Maximum immunity effectors: Mechanisms and animal performance limitations\textsuperscript{1,2}

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Primary Audience: Researchers, Feed Nutritionists, Poultry Producers

SUMMARY

The immune system, as with any other system in the body, requires several nutrients for normal development and function, such as carbohydrates, amino acids, fatty acids, vitamins, and minerals. Theoretically, for any nutrient, increasing the dietary levels of a particular nutrient can be expected to improve immune parameters and poultry performance linearly up to a certain level of inclusion, followed by a plateau and negative effects at higher doses. Under practical situations, increasing the dietary levels of a particular nutrient fails to improve the immune parameters and poultry performance expected in the linear phase. This article reviews the limitations that restrict application of the nutritional immunology concept developed under experimental conditions to much different field conditions. It may not be beneficial to alter a host immune response; further, maximizing the immune response may not be ideal in every situation. The immune system is a balance between different arms of the immune response, and strengthening one arm of the balance might impair the other arm of the balance. Also discussed are the interactions within and between dietary nutrients and host physiology, which ultimately determine the net effect of nutrients on the immune parameters. It is critical that we understand the limitations of nutritional immunology before applying its concepts in attempts to improve poultry performance.

Key words: immune system, nutritional immunology, performance limitation

INTRODUCTION

The immune system, as with any other system in the body, requires several nutrients for normal development and function, such as carbohydrates, amino acids, fatty acids, vitamins, and minerals. The minimal or adequate requirements for several of the above essential nutrients have been identified, at least from the poultry production perspective, by the NRC [1]. The NRC recommendations are based mostly on poultry production parameters such as BW gain, breast muscle yield, or number of eggs produced. Because a healthy immune system is...
Indispensable for achieving high production parameters, as observed in modern poultry production, it could be assumed that the requirements defined by the NRC would likely meet the needs of the immune system. However, NRC requirements are determined in birds raised in a controlled laboratory environment, where infection is the least common. In addition, various nutrients have been identified that are not defined as essential by the NRC but that can still modify an immune response during infections. The ability and efficiency of several nonessential nutrients to modify an immune response becomes important in commercial poultry conditions, where infections and inflammation are common. Accordingly, several researchers have identified a multitude of nutrients that can alter the immune response in a beneficial way and ultimately increase production parameters during or after an immune response.

Theoretically, for any nutrient, increasing the dietary levels of a particular nutrient can be expected to improve the immune parameters linearly up to a certain level of inclusion, followed by a plateau and then negative effects at higher doses. It has been claimed or insinuated that including such immunomodulating nutrients in a poultry diet can be expected to increase poultry production parameters. Unfortunately, the relationships among the inclusion levels of dietary nutrients, immune parameters, and production performance are much more complicated interactions than they appear on the surface. In this article, I review the interactions between dietary immunomodulating nutrients and production performance, the mechanisms that dictate the interactions, and the limitations of applying the interactions to increase the production parameters or performance of the bird. This article is focused on the following:

1. Altering the immune response by dietary nutrients, and whether it is ideal for all situations;
2. Nutrients altering the balance in immune response, and the counterbalance by body physiology;
3. The nutrient reserve capacity;
4. The prioritization of nutrients; and
5. Interactions between nutrients.

**Altering the Immune Response by Dietary Nutrients: Is It Ideal for All Situations?**

**Immune Response Is a Balance Between Cells with Opposing Effects.** The functional immune system in avian species is composed of several cell types, which can be broadly classified into cells involved in innate and adaptive immune responses. Macrophages, neutrophils, natural killer cells, T helper (Th) 1 cells, Th2 cells, B cells, and dendritic cells are some of the major immune cells. The immune system directly protects the host by using an array of inflammatory mediators, such as proinflammatory cytokines, perforins, granzymes, reactive oxygen species, reactive nitrogen species, nitric oxide, superoxides, and other effector molecules that can destroy the pathogen. On the other hand, anti-inflammatory cytokines and enzymes of antioxidant defense act to restrict the effect of inflammatory mediators. It is important to understand why an animal that is undergoing an infection challenge produces anti-inflammatory mediators that might increase the risk of unsuccessful pathogen clearance.

Nutritional and physiological costs are associated with an immune response [2], and immune responses are expensive to the host in terms of energy requirements [3]. Maintaining a functional immune system and mounting an immune response are nutritionally demanding processes that necessitate trade-offs among competing energy demands for growth, reproduction, parental and mating effort, temperature regulation, and work [4]. Chicken BW gain or egg production decreases within 24 h after an infection. Thus, although the immune system has a beneficial effect on the host by fighting the pathogen, the energetic cost associated with a normal immune response or a hyperactive immune response requires that the animal have some inherent adaptive mechanism to restrict the production and action of inflammatory mediators of an immune response. An optimal immune response involves a proper balance between the pro-inflammatory and anti-inflammatory mediators. Understanding the effect of the above dichotomy is essential for understanding why some immune-enhancing nutrients fail to translate into increased poultry performances.
Strengthening One Part of the Balance Might Impair the Other Part of the Balance.

Altering the balance between different parts of the immune system will have consequences that may not be beneficial in all situations. It has been well studied that fish oil alters the balance between Th1 and Th2 immune responses. The Th1 cells are directed against intracellular pathogens (viruses, bacteria, and cancer cells), whereas the Th2 cells are directed against extracellular pathogens (bacteria and parasites). In a simplistic model, Th1 cells are considered overactive (or overaggressive) compared with Th2 cells; hence, it has been suggested that nutrients that promote Th2 responses are able to increase production performance in poultry. Accordingly, several of the nutrients that can upregulate a Th2 response have been promoted to increase production performance in poultry during an immune response. For example, fish oil or n-3 fatty acids have been widely shown to upregulate Th2 responses in several species, including poultry. Including fish oil during an immune challenge increases BW gain compared with including corn oil (which increases Th1 responses) in broiler chickens [5].

Altering the balance between different parts of the immune response may have unintended detrimental consequences in some situations. The negative effects of altering the Th1-Th2 balance through the use of pharmaceutical compounds have been well documented [6]. In mice, dietary fish oil decreases CD8+ cell numbers, increases regulatory T-cell numbers, and induces severe colitis and adenocarcinoma formation [7]. Fish oil supplementation, however, decreases the incidence of cardiovascular diseases and increases the susceptibility to intracellular pathogens [8]. In other words, fish oil decreases the efficiency of the Th1 response and increases the efficiency of the Th2 response. Similar results have been reported in poultry production. Dietary n-3 fatty acids significantly increased incidences of tumors, both spontaneous and from Marek’s disease virus, while decreasing the incidence of septicemia [9]. Similar alterations in one part of the immune system causing unintended upregulation or downregulation of the other part of the immune system is a limitation to applying immune enhancers to increase production. Thus, a nutrient might ultimately change the spectrum of disease in a population. Inclusion of a particular nutrient to increase production performance should take into account the likely pathogen population in that particular location.

Maximizing the Immune Response May Not Be Ideal. Any nutrient that increases the pro-inflammatory arm of the immune response might increase the chance of successful pathogen clearance but might also decrease production performance, at least in the short term. Any nutrient that increases the anti-inflammatory part of the immune response and increases production performance in the shorter term might increase the risk of host susceptibility to pathogen infection in the longer term. In other words, it is not possible to define any set values for an optimal immune response that will include different situations. Studies with n-3 fatty acids have repeatedly shown both beneficial and detrimental effects in different situations (Table 1). Other examples include the increased numbers and activity of regulatory T cells in the mucosal-associated lymphoid tissues of animals fed prebiotics [10]. Although increasing regulatory T cells might actually help alleviate allergy, regulatory T cells dampen T effector cell functions, which may, in fact, increase incidences of gut infections.

The optimal immune response will depend on the specific circumstances and infection status of the host [11]. An optimal immune response should be based on specific situations that should take into account environmental and nutritional factors, the age of the bird, infection status, and pathogen virulence. Optimal immune responses are context specific; hence, the maximum immune response is not necessarily ideal for poultry production in terms of BW gain or egg production [12].

Nutrients Altering the Balance in Immune Response: Counter Balance by Body Physiology

The ultimate goal of the immune system is to eliminate the pathogen with minimal damage to the host, and the immune system achieves this goal by using a unique combination of different components of the immune system that will cost the least to the host in terms of nutrient re-
The immune system has evolved to include several inherent balances and counter-balances that regulate it. For example, at the end of the acute phase response, the host downregulates the inflammatory mediators by upregulating anti-inflammatory mediators or enzymes of antioxidant defense to protect the host from tissue damage. This inherent built-in balance system will limit the efficiency of a nutrient to successfully alter the immune outcome and, consequently, the production parameters. This variable physiological response by the host to dietary nutrients is often manifested as a biphasic response to nutrients.

Several nutrients have been shown to have biphasic immunomodulatory effects, at least in vitro. For example, lutein [13], lipoic acid [14], arginine [15], and several polyphenols [16] have biphasic effects on the immune system. Lutein supplementation at a lower dose decreases inducible nitric oxide synthase (iNOS) messenger RNA, but at a higher dose, lutein increases iNOS messenger RNA in chicken macrophages [13]. Lipoic acid supplementation at a lower dose increases IL10 production, but at a higher dose, it decreases IL10 production [14].

The biphasic effects of arginine on the immune system have been well studied. Arginine is a common component of several immune supplements. Arginine is metabolized either by iNOS or by arginase 1, enzymes that are stimulated by Th1 or Th2 cytokines, respectively [17]. Arginine metabolized through the iNOS pathway will produce nitric oxide and stimulate other proinflammatory pathways that are detrimental to cell proliferation, collagen formation, and wound closure. On the other hand, arginine metabolized through the arginase 1 pathway contributes to protein synthesis, cell signaling, differentiation, proliferation, and collagen synthesis. It is interesting that pathogens also produce arginase (to deplete arginine) to limit nitric oxide production by the host, which contributes to pathogen survival within the host. Further, arginine depletion by the pathogen will impair T-cell proliferation [18]. Thus, the net effect of arginine on the immune system is dependent on the inflammatory status and the availability of arginine to the different cells of the immune system. A similar biphasic response to tryptophan has been well studied [19].
To summarize, the effect of a particular nutrient on the immune system is determined by factors other than the nutrient itself. One such factor is how the body responds to the nutrient of interest and how the physiology applies the different balances or counterbalances to alter the effects of nutrients on the immune system. The effect of the body’s physiological response to the nutrient will limit the ability of the nutrient to modify the immune response.

**Nutrient Reserve Capacity**

Poultry and animals store several nutrients in the body. For the purpose of this article, the nutrient reserve capacity is defined as the amount of time required to deplete the nutrient from the body. This physiological capacity will have practical implications especially for nutrients that act by replacing other nutrients in the body. For example, one major pathway through which n-3 fatty acids act is by replacing n-6 fatty acids in different tissues, especially in immune tissues. Omega-3 fatty acids replace arachidonic acid in phospholipid pools of immune cells and thereby inhibit cyclooxygenase and lipoxygenase enzymes from reducing eicosanoid synthesis by platelets and macrophages [20]. Replacing one nutrient with another nutrient is a complex process that includes several factors, such as the relative dietary concentrations of both nutrients of interest, the type of cell studied, the age of the animal, and the relative prioritization of nutrients to different organs of interest. Such thresholds and kinetics of one nutrient replacing another nutrient have not been identified for all nutrients of interest in poultry species.

Thresholds and kinetics of fatty acid replacement in different cellular compartments have been studied extensively in humans [21], rats [22], lambs [23], and chickens [24, 25]. In all the studies, the ratio of n-6 to n-3 fatty acids determines the rate of replacement of n-6 fatty acids by n-3 fatty acids. Although n-3 fatty acids can replace n-6 fatty acids in several immune organs, up to even 50% of n-6 fatty acids, n-3 fatty acids have never been able to replace n-6 fatty acids completely in any of the immune cells studied. Specifically, arachidonic acid content reaches a critically minimal level by wk 2 of feeding a diet high in n-3 fatty acids, and any additional feeding of n-3 fatty acids fails to further replace arachidonic acid [22]. Similarly, other beneficial fatty acids, such as conjugated linoleic acid and γ-linolenic acid act by replacing arachidonic acid. Some of the immunomodulating amino acids compete with each other for preferential enrichment of immune cells. This competition among different opposing nutrients for space in the immune cells will determine the net outcome of the immune response. The time it takes to replace any particular nutrient and the level of replacement will limit the ability of that particular dietary nutrient to modify an immune response.

**Prioritization of Nutrients**

Poultry and animals regulate the availability of nutrients to different organs by upregulating or downregulating nutrient transporters or by maintaining several isoforms with different affinity constants ($K_m$). Allocation of nutrients to different tissues is dependent on the physiological status of the host. Under situations of no immunological stimulus, the immune system has a lower priority for nutrients than does muscle, bone, or the brain [26]. The lower priority of the immune system for nutrients during situations of no inflammation is evident from studies with animals raised in germ-free conditions. The immune system is normally underdeveloped in animals raised in germ-free conditions. In the animal production industry, producers ensure that an animal is maintained under situations of the least inflammatory challenge. The lower priority of the immune system for nutrients during noninflammatory conditions is a limitation that will restrict efficient enrichment of the immune system with the nutrient of interest. This is particularly relevant for nutrients that are involved in cell proliferation and multiplication, such as amino acids.

For example, sulfur-containing amino acids can be incorporated into protein either through L-cysteine transfer RNA synthetase or through γ-glutamyl cysteine synthetase to synthesize glutathione, a metabolite of interest to the antioxidant defense. The $K_m$ of L-cysteine transfer RNA synthetase is 10 times lower than the $K_m$ of γ-glutamyl cysteine synthetase [27]. Under noninflammatory conditions, almost all sulfur-containing amino acids are directed toward pro-
tein synthesis rather than glutathione synthesis, but under inflammatory situations, 30 to 65% of the sulfur-containing amino acids are directed toward glutathione synthesis, presumably to modify the antioxidant defense [28]. Thus, the antioxidant-synthesizing potential of sulfur-containing amino acids, and hence their immunomodulatory effects, is determined by the animal prioritizing the nutrient through the l-cysteine transfer RNA synthetase or γ-glutamyl cysteine synthetase pathway. Another example is tryptophan metabolism. Tryptophan can be metabolized through indoleamine 2,3-dioxygenase (IDO), a tryptophan-catabolizing enzyme. Interferon γ, tumor necrosis factor α, and several other proinflammatory signals induce IDO [29], the end result of which is decreased concentrations of tryptophan, an essential amino acid, in the local microenvironment of T cells and the production of several immunosuppressive by-products. However, under situations of no inflammatory stimulus, approximately 95% of tryptophan is preferentially oxidized through tryptophan 2,3-dioxygenase, the action of which may not be immunosuppressive. The use of tryptophan as an immunosuppressor will be efficient only during inflammatory phases because of the prioritization of tryptophan to the liver to be metabolized through tryptophan 2,3-dioxygenase rather than to dendritic cells and macrophages to be metabolized through the IDO pathway. Thus, by prioritizing the nutrients to different compartments, the physiology of the body will limit the ability of dietary nutrients to modify an immune response because the physiology of the body will ultimately determine the metabolic pathway of the nutrient under study.

Interactions Between Nutrients

Most of the research with nutrients modifying an immune response has been conducted with individual nutrients. In experimental conditions, typically a particular nutrient will be added at different doses to a basal diet with no or minimal content of the particular nutrient. This experimental design assumes that the basal diet represents field conditions. Unfortunately, feed formulation in the poultry industry is driven by least-cost formulation, wherein feed ingredients are changed frequently. Poultry feed formulators commonly change almost every component of the feed formulation, and new ingredients are included to reflect market situations. Thus, the basal diet studied in the laboratory may not be the best representation of feed ingredients used in field conditions. Interaction between the nutrient under study and other feed ingredients will limit the effect of the particular nutrient on the immune response.

Interactions between nutrients have not been studied extensively. The effect of dietary lutein on the immune response of the chicken [30] and Japanese quail [31] is dependent on the dietary fat level. Dietary antibiotics alter the efficiency of prebiotics in modifying the immune response of the host [32]. Such interactions between nutrients raise intriguing possibilities. For example, what would be the outcome if vitamin D, a known immune suppressor [33], were combined with a known immune stimulator, such as lectin? Or what would be the outcome if a known immune suppressor were combined with another known immune suppressor? Would there be an additive or synergistic effect? Would there be an antagonistic effect?

The possible interactions between different nutrients and different components of the immune system are mind-boggling and, for all practical purposes, are impossible to quantify. However, it is such interactions that determine the net effect of individual nutrients on the immune system and limit the use of a particular nutrient to modify poultry production performance.

CONCLUSIONS AND APPLICATIONS

1. Several nutrients clearly have immunomodulatory effects.
2. Many of them have been studied and characterized concerning their mechanisms of action both in vitro and in vivo.
3. There are limitations in extrapolating the observations of in vitro and in vivo experiments conducted under experimental trial conditions to field situations.
4. Understanding the limitations of nutritional immunology is critical to applying it to maximize poultry production.
REFERENCES AND NOTES


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