Vitamin E and Fertility in the Poultry Birds;
Deficiency of Vitamin E and its Hazardous Effects

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Abstract

Vitamin E is found in high quantities in vegetable oils and has multiple functions in humans and animals including egg production, egg fertility, hatchability, sperm motility and transport, conception rate and post natal growth. Its main function is to protect cells from oxidative stress. Various studies have reported that vitamin E deficiency causes reduced fertility in humans and avian species. However, very few studies have addressed the effects of the supplementation of vitamin E and its deficiency on the fertility of domestic animals, especially poultry. Therefore, our aim was to thoroughly explore the effects of the supplementation of vitamin E on the fertility of poultry birds in order to understand the beneficial role of vitamin E in the maintenance of sperm and egg qualities. The available findings suggest that a moderate amount of vitamin E supplementation in poultry nutrition significantly protects sperm and egg quality in male and female birds respectively, via decreasing the lipid peroxidation in sperms/semen and eggs. This review may provide important scientific information to better understand the effects of vitamin E supplementation on fertility functions in the avian species.

Keywords: Poultry; Fertility; Vitamin E; Antioxidant; Oxidative stress

Introduction

Nutrition plays an important role in enhancing reproductive efficiency of all animals. Vitamins are vital organic compounds that can be obtained in high quantities from vegetable oils. Thirteen vitamins named alphabetically vitamin A to vitamin K have been discovered. In the early 1920s, fat-soluble vitamin E was discovered by Evans and Bishop [1]. Vitamin E is a generic term for a group of tocopherols and tocotrienols. Among the four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ) discovered, α-tocopherol is the most biologically active form and available in high quantities from vegetable oils, unprocessed cereal grains, and nuts [2,3]. Numerous reports have revealed the nutritional significance of vitamin E in humans, laboratory animals and avian species. Vitamin E is absorbed through the lymphatic pathway and transported into the circulation in association with chylomicrons [3]. After absorption, vitamin E is stored mainly in the liver. It is incorporated in lipid storage organelles and plasma membranes due to its fat-soluble properties, therefore it is also widely distributed throughout the body [3]. Vitamin E have interaction with several other dietary components, including selenium, sulphur-containing amino acids, polyunsaturated fatty acids and synthetic antioxidants [4].

Vitamin E possess several biological characteristics such as antioxidant, regulation of heme biosynthesis, transport of amino acids and lipids in the intestine [4,5]. Vitamin E is also involved in iron metabolism, steroidogenesis and stimulation of humoral and cellular immune responses against infectious diseases [6,7]. Vitamin E is essential in egg production, egg fertility, hatchability and sperm motility. But, Vitamin E deficiency causes disorders in nervous system, skeletal system, circulatory system, muscular system, cardiovascular system, immune system and reproductive system [7,8]. In addition, vitamin E deficiency also disturbs the function of liver, kidney and lung. Vitamin E deficiency can increase the risk of infection, ischemic heart disease and breast cancer that may promotes susceptibility to dietary and environmental stress in humans and animals [8]. The above mention negative effects of vitamin E deficiency produces huge economic losses in the poultry industry. Several findings from animal studies show that vitamin E has low toxicity and it still has not been classified as mutagenic, carcinogenic, or teratogenic.

Vitamin E is essential for humans and livestock species. Vitamin E deficiency causes abnormal spermatogenesis in males.
and failure to retain zygotes and fetal resorption in females birds [6,8]. Reproductive functions are crucial for healthy offspring and species survival of all animals. Very few studies have focused the role vitamin E in the poultry birds and its possible consequences. Therefore, the objective of this paper was to evaluate the effects of the supplementation vitamin E on fertility in the poultry species; deficiency of vitamin E and its possible hazardous effects.

**Why vitamin E is important in poultry nutrition ...?**

Dietary vitamin E supplementation increases the resistance of animals against infectious diseases and therefore, it is very important to estimate the optimum allowances of vitamins for poultry, lab animals and livestock [9]. Globally, poultry farming has been increasing for the purpose of meat and egg production. Poultry feeds are largely supplemented with different ingredients including carbohydrates, proteins, lipids, minerals, and vitamins to meet the basic nutritional requirements of the birds. The standard supplementation of proteins, carbohydrates, lipids, minerals, and vitamins supports the normal growth of the birds. In addition, the standard dietary supplementation with certain minerals and vitamins increases the birds immune response against various diseases [10]. Vitamin E requirement in the poultry feed is highly variable and depends on several factors such as the concentration and type of fat, the concentration of selenium, and the presence of pro-oxidants and antioxidants in the diet [11].

**Vitamin E requirements for poultry birds**

National Research Council's (NRC) Committee on Animal Nutrition (USA) has provided the nutrient requirements for poultry species. According to NRC recommendations, poultry feed can be supplemented with 101U of vitamin E per kg feed (1 IU=0.67mg dl-α-tocopheryl acetate) for chickens aged up to 6 weeks, 51U/kg feed for chickens aged over 6 weeks, 121U/kg feed for turkeys aged up to 8 weeks, and 101U/kg feed for turkeys aged over 8 weeks. For Japanese quail and ducks feed can be supplemented with 121U/kg feed and 101U/kg feed respectively for starting and growing birds. The dietary recommendations of vitamin E for poultry species during laying and breeding vary slightly [11]. Vitamin E deficiency causes various types of disorders in avian species including nutritional muscular dystrophy, erythrocyte hemolysis, and exudative diathesis (affects capillary walls) (Figure 1). Moreover, vitamin E deficiency results in lipid membrane peroxidation, affecting hepatic mitochondria and microsomes, as well as cerebellar encephalomalacia (brain disorder) in the birds [12,13]. Furthermore, vitamin E deficiency impairs feather development in the chickens [13]. Vitamin E deficiency causes gizzard myopathy in turkeys and ducks, and lead to accumulation of ceroid in turkeys [13].

**Vitamin E supplementation and fertility functions in male birds**

**Role of vitamin E as antioxidants:** Several reports have exposed the benefits of dietary vitamin E on fertility in different animal species. Currently, we have reviewed the effects of vitamin E supplementation on fertility functions in male birds. Male fertility depends on semen and sperm qualities, including the volume of semen, concentration of sperms, sperm motility, sperm viability, sperm forward progression, and the fertilization capacity of sperm. Several environmental factors and endocrine disrupting chemicals can affect the sperm and/or semen quality via enter the body through diet, respiration, and skin contact [14,15].

Dietary supplementation of minerals, vitamins, and antioxidants can reduce the above mention adverse effects. The addition of an antioxidant in poultry diet is required in order to avoid peroxidation of the fatty acids [16]. A study has reported that adult male White Leghorn (WL) chickens fed diets containing high levels of linoleic acid (7.3%) and low levels (4.3mg/kg feed) or high (166.3mg/kg feed) concentration of vitamin E for 25 weeks trials. The results showed that the males given diets high in linoleic acid and low in vitamin E showed impaired sperm concentration and fertilizing capacity [9].
On the other hand, diet supplementation with a high amount of vitamin E prevented these adverse effects. The above diet had no effects on semen volume, hatchability of fertile eggs, and weight of the testes. Moreover, the above authors recommended that vitamin E acts as an antioxidant that protects chickens against encephalomalacia by preventing the breakdown of linoleic acid to 12-oxo-cis-9-octadecenoic acid (lipoic acid). Encephalomalacia is a vitamin E deficiency disease, which readily occurs in chickens fed on a diet containing high levels of polyunsaturated fatty acids of the linoleic acid series and low concentration of vitamin E [9]. A study has stated that White Leghorn (WL) roosters fed a diet containing 12% dlα-tococyclate had significantly reduced fertility under 16 weeks experiments [17]. On the other side, the sperm fertilizing capacity was significantly increased when the roosters fed a diet containing 12% dlα-tococyclate mixed with 200mg/kg dlα-tocopherol acetate.

Avian spermatozoa are rich in polyunsaturated fatty acids (docosatetraenoic acid and arachidonic acid) [18,19]. The high concentration of polyunsaturated fatty acids provide sperm membrane flexibility, which plays an essential role in sperm mobility and sperm-egg fusion [20]. Conversely, avian sperm are sensitive to reactive oxygen species (ROS) due to high levels of polyunsaturated fatty acids that cause male infertility [19,20]. Therefore, it is necessary to add vitamin E in poultry nutrition to increase antioxidant status in semen or spermatozoa which is essential for the prevention of male infertility.

A study by Zanini et al. [21] in WL roosters (30-week-old) has reported that male birds fed a basal diet high in fish and/or soybean oil mixed with or without vitamin E supplementation (30, 200, or 400mg/kg). The roosters fed with fish/soybean oil expressed the lowest antioxidant status in the semen after 38 weeks experiment. However, the supplementation of vitamin E in mixed with fish and/or soybean oil diet had increased semen volume, motility, and sperm potency under 38 weeks trial.

In another study, male turkeys given a basal diet supplemented with 60mg/kg of α-tocopherol acetate supplemented with 2% fish oil 40 to 60 weeks of age [22]. The result indicated that α-tocopherol status in the turkey semen was increased two-fold. A similar study has reported that male chicks (Indian native Kadaknath) fed a basal diet supplemented with 10, 100, or 200mg/kg dlα-tocopherol acetate from day old to 30 weeks of age. The trial revealed that the proportion of abnormal and dead spermatozoa was significantly lowered and the fertilizing capacity was significantly higher in birds fed with 100mg/kg of vitamin E supplementation [23]. Furthermore, birds fed 100mg/kg of vitamin E had higher status of vitamin E in semen and spermatozoa than those birds supplemented with 10mg/kg of vitamin E [23]. These studies suggest that increased antioxidant status of semen based on increased concentration of antioxidant in the birds diet. Among the different concentrations of vitamin E, 7.5IU/kg diet showed the best result in protecting the male fertility in the birds [24]. The available data suggest that a moderate level of vitamin E supplementation is best for maintaining the fertility in male chickens and quail [23-25].

Vitamin E interaction with selenium: Selenium is a component of selenoproteins (glutathione peroxidase) which protect sperms against oxidative stress [26,27]. Various studies have revealed that vitamin E interacts with selenium. But, both are essential in diets because they play a role in the maintenance of reproductive hormonal functions as well as in the reduction of free radicals and reactive oxygen species (ROS) [9]. Khan et al.[28] has described that Rhode Island Red (RIR) males birds fed a basal diet supplemented with vitamin E (20 or 200mg/kg diet) and selenium (0.3mg/kg diet) had significantly increased the activity of glutathione peroxidase in testes, semen, and sperms [28]. Another reports showed that geese given vitamin E supplemented diet exhibited lower semen quality, egg production, fertility and hatchability rates as compared to chickens and quail. Several authors revealed that White Koluda male geese (3 year old) fed with basal diet and an experimental diet supplemented with 0.3mg/kg feed of selenium and 100mg/kg feed of vitamin E, the result showed that the males fed on the experimental diet had significantly increased semen volume and sperm concentrations in the semen (1.5- and 1.7-fold, respectively) during the next reproductive season and the level of lipid peroxidation was also significantly decreased in males fed on the experimental diet [29].

Avian study by Jerysz and Łukaszewicz [29] suggests that combined supplementation with selenium and vitamin E enhances the reproductive functions of avian species that show low reproductive behavior. The addition of antioxidant is not only important for lipid-containing poultry feed but also necessity for any basal diet in order to maintain fertility. Lin et al. [30] has found that day old male chicks (Taiwan breed) fed with maize/soybean diets for 23 weeks of age. After 23 weeks, the birds were given maize/soybean diets supplemented with 80mg/kg feed of dlα-tocopherol acetate for 52 weeks of age. Males fed with the vitamin E supplemented diet had significantly higher sperm viability, sperm motility, and sperm concentration in the semen. This study suggests that a maize/soybean containing diet is inadequate for maintaining the fertility functions in male birds; therefore the addition of vitamin E is required in the poultry diet.

Vitamin E deficiency and male reproductive functions: Molting (replacement of feathers) in birds is a natural periodic occurrence once a year. Molting is commonly associated with hens. But, some studies reported that molting improves semen quality in males [20,31]. Khan et al. [28] induced molting in male birds with a basal diet containing 3000mg zinc oxide/kg feed. After two weeks of molting, the birds were fed with a basal diet mixed with vitamin E supplementation (100IU/kg feed) for 3 weeks. The results indicated that the semen volume, sperm motility, and sperm capacity in fertilizing eggs were significantly increased in vitamin E supplemented male birds as compared to the control group. Various authors have demonstrated that vitamin E deficiency impairs male reproductive functions. It has been identified as an anti-sterility vitamin, and its deficiency or long-term omission in

the diet causes abnormal spermatogenesis and affects the semen qualities, including sperm viability, sperm motility, and sperm capacity [29,30]. Previous studies reported that Rhode Island Red (RIR) males fed a vitamin E deficient diet resulted in testicular degeneration under 2 years trial [16]. The above studies have reported that dietary vitamin E supplementation protects semen quality by preventing the breakdown of polyunsaturated fatty acids, which can cause oxidative damage. The recommended quantity of vitamin E in poultry diet for maintaining male fertility can vary, depending on the various research consequences. Regarding to vitamin E concentration, Khan et al. [28] and various others authors have suggested that a poultry diet should contain 10mg vitamin E/ kg of feed to maintain fertility of poultry birds [23-25,28].

**Vitamin E supplementation and fertility functions in female birds**

**Vitamin E as an important ingredient:** The fertility functions in females are crucial for successful production of healthy offspring. Specifically, in the poultry species the daily egg production, egg quality, egg weight, yolk weight, albumin weight, egg fertility and hatchability are the most important factors that determine healthy offspring. The number of fertile eggs produced for hatching shows the ultimate profitability of laying hens [20]. The nutrients required for embryo development are derived from the yolk and albumin stored in the eggs. A chicken egg contains significant amounts of nutrients such as carbohydrates, proteins, lipids, vitamins, and trace elements. Dietary composition can increase or decrease the nutritional profile of eggs. Chickens fed with a standard diet have stable the concentrations of total lipids and antioxidants in the eggs [32,33]. A similar study in males has stated that WL hens fed with a basal diet high in linoleic acid (7%) and low in vitamin E (20IU/pound feed) had decreased egg production from 78% to 25% during 8 weeks of experiment. Only 37% of the eggs were fertile, and none of the remaining fertile eggs was hatched [16]. However, when hens were given a basal diet containing high amounts of both linoleic acids (7%) and vitamin E (100IU/pound feed) had improved egg production reached up to 57% and 76% percent of the eggs were fertile and 67% of the fertile eggs were hatched. The findings obtained from males study suggest that vitamin E protects female fertility by preventing the breakdown of linoleic acid, which lead to oxidative damage [16]. In addition, when hens fed a basal diets containing low linoleic acid do not require additional vitamin E and/or antioxidants for maintenance of fertility, egg production, and hatching of fertile eggs [16]. While, on the other side, various authors observed that WL hens fed a basal diet containing 12% dilauryl succinate (similar effects as linoleic acid), the eggs fertility and the hatchability of fertile eggs were decreased. However, some authors suggest that these effects can be prevented by supplementation of 200mg/kg feed of dl-α-tocopheryl acetate to basal diet, containing 12% dilauryl succinate [9].

**Vitamin E and female reproductive system:** Dietary Vicine found in high quantities in faba beans (Vicia faba L.) and influence the metabolism of laying hens. Dietary vicine causes peroxidation of cellular membrane, which cause the abnormal lipid transport and a reduce fertility. A study reported that laying hens (WL) fed a basal diet containing vicine showed decreased fertility, hatchability and egg weight. However, the supplementation of vitamin E had slightly increased the egg weight and protected fertility and hatchability of eggs [34]. Clearly, this study shows that vitamin E as an antioxidant plays an important role in reducing the adverse effects of toxic compounds like vicine. Therefore, the above studies suggest that an adequate amount of vitamin E is necessary in the poultry diet for maintaining female fertility.

Another study in female chicks (Taiwan Native) has investigated that female fed with a maize/soybean diet for 17 weeks of age. After 17 weeks, the birds were allowed to feed on a maize/soybean diet supplemented with 80mg/kg feed of dl-α-tocopheryl acetate for up to 46 weeks of age [32,33]. The result indicated that hens fed with the vitamin E supplemented diet showed an improved egg production and egg mass. Similarly, diet supplemented with vitamin E had improved hatchability (over 13%) and fertility (over 7%). In another study, Japanese quail were given a soybean meal diet supplemented with low levels of vitamin E (2IU/kg diet) during 35 weeks experiment. The result showed that the percentage of fertile eggs was significantly decreased and the hatchability of fertile eggs was severely affected. But, no other clinical symptoms were observed [35]. The above study proposed that diet containing vitamin E given to quail was much lower in Vitamin E concentration that it was inadequate for supporting normal reproduction in quail.

**Vitamin E requirements for female birds:** In poultry species, the sperms are stored for a short period in male reproductive tract (vas deferens). Breque et al. [36] indicated that vitamin E has a positive effect on the antioxidant status of sperm storage sites in hens [36]. Specifically, the proportion of sperms found at utero-vaginal junction significantly correlate with the sperms found at perivitelline layer of eggs in avian species [37]. Additionally, Kihlare et al. [38] suggest that hen's egg achieves 100% of fertility when the perivitelline layer contains 1440 or more sperms. Taken together, it is well known that vitamin E deficiency significantly affects the fertility of poultry species [9]. Thus, chicks show symptoms of vitamin E deficiency should increase vitamin E supplementation in the maternal diet to avoid the hazardous effects in chickens. Furthermore, the addition of vitamin E in feed should be correlated with the amount of dietary selenium and polyunsaturated fatty acids. If the poultry diet contains a sufficient amount of selenium and is not rich in polyunsaturated fatty acids then 20mg vitamin E/kg diet is required for maintaining fertility of breeding hens [9]. In addition, Rengaraj and Hong [9] suggested an amount of more than 500μg vitamin E/kg to ensure the hatching of healthy chicks [9]. The effects of vitamin E supplementation on male and female fertility in poultry birds are shown in Figure 2. Therefore, by reviewing several reports of male and female birds, it has been documented that feeding the birds with 100mg vitamin E/kg diet may prevent the problems of vitamin E deficiency (Figure 3) [39-42].
Conclusion and Recommendation

In this review, we have explored the effects of dietary supplementation of vitamin E on fertility function of the poultry birds. Vitamin plays an essential role in the reproductive system of male and female birds; therefore, this review suggests that a moderate level of vitamin E supplementation in the poultry diet maintains the fertility functions in male birds, including sperm motility, sperm viability, semen volume, sperm concentration and sperm capacity. Moreover, a moderate amount of vitamin E supplementation significantly maintains fertility function in female birds, including egg production, egg fertility and egg hatchability. It is well addressed that vitamin E maintains fertility in male and female poultry birds due to its antioxidants property as a defense mechanism against oxidative damage. In addition, the vitamin E deficient diet impairs male and female fertility in the birds. Therefore, it is recommended that moderate level of vitamin E should be used in the poultry nutrition to avoid the adverse effects.

However, this review suggests a few future perspectives for research on poultry birds;

1. Several studies have focused the effects of vitamin E on semen and sperm qualities in males, and egg qualities in females’ birds. However, further work need to be elucidated on the effects vitamin E supplementation on Leydig cells and Sertoli cells in males, and on theca cells and granulosa cells in females birds.

2. In avian species, the effects of vitamin E deficiency or supplementation on male and female steroidogenesis are not well understood.

3. Especially, the effects of vitamin E deficiency or supplementation on fertility-associated specific genes and proteins need to be elucidated.

4. Various studies suggested a moderate amount of vitamin E (75–100 mg/kg diet) for the maintenance of fertility in male and female poultry birds. Regarding to above studies, the National Research Council’s (NRC) Committee on Animal Nutrition should establish a recommended safe concentration for poultry species.
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