.6	83.1	83.2	76.8	76.8
Ser	82.4	81.3	78.1	79.4	80.6	81.5	73.9	74.5
Tyr	85.4	84.5	82.3	83.0	84.6	85.0	79.0	79.0

[1] Challenged birds were gavaged with the respective dose of sporulated oocysts of E. acervulina on d 15 post-hatch.
[2] Challenged birds were gavaged with 6.33 x 105 sporulated oocysts of E. acervulina on d 15 post-hatch.

In another study (Table 1), E. acervulina infection decreased AID of most of the amino acids without many effects of high supplemental amino acids (Rochell *et al.*, 2017b). The results from these two studies clearly show that Eimeria infection impairs the digestive process and in turn reduces the performance of the birds. Additionally, both studies by Rochell did not account for EAA losses occurred due to the infection, which is expected to be higher in infected chickens as a result of increased leakage of plasma proteins into the lumen (Prescott *et al.*, 2016), increased mucogenesis (Collier *et al.*, 2008), and higher epithelial turnover rate (Fernando and McCraw, 1973; Gottardo *et al.*, 2016). Moreover, a measure of the EAA losses attributed to coccidiosis and/or NE would be valuable in order to formulate diets with more optimal amino acids concentrations when necessary.

The move from formulating diets on total amino acids basis to digestible amino acids, especially when EAA losses are considered, is essential to maximize the use of ingredients and reduce excretion of nutrients into the environment (Ravindran and Hendriks, 2004; Adedokun *et al.*, 2016). Adedokun *et al.* (2016) studied the effect of a coccidiosis vaccine on the ileal EAA losses and standardized ileal amino acid digestibility (SIAAD) in broilers on d 21 and 42. The

authors observed that coccidiosis challenge increased the EAA losses on d 42, but not on d 21. On the other hand, SIAAD was reduced by the challenge only on d 21 while it increased with age in challenged birds. Furthermore, it was observed that the challenge reduced the AID (without correction for EAA losses) of most of the indispensable amino acids on d 21, without any effect on d 42; yet, the challenge decreased AID and total utilization of dry matter (DM), nitrogen, and energy only on d 21 (Adedokun *et al.*, 2016). Thus, it is clear that the determination of EAA losses may change the results and interpretation of the data.

A point to be taken into consideration is the fact that Eimeria infection usually leads to a reduced feed intake (FI; Kipper *et al.*, 2013; Rochell *et al.*, 2016, 2017b; Adedokun *et al.*, 2016). Indeed, different Eimeria species differentially affect FI as demonstrated in a meta-analysis by Kipper *et al.* (2013). For instance, the reduction in FI should be compensated with an increase in feed/nutrient intake by 4.3, 5.9, and 2.2% in chickens challenged with E. acervulina, E. maxima, and E. tenella, respectively. Nevertheless, it can be assumed that the FI is also influencing EAA losses (Adeola *et al.*, 2016), which could explain the results obtained by Adedokun *et al.* (2016), wherein the challenge reduced FI on d 21 and numerically decreased the EAA losses but did not reduce FI on d 42 when the EAA losses were higher in challenged birds. Therefore, two explanations for the findings of Adedokun *et al.* (2016) could be elaborated. First, lower FI would lead to a decreased secretion of endogenous digestive enzymes and mucus (Adedokun *et al.*, 2011) and consequently lower EAA losses. Secondarily, lower FI could lead to a lower state of intestinal inflammation, known as feed-induced immune response (FIIR) that may increase the expenditure of nutrients (Kogut, 2017) in modern strains of broiler chickens.

Even though studies relating FI, intestinal inflammation, and EAA losses are scarce, the excess of nutrient intake such as fat and carbohydrates may lead to metabolic inflammation, including the inflammation of intestinal tissues (Kogut *et al.*, 2018), that could increase the flow and waste of amino acids and influence the measurements of amino acid digestibility. This phenomenon may be linked to the modern genetic/management practices of growing broilers that show a higher incidence of metabolic diseases as a result of chronic low-grade inflammation (Kogut *et al.*, 2018). Indeed, the excess of nutrients from diets can be recognized by immune receptors (Arsenault *et al.*, 2017) and induce chronic inflammation. Therefore, the type of the bird used for digestibility estimations must also be taken into consideration due to the differences of digestive processes. For instance, the flow of endogenous nitrogen and total amino acids did not differ between broilers, roosters, and layers, but, the flow of specific amino acids such as Ser, glutamic acid, proline, alanine, isoleucine, tyrosine, Arg and methionine differed between these classes of birds. Regardless of the bird type, glutamic acid, followed by aspartic acid, proline, Ser, Gly and Thr were the most abundant amino acids in the endogenous protein (Ravindran and Hendriks, 2004). The authors attributed the differences in the EAA flow to different physiological status among these three classes of chickens (28 d old broilers vs. 70-wk-old roosters and layers), and to the differences in the digestive and absorptive processes between broilers, layers, and roosters (Ravindran and Hendriks, 2004). However, data regarding FI of the birds is not presented which could also help draw further conclusions. Still, it would be important to determine the differences in EAA losses in different classes of birds (broilers, roosters, and layers) when subjected to an Eimeria challenge.

Factors inherent to the diet can also affect the flow of EAA in the intestine such as protein and amino acid concentration, fiber, and phytate (Adedokun *et al.*, 2011). For instance, low quality protein may be a stimulus for higher secretion of enzymes by the pancreas, which undergoes continuous enzymes secretion. Even though these secreted enzymes may be a substrate for microbial fermentation in the lower intestine, much of it will represent EAA losses (Siriwan *et al.*, 1993). Dietary soluble fiber is also recognized to increase intestinal transit time, delay gastric empty and glucose absorption, increase pancreatic secretion, and slow absorption which enhances DM flow and endogenous losses, leading to decreased nutrient digestibility (Montagne *et al.*, 2003). Nevertheless, increasing concentration of phytic acid in the diet also increases the losses of EAA and nitrogen in the terminal ileum of chickens, and the inclusion of phytase minimizes this effect (Cowieson and Ravindran,

2007). Therefore, all these factors must be considered when analyzing the EAA flow in the intestine to improve the consistency of data generated regarding the digestibility of specific amino acids, which will allow the formulation of diets that better meet the nutritional requirement of the broiler chicken during specific situations. Further insights into the role of some amino acids in ameliorating the EAA losses in broiler chickens raised under challenge and non-challenged conditions will be provided later on.

AMINO ACIDS AND INTESTINAL FUNCTION

The function of amino acids in maintaining the integrity and function of the intestine has been frequently discussed in poultry and other animals (Kidd and Kerr, 1996; Wang *et al.*, 2009; Bortoluzzi *et al.*, 2018). Of particular interest are the amino acids known for having effects on metabolic functions such as those involved in the proper functioning of the immune system and in the intestinal mucosal repair processes (Bequette, 2003). Higher concentration of some amino acids, from highly available sources, may help compensate for malabsorption during periods of intestinal challenge (Bortoluzzi *et al.*, 2018). For example, specific situations may increase the dietary requirement of specific amino acids as shown by Corzo *et al.* (2007) in the case of Thr. Indeed, Thr may have its use increased during enteric challenge since it is major constituent of the mucins secreted into the intestine which act as a barrier to infections and antigen materials presented to the gut (Bequette, 2003). Mucins are generally resistant to digestion and so endogenous losses of this amino acid might represent a waste path (Bequette, 2003). Glutamine, a nonessential amino acid, is regarded as a conditionally essential nutrient under stressful conditions (Wang *et al.*, 2009), and has been shown to reduce the severity of NE lesions in broiler chickens (Xue *et al.*, 2018). Arginine has fundamental roles in many metabolic and physiologic pathways of the intestine (Gottardo *et al.*, 2016; Rochell *et al.*, 2017b), on the enhancement of intestinal barrier functions and on vascular development (Wang *et al.*, 2009). Zhang *et al.* (2018) have recently demonstrated the role of Arg in normalizing the ileal microbiota of chickens challenged with C. perfringens in terms of microbial composition and function.

Sulfur amino acids (Methionine (Met) and Cysteine (Cys)) and their metabolites are also of great interest for maintaining intestinal integrity and function, and therefore may reduce the endogenous losses of amino acids within the intestine. For instance, the metabolism of Met and Cys produces glutathione (GSH), homocysteine (Hcy), and taurine (Tau; Wang *et al.*, 2009), and may be critical for proper functioning of rapid proliferating cells such as enterocytes (Shaw and Chou, 1986; Wu, 2009). In a work by Stoll *et al.* (1998) it has been shown that only 48% of the Met, and 38% of Thr intake was recovered in the portal blood of piglets, meaning that 52, and 62% of each amino acid, respectively, was metabolized in the intestine before being absorbed, either by the enterocytes or by the microbiota. Tryptophan is also regarded for having essential roles on the metabolism of the GIT function, mainly through the function of its metabolites (Keszthelyi *et al.*, 2009). Serotonin, N-acetylserotonin (NAS), melatonin, and anthranilic acid (ANS) are the main products derived from the metabolism of Trp, and possess a wide array of functions, including antioxidant, inhibition of pro-inflammatory cytokines, and improvement in immune functionality (Wu, 2009). Recent work published by Liang *et al.* (2018) showed that Trp supplementation for piglets modulated the intestinal microbiota by reducing the abundance of opportunistic pathogens, increasing beneficial groups of bacteria, and enhancing the microbial diversity of the hindgut. Yet, Trp supplementation suppressed the expression of the pro-inflammatory cytokine, IL-8, in the colonic tissue of piglets, which may represent a lessened inflammatory response and, therefore, lower endogenous losses. In laying hens, Trp showed positive effects by modulating the cecal microbiota towards an increase in beneficial groups of bacteria such as Lachnospiraceae (Khattak and Helmbrecht, 2019).

The metabolic functions of dietary essential amino acids used by intestinal tissues influence their availability for growth, and their requirement (Stoll, 2006). The amino acid metabolism in the intestine of piglets is high (Table 2), and it would be useful to know if poultry species have the same rate of metabolism.

Table 2: Effect of E. acervulina dosage inoculation in 21-d-old broiler chickens fed a regular diet, and effect of coccidia infection to 21-d-old broiler chickens fed low or high supplemental amino acid diets on the apparent ileal digestibility of amino acids. Adapted from Rochell *et al.* (2016, 2017b).

Amino acid	Percentage of dietary intake	Amino acid	Percentage of dietary intake
Arginine	147	Cysteine	69
Histidine	71	Glutamate	3
Isoleucine	66	Glutamine	-16
Leucine	64	Glycine	69
Lysine	55	Serine	66
Methionine	69	Tyrosine	71
Phenylalanine	63		
Proline	59		
Threonine	50		
Tryptophan	75		
Valine	65		
Alanine	154		
Asparagine	74		
Aspartate	5		

According to Stoll *et al.* (1998) there are three possible explanations for the fact that less amino acids appear in the portal blood vs. their disappearance from the lumen. First, oligopeptides produced during digestion could be absorbed instead of free amino acids; second, the removal of mesenteric arterial amino acids by the portal-drained viscera could underestimate the quantity of dietary amino acids that appear in the portal blood; and the third explanation relates to the possibility of catabolism of amino acids in the enterocytes, which could contribute to amino acids deficiency. Indeed, Stoll *et al.* (1998) found that, on average, one third of the amino acids ingested by animals are metabolized by the intestinal mucosa, which reiterates the importance of amino acids as sources of energy for intestinal epithelial cells. As a result, the metabolism of many essential and nonessential amino acids generates metabolites that are important for overall metabolic functions, such as nitric oxide (NO) from Arg, betaine, choline, and Tau from Met, among others, as reviewed by Wu (2009).

The differences in apparent digestibility of various protein sources among diets is greatly influenced by the rate of EAA losses (Stoll *et al.*, 1998). Besides the aforementioned factors that may impact EAA losses in the intestine, the differences in metabolism of specific amino acids by the intestinal epithelial cells and luminal microbiota must be considered. There is a positive correlation between the catabolism of amino acids during their first-pass through the intestine and the intestinal mass (Stoll *et al.*, 1998). Factors that affect the mass of the intestine such as inflammation, may exert an important influence on the dietary requirement of amino acids (Wu, 1998), on the digestibility, and on EAA losses. Because of the beneficial role of amino acids in protecting intestinal tissues against injuries, it is reasonable to argue that they are able to mitigate, direct or indirectly, the losses of EAA stimulated by enteric challenges. From this point forward, we will focus on Thr, Arg, Gln, SAA, and Trp, their metabolism within the intestinal cells, and their effects in maintaining the intestinal integrity and possibly reducing the EAA losses in the intestine.

Threonine

The incorporation of Thr into endogenous secretions that may be fermented in the lower intestine represents a nutritional loss potentially exacerbated if the amino acid is also oxidized by the mucosal cells to provide energy (Stoll, 2006). A technique that measures the differences in amino acid intake and their appearance in the portal blood has been utilized

to determine the usage of amino acids by the GIT in piglets (Stoll., 1998). It has been shown that 62% of the Thr intake is retained by the intestine on the first pass and 90% of the metabolized Thr is catabolized or secreted as mucin (Stoll *et al.*, 1998), which shows the high demand of Thr by the GIT of animals. Threonine is typically classified as the third most limiting amino acids in diets of broiler chickens after Met and Lysine (Lys; Corzo *et al.*, 2007). The requirement of Thr is high when compared to other amino acids mainly because of its secretion in the form of intestinal mucins (Fernandez *et al.*, 1994). Thus, the demand for Thr by the intestinal tissue may be higher in older birds, as the mass of the GIT increases, and in situations where the synthesis of mucin is increased such as challenge conditions or when using diets with high content of fiber (Montagne *et al.*, 2003). The digestibility of Thr is often lower than the average digestibility of protein and other amino acids (Rochell *et al.*, 2016, 2017b; Adeola *et al.*, 2016) which may be explained by the losses of Thr into the GIT that is usually higher compared to other amino acids (Ravindran *et al.*, 2004; Golian *et al.*, 2008; Soleimani *et al.*, 2010), and underestimates the concentration of the absorbed Thr by the intestine.

Threonine is an essential amino acid for broilers (Kidd and Kerr, 1996). The effect of Thr in improving the intestinal function could indirectly minimize the endogenous losses of amino acids and improve their digestibility. A study by Faure *et al.* (2005) aimed at looking at the effects of Thr restriction on the production of mucins and total intestinal mucosal proteins in rats. It was observed that rats fed 30% of the dietary requirements of Thr showed a drastic reduction in mucin synthesis in all evaluated intestinal sections; yet, no effects of the Thr reduction were observed on the total mucosal protein synthesis. The authors explain that this effect occurred at the translational level because the mRNA levels of MUC2 and MUC3 (the main genes involved in mucin production) were similar among treatments, and no compensatory mechanism operated to mobilize Thr from the body to supply the intestine was observed (Faure *et al.*, 2005). Beyond its functions in protein synthesis, studies have demonstrated the possibility of using Thr in diets to manipulate the different components of the immune system (Wang *et al.*, 2006; Faure *et al.*, 2007; Zhang *et al.*, 2014, 2016, 2017). It has been demonstrated that Thr supplementation has beneficial effects on both humoral (Zhang *et al.*, 2014) and innate immunity (Zhang *et al.*, 2017), mainly by modulating the expression of proinflammatory cytokines and immunoglobulins, while Thr deficiency impaired BW gain and increased Eimeria oocyst shedding by impairing intestinal morphology, intestinal integrity, and immune functionality (Zhang *et al.*, 2016a).

Star *et al.* (2012) showed that the requirement of Thr increased in broiler chickens induced to a subclinical NE (0.63 in uninfected vs. 0.67 or 0.69 of Thr:Lys ratio in infected chickens), even though higher dietary Thr concentration did not prevent the incidence and severity of the infection. Similarly, Chen *et al.* (2018) showed that Thr supplementation was able to attenuate the inflammatory response induced by an LPS injection in broiler chickens to enhance the intestinal barrier function by increasing villus height in the jejunum and ileum; and to upregulate the expression of tight junction proteins in the ileum. Despite the lack of data regarding the influence of Thr supplementation on amino acid digestibility and EAA losses, one could assume that the beneficial effects of supplementing higher concentration of Thr to broiler diets also rely on the improvement of the micro-architecture of the intestine. For instance, while supplementing extra amounts of amino acids to chickens challenged with a coccidiosis vaccine, Adedokun *et al.* (2016) observed beneficial effects on birds' performance, even though DM matter and nitrogen digestibility, and metabolizable energy of the diet were not restored by the supplementation. Nevertheless, Thr supplementation increased villus height and number of goblet cells in the jejunum and ileum (Chen *et al.*, 2017), and stimulated the proliferation of enterocytes in the jejunum of chickens (Gottardo *et al.*, 2016).

Additionally, in a study by Wils-Plotz *et al.* (2013) an increased concentration of Thr (1.8 vs. 5.3 g/kg) had beneficial effects on overall growth performance of broiler chickens under E. maxima challenge, and downregulated the expression of proinflammatory cytokines in the cecal tonsils and ileal mucosa. Yet, these authors reported that the deleterious effect of the challenge was more evident when the birds were supplemented with 0.7% of soluble fiber (pectin), most likely due to its antinutritional effects in increasing the viscosity of the intestinal digesta. These results corroborate the fact that infection and fiber contribute towards an increment in the losses of EAA, and higher concentrations of amino acids such as Thr are necessary to mitigate these effects. Nonetheless, it would be useful to have results of amino acid digestibility

in similar studies as the one conducted by Wils-Plotz *et al.* (2013). As a conclusion, sanitary conditions in which the chickens are raised, immune stimulation, and dietary composition, i.e., presence of antinutritional factors, may negatively influence the digestibility of dietary protein and increase EAA losses, and Thr may help overcome these effects.

Arginine

The requirements of Arg in broiler chickens are considerably variable depending on the growth rate, strain, and sanitary conditions. As the urea cycle is not functional in birds they are incapable of synthesizing de novo L-Arg (Sung *et al.*, 1991), and therefore Arg has to be supplied through the diet. Birds lack the enzyme carbamoyl phosphate synthetase which catalyzes the first step of ammonia detoxification and leads to the production of urea and Arg. Birds also depend on the supplementation of dietary Arg for the formation of ornithine, which in mammals is obtained from glutamic acid (D'Mello, 2003). The synthesis of ornithine is essential for the formation of proline and polyamines (spermidine, spermine, and putrescine), that are directly associated with growth and cellular differentiation of the intestine (Fernandes and Murakami, 2010), and are responsive to dietary Arg. Low production of polyamines from Arg inhibits proliferation, migration, and apoptosis of intestinal cells (Fernandes and Murakami, 2010). As such, Arg is considered a trophic agent that stimulates intestinal development (Uni *et al.*, 1998).

In the enterocytes of pigs, Gln can be used as precursor for the formation of Arg (Wu and Morris, 1998), which does not occur in the chick enterocyte due to the lack of the enzyme pyrroline-5-carboxylate (P-5-C) and ornithine carbamoyltransferase (OAT) which elucidates the mechanism for the nutritional requirement for Arg in broilers (Wu *et al.*, 1995). Stoll *et al.* (1998) demonstrated that the appearance of Arg in the portal blood of piglets was superior from what was supplied by the diet, providing evidence for the important role of Arg synthesis in the intestine of young piglets (Flynn and Wu, 1996). On the other hand, Arg was substantially catabolized by the intestine of germ-free or conventional rats (Windmueller and Spaeth, 1976). These authors observed that only 60% of Arg was recovered intact in the blood, and the remaining was catabolized to generate Arg metabolites, essential for a well-functioning of the GIT. Although there are few publications discussing the metabolism of Arg in the intestine of chicks (Tamir and Ratner, 1963; Kadirvel and Kratzer, 1974; Wu *et al.*, 1995), this subject still remains understudied in poultry species, specially when considering the influence of Arg supplementation and its metabolism on intestinal EAA losses and on amino acids digestibility.

The endogenous losses of Arg have been demonstrated in non-challenged and coccidiosis challenged chickens (Ravindran *et al.*, 2004; Adedokun *et al.*, 2007, 2016; Soleimani *et al.*, 2010; Rochell *et al.*, 2016). Adedokun *et al.* (2007) showed that the ileal endogenous loss of Arg is highly influenced by the age of the chickens. Indeed, Arg flows in the ileal digesta was 437 on d 5, 156 on d 15, and 168 mg/kg of DM intake on d 21. Additionally, while looking at EAA losses due to coccidiosis, Adedokun *et al.* (2016)observed that the flow of ileal Arg was 634 vs. 463 mg/kg of DM intake (unchallenged and challenged birds, respectively) on d 21, and 434 vs. 834 mg/kg of DM intake (unchallenged and challenged birds, respectively) on d 42. Curiously, the loss of the other essential amino acids on d 21, except Met, remained unaltered or increased (in the case of Trp) due to coccidiosis infection. On the other hand, the AID of Arg, as most of the other essential amino acids, decreased in challenged birds on d 21, but not on d 42 (Adedokun *et al.*, 2016). The authors explain this phenomenon by the fact that on d 21 the birds are more susceptible to coccidiosis infection which is responsible for the destruction of the intestinal mucosa, for the reduction of the flow of EAA, and for the lowered AID of amino acids. On the contrary, on d 42, the infection did not cause a large destruction of the mucosa but increased the flow of EAA without affecting the AID measure, as it does not account for the endogenous losses. Another point to be considered is the FI that was decreased by the challenge on d 21, but not on d 42 which suggests that the loss of Arg is influenced by the FI in a larger extent when compared to the other essential amino acids (Adedokun *et al.*, 2016).

Dietary supplementation of Arg has been used in broilers with the objective of minimizing the impact of enteric infections on the intestinal function (Allen and Fetterer, 2002; Tan *et al.*, 2014; Gottardo *et al.*, 2016; Laika and Jahanian, 2017; Rochell *et al.*, 2017a), and potentially improve the digestion and absorption of dietary nutrients. Tan *et al.* (2014) observed a beneficial effect of supplemental Arg (11.1, 13.3, 20.2 g/kg) in coccidial vaccine challenged broilers by

improving the intestinal structure (villus height), increasing sucrase and maltase activity, and down-regulating the expression of inflammatory markers, which may reflect a better use of nutrients and explain the beneficial effects of Arg on the growth performance of challenged chickens. In a recent study by Laika and Jahanian (2017) it was observed that 105 and 110% of the standard recommendation (10.2 g/kg for finisher phases) of Arg improved weight gain and FCR of coccidia challenged broilers during the finisher phase (28 to 42 d); yet, higher Arg reduced the thickness of the muscular layer in the jejunum of infected birds which suggests a lower inflammatory process. The potential anti-inflammatory role of Arg showed by these latter authors suggests that Arg reduces the intestinal EAA losses by reducing the intestinal inflammation, mainly through the suppression of the TLR-4 pathways (Tan *et al.*, 2014), and the maintenance cost.

Arginine is also the only precursor for NO synthesis, a key metabolite with several biological functions, including vasodilation, cytotoxicity mediated by macrophages, inhibition of platelet activation, adhesion, and aggregation, and it is one the most important regulating molecules of the immune function (Hibbs *et al.*, 1988; Fernandes and Murakami, 2010). Arginine supplementation has been shown to increase plasma levels of NO in chickens (Khajali *et al.*, 2011), and may have an important role in eliminating intracellular protozoans such as Trypanosoma cruzi (Vespa *et al.*, 1994). However, high NO synthesized from Arg can have deleterious effects due to its effect in stimulating apoptosis (Tan *et al.*, 2014). It is expected that Arg may be depleted during a coccidiosis event due to the production of NO, impairing the growth performance of broilers (Rochell *et al.*, 2017a). Indeed, coccidiosis dramatically increased plasma NO concentration, which may have exacerbated the need for Arg; yet, the impact of infection on the growth performance was lowered by Arg supplementation (0.74 vs. 1.23% of digestible Arg) in broilers (Rochell *et al.*, 2017a). Therefore, Arg supplementation above that required for growth, may be a useful strategy to help chickens to cope with intestinal infections and sustain adequate immune stimulation and nutrient utilization.

Glutamine

Glutamine is an α-amino acid with a chemical structure similar to that of the glutamic acid (Glu), except for the fact that the carboxylic acid is replaced by an amide. Glutamine is the most abundant amino acid in intra and extracellular fluid compartments (Rao and Samak, 2012), synthesized from Glu and ammonia through the action of the enzyme glutamine synthetase. It is a conditionally essential amino acid which means that there is endogenous production which may not be enough during stressful conditions, when the demand for Gln increases. The small intestine plays an important role in the metabolism of Gln. As reviewed by Wu (1998) most of the dietary Gln does not enter the portal circulation and is not available for extraintestinal tissues due to its high metabolic utilization by the enterocytes. Beyond its potential function in reducing the atrophy of the intestinal

mucosa, Gln can be hydrolyzed to Glu and ammonia, and Glu can be used as precursor for the synthesis GSH, important molecule of the antioxidant system (Windmueller and Spaeth, 1975). Intestinal epithelial cells are supplied with Gln from the dietary and blood polls; however, the presence of Gln or Glu in the diet depresses the utilization rate of Gln from the blood (Windmueller and Spaeth, 1975). Unlike other animals, in vitro studies by Windmueller and Spaeth (1975) showed that in chicken enterocytes the addition of 2.5 mM of Gln stimulated O2 uptake, but 6.7mM was inhibitory; yet, the rate of Gln metabolism was only 20% of that obtained with rat enterocytes, although it showed cellular capacity to synthesize, as well as to degrade, Gln. In another study, the main products of Gln metabolism in chick enterocytes were CO2, NH3, Glu, and from Glu, alanine and aspartate were obtained (Wu *et al.*, 1995), with a pattern of Gln metabolism similar to other rapid proliferating cells such as tumor cells. As explained, the use of Gln by the intestine of the chicken is naturally high, and may increase during events of rapid cell turnover, such as coccidiosis.

Using porcine intestinal cells, it has been reported that supplementation of physiological concentration of Gln to the culture medium stimulates protein synthesis and inhibites protein degradation, not attributable to ammonia production, but mediated through the activation (phosphorylation) of two downstream proteins of the mTOR signaling pathway (4EBP1 and S6K1) that promote polypeptide formation (Xi *et al.*, 2012). Similar results were observed by Yi *et al.* (2015) supporting the view that Gln enhances intestinal protein synthesis and development by its action on the mTOR signaling

pathway. Additionally, Yi *et al.* (2014) reported that Gln upregulated the expression of several genes encoding nutrient transporters in porcine intestinal cells which may improve the absorptive function of enterocytes; yet, it elevated the expression of zonula occludens-1, a tight junction protein, that maintains the intestinal barrier function. These findings provide evidence to the hypothesis that Gln exerts its beneficial effects on the intestinal health and growth by changing the molecular profile of several genes and proteins, which in turn support improved intestinal function, nutrient digestion and absorption. Although one can expect the same pattern of response to be observed in poultry species, to our knowledge, is no such information is available on chickens.

There is increasing evidence showing that Gln supplementation in the diet of birds enhances growth performance by stimulating the development of the GIT (Bartell and Batal, 2007; Luquetti *et al.*, 2016; Fernandes *et al.*, 2018; Pereira *et al.*, 2019). For example, 1 and 1.5% of Gln supplemented to the diet of broiler chickens increased the relative weight of the duodenum and jejunum, and increased villus height and surface area of both sections (Moghaddam and Alizadeh-Ghamsari, 2013). In another study by Pereira *et al.* (2019) the inclusion of Glu to the diets of laying hens increased the number of proliferating cell nuclear antigen (PCNA) positive cells, and that higher Glu supplementation (3.28 and 3.48%) resulted in PCNA positive cells along the entire length of the villus. Furthermore, Wang *et al.* (2008) reported that 1% of Gln supplementation reduced the negative effects of weaning in piglets by improving BW gain, increasing Gln concentration in the plasma and jejunum, and lowering the oxidative stress generated by the weaning; yet, Gln supplementation enhanced the antioxidant capacity in the jejunum by downregulating genes expected to stimulate oxidative stress, and by upregulating genes related to transcription regulation, defense against pathological microorganisms, regulation of nutrient metabolism and cell growth. These observations help elucidate how Gln prevents atrophy of the intestinal mucosa and improves nutrient utilization in stressed animals (Wang *et al.*, 2008).

Additionally, Gln has been used as a nutritional strategy to mitigate the impact of delayed placement in young chicks (Zulkifli *et al.*, 2016; Gilani *et al.*, 2018) and enteric infections such as Salmonella (Fasina *et al.*, 2010), coccidiosis (Luquetti *et al.*, 2016; Fernandes *et al.*, 2018), and necrotic enteritis (Xue *et al.*, 2018). Zulkifli *et al.* (2016) observed a positive effect of Gln and Glu supplementation on the intestinal morphology of broiler chickens, which reflected in an improved performance at 42 d of age, even though the delayed placement did not affect these variables. Similarly, supplemental Gln improved FCR of chickens from 1 to 14 d of age, and positively affected jejunal and ileal morphology. Besides its beneficial effects on the performance and intestinal morphology, Gln supplementation reduced the lesions characteristic of NE in broilers and reduced serum uric acid

concentration, indicative of better utilization of amino acids (Xu *et al.*, 2018). Interestingly, removing Gln from the finisher diet (d 28 – 35) negatively affected BW gain, but not FCR. Furthermore, as reviewed by Adedokun *et al.* (2011) the endogenous losses of Glu are the highest among the nonessential amino acids evaluated, and its ileal digestibility decreased by 17% in broilers challenged with a coccidiosis vaccine containing E. acervulina, E. mivati, E. maxima, and E. tenella (Adedokun *et al.*, 2016), and by 3% when using only E. acervulina infection (Rochell *et al.*, 2016). Thus, Gln supplementation may also be necessary in events of enteric challenges to account for its decreased digestibility and high loss within endogenous secretions.

Sulfur amino acids

Methionine is considered the first most limiting amino acid in diets of broiler chickens due to its low concentration in plant protein sources and due to the high requirement for protein synthesis and feather growth. Methionine has several roles in poultry including protein synthesis, methyl donor and formation of S-adenosylmethionine (SAM; Bunchasak, 2009). In vivo studies have shown that the intestinal metabolism of dietary SAA is nutritionally important for normal intestinal mucosal growth (Burrin and Stoll, 2007; Bauchart-Thevret *et al.*, 2009; Ding *et al.*, 2014; Olsen *et al.*, 2018). The GIT metabolizes a major part of the dietary Met and its main metabolic fate is transmethylation to Hcy via SAM, the principal biological methyl donor in mammalian cells and a precursor for polyamine synthesis and transsulfuration to Cys (Finkelstein, 2000; Burrin and Stoll, 2007) The GIT accounts for 25% of whole-body transmethylation and

transsulfuration and it is a site of net Hcy release. Moreover, Stoll *et al.* (1998) showed that nearly 52% of the Met consumed by piglets is retained and metabolized by intestinal tissue; yet, the requirement of Met of piglets was 30% higher when fed orally compared to parenteral nutrition (Shoveller *et al.*, 2003) showing that the requirement of Met by the intestine is high and contributes significantly to the overall requirement of this amino acid, which may increase during enteric infection episodes.

The production of Hcy within intestinal mucosa may contribute to the inflammatory response and may exert its effects in destroying the epithelial barrier, increasing the intestinal permeability of rats with induced colitis (Ding *et al.*, 2014). These authors found that the Hcy concentration in plasma and colonic tissues of rats with colitis was increased significantly, which might be associated with the long-term decrease in FI and the loss of vitamin supplements due to severe diarrhea. The decreased concentration of plasma folic acid and Vitamin B12 reduces the transsulfuration of the Hcy in the liver to form cystathionine and ultimately Cys, that may be used for the synthesis of protein, Tau, or GSH (Brosnan and Brosnan, 2006; Olsen *et al.*, 2018). Homocysteine can also be methylated back to Met via the remethylation pathway. The combination of transmethylation and remethylation pathways comprises the Met cycle which occurs in most cells (Bauchart-Thevret *et al.*, 2009).

Cysteine derived from the diet and Met transsulfuration is a functional constituent of antioxidant systems and impacts several elements of redox status that regulate epithelial intracellular signaling, proliferation and survival (Mastrototaro *et al.*, 2016). Oxidative stress is known to increase Met transsulfuration to meet the increased Cys demand for cellular GSH synthesis. In such situations, Cys may be used for acute-phase proteins synthesis but also for synthesis of GSH which is the most important intracellular antioxidant of the body (Lai *et al.*, 2018). Indeed, in association with Trp and Cys, Met is the most susceptible amino acid to suffer oxidation by radical oxygen species (ROS; Levine *et al.*, 1999; Mastrototaro *et al.*, 2016).

Maintaining normal GSH concentration is essential for most tissues, especially the intestine, which is constantly challenged by luminal toxins and oxidants derived from the diet as well as endogenous generated ROS (Bauchart-Thevret *et al.*, 2009; Mastrototaro *et al.*, 2016). Therefore, the functional role of Met in the GIT, especially its antioxidant effect, may be key for maintaining the health of the GIT of rapid growing animals and consequently improving their growth (Chen *et al.*, 2014). This suggests that the metabolic requirement of Met and Cys of the gut may be increased in conditions such as inflammatory bowel disease and enteric infections (Lai *et al.*, 2018). It is unknown whether Met transsulfuration can affect Cys availability and epithelial cell function. There is clear evidence in HepG2 cells (a human liver cancer cell line) that oxidative stress increases transsulfuration and incorporation of 35S-Met into GSH and that cystathionine b-synthase activity is coordinately regulated by its production via a redox-sensitive mechanism (Mosharov *et al.*, 2000). It may result in an increased Cys requirement, with a demand for Cys thus exceeding the body's capacity of Cys production.

The dysfunction of the intestinal barrier caused by weaning in piglets leads to intestinal atrophy and diarrhea. Su *et al.* (2018) found that the supplementation of Met above the requirement in the diets of post-weaning piglets improved small-intestinal mucosa villi architecture, upregulated the expression of occludin, and increased GSH concentration in the jejunum. Chen *et al.* (2014) showed greater abundance of occludin and a significant reduction in the activity of caspase-3 level (apoptotic protein) in the jejunum of pigs supplemented with L-Met. Additionally, dietary Met may play a role in the control of some pathogenic microorganisms in the intestine. The dietary supplementation of broilers with 0.8% of Met reduced the population of C. perfringens, Streptococcus, and coliforms, and significantly increased Lactobacillus populations (Dahiya *et al.*, 2007), suggesting that reducing the concentration of crude protein and supplementing the diet with crystalline amino acids may be beneficial in attenuating the impact of enteric pathogens.

Studies have shown that coccidiosis reduces the ileal digestibility of Met and Met + Cys (Rochell *et al.*, 2016; Adedokun *et al.*, 2016). Adedokun *et al.* (2016) observed that a coccidial vaccine challenge reduced the ileal digestibility of Met in

approximately 10%, and Rochell *et al.* (2016) found that increasing the infecting dose of E. acervulina linearly reduced the ileal digestibility of Met. Additionally, as reviewed by Adedokun *et al.* (2011) the endogenous flow of Met ranged from 50 to 101 mg/kg of DM intake. However, Adedokun *et al.* (2016) found higher values which increased even more in broilers under a coccidial vaccine challenge (134 in non-challenge vs. 259 mg/kg of DM intake in challenged birds on d 42). On the other hand, Lai *et al.* (2018) showed that high dietary Met concentration, ranging from 0.45% to 0.68%, enhanced growth performance of 42 d old chickens fed diets containing narasin as a coccidiostat and challenged with E. tenella, but did not influence the growth of chickens when they were vaccinated against coccidiosis. They suggested that higher Met concentration was beneficial to meet the deficiency of Met generated by the challenge as narasin was unable to control the infection, whereas vaccination was able to elicit an immune response against the E. tenella challenge. Therefore, it seems evident that enteric infections increase the requirement of Met which may be due to an increased intestinal loss and impairment in Met digestibility.

The availability of SAM as a precursor for methylation reactions and for production of polyamines plays a key role in chronic inflammation and is strongly correlated with epigenetic alterations. While polyamines have antioxidant and anti-inflammatory effects and protect genes and cells against harmful stimuli, they may also affect gene methylation when its synthesis is overstimulated, as in an enteric inflammation event (Soda, 2018). A decrease in Met intake or a deficiency in folate may disrupt Met metabolism and have an impact on the intestinal SAM pool and hence polyamine synthesis, which clearly emphasizes the importance of dietary sulfur amino acid intake to maintain a normal intestinal growth and function (Bauchart-Thevret *et al.*, 2009). Besides its role as a constitutive precursor for protein synthesis, the role of Met as a precursor for SAM synthesis may have greater regulatory importance for cell function and survival given its singular role in methylation reactions and control of gene expression. All these observations strongly suggest that Met and Cys play a key role on the intestinal mucosal growth associated with a regulatory redox status, and intestinal epithelial cell function. However, there is a lack of studies looking at the effects of SAA in reducing the EAA flow into the intestine due to enteric infections, and how Met could ameliorate these effects.

Tryptophan

In the body, ingested Trp is used for protein synthesis and its catabolism occurs mainly in the liver through the serotonin and kynurenine metabolic pathways (Yao *et al.*, 2011). Approximately 95% of Trp is degraded through

the kynurenine pathway, which is mainly regulated by two rate-limiting enzymes: tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), while only approximately 3% is metabolized into serotonin throughout the body, and the rest is degraded by the gut microbiota to produce indole and its derivatives (Richard *et al.*, 2009). A wide range of physiological roles have been identified for Trp. In the last few years, studies have also suggested an influence of dietary Trp on the intestinal and peripheral inflammation, intestinal immune response, and the connection between Trp metabolism and the gut microbiota (Bai *et al.*, 2017; Gao *et al.*, 2018).

Indoleamine 2,3-dioxygenase expression can be induced by pro-inflammatory cytokines and immune-regulating factors (Bai *et al.*, 2017). Activated IDO accelerates the degradation of serotonin into formyl-5-hydroxykynuramine (keszthelyi *et al.*, 2009) which yields ROS byproducts that can inhibit the function of lymphocytes and promote apoptosis (Bai *et al.*, 2017). Nevertheless, IDO is responsible for the conversion of Trp to kynurenine, that functions as an endogenous ligand of the aryl-hydrocarbon receptor (AhR) transcription factor, found in intestinal immune cells. The AhR is a cytosolic ligand-activated transcription factor that mediates xenobiotic metabolism, is a critical regulator of immunity and inflammation, is involved in the fine-tuning of adaptive immunity and mucosal barrier function, maintenance of intestinal homeostasis, and carcinogenesis (Hubbard *et al.*, 2015; Korecka *et al.*, 2016).

A Trp rich diet can activate AhR and subsequently increase colonic IL-22 mRNA expression. During acute enteritis, Trp supplementation protects the epithelial layer and prevents the intestinal inflammation mediated by AhR signaling (Hashimoto *et al.*, 2012). Both, AhR and IDO, play crucial roles in connecting microbial Trp catabolism and host

endogenous Trp metabolites with regulatory T-cell function (Lanis *et al.*, 2017). When attempting to counteract tissue damage, high expression of IDO by intestinal mononuclear cells can mediate anti-inflammatory and immunosuppressive effects on the intestinal mucosa (Le Floc'h *et al.*, 2011) by regulating host immunomodulatory activity via kynurenine production, mucosal amino acid nutrition, mucosal immune reactivity, and gut microbial community metabolism (Dai and Zhu, 2010; Lanis *et al.*, 2017).

In the intestine, activation of AhR is essential for the maintenance of intestinal intraepithelial lymphocytes, a subset of T cells present in the intestinal epithelium that are involved in the maintenance of intestinal epithelial integrity and localized immune quiescence. Yu *et al.* (2018) investigated the mechanisms of AhR activation in the maintenance of intestinal barrier function and found that AhR activation ameliorates tight junction barrier dysfunction by regulating both the expression and distribution of tight junction proteins. Disruption of this barrier induces an increased permeation of toxic luminal antigens into the lamina propria, triggering an inflammatory cascade and promoting intestinal inflammation, as well as the development of a variety of intestinal diseases (Turner, 2009). More recently, a number of studies have shown that gut microbial species produce a variety of Trp catabolites by various metabolic pathways (Roager and Licht, 2018; Waclawiková and El Aidy, 2018). While bacterial products of protein degradation are generally associated with detrimental effects, new research suggests that microbial Trp catabolites may also have a positive impact on the host physiology. Because indole has been known for more than 100 years, numerous indoles producing species have been identified (Roager and Licht, 2018).

It is notable that other branches of Trp metabolism, such as the generation of serotonin, can be also increased during intestinal inflammation and implicate a common metabolic process that promotes the depletion of local Trp levels. This implies a strong competition between serotonin synthesis and the first downstream metabolites from the kynurenine pathway for the available Trp. In inflammatory conditions, more kynurenine is produced on the expense of serotonin. In fact, in a state of inflammation, not only high levels of proinflammatory cytokines are produced but also altered levels of neurotransmitters, such as serotonin. Thus, these metabolic regulations provide an important example of the intricate balance between inflammation and tissue metabolism of Trp (Lanis *et al.*, 2017).

Tryptophan regulates the immune function in livestock in a range of physiological conditions. In animals under stress and showing inflammation, the degradation of Trp is accelerated to promote the differentiation of lymphocytes, the production of immunoglobulins, and to improve humoral immunity (Bai et al, 2017). For instance, during an inflammatory process caused by an infection or trauma, there is synthesis of acute phase proteins in the liver . Due to acute phase proteins' high concentration on Trp, the amino acid availability for the synthesis of kynurenine may be reduced (Le Floc'h *et al.*, 2004; Bai *et al.*, 2017). Additionally, as reviewed by Adedokun *et al.* (2011) the intestinal endogenous flow of Trp varied from 50 to 95 mg/kg of DM intake in broilers; yet, Adedokun *et al.* (2016) reported that the endogenous losses of Trp due to a coccidial challenge increased by 10% on d 21, and by 35% on d 42. Thus, during inflammatory processes, Trp may become a limiting factor for the synthesis of body protein, growth, and other metabolic process (Le Floc'h and Seve, 2007).

In a recent study conducted with weaning piglets (Liang *et al.*, 2019) it was shown that supplementation of 0.2 and 0.4% of Trp enhanced the intestinal barrier function by increasing the expression of secretory immunoglobulin A (sIgA) and porcine β-defensine; yet, Trp upregulated the expression of tight junction proteins in the jejunum. On the other hand, Trp modulated the jejunal microbiota by increasing the diversity of the microbiota, reducing opportunistic pathogenic species and increasing Lactobacillus species (Liang *et al.*, 2019). The authors explain these findings by arguing that the enterocytes do not metabolize dietary Trp, and that the intestinal microbiota possess the ability of catabolizing this amino acid, thus producing metabolites that regulate the intestinal microbiota diversity and benefit the host. Additionally, Liang *et al.* (2018) also reported a modulation of the intestinal microbiota in weaning piglets by Trp, improved intestinal barrier function, activated AhR signaling pathway, and modulation of the intestinal immune response. Even though studies relating the effects of dietary Trp and intestinal homeostasis in broiler chickens are scarce, supplementation of Trp could

be a therapeutic approach to ameliorate the inflammation of the intestinal mucosa by modulating the intestinal microbiota, the intestinal barrier function and immune system and hence reducing the EAA losses of Trp and other amino acids.

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Evonik Operations GmbH
Animal Nutrition Business Line

animal-nutrition@evonik.com
www.evonik.com/animal-nutrition