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Immunomodulating effects of vitamin E in broilers

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Modern commercial broilers appear to have compromised immunocompetence, higher mortality and lower resistance to stressors. To overcome these, dietary manipulation seems to be the easiest approach, and this has been supported by research investigations conducted over the last few decades. The goal of enhancing the immune system of broilers is laudable for both economic and welfare reasons. Vitamin E (VE) is now well accepted as nature's most effective lipid-soluble, chain-breaking antioxidant. This antioxidant vitamin has been shown to improve both cell-mediated and humoral immunity in broiler chicks. However, the effect of this vitamin depends upon dose, age and genetics of the broiler chicks. Several mechanisms have been postulated for the beneficial effects of this vitamin. In this review, several aspects of the immunomodulatory effects of VE are reviewed.

Keywords: broilers; immunity; vitamin E

Introduction

The broiler industry is increasingly dependent on medication for the control of diseases to realise the objective of selective breeding for high growth rates (Boa-Amponsem *et al.*, 2000). Reliance on medication has caused animal and human welfare concerns due to possibility of pharmacological residues in animal products, resistance of pathogenic organisms and environmental effects. Broiler chicks are generally raised for five to seven weeks and then marketed. During this period, chicks increase their body weight from approximately 40 g to 2.5 kg (Erf *et al.*, 1998). Modern nutritional and farming strategies have been designed to produce broilers with high potential for growth, yield and feed efficiency that have resulted in compromised health status. Improving yield and reducing age at market weight are inevitable in the economics of broiler production (Siegel *et al.,* 2000). The environmental conditions of poultry farms are usually not sufficient in terms of what is required for the optimum health of these rapidly

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growing chicks. Under such conditions, broilers are exposed to pathogens and other environmental stressors at an age when they are not fully immunocompetent. Additionally, fast growth, which has been negatively correlated with immune status in chickens, imposes another challenge on these broilers (Erf *et al.,* 1998). Early in life, broilers are not fully equipped with a competent immune system and rely substantially on maternal immunoglobulins and their innate immune system to combat environmental pathogens.

The ban of some prophylactic feed ingredients used to improve bird health and efficiency has driven the study of other ingredients that could promote optimal nutrient utilization and favour the full genetic expression of their potential (Siegel *et al.*, 2000). The rapid growth seen in commercial broilers may have changed the nutrient requirements of these lines, especially for micronutrients. Enhancement of the immune system of chickens has been sought through vaccination programs, breeding, nutrition and husbandry practices (Yang *et al.*, 2000). One of the many approaches proposed to enhance immune response is to supplement the ration with vitamins which can modulate immune responses due to their extensive involvement in structural components and molecular mechanisms.

Vitamins are defined as a group of complex organic compounds present in minute quantities in natural food that are essential to normal physiological functions and therefore lack of vitamins in the diet causes deficiency diseases and poor performance. Vitamin E (VE) is a fat soluble vitamin of plant origin and is essential for the integrity of the reproductive, muscular, circulatory, nervous and immune systems (Leshchinsky and Klasing, 2001). It is most likely that VE, like other nutritional factors, affects the development and maintenance of immunocompetence through multiple functions; by acting directly on immune cells, or by indirectly affecting metabolic and endocrine parameters, which in turn influence the immune system (Gershwin *et al.*, 1985; Leshchinsky and Klasing, 2001). The typical clinical signs of VE deficiency in chickens include exudative diathesis, nutritional muscle dystrophy, encephalomalacia, retarded growth and poor reproductive performance (Niu *et al.*, 2009).

VE is a term used to denote two compounds; tocopherols and tocotrienols. After ingestion, VE is hydrolyzed and absorbed through the intestinal epithelium in its non-esterified form. It is readily incorporated into the cellular membrane where it promotes integrity of the bilayer. VE has a number of different but related functions. One of the most important functions is its role as an intracellular antioxidant. In this capacity, it prevents lipid peroxidation of polyunsaturated fatty acids within the cell, thus protecting the cell against the toxicity of free radicals (Khan, 2011). According to global standards (NRC, 1994), the recommended dose of VE ranges from 5 to 25 IU/kg of feed is needed for the normal performance of birds. However, at higher doses, VE has been found to improve the performance of birds exposed to various diseases, suggesting an immune potentiating role of VE.

Environmental stress is the major modifier of VE action. Nutritional stress, temperature extremes, overcrowding, noise and transportation may predispose birds to infectious diseases by inhibiting defence mechanisms. A stress-induced infection may cause diarrhoea and decrease feed utilization, thus further leading to decreased immunocompetence. Intensive genetic selection for rapid weight gain and feed efficiency has led to compromised avian immunity in some aspects. Previously, Marsh *et al.* (1986) reported that deficiency of VE depressed bursal, thymic and spleen growth. Additionally, reductions in chicken circulatory lymphocytes have also been demonstrated by Dietert *et al.* (1983), as a consequence of marginal dietary VE. Gore and Qureshi (1997) suggested that the availability of this vitamin in suitable amounts seems to be necessary for beneficial effects on the ontogeny of the immune response of the birds.

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A brief overview of the avian immune system

Two functional immune aspects of the avian immune system; humoral and cell-mediated provide the basis for the prevention and protection against micro organisms. Humoral immune response is the adaptive function of the immune function through which antibodies are produced in response to antigenic challenge. Cell-mediated immunity involves immune mechanism(s) by which cells infected with a foreign agent, such as bacteria, are destroyed (Qureshi *et al.*, 1998). Broadly, the avian immune system is comprised of two broad structural categories; lymphoid and non-lymphoid systems. Lymphoid components are further composed of bursa of fabricius and thymus, where the development and differentiation of B-lymphocytes and T-lymphocytes occur respectively (Glick, 1995). Bursa of fabricius and thymus are considered to be the primary lymphoid organs, whereas the spleen is usually termed a secondary lymphoid organ. The non-lymphoid parts of the immune system include cells that provide a non-specific immunological defence to the host (Qureshi *et al.*, 1998). Blood monocytes and tissue macrophages, which are unique due to their ubiquitous distribution throughout body fluids, organs and cavities, are the primary players in this category. After an encounter with foreign antigens such as bacteria, macrophages bind, engulf and degrade them (Weinstock *et al.*, 1989). Furthermore, in avian species, the non-lymphoid cells with phagocytic potential include heterophils, counterpart of neutrophils in other mammals, and thrombocytes (Qureshi *et al.*, 1998).

The immune system has one of the most complex molecular and cellular interactions, encompassing the humoral and cell-mediated immune systems, in both of which lymphocytes are the primary cells lines. Antibodies are produced in the secondary lymphoid tissues, which include, in addition to the spleen and lymph nodes, the bone marrow, caecal tonsils and other lymphoid tissues distributed throughout the body, particularly in the respiratory, digestive and urogenital systems. At hatching, the immune system of the birds is only partially developed with the presence of thymus and bursa, however, the less important immune organs such as spleen, caecal tonsils, Meckel's diverticulum and lymphoid tissues are still incomplete (Dibner and Richards, 2004). The crucial roles of monocytes and macrophages in host immune systems make them indispensable factors in humoral and cell-mediated immunity. Through the process of phagocytosis, macrophages take part in the elimination of necrotic cells, tissue debris and invading micro organisms (Qureshi *et al.*, 1998) and are involved in antigen presentation and the secretion of cytokines, prostaglandins and free radicals.

Interaction of vitamin E and immunity

CELL-MEDIATED IMMUNITY

Historically macrophages are regarded as little more than the garbage-collecting or recycling component of the immune system (Dietert and Golemboski, 1998). Macrophages, being mobile scavenger cells, participate in innate immunity by serving as phagocytic cells. These cells arise in the bone marrow and subsequently enter the blood circulation as blood monocytes (Qureshi *et al.*, 2000). Being the first line of immunological defence, macrophages, therefore, perform an important role as antigen processing and presentation, secreting several kinds of cytokines and metabolites. Gore and Qureshi (1997) found that 10 IU VE significantly increased Sephadex-elicited inflammatory exudate cells and the percentage of phagocytic macrophages in broiler chicks injected *in ovo*. Niu *et al.* (2009) found that abdominal exudate cells and the percentage of macrophages were significantly increased in response to 200 mg/kg feed of

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VE in heat stressed broilers. Konjufca *et al.* (2004) fed 110 and 220 mg of VE/kg feed and found that the number of SRBC phagocytosed per macrophage were higher in three weeks old broilers compared with age-matched controls. Qureshi *et al.* (2000) suggested that the process of phagocytosis by macrophages is a membrane-mediated phenomenon, maintained by the availability of higher levels of VE needed for the integrity for phagocytosis. VE accomplishes this via its antioxidant properties, or by down-regulating prostaglandin synthesis, elevated levels of which are known to be immunosuppressive.

The heterophil to lymphocyte ratio (H:L) has been used as a reliable indicator of stress in birds (Mashaly *et al.*, 2004). The proportion of blood H:L is widely used to evaluate the presence of inflammation and has been well characterized in a variety of models of avian inflammation (Leshchinsky and Klasing, 2001). Heterophils are phagocytic cells whose main role is protection against invading micro organisms. The H:L ratios of approximately 0.2, 0.5 and 0.8 indicate low, medium and high degrees of stress respectively (Gross and Siegel, 1983). Additionally the influx of heterophils is the first line of cellular defence in the avian respiratory tract because of the absence of resident populations of pulmonary macrophages (Boa-Amponsem *et al.*, 2000). Boa-Amponsem *et al.* (2000) reported increased H:L in response to higher doses (300 mg/kg feed) of VE in broiler chicks. Leshchinsky and Klasing (2001) observed decreased heterophils due to moderate levels of supplemental VE (50 IU/kg). Leshchinsky and Klasing (2001) recorded lowest heterophil levels at 50 IU/kg of VE after challenge with lipopolysaccharide injections. The number of heterophils increased during mild or moderate stressful conditions, hence the H:L ratio could be used to detect the presence of physiological stress which may result in heterophilia and basophilia in fowls.

Erf and Bottje (1996) observed the effects on the lymphocyte populations in broilers in response to 50 and 100,000 IU/t feed of VE on day of hatch. They found elevated percentages of CD4+ cells in the thymus and spleen and increased CD4:CD8 ratios in the blood at seven weeks of age. The CD4+ is a key player as helper cells in antibody production. Erf *et al.* (1998) supported the view that dietary VE supplementation in the form of α-tocopherol acetate, significantly affected T cell differentiation in the thymus and spleen in broiler chicks. A level of 87 mg/kg of VE increased the percentage of mature T helper cells (CD4+CD8–) in the thymus and spleen in seven week old broilers.

Boa-Amponsem *et al.* (2000) recorded decreased coetaneous basophilic hypersensitivity (CBH) responses with feeding 10 mg/kg VE. A similar result was found in the study of Sakamoto *et al.* (2006), who concluded that VE reduced ($P = 0.05$) CBH with a level of 10 mg/kg allowing higher cell proliferation as compared to 500 mg/kg.

Upon suitable stimulation, many mammalian cells are capable of *de novo* expression of inducible nitric oxide synathase (NOS), and once expressed this enzyme produces large amounts of nitric oxide (NO) for prolonged periods of time. NO plays an important role in diverse physiological and cellular processes including inflammation and host defence, neurotransmission, vasodilatation and platelets inhibition. NO produced by activated macrophages is an important immunomodulatory molecule and is cytotoxic to tumour cells and micro organisms (Gore and Qureshi, 1997). Chicken macrophages have been reported to be capable of producing NO under various immunological stimuli (Hussain and Qureshi, 1997). Post-hatch NO production was significantly higher when VE (10 IU) was injected into the amnion of broiler chicks three days prior to hatch (Gore and Qureshi, 1997). The exact mechanism of the increased NO is not fully known. Gore and Qureshi (1997) suggested that VE may increase the affinity of macrophage membrane receptors for the activation of stimuli such as lipopolysaccharide, or increase the nitric oxide synathase activity resulting in more NO production.

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Humoral immunity

We know that binding antibodies to an antigen initiates mechanisms that include the promotion of phagocytosis at the site of infection, activation of the complement cascade, followed by an inflammatory response, phagocytosis and initiation of antibody dependent cellular toxicity executed by monocytes, neutrophils and natural killer (NK) cells. Boa-Amponsem *et al.* (2000) found an increased response of antibody titre against SRBC in response to low (10 mg/kg) and high (300 mg/kg) doses in broiler chicks measured at two different intervals. Gore and Qureshi (1997) found higher antibody responses against SRBC, when VE was injected at the rate of 10 IU during embryonic development. Leshchinsky and Klasing (2001) reported improved antibody levels to SRBC, when birds were supplemented with 50 IU of VE/kg compared to those supplemented with 200 IU/kg of VE.

There is evidence that VE facilitates isotopes to switch from IgM to IgG, which is typical of memory response (Tanaka *et al.*, 1979). Birds respond to antigenic stimulation by generating antibodies. Boa-Amponsem *et al.* (2000) found increased IgG and IgM in response to 10 and 300 mg/kg of VE in three different genetic stocks of broiler chicks, measured at six and twenty days post-injection of SRBC. Gore and Qureshi (1997) injected VE (10, 20 and 30 IU) into the amnion of broiler embryos three days pre-hatch, and noted the response post-hatch. When challenged at seven days post-hatch, IgM was significantly high against SRBC for birds receiving 10 IU VE. Niu *et al.* (2009) found significantly higher IgG and IgM production in response of 200 mg/kg dietary VE in broilers reared under heat stress. Leshchinsky and Klasing (2001) found dose dependent increases in antibody production in response to attenuated IBV (infectious bronchitis virus) between 0 and 25 IU/kg of supplementation of VE. In the same experiment, antibody levels of SRBC were higher in birds supplemented with 50 IU/kg.

Factors affecting immunity in broilers

Although a considerable body of evidence have demonstrated the beneficial effect of VE supplementation on the immune system of broilers, several studies have failed to show improvement in disease resistance or immunocompetence due to dietary VE supplementation. Qureshi and Taylor (1993) and Marsh *et al.* (1981) reported that VE supplementation (100 or 250 IU/kg of diet) did not affect antibody production in broilers, although it increased the number of macrophages (Qureshi and Taylor, 1993). No beneficial effect on mortality and pathological lesions induced by *E. coli* was found in poults supplemented with 12 and 300 IU/kg of dietary VE (Sell *et al.*, 1997). Friedman *et al.* (1998) found that intake of VE at levels exceeding NRC recommendations by as much as 15 fold (NRC, 1994), impaired antibody production in both chicks and turkeys. Konjufca *et al.* (2004) did not find any effect of VE supplementation in terms of greater macrophage engulfment percentages in response to 110 and 220 mg/kg of diet in five and seven week old broilers, when elicited by SRBC, although positive results were recorded at three weeks of age. Leshchinsky and Klasing (2001) reported that VE doses in the range of 25 to 50 IU/kg were immunomodulatory but higher levels (100 and 200 IU/kg of feed) were less effective. The question then arises: why VE is beneficial in some cases but failed to produce the expected results in others?

In experimental models, many parameters of the immune system, including resistance to infection, specific antibody production, numbers of antibody-producing cells, *in vitro* mitogenic responses to lymphocytes and the phagocytic index are altered by diets that are supplemental or deficient in VE (Leshchinsky and Klasing, 2001). The effects of VE

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appear to be influenced by age, dietary level, measurement criteria and the genetic stock of the birds (Gore and Qureshi, 1997; Erf *et al.*, 1998; Boa-Amponsem *et al.*, 2000). By comparing commercial genetic lines of broilers, it was observed that 1991 strains were less efficient in antibody production than 1957 strains (Sakamoto *et al.*, 2006). Boa-Amponsem *et al.* (2000) investigated cell-mediated and humoral immune response in three pure lines of broiler chicks by feeding either 10 or 300 mg/kg of VE. In this experiment, it was found that antibody titre against SRBC was not only dose-dependent but also showed variations in different genetic stocks. Similarly, production of IgM and IgG were affected by dose, genetic stock and the measurement criteria. Similar results were recorded in terms of H:L and CBH responses by the same authors. Yang *et al.* (2000) demonstrated that the age of the broilers and dosage of VE greatly contributed to the differences in findings in the same set of experiments. Qureshi *et al.* (1998) reported that although the avian immune system is structurally and functionally unique, it is directly influenced by physiological, genetic, nutritional and environmental factors. Cheema *et al.* (2003) concluded that genetic selection for improved broiler performance has resulted in a decreased adaptive immune response but an increase in the cell-mediated and inflammatory responses.

Siegel *et al.* (2000) reported that the effect of high levels of VE up to 12 days of age in broilers were age, sex and line specific. For example, the high levels affected body weight, breast yield in females but had no effect in males. Higher levels of VE reduced mortality at day 22 but had no effect in 41 day old broilers. An influence of the animal's sex has also been reported, whereby a significant negative correlation between phagocytic activity and T-cell-mediated response was found in female birds of White Leghorn chicken lines, which was not seen in male lines of the same genotype (Cheng and Lamont, 1988). To find the effect of genetic selection for improved broiler production, Cheema *et al.* (2003) compared the immunocompetence of 2001 Ross 308 broiler strain and the 1957 Athens Canadian Random Breed Control (ACRBC) when they were given diets representative of those that were being used in those years respectively. The ACRBC strain showed greater antibody response against SRBC than 2001, while Ross 308 birds had greater phytohemagglutinin-P (PHA-P) induced toe-web swelling response, higher inflammatory exudates cell number as well as SRBC phagocytosed macrophages.

It is interesting that, in a long term selection experiment, two lines derived from the same population were different not only in antibody response to SRBC as growing chicks but also in peak and persistence of antibody resistance to the same antigen as adults (Yang *et al.*, 1999; Boa-Amponsem *et al.*, 1997; Yang *et al.*, 2000). Siegel *et al.* (2000) reported that performance of poults from older dams, in terms of body growth, mortality and immunocompetence, were superior to those from young dams. Previous studies have shown that stress caused by handling birds during blood collection could influence the immune status of the birds (Leshchinsky and Klasing, 2001). It seems that immune parameters are so sensitive to stress that even handling during laboratory studies could contribute to variations in immune responses. According to Friedman *et al.* (1998), most of the so-called antioxidants including alpha-tocopherol, carotenoids and ascorbate act both as antioxidants and pro-oxidants under conditions determined by their concentration, redox potential and the chemistry of the cell. Increasing concentrations of tissue tocopherol as produced by high diet in chicks induces oxidative changes that are detrimental to some immune parameters being fully expressed.

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Immunomodulating mechanisms of vitamin E

VE has been reported to protect the cells involved in the immune response against oxidative damage and enhance the function and proliferation of these cells. Although the exact mechanism is not understood, its bioactivity is mainly associated with its antioxidant potential. As an antioxidant, VE scavenges free radicals produced as a result of lipid peroxidation during normal metabolic state and inflammation. VE affects free radical-mediated signal transduction events and ultimately modulates the expression of genes that are required by free radical signalling (Packer and Suzuki, 1993). Leshchinsky and Klasing (2001) suggested that the immunomodulatory effect of VE corresponds to the level necessary for the inhibition of lipid peroxidation and protection of mitochondria and microsomes of the liver against oxidative stress. As a primary antioxidant of cell membranes, VE takes part in the prevention of peroxidation of fatty acid that can act as immunoregulatory molecules that mediate cellular communication, membrane fluidity and second messenger elaboration, while the effect of VE may be immunoregulatory in itself (Leshchinsky and Klasing, 2001). It is known that VE works in integrated fashion with other cellular antioxidants such as ascorbate and glutathione systems. During encounters with free radicals, VE becomes deficient of electron which is compensated by ascorbic acid. Thus VE molecule is again available to function as an antioxidant.

Gore and Qureshi (1997) suggested that higher doses of VE may maintain the macrophage membrane integrity needed for phagocytosis or as an antioxidant may prevent the oxidation of arachidonic acid involved in the biosynthesis pathway of prostaglandins which has immunosuppressive effects at elevated levels (Sheffy and Schultz, 1979). Another effect of immune potentiating of VE may be explained by its role in down regulating prostaglandin (PG) production by antagonizing the lipid peroxidation of arachidonic acid and limiting the entry of precursor into the PG cascade (Gore and Qureshi, 1997). In support to this view, Likoff *et al.* (1981) demonstrated that, at the level of 300 mg/kg feed, VE enhanced the phagocytic function in chickens which was due to decreases in endogenous PG as compared to the unsupplemented control. VE is believed to exert regulatory actions on the immune system as well as minimizing the level of pathology resulting from the cytotoxic immune response (Klasing, 1998). According to Sakamoto *et al.* (2006) VE decreases synthesis of prostaglandins, leukotriens and cytokines, which regulate the inflammatory response, thereby reducing damage caused to the tissue by inflammatory reactions.

Konjufca *et al.* (2004) suggested that macrophages have receptors on the plasma membrane for the Fc portion of antibodies. These receptors enhance the phagocytosis of antibody-opsonised antigens by macrophages. Hence the observed increase in phagocytosis by macrophages of the VE-fed broilers appears to be the result of increased expression of these receptors on macrophage membranes. In many cases, the protective immune response was increased by VE supplementation as evidenced by increased survival of chickens against *Escherichia coli*, *Brucella abortus*, *Pasteurella anatipestifer* (Tengerdy and Nockels, 1975), *Eimeria tenella* (Colnago *et al.*, 1984), and Newcastle disease virus infections (Boren and Bond, 1996). Erf *et al.* (1998), while explaining the role of dietary VE on production of T cells, suggested that the beneficial effect of VE is related to the support of this vitamin in cell survival within the thymic environment, which has been previously reported to decrease oxidative DNA damage in human lymphocytes. Another possible immunomodulatory mechanism of VE is the modulation of arachidonic acid metabolism via cyclo-oxgenase and lipoxygenase pathways which lead to the synthesis of prostaglandins and leukotriens, respectively (Leshchinsky and Klasing, 2001).

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Conclusions

Although a considerable body of evidence supports the beneficial effects of VE supplementation, the dose-response relationship between VE levels and indices of immunocompetence remains to be elucidated. Most studies have evaluated only two levels (high and low, usually three to 20 times higher than NRC) of VE supplementation to a basal diet. Consequently, it is not possible to make recommendations on the optimum level of dietary VE for immunocompetence. The NRC (NRC, 1994) recommended level of VE does not consider immunocompetence, although this level is sufficient to prevent oxidative damage. Moreover, there is a paucity of research reports delineating specific requirements of VE for a given immune function.

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