Nutritional modulation to enhance immunity in chickens

Prevention is better than cure, as they say. Many nutrients – energy, amino acids, vitamins and minerals – play different but significant roles in the immune response and so can contribute to keeping birds in good health, without the need for medication. — ***Dr S.V. Rama Rao, M.V.L.N. Raju and D. Nagalakshmi***

Presently, the aim of commercial poultry breeding is to achieve higher body weight and maximum egg production per unit of feed intake. However, there is a negative correlation between production and immunity in chickens as a result of the conflict between production and immunity, i.e. maturation and function of the immune system. Accommodation of all the physiological demands within the limited resources, i.e. nutrients, available to birds may be the factor responsible for the negative relationship between performance traits and immunity. The genotypes with the maximum bodyweight exhibit lower immunity, as indicated by *E. coli* lesion score and cellular immunity antibody titres, compared to those having lower body weights. Therefore, the possibility of breakdown of the immune system in commercial chicken crosses is more evident nowadays than before.

In addition to genetic selection, certain non-genetic factors like dietary nutrient concentration also modulate the expression of the genes responsible for immuno-responsiveness by altering the maturity of the immune system and magnitude of antibody production.

Defence mechanism in chickens

Under intensive farming conditions, the poultry environment contains ubiquitous micro-organisms that continuously challenge the bird's immune system. Generally, the invading pathogen will be attacked by antibodies, which will neutralise, weaken and inactivate the pathogen and finally, phagocytic cells will engulf the invader. The mechanism is quite effective in controlling extra-cellular pathogens, such as bacteria. For the intracellular pathogens – viruses – cell-mediated immunity (CMI) plays a key role. The CMI protects the host by destroying the cells that harbour the pathogen with the help of cytotoxic T-lymphocytes. Against invading pathogens, the immune system produces a variety of compounds like acute phase proteins (APP), proteolytic and hydrolytic enzymes, oxygen radicals and nitrogen derivatives, which destroy the invader or infective cells.

Nutrition and immunomodulation

Nutrient recommendations are typically developed using indices of productivity such as growth, egg production and feed efficiency. The criteria for adequacy of immunocompetence are often ignored. Nutrients also influence the maturity of the immune system and magnitude of the antibody. During the acute phase of the immune response, the greatest nutritional need is for the synthesis and release of APP by the liver. The process requires more energy and amino acids than are normally needed for responding leucocytes. Interactions among various nutrients and imbalance or toxicity of nutrients lead to disturbances in normal physiology of the bird, with consequent immunosuppression in chickens.

Energy

Variations in concentration of energy in the diet modulate the immune response in birds, probably due to the change in intake of nutrients, which influence the immunity. Energy intake regulates the activity of the immune cells and activity of certain hormones, e.g. thyroxin, corticosteroids, growth hormones, glucagons, catecholamines, which influence immunity. Variation in the level and composition of dietary fat also influences the immune response in chickens by altering the structure of the cell membrane and modulating the synthesis of prostaglandins. Mortality associated with *E.coli* and *Mycobacterium tuberculosis* was reduced by increasing the level of fat from 3% to 9% of the diet. Antibody titre against sheep red blood cells (SRBC) antigen was markedly increased with supplemental tallow at 6% in the chick diet. Higher levels of unsaturated fatty acids enhance immune function by stimulating macrophages.

Requirements and allowances for nutrients influencing immunity in broilers

Nutrient	NRC, 1994*	For immunity	Toxic level
Energy, kcal/kg	3200	Excess energy depresses nutrient intake Supplemental fat & unsaturated fat increase immunity	
Protein, %	23	High protein level beneficial against *E. coli* Low protein reduces the risk of coccidiosis	
Methionine, %	0.50	0.60-0.80	>1.00**
Arginine, %	1.25	-	>2.50**
Threonine, %	0.80	-	>1.80**
Lysine, %	1.20	-	>2.40**
Valine, %	0.90	Lower valine: leucine+isoleucine ratio desirable	>1.80**
Vitamin A, IU/kg	1500	8000-15000	2 million
Vitamin D3, ICU/kg	200	2500-3500	0.3m
Vitamin E, mg/kg	10	200-300	40,000
Vitamin C, mg/kg	-	200-400	
B-complex vitamins	-	Double concentration is beneficial	
Sodium, %	0.20	0.30-0.40	0.90
Chloride, %	0.20	0.20-0.25	1.50
Zinc, mg/kg	40	80-100	1500
Manganese, mg/kg	60	100	4000
Selenium, mg/kg	0.15	0.25-0.50	10
Copper, mg/kg	8.0	100-150	800
Iron, mg/kg	80	150-300	4500

* National Research Council, 1994
** Excessive level depends on concentration of other amino acids in the diet
Note: The concentration of nutrients indicated for immunity is based on published research data. These levels may be beneficial during disease outbreaks, stress, feed toxins, immunisation etc. Although the levels indicated for immunity are well below the toxic levels, interactions between nutrients need to be considered before increasing the level of any particular one.

Protein

The growth of bursa and thymus are relatively faster than the bird's body growth. Therefore, it is important to supply the required quantity of protein, particularly during the early growth phase. Deficiency of protein at this stage leads to the improper development of lymphoid organs. Several research workers have suggested that there is a higher amino acid requirement for immunity than for growth. However, the influence of level of protein in diet on severity of disease depends on the type of infective organism. The lesion score to *E. coli* inoculation decreased with the increase in the protein level (18, 20.5 and 23%) in broiler diets. With coccidiosis, the mortality decreased from 32% to 8% in chickens fed protein-deficient diets compared to those fed a normal protein level.

High dietary protein increases the activity of trypsin in the chicken gut. A high level of trypsin in the gut leads to a faster release of coccidia from oocysts, which will aggravate the disease symptoms.

Dietary **methionine** levels in excess of those required for maximum growth are essential for maximising immunity. Methionine is required by the thymus-derived T-cell function. Methionine deficiency produces severe lymphocyte depletion and atrophy of the bursa and an increased susceptibility to Newcastle disease and coccidiosis.

Cystine supplementation also stimulates cellular and humoral immunity (70 to 84% as effective as methionine.).

Deficiency (16 to 50%) of branched-chain amino acids, i.e. **isoleucine, leucine** and **valine**, reduces the antibody titres against SRBC in broilers. Immunoglobulins contain a high concentration of valine and threonine. A deficiency of either of these amino acids reduces the immune response in chickens. A higher ration between leucine to valine + isoleucine reduces immunity due to structural antagonism between the three amino acids. The absorption of valine and isoleucine are inhibited by a high leucine content in the diet.

Increasing the dietary concentration of **lysine** improved the haemagglutination and agglutinin titres, and IgG and IgM levels.

Arginine is a substrate in the synthesis of nitric oxide, a cytotoxic product that is helpful in phagocytic activity of macrophages and kills bacteria and intracellular parasites.

Vitamins

Vitamins act as co-factors in several metabolic functions in immune reactions and therefore, deficiencies of vitamins cause impairment of immunity. Generally, higher levels of vitamins than the current recommendations will increase the immune response.

Retinal

This vitamin is important for maintaining the

cellularity of the lymphoid organs and epithelial tissues and for enhancing both cellular and humoral immunity. Vitamin A helps in maintaining the mucous membrane of natural orifices in healthy condition to prevent the invasion of micro-organisms. Vitamin A directs differentiation and development of B-lymphocytes. The concentration of vitamin A in the diet modulates the expression of retinoic acid receptors on lymphocytes in chickens. The production of immunosuppressive agents (hydrocortisones) is reduced with higher levels of vitamin A in the diet. Furthermore, deficiency of vitamin A causes keratinisation of basal cells of the bursa and impairment in the response of T-lymphocytes. Therefore, deficiency of vitamin A impairs immunity by producing defective T, B-lymphocytes, impaired phagocytosis and reduced resistance to infection. Increased morbidity due to Newcastle disease virus has been reported due to a deficiency of vitamin a in the diet. The requirement for vitamin A for maximum immunity, i.e. lymphoid organ weight, was higher than for the bodyweight gain in the chicken. An increase in vitamin A from 12850IU to 42850 or 74045IU/kg decreased mortality due to *E. coli*, and CRD in chickens and increased the rate of clearance of the pathogen from the blood. However, the beneficial effect of higher levels of vitamin A depends on the concentration of other fat-soluble vitamins in the diet. An excessive level of vitamin A interferes with the utilisation of vitamins D and E.

The administration of 60IU of vitamin A per chick per day during a severe attack of coccidiosis reduced mortality from 100% to almost zero. However, practical chick and young layer diets should contain 4000 and 2000IU/kg, respectively. To minimise stress damage and also to prevent immune suppression, dietary vitamin A levels should be increased to ten times the normal requirement. A combination of vitamin A (14000IU/kg) and zinc (65mg/kg) has been shown to enhance growth and both humoral and CMI immunity in chickens.

Cholecalciferol

A receptor for 1,25-dihydroxy vitamin D3 is present in peripheral monocytes. Vitamin D3 is necessary for the differentiation of pro-monocytes and monocytes to macrophages and the proper phagocytic and cytotoxic activity of the macrophages. CMI decreased significantly in broiler chicks fed diets without any supplemental vitamin D3.

Tocopherol and selenium

The immune system stimulated by infection or vaccination – especially under stress conditions – is prone to damage by peroxides and superoxides. Vitamin E and selenium protect the immune system under this situation. Vitamin E protects the cell by keeping the lipoprotein fraction together in cell membranes and reduces the oxidative changes developed by CMI. Vitamin E also enhances humoral immunity by favourably altering the proliferation and ratio of T-helper cells. Selenium in glutathione peroxidase together with vitamin E acts as an antioxidant and reduces the concentration of free radicals in cells. Vitamin E and selenium play important roles in lymphocyte blastogenesis. Deficiency of these nutrients reduces the phagocytic activity of the macrophages.

The NRC recommendation for vitamin E (at 20mg/kg) is way below the level of 300mg/kg that has been shown to increase the immune response and decrease mortality to *E. coli*, Newcastle disease, infectious bursal disease and coccidiosis. Increasing vitamin E supplementation to 250mg/kg was reported to prevent a drop in egg production in layer exposed to heat stress. The beneficial role of higher levels (up to 300mg/kg) of vitamin E on immunity is mediated through increasing the maturation of T-cells. The same high level of vitamin E reduced the lymphoid organ levels of prostaglandins, which cause suppression of CMI, and thus improved antibody production. Both humoral and CMI responses in immunised chickens increased significantly with supplemental vitamin E alone (200 vs. 80mg/kg) or in combination with selenium (300mg/kg vitamin E and 1mg/kg selenium). Supplementation of vitamin E (0.03%) in a breeder diet or by injection (2.5-30.0mg/egg) to fertile eggs was reported to increase the immune response of chicks. Similarly, incorporation of vitamin E in oil adjuvant vaccines (Newcastle disease and infectious bursal disease) induced more rapid and higher antibody titres than the controls.

Ascorbic acid

The synthesis of vitamin C is inadequate in newly hatched chickens and in adult birds subjected to severe stress. Vitamin C enhances both humoral (SRBC and Newcastle disease) and CMI responses and increases the resistance of birds to *E. coli*, *Mycobacterium avium*, Newcastle disease, infectious bursal disease, Marek's disease etc. Vitamin C, through its antioxidant properties, maintains the stability of leukocyte membranes. It is also essential for the optimum functioning of neutrophils /granulocytes and thereby, it enhances the phagocytic activity of the neutrophils. Vitamin C protects birds under heat stress by reducing the synthesis of glucocorticoids. The immune suppression caused by corticosterone and thermal stress was minimised by vitamin C supplementation (0.1%) in chickens. Variation in the beneficial effects of vitamin C supplementation has been attributed to the poor stability of the vitamin in some forms under poor but practical storage conditions.

Vitamin B complex

B-complex vitamins play an important role in intermediate cell metabolism as co-factors for several enzymes involved in various metabolic reactions. Among the B-complex vitamins, vitamin B6 is has been widely studied for its effects on immunity. It is important in the development and maintenance of lymphoid tissue. Deficiency of vitamin B6 (0.95 and 1.48mg/kg) reduced the

antibody response to SRBC and the production of IgG and IgM. Under conditions of heat stress, the administration of vitamins B2, B6 and B12 has had positive effect of chicken immunity.

Minerals

Certain minerals play an important role in immunomodulation through their effects on osmoregulation and by acting as co-factors and enzymatic catalysts and also by optimising hormone function. Dietary concentrations of sodium (Na), chloride (Cl), zinc (Zn), selenium (Se), manganese (Mn), copper (Cu), iron (Fe) and cobalt (Co) have all been shown to influence immune response. Generally, the inorganic forms of the minerals are less well absorbed than the organic/ chelated form. Therefore, a higher immune response has been observed when chelated minerals are supplemented in the diet.

Sodium and chloride

Na and Cl in addition to potassium (K) play a key role in maintaining osmotic balance in extra- and intra-cellular fluids. During salt deficiency, chickens retain Na and Cl in the plasma, in addition to other plasma constituents, which may result in decreased immune response in birds fed low Na and K diets. Generally, antibody titres increased with higher levels of Na (0.14%) or Cl (0.21%) in the diet. The humoral response decreased with less than 0.14% Na and 0.17% Cl. Supplementary salt (NaCl; 0.25, 0.50 and 0.75%) improved antibody titres against SRBC. However, excessive levels of Cl (0.25 and 0.36%) reduced the antibody response even at a higher Na level in the diet (0.24 or 0.40%). Increasing Cl as an immunomodulator under conditions of heat stress should be used only with great care.

Zinc

The role of Zn on immunity is mediated through increasing the thymocyte and peripheral T-cell count; the activity of natural killer cells (NKC) and neutrophils; macrophage production and antibody production; production of interferon; and reducing viral penetration. Zn is also required for the proper functioning of thymulin, which is involved in lymphocyte development, and metalloenzymes, e.g. DNA and RNA polymerases. Deficiency of Zn impairs interleukin-2 production, which plays a role in CMI, as well as through its role in maintaining the integrity of the lymphoid organs and T-cell function. A Zn deficiency in a breeder diet decreases antibody titres to SRBC in their offspring. Conversely, the antibody response increased significantly in the progeny was Zn was supplemented in the breeder diet (38-160mg/kg). A few studies have indicated no significant benefits of supplementing Zn up to 220mg/kg diet of breeders on humoral immunity or CMI. The discrepancy between the reports may be due to differences in the concentrations of Zn and other trace minerals in the basal diets used in the trials. Supplementation of Zn in the form of a methionine chelate to a breeder diet was more beneficial in the development of immune system organs and increasing antibody titres to SRBC and CMI in the progeny and specific antigens, e.g. *Salmonella enteritidis, E.coli*, in the parents.

Manganese

Mn plays an important role in the development, repair and maintenance of epithelial tissues. Mn-dependent super oxide dismutase (SODM), present in the mitochondria, inactivates free radicals produced within the cell. Organic forms of this mineral help to reduce the incidence of cellulitis and increase the antibody response to infectious bursal disease, infectious bronchitis and Newcastle disease virus in breeders.

Copper

During the acute phase of an immune response, liver cells produce and secrete APP, which gives protection to birds against infection. Ceruloplasmin is one significant APP in chickens, and it needs Cu as a co-factor. Ceruloplasmin protects the bird by removing the free radicals produced during phagocytosis. Thus, the Cu requirement increases during infection. Cu- and Zn-dependent SODM in the cytosol inactivates the free radicals. Generally, the requirement for Cu by chicks is higher when they are experiencing an acute phase response than when they are healthy. To increase bird health and well-being, additional levels of Cu (125-250mg/kg) can be supplemented to the diet. Cupric chloride is more effective in enhancing intestinal health and acting as an antimicrobial agent against *E. coli* and salmonella than copper sulphate. Providing Cu in a chelated form with amino acids is said to offer promise.

Iron

The role of Fe in immunity can be appreciated by the sudden fall in Fe concentration in the serum during the early phase of infection. Fe supplementation has been shown to increase the bactericidal activity of the macrophages in the liver and spleen of affected chicks. The survival rate increased in chicks inoculated with *Salmonella gallinarum* with additional supplementation of 100mg/kg Fe in diets containing 200mg/kg Fe.

Cobalt

The beneficial effects of cobalt on immunity have been attributed to it positive effects on protein synthesis and the function of the lymphoid organs. Supplementary feeding of 0.1 or 0.5mcg/kg bodyweight enhanced the host defence function against infection or Newcastle disease vaccination in broiler chickens.

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