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Application of Nutritional Immunology in the Mitigation of Economic and Production losses in the Poultry Industry Associated with Food-borne pathogens, Coccidiosis, and Necrotic enteritis.

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INTRODUCTION

Sub-therapeutic doses of antibiotics were used in poultry production since the 1950s for improved production, prophylaxis, and animal welfare. Extensive and indiscriminatory use of antibiotics led to the emergence of antibiotic resistance in food-borne pathogens of public health significance (Jones & Ricke, 2003). As per the United States Centre for Disease Control, more than 2.8 million infections in 2019 were caused by multidrug resistant bacteria. Due to public health concerns, the use of antibiotic growth promoters in livestock production was prohibited by Sweden and Denmark in 1986 and 1998 respectively (Hammerum et al., 2007). The European Union banned the use of antibiotics except for coccidiostats and histomonostats in livestock production effective from January 1, 2006 (Anadón et al., 2018). In 2013, the United States Food and Drug Administration recommended voluntary regulation on the use of medically important antibiotics in food animal production (Sneeringer et al., 2015). Regulations on the use of in-feed antibiotic growth promoters led to the reemergence of poultry pathogens that were otherwise manageable. An increase in the consumer preference for organically raised and antibiotic-free poultry products has necessitated the need to find an alternative to antibiotics in commercial poultry production. Several potential alternatives are currently available in the market such as probiotics, prebiotics, synbiotics, phytobiotics, engineered peptides, enzymes, organic acids, egg yolk immunoglobulins, bacteriophages, vaccination, and nutraceuticals (Low et al., 2021). This review aims at introducing the recent progress in the field of nutritional immunology in the prevention and control of enteric diseases of poultry with special emphasis on food-borne pathogens, coccidiosis, and necrotic enteritis.

FOOD-BORNE PATHOGENS OF POULTRY ORIGIN
(Mead et al., 2000; Scallan et al., 2011)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Poultry product</th>
<th>Food-borne percentage</th>
<th>Contribution to total cases of food-borne infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Undercooked meat and poultry, Cross contamination</td>
<td>80%</td>
<td>9%</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Raw or undercooked eggs, and poultry</td>
<td>94%</td>
<td>11%</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Meat and meat products</td>
<td>100%</td>
<td>10%</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Ready to eat deli-style poultry, chicken salad</td>
<td>99%</td>
<td>0.03%</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>Undercooked meat</td>
<td>68%</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

**NUTRITION AND POULTRY IMMUNE SYSTEM**

Nutrition has a multidimensional complex relationship with the host, pathogen, and commensal microbes. It is now clearly established that besides the conventional role of nutrition in physiology and metabolism, nutrition can target and improve specific body functions of the host. Dietary ingredients and the level of different nutrients can modulate the immune response of birds to different infectious diseases. The mechanisms by which nutrition modulates the immune status of the bird are discussed below and summarized in Figure 1.

1. Nutrition is critical for the development and optimum functioning of the immune system. For example, the trace element selenium is involved in diverse cellular metabolic pathways including the maintenance of the redox balance of the cell (Dalgaard et al., 2018). Similarly, zinc is a trace mineral essential for maintaining the integrity of innate immune cells. In a study conducted by Bartlett & Smith et al., (2003) it was observed that zinc supplementation significantly increased the total number of abdominal exudate cells which are essentially peritoneal macrophages and polymorphonuclear cells. Zinc supplementation also improved the phagocytic ability of macrophages (Bartlett & Smith, 2003)
2. Nutrients act as a substrate for the production of immune effector molecules. It is reported that selenium supplemented at 0.3mg/kg of diet significantly increases the serum IgG and IgM levels in broiler chickens (Cai et al., 2012).

3. Nutrients act as limiting factors inhibiting the proliferation of pathogens and the deficiency is exacerbated during the acute phase of an immune response. For example, the element iron is essential for the growth and proliferation of bacteria and a successful pathogen develops several mechanisms to scavenge iron from the host. Iron is also essential for the host immune response, particularly for the cell mediated immune response (Jarosz et al., 2016). Hence, the iron status has an influence on the immune status of the host.

4. Vitamins and trace minerals act as secondary messengers regulating the immune response. For instance, vitamin A supplementation was reported to increase the mRNA expression of vitamin D receptors in broiler chickens (Yuan et al., 2014). Immune cells such as macrophages express the receptor for vitamin D. Vitamin D regulates the phagocytic potential of the macrophages (Aslam et al., 1998) which is the primary defense mechanism of the innate immune system.

5. Nutrients such as Vitamin C regulate the endocrine system which in turn modulate the immune status of the bird. Vitamin C is important for the biosynthesis and bioconversion of catecholamines (Combs Jr & McClung, 2016). Vitamin C supplementation during stress conditions is found to reduce the level of stress hormones in broilers. Though the mechanism is not completely elucidated, Vitamin C supplementation is reported to improve the immune system function in broilers by its effect on phagocytes, lymphocytes, secretion of cytokines, antioxidant activity, and regulation of gene expression (Sorice et al., 2014).

6. The physical and chemical characteristics of the diet can modulate the gut microbiome and consequently affect the host immune system. Different classes of feed additives such as probiotics, prebiotics, synbiotics, essential oils, and organic acids are reported to positively regulate the host immune system (Fathima et al., 2022) possibly by their effect on the gut microbiota.

Besides the conventional formulation of poultry feed to meet the nutritional requirements, it can be enriched with ingredients that have health benefits for the host. Such ingredients or food...
components with the potential for enhancing the health and wellbeing of the host and reducing the incidence of diseases are termed functional foods or nutraceuticals (Figueroa-González et al., 2011). Different feed components and additives such as probiotics, prebiotics, synbiotics, organic acids, feed enzymes, omega-3 fatty acids, vitamins, essential oils, and amino acids which directly or indirectly regulate the host immune system are essentially functional food components (Amalaradjou & Bhunia, 2012) and are used as alternatives to antibiotics in poultry feed. Some of the commercially available products that employ nutritional immunology in the control of enteric diseases in poultry are reviewed below.

![Figure 1. Interaction between nutrition and immune system of the birds. Created with Biorender.com](https://scholarworks.uark.edu/panc/vol2022/iss1/5)

**PROBIOTICS**

The avian gastrointestinal tract is colonized by microbes shortly after hatch and the Gut-associated lymphoid tissue (GALT) is constantly exposed to the structural components of the gut microbiota. This interaction between the GALT and gut microbiota leads to the diversification of

https://scholarworks.uark.edu/panc/vol2022/iss1/5
the B-cell and T-cell repertoire (Macpherson et al., 2000). Probiotics, otherwise known as direct-fed microbials, are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Joint FAO/WHO Working Group & Joint FAO/WHO Working Group, 2002). Probiotics promote gut microbial homeostasis and positively modulate the host immune system (Fathima et al., 2022).

Probiotics and genetically modified probiotics are potential alternatives to antibiotics in the control of food-borne pathogens and specific enteric pathogens of poultry. The principal mode of action of probiotics are 1. competitive exclusion, wherein the administered probiotics colonize the intestinal tract of the host and prevent the further attachment and colonization by pathogenic microbes (Patterson & Burkholder, 2003) 2. Production of antimicrobial peptides such as bacteriocins which are bactericidal to a closely related group of microorganisms (Todorov, 2009) 3. Immunomodulation by the interaction of probiotic microbes with the GALT of the host (Ajuwon, 2016) 4. End-products of anaerobic fermentation by the probiotic bacteria such as organic acids and short chain fatty acids promote the host gut health and integrity (Ajuwon, 2016). The intestinal lamina propria harbors B1-cells which are plasma cells that produce antibodies with low affinity and broad specificities called natural antibodies (Berland & Wortis, 2002).

Probiotic supplementation in birds is demonstrated to significantly improve the systemic antibody response and phagocytic ability of macrophages. In a field study conducted by Smialek et al., (2018) it was observed that Lactobacillus-based probiotic supplementation in broiler chickens induced significantly higher total and vaccine-specific antibody titers throughout the production cycle. It was also found that probiotic supplementation significantly reduced intestinal colonization and environmental contamination with Campylobacter spp. in poultry houses (Smialek et al., 2018). Similarly, supplementation of probiotics or cell-free supernatant of probiotics is also demonstrated to significantly reduce the contamination of poultry meat with Salmonella (Al-Zenki et al., 2009) and Clostridium perfringens (Hamad et al., 2020).

Although probiotics are “generally recognized as safe” (GRAS) by the United States Food and Drug Administration (Szkaradkiewicz & Karpinski, 2013), comprehensive characterization of the candidate probiotic bacteria, safety assessment, and establishment of an effective dose against specific pathogens in poultry should be done before commercialization. Due to the risk of transfer of antimicrobial resistance genes, probiotic bacteria are being replaced by inactivated probiotics, cell-free supernatant, and soluble non-viable metabolites of probiotic bacteria which
are collectively called postbiotics (Abd El-Ghani et al., 2022). In the near future, with the recent advancement of technology, probiotic bacteria can be genetically engineered to be compatible with the different breeds of poultry and to address the specific situation of concern or to produce a specific bioactive peptide capable of effectively controlling the various enteric diseases of poultry.

**PREBIOTICS**

Prebiotics are defined as “selectively fermentable feed ingredients that positively modulate the composition and/or metabolism of the gastrointestinal microbiota for the benefit of the host” (Gibson et al., 2010). Prebiotics most frequently used in the poultry industry are complex carbohydrates such as fructooligosaccharides, inulin, glucooligosaccharides, oligochitosan, mannanoligosaccharide, stachyose, xylooligosaccharide, and maltooligosaccharides (Hajati & Rezaei, 2010; Patterson & Burkholder, 2003). These compounds are selectively utilized by and promote the growth of certain endogenous bacteria such as *Lactobacillus spp.* and *Bifidobacterium spp.* owing to their ability to break down the β-linkages in complex carbohydrates (Kaplan & Hutkins, 2000).

Prebiotics exert their beneficial effect on the host by 1. Acting as adjuvants that stimulate the immune response by binding to the mannose receptor C-type lectins expressed by dendritic cells and macrophages (Sheng et al., 2006) 2. Directly binding to the type-I fimbriae of enteric pathogens such as *Escherichia coli* and *Salmonella* inhibiting their attachment to the intestinal epithelial cells (Shashidhara & Devegowda, 2003) 3. Fermentation products of complex carbohydrates such as acetate, propionate, and butyrate inhibit epithelial cell invasion by pathogens (Fukuda et al., 2011).

The inhibition of pathogen colonization in poultry by prebiotic supplementation by the binding of MOS to type-I fimbriae is demonstrated by several studies. In a study conducted by Yang et al., (2008) it was demonstrated that supplementation of MOS in a broiler diet significantly reduced the coliform counts in the ileal lumen and mucosa-associated coliforms in the small intestine (Yang et al., 2008b). In another study by the same group, supplementation of FOS was demonstrated to significantly increase the abundance of lactic acid bacteria and increase the concentration of lactic acid concentration in the ileum of chickens. The acidic environment produced by the organic acids prevents colonization by enteric pathogens as evidenced by the lower number of *C. perfringens* in the prebiotic supplemented birds (Yang et al., 2008a). Thus,
prebiotic supplementation in chickens favors the proliferation of beneficial bacteria, inhibits the colonization by pathogenic bacteria, and improves the overall immune status of the bird. These characteristics make prebiotics a potential alternative to antibiotics in poultry production.

**SYNBIOTICS**

Synbiotics are synergistic combinations of probiotics and prebiotics- the prebiotic component favors the implantation and survival of the probiotic bacteria in the gastrointestinal tract of the host (AT & Action, 1999). The prebiotic provides the specific substrate for the fermentation of the probiotic thus enhancing the survivability of prebiotics in the gastrointestinal tract. Synbiotics exhibit the beneficial effects of both probiotics and prebiotics, characterized by improved growth performance, intestinal integrity, resistance to enteric pathogens, and immune status of the bird (Awad et al., 2008). Synbiotic supplementation improves the body weight and body weight gain of broiler chickens and hence is an alternative candidate to antibiotic growth promoters in the poultry industry (Awad et al., 2008).

In a study conducted by our group using a symbiotic product containing four bacterial strains and the FOS as the prebiotic component, it was found that symbiotic supplementation significantly improved the immune response of the birds to experimental *Salmonella* Enteritidis infection in layer chicken (Luoma et al., 2017). Synbiotic supplementation is also demonstrated to significantly increase the anti-*Salmonella* IgA titers in chickens (Markazi et al., 2018).

Apart from the effect on bacterial pathogens, synbiotics also exert an inhibitory effect on poultry intestinal parasites such as *Eimeria*. *Eimeria* infection damages the host intestinal tissue, increasing intestinal permeability and permitting the passage of toxins across the gut barrier. Synbiotics improve the gut health of the host characterized by increased villi height, integrity, and uniformity (Loddi et al., 2002) and positively modulate the local systemic immunity (Ferket et al., 2002). Synbiotics thus alleviate the intestinal damage caused by *Eimeria* infection in poultry.

In a study conducted by Ghasemi et al., (2010) it was reported that symbiotic supplementation significantly reduced the oocyst shedding and lesion scores in broiler chickens under an experimental *Eimeria* challenge (Ghasemi et al., 2010). Therefore, symbiotic supplementation is an effective strategy to control the incidence and severity of coccidiosis and subsequent infections with other enteric pathogens of public health significance such as *C. perfringens*, *Salmonella*, and *E. coli* in poultry.

**PHYTOBIOTICS**
Phytobiotics (also known as phytogenics or botanicals) are defined as natural, residue-free, relatively non-toxic, plant-derived bioactive compounds such as alkaloids, glycosides, terpenoids, carotenoids, saponins, and phenolics (Shad et al., 2014). Phytobiotics improve the overall health and production of poultry due to their antimicrobial, antiparasitic, antioxidant, and immunostimulant properties (Hafeez et al., 2020).

Essential oils are volatile secondary metabolites derived from aromatic plants and are one of the most commonly used class of phytobiotics due to their outstanding antimicrobial and antioxidant activity (Lin et al., 2018). In a study conducted by Lin et al., (2019) thyme oil was found to reduce the Salmonella Typhimurium on poultry meat from 6.64 Log CFU/g to 1.15 Log CFU/g (Lin et al., 2019). Considering the contribution of poultry products to the incidence of foodborne illnesses, thyme oil, and other essential oils can be an effective method to reduce the abundance of pathogens on fresh meat and increase their shelf life.

Tannins are water-soluble polyphenolic substances derived from plants. Tannins are astringents, that is, they are capable of precipitating proteins. The antimicrobial activity of tannins can be attributed to their direct action on microbial cell metabolism by the inhibition of oxidative phosphorylation, altering the membrane permeability, precipitation of proteins, prevention of biofilm formation, and antioxidant activity (Liu et al., 2013). Tannin supplementation during experimental necrotic enteritis challenge significantly improves the mortality, lesion score, and feed conversion efficiency in broiler chickens (McDougald et al., 2008). Similarly, plant extracts are found to possess antimicrobial activity against E. coli O157:H7 (Liu et al., 2013), Coccidia, C. perfringens (Díaz Carrasco et al., 2016), Salmonella (Nair et al., 2020), and Campylobacter (Valtierra-Rodríguez et al., 2010) in poultry and poultry products. A more comprehensive understanding of the mode of action of these plant-derived compounds can help utilize their maximum potential as antibiotic alternatives.

**ORGANIC ACIDS**

Organic acids are weak carboxylic acids that dissociate partially in the water. Organic acids such as formic acid, propionic acid, and their salts are used as preservatives in poultry feed for decades and are generally considered safe (Lückstädt, 2014). Short-chain fatty acids with a carbon chain length of C1-C7 such as citric acid, lactic acid, fumaric acid, tartaric acids, malic acid, and sorbic acid particularly possess antimicrobial activity (Shahidi et al., 2014). Organic acid
supplementation is known to exert several beneficial effects on the host such as improved growth performance, immune response, digestibility of nutrients, and antimicrobial activity. The postulated mechanisms by which the organic acids exert their beneficial effects are: 1) Organic acids reduce the pH of the gastrointestinal tract which promotes the conversion of pepsinogen to pepsin (Samanta et al., 2010) 2) Organic acids act as a readily available source of energy for the enterocytes (Poirier et al., 1996) 3) The non-dissociated form of organic acids are capable of penetrating the bacterial cell membrane, and reducing the intracellular pH, leading to the disruption of normal metabolic activity of the microbial cells causing cell death (Van Immerseel et al., 2006) 4) Organic acids stimulate the innate and adaptive immune response in chickens (Ebeid & Al-Homidan, 2022).

Based on the concentration, organic acids can be bacteriostatic or bactericidal. The chain length also influences the antimicrobial activity of the organic acids. For instance, medium chain fatty acids are found to be more effective against Salmonella compared to short chain fatty acids (Van Immerseel et al., 2006). In a study conducted by Samanta et al., (2010) it was demonstrated that the efficacy of organic acids in reducing the abundance of total coliforms in poultry was comparable to that of antibiotic growth promoter bacitracin. Also, the growth performance of the birds supplemented with organic acid was significantly better than the bacitracin supplemented birds (Samanta et al., 2010). Similarly, using organic acids in the marinades of poultry products such as breast fillets was found to reduce the C. jejuni load. It is of particular interest that the study used the C. jejuni strain 305A which is an acid-tolerant strain (Birk et al., 2010). Hence, the study examines the antimicrobial value of organic acids in the worst-case scenario. Due to the preserving ability and safety of use, organic acid supplementation in poultry feed is a prospective alternative to in-feed antibiotic growth promoters.

**VITAMINS**

Vitamins are organic compounds usually required in small quantities for a wide range of metabolic functions of the body and maintenance of normal growth and health conditions. Vitamins can be classified into fat soluble (Vitamins A, D, E, and K) and water soluble (Vitamin B complex and C). Among these, vitamin A, D, E, and C have the greatest influence on the immune system. Vitamin A deficiency negatively impacts antibody production and T-cell responses in chickens (Sklan et al., 1994). In a study conducted by Yuan et al., (2014) it was demonstrated that supplementation of 5,000 IU/kg of vitamin A significantly increased the
antibody titer in response to Newcastle Disease vaccination while supplementation of vitamin A at a dose greater than 20,000 IU/kg significantly decreased. This result suggests that vitamin A is immunostimulatory at lower doses and anti-inflammatory at higher doses (Yuan et al., 2014). Vitamin A is also essential for the maintenance of gut mucosal integrity. Vitamin A supplementation during experimental coccidial infection significantly reduces mortality and improves the growth performance of poultry (Dalloul et al., 2002; Erasmus et al., 1960). Vitamin A supplementation also upregulates the mRNA expression of vitamin D receptors in the intestinal mucosa of chicken (Yuan et al., 2014).

Vitamin D (cholecalciferol or 1,25-dihydroxycholecalciferol) is reported to have a profound effect on the immune system of poultry. Vitamin D is also a transcriptional regulator of various genes and maintains the mineral homeostasis of the body (Akimbekov et al., 2020). Though the mechanism by which vitamin D exerts its modulatory effect on the immune system is not completely elucidated, vitamin D is reported to suppress leukocyte infiltration into inflammatory sites and secretion of proinflammatory cytokines (Jadhav et al., 2018). In-ovo injection of vitamin D is reported to ameliorate the intestinal pathology associated with coccidial infection in broilers (Fatemi, Seyed Abolghasem et al., 2022). This effect can be due to the suppression of the proinflammatory pathway and stimulation of the anti-inflammatory pathway by vitamin D (Fatemi, S. A. et al., 2021), thus reducing the tissue damage associated with coccidiosis and inflammation.

Vitamin E (D-α-Tocopherol) supplementation in the poultry diet enhances the immune response and resistance to enteric diseases by improving the phagocytic capacity of the macrophages, improving humoral immune response (Muir et al., 1977), immune cell populations, cytokine production, and antioxidant activity (Leshchinsky & Klasing, 2003). Dietary supplementation of vitamin E in poultry feed during *E. coli* infection is reported to significantly increase antibody production, phagocytosis by macrophages, and reduce the mortality associated with *E. coli* infection (Tengerdy & Brown, 1977). Similar results were observed in response to *Salmonella* Typhimurium immunization of chickens. Vitamin E supplementation significantly increased the anti-*Salmonella* Typhimurium IgA titers in the bile and intestinal mucosa of chickens (Muir et al., 1977). Vitamin E can also be used as an immunopotentiator in vaccines to improve vaccine efficiency (Singh, 2009).
Vitamin C, otherwise known as ascorbic acid, possesses antioxidant, immunomodulatory, and anti-inflammatory properties. Vitamin C supplementation in broilers is found to decrease the hepatic translocation of _Salmonella_ Enteritis to the liver of experimentally infected birds. Vitamin C also significantly increased the jejunal secretory IgA concentration and serum antioxidant capacity in infected birds (Gan et al., 2020). Vitamin C enhances lymphocyte proliferation in response to ConA stimulation following heat stress. It also plays an important role in B-lymphocyte proliferation and humoral immune response characterized by significantly increased antibody titers in response to vaccination (Amakye-Anim et al., 2000).

Vitamins alone or in combination with trace minerals (Colnago et al., 1984) or amino acids (Perez-Carbajal et al., 2010) can be used to modulate the immune response of chickens in response to vaccinations and infections.

**AMINOACIDS**

Amino acids are structural and functional units of proteins that play vital role physiological roles. Amino acids can be broadly classified as essential which are not synthesized by the body in adequate amounts to meet the metabolic requirements and non-essential which are synthesized in the body (Debnath et al., 2019). Amino acids that play a regulatory role in the metabolic pathways are termed functional amino acids. Functional amino acids include essential amino acids, conditionally essential amino acids, and certain non-essential amino acids such as arginine, glycine, glutamate, glutamine, and proline (Wu, 2013). The acute phase immune response to pathogen invasion is an anabolic process that increase the nutrient requirement of the host by several folds, particularly the amino acid requirement (Grimble, 2006).

Methionine is an essential amino acid and, the first limiting amino acid in the poultry diet. Methionine plays a key role in various physiologic and metabolic functions such as precursors for the synthesis of other amino acids such as cysteine, protein synthesis, synthesis of glutathione, elimination of free radicals, and methylation of DNA (Elnesr et al., 2019). Though methionine supplementation along with the anti-coccidial narasin was found to significantly improve the weight gain and attenuate the pathological effect of _Eimeria_ in broiler chicken, no significant effect was observed with anti-coccidial vaccination (Lai et al., 2018). Methionine supplementation during _Eimeria_ challenge is reported to improve intestinal histomorphology, but adversely affected the production parameters (Teng et al., 2021). The inconsistencies in the results can be due to the different study designs, chicken breeds, and _Eimeria_ species used.
Arginine is an essential amino acid in poultry and is the precursor for the synthesis of immune effector molecules such as polyamines and nitric oxide (Brand, 1987). Arginine also promotes the proliferation and survival of the enterocytes and lymphocytes and attenuates inflammatory responses (Gogoi et al., 2016). Arginine supplementation is reported to ameliorate the histomorphological damages associated with Salmonella Typhimurium infection. Arginine supplementation also upregulated the expression of interferon-γ (IFN-γ) which is important for the clearance of Salmonella. Apart from upregulating the expression of IFN-γ, arginine supplementation favors the anti-inflammatory pathway (Zhang et al., 2020) and hence ameliorates the damages caused by Salmonella infection.

Other amino acids such as threonine (Debnath et al., 2019), glutamine (Fasina et al., 2010), and tyrosine (Skoufos et al., 2019) are also reported to decrease the proliferation of food-borne pathogens such as Salmonella, Campylobacter, E. coli, and Clostridium perfringens in poultry. These amino acids also ameliorate the pathological effects of the infection, thus improving production and reducing associated economic loss.

CONCLUSION

To maintain the production performance of poultry and safety of poultry products with the rising demand for a cheap source of protein, it is essential to find an alternative to antibiotics. Nutritional immunology can be used as a tool for the manipulation of the physiology and immune response of the host. However, due to the inconsistencies in the results obtained from various studies, there is a need for standardization and identification of the appropriate solution for a specific problem as to which is best for what. Due to the varied functions of nutrients on the body, it would be difficult to target a particular response unlike in the case of antibiotics. The potential of immunomodulating nutrients in nutritional imprinting and genetic selection is an area to be explored in the poultry industry.

References


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