Centennial Review: Effects of vitamins A, D, E, and C on the chicken immune system

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ABSTRACT Vitamins are nutritional elements which are necessary for essential activities such as development, growth, and metabolism of cells. In addition to these conventional functions, vitamins A, D, E, and C have vital roles in normal function of the immune system as their deficiency is known to impair innate and adaptive host responses. By altering transcription of multiple immune system genes and contributing to antioxidant activities, these vitamins influence the immune system in different ways including modulation of cell-mediated and antibody-mediated responses, immunoregulation, and antiinflammatory effects. Furthermore, supplementation of these vitamins to poultry may assist the immune system to combat microbial pathogens while reducing detrimental effects associated with stress and enhancing responses to vaccines. In this article, the relationship between the chicken immune system and vitamins A, D, E, and C is reviewed, and evidence from the literature pertaining to how these vitamins exert their antiinflammatory, regulatory, and antimicrobial effects is discussed.

Key words: vitamin A, vitamin C, vitamin D, vitamin E, chicken immune system

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INTRODUCTION

In view of the high costs of disease treatment and their potential negative consequences on health, prevention has always been preferred over cure (Rheinberger et al., 2016). Considering the essential role of the immune system in disease prevention and optimal growth, a pivotal objective in poultry production is to produce chickens with competent immune systems. This helps in defence against pathogens and results in stronger response to vaccines. The critical roles of vitamins on normal function of the immune system have been extensively studied and are well understood. Insufficient vitamin levels may also lead to dysfunction of the immune system, which can result in increased rates of infection or inflammation and ultimately decreased growth (Koutsos et al., 2006). Among all vitamins, vitamins A, D, E, and C have been demonstrated to have the greatest effects on immune system function through a variety of mechanisms. Important for maintaining epithelial cell integrity, vitamin A contributes to many immune-related functions, such as enhancement of mucosal immunity and reduction of free radicals in chickens and mice (Stephensen et al., 1996; Kam et al., 2012; Lucas et al., 2014). Another feature of this vitamin is the induction of opposing effects in a dose-dependant manner, as it has been shown to be antiinflammatory in high doses and immunostimulatory in lower doses in chickens and mice (Yuan et al., 2014). Vitamin D is also well known for its antiinflammatory effects as it reduces the level of proinflammatory cytokines such as interleukin (**IL**)-1β in chicken macrophages (Shojadoost et al., 2015) and interferon (**IFN**)-γ and IL-2 in human T cells (Rigby et al., 1987). Moreover, vitamin E induces strong antioxidant and antiinflammatory effects (Khan et al., 2012), and also increases the number and functionality of immune system cells and stimulates antibody release in response to chicken vaccination (Adolfsson et al., 2001; Khan et al., 2014). Vitamin C is also well known for its antioxidant and antiinflammatory effects, which makes it beneficial in cases of oxidative stress, infection, and inflammation in chickens (El-Senousey et al., 2017).

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This review provides insights into the effects of 4 important vitamins, A, C, D, and E on the poultry immune system. For each vitamin, specific immunomodulatory roles have been described and include effects on innate antimicrobial responses, cell-mediated and antibody-mediated immune responses to infection and vaccination, in addition to regulatory/antiinflammatory responses.

VITAMIN A

Vitamin A refers to a group of fat-soluble and brightly pigmented molecules that includes retinol, retinal, retinoic acid (**RA**), and several pro-vitamin A carotenoids, which are delivered in the diet and converted to retinoid within the intestine and in other tissues, including the liver (Koutsos et al., 2003). Among the 700 known carotenoids, only 50 have pro-vitamin A activity with β-carotene, β-cryptoxanthin and α-carotene being the major pro-vitamin A molecules (Romanchik et al., 1995). Carotenoids have a wide range of functions, including immune-regulatory and immune-stimulatory functions in addition to antioxidant, antimutagenic and anticarcinogenic properties (Bendich, 1989; Møller et al., 2000; Mora et al., 2008). Unlike plants and microorganisms, which are able to synthesize carotenoids from acetyl-coenzyme A through a series of well-defined condensation reactions, avian and mammalian species require carotenoids in their diets as they lack this capability (Olson, 1989).

In the embryonic stage of chicken development, vitamin A is supplied by the yolk; however, after hatch, this must be replaced by dietary sources (Uni et al., 2000). Although dietary supplementation of carotenoids in poultry production has focussed on influencing pigmentation of poultry products (Hencken, 1992), the importance of vitamin A in the poultry diet has since become well recognized. This is because of production losses associated with vitamin A deficiencies, including impaired growth, ruffled feathers, weakness, xeropthalmia, impaired egg production, and depressed immunity (Aydelotte, 1963; Chun et al., 1992; Sklan et al., 1994). Additionally, the requirement for optimal levels of vitamin A in hatching eggs has been emphasized as chicks hatched from carotenoid-depleted eggs have been shown to exhibit impaired immune responses, marked by increased signs of systemic inflammation (reduced body weight, increased copper and haptoglobin, and reduced zinc levels in serum, and increased Bursa of fabricius, thymus, and spleen weight) following lipopolysaccharide (**LPS**) administration. These observations suggest that vitamin A plays a critical role in the development of immune system function post hatch (Koutsos et al., 2006). One of the 600 naturally occurring carotenoids known to date is lutein which acts as an antioxidant in tissues. Dietary supplementation of lutein (at 50 mg/kg) has been shown to affect oxidative and inflammatory parameters (Shanmugasundaram and Selvaraj, 2011). Compared with some other vitamins, many biological properties of vitamin A, including the mechanisms by which vitamin A affects the immune system, are well defined and are mediated through interactions of RA with nuclear-hormone receptors in immune system cells (Mora et al., 2008). The general effects of vitamin A on the immune system has been highlighted in Figure 1.

Vitamin A and Immunomodulation

Antiinflammatory Effects Regulation and control of inflammatory processes are important in blunting the consequences of immune responses to infection and maintaining production in chickens. Vitamin A and its active metabolite, RA, have been shown to produce antiinflammatory effects in different species exerted through different mechanisms. Retinoic acid aids the differentiation of bone marrow human macrophages from M1 to M2, transforming the proinflammatory effects of the former to antiinflammatory functions of the latter (Vellozo et al., 2017). In another study, RA-treated mice showed reduced expression of the immunostimulatory cytokine, IFN-γ, by mice natural killer cells (Chang and Hou, 2015). Dendritic cells (**DC**) are also affected by vitamin A. Generally, RA exerts opposite effects on DC, as it has been shown to induce mouse intestinal DC to exhibit antiinflammatory effects ($CD11b^+CD8^-$DC instead of $CD11b^-CD8^+$DC) (Klebanoff et al., 2013). Yet, in the presence of IL-15, RA induced mice DC to produce proinflammatory cytokines such as IL-12 and IL-23, an indication of RA's differential effects on the immune system during inflammation or infection (Coombes et al., 2007; Sun et al., 2007; Scott et al., 2011). The opposing effects of vitamin A and its derivatives in chickens or other species seems to be largely affected by the dose or rate of inclusion in feed, as stimulatory or modulatory effects are incurred when used in relatively lower or higher levels, respectively. In broiler breeders, vitamin A has been shown to enhance anti-Newcastle Disease Virus (**NDV**) vaccine antibody titers when administered at a level of up to 20,000 IU/Kg, however, increasing this dose to 35,000 IU/Kg led to decreases in antibody titer (Yuan et al., 2014). These findings suggest that a comprehensive experiment with different doses is needed to determine optimum dietary vitamin doses. In such an experiment, it would be necessary to study the effects of age, breed, and the immune status of the birds.

Effects of Cell-Mediated and Antibody-Mediated Responses Vitamin A deficiency impacts the function of cells of the innate and adaptive immune systems. For example, vitamin A deficiency in rats has been shown to be associated with decreased phagocytic and intracellular bactericidal activities of peritoneal macrophages and polymorphonuclear leukocytes (Ongsakul et al., 1985; Wiedermann et al., 1996). Along similar lines, inducing vitamin A deficiency in chickens was shown to impair the function of peritoneal macrophages in both healthy and NDV-infected birds (Sijtsma et al., 1991). Furthermore, vitamin A has been shown to modulate T cell number and function in chickens (Garbe et al., 1992). Broiler chickens fed low levels of vitamin A have been shown to exhibit lower $CD4^+$:$CD8^+$ ratios

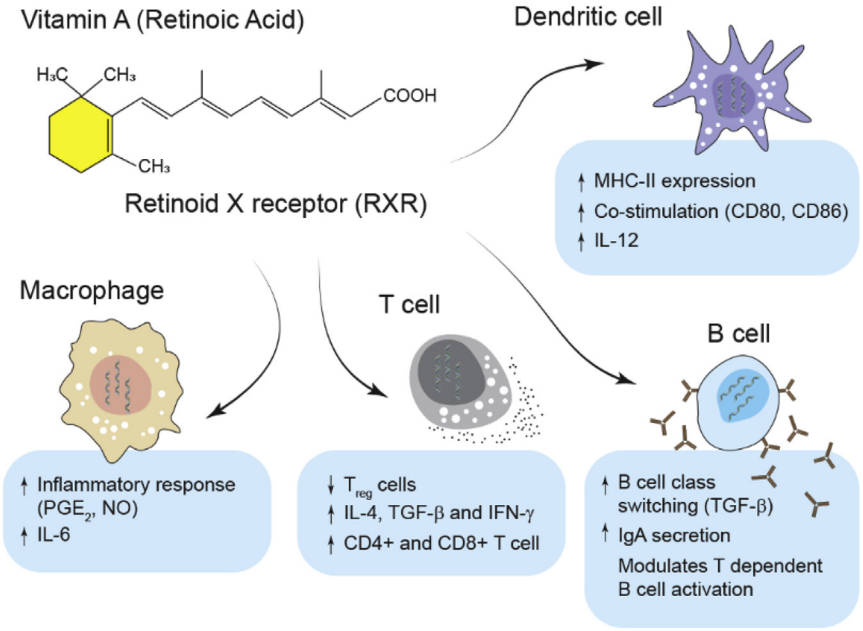

Figure 1. Effect of vitamin A on the immune system: Vitamin A (Vit A; retinoic acid) is a fat-soluble vitamin. Passive diffusion is the main mechanism by which immune system cells acquire Vit A. Post entry, Vit A binds to its nuclear receptor, Retinoid X receptor (RXR), which modulates specific immune system cells subset. Vit A activates (upregulation of MHC-II, CD-80, CD86, IL-12) innate immune cells (macrophages and dendritic cells). In mucosal sites such as lungs and gut, treatment leads to an increase in mucin and secretory IgA antibody production. The activity of this vitamin is highly dose dependent, as low and high doses induce inflammatory and antiinflammatory responses respectively. Abbreviation: IL, interleukin.

compared with birds fed with optimal or excess vitamin A in the diet (Lessard et al., 1997; Dalloul et al., 2002). These authors also showed a reduction in lymphocyte responses to mitogens in birds fed diets with 15,000 IU/kg of vitamin A compared with 400 IU/kg, suggesting that vitamin A supplementation can affect T cell populations, their cytokine production profiles, and the type of immune response that is mounted by T cells. Furthermore, a significant reduction in multiple intraepithelial lymphocyte subsets, particularly $CD8^+$ T cells, has also been observed in the intestine of broiler chickens fed diets deficient in vitamin A (Dalloul et al., 2002).

A study by Lessard et al. (1997) showed that in chickens received a diet deficient in vitamin A, lymphocyte responses to concanavalin A (**ConA**), phytohemagglutinin (**PHA**), and pokeweed favored development of T helper cell (**Th**)2 immune responses while chickens received a higher level of vitamin A and showed higher Th1 cytokine responses. In contrast, studies in mice and rats have shown that diets deficient in vitamin A led to suppression of Th2 responses, thus skewing toward Th1 responses (Carman and Hayes, 1991; Wiedermann et al., 1993). However, it is important to note that immune response phenotypes in mice are affected by the genetic line used. In the above study, C57BL/6 mice were used, which have an inclination toward eliciting Th1 responses while BALB/c mice tend to exhibit Th2 responses to test antigens. Interestingly, antigen-specific T lymphocyte proliferative responses were significantly reduced in chickens when fed a diet deficient in vitamin A, although these responses were restored to levels exhibited in control birds when their feed was supplemented with vitamin A in the form of pellets (Friedman and Sklan, 1989). This indicates the beneficial effects of retinol on antigen-specific T cell responses to ovalbumin and bovine serum albumin (**BSA**). In support of this observation, a previous study showed that mitogenic responses in splenocytes to ConA are augmented when chickens are fed sufficient or excess levels of dietary vitamin A compared with control birds that received a vitamin A–deficient diet (Lessard et al., 1997). Subsequently, Dalloul et al. (2002) reported that ConA stimulation of splenocytes resulted in significantly reduced lymphocyte proliferation in vitamin A–deficient broiler chickens. In addition to these findings, a few studies have highlighted the role of vitamin A in antiviral T cell responses. For example, chickens fed a vitamin A–deficient diet and also infected with NDV demonstrated decreased cytotoxic activity of $CD8^+$ T cells, resulting in impaired recovery from viral infection (Sijtsma et al., 1990). Vitamin A deficiency has also been shown to impair serum IFN-γ quantity, suggesting potential implications in the ability of chickens to resist infections (Dalloul et al., 2002). Taken together, these findings suggest that vitamin A supplementation in chicken diets not only augments T cell proliferation but also modifies their effector functions against infectious agents.

Vitamin A has also been reported to play a critical role in immunoglobulin synthesis. Studies have suggested that dietary deficiency of vitamin A is associated with impaired IgA and IgG synthesis, but not IgM. In chickens, there is evidence that dietary deficiency of

vitamin A can lead to significantly diminished antibody responses to BSA and NDV vaccines (Davis and Sell, 1989; Sklan et al., 1994). Similarly, vitamin A–deficient rats demonstrated significantly lower antibody responses to ovalbumin (Friedman and Sklan, 1989). Along similar lines, chickens fed diets deficient in vitamin A and also challenged with *Escherichia coli* produced lower levels of serum anti-*E. coli* IgG antibodies compared with chickens fed sufficient dietary vitamin A (Friedman et al., 1991). Although these findings imply an important role for vitamin A in avian antibody–mediated immune responses to infectious diseases, some findings have since raised ambiguity about this relationship. For example, Lessard et al. (1997) reported elevated antibody responses to NDV vaccine antigens in birds fed diets deficient in vitamin A compared with birds that received diets with sufficient or excess vitamin A. Similarly, introduction of vitamin A dietary deficiency resulted in higher magnitude antibody responses following coccidial infection in chickens (Dalloul et al., 2002).

Vitamin A is important in mucosal immune regulation and epithelial cell differentiation. The role of vitamin A in mucosal immunity was established with the discovery of genes that encode retinaldehyde dehydrogenase isoforms in DC within mammalian mesenteric lymph nodes and Peyer's patches (Iwata et al., 2004). Retinaldehyde dehydrogenase belongs to the aldehyde dehydrogenase family of proteins and catalyzes the synthesis of RA from retinaldehyde. In chickens, vitamin A deficiency has been associated with morphometric changes and severe defects in immune system function and mucosal integrity leading to increased susceptibility to infections (Uni et al., 1998). For example, vitamin A deficiency may be associated with reduced numbers of goblet cells and decreased expression of sucrose-isomaltase and aminopeptidase in intestinal villi cells of broiler chickens, ultimately affecting differentiation and maturation of cells in the gastrointestinal tract (Uni et al., 2000). Goblet cells are specialized mucus-producing enterocytes (Smith et al., 2014), and sucrose-isomaltase and aminopeptidase activity are known markers of brush border enzymatic activity associated with intestinal cell differentiation and maturation (Weiser, 1973; Traber et al., 1991). In early posthatch stages of life in chickens, immune system development is associated with an elevated production of reactive oxygen species (**ROS**), and carotenoids have been suggested to play an important immunomodulatory role by trapping and quenching free radicals (Bendich, 1989; Møller et al., 2000; Lucas et al., 2014).

One of the contributing factors to maintenance of mucosal immunity is IgA. In mice, deficiency in vitamin A has been shown to have impaired IgA responses in the lungs and saliva, in addition to reducing levels of influenza A-specific IgA secreting plasma cells in the salivary glands (Stephensen et al., 1993, 1996; Gangopadhyay et al., 1996). In chickens fed vitamin A–deficient diets and infected with NDV, significantly lower bile IgA levels were observed compared with those fed sufficient amounts of dietary vitamin A (Sijtsma et al., 1991; Rombout et al., 1992). This observation highlights the importance of vitamin A in chicken diets for production of optimal IgA levels to facilitate antimicrobial mucosal defense.

Role in Antimicrobial Immunity

The role of vitamin A in the protection of chickens against pathogenic infections was established more than 40 yr ago (Bang et al., 1973; Tengerdy and Nockels, 1975). The small intestine is greatly impacted during vitamin A deficiency as it is a tissue that undergoes rapid cell proliferation and differentiation (Uni et al., 2000). Chickens fed diets deficient in vitamin A and challenged with either *Eimeria acervulina* or *Eimeria tenella* showed higher mortality rates compared with chickens fed diets sufficient in vitamin A (Erasmus et al., 1960). Dalloul et al. (2002) also showed substantially fewer *E. acervulina* oocysts in birds fed 8000 IU of excess vitamin A in the diet compared with birds without vitamin A supplementation. In broiler chickens fed carotenoid-enriched (β-carotene, lycopene, zeaxanthin, and lutein) transgenic corn and challenged with *E. tenella*, normal growth and lower fecal oocyst counts were observed compared with birds fed a normal corn variety (Nogareda et al., 2015). The proposed mechanism for vitamin A deficiency–mediated increased oocyst counts included reduced function of $CD4^+$ intraepithelial lymphocytes and $CD8^+$ T cells in addition to impaired maintenance of epithelial tissues (Dalloul et al., 2002).

In chickens subcutaneously challenged with *E. coli*, increased susceptibility was evident as higher mortality and morbidity were seen in birds fed diets deficient in vitamin A (Friedman et al., 1991). Similarly, in turkeys, dietary deficiency of vitamin A was associated with higher mortality and altered mucosal integrity after inoculation with *Pasteurella multocida* (Aye et al., 2000). Studies in chickens on vitamin A deficiency and susceptibility to NDV have previously been conducted as a model for the relationship between measles and vitamin A in humans. In 1 study, vitamin A deficiency resulted in higher morbidity in chickens infected with NDV; additionally, NDV infection was shown to reduce marginal plasma vitamin A to levels regarded as deficient (Sijtsma et al., 1989). Other kinds of viral infections have also been shown to influence vitamin A levels in chickens, including infectious bronchitis virus (**IBV**) and reovirus, which primarily affect the respiratory and intestinal tract, respectively. Infection with IBV and reovirus in chickens while receiving normal or marginal intakes of vitamin A resulted in lowered plasma retinol levels. This effect was more pronounced in birds fed a diet that was marginally deficient in vitamin A (West et al., 1992). Collectively, the above studies demonstrate that supplementation of poultry diets with sufficient or possibly increased quantities of vitamin A could contribute to improved health status of chickens. Immunomodulatory and antimicrobial effects of vitamin A in chickens are summarized in Table 1.

Table 1. Effect of vitamin A on chicken immune system and microbial pathogens.

Effect on the immune system and microbial pathogens	Dose	Host	Reference
Increase anti-NDV antibody	20,000 IU/kg	Broiler breeder	Yuan et al., (2014)
Decrease anti-NDV antibody	35,000 IU/kg	Broiler breeder	Yuan et al., (2014)
Lower $CD4^+$:$CD8^+$ ratios, reduction of intraepithelial lymphocytes, reduced lymphocyte proliferation, impair serum, IFN-γ quantity	Deficiency: Not supplemented with vitamin A	Broiler chickens	Dallul et al., (2002)
Lower CD4:CD8 ratios, increase anti-NDV antibody, developed Th-2 immune response	Deficiency: 400 IU/kg	Broiler chickens	Lessrad et al., (1997)
Developed Th-1 immune response	15,000 IU/kg	Broiler chickens	Lessrad et al., (1997)
Lower levels of serum anti-*E. coli* IgG antibodies, decreased T cell proliferation	Excess vitamin A (1,000 mg/kg) or depleted	Broiler chickens	Friedman et al., (1991)
Higher mortality due to *E. coli* infection	Vitamin A		
Higher mortality due to coccidiosis infection	Deficiency: 800 IU/kg	Broiler chickens	Erasmus et al., (1960)
Increased mortality and damage to mucus membrane, such as metaplasia when challenged with *Pasturella multocida.*	Deficient diet	Turkey	Aye et al., (2000)
Increased mortality when challenged with NDV (*Lasota* strain)	Deficiency: 120 RE/kg	Broiler chickens	Sijtsma et al., (1989)

Abbreviations: 1IU, 0.3 mcg retinol; IFN-γ, interferon-γ; NDV, Newcastle Disease Virus; RE, retinoic equivalent; Th2, T helper 2.

VITAMIN D

Vitamin D is a fat-soluble vitamin which is obtained by either producing it in the skin upon exposure to sunlight or by supplementation in the feed. Dietary vitamin D is absorbed by the small intestine, bound by vitamin D binding protein or albumin in the blood, and then rapidly taken up by the liver. Here, it is hydrolyzed by enzymes such as 25-hydroxlyase to form 25-hydroxy-vitamin D_3 (**25(OH)D_3**), which is the circulating or stored form of vitamin D in the body (Holick and Clark, 1978). Importantly, 1, 25-dihydroxyvitamin D_3 (**1,25(OH)$_2$$D_3$**), the biologically active form of vitamin D that is responsible for most, if not all of the biological actions of vitamin D, is produced in the kidneys by hydroxylation of 25(OH)D_3 with the aid of 1α-hydroxylase (Prosser and Jones, 2004; Christakos et al., 2016). It is interesting to note that 1α-hydroxylase is also produced by macrophages which are upregulated during inflammatory reactions, thus assisting in increased production of 1,25(OH)$_2$$D_3$ (Vanherwegen et al., 2017). In poultry, because vitamin D binding protein cannot bind to vitamin D_2 effectively, it is inadequate for biological function in chickens (Belsey et al., 1974; LeVan et al., 1981; Hoy et al., 1988). The active metabolite of vitamin D, (1,25(OH)$_2$$D_3$), is a pleiotropic molecule that acts in multiple biological processes including the regulation of bone and mineral metabolism as well as modulation of immune responses (Schwarz et al., 2012). The major and conventional function of 1,25(OH)$_2$$D_3$, also referred to as calcitriol, is assisting the formation of bone and egg shell through the regulation of calcium and phosphorus homeostasis in the body by influencing intestinal and renal absorption (DeLuca and Schnoes, 1983; Underwood and DeLuca, 1984; DeLuca, 1988; de Matos, 2007; Plum and DeLuca, 2009).

The cellular intake and biological activities of 1,25(OH)$_2$$D_3$ are mediated by the vitamin D receptor (**VDR**), which is expressed by many cells including those of the immune system (Veldman et al., 2000). It is understood that immune system activation elevates cellular VDR expression, suggesting an important role for vitamin D in the activation and regulation of host immune responses (Christakos and Norman, 1979; Yang et al., 1993; Veldman et al., 2000; Evans et al., 2004). In the context of chickens, we have previously shown that LPS-stimulated macrophages upregulate expression of the VDR (Shojadoost et al., 2015). Furthermore, high concentrations of 1,25(OH)$_2$$D_3$ can be found in lymphoid microenvironments (Overbergh et al., 2000; Veldman et al., 2000; Chen et al., 2007), further suggesting an immunological role for vitamin D.

It is also of note that absorption of vitamin D_3 seems to be dependant on the levels of vitamins A and E in the diet. For example, a previous study showed that supplementation of the chicken diet with higher levels of vitamins A and E can compete with the absorption and bioavailability of vitamin D_3 and thus can result in decreased body weight, bone ash, and higher incidences of hypocalcemia and rickets (Aburto and Britton, 1998a,b; Aburto et al., 1998). To this end, it is necessary to take into consideration the factors that influence vitamin D uptake from the gut while formulating poultry diets. The general effects of vitamin D on the immune system has been highlighted in Figure 2.

Vitamin D and Immunomodulation

Antiinflammatory Effects Intensive livestock production puts poultry under great performance stress that can result in compromised immune function leading to increased susceptibility to infectious agents and inflammation. Inflammatory responses are often mediated by the production of IL-1β and IL-6, and regulating these cytokines has been suggested to provide beneficial effects on the growth and production of birds (Johnson, 1997). We have previously shown that treatment of LPS-stimulated chicken macrophages with 1,25(OH)$_2$$D_3$ can

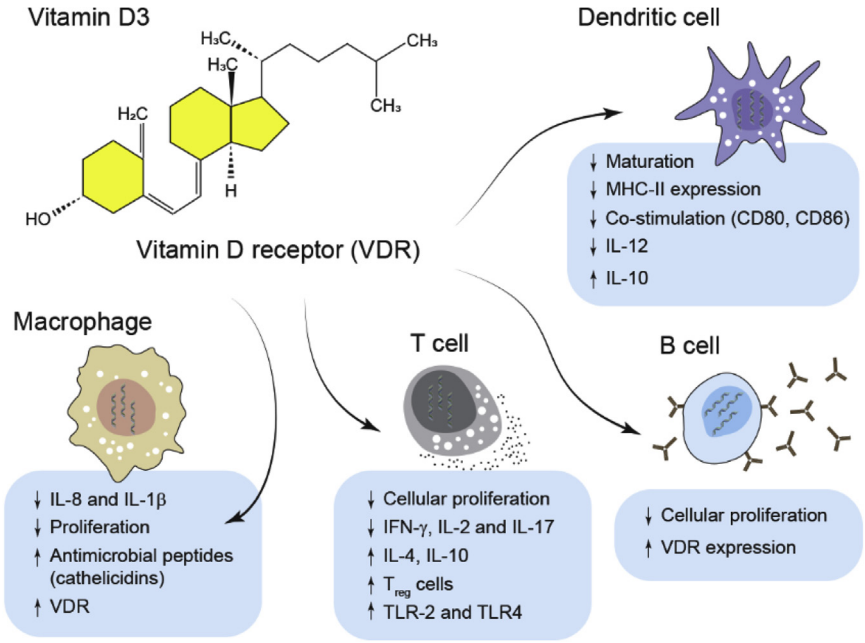

Figure 2. Effects of vitamin D on the immune system: Vitamin D (Vit D) is a fat-soluble vitamin and the main functional isoform is Vit D3. Passive diffusion is the main mechanism by which immune system cells acquire Vit D3. Post entry, Vit D3 binds to its nuclear receptor, Vitamin D Receptor (VDR) where it has a broad modulatory activity on immune system cells. Treatment with Vit D3 increases the host cell ability to express TLR-2 and TLR-4 leading to an increase in antimicrobial peptide expression (cathelicidins). Furthermore, it exerts antiinflammatory effects by downregulating macrophage and dendritic cell activity (decrease IL-8 and IL-1β expression) as well as T cell activation (increased IL-10 and decrease in IFN-γ, IL-2 and IL-17 expression) and an increase in Treg cells (regulatory activity). Abbreviations: IL, interleukin; TLR, Toll-like receptor.

result in downregulation of IL-1β expression (Shojadoost et al., 2015). It has also been shown that LPS treatment of bovine mononuclear cells resulted in elevated expression of 1-α hydroxylase, an enzyme that mediates the production of $1,25(OH)_2D_3$ from $25(OH)D_3$ and thus assists in the regulation of cellular inflammatory responses (Nelson et al., 2011). Along similar lines, levels of IL-1β transcripts were found to be reduced in the livers of LPS challenged broiler chickens that received $25(OH)D_3$ compared with those that did not receive $25(OH)D_3$ (Morris et al., 2014).

Zhang et al. (2012) reported a reduction in proinflammatory cytokine production (IL-6 and TNF-α) in LPS-stimulated human mononuclear cells treated with physiological doses of $1,25(OH)_2D_3$ and $25(OH)D_3$. This phenomenon was attributed to upregulation of MKP-1, which is a regulator of proinflammatory cytokine production. Other mechanisms have also been suggested for the antiinflammatory effects of vitamin D, such as an upregulation of IkBα, which in turn inhibits NF-kB P65, a cascade that has been shown in cells treated with $1,25(OH)_2D_3$ (Chen et al., 2013). In addition to inhibition of inflammation, other evidence has shown that $1,25(OH)_2D_3$ exerts important immunomodulatory effects. For example, Penna et al. (2005) showed that in vitro treatment of human DC with $1,25(OH)_2D_3$ resulted in enhanced induction of T regulatory (**T reg**) cells (Penna et al., 2005). Taken together, the available evidence indicates that vitamin D can benefit the host by exerting immunomodulatory effects, highlighted by antiinflammatory capabilities.

Effects on Cell-Mediated and Antibody-Mediated Responses The critical role vitamin D plays in regulating T and B cells is evident as VDR expression is increased significantly in activated T and B cells. Compared with their resting condition, VDR stimulation results in activation of more than 500 genes, affecting the proliferation, differentiation, and function of these cells (Prietl et al., 2013). In general, activation of the VDR and production of $1,25(OH)_2D_3$ by immune system cells, which is induced by some Toll-like receptor (**TLR**) ligands (such as LPS), results in upregulation of human Th2 and T reg cells and downregulation of Th1 cells (Gil et al., 2018). Studies investigating the immunomodulatory effects of $1,25(OH)_2D_3$ show that administration can result in a reduction of T cell proliferation and T cell–mediated IFN-γ production in human and chicken derived T cells (Rigby et al., 1987; Boodhoo et al., 2016). Furthermore, Boodhoo et al. (2016) showed that the regulatory effects of $1,25(OH)_2D_3$ were mediated by the inhibition of IL-2 production but not because of cell death or apoptosis. It was also shown that while IFN-γ production of $1,25(OH)_2D_3$–treated $CD8^+$ T cells was diminished, the cytotoxic ability of these cells remained unaffected (Boodhoo et al., 2016). These observations suggest that $1,25(OH)_2D_3$ can regulate cellular responses via cytokine production; however, the effects do not seem to come at the cost of disrupting the cytotoxic function of these cells. In support of this, others also have shown that in cases of chronic disease (such as immunopathological disorders), vitamin D regulates the proliferation of T cells. Additionally, VDR

expression is mostly observed in activated T cells but not in resting cell populations (Cantorna and Waddell, 2014). To this end, a previous study showed that mouse $CD4^+$ T cells treated with $1,25(OH)_2D_3$ produced significantly less IFN-γ and IL-17 compared with cells from mice that lacked VDR expression (Cantorna et al., 2015). There are also reports in laboratory animal models that highlight the ability of vitamin D to modulate adaptive immune responses through Th cell development via cytokine responses; IL-4, IL-5, IL-13, and IL-10 represent cytokines upregulated by vitamin D, whereas IL-17 and IL-12 have been found to be downregulated (Prietl et al., 2013; Gil et al., 2018).

In addition to mice and other animal models, vitamin D in chickens also has demonstrated enhanced cell- and ab-immune responses. In the context of antibody responses, Vazquez et al. (2018) evaluated the effect of vitamin D_3 and/or $25(OH)D_3$ supplements on immune responses of Ross 308 broiler chickens. Serum antibody levels induced by an NDV vaccine as well as total and nonspecific intestinal IgA levels were enhanced in chickens that received higher amounts of vitamin D_3 (5,000IU/kg) combined with 69 mg/kg of $25(OH)D_3$ (Vazquez et al., 2018). It was also shown in another study that supplementation of broiler chicken diets with 2,000 IU/kg of vitamin D_3, which is higher than conventional dietary levels (NRC level=200IU/kg), resulted in higher antibody titers to NDV vaccine antigens (National Research Council, 1994; Gómez-Verduzco et al., 2013). Additionally, levels of peripheral blood $CD3^+CD8^+$ T cells were significantly increased in chickens supplemented with vitamin D_3 and $25(OH)D_3$ (Vazquez et al., 2018). A previous report revealed that chickens fed a $25(OH)D_3$ supplemented diet (69 μg/kg) exhibited enhanced delayed-type hypersensitivity reactions to PHA (Gómez-Verduzco et al., 2013). In support of this finding, it has also been shown that delayed-type hypersensitivity responses to the mitogen, PHA, were significantly reduced in vitamin D_3–deficient chickens (Aslam et al., 1998).

Role in Antimicrobial Immunity

In the context of microbial infections, vitamin D plays an essential role in augmenting pathogen-specific immune responses, specifically, $1,25(OH)_2D_3$ has been shown to increase phagocytosis, differentiation, and antimicrobial peptide production in macrophages (Deluca et al., 2008). Further, an important role of vitamin D in antimycobacterial responses in cases of human tuberculosis has been emphasized (Martineau et al., 2011). Expression of the VDR is upregulated in macrophages infected with *Mycobacterium tuberculosis*, and treatment of infected macrophages with vitamin D results in augmented cellular activation and intracellular killing via production of cathelicidin, an antimicrobial peptide (Sato et al., 2013). A study also showed that in broilers challenged with *Salmonella enterica* serovar Typhimurium at 7 and 14 d of age while also fed a diet supplemented with $25(OH)D_3$, significantly higher levels of *Salmonella*-specific IgG were observed at 21 d of age (Chou et al., 2009). Additionally, receiving higher levels of $25(OH)D_3$ in feed (100 μg/kg) resulted in upregulation of IL-10 gene expression in chickens challenged with coccidial oocyts (Morris et al., 2015). This finding emphasizes the role of vitamin D in regulation of immune responses. In another study, cathelicidin gene expression was upregulated in the spleens of chickens that received calcium- and phosphorus-deficient diets but were supplemented with vitamin D (Rodriguez-Lecompte et al., 2016). The same researchers also reported upregulation in expression of TLR2 and TLR4 in addition to Th2 genes. Moreover, there is evidence that a vitamin D deficiency reduces cellular responses to antigens, for example, in chickens challenged with sheep red blood cells (**SRBC**), thymic weight, and quantities of abdominal macrophages that phagocytize SRBC were reduced in chickens fed a vitamin D–deficient diet (Aslam et al., 1998).

Collectively, it is reasonable to infer that dietary supplementation of vitamin D in chickens can offer beneficial effects, including reductions of inflammatory responses as well as enhancement of cell-mediated and antibody-mediated immune responses, contributing significantly to enhancing antimicrobial defenses. Immunomodulatory and antimicrobial effects of vitamin D in chickens are summarized in Table 2.

VITAMIN E

Vitamin E is a fat-soluble antioxidant, and 4 different tocopherols, α, β, γ, and δ, exist as the functional forms of this vitamin. α-tocopherol is the most commonly found form in nature and is considered the most biologically active (Weiser et al., 1996). The effect of vitamin E as an antioxidant has been well established against the detrimental effects of free radicals in maintaining cell integrity during normal cell metabolism and inflammations (Khan et al., 2012).

In poultry production, vitamin E supplementation is essential for maintaining fertility and hatchability in parent stocks. It also has a crucial role for prevention of nutritional encephalopathy and myopathies in chickens and turkeys (Klasing and Korver, 2020). Supplementing poultry feed with vitamin E (or other antioxidants) is essential, especially when oxidizable fats are included in the feed, because upon oxidation, these fats release metabolically harmful free radicals that affect poultry health and production (Engberg et al., 1996; Lu et al., 2014b). Vitamin E supplementation of chicken feed also prevents the oxidation of lipids in unsaturated fatty acids (Rama Rao et al., 2011); because of this, the amount of active vitamin E that reaches the intestine for absorption can be reduced in poultry diets high in unsaturated fat (Villaverde et al., 2008). Under these circumstances, the antioxidant status in poultry can be decreased as a result of an increase in lipid peroxidation (Rama Rao et al., 2011). In a recent study, supplementation of layer hen feed with 30 IU/Kg of vitamin E resulted in a significant increase of serum superoxide

Table 2. Effect of vitamin D on chicken immune system.

Effect on the immune system and microbial pathogens	Dose	Host	Reference
Increased anti-NDV antibodies and total and nonspecific intestinal IgA levels, increased peripheral blood $CD3^+CD8^+$ T cells	5,000 IU/kg combined with 69 µg/kg of 25(OH)D3	Broiler chickens	Vazquez et al., (2018)
Higher antibody titers to NDV vaccine antigens	2,000 IU/kg	Broiler chickens	Gómez-Verduzco et al., (2013)
Enhanced DTH reactions to phytohemagglutinin	69 mg/kg of 25(OH)D3	Broiler chickens	Gómez-Verduzco et al., (2013)
Reduced cellular responses to antigens in chickens challenged with SRBC. Reduced thymic weight and quantities of abdominal macrophages	Not supplemented with vitamin D_3	Broiler chickens	Aslam et al., (1998)
Higher levels of Salmonella-specific IgG	69 µg/kg of 25(OH)D3	Broiler chickens	Chou et al., (2009)
Upregulation of IL-10 gene expression	100 µg/kg of 25(OH)D3	Broiler chickens	Morris et al., (2015)
Upregulation in expression of TLR2 and TLR4 in addition to Th2 genes.	69 and 275 µg/kg of 25(OH)D3	Broiler chickens	Rodriguez-Lecompte et al., (2016)

Abbreviations: DTH, delayed-type hypersensitivity; IL-10, interleukin-10; SRBC, sheep red blood cells; TLR, Toll-like receptor; Th2, T helper 2.

dismutase and glutathione peroxidase, both of which are enzymes with antioxidant activities (Liu et al., 2019). The general effects of vitamin E on the immune system have been highlighted in Figure 3.

Vitamin E and Immunomodulation

Antiinflammatory Effects In addition to its antioxidant activity, vitamin E is well-recognized for its ability to regulate inflammatory responses. For its antiinflammatory activities, α-tocopherol inhibits activity of cyclooxygenase 2, which in turn reduces conversion of arachidonic acid to prostaglandins such as prostaglandin E2, as an inflammatory mediator (Lewis et al., 2019). It is well established that chronic inflammation is associated with disease conditions such as atherosclerosis in mice (Libby, 2002) and cancer (Balkwill and Mantovani, 2001) and neurodegenerative diseases such as Alzheimer's in humans (McGeer and McGeer, 2001). This indicates that vitamin E can potentially help in the prevention or control of these conditions in addition to other long-term inflammatory conditions (Jiang, 2006). Interestingly, the combination of both α and γ-tocopherols has shown to be more effective in controlling inflammation compared with either antioxidant administered alone (Reiter et al., 2007).

Vitamin E–mediated regulation of inflammation has been demonstrated in chickens. Specifically, it has been shown that when broiler chickens receive 220 IU/kg of

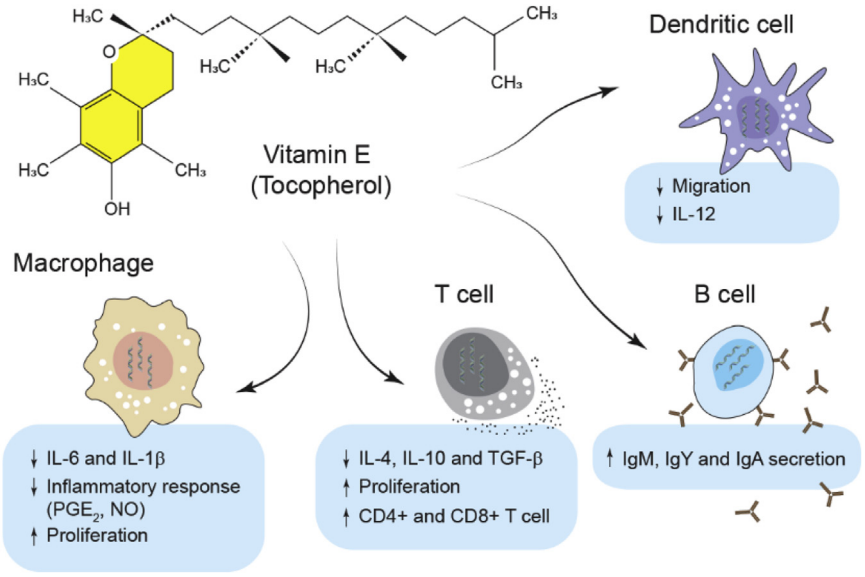

Figure 3. Effect of vitamin E on the immune system. Vitamin E (Vit E; Tocopherol) is a fat-soluble vitamin that leads to an increase of serum superoxide dismutase and glutathione peroxidase enzymes which has functional roles as antioxidant. Treatment with vitamin E leads to proliferation of T cells (both CD4+ and CD8+), leading to activation of immune system and decreasing cytokines such as IL-4, IL-10, and TGF-β. In addition, treatment with Vit E leads to B cell activation and subsequent increases in IgM, IgY, and IgA antibody secretion to help induce immunity against infections, as observed in an increase in anti-NDV and anti-IBV antibodies. In general, Vit E confers a dose-dependent antiinflammatory response based on reduction in IL-1β, IL-4, IL-6, IL-10, TGF-β, and PGE_2 production. However, Vit E treatment leads to a general increase in lymphocyte populations in the thymus, plasma, spleen, and gut tissue (jejunum, cecal tonsils, and ileum). Abbreviation: IL, interleukin.

vitamin E in feed, levels of IL-6 mRNA in the spleens of LPS-inoculated chickens were significantly decreased (Kaiser et al., 2012). This study suggested that vitamin E controls inflammatory responses when proinflammatory cytokine production is elevated, normally occurred during infectious conditions and particularly after infection with Gram negative bacteria that use LPS as one of their virulence factors. Along the same lines, a recent study showed that supplementation of broiler chicken feed with vitamin E resulted in a dose-dependent decrease of both inflammatory (IFN-γ, IL-1β, and IL-6) and antiinflammatory cytokines (IL-4, IL-10, and TGF-β) in the jejunum (Pitargue et al., 2019). The results were attributed to vitamin E's essential role in balancing cytokine responses, which could be critical in cases of inflammation. Furthermore, a previous study showed that feeding broiler chickens a blend of antioxidants (ethoxyquin and propyl gallate) along with 200 IU/kg of vitamin E reduced histological inflammatory scores that were induced by oxidized soybean oil (Lu et al., 2014a). It is noteworthy that soybean and other vegetable oils are routinely added to chicken feed to increase its energy content. Vitamin E feed supplementation can also be beneficial for chickens raised in stressful environmental conditions. For example, a recent study showed that when broiler chickens were kept under heat stress conditions, those fed vitamin E supplemented feed (100 mg/kg) demonstrated significantly reduced liver expression levels of IL-6 and heat shock protein 70 compared with controls (Jang et al., 2014). Taken together, it is clear that vitamin E can benefit the health of chickens via antiinflammatory effects.

Effects on Cell-Mediated and Antibody-Mediated Responses Several studies in humans have demonstrated that vitamin E has certain beneficial effects in boosting immunity against infectious diseases and cancer (Moriguchi and Muraga, 2000). Mechanistically, vitamin E has been shown to augment IFN-γ production and induce proliferation of cells of the immune system in addition to modulating chemotaxis and bactericidal properties of polymorphonuclear cells (Boxer, 1986). Vitamin E, therefore, appears to boost both cell-mediated and antibody-mediated responses to antigens.

Studies conducted in chickens have shown that dietary supplementation of vitamin E can augment lymphocyte- and monocyte-mediated responses both quantitatively and qualitatively. For example, following infectious bursal disease virus (**IBDV**) vaccination in chickens fed 80 IU/kg or 40 IU/kg of vitamin E–supplemented feed, birds that received the higher dose of vitamin E had significantly more peripheral blood $CD4^+$ and $CD8^+$ T cells (Abdukalykova et al., 2008). Similarly, Khan et al. (2008) also found increased lymphocyte populations in the thymus and increased plasma cell numbers in the spleen, cecal tonsils, and ileum in broiler chickens fed higher levels of vitamin E (Khan et al., 2008). In support of these findings, Abdukalykova and Ruiz-Feria (2006) found that inclusion of arginine in a vitamin E–supplemented diet in broiler chickens enhanced responses to PHA as assessed by the cutaneous basophil hypersensitivity test. Although these observations undoubtedly show that vitamin E can effectively augment T and B cell responses in chickens, the molecular mechanisms are yet to be unravelled. Possible mechanisms highlighted in mice suggest that vitamin E aids T cells by increasing production of IL-2, enhancing cell proliferation and through prevention of activation-induced cell death, by diminishing their expression of Fas-ligand (Adolfsson et al., 2001; Li-Weber et al., 2002). In addition to lymphocytes, macrophage responses are also enhanced by feeding chickens a diet supplemented with vitamin E. In this context, Konjufca et al. (2004) found elevated numbers of peritoneal macrophages that displayed an increased ability to opsonize SRBC in 3-week-old chickens fed higher amounts of dietary vitamin E. Because macrophages employ oxidative bursts that release free radicals, vitamin E may affect macrophage cell viability and function by regulating levels of these products to maintain normal cell functions (Khan et al., 2014).

Recent studies have demonstrated the effectiveness of supplementing chicken diets with vitamin E to enhance vaccine-specific antibody responses. One such study in broiler breeder males showed that vitamin E feed supplementation (100IU/kg) resulted in increased antibody titers to an IBV vaccine (Khan et al., 2014). These authors also addressed the effects of vitamin E supplementation on antibody responses to NDV vaccination. Specifically, broiler chickens immunized with an NDV vaccine and fed a diet supplemented with 200IU/kg of vitamin E and 0.2 mg/kg of selenium developed significantly higher vaccine-specific antibodies compared with controls (Singh et al., 2006). Similarly, immunization with SRBC resulted in significantly elevated antibody quantities after both primary and secondary immunizations in birds that received vitamin E in their diet (Niu et al., 2009; Habibian et al., 2014).

The quantity of antibodies transferred from breeder hens to progeny plays an important role in protection against pathogens in the first week of life. The effect of additional (more than in usual practice) vitamin E supplementation in parent stock diets has been studied to determine the effects on passive antibody transfer. To this end, when broiler breeder hens received vitamin E at 150 IU/kg or 450 IU/kg in feed before inoculation with *Brucella abortus* antigens, the progeny from birds that received more vitamin E showed higher antigen-specific antibody titers (Jackson et al., 1978). Haq et al. (1996) showed that when breeder hens were fed 0.03% total vitamin E in their diet for 3 wk before vaccination against NDV, their progeny showed higher antibody levels compared with control birds at 1 and 7 d of age. This evidence suggests that vitamin E supplementation in breeder diets has beneficial implications for chicks in the context of passively transferred antibody-mediated immunity against infectious diseases. These observations suggest that vitamin E enhances antibody responses to vaccine antigens; however, the underlying mechanisms are yet to be

understood. One possible explanation is that the antioxidant property of vitamin E improves cellular functions resulting in plasma cells that are adequately programmed for enhanced antibody production. However, owing to the effects that vitamin E has on T cells, it is difficult to know whether vitamin E directly increases antibody production by altering B cells or indirectly via T cells (Lee and Han, 2018).

Some studies have also examined the *in ovo* effects of vitamin E. Gore and Qureshi (1997) found that inoculation of vitamin E (10 mg) into embryonated chicken eggs increased cell-mediated and antibody-mediated responses in hatched chickens (Gore and Qureshi, 1997). These authors showed that vitamin E receiving hatched chickens had higher phagocytic activity when inoculated with SRBC at 7 d old and that their macrophages produced more nitric oxide when stimulated with LPS. Higher antibody titers to SRBC were also seen in these chickens at 14 and 21 d old. Gore and Qureshi. (1997) also examined the effect of injecting 3 different doses of vitamin E into embryonated turkey eggs 3 d before hatching. When eggs were injected with 20 and 30 IU, hatchability decreased significantly, while injection with 10 IU resulted in slightly higher hatchability. Additionally, following inoculation on day 7 of age, total IgM antibody levels against SRBC in the hatched turkey poults were higher than in controls 7 and 14 d postinoculation. The number of phagocytic macrophages at 7 wk posthatch were also significantly higher in the group injected with 10 IU of vitamin E. Similar results were observed following *in ovo* vitamin E inoculation in broiler chickens and turkey poults; however, IgG responses to SRBC injection were significantly increased in broilers, while unchanged in turkey poults. Phagocytic activity of both broilers and turkey poults were similarly increased when 10 IU of vitamin E was administered during embryonic development.

Role in Antimicrobial Immunity

The role of vitamin E in resistance against infectious disease has been well studied in mammalian species, and similar evidence exists in chickens. For example, Colnago et al. (1984) reported that when broiler chickens were fed a diet containing 100 IU/kg of vitamin E in feed and infected with *E. tenella* (cecal coccidiosis) oocysts, the chickens showed substantial resistance to disease indicated by lower mortality and increased weight gain. However, such resistance to *E. tenella* infection was not observed when γ-tocopherol was used as the source of vitamin E (Allen et al., 1998). This effect is likely associated with the lower biological activity of γ-tocopherol compared with α-tocopherol (Tran and Chan, 1992). In the context of coccidiosis infections, Perez-Carbajal and colleagues (2010) found that feed supplementation with vitamin E along with arginine could enhance the phagocytic activity of chicken heterophils and monocytes. Similar cellular effects were also observed when birds were infected with *S. enterica* serovar Typhimurium. (Liu et al., 2014). The effect of 30 IU/kg of vitamin E feed supplementation was assessed considering antibody levels, proinflammatory cytokines, and mortality of laying hens challenged with *Salmonella enteritidis*. The levels of IgA, IgM, and IgY increased at 2 wk after challenge, whereas IL-1β, IL-6, and mortality decreased in vitamin E–supplemented birds (Liu et al., 2019).

Although limited, there is some evidence suggesting that vitamin E aids in resisting viral infections in chickens. For example, when chickens with subclinical IBD were fed vitamin E (178 IU/kg), higher weight gain and reduced mortality were observed (McIlroy et al., 1993). The immunostimulatory properties of vitamin E seem to play a crucial role in augmenting avian host resistance to infectious diseases. Based on available evidence, it can be suggested that dietary vitamin E in chickens not only modulates inflammatory responses but also enhances adaptive immune responses and thus contributes to antimicrobial immunity. Immunomodulatory and antimicrobial effects of vitamin E in chickens are summarized in Table 3.

VITAMIN C

Vitamin C, known as L-ascorbic acid (**AA**), is a water-soluble vitamin that is synthesized from glucose (Sahin et al., 2003). Unlike fat-soluble vitamins, vitamin C is not stored in the body, and elevated dietary intake of vitamin C results in decreased absorption and rapid excretion by the kidneys (Johnston et al., 2006). It has notable antioxidant properties because of its ability to donate electrons, and it protects the integrity of many cells, including lymphocytes, against damage from free radicals generated in response to infection or toxins (Nimse and Pal, 2015). Poultry, unlike humans, can synthesize vitamin C endogenously thanks to the L-gulonolactone oxidase enzyme, which is present in the renal tissue where it converts l-gulono-g-lactone into ascorbic acid (Hooper et al., 2001). However, under stressful conditions such as beak trimming, vaccination, transportation, thermal stress, or infection, requirements for vitamin C are increased (Gross, 1992; Wu et al., 2000; Hooper et al., 2001; Abidin and Khatoon, 2013). Therefore, supplementation with vitamin C may alleviate adverse effects associated with stressful conditions. In this section, the immunomodulatory effects of vitamin C are discussed in addition to highlighting its ability to increase host resistance to infectious diseases in poultry. The general effects of vitamin C on the immune system has been highlighted in Figure 4.

Vitamin C and Immunomodulation

Antiinflammatory Effects There is some evidence that vitamin C can modulate inflammatory responses in poultry. It has been reported that dietary supplementation of vitamin C can alleviate the expression of inflammatory cytokines in response to oxidative stress (El-Senousey et al., 2017). In this study, dietary supplementation with vitamin C significantly decreased

Table 3. Effect of vitamin E on chicken immune system and microbial pathogens.

Effect on the immune system and microbial pathogens	Dose	Host	Reference
Decrease of IL-6 mRNA	220 IU/kg	Broiler chickens	Kaiser et al., (2012)
Reduced liver expression levels of IL-6 and heat shock protein 70 (HSP70)	100 IU/kg	Broiler chickens	Jang et al., (2014)
More peripheral blood CD4+ and CD8+ T cells	80 IU/kg	Broiler chickens	Abdukalykova et al., (2008)
Increased lymphocyte populations in the thymus and increased plasma cell numbers in the spleen, cecal tonsils, and ileum	447 IU/kg	Broiler chickens	Khan et al., (2008)
Elevated numbers of peritoneal macrophages	164 and 328 IU/kg	Broiler chickens	Konjufca et al., (2004)
Increased antibody titers to an IBV vaccine	100 IU/kg	Broiler breeder	Khan et al., (2014)
Increased anti-NDV antibody	200IU/kg	Broiler chickens	Singh et al., (2006)
Lower mortality and increased weight gain in chickens challenged with *E. tenella*	100 IU/kg	Broiler chickens	Colnago et al., (1984)
IgA, IgM, and IgY increased at 2 wk after challenge with *S. enteritidis*, while IL-1β, IL-6, and mortality decreased	30 IU/kg	Laying hens	Liu et al., (2019)
Higher weight gain and reduced mortality when challenged with IBDV	178 IU/kg	Broiler chickens	McIlroy et al., (1993)

Abbreviations: IL-6, interleukin-6; IBV, infectious bronchitis virus; IL-1β, interleukin-1β; IBDV, infectious bursal disease virus.

mRNA expression levels of IL-1β, IL-6, and IFN-γ in the spleens of broiler chickens subjected to oxidative stress induced by dexamethasone. Additionally, El-Senousey et al. (2018) evaluated the effect of *in ovo* injection of AA on immune system–related gene expression in the spleen of newly hatched broiler chickens. It was found that *in ovo* injection with 3 mg of AA on day 18 of embryonic incubation resulted in decreased expression of IL-1β and IL-6 compared with controls. Therefore, the authors suggested that the immunomodulatory activity of AA following *in ovo* injection could perhaps be because of its ability to scavenge ROS, alleviating inflammatory responses.

Effects on Cell-Mediated and Antibody-Mediated Responses Wu et al. (2000) studied the immunomodulatory effects of dietary vitamin C supplementation on the immune response in broiler chickens infected with IBDV. In this study, supplementation with AA significantly increased IL-2 production in splenocytes and thereby promoted the proliferation and

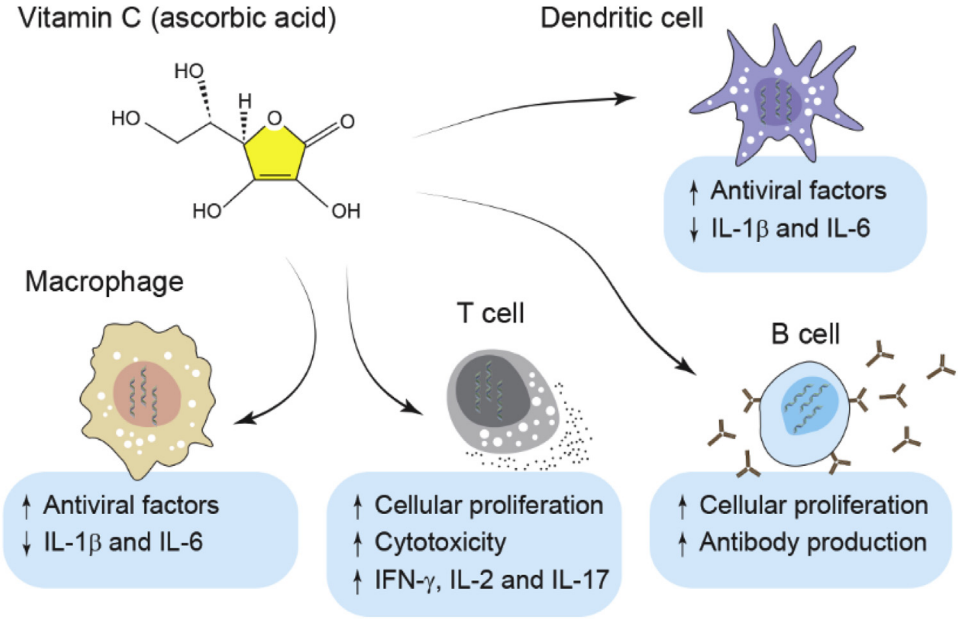

Figure 4. Effect of vitamin C on the immune system. Vitamin C (Vit C; Ascorbic acid) is a water-soluble vitamin which are biosynthesized by chickens. Although Vit C is naturally biosynthesized by chickens, in cases of infection/inflammation, the level of this vitamin decreases in the body, which necessitates supplementation of precursor or bioactive molecules in the feed. Vit C has antioxidant activities and treatment with this vitamin leads to antiinflammatory activities marked by a reduction in IL-1β and IL-6. However, innate immune system cells are more responsive to viral infection by producing and responding to antiviral factors. This heightened antiviral function is supported by an increase in B cell proliferation and activation as observed by an increase in secretory IgM production and anti-IBDV antibodies. Vit C treatment leads to an increase in T cell proliferation and killing ability supported by an increased IFN- γ, IL-2, and IL-17 expression. Abbreviation: IL, interleukin.

Table 4. Effect of vitamin C on chicken immune system and microbial pathogens.

Effect on the immune system and microbial pathogens	Dose	Host	Reference
Decreased mRNA expression levels of IL-1β, IL-6 and IFN-γ in the spleens	200 mg/kg	Broiler chickens	El-Senousey et al., (2017)
Decreased expression of IL-1β and IL-6	3 mg/egg	Embryonated chicken eggs	El-Senousey et al., (2018)
Increase IL-2 and lymphocytes proliferations	1,000 part per million (ppm)	Broiler chickens	Wu et al., (2000)
Increased CD8(+) in spleen and IgM (+) cells in bursa	1,000 ppm	Broiler chickens	Wu et al., (2000)
Increased antibody against IBDV	1,000 ppm	Broiler chickens	Amakye-Anim et al., (2000)
Higher antibody against SRBC and an NDV vaccine	200 mg/kg	Broiler chickens	Panda et al., (2008)
Decreased mortality caused by *Salmonella gallinarum*	1,000 mg/kg	Broiler chickens	Hill and Garren, (1958)
Decreased the incidence of *E. coli* infection	330 mg/kg	Broiler chickens	Gross et al., (1988a)
Decreased mortality caused by co-infection of NDV, *E. coli* and *M. gallisepticum*	100 mg/kg	Broiler chickens	Gross et al., (1992)

Abbreviations: IL-1β, interleukin-1β; IL-6, interleukin-6; IFN-γ, interferon-γ; IL-2, interleukin-2; IBDV, infectious bursal disease virus; NDV, Newcastle Disease Virus; SRBC, sheep red blood cell.

differentiation of T lymphocytes. Furthermore, an in vitro study that evaluated the effects of AA treatment on heterophil function against *Staphylococcus* bacteria was performed with interesting results (Andreasen and Frank, 1999). The findings revealed that while no significant differences were observed when examining heterophil phagocytosis or random migration, treatment with AA increased the bacterial killing ability of heterophils, indicating the possibility of preventing poultry staphylococcal infections via AA supplementation. In addition, Puthpongsiriporn et al. (2001) demonstrated that supplementing hen diets with 1,000 ppm of vitamin C–enhanced in vitro lymphocyte proliferation responses to ConA following heat stress.

Considering the role of vitamin C in the differentiation and proliferation of B lymphocytes and its protective role against free radicals, it is likely that AA supplementation in poultry diets can influence antibody-mediated immune responses (Prinz et al., 1980; Feigen et al., 1982). The beneficial effects of vitamin C on antibody-mediated immune responses in poultry have been reported in a few studies. Lohakare et al. (2005) investigated the effect of various levels of vitamin C supplemented feed (50–200 ppm) on postvaccination antibody titers against IBDV and NDV. The results demonstrated that antibody titers against IBDV significantly increased in birds that received 200 ppm of AA compared with groups that received less, demonstrating the beneficial effects of AA for enhancing antibody-mediated immunity in broilers. In contrast, Bhatti et al. (2016) demonstrated that supplementation of poultry diets with vitamin C alone did not significantly influence antibody-mediated immune responses against NDV and IBDV; however, vitamin C supplementation alone or combined with vitamin E led to nonsignificant increases in antibody titers. In a broiler study, the effect of vitamin C supplementation on antibody-mediated immune responses in response to IBDV vaccination was investigated (Amakye-Anim et al., 2000). When compared with the control group, dietary AA supplementation at 1,000 ppm significantly increased antibody titers against IBDV 14 d after vaccination. The authors concluded that AA may alleviate lysis of bursal lymphocytes caused by the IBDV vaccine, increasing antibody producing cell numbers and antibody production. There is also evidence showing that dietary supplementation with vitamin C can improve antibody-mediated responses in birds under stress conditions. In a study conducted on laying hens, the immunomodulatory effects of vitamin C in white leghorn birds (44–56 wk) subjected to heat stress were investigated (Panda et al., 2008). Birds fed a diet that contained 200 mg/kg of vitamin C showed higher antibody-mediated immune responses against SRBC and an NDV vaccine. In contrast, Mirfendereski and Jahanian (2015) included AA (500 ppm) in poultry diets and examined antibody-mediated immune responses against NDV vaccines in laying hens reared at a high stocking density. Results showed that high stocking densities significantly decreased anti-NDV vaccine antibody responses at both day 7 and 14 postvaccination; however, antibody responses against NDV were not affected by vitamin C supplementation. Gan et al. (2018) investigated the effects of vitamin C dietary supplements on AA synthesis and tissue transportation capacity in laying hens. The results demonstrated that vitamin C supplementation significantly decreased synthase activity of L-gulonolactone oxidase, suggesting the presence of an internal feedback mechanism in poultry that regulates vitamin C biosynthesis. On the other hand, AA supplementation increased splenic AA concentration and serum IgG levels against BSA. It was concluded that supplementation of AA can improve the health of laying hens by enhancing immunity and antioxidant capacity. Inconsistent results regarding the effects of vitamin C on immune responses in poultry could be related to multiple experimental variables, including the dose of vitamin, vaccination regimen, and environmental conditions applied in different studies. In conclusion, dietary supplementation of AA might be beneficial for the immune system of poultry, especially in face of stress or

infectious disease; nevertheless, the underlying mechanisms beyond general antioxidant effects remain to be elucidated.

Role in Antimicrobial Immunity

Infections with pathogenic microorganisms are often associated with activation of phagocytes and increased production of ROS by these cells (Padayatty et al., 2003). Reactive oxygen species are free radicals derived from molecular oxygen and are involved in the deactivation and killing of viruses and bacteria (Yang et al., 2013). However, excess amounts of ROS in response to infection can be harmful for host cells (Kohchi et al., 2009). As a potent antioxidant, ascorbic acid plays a critical role in protecting host cells from free radicals and limits the damaging effects of ROS (Fukumura et al., 2012; Barrita and Sanchez, 2013). There is some evidence that infectious diseases can affect vitamin C levels in poultry, resulting in elevated requirements for antioxidants (Panda et al., 2008; Ahmadu et al., 2016; Zhang et al., 2019). Increased ROS production following an immune response against pathogens may explain the decrease in vitamin C levels observed following infectious diseases (Kawashima et al., 2015; Zhong et al., 2017). Previous studies have reported that infectious diseases in chickens reduce levels of AA in plasma and tissues. For example, Hill and Garren (1958) demonstrated this after infection of chickens with *Salmonella gallinarum*; however, it was also found that dietary supplementation with vitamin C (1,000 mg/kg) prevents depletion of AA and reduces mortality. Kechik and Sykes (1979) studied the effect of intestinal coccidiosis (*E. acervulina*) on blood plasma and tissue AA concentrations in chickens. It was shown that intestinal coccidiosis significantly decreased AA concentrations in the small intestine, liver, adrenal glands, and in blood plasma. Results of different studies have shown that dietary supplementation of vitamin C may contribute to resistance to infectious disease in poultry. As an example, the effects of diets containing various levels of vitamin C (0–880 mg) in 6-week-old Leghorn chickens subjected to air-sac challenge with *E. coli* were investigated (Gross et al., 1988a). Results showed that dietary supplementation with AA (330 mg/kg) significantly decreased the incidence of infection compared with the control group. However, levels above and below 330 mg/kg of AA were found to be less effective. Furthermore, Gross (1988b) studied the effects of AA in chickens subjected to environmental stress and challenged with *E. coli.*, using heterophil to lymphocyte (**H**/**L**) ratios as a biological index of stress status in broiler chickens. It was shown that when stress was low (H/L ratio = 0.33), the incidence of severe lesions in birds fed a diet that contained AA was significantly lower compared with the control group. However, at higher levels of stress (H/L ratio = 0.53 or more), AA supplementation did not alleviate the severity of infection. In a follow-up study, Gross (1992) evaluated the effects of dietary AA in chickens challenged with bacteria and viruses. In birds fed a diet that contained 100 mg/kg of AA, increased resistance to co-infection with NDV, *Mycoplasma gallisepticum,* and *E. coli* was observed. Collectively, supplementing vitamin C in poultry diets may alleviate lymphocyte damage caused by ROS in addition to providing beneficial resistance and immune response to infection. However, further research is required to clarify the role of vitamin C in prevention and resistance to infectious disease in poultry. Immunomodulatory and antimicrobial effects of vitamin C in chickens are summarized in Table 4.

CONCLUSION

As can be seen, vitamins A, D, C, and E have measurable effects on function of the immune system. Their inclusion in poultry feed is not only essential for efficient growth and health but also for maintenance and enhancement of immune system function. These effects include enhancement of innate responses against microorganisms, more efficacious adaptive immune responses in response to infection and vaccination, and regulation of inflammatory responses. Nevertheless, many questions still surround dietary administration of vitamins to poultry. For example, research has shown inconsistent results concerning dose–response relationships for some vitamins. Nonetheless, as more data are produced concerning the poultry immune system and vitamin-related effects, poultry health and production has a chance to further increase as dietary vitamin administration practices are further optimized.

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DISCLOSURES

The authors declare no conflict of interest.

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