Amino acid nutrition and gut health in poultry

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Abstract

The intestine is not only critical for the absorption of nutrients, but also interacts with a complex external environment. Most foreign antigens enter the body through the digestive tract. A healthy gut has a well-coordinated immune system that must accommodate commensal microbiota while inhibiting the colonization and proliferation of harmful pathogens. Dietary amino acids are major fuels for the small intestinal mucosa, as well as important substrates for synthesis of intestinal proteins, nitric oxide, polyamines, and other products with enormous biological importance. Recent studies indicate positive effects of amino acids on gut integrity, growth, and health in animals and humans. In modern commercial intensive feeding practices, the incidence of intestinal diseases such as coccidiosis and necrotic enteritis may increase worldwide due to mounting pressure to limit the use of subtherapeutic antibiotics as growth promoters or ionophores for coccidial suppression/prevention in the diets of broilers. For this reason, amino acid nutrition now considered to be beneficial to modulate the intestinal physiology, immunology, and microbiology of broilers.

Background

Gut health is a complex area combining nutrition, microbiology, immunology and physiology. The primary function of the gastro intestinal tract (GIT) is efficient digestion and absorption of nutrients. It is the most important route of entry for foreign antigens, including food proteins, natural toxins, commensal gut flora, and invading pathogens (LI et al. 2007). The intestinal tract is also one of the largest lymphoid organs in the body, and consists of immune cells in organized gut-associated lymphoid tissues (Field et al. 2002). To support these functions, a better understanding is required on the intestinal immune system and its relationship with the microbial community as a distinct organ with specific nutrient needs. Enteric challenges such as coccidiosis or necrotic enteritis may alter the development of the immune response, and certain nutrients such as amino acids may become limiting factors to produce key proteins required for appropriate immune function. Amino acids are building blocks for proteins and also key regulators of fluxes through major metabolic pathways. Presently the main focus is reduced protein levels with supplemental amino acids for gut health, economic and environmental and bird welfare (Moss et al, 2018). The purpose of this article is to review current information regarding the critical role of amino acids to maintain gut integrity and immune function.

Threonine (Thr)

Thr is considered as the third limiting amino acid in a corn- soybean based feed in broiler chickens. Thr plays a vital role in synthesis of mucin and thus maintenance of gut integrity (Bertolo et al. 1998). Mucin is secreted by the goblet cell as a functional protein in mucus, is glycoprotein in nature and composed of polypeptides of which core protein composed of Pro- Thr- Se sequence. Thr is the major component of intestinal mucin in animals, representing approximately 30% of its total amino acid content (Faure et al., 2002). Therefore, factors that induce mucin secretion may increase dietary Thr requirements, such as bacterial load, which can influence endogenous amino acid flow through mucin production (Adedokun et al., 2012) and decrease its availability for growth; for instance, based on body weight gain, the requirement of Thr from 21 to 42 d was 0.77% in broilers raised on used litter vs. 0.74% in broilers raised on new litter (Corzo et al., 2007). Thus, under inflammatory conditions, Thr availability may become limited for the synthesis of intestinal mucins, which leads to an impairment of gut barrier function. Consequently, an increase in dietary provision of Thr and other amino acids can promote mucin synthesis and re-equilibrate the gut microbiota to favor intestinal protection and mucosal healing (Faure et al. 2006).

Thr is also a major component of immunoglobulins (Ig), particularly IgA secreted by the intestinal mucosa and accounts for more than 2/3 of all Ig in the body (Slack et al., 2012). IgA is essential for maintaining intestinal homeostasis by preventing the attachment and entry of bacteria in intraepithelial cells, or eliminating bacteria from the basolateral space to the lumen (Brisbin et al., 2008). The dietary Thr requirement was studied in ducks from 15 to 35 d of age and observed that serum natural IgY (with IgM, the predominant Ig in the serum of chickens and ducks) increased linearly when dietary Thr increased, even though Thr had no effect on villus height, crypt depth, goblet cells, or MUC2 gene expression (Zhang et al. 2014). Thr supplementation changes the microbial balance in the intestine and modulates the immune system by increasing IgA secretion and down-regulating the expression of the inflammatory genes INF-γ and IL-1 β (Chen et al., 2016). Star et al. (2012) showed that in subclinical necrotic enteritis infection, a dietary Thr: Lys ratio of 0.67 promoted better body weight gain than a ratio of 0.63 in infected chickens, without improvements in the incidence or severity of lesion scores. As stated by Faure et al. (2007), in pathological situations, the defense and repair will increase the demand for amino acids, especially Thr, and if the extra requirement for Thr is not met by the diet, muscle protein will be mobilized. Thr supplementation to a low crude protein diet restored the bacteria diversity and increased the frequency of beneficial populations of bacteria in the cecum of laying hens (Dong et al., 2017).

Arginine (Arg)

Poultry lack key enzymes involved in de novo Arg synthesis. The genetic material of birds does not encode for the enzyme carbamoyl phosphate synthetase, which catalyzes the first step of ammonia detoxification involved in the production of citrulline from ornithine (Tamir and Ratner, 1963). Citrulline can ultimately be converted to Arg through urea cycle enzymes, and as such, citrulline can spare dietary Arg in chickens (Klose and Almquist, 1940). Additionally, chickens lack the enzymes necessary for citrulline production in the small intestine (Wu et al., 1995). Again chickens have a very high activity of kidney arginase compared with mammals (Tamir and Ratner, 1963), so dietary supply must account for this degradation as well. L-Arg is an essential substrate for the synthesis of molecules, including nitric oxide (NO), polyamines, and creatine (Wu and Morris 1998). In a pioneering "in vitro" study Yuan et al. (2016), observed that the supplementation with L-arginine in the intestinal cells of cultured poultry resulted in increased gene expression of the TOR signaling pathway (same function as mTOR in mammals). Arg also stimulates secretion of insulin-like growth factors (Fernandes and Murakami, 2010). NO, a key metabolite with several biological functions, such as 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). Depletion of Arg may be observed in coccidial-infected chickens, due to the high expression of inducible NO synthase (iNOS) in an attempt to limit the replication of Eimeria in the intestinal epithelia (Tan et al., 2014a). Polyamines are essential for the development of the intestine in newborn chicks (Loser et al., 1999), which may explain the positive effects of Arg supplementation on performance and small intestine morphology of one-week-old broiler chickens (Murakami et al., 2012). Polyamines can also stimulate proliferation, migration, and apoptosis of intestinal cells (Ruemmele et al., 1999). Therefore, Arg, a key precursor of polyamines, may be considered as a trophic substance by supporting the mitotic process in the crypt-villus region to increase the number of cells and the size of the villus (Uni et al., 1998). It is not yet fully understood whether Arg directly affects goblet cell or enterocyte replication; however, mucosal density increased linearly with increasing dietary Arg concentration (Tan et al., 2014a), which can be considered as an indirect effect of the polyamines. In a study by Tan et al. 2014a, coccidiosis shown to downregulate the expression of MUC-2 and IgA, but upregulated β-Defensin-8 and inflammatory genes (iNOS, IL-1β, IL-8, TLR4) mRNA expression. Meanwhile, Arg linearly diminished the expression of TLR4, suggesting that the anti-inflammatory effect of Arg is via suppression of the TLR4 pathway, which was again verified when the inflammation was stimulated by lipopolysaccharide (Tan et al., 2014b). Arg increases the serum levels of IFNα, IFNγ and IgG, confirmed by histopathological examination of the bursa and spleen of broilers chickens feed with 2.5 times NRC level of Arg (EMADI, et al., 2011).

Sulfur amino acids – Methionine (Met) and Cysteine (Cys)

The major end products of Met and Cys metabolism are glutathione (GSH), homocysteine (Hcy) and taurine (Tau), which play important roles in the intestinal immune response (Grimble 2006). Cys is the precursor of glutathione (GSH) and hydrogen sulphide (H_2S), a signalling molecule in animal cells that is positively correlated with Glu concentrations in the liver, spleen and muscle. This plays an important role in regulating cellular signalling pathways in response to immunological challenges (Li et al. 2007). GSH, which consists of glycine, Glu and Cys, is the major intracellular low-molecular-weight thiol and plays important roles in antioxidant defense, nutrient metabolism and cytoprotective events (Wu et al. 2004a). GSH in the gut lumen and enterocytes is of critical importance in maintaining normal intestinal function, in part, by protecting epithelial cells from damage by electrophiles and fatty acid hydroperoxides (Aw et al. 1992). GSH is also essential for normal intestinal function and a deficiency increases the susceptibility to carcinogenesis and oxidative injury. GSH redox cycle is considered to be a key intracellular antioxidant mechanism to promote intestinal hydroperoxide removal and reduce lymphatic peroxide transport in vivo (Aw and Williams, 1992) and in vitro (Wingler et al. 2000). Thus, administration of specific dietary substrates and precursors for GSH synthesis is an effective strategy to improve gut mucosal functions and may prevent or treat intestinal diseases (Wu et al. 2004a). Because a portion of dietary Met is normally converted to Cys, dietary Cys can spare, reduce, or replace a portion of the requirement for Met by as much as 50%-80% in birds

(Shoveller et al. 2003). Increasing total Met levels from 0.35 to 1.2% in the diet of chickens infected with Newcastle Disease virus markedly enhanced immune responses: T-cell proliferation, plasma IgG levels, leucocyte migration and antibody titres (Li et al. 2007). Therefore, in reduced protein diets, total sulphur-containing Amino acid (TSAA) requirement may be much higher than growth requirement.

Branched chain amino acid (BCAA)

Leucine (Leu), Valine (Val) and Isoleucine (Ile) commonly referred to as BCAA due to their unique branched chain structure. BCAA play a crucial role to serve as an N source to synthesize glutamate and glutamic acid (Glu), which are important in the gut and immune system (Bao, 2020). BCAA promote muscle protein synthesis and reduce protein catabolism (Mattick et al. 2013). Mattick in an experiment also stated that BCAA could reduce oxidative stress through influencing metabolism that increases defence mechanisms (Mattick et al. 2013). Leu activates mammalian rapamycin (mTOR) to stimulate protein synthesis (Wu 2010). Leu known to exert a greater effect on immune function than other BCAA members and a deficiency of BCAA will result in immune impairment and less antibody production (Li et al. 2007).

Other Amino acids

Alanine (Ala) is a major substance for the hepatic synthesis of glucose, the major source of energy for leucocytes and supplementation of Ala also known to prevent apoptosis, enhance cell growth and augment antibody production (Li et al. 2011). During inflammation, the increase in immune cell protein synthesis may require great quantity of tyrosine (Tyr), phenylalanine (Phe) and tryptophan (Trp) (Le Floc'h et al. 2004). Glu and aspartate (Asp) are major metabolic fuels for enterocytes and regulate intestinal and neurological development and function (Wu 2014). Glu activates chemical sensing in the intestinal tract and may inhibit degradation of both essential and non-essential AAs. Most Glu needs to be synthesised endogenously and may be synthesised from Arg, proline (Pro) and histidine (His) (Brosnan and Brosnan 2013). Under heat stress, increased Pro secretion via saliva may increase the requirement of Glu. Ornithine (a product of Arg, Pro and Glu) is the immediate precursor for polyamine synthesis, which is essential for proliferation, differentiation and repair of intestinal epithelia cells, especially when these have been damaged by pathogen toxins (Wu 1998).

Trp also has positive effects on immune and antioxidant responses in poultry. Trp leve above NRC recommendation (0.3 and 0.5%) improve the anti-oxidant status, humoral and cellular immunity in broiler chicken during 7 to 21 days of age (MUND et al., 2019). Furthermore, the supplementation of the combination of Arg and Trp above the NRC (1994) requirements (2.0 times the NRC level of Trp and 2.5 times the NRC level of Arg) promotes a positive immunomodulatory effect on the innate (IFNα), cellular (IFNγ) and humoral (IgG) immune responses of broilers chickens challenged with the live intermediate plus strain of with infectious bursal disease vaccine (IBDV) and also this combination improve growth performance and serum parameters. In this study, the responses for the combination of the two amino acids were significantly better in relation to the supplementation of each isolated amino acid, even at levels higher than those recommended by the NRC.

Conclusions

Gut health is of major importance, as the gut plays a vital role in barrier defense in addition to digestion, absorption and metabolism of nutrients. Thus, it is required to emphasize more on maintaining or improving gut integrity and function under stress conditions and to avoid an unbalanced supply of amino acids which can affect the immune system adversely. Therefore, an ideal dietary amino acid profile is crucial for broiler chicken gut health and growth, especially in the present scenario of antibiotic free situations. Ideal amino acid profile in a ratio to dietary lysine (the first limiting amino acid) should be maintained i.e. when dietary lysine concentration is higher, both essential and non-essential amino acid concentrations need to be increased accordingly to maintain growth and meet the needs of essential functions, including maintaining the gut wall and immunity.

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