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Chapter

Advances in the Nutrition of Functional Amino Acids in Healthy and Immunologically Challenged Birds

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Abstract

The effects of some functional amino acids (AAs) such as arginine, threonine, and methionine on the development of immune and digestive capacities in poultry were reviewed. The information was examined analytically to identify the source of the AA requirements, growth potential, type of housing, and type and degree of immune challenge applied. Regardless of these factors, the level of functional AA required to stimulate the immune or digestive response was higher than that required to maximize the productive performance. The implications section describes the main obstacles to integrating and applying the concept of functional AA in conventional diet formulation, and its use in birds raised under commercial conditions experiencing different types and degrees of immune stressors. It is necessary to develop a profile of functional AAs and establish their strategic use during or after immunological challenge situations to aid in the recovery of productive parameters to prechallenge levels.

Keywords: functional amino acids, growth, immune response, digestive function

1. Introduction

The establishment and reevaluation of nutrient requirements in poultry is a dynamic process due to constant increases in feed consumption, growth rate, and the amount of dietary protein, and amino acid (AA) transformed into body protein in chicken meat over time, as a result of genetic selection programs [1–3]. The advancement of each of these factors necessitates the constant labor of AA to maintain high levels of productivity, especially when we consider that in modern animal production, new concepts such as sustainability in meat production are emerging, as a result of the constantly increasing societal demand for food production through environmentally friendly practices.

Decades ago, one of the responses to achieving high levels of efficiency in the transformation of feed into high-quality protein was the development of the concept of the ideal AA ratio, which seeks to maximize efficiency.
However, when comparing the AA profiles recommended by various sources, there is a significant difference between them. For example, among the main essential AA in poultry, arginine (Arg), methionine (Met), threonine (Thr), and tryptophan ratios of 94–120, 36–46, 58–73, and 14–20 have been proposed over time. One reason for these disparities is that these available AA ratios were proposed over 50 years, from 1965 to 2014 [3, 4]. During this time, significant advances in broiler growth have been made, as well as the development of other concepts such as the use of digestible AA ratios, in conjunction with a steady improvement in the nutritional quality of vegetable feedstuffs through selection, and an ever-increasing amount of new information on the composition and availability of nutrients in feed ingredients.

Another strategy, in addition to the use of ideal AA ratios, to improve broiler production efficiency is the use of low-CP diets supplemented with available feed-grade amino acids [3, 5, 6] to fulfill the AA requirements according to different recommendations [7, 8], ensuring the birds productivity at least at the same level shown by birds. Furthermore, using low-CP rations improves nitrogen efficiency by avoiding excess nitrogen excreted in the form of uric acid, reducing environmental pollution caused by nitrogen and ammonia emissions, and lowering the carbon footprint of feed manufacturing through changes in the type and amount of raw material included in the feeds.

While these feeding strategies are still being refined in terms of research and practical application, they must keep up with changes in broiler genetic potential. Furthermore, they must adjust to new nutritional concepts, such as the use of functional AA. Functional AAs are defined as AAs that participate and regulate key metabolic pathways that improve organism health, survival, growth, development, lactation, and reproduction [4, 9]. Functional AA's descriptive roles in nutrition and health, as well as the metabolic pathways involved, have been documented [4]. In recent years, there has been a large number of publications on this topic, as well as several outstanding reviews on the use of functional AA to improve the immune response and digestive capabilities in chickens, including the embryonic developmental stage and the growth stage on the farm [10–12]. According to a recent review [12], AA supplementation strategies can positively contribute to immune and gut health. In the present chapter, an attempt was made to analyze the available information on the use of Arg, Thr, and Met as functional AA aimed at establishing a pattern between the improvement in immune response and digestive physiology with improvements in broiler growth, estimate an AA requirement, and discuss the implications regarding the readjustment of feeds based on functional AA formulation and their application in broilers kept in commercial settings.

2. Arginine

Arg is known as an essential amino acid (AA) for birds due to its inability to synthesize Arg, so it must be supplemented in their diet [13]. Arg plays an important function in serving as the building block of proteins and polypeptides and fulfills several physiological roles through the regulation of key processes such as maintenance, growth, reproduction, and immunity. The recommended dietary Arg levels for optimum growth performance in broilers varies from 1.25 to 1.10% for starting and growing birds [7] and from 1.37–1.43 and 1.0–1.1 for starting and finishing birds, respectively [8]. There are also dietary Arg recommendations for different commercial strains of broilers. In addition to this, it has been documented that the addition of
Arg either in ovo or dietary Arg above the recommended level improves the digestive physiology and the cellular and humoral immune responses in nonchallenged and challenged birds; hence Arg has gained the distinction of belonging to the group of functional nutrients. The readers are referred to several comprehensive reviews about this topic [14–18]. In the next section, the recent findings on Arg feeding from in ovo to unchallenged and challenged broilers will be presented with emphasis on simultaneous effects on immunity/digestive physiology and productivity.

In ovo feeding (IOF) of Arg. IOF of Arg has been shown to boost glucose synthesis in the liver, which correlates with enhanced glucose 6-phosphatase activity at hatch [19, 20]. In this regard, it has been shown that IOF of Arg improved posthatch growth performance in chicks, and the effects of Arg have been linked to glucose synthesis and hormone production. Furthermore, supplementing with Arg has been proven to improve gut morphology (a sign of gut health), implying that it may affect the metabolism of this oxygen-demanding tissue [21]. IOF of Arg also stimulates the intestine mucin gene expression at 18th d of incubation and 14th d posthatch, as well as the IL-6 and IFN-γ humoral gene expression in 26-days-old birds [22]. In this section, the available literature was examined to find the best dosage of Arg that improves the immune response and gut morphology and that leads to enhanced growth performance in posthatch broilers.

In some experiments, the Arg IOF at day 14th of incubation has been evaluated. IOF of 35 mg Arg/egg depressed hatchability but increased the body weight of alive broilers at 11, 24, and 42 days of age; Arg inclusion increased the length of jejunum and ileum at 42 d of age and led to the greatest villus height and crypt depth in jejunum at 11 days of age [23]. In a similar experiment, Arg reduced hatchability again but increased the body weight in 42 days old broilers, and the relative weight of spleen and bursa of Fabricius at 11 days posthatch and antibody titer against SRBC at 30 days posthatch [24].

In several recent experiments, lower levels of IOF (0.6 mg Arg/egg) at 17.5 d of incubation have been tested. A summary of the results indicates that Arg IOF did not negatively affect the hatchability and improved the body weight at 7 and 21 d posthatch, and the ADWG from 1 to 21 d of age; Arg also increased the weights of digestive organs, the activities of digestive enzymes, alkaline phosphatase, maltase, and sucrase in the jejunum, the mRNA expressions of jejunal sensing receptors of taste and nutrient transporters of solute carriers [21]. Arg also increased the absolute weights of lymphoid organs, the activity and the mRNA expression and protein abundance of iNOS, the contents of IL-2, IL-4, and sIgA, the mRNA expressions of TLR-2 and TLR-4 in intestinal mucosa and serum; conversely, Arg decreased the iNOS promoter methylation percentage in jejunal mucosa [25].

In other reports, it was found that IOF of 0.6 mg Arg increased the weight of embryos at 19 days of incubation and the ADWG in chicks from 1 to 7 days posthatch [26], and the ADWG of broilers from 1 to 21 days and from 1 to 42 days of age [27]. Arg also increased the duodenum activities of alkaline phosphatase, maltase, sucrase, and inducible nitric oxide synthase of 7–days-old posthatch broilers, and the villus height and the ratio of villus height to crypt depth in duodenum of broiler embryos and posthatch birds and increased the density of goblet cells [26, 27]. The hatchability was high and similar to the control group in Arg supplemented eggs. Other benefits of Arg were the increased percentage of proliferating cell nuclear antigen positive cells of villus, and the mRNA expressions of mucin-2, claudin-1, and zonula occludens-1 and -2 in jejunal mucosa of 21-day-old broilers [27]. Furthermore, IOF of 0.6 mg Arg/egg increased the relative weight of breast muscle.
Broiler Industry

at hatch and 7, 14, and 21 days posthatch, and increased the concentration of some essential AA in the breast muscle such as Thr, valine (Val), phenylalanine (Phe), lysine (Lys), and Arg at hatch and 21 days posthatch [20]. Some studies have also been published using higher levels of IOF Arg with positive results. The IOF of 2.5 mg Arg/egg at 18 days of incubation resulted in similar hatchability to the control group, and in higher chick weight at hatch and lower transit weight lost from the hatchery to the farm; Arg also improved the body weight and ADWG in broilers up to 21 days of age [28]. When using even higher levels of IOF Arg (11 and 22 mg Arg/egg) at 18 days of incubation, the hatchability was similar to the control group, and greater body weight in chicks at 7 days posthatch was reported with 11 mg Arg; improved development of duodenal villi in 7-days-old chicks and enhanced cell-mediated immune response after 24 and 48 h in 28-days-old broilers was observed with 11 and 22 mg Arg [29].

A summary of the results indicates that IOF of 0.6 mg Arg/egg at 175 days of incubation increased the growth performance of broilers up to 42 days of age, which could be explained by the enhanced immune humoral and cellular response and the early maturation of the digestive capabilities.

In several studies, the stimulatory properties of increasing levels of dietary Arg on the immune and digestive systems have been documented in broilers kept in nonchallenged and under-challenged conditions [30–33]. From this, a number of studies have been published that have evaluated the productive performance along with the immune and digestive responses to high levels of Arg supplementation. These studies, while few, may provide insight into whether improvements in the immune and digestive responses can be associated with increased productivity at the same level of Arg supplementation.

In nonchallenged, 1–28 days of age broilers kept in cages and fed 1.48% (considered a normal level in corn/soybean meal diets) and 1.58 dietary Arg (keeping an Arg:Lys ratio of 1.20), no differences in growth performance were found, whereas the addition of 1.58% Arg increased the percentage of mucosa T helper (CD4+TCRβ1+) and T cytotoxic (CD8+CD28+) [34]. In nonchallenged chicks housed in floor pens from 1 to 21 days of age and fed increasing levels of Arg [1.00, 1.125, 1.250, 1.375, and 1.50% of NRC [7] recommendations for Arg requirements], the performance was improved at 1.25% Arg, while the relative weight of thymus increased in a nearly linear manner, and the cell-mediated immune response to phytohemagglutinin P and antibody titer against NDV increased linearly up to 1.375% Arg [35]. Similarly, using increased Arg levels (0.86, 1.31, 1.76, 2.21, and 2.66%, based on the recommended Arg requirement by NRC [7]), the ADWG and FCR were improved at 1.31% Arg, whereas serum total immunoglobulins and IgA increased up to 1.76 and 2.21% dietary Arg, respectively [36]. Furthermore, low growth potential chicks fed increasing total Arg levels (0.85, 0.97, 1.09, 1.21, and 1.33%, based on the nutritional requirements for Qingyuan partridge chickens) for 30 days showed maximum ADWG and FCR at 0.97% Arg, while mucosal jejunum IgG and ileum sIgA increased linearly up to 1.21% [37].

In challenged broilers vaccinated against Salmonella enteritidis at nine days of age and kept in cages from 1 to 28 days of age, similar performance was observed with 1.48 and 1.58% dietary Arg (keeping an Arg:Lys ratio of 1.20); however, increased suppressors monocytes (Kul+MHCII−) were found in birds supplemented with 1.58% Arg [34]. In broilers fed diets: deficient, normal, and excessive in Arg (1.05, 1.42, and 1.90% according to NRC [7]), kept in cages and challenged with an Escherichia (E.) coli lipopolysaccharide (LPS), showed higher ADWG and FCR at 1.42 and 1.90% Arg, respectively, after the challenge; depressed TLR4 and NF-kB in cecal tonsils relative
mRNA expression at 1.42% Arg and PPAR-γ in spleen and IL-1b in cecal tonsils relative mRNA expression at 1.90 Arg were observed after the challenge [38].

In several studies, the coccidiosis challenge has been used as a mean to demonstrate the functional benefits of Arg. A summary of some studies is given below:

Broilers from 1 to 26 days of age allocated in metabolic cages fed increasing levels of dietary Arg (1.04, 1.14, 1.24, 1.34, and 1.44%) and challenge with Eimeria sporulated oocysts at 12 days of age, showed better ADWG and FCR at 14 days postchallenge at 1.14% Arg. Higher levels of Arg (1.34%) improved the intestinal permeability at five d postchallenge and the tight junction proteins zonula occludens-1 and zonula occludens-2 at six d postvaccination, while the addition of 1.44% Arg increased the zonula occludens-1 and zonula occludens-2 at 14 d postvaccination [16]. In chicks also housed in cages from 1 to 21 days, added with 1.11, 1.33, and 2.01% dietary Arg and challenged with a coccidiosis vaccine at 14 d of age, showed similar ADWG regardless of the dietary Arg level and lower FCR at 1.33% Arg. Increased sucrase, slgA, and relative IL-1RI mRNA expression and reduced abundance of TLR4 and MyD88 in jejenum at 7 days postchallenge were observed at 1.33% Arg, and increased mucosal density in the jejunum was observed at 2.01% Arg at 7v postchallenge [38].

In floor-pens reared broiler given 100, 105, and 110% of the standard recommended values of dietary Arg for Ross broilers, and challenged with a mixture of Eimeria species from 16 to 20 days of age, addition of 105 and 110% Arg, prevented depressed ADG in coccidia-infected broiler chickens during the finisher period. The FCR was improved at 110% Arg supplementation during the grower and finisher periods. Increased villi height to crypt depth ratio at 105% Arg and increased villi surface area at 110% Arg were found, as well as a linear decrease in fecal oocyst count [39].

Broiler chicks reared in pens fed 100, 125, and 150% Arg levels, according to Ross recommendations, and infected with Eimeria on day 21, showed better ADWG, FI, and FCR from 22 to 42 days of age at 125% dietary Arg (starter 1.71%, grower 1.54%, and finisher 1.375%); furthermore, at 125 and 150% dietary Arg, increased levels of serum NO and proinflammatory cytokine concentrations (IL-1β, IL-2, IL-6, TNF-α, IFN-γ) and reduced fecal oocysts were found [40].

In two additional studies with broilers subjected to viral challenges, it was observed that productivity and immune responses were improved with higher levels of Arg than recommended. Broiler chickens fed diets exceeding by 2.5 times, the recommended NRC levels (starter 1.34 vs. 3.35, grower 1.13 vs. 2.8, and finisher 1.1 vs. 2.58), and challenged with an intermediate plus strain of IBD virus (10-fold greater than normal vaccination doses) at 28 days of age, showed enhanced body weight, ADWG, and FCR, as well as higher serum level of IFNα, IFNγ, immunoglobulin G, and lower lesion scores in the bursa and spleen compared to the control birds [41]. In the same way, broiler chicks fed 2% supplementary dietary Arg and vaccinated and challenged against hydropenicardium syndrome virus showed higher body weight, lymphoproliferation, and cutaneous basophil hypersensitivity reactions, lymphoid organ weights, and highest survival rate compared to unvaccinated non-Arg supplemented chicks [42].

Results in four available studies, in which nonchallenged broilers were fed increasing dietary Arg concentration, indicate that the Arg needed to stimulate the immune system was higher than that needed to improve the growth performance. These results were irrespective of the basis of Arg formulation, the growth rate of the birds, and the type of housing (cages or floor pens).

The information also denotes that in four out of eight studies available, in which challenged broilers were fed increasing dietary Arg concentration, the Arg needed to
stimulate the immune system was also higher than that needed to improve the growth performance. These results were irrespective of the basis of Arg formulation, the growth rate of the birds, the type of housing (cages or floor pens), and the type and degree of challenge.

It was found that in three out of four studies, in which the growth performance and immune and digestive responses were enhanced at the same Arg levels, the Arg levels were higher than those recommended for optimum growth; it is noteworthy that in one of these studies, ADWG and FCR were improved in 1–49-days-old broilers with Arg levels 2.5 times higher than recommended.

3. Threonine

Thr is ranked as the third limiting AA [2, 4] and is very important for the synthesis and maintenance of proteins in the body. About 30–50% of Thr, as well as some other amino acids, is directly used by the small intestine and is not available for extraintestinal tissues. Thr has special importance as an essential nutrient because, compared with other AA, it has the highest metabolism in the portal-drained viscera. One of the primary fates of absorbed Thr is the synthesis of intestinal proteins, which are mainly secreted into the lumen as mucus, whereby protecting the gut from pathogens and antinutritional factors. Mucins are particularly rich in Thr, proline, and serine, with Thr representing as much as 28 to 40% of its total AA profile [43].

The recommended dietary Thr levels for optimum growth performance in broilers varies from 0.80 to 0.68% for starting and growing birds [7] and from 0.85–0.89 and 0.65–0.68 for starting and finishing birds, respectively. Further to this, it has been demonstrated that supplementation of Thr either in ovo or dietary Thr above the recommended level improves the digestive physiology and the cellular and humoral immune responses in nonchallenged birds and those subjected to different immune challenges [43]. IOF of Thr has shown to increase the expression profile of growth factors and immunity-related genes, including higher mucin gene expression on incubation day 18, higher expression of mucin gene on day 14 postinoculation, higher humoral expression of IL-6 and TNF-α, and higher IL-12 cellular gene expression in 26-days-old broilers [22]. In the next section, the recent findings on Arg feeding from in ovo to unchallenged and challenged broilers will be presented with emphasis on simultaneous effects on immunity/digestive physiology and productivity.

In several experiments, the IOF of Thr at day 14th of incubation enhanced various immune and digestive responses. IOF of 20 or 30 mg Thr/egg improved the ADWG of broilers from 14 to 28 days of age and enhanced the humoral response to sheep red blood cells; there was a tendency for digestive enzyme activities in proventriculus, jejunum, and pancreas to be higher in Thr-injected chicks at 21 days of age [44]. IOF of 25 mg Thr/egg increased the body weight of broilers at 11, 24, and 42 d and the FI from 1 to 42 days of age; Thr also enhanced the ileum villus height in 11-days-old chicks and the relative weight of the jejum and ileum and the length of the jejum in 42-days-old broilers [23]. IOF of 25 mg Thr/egg also increased the ADWG and FI in broilers from 1 to 42 days of age and the antibody titer against sheep red blood cells in broilers at 30 days posthatch [24]. In both studies, hatchability was similar to the control group.

In few experiments, the IOF of Thr in the last days of incubation has been also evaluated. IOF of 10.5, 21.0, 31.5, and 42 mg Thr/egg on day 17.5 of incubation
improved the chick hatch weight and growth performance from 1 to 21 days of age; Thr increased the villus height, villus height: crypt depth ratio, and villus area at hatch and 21 days posthatch. At hatch, all Thr levels increased the expression of MUC2 and PepT1 compared to the control group [45]. IOF of 15, 30, and 45 mg Thr/egg at 18th embryonation d increased the ADWG in broilers up 21 days posthatch, and the FCR was improved at 45 mg Thr; Thr increased the thymus weight (d0), bursa weight (d3), spleen weight (d3 and d7), whereas quadratic effect was observed on weights of bursa, thymus, and spleen at d21. IOF of Thr also increased the weights of gizzard, intestine, and liver at hatch, proventriculus at d7, as well as intestine and liver at d21 [46].

A summary of the results indicates that IOF of Thr at 14 and 17.5–18 days of incubation increased the growth performance of broilers up to 42 days of age, which could be explained by improved immune responses, but especially by increasing the development of the digestive capabilities. The best dosage for IOF of Thr appears to be around 25 mg/egg. In all cases, a high hatchability is maintained.

During the growth out of broilers, there are several studies of Thr supplementation as functional AA in nonchallenged conditions. Ross male broilers fed diets containing 0.8% (NRC [7] requirement), 0.87% (average of NRC and Ross requirement), 0.94% (Ross requirement), and 1.01% (more than Ross requirement) Thr had improved growth responses as dietary Thr increased from 0.8% to 0.87%; similarly, the villi height, crypt depth, and villi surface increased as dietary Thr increased from 0.8% to 0.87% [47]. In broilers from 1 to 21 days fed increasing standardized ileal digestible Thr levels from 0.4 to 1.1%, it was reported that ADWG was higher at 0.84–0.89% Thr, while the villus height in duodenum, jejunum, and ileum were increased linearly up to 1.1% Thr [48]. In broilers from 1 to 21 days of age fed 0.79, 0.87, and 1.07% Thr showed no differences in growth performance due to the supplementation of Thr; opposite to this, Thr supplementation increased the relative weight of spleen and thymus. Thr supplementation linearly increased the intestinal villus height, the ratio of villus height to crypt depth, as well as the goblet cell density and the jejunal immunoglobulin G and M. At the highest Thr supplied, the ileal secretory immunoglobulin A content and mucin-2 mRNA expression were increased, while the mRNA abundances of interferon-γ and interleukin-1β in the ileum were downregulated [49].

In broilers reared in floor pens and fed increasing dietary Thr levels (starter from 0.69–1.21% and grower 0.62–1.12% Thr), which correspond to 85–150% of NRC [7] recommendations, the ADWG and FCR were improved at 100% Thr, whereas the villus height in duodenum and jejunum, crypt depth in duodenum, and villus height/crypt depth ratio in jejunum were increased a 150% Thr in 21-days-old broilers, and the villus height and villus height/crypt depth ratio in jejunum were increased a 125% Thr in 42-days-old broilers [50]. Floor pen reared broilers fed increasing levels of dietary Thr (starter from 0.94–1.22% and grower from 0.74–0.96% Thr), equivalent to 100–130% of Ross 308 recommendations, had higher growth performance at 110% Thr inclusion, but the antibody titers against NDV and SRBC increased up to 120% Thr supplementation [51]. In two floor pen experiments using slow-growing broilers and a basal feed formula that met the requirements mentioned by Rostagno et al. [8] and added with increasing levels of digestible Thr, it was estimated that the lowest FCR was reached at 0.762 and 0.767 for starter and grower broilers, respectively, while the production of intestinal mucin was highest at 0.697% Thr in the starter phase [52].
In another study, in which broilers from 1 to 21 days of age were fed diets to match the Thr supply to 100% NRC specification, and from 100 to 130% Thr of Vencob-400 strain specification, the ADWG was highest at 100% Thr of Vencob-400 strain specification (0.87% Thr); the villus height, crypt depth, villus surface area, goblet cell number/villus, villus width, and goblet cell density were higher at 120% Thr and the weight of bursa and thymus, the total immunoglobulins, titers against Newcastle disease virus, lymphocyte proliferation, and neutrophil phagocytic activity were increased linearly up to 130% Thr [53]. Broilers fed dietary Thr levels that matched 100, 110, and 120% of NRC recommendation and kept in floor pens from 1 to 35 days of age showed enhanced ADWG and FCR at 110% Thr as well as higher villus height, lower crypt depth, greater VCR, greater weight of thymus and bursa, and greater infectious bursal disease titer [54]. Similarly, 1–21 days of age broilers fed dietary Thr level of 100, 120, and 140% of the NRC recommendation had improved performance ADWG and FCR at 120% Thr; anti-SRBC titer were increased at 120% Thr, and the jejunal crypt depth increased and the jejunal and ileal crypt width decreased at 140% Thr [55].

Some experiments were carried out using increasing dietary Thr addition in broilers under bacterial and coccidial challenges. Broilers from 1 to 10 days of age fed two dietary Thr levels (0.857 and 0.956%) and challenged with Salmonella Enteritidis at 2 days of age showed no difference in performance, but the intestinal integrity was improved in chicks fed the higher Thr level, including higher villus height, villus:crypt ratio, and goblet cell counts in the jejunum and ileum [56]. In the same way, broilers from 1 to 10 days of age fed two dietary Thr levels (0.81 and 1.00%) and challenged with Salmonella Enteritidis at 2 days of age showed no difference in performance, but had increased villus height and villus: crypt ratio in the duodenum [57]. Broilers of 1–21 days age kept in cages and fed two levels of dietary Thr (0.784 and 1.084%), undergoing a challenge using E. coli LPS from 17 to 21 days had improved ADWG and FCR at the higher Thr level and reduced serum IL-1β, and TNF-α, IFN-γ in jejunal mucosa and L-1β in ileal mucosa [58]. In three floor-pen experiments, different Thr-to-Lys ratios (from 0.56 to 0.77) were evaluated (as standardized digestibility) in the diets of broilers subjected to a subclinical Clostridium infection at nine d of age; from 9 to 37 days of age, the ADWG in broilers fed the high dietary Thr was increased, but the intestinal damage (incidence and lesion severity) was not affected by Thr supplementation [59].

The results indicate that in eight out of 12 studies, in which nonchallenged and challenged broilers were fed increasing dietary Thr concentrations, the Thr needed to stimulate the immune and digestive system was higher than that needed to improve the growth performance. These results were irrespective of the basis of Thr formulation, the growth rate of the birds, the type of housing (cages or floor pens), and the type and degree of challenge.

4. Implications

According to the literature reviewed, the level of AA required to stimulate the immune and digestive systems in unchallenged and challenged chickens is higher than that required for optimum growth performance; however, from a practical standpoint and the formulation of commercial diets, there is not enough information to confirm any benefits of adding functional AA, especially when issues such as sustainable
poultry production, in which the economic return and environmental concerns are key components, come across.

The promotion of concepts such as phase feeding, an ideal AA profile, the addition of AA on a digestible basis, and the use of low-CP diets supplemented with crystalline AA in modern feed formulation aims to maintain high levels of productivity while having a low environmental impact. The recommendations on the required levels of AA in each specific situation have been established by taking into account the stages of development, environmental conditions, management, and degree of immunological challenge due mainly to the presence of infectious agents. All of this is done to ensure that the birds consume the amount and proportion of AA that best suits their maintenance and growth needs while avoiding any excess or deficiency of AA.

The incorporation of functional AA into practical formulation is complex, owing to the fact that levels of AA above the established requirement for growth must be included. When this occurs, an AA imbalance may exist, affecting digestion, absorption, and metabolism of AA from the same group, as has been demonstrated with dibasic AA such as Arg and Lys. This could result in a deficiency of one or more AAs from the same group, resulting in the deamination of all AA not required in the various metabolic processes, in order to eliminate excess nitrogen in the form of uric acid, resulting in the excretion of excess nitrogen through urine. At the same time, significant amounts of energy associated with uric acid synthesis would be excreted. This problem has not been addressed in the literature.

Experiment models in animals subjected to various challenges attempt to simulate what happens in commercial farms, where animals are exposed to various sources of stress as well as viral, bacterial, and parasitic infectious agents. During the growthout process, the main factors that cause immune challenges (dietary components, management, environment, and infectious agents) can be present simultaneously and sequentially. If experimental and field challenges elicit the same level of immune stimulation and type of immune response, implying that the stimulatory effects of functional AA are similar in both scenarios, it is important to note that in AA nutrition, the ultimate response to AA additions is measured by the productive response. This implies that perhaps, with the information at hand, the use of higher levels of AA beyond the levels necessary to maximize growth and FCR is questioned. In other words, in challenged birds, the use of higher than recommended levels of AA to stimulate a greater immune and digestive response is not worthwhile if this is not reflected in increased growth.

This controversy could probably be explained by drawing on much of the information already known about the metabolic effects of immune challenges to redirect AA to protective functions involving various humoral and cellular mechanisms. To increase the supply of AA, body protein will be broken down and production performance will decrease. This is necessary since the entire immune response process requires amounts and proportions of AA that vary for each type of response. In contrast to this, in most of the reviewed studies, functional AAs have been evaluated individually, or adjusted to a profile to cover the growth recommendations, but in very narrow ranges. This is explained by the difficulty of adjusting the amount and profiles of AA when feeds are balanced with AA concentrations far above the normal requirement, especially in low-CP diets. In addition, the lack of information of a proper AA profile for immune-challenged situations makes this task more difficult.
It is noteworthy that several authors have hypothesized that the addition of synthetic AA would particularly improve the animals’ immune response against intracellular pathogens. If this hypothesis is confirmed, the use of functional AA could play a critical role in pathogen reduction and, as a result, in the spread of antimicrobial resistance factors. These advantages should be confirmed in farm animals that are normally subjected to acute and chronic stressors, which may be concurrent and synergistic. It is critical at this point to determine whether episodes of immunosuppression caused by stress can be overcome by functional AA.

It is also unclear whether functional AA should be used continuously or only on a case-by-case basis, particularly when birds are stressed or when there are conditions that increase the risk of disease. If the application is strategic, it should be specified the best moment and the period they should be supplemented.

It is also possible that functional AA should be supplemented during or after an immunological challenge to aid in the recovery of affected individuals and to restore productive parameters to prechallenge levels.

5. Conclusions

The use of functional AA such as Arg, Thr, and Met to improve the health and productivity of birds exposed to immune challenges is promising. It has been proposed that functional AA can help the immune system fight intracellular pathogens. It is necessary to determine whether episodes of immunosuppression caused by stress can be overcome by functional AA in field-raised birds, as well as to define the strategic use to reduce disease risk. It is also possible that functional AA should be supplemented during or after an immunological challenge to help affected individuals recover and return productive parameters to prechallenge levels.

Conflict of interest

The authors declare no conflict of interest.
Advances in the Nutrition of Functional Amino Acids in Healthy and Immunologically...
DOI: http://dx.doi.org/10.5772/intechopen.101895

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Broiler Industry


