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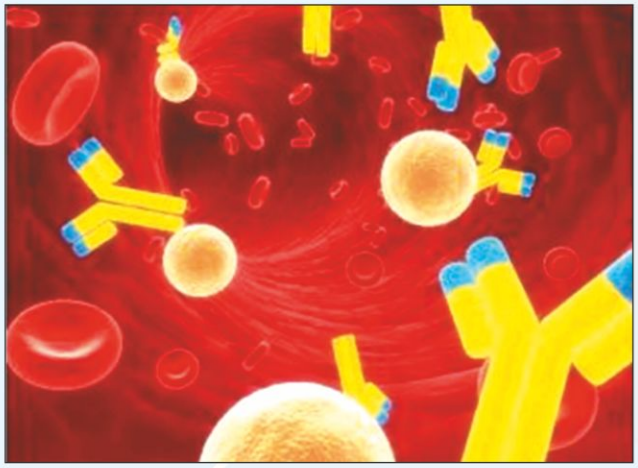
Nutritional approach to unleash potential under stress conditions

With high density confinement rearing of birds, an additional important role of nutrition is that birds are not only fed for production or reproductive performances but must also be fed to minimize infectious disease and their concomitant stresses. In context of Poultry Industry, problem of immunosuppression has been felt to be prominent due to various factors *viz.*, managemental conditions, nutritional status, intensive production system, high density rearing and infectious diseases. Therefore, it is highly essential to find ways and means for enhancement of immune response by nutritional manipulation. Substantial information is available in literature to indicate that administration of certain vitamins, minerals, amino acids and their different combinations to mammals and chicken in excess of their supposed requirements enhances their disease resistance. This increased resistance has been attributed to significant stimulation of humoral and cellular immunity and phagocytosis. Since, the use of antibiotics has been limited, better use of supplementary immuno-stimulatory nutrients has to be made in poultry feeding.

The relationship between stress and chronic disease has been difficult to establish due to the fact that stress can both increase and decrease disease resistance based on many interacting factors including the type and degree of stress as well as the individual perception of, or response to, the stressor (Biondi and Zannino 1997, Glaser *et al.* 1999, Salak-Johnson and McGlone 2007).

The immune system benefits greatly from proper nutrition of the bird. Not only does the immune system



benefit directly from proper nutrition, but indirectly proper nutrition will also prepare the bird for periods of stress, reducing the adverse effects of stress and enhancing recovery from stressful periods. Therefore, in many instances, proper nutrition lessens the immune suppression associated with the stress response in the bird. The immune system of the bird can be influenced by nutrition in several ways:

1. Anatomical development of lymphoid tissues
2. Mucus production
3. Synthesis of immunologically active substances
4. Cellular proliferation
5. Cellular activation and movement
6. Intracellular killing of pathogens
7. Modulation and regulation of the immune process

Factors related to the genetics of poultry, the frequency of their exposure to pathogens, the virulence of the pathogens, and the efficacy of vaccination programs are predominant detriments of the incidence of infectious diseases in poultry flocks. However dietary characteristics can modulate a bird's susceptibility to infectious challenges and subtle influences due to the level of nutrients or the types of ingredients may at times be of critical importance. The bird's susceptibility to an infectious challenge can be subdivided into two components, resistance and resilience.

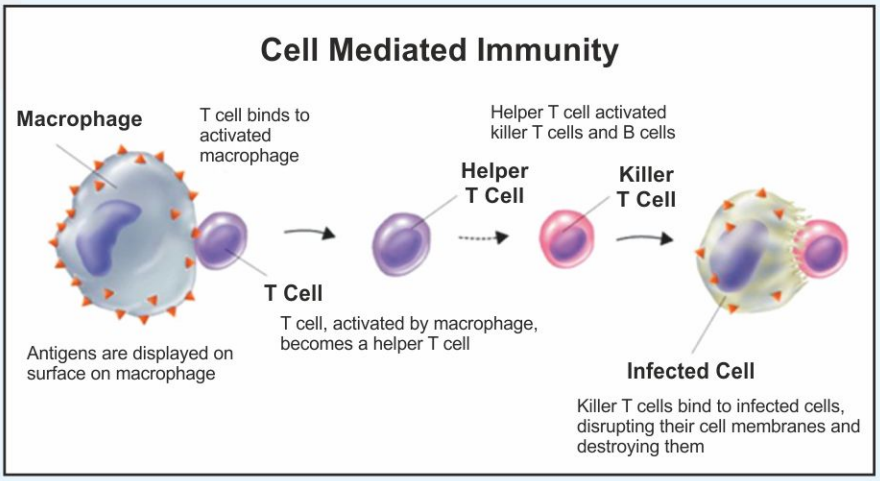
Resistance refers to the capacity of a variety of anatomical and physiological systems, including the immune system, to exclude pathogens.

Resilience refers to the capacity of the bird to maintain productivity (e.g. growth, feed efficiency, egg production) during an infectious challenge.

The role of nutrition in maximizing resilience is only now being appreciated and this relationship deserves future attention by poultry stake holders. There are probably many situations in which diets that optimize resistance to infectious challenges are not optimal for resilience and maximal profitability (Cook, 1996; Klasing, 1997). However, in many cases it is not known whether the requirement values that maximize productivity in healthy, unchallenged birds are optimal for immuno-competence and disease resistance. An understanding of the mechanisms through which nutrition influences the immune system is necessary to appreciate the many complex interactions between diet and infectious diseases. Several recent reviews of nutrition and immunity provide an excellent survey of nutrition and immunity, including the impact of toxic components (mycotoxins) that may contaminate the feed (Cook, 1991, 1996; Latshaw, 1991; Dietert *et al.*, 1994).

Mechanisms of Nutritional Modulation of Resistance

The mechanisms of nutritional modulation of resistance to infectious disease are divided into seven categories. Obviously these categories are overlapping and nonexclusive. A single nutrient may impact the immune system by several of the general mechanisms that are described as well as mechanisms not listed. The first six of



Mechanisms of Nutritional Modulation of Immune Responses

Mechanism	*Nutrients*
Effects on the development of the immune system	Linoleic acid, iron, vitamin A
The supply of substrates to the immune system	All nutrients
Nutritional immunity	Iron, Biotin
Changing the hormonal settings	Energy, protein
Direct regulatory actions of nutrients on the immune system	Vitamins A, D, E, polyunsaturated fatty acids(PUFA)
Reduction of pathology	Vitamin E
Physical and chemical actions of feeds in the intestines	Fiber, oxidized lipids, lectins

these mechanisms relate to the effect of nutrients on the immune system, whereas the last mechanism considers several non-immunological aspects of the diet. The first three categories consider the role of nutrients as substrates for the replication and function of cells. A substrate role of nutrients is necessary for the initial development of the immune cells and tissues (Mechanism 1) and during an actual immune response so that responding cells can divide and synthesize and their supply is contraindicated during an infection (Mechanism 3). Though diet may influence processes important to immunity by providing building blocks (substrates) for the construction of cells and molecules, other mechanisms may be even more important for dietary modulation of the immune system. These include direct regulatory actions of nutrients on the leukocytes that respond to infectious challenges (Mechanism 4), as well as indirect effects that are mediated by nutritional modulation of the classical endocrine system (Mechanism 5). Furthermore, nutrition may impact the level of pathology resulting from the killing pathways of the immune system (Mechanism 6).The diet may also impact the incidence of infections by its functional characteristics in the lumen of the gastrointestinal tract. For example, physical and chemical aspects of the diet can modify the populations of microorganisms in the gastrointestinal tract, the capacity of pathogens to attach to enterocytes, and the integrity of the intestinal epithelium (Mechanism 7).

Impact on the Development of the Immune System

The developmental events important for immuno-competence begin in the embryo and continue during the 1st week following hatching (Gobel, 1996; Ratcliffe *et al.*, 1996). The 1st week of life is a period of rapid expansion of leukocyte populations, seeding of lymphoid organs, and educational events that produce the unique clones of lymphocytes that will mediate immunity later in life. It is not surprising that this is a critical period during which nutritional deficiencies or excesses may impact the immune system. In general, chronically severe deficiencies of micronutrients are more debilitating to the development of the immune system than macronutrients such as energy and protein. Nutrient deficiencies that are especially damaging to development of the immune system include linoleic acid, vitamin A, iron, selenium, and several of the B vitamins (Cook 1991; Latshaw, 1991; Dietert *et al.*, 1994).

Embryonic development of the chick is known to be very sensitive to vitamin A deficiencies and it has been known for many years that chicks hatched from vitamin A-deficient hens have impaired immunity and decreased resistance to a wide variety of infectious diseases. The dietary level of vitamin A that maximizes growth and feed efficiency of broiler chickens (500 mg/kg) is insufficient for optimal development of the immune system. The level needed for maximal growth and efficiency in clean University facilities was used to set the NRC requirement, yet an amount that is10- to 20-fold higher is necessary to maximize immuno-competence of the young broiler chick (Sklan *et al.*, 1994; Friedman and Sklan, 1997). However, excess of vitamin A can also impair immuno-competence, probably by causing secondary deficiencies of other fat soluble vitamins (Veltman *et al.*, 1984; Friedman and Sklan, 1997). Clearly the immune system is sensitive to deficiencies as well as excesses of nutrients. The mechanism through which vitamins such as A and D effect the development of the immune system is probably through direct influences on differentiation decisions of precursor cells (Kline and Sanders, 1993; Woods *et al.*, 1995).

Micronutrient nutrition of the hatchling is very dependent upon the nutrition of the laying bird (Naber and Squires, 1993). The level of stores of most of the vitamins and trace minerals in the hatchling are highly correlated with the level in the breeder's diet. Sufficient stores may completely buffer the young chick against severe dietary deficiencies during the critical early weeks when the immune system and the intestines develop. Thus, the role of nutrition on the developmental events of the immune system must consider the diet of the hen as well as the diet of the chick.

Substrate Supply

Defense against an infectious challenge requires a highly integrated response by the immune system. From a nutritional viewpoint, substrates (*e.g.* amino acids, energy, enzyme co-factors) are needed to support the clonal proliferation of antigen-driven lymphocytes, the recruitment of new monocytes and heterophils from bone marrow, the synthesis of effector molecules (*e.g.* immunoglobulins, nitric oxide, lysozyme, complement), and communication molecules (*e.g.* eicosanoids, cytokines). A quantitative summation of the nutritional costs of maintaining the immune system and the additional costs of a vigorous immune response against a challenge is not known for any species.

A rough estimation of the size of the immune system including bone marrow components can be obtained by summing the number of leukocytes in the body. Such an exercise reveals that a little over 0.42% of the body is made up of leukocytes and their progenitors. Total anti body found in the serum contribute less than 0.1% of the body weight of a chicken. Connective tissue components that are dedicated to immune-surveillance functions such as reticular, dendritic, and stromal cells should also be considered in these calculations, but accurate quantitative estimates of their contribution are not yet available. Even if these accessory cells contribute similar mass to the immune

system as leukocytes, the cellular components of the immune system probably does not exceed 1% of the body weight. Inclusion of the extracellular fluids and collagen and other structural components found in lymphoid organs brings the contribution of the immune system to the body weight of a human up to 3 to 4% (Elgert, 1996; Roitt, 1997). Many of the cells of the immune system are long-lived (life span of weeks to months), including dendritic cells, macrophages, and many lymphocytes. Other cells are very short lived, such as heterophils, which live for only a few days, and lymphocytes found in the thymus, bursa, and germinal centers, which undergo apoptosis soon after cell division. In adult animals, leukopoiesis in bone marrow occurs at a rate of 0.02% of body weight/d and lymphocytes in other tissues (*e.g.* thymus, spleen) contribute almost twice this amount for the purpose of replacing leukocytes lost due to normal immune-surveillance. Rates of leukopoiesis in the bone marrow increase by about two fold during acute systemic infections.

Normal rates of immunoglobulin synthesis in a young chicken are less than 0.02% of body weight/day. Immunoglobulin synthesis is probably higher in laying hens because of daily secretion into egg yolk; yet even in the hen, daily total immunoglobulin synthesis is probably well less than 0.05% of body weight/day. Among the immunoglobulin classes, rates of IgA synthesis are high relative to circulating concentrations in the blood because most is secreted onto the epithelial surfaces.

Although the rate of synthesis of specific immunoglobulins increases dramatically during a disease challenge, the rate of synthesis of total immunoglobulins increases only moderately. Hyperimmunization, for example, results in about a 25% increase in serum immunoglobulin (Leslie and Clem, 1970). Based on serum concentrations and half-life estimates, other humoral components contribute an order of magnitude less to daily synthesis than do immunoglobulins. This analysis of the size of the immune system and rates of its processes suffers from the use of cross-species data and lacks attention to specific nutrients that may be concentrated in leukocytes or preferentially utilized by them (*e.g.* Arginine and Glutamine). Even with these qualifications in mind, it is apparent that the amount of substrate resources (nutrients) needed by the immune system is very low relative to needs for growth or egg production.

For example, the weight of new leukocytes and immunoglobulins normally produced each day (about 800 mg/kg body weight) appears to be less than 1% of the total increase in body weight of a 2-wk-old broiler chick and less than 10% of the amount of breast muscle synthesized each day. Even if an infectious challenge increases the rate of leukopoiesis by considerably more than the twofold estimates that have been reported, it is doubtful that the immune system would be a significant consumer of nutritional resources. It is often stated that the depression in performance that is associated with an immune response is due to the diversion of nutrients away from growth or egg production to be used by the immune system. Given the above quantitative analysis, this statement cannot be true. However, the immune system is not the only system that increases nutrient use during an infectious challenge. Many infections are accompanied by an acute phase response, which is characterized by the synthesis of acute phase proteins, fever, accelerated whole-body protein turnover, and high rates of hepatic gluconeogenesis. The acute phase response includes changes in metabolism and nutrient fluxes in all organ systems of the body, especially liver and muscle. Although descriptive information on the acute phase response of chickens has been detailed (Klasing and Johnstone, 1991) the nutritional demands of this response are not well described. Clearly the acute phase response is a process that is both nutrient liberating (skeletal muscle catabolism) and nutrient consuming (acute phase protein synthesis, fever). Given the marked increase in hepatic demand for amino acids to support gluconeogenesis and for acute phase protein synthesis, it is likely that the amino acid costs of an acute phase response are considerably greater than the relatively minute needs of leukocytes that respond to an infectious challenge (*i.e.* the immune response). For several nutrients (*e.g.* zinc, iron, copper, lysine), it is known that the amount liberated is sufficient to meet the needs of both acute phase and the immune responses (Klasing and Barnes,1988; Laurin and Klasing, 1990; Koh *et al.*, 1996). Given that the acute phase response is likely to be a much larger consumer of nutrients during an infectious challenge than the immune system itself, future studies on the impact of nutrition on resistance of poultry should include measures of the adequacy acute phase response, such as production of acute phase proteins.

Nutritional Immunity

The immune system coordinates a rapid flux of several nutrients out of body fluids and into intracellular storage pools with the result of nutritionally starving some types of pathogens. For example, transferring production by the liver increases dramatically during the acute phase response of broiler chickens and laying hens (Hallquist and Klasing, 1994). This iron binding protein mediates the transfer of iron out of blood plasma and into the liver where it is less available to pathogens. Feeding high levels of EDTA to chicks increases the deposition of iron into tissues and increases plasma concentrations. This predisposes

chicks to increased mortality following a challenge with Escherichia coli (Tufft and Nockels, 1991). A redistribution of zinc is mediated by interleukin (IL)-1 during the acute phase response, due to enhanced synthesis of hepatic metallothionein (Klasing, 1984); however it is not clear if this is important in limiting the availability to pathogens of this essential nutrient. Avidin is secreted by stimulated chicken macrophages, suggesting that the sequestration of biotin aids in starving pathogens of biotin at the site of infections (Korpela, 1984).

Direct Regulatory Actions

An immune response requires extensive communication between a wide variety of cell types. Several nutrients play important roles by modulating the release of communication molecules or by changing the reactivity of leukocytes to extracellular signals. Polyunsaturated fatty acids (PUFA) provide a good example of nutrients that influence the immune system by affecting intercellular communication. The amount, type, and ratio of dietary PUFA determines the types of fatty acids that are incorporated into cell membranes and consequently the fluidity of the membrane and the types of eicosanoids that are released as communication molecules (Korver and Klasing, 1995, 1997). Linoleic acid is a member of the n-6 series of PUFA and a principle fatty acid in cereal grains and soybeans. It is elongated to Arachidonic acid and incorporated into cell membranes. A phospholipase-dependent communication cascade causes the release of arachidonate and its conversion into metabolically active prostaglandins, leukotrienes, and thromboxanes. Similarly, dietary n-3 PUFA can be incorporated into cell membranes. Upon release during cell signaling, the n-3 PUFA modify the amounts and types of eicosanoids that are produced causing changes in cell communication. In broiler chickens for example, dietary fish oil, which is high in n-3 PUFA, modulates IL-1 and prostaglandin E (PGE) release during an inflammatory response. The modulatory effects of dietary n-3 fatty acids are reflected in increases in antibody responses to antigens but decreases in mitogen-induced proliferation of lymphocytes (Fritsche *et al.*, 1991; Korver and Klasing, 1997).

Vitamins A, E, and D have regulatory roles in cells of the immune system. A similar influence appears to occur in chickens; vitamin E dampens the release of prostaglandins and modulates cytokine release from stimulated leukocytes (Romach *et al.*, 1993) and vitamin A, acting through the retinoic acid receptor, increases antigen specific responses in T lymphocytes (Halevy *et al.*, 1994).

The immune system does not function independent from other physiological systems, but is highly integrated with normal metabolism and physiology. Cells of the immune system have receptors for a wide variety of hormones that are normally regulated by the diet (*e.g.* insulin, insulin-like growth factors, glucagon, thyroxin, catecholamines, corticosterone). The tone of the endocrine system may have acute influences that modulate the magnitude or type of immune responses as well as chronic influences that impact important developmental events (see reviews by Marsh, 1995, 1996). It is likely that nutritionally responsive hormones mediate the acute immunoregulatory impact of feed deprivation or over consumption as well the chronic impact of the plane of nutrition on the susceptibility of broiler breeders to infectious diseases.

Both cell-mediated and antibody responses are enhanced in chicks that have been deprived of feed for short periods of time (12 to 24 hours), presumably due to a permissive endocrine climate for these responses (Klasing, 1988). Longer periods without feed result in greatly elevated corticosterone levels and eventually impair both antibody and cell-mediated immune responses. For example, Leghorn hens deprived of feed for 14 days have decreased peripheral blood CD4+ helper T lymphocytes, impaired cell-mediated immunity, and increased susceptibility to Salmonella enteritis challenges (Holt, 1992; Holt *et al.*, 1994).

Chronic feed restriction is a commonly used practice in rearing and managing broiler breeders as a way to enhance productivity and livability (Katanbaf *et al.*, 1989; O'Sullivan *et al.*, 1991). Restriction feeding regimens have been shown to improve resistance to a variety of infectious diseases. Some of the improvement may be due to increased humoral immunity and an inhibition of the involution of the thymus and bursa that is normally associated with aging.

Reduction of Pathology

The activation of cellular components of the immune system, such as cytotoxic T cells, natural killer cells, macrophages, and heterophils results in the elaboration of a wide variety of destructive molecules into the surrounding microenvironment. For example, stimulated chicken macrophages and heterophils release reactive oxygen intermediates, nitrous oxide, and catabolic enzymes into their phagocytic vacuoles and into the extracellular environment. These defensive agents are cytotoxic and kill bacteria, parasites, and infected host cells. They also may cause damage to uninfected host cells that are in the immediate vicinity. Eimeria maxima infection causes sufficient production of nitric oxide that its concentration rises not only in the intestinal mucosa of the infected area, but also in the blood (Allen, 1997).

Widespread damage due to reactive oxygen and nitrogen intermediates may be minimized by a variety of mechanisms, including reinforcement of anti-oxidant mechanisms of host cells due to local and systemic stress signals and the protective effects of several of the acute phase proteins secreted from the liver.

Local antioxidant defense is presumably facilitated by adequate vitamin E in cell membranes and by high levels of vitamin C in the cell cytosol. A deficiency of vitamin E or selenium results in peroxidation of lipids in the cell membranes and increased signs of functional damage during the inflammatory response induced by Salmonella minnesota's lipopolysaccharide (Sword *et al.*, 1991). Vitamin C reduces the lesions associated with Newcastle disease virus and Mycoplasma gallisepticum infection and by an Escherichia coli infection (Gross and Bailey, 1995). In that stressed host, cells release cytokines and eicosanoids that down-regulate the immune response, minimizing their injury and theoretically permit more sustained and vigorous immune responses and increased resistance.

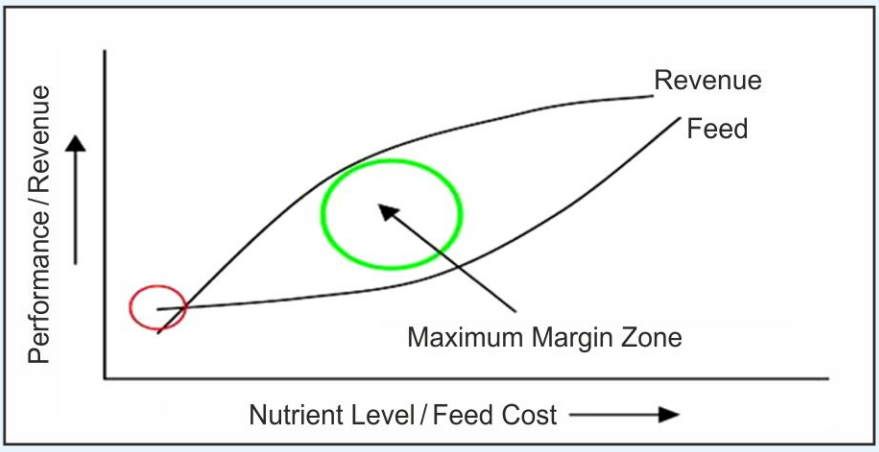
Physical and Chemical actions of Feeds

Feed accounts for 65-70% of the total costs in animal production. Any operation must therefore have clear targets how to optimize feed efficiency and reduce feed cost and work daily towards those targets. The sharp rise in feed ingredient prices in general, and soy bean meal in particular has forced producers to refocus on what they spend on feeding, to raise efficiency targets and to go the extra mile for converting feed protein more efficiently into lean gain.

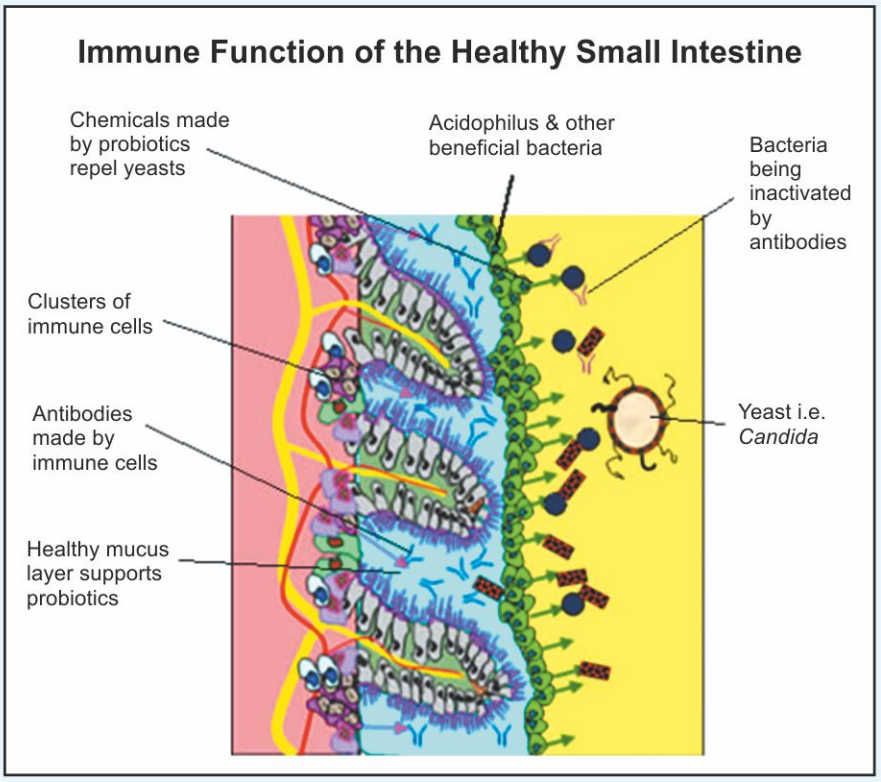
The figure shows the relation between dietary nutrient concentration and feed cost, performance and revenue. Both revenue and feed cost increase with higher nutrient density in the diet (Waller, 2007). The green zone marks the area with maximal difference between the revenue curve and feed cost curve. When setting the nutrient levels in this range, the margin for 'revenue over feed cost' is highest. As feeding cost is a major production issue, this is also likely to represent the area with maximal net profit. A consistent production system with accurate data on animal performance and carcass value are needed to fine-tune the system. This basic information is available in most broiler operations, as bird genetics and the overall production system are well standardized. Many swine fattening farms also have such data available to define the nutrient concentrations which are most suited for their genetics and the market conditions they operate under.

The intestines are the primary battleground between potential pathogens and the immune system. This is evidenced by the large number of pathogenic and nonpathogenic organisms located in the intestinal lumen (15×10^{13}/kg body weight; Roitt, 1997), the focusing of host defenses along the intestinal epithelium (*i.e.* the gut-associated lymphoid tissue), and the high incidence of entero-pathogenic diseases in all animals, including poultry. The intestinal epithelium must maintain exceptionally high physical integrity to prevent the bulk transport of pathogens into the body, but it must be sufficiently thin to actively transport nutrients. Physical and chemical attributes of the diet can modify the populations of microorganisms in the gastrointestinal tract, the capacity of pathogens to attach to enterocytes, and the integrity of the delicate intestinal epithelium.

Dietary factors that impact the microbial ecology of the intestines include the amount of fiber, the viscosity of the fiber, and fats that are refractory to digestion. For example barley, which is high in non-starch polysaccharides(NSP), is associated with increased presence of Clostridium perfringens, and increased incidence of necrotic enteritis (Hofshagen and Kaldhusdal, 1992; Kaldhusdaland Hofshagen, 1992). Rye is also high in non-starch polysaccharides and



Effect of dietary nutrient concentration on feed cost, performance and revenue (Waller,2007)



increases the incidence of necrotic enteritis (Riddle and Kong, 1992), enhances the attachment of enterococci to the intestinal wall (Untawale and McGinnis, 1979) and causes a several log increase in the numbers of anaerobic bacteria including a Clostridial-like organism (Wagner and Thomas, 1978). Other dietary components besides fiber influence the microbial ecology of the intestines, for example, unstabilized rancid fat in the diet increases numbers of E. coli and lowers numbers of Lactobacilli (Dibner *et al.*, 1996).

Role of trace minerals in immunomodulation

A deficiency in one or more of these elements can compromise immunocompetence of an animal (Beisel, 1982; Suttle and Jones, 1989). The trace metals that have been associated with an improvement in immunity, or functions that support immunity, are Zinc, Manganese, Copper, and Selenium. The first level of defense in the immune system is the skin. Zinc and manganese are key elements for maintaining epithelial tissue integrity.

Zinc plays an important role in poultry, particularly for layers, as a component of a number of metallo-enzymes such as carbonic anhydrase which is essential for eggshell formation in the hen's shell gland. Other important zinc metalloenzymes in the hen include carboxypeptidases and DNA polymerases. These enzymes play important roles in the bird immune response, in skin and wound healing, and for hormone production (testosterone and corticosteroids). Classic deficiency symptoms of a zinc deficiency in poultry could include a suppressed immune system, poor feathering and dermatitis, infertility and poor shell quality.

Manganese needs by the gastrointestinal tract, lungs and several other tissues in the bird are increased during an immune response. The reason for this is that manganese serves as a cofactor of superoxide dismutase and ameliorates damage induced by the immune response itself. However, there is very little change in the circulating manganese levels in the plasma during stress (Klasing, *et al.*, 1991).

Copper functions in the immune system through the following: energy production, neutrophil production and activity, antioxidant enzyme production, development of antibodies and lymphocyte replication (Niederman *et al.*, 1994; Nockels, 1994). The importance of copper for maintaining the functions of the immune system has been demonstrated in several studies. In vitro activities of T- lymphocytes and neutrophils isolated from adult male rats chronically fed a diet marginally low in copper were significantly suppressed without marked alterations in traditional indicators of copper status (Hopkins and Failla, 1995). Increased incidence of scours, occurrence of abomasal ulcers shortly after birth and respiratory problems have both been attributed to inadequate copper levels in newborn calves (Naylor *et al.*, 1989; Smart *et al.*, 1986).

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Selenium is a very unique trace mineral in the chicken's diet in that its inclusion rate is regulated and limited by the FDA. Selenium is considered a heavy metal in manure and is limited in its soil application. Selenium was recognized for its toxicity in animal diets before its essentiality was established.

Selenium is an important constituent of the enzyme glutathione peroxidase. Glutathione peroxidase functions in the cell as its first line of defence against oxidation. Other selenoproteins in poultry play an important role in prevention of exudative diathesis, normal pancreatic function, and fertility. Levels of selenium supplementation are limited by the FDA to only 0.30 ppm in poultry diets. Levels of selenium in feedstuffs for poultry can vary considerably dependent on soil content of selenium the crops are grown on. Often times, total selenium of poultry diets would reach levels of 0.40 to 0.50 ppm when corn and soybean levels are combined with 0.30 ppm supplementation levels. These high levels can to be beneficial to the immune status and performance of poultry flocks without being toxic. Dietary selenium works with Vitamin E in boosting the immune status of poultry.

Role of trace minerals in immuno-modulation (Swine)

Trace minerals such as manganese, iron and zinc are essential for swine mineral nutrition as they are involved in many digestive, physiological and biological processes that directly affect fertility and health of sows and newborn pigs, and carcass quality of fattening pigs. When trace minerals are not provided in the proper levels, digestion, immune system, hormone production, bone integrity and skin health can be seriously affected.

Several minerals are included in the regulation of the pig's immune system. The role of selenium in protecting biological membranes from oxidative degeneration was established many years ago (Lessard *et al.*, 1991; Oldfield, 2003).

Dietary selenium is, therefore, necessary to obtain maximal immunity in pigs, but to avoid toxicity due to over supplementation the inclusion of selenium in diets fed to livestock is regulated by FDA and cannot exceed 0.3 ppm. However, recent research indicates that organic sources of selenium are better utilized by pigs than inorganic sources (Mahan and Parrett, 1996; Mahan *et al.*, 1999). As a consequence, to obtain maximum improvements in the immune system, it may be advisable to use organic selenium, rather than inorganic sources of selenium.

Copper sulfate is usually added to nursery diets at concentrations between 150 and 250 ppm, although the nutritional requirement for copper is much lower.

Likewise, zinc oxide is often added to starter diets at levels of 2,000 – 4,000 ppm, which is much higher than the nutritional requirement for zinc. However, the inclusion of these minerals at high concentrations in diets fed to nursery pigs has been shown to reduce scouring and to control post-weaning diarrhea without causing any toxicity symptoms (Poulsen, 1995; Goransson, 1997). These effects may be caused by the ability of zinc and copper to reduce concentrations

Table: **Dietary requirements for trace elements (per kg diet)***

Body weight (kg)	**Piglet** ***<20***		**Growing pig** ***20-50***		**Finishing pig** ***50-120***		**Breeding sow**	
Source	***ARC[1]***	***NRC[2]***	***ARC[1]***	***NRC[2]***	***ARC[1]***	***NRC[2]***	***ARC[3]***	***NRC[2]***
Zinc (mg)	50	100	50	60	50	50	50	50
Manganese (mg)	16	4	16	2	16	2	15	20
Iron (mg)	60	100	–	60	–	50	60	80
Copper (mg)	4	6	4	4	4	3.5	5	5
Iodine (mg)	0.16	0.14	0.16	0.14	0.16	0.14	0.5	0.14
Selenium (mg)	0.16	0.3	0.16	0.15	0.16	0.15	0.15	0.15

*Values represent the highest concentrations quoted
[1]ARC (1981) per kg dry matter
[2]NRC (1998) 90% dry matter
[3]AFRC (1991) 90% dry matter

Table: **Range of dietary minerals addition in several EU countries (per kg feed) (Whittemore *et. al.* 2002)**

Body weight (kg)	Piglet *<20*	Growing pig *20-50*	Finishing pig *50-120*	Breeding sow
Zinc (mg)	100 - 200	100 - 200	70 - 150	80 - 125
Manganese (mg)	40 - 50	30 - 50	25 - 45	40 - 60
Iron (mg)	80 - 175	80 - 150	65 - 110	80 - 150
Copper (mg)	6 - 18	6 - 12	6 - 8	6 - 20
Iodine (mg)	0.2 - 1	0.2 - 1.5	0.2 - 1.5	0.2 - 2.0
Selenium (mg)	0.2 - 0.3	0.15 - 0.3	0.2 - 0.3	0.2 - 0.4

of coliform bacteria in the intestinal tract of weanling pigs (Namkung *et al.*, 2006).

Both minerals have also been shown to have growth promoting effects of the same magnitude as what is usually expected from antibiotic growth promoters (Hahn and Baker, 1993; Smith et al., 1997; Hill *et al.*, 2000)

Role of vitamins in immunomodulation

Vitamin A:

Low and very high dietary vitamin A decreases body weight gain in broilers. Low dietary vitamin A causes depression in vitro T-Lymphocytes responses and in vitro antibody production to defined protein antigens. Excess vitamin A intake also decreases immune responses. Maximum T-cell proliferative responses to antigen have been observed at vitamin A levels considerably above NRC (1984) recommended level. Vitamin A is required for intestinal absorption of zinc (Zn) in poultry while zinc influences vitamin A utilization by affecting retinol binding protein (RBP) synthesis and release from liver.

Recent studies in broiler chickens have shown to have a detrimental effect on lymphoid tissues in vitamin A deficient chickens. Chickens receiving 0.2 µg vitamin A /g in their diet have relatively smaller bursa of Fabricius than the chicks receiving 2.0 µg vitamin A/g of feed. Thymus weight is only decreased with total vitamin A deficiency.

Following primary immunization, the chickens deficient in vitamin A show the lowest antibody titer. The difference in antibody titer is the maximum on 7th day post-immunization. Supplementation of Vitamin A either on the day of vaccination or few days afterwards increased antibody titer. It has been demonstrated that the optimum HI titer against Newcastle disease was obtained when the feed contained 20,000 I.U. Vitamin A per kg of feed.

Biotin:

Biotin is required in several enzymes particularly for trans-amination and decarboxylation of amino acids. It has been demonstrated that double the NRC requirement of biotin is required for optimum antibody production in infected flocks.

Vitamin B6:

Pyridoxine (vitamin B6) is essential for the development and maintenance of lymphoid tissues. Pyridoxine deficient birds exhibit reduced capacity to synthesize DNA and there is therefore an adverse effect on cell multiplication and the immune function. The marginal deficiency of vitamin B-6 (0.95%) results in a significant reduction in antibody levels. However, it has been demonstrated that marginal B-6 deficiency alone does not severely impair immune response during the first four weeks of age.

Vitamin C:

Under normal conditions, Vitamin C (Ascorbic acid) is synthesized in sufficient amounts by all species of poultry. However, under prolonged exposure to stress the ascorbic acid utilization may exceed the ability of chicken and turkeys to synthesize ascorbic acid. It is observed that ascorbic acid supplementation increases the HA level from 4 to 6 days post-vaccination. The mechanism by which ascorbic acid ameliorates steroid mediated immuno-suppression is either by reducing adrenal synthesis of corticoids or by protecting the lymphoid tissues.

The optimum vitamin supplementation levels are given in the table below.

Vitamins (added to air-dried feed)	*Replacement pullets*	*Laying hens*
Vitamin A (IU/kg)	7000–10000	8000–12000
Vitamin D3 (IU/kg)	1500–2500	2500–3500
Vitamin E (mg/kg)	20–30	15–30
Vitamin K3 (mg/kg)	1–3	2–3
Vitamin B1 (mg/kg)	1.0–2.5	1.0–2.5
Vitamin B2 (mg/kg)	4–7	4–7
Vitamin B6 (mg/kg)	2.5–5.0	3.0–5.0
Vitamin B12 (mg/kg)	0.015–0.025	0.015–0.025
Niacin (mg/kg)	25–40	20–50
Pantothenic acid (mg/kg)	9–11	8–10
Folic acid (mg/kg)	0.8–1.2	0.5–1.0
Biotin (mg/kg)	0.10–0.15	0.10–0.15
Vitamin C (mg/kg)	100–150	100–200
Choline (mg/kg)	200–400	300–500

Vitamin D:

Vitamin D is critical for proper bone development in poultry. Research has elucidated negative effects on broiler cellular immunity as affected by vitamin D deficiency (Aslam *et al.* 1998). In female broilers a diet devoid of vitamin D or a diet containing 800 IU/kg of cholecalciferol had depressed cellular immunity as measured by cutaneous basophil hypersensitivity response to phytohemagglutinin-P, depressed thymus weight, and depressed macrophage function. However, although SRBC is a T-dependent antigen, differences in primary or secondary responses did not occur (Aslam *et al.*, 1998).

Vitamin E:

Vitamin E enhances specific humoral and cell-mediated immune responses as well as native resistance to disease, particularly phagocytosis. Supplemental levels of vitamin E have an immuno-stimulatory effect, increase delayed hypersensitivity and affect mitogenic responsiveness. Dietary supplementation of Selenium at levels above those recommended as nutritional requirements (0.1 ppm) enhances the primary immune responses. Vitamin E and Se appear to participate in similar nutritional and bio-chemical relationships. Supplementation of vitamin E in the diet of chicks enhances humoral immunity, which may be due to destruction of peroxides by vitamin E. Vitamin E and Se play a role in protecting against oxidative damage. Free radicals are scavenged by vitamin E as a first line of defense and then glutathione peroxidase of which Se is a part destroys any peroxides formed before they can damage the cell. Nutritional deficiencies of vitamin E or Se or both impair immune function as measured by humoral response to sheep red blood cells in young chicks.

Accordingly studies have been conducted with various levels of Se, vitamin E and their combinations to examine the effect on performance and immune response of broilers. Effect of supplementation of vitamin E, selenium and their combinations suggested that maximum body weight gain and best efficiency of feed utilization were observed in broilers fed diets containing 0.50 mg/kg Se and 300 IU/kg vitamin E. Significantly higher antibody titres (HI and ELISA) at 10 day PI were attributed to 0.06 mg/kg and 150 IU/kg Se and vitamin E respectively. Hence, optimum growth and immune response may be achieved at supplemental level of Se of 0.06 mg/kg and vitamin E at 150 IU/kg. The vitamin E level is higher than that recommended by NRC (1984, 1994).

Role of AA in immunomodulation

Amino acids are required for the synthesis of a variety of specific proteins (including cytokines and antibodies) and regulate key metabolic pathways of the immune response to infectious pathogens. Adequate dietary provision of all amino acids is necessary for sustaining normal immuno-competence and protecting the bird from a variety of diseases. In commercial diets, Lysine is limiting behind TSAA. Arginine ranges from third to fifth limiting depending on nutrient requirements and the dietary ingredients used. Although Lysine has been implicated in the antiviral immune response, research evaluating the impact of Lysine and chicken immunity is sparse. However, a deficiency of Lysine did not impair the ability of the bird to produce circulating IL-1 during immunologic stress (Klasing and Barnes, 1988).

Branched-Chain Amino Acids

Bhargava *et al.* (1971a) conducted experiments measuring growth and antibody production to Newcastle disease virus in chickens fed various levels of Valine In both studies, Valine need for antibody production was higher than that of growth. Konashi *et al.* (2000) evaluated a severe dietary reduction (50% of the control level) in the branched-chain amino acids and noted a reduction in the relative size of the thymus and bursa. The specific branched-chain amino acid that is most important for immune organ development (Valine, Isoleucine, or Leucine) in the former study is unknown because the 3 were reduced in the diet concomitantly.

Threonine

Threonine is an important limiting amino acid in livestock and poultry, which is often added to the feed as feed additive. Threonine is usually the second limiting amino acid for pig and the third for poultry. Like lysine, to which it acts as complementary, threonine is an essential amino acid for body protein deposition and growth. Thus, deficiency in threonine affects the utilization of dietary lysine and consequently the animal growth. Threonine is needed in gastrointestinal mucin production, and thereby involved in immune response. It has also been shown to improve livability of heat-stressed broilers (Lemme, 2001). Sanitary status and animals' environmental conditions are factors of variation of the Threonine : Lysine requirement.

Antibody titer against IBD and ND were influenced by interaction of threonine and methionine. On 7th day after IBD challenge, ND antibody titer of the broilers receiving the highest level of methionine and threonine was significantly higher than that of other treatment groups. (University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia)

Through protein synthesis and cellular signaling mechanisms, addition of threonine to the culture medium prevented apoptosis, stimulated cell growth and promoted antibody production in lymphocytes (Duval *et al.*, 1991). The Threonine need in chickens for antibody production to Newcastle disease virus was found to be higher than that for growth (Bhargava *et al.*, 1971b). However, Takahashi *et al.* (1994) fed broilers diets deficient in Threonine and observed reduced growth but not reduced antibody responses or lymphoid organ development. This work is in agreement with that of Kidd *et al.* (2001b) in that dietary Threonine does not influence immune organ development in young broilers. In addition, Kidd *et al.* (1997b) evaluated cellular and humoral immunity in chicks fed diets ranging in Threonine (0.68 to 0.86% of diet) and noted no improvements in immunity.

Tryptophan

It is essential amino acid & has emerged as a regulator of many immunological and physiological processes. Its plasma concentration declines in animals suffering from different illnesses and inflammations (induced or natural), suggesting an increased utilization of the amino acid in such instances (Le Floc`h *et al.*, 2004). IBDV primarily impairs the humoral immune response which is followed by sever immune-suppression due to down regulation of T cells and macrophages.

Conclusion

Feed accounts for 65-70% of the total costs in animal production. Any operation must therefore have clear targets to optimize feed efficiency and reduce feed cost. The high density confinement rearing of birds with limited use of antibiotics has made it essential to find a solution for enhancement of immune response by nutritional manipulation. Not only does the immune system benefit directly from proper nutrition, but indirectly proper nutrition will also prepare the bird for periods of stress, reducing the adverse effects of stress and enhancing recovery from stressful periods. The mechanisms of nutritional modulation of resistance to infectious disease are divided into seven categories. Obviously these categories are overlapping and nonexclusive.

A rough estimation of the size of the immune system reveals that a little over 0.42% of the body is made up of leukocytes and their progenitors. Total anti body found in the serum contribute less than 0.1% of the body weight of a chicken. Many of the cells of the

immune system are long-lived (life span of weeks to months), including dendritic cells, macrophages, and many lymphocytes. Other cells are very short lived, such as heterophils, which live for only a few days, and lymphocytes found in the thymus, bursa, and germinal centers, which undergo apoptosis soon after cell division. Normal rates of immunoglobulin synthesis in a young chicken are less than 0.02% of body weight/day. Immunoglobulin synthesis is probably higher in laying hens because of daily secretion into egg yolk; yet even in the hen, daily total immunoglobulin synthesis is probably well less than 0.05% of body weight/day.

Nutrient deficiencies that are especially damaging to development of the immune system include linoleic acid, vitamin A, vitamin D, Iron, Selenium, and several of the vitamins B. Vitamins A, E, and D have regulatory roles in cells of the immune system. A similar influence appears to occur in chickens; vitamin E dampens the release of prostaglandins and modulates cytokine release from stimulated leukocytes. The level of reserves of most of the vitamins and trace minerals in the hatchling are highly correlated with the level in the breeder's diet.

Vitamin E enhances specific humoral and cell-mediated immune responses as well as native resistance to disease, particularly phagocytosis. Supplemental levels of vitamin E have an immuno-stimulatory effect, increase delayed hypersensitivity and affect mitogenic responsiveness.

The intestines are the primary battleground between potential pathogens and the immune system. The intestinal epithelium must maintain exceptionally high physical integrity to prevent the bulk transport of pathogens into the body, but it must be sufficiently thin to actively transport nutrients.

The trace metals that have been associated with an improvement in immunity, or functional in supporting immunity, are Zinc, Manganese, Copper, and Selenium. The first level of defense in the immune system is the skin. Zinc and manganese are key elements for maintaining epithelial tissue integrity. Copper functions in the immune system through the following: energy production, neutrophil production and activity, antioxidant enzyme production, development of antibodies and lymphocyte replication. Selenium is an important constituent of the enzyme glutathione peroxidase.

In commercial diets, Lysine is limiting behind TSAA. Arginine ranges from third to fifth limiting depending on nutrient requirements and the dietary ingredients used. Although Lysine has been implicated in the antiviral immune response, research evaluating the impact of Lysine and chicken immunity is sparse. The specific branched-chain amino acid that is most important for immune organ development (Valine, Isoleucine, or Leucine) in the former study is unknown because the 3 were reduced in the diet concomitantly.

*References available on request.

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AVITECH NUTRITION PVT LTD
(An ISO 9001 Certified Company)
GP-37, Udyog Vihar, Sector-18, Gurgaon-122001 Haryana, India.
Tel: +91-124-4011147, 4278511/12 Fax: +91-124-4013620
Email: info@avitechnutrition.com Website: www.avitechnutrition.com