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Role of amino acids in the immune system: A special focus on broilers

A study published in 2003 comparing the nutrition and genetics of broilers from 1957 to a commercially available broiler strain, estimated that within less than 50 years of development, broilers were reared to maturation in a third of the time (Havenstein *et al*., 2003). This shows clear evidence that improvements in genetics, nutrition and management of rearing meat-producing chickens have continually improved the growth rate of birds.

However, the effects of this rapid growth rate on immune function and nutrient requirements of the immune system, have not been well defined.

To date, growth and feed efficiency curves in clean experimental conditions have been used as basis for defining the amino acid requirement of the broiler in requirement studies. A growing body of research suggests that this definition of requirement may be too narrow, as the requirement for specific amino acids in terms of optimum growth and immune function may be above those required for growth alone.

To best prevent detriments to growth performance associated with disease, it is advisable to ensure the animal has a strong, fast and adaptable immune system that can efficiently and swiftly manage threats. It is important to identify if nutrient requirements change when an animal undergoes a disease challenge.

Immune system and metabolism

To understand how nutrient recommendations may change if both muscle deposition and immune function are included in the equations that define requirements, the interactions between metabolism and immunity need to be investigated. It may not be as simple as increasing all amino acid levels in feed formulations in order to provide sufficient quantities for maintenance of growth and health. In addition, the effects of immune system activation on metabolism demonstrate the importance of maintaining broiler health in order to optimise performance and subsequently cost-efficient production.

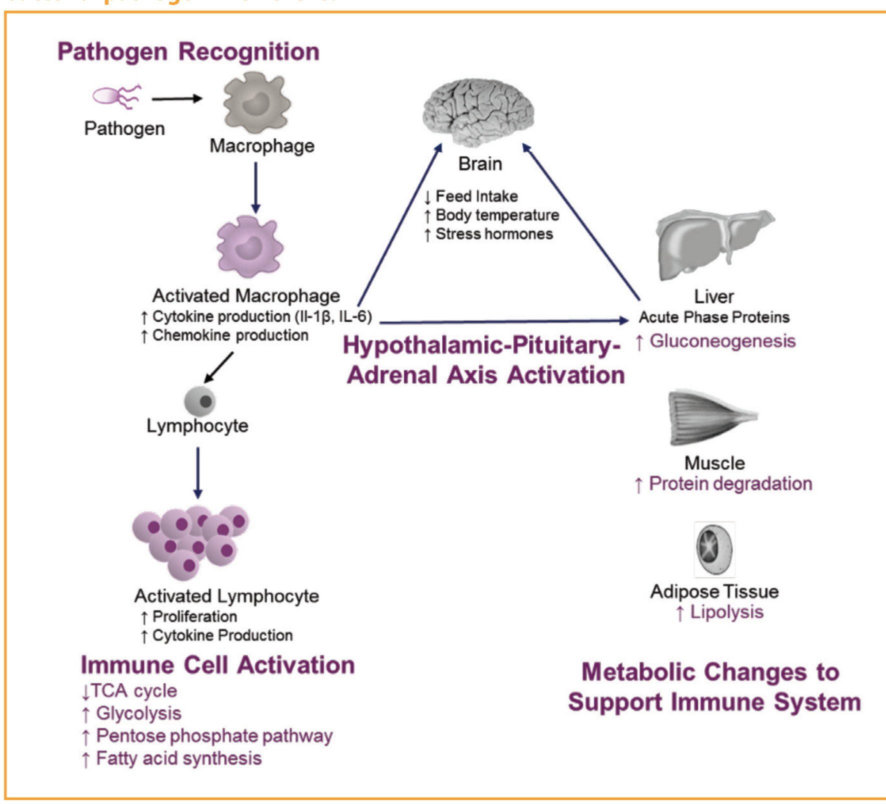
It has been well described that acute activation of the immune system often results in the initiation of inflammatory pathways that reduce feed intake (*Figure 1*).

When the broiler immune system is stimulated by a pathogenic organism, the efficiency of feed conversion into protein deposition is significantly impacted.

Methionine requirements

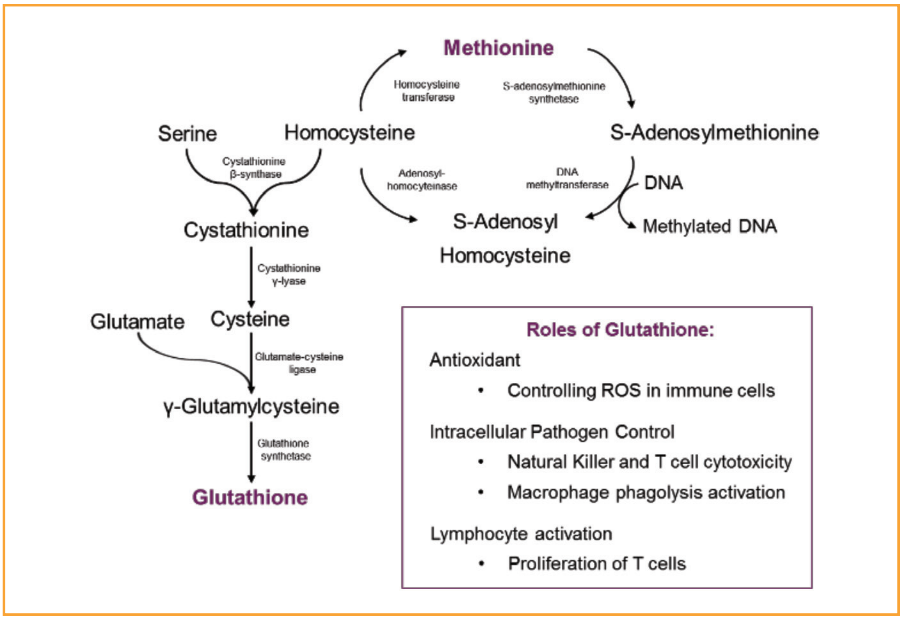
Polyamine synthesis: Polyamines are required within individual cells for many important processes involved in rapid cell proliferation, including transcription, mRNA stabilisation and translation. Polyamines can be vital for proper viability of cells (membrane stability and DNA stabilisation) as well as cell

Figure 1: The metabolic pathways involved in a primary immune response to a bacterial pathogen in chickens.



Upon recognition of a pathogen macrophages become activated to produce cytokines. This, in turn, activates lymphocyte populations and signals the liver to produce acute-phase proteins. The cytokine and acute-phase protein milieu activate the hypothalamic-pituitary-adrenal axis, which may reduce feed intake and induce fever. Metabolic pathway prioritisation within immune-activated immune cells changes to meet the needs of cytokine production and proliferation, while global metabolic changes provide substrates required for immune function.

Figure 2: The role of methionine in the synthesis of glutathione, as well as the various roles of glutathione in immune function.



Adapted from Gallus gallus specific pathways on the KEGG PATHWAY database 'Cysteine and methionine metabolism' and 'Glutathione metabolism', Kanehisa, 2002.

functions, including cell-receptor binding. Methionine is an essential precursor for the synthesis of polyamines (putrescine, spermidine and spermine). The synthesis of these polyamines is generally upregulated in inflammation; however, so is the catabolism of these polyamines.

Oxidative stress: Glutathione in a reduced state is an important antioxidant as it acts as an electron acceptor for free radicals. Cysteine is a precursor for this antioxidant that can be supported through the degradation of methionine in the homocysteine pathway (*Figure 2*). The availability of these sulphur amino acids (SAAs), methionine and cysteine, may therefore be increasingly important in situations where oxidative stress occurs.

Oxidative stress may occur in the initiation of an innate immune response. The production of reactive oxygen species, such as nitric oxide, is utilised by innate cells, such as granulocytes and macrophages, to kill ingested pathogens. These free radicals that are produced to kill pathogens must be regulated so they do not induce oxidative stress that can impact healthy host cells; glutathione production can play a role in maintaining the redox balance within these innate immune cells that produce reactive oxygen species.

Immune function in broiler chickens: Several studies have shown that the dietary requirement for SAAs for growth may not be meeting the requirement for immune function. Tsiagbe *et al*. (1987) conducted a series of trials to compare the growth performance and immune response of broilers with diets that included different SAA levels. They found that optimal growth performance was reached at a lower dose than was observed for immune function optimisation, as measured by total antibody and T cell proliferation response. Additional dietary SAA above the requirements for broiler growth may also support innate immunity.

Arginine requirements

Immune cell production: Immune cell progenitors all arise from haematopoietic stem cells in the bone marrow. The progenitor cells that will become T cells move from the bone marrow to the thymus to finish their training, while the progenitor cells that will become B cells move to the bursa of Fabricius to complete their maturation. Arginine has been found to act as a secretagogue, stimulating the release of hormones, such as growth hormone, from the pituitary gland in the brain.

Innate immune cell functions: Macrophages are innate immune cells best known for their ability to eat relatively large components, such as live bacterial pathogens or dead cell debris, through a process known as phagocytosis. However, these cells have several phenotypes that support diverse functions. The differentiation of macrophages into specific phenotypes is largely dependent on the way they utilise arginine (*Figure 3*).

Adaptive immune cell functions: T cells are members of the adaptive immune system. Arginine has been shown to be very important in both maintaining the viability of T cell receptors, in regulating proliferation of T cells after activation, and in the long-term survival of memory T cell subsets.

Importance of arginine to broiler immune function: The role of arginine in immune development and function has been specifically studied for broiler chickens and confirms the hypothesised importance of the amino acid in immune function based on multi-species literature reviews and broiler specific pathway analysis. Additional dietary arginine above growth requirements has been observed to improve spleen and bursa development, increase populations of both innate and adaptive immune cells, and improve antibody responses.

In an *Eimeria* spp. and *Clostridium perfringens* co-infection challenge model, additional dietary arginine was able to ameliorate intestinal damage from the disease model, increase both inflammatory and regulatory cytokine expression, and reduce the abundance of *C. perfringens* in the intestines. Unlike the parasite challenge studies, these bacterial pathogen challenge models suggest that additional dietary arginine may directly assist in anti-bacterial immune responses.

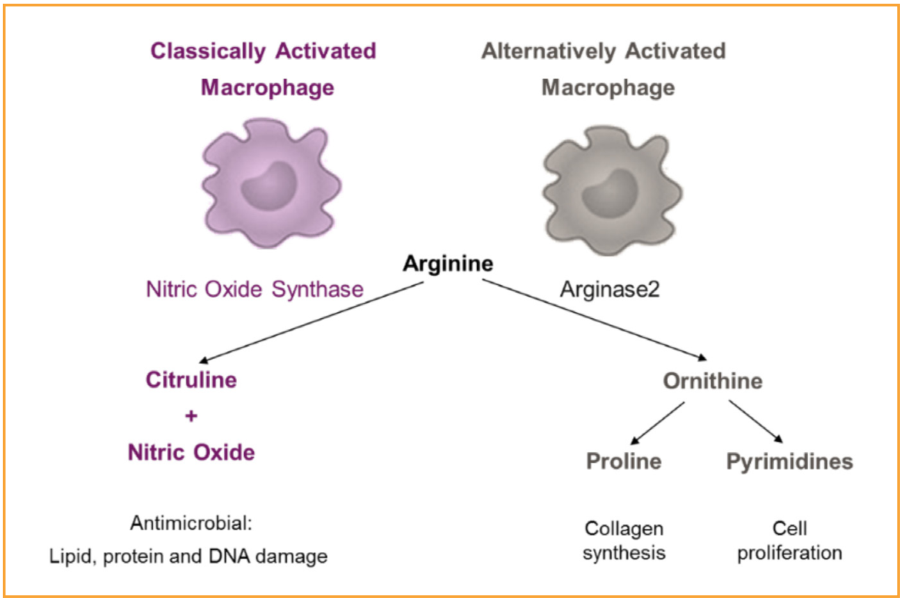
Arginine has also been reported to be vital in the immune response to viral diseases of poultry. Increasing dietary arginine in layers challenged with infectious bronchitis was shown to increase the numbers of innate immune cells in both the blood and respiratory tract.

The roles of arginine in immune function are diverse and involved in development, activation and maintenance of immune function. It is thus unsurprising that the requirement for arginine is reported to increase in various kinds of immune system challenges of poultry, including parasitic, bacterial and viral infections.

Threonine requirements

Mucin production: The greatest borders between the inside of the body and potentially harmful agents in the

Figure 3: The role of arginine in the function of classically activated macrophages for antimicrobial activity, and of alternatively activated macrophage for wound healing mechanisms.



Adapted from Muraille et al. (2014) with Gallus gallus specific pathways from the KEGG PATHWAY database 'Arginine and proline metabolism' (Kanehisa, 2002).

environment are the mucosa-associated lymphoid tissues. Many of these borders are lined with a layer of thick, protective mucus to provide a barrier between micro-organisms in the lumen and the epithelial cells lining the lungs or gut. The mucus layer provides a physical barrier for bacterial infections.

Mucus is comprised of mucins, which are glycosylated proteins containing a proline-threonine-serine-rich region that can comprise 50% of the protein structure. For some secreted mucins, the threonine levels have been estimated as high as 11% in some species. It is therefore logical that restriction of dietary threonine has been shown to inhibit mucin production.

Environmental triggers such as pathogen infections, or bacterial fermentation in the gut occurring in the presence of high fibre diets, can stimulate increased mucus production and subsequently threonine requirements.

Antibody production: Antibodies, also known as immunoglobulins, are an important component of the immune system of mammalian and avian species. Threonine has been implicated in mammalian studies as being a major component of antibodies and was estimated to be as high as 10% of milk immunoglobulins.

Importance of threonine to broiler immune function: It has already been reported that requirements for threonine in broilers may be largely dependent on feed ingredients, rearing environment, and pathogen exposure. For example, high fibre diets have been shown to increase the threonine requirement, which is postulated to be due to both increased bacterial fermentation in the gut and/or mucus sloughing.

Vaccination may also increase the threonine requirement of broilers to support antibody response, as increased threonine above maintenance requirements was shown to improve both non-specific antibody responses and production of antibodies specific to Newcastle disease, post-vaccination.

Tryptophan requirements

Tryptophan as a substrate for serotonin synthesis: The production of serotonin is probably one of the best-studied examples of the uses of tryptophan beyond growth performance, especially for broilers. Serotonin is synthesised from tryptophan and is a neurotransmitter involved in gastrointestinal motility, wound healing and mediating stress responses. Increased serotonin production has been linked to a reduction in aggressiveness and fear responses, which could help reduce the risk of injury during handling or when animals are housed at a high stocking density.

Stress and acute inflammation also initiate stress hormone release through the hypothalamic-pituitary-adrenal axis (*Figure 1*), which is negatively correlated to serotonin activity. It could therefore be that higher levels of serotonin, and therefore tryptophan, are required to mediate the stress hormone response to environmental stimuli.

Importance of tryptophan for broiler immune function: The best studied effects of dietary tryptophan beyond broiler growth relates to the production of serotonin, which may be because the serotonergic system and its development has been well defined in chickens for several decades. Increasing dietary tryptophan has been observed to increase serotonin, decrease stress-related hormones such as corticosterone, and reduce aggressive behaviour or fear responses in chickens.

Role of other amino acids

Branched chain amino acids (BCAAs) have been reported in mammalian studies to be involved in immune function. For example, leucine is important in pathways initiating lymphocyte cell proliferation, as well as active functions of cytotoxic T cells and natural killer cells. Another BCAA, isoleucine, has been implicated in the production of antimicrobial peptides in the gut, while valine may improve innate immune function. However, BCAA studies in broilers are very limited.

Summary

The amino acid requirements for immune development, vaccination response, parasite control, bacterial response, anti-viral immunity, and immune regulation could vary quite significantly as the cells and pathways involved in these different arms of the immune system are diverse. In addition, for enteric pathogens the utilisation of amino acids in the lumen by the pathogen itself may alter the availability to the animal and intestinal damage could affect digestibility.

Nutritional strategies to support the development of a healthy immune system and prevent disease should be considered as an area with great potential in order to reduce the cost of disease to broiler performance and therefore the broiler producer.

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