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Influence of in-ovo administration with vitamin E and ascorbic acid on the performance of Muscovy ducks

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Abstract

Nutrient administration in-ovo could be considered as an alternative method to improve hatchability and duckling weights followed by better economic performance. On the 12th day of incubation, fertile duck eggs (n= 500) were distributed into 5 groups: uninjected control; 0.1 ml corn oil; 0.1 ml corn oil plus 10 mg vitamin E; 0.1 ml saline; and 0.1 ml saline plus 3 mg ascorbic acid. In-ovo injection of vitamin E but not ascorbic acid resulted in higher hatchability percentage compared to the uninjected control; however this was not statistically confirmed. In-ovo injection of either vitamin E or ascorbic acid resulted in significantly ($P < 0.05$) higher body weights at hatch, final body weights, and feed intakes than the uninjected control group. However, both male and female ducklings hatched from eggs injected with either vitamin E or ascorbic acid had better feed conversion during the starting period only. The carcass characteristics and relative lymphoid organ weights did not differ ($P < 0.05$) between the in ovo injected groups and the uninjected controls. In-ovo injection of either vitamin E or ascorbic acid resulted in significant increase ($P < 0.05$) in the geometric mean of the 1st antibodies titers of males and the 1st and 2nd antibodies titers of females compared to the uninjected control. It could be concluded that in-ovo injection of vitamin E and ascorbic acid improve the embryonic and post-hatch growth of Muscovy ducks.

Key words: In-ovo, Muscovy ducks, Growth, Vitamins

Introduction

The subsequent development of avian embryos and hatched chicks are influenced by the yolk nutrient status (Al-Murrani, 1982). Many nutrients have important structural, physiological, and immunological roles in avian embryogenesis and growth performance. In-ovo injection of nutrients may help overcome any constraint of inadequate egg nutrition. During early development, there is rapid oxidative metabolism that leads to the production of large quantities of free radicals in many tissues, making them more susceptible to oxidative damage. Antioxidants are a critical defense against these free radicals. The developing embryo may use antioxidants found in the yolk in order to reduce the impact of free radicals. Vitamin E has received the most attention over the past few years relative to immunity and more recently meat quality. Increasing egg vitamin E content in order to maximize the vitamin E available to the embryo and newly hatched chick becomes of interest either through dietary supplementation of vitamin E to breeder hens or directly via injection into the egg yolk (Cherian et al., 1997; Surai et al., 1999). Exogenous vitamin E administered in-ovo at a time of increased fatty acid oxidation, which begins at day 14 of incubation (Cherian et al., 1997), could be beneficial in reducing the production of free radicals that cause serious damage to the highly poly un-saturated fatty acids of cellular membranes (Cherian and Sim, 1992, 1997).

On the other hand, there are several reports regarding the effect of ascorbic acid as anti-stress agent on the productive performance parameters of chickens such as growth and reproductive traits, including fertility and hatchability. Ascorbic acid is synthesized by the embryo (Surai et al., 1996; Wilson, 1990) and the accumulation of this water-soluble antioxidant by the developing tissues will be independent of the dietary intake by the breeding hen. On the other hand, there were clear differences between the species (turkey, goose, duck, and

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chicken) concerning the concentrations of ascorbic acid especially by the brain during the development (Surai et al., 1998). Additionally, incubated eggs and their embryos may be subjected to stress caused by excessive production of metabolic heat during the latter part of egg incubation (Tullett, 1990), producing some internal environmental stress for embryo, which may be not suitable for the embryo to hatch successfully. In-ovo injection of ascorbic acid as an anti-stress agent may be beneficial. Our objective is to examine the effect of in-ovo injection of vitamin E or ascorbic acid on the performance of Muscovy ducklings.

Materials and Methods

Vitamin E and ascorbic acid preparation

Vitamin E was prepared as a solution of 1 g of water dispersible vitamin E (E-Viton®; 100mg alpha tocopheryl acetate per one capsule; Kahira Pharmaceutical and Chemical Industries Company, Egypt) per 10ml of corn oil (vitamin E solvent according to Ameenuddin and Sunde, 1984) and 0.1 ml from this solution is equivalent to 10 mg of vitamin E (Schaal, 2008). Ascorbic acid was prepared as a solution of 3 g of ascorbic acid (99% ascorbic acid®; SD Fine-Chem-Limited, India) in 100ml 0.9% saline solution (ascorbic acid solvent) and 0.1ml from this solution is equivalent to 3mg of ascorbic acid (Zakaria and Al-Anezi, 1996).

Eggs incubation and injection

Incubated fertile duck eggs (n= 500) were obtained from Muscovy breeders ducks fed on adequate nutritional diet. Fertility was verified by candling with a hand ultraviolet lamp at day 12 of incubation, Eggs were distributed into five groups of 100 eggs each. Eggs were incubated at 37 to 37.5°C and 70% relative humidity during the first 32 days of incubation. Eggs were turned automatically every hour until the 32nd day. All eggs were transferred to the hatchery at the end of the 32nd day of incubation, and placed in hatching boxes at 37°C temperature and 70-75% relative humidity until hatching occurred between days 35 and 36. On the 12th day of incubation, the groups of fertile eggs were distributed into five groups: uninjected control; 0.1 ml corn oil; 0.1 ml corn oil plus 10 mg vitamin E; 0.1 ml saline; and 0.1 ml saline plus 3 mg ascorbic acid. The treatment solutions or sham control were injected into the yolk of the 12-day-old embryo which was identified by candling with a hand ultraviolet lamp, through a pinhole made at the broad end of the egg, using a 25 mm needle. Prior to in-ovo injection the injection site was disinfected with 70% ethanol and the solutions were warmed to 30°C. The pinhole site was sealed with sterile paraffin wax immediately after injection. The injected eggs were returned to the incubator after injection.

Duckling management and feeding

All hatched ducklings from each treatment were weighed and sexed to males and females subgroups. The ducklings in each treatment group either males or females were randomly assigned to 4 replicates of 8 to 10 ducklings depending upon the hatch size, each replicate with a pen in each treatment. All ducklings were reared under similar managerial and hygienic conditions. The ducklings were raised in clean, well-ventilated, previously disinfected room. A continuous lighting program was maintained throughout the nine weeks experimental period. Temperature was adjusted at 32°C ± 2 in the first week and then reduced by 2°C each successive week then maintained at 22°C ± 2. The ducklings of all groups were vaccinated once against Avian Influenza with 0.50 ml single dose of Reassortant H5N1 Avian Influenza vaccine (Re-1 vaccine) subcutaneously in the lower back of the neck at the 14th day old (Middleton et al., 2007). Ducklings of different experimental groups fed on basal starter (0-3 weeks), grower (3-6 weeks) and finisher (6-9 weeks) rations as shown in Table (1). Basal rations were formulated to meet the nutrients requirements for Muscovy ducks as recommended by French Group Company, Sadat City, Egypt (Strain' origin). Nutrients compositions of the used rations were calculated according to the feed composition tables given by NRC (1994). The ducklings were fed ad libitum on dry mash ration and fresh clean water was constantly available.

Growth parameters

After hatching, the body weights (BW) of the ducklings in each group were recorded. The body weight of individual ducklings in each group and feed consumption of each pen were recorded at 3, 6 and 9 weeks of age. The feed conversion ratio (FCR) was calculated accordingly.

Sampling

Blood samples were taken from tibial vein of eight birds in each group (4 males and 4 females) at 5 and 9 week of age by needle under aseptic precaution. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 15 minutes for determination of antibodies titer against Avian Influenza disease virus by Haemag glutination Inhibition test (Thayer and Beard, 1998). At the end of the experimental period (9 weeks of age) 8 birds (4 males and 4 females) were selected from each treatment group and were slaughtered after being fasted for 12 hours. After

slaughter and complete bleeding, the birds were dressed. The carcass and some other components (liver, gizzard, visible fat, heart, bursa of Fabricius, spleen, and thymus) were weighed.

Dressing percentage = [(Dressed carcass weight/Live body weight) × 100].

Relative organ weights were calculated as percentages of body weight = [(Organ weight/Body weight) × 100].

Total edible parts were calculated as percentage of body weight = [(Weight of liver + gizzard + heart + visible fat)/Body weight) × 100].

Statistical Analysis

The obtained data were presented as means ± SE. Analysis of variance (ANOVA) was used to test the significance of the difference between different treatments and statistical differences were established using a Duncan's Multiple Range Test (Duncan, 1955) at the level of $P < 0.05$.

Table 1. Composition and nutritional levels of experimental diets.

Ingredients (%)	Ration		
	Starter	Grower	Finisher
Ground yellow corn	61.40	67.00	68.10
Soya bean meal (44 % CP)	30.00	23.40	20.00
Corn gluten meal (60 % CP)	4.30	5.00	5.00
Vegetable oil[1]	0.40	0.70	3.00
Mono calcium phosphate[2]	1.36	1.42	1.36
Ground limestone	1.73	1.72	1.54
Salt (NaCl)	0.31	0.30	0.40
Mineral and Vitamin Premix[3]	0.30	0.30	0.40
L-Lysine[4]	0.10	0.10	0.15
L-Methionine[5]	0.10	0.10	0.10
Calculated composition			
ME MJ/Kg diet	12.2	12.6	13.1
Crude protein (g/kg)	211.0	191.0	177.0
Calcium (g/kg)	9.7	9.6	8.7
Available phosphorus (g/kg)	4.2	4.2	4.0
Lysine (g/kg)	10.8	9.3	8.8
Methionine (g/kg)	4.6	4.4	4.2
Methionine + Cystine (g/kg)	8.1	7.7	7.3

1Vegetable oil composed of a mixture of soybean, cotton seed and sunflower oils.

2Monocalcium phosphate; 21% phosphorus, and 15% calcium.

3Minerals and Vitamins mixture (Pharma Mix). Each 3 kg contain: 12.000.000 I.U. Vitamin A, 2.500.000 I.U. Vitamin D3, 10.000 mg Vitamin E, 2.00 mg Vitamin K3, 1.000 mg Vitamin B1, 5.000 mg Vitamin B2, 1.500 mg Vitamin B6, 10 mg Vitamin B12, 30.000 mg, Niacin 1.000 mg, Folic acid, 50 mg Biotin, 10.000 mg Pantothenic acid, 10.000 mg Copper, 10.000 mg Iodine, 100 mg Selenium, 30.000 mg Iron, 60.000 mg Manganese, 50.000 mg Zinc, 100 mg Cobalt, CaCo3 add to 3000 gm.

4L-lysine: 78% produced by Archar Daniels Medland Company De Caur I.L. made in U.S.A. (ADM).

5DL-methionine: 99% Canadian registration number 990137 Guaranteed analysis, DL-Methionine 99%.

Results and discussion

Hatchability and duckling weight

The actual process of hatching a duck is complicated and there are many factors influencing the normal procedure. Poor breeder hen nutrition can result in embryo mortality at all times during incubation. The hatchability and embryonic mortality of fertile eggs were influenced by the type of injected substance into the eggs. In-ovo injection of vitamin E (78%) into the yolk of fertilized Muscovy duck eggs at the 12th day of incubation increased hatchability percentage as compared to uninjected control and oil injected group (72%). However, in-ovo injection of saline solution with or without ascorbic acid decreased hatchability percentage (68%) in comparison with the uninjected control (74%). Exogenous vitamin E administered around day 14 of incubation (the time of intense fatty acid oxidation) may be beneficial in reducing the production of free radicals that cause a serious damage to the cellular membranes (Cherian and Sim, 1992, 1997; Cherian et al., 1997) and increased lipid utilization for energy production in order to improve hatchability (Schaal, 2008). Therefore, this increase in hatchability percentage after in-ovo injection of vitamin E might be due enhancement of the antioxidant status of the eggs (Surai, 2000) and/or the vitamin E may protect the protein and lipids from oxidation (Puthpongsiriporn et al., 2001). The reduced hatchability and increased embryonic mortality percentages with in-ovo injection of ascorbic acid might be due to the

relatively high dose of ascorbic acid during this injection time (12th day of incubation) or the site of injection (yolk), since Zakaria and Al-Latif (1998) demonstrated that either premature or excessively high doses of ascorbic acid injected in-ovo can cause worsening of chick hatchability. Moreover, greatest improvement in hatchability was obtained when Pekin ducks eggs were injected with 6 mg/egg of ascorbic acid into the air cell on the 20th day of incubation (Nowaczewski et al., 2012). This decrease in hatchability after in-ovo injection of saline with or without ascorbic acid may also probably due to the varied osmolality of the injection solution, since the osmolality of the injected solution was a critical factor affecting hatchability of turkey (Ferket et al., 2005). On the other hand, Zakaria and Al-Anezi (1996) found that injection of ascorbic acid at a dose 3 mg on day 15 of incubation in broilers' eggs improved hatchability, and therefore in-ovo injection of ascorbic acid may become effective and successful when it is carrying out before incubation by eggs dipping in ascorbic acid solution (Shafey, 2002) or by its injection during the latter period of incubation, when there is a risk of heat stress (Tullet, 1990).

In-ovo injection of vitamin E (53.23g) or ascorbic acid (52.20g) significantly increased ($P < 0.05$) body weights of ducklings at hatch compared to the uninjected control (45.95g). On the other hand, there was no significant difference between the uninjected control and sham groups (47.10g and 44.10g for oil and saline injected groups, respectively). This higher body weight might be due to the prevention of hydro peroxides impacts and more energy production by vitamin E injected eggs which enhances the embryonic growth (Schaal, 2008). However, Schaal (2008) reported different results when they injected the same dose into fertile chicken eggs at the 14th day of incubation. Similarly, this is contrary to the result of Bhanja et al. (2007) whose injected 0.5 IU of vitamin E into the yolk of fertile chicken's eggs. These conflicting results may be due to the late injection time in the former study and to the lower dose in the latter. However, in the case of ascorbic acid these results are in agreement with Bhanja et al. (2007) and Zakaria and Al-Anezi (1996) who found an improved hatchling weight by in-ovo injection of ascorbic acid into broiler's fertile eggs.

Growth parameters

It was documented that vitamin E has an important role in maintaining normal rate of growth of chicks (Marsh et al., 1981) and improving broiler performance under commercial condition (Kennedy et al., 1992). In-ovo route of vitamin E supplementation enhances the post-hatch performance as compared with the traditional means of dietary feeding, which requires more time to build up the vitamin E levels necessary for optimal performance (Gore and Qureshi, 1997). The obtained results revealed that the ducklings from the eggs injected with vitamin E significantly exhibited heavier body weights for both males and females than those of the uninjected control and sham group during the experimental periods (Table 2). The heavier body weights of vitamin E injected group may be due to the vitamin E requirement for early post-hatch growth (Bhanja et al., 2007). These results agreed with those obtained by Hossain et al. (1998) who observed that higher final body weight at 42 days with broilers hatched from eggs injected with 2.5 or 5 mg of vitamin E, and Bhanja et al. (2006 and 2007) who reported that body weight of broiler chickens was more at 14 and 28 day of age in vitamin E injected group than the uninjected control. In-ovo injection of 3 mg ascorbic acid significantly increased body weight of males than those of the uninjected control and sham group. The same result was recorded with the females of the same treatment except during the finishing period; there was no significant difference (Table 2). The heavier body weights of ascorbic acid injected group may be due to the important role of ascorbic acid during incubation in the subsequent growth of broiler chickens to market weight (Zakaria et al., 1998) with relate to the limited capacity of ascorbic acid biosynthesis in male chicks (Hornig and Frigg, 1979). Furthermore, the rapid growth rates of male broiler considered as a stressful condition and consequently increases ascorbic acid requirement. The obtained results are in consistent with the results of Zakaria et al. (1998) and Zakaria (2001), they recorded that in-ovo injection of 3 mg ascorbic acid at the 15th day of incubation resulted in greater body weight of males and no significant differences were observed for female broiler chickens. In our study, there was significantly decrease in the body weight of males and females injected with either saline or corn oil during the starting period only.

In-ovo injection of vitamin E increased feed intake of males and females than the uninjected control (data not shown). While, it caused significant decrease in feed conversion of male and female ducks except during the starting period, which wasn't affected for males and improved for females as compared to the uninjected control and sham group (Table 3). These results were in agreement with the results of Bhanja et al. (2006)

who found that vitamin E injected birds had significantly better feed conversion than the un-injected control birds during 0-14 days of age, and Bhanja et al. (2007) who observed that no significant difference in feed conversion between vitamin E injected birds and un-injected control birds until 28 days of age. In-ovo injection of ascorbic acid increased feed intake of male and female ducks except during the finishing period for females as compared to uninjected control and sham group (data not shown). Moreover, in-ovo injection of ascorbic acid caused a significant decrease in feed conversion of male ducks except during the starting period and no significant changes in females except during the finishing period; it significantly decreased as compared to the control group (Table 3). This higher feed intake of ducklings hatched from eggs injected with either vitamin E or ascorbic acid may attribute to greater body weight as feed intake is a function of live body weight.

Table 2. Body weight (g) of male and female Muscovy ducklings hatched from eggs injected with vitamin E and ascorbic acid in different growth periods (mean ± SE).

Group Period	Control	Vitamin E	Corn oil	Ascorbic acid	Saline
			Male		
Starting	551.6 ± 12.8^{c}	845.5 ± 6.1^{a}	466.3 ± 5.8^{d}	784.1 ± 11.9^{b}	404.3 ± 15.4^{e}
Growing	2196.6 ± 41.2^{b}	2588.7 ± 17.1^{a}	2253.3 ± 16.8^{b}	2640.0 ± 29.5^{a}	2238.9 ± 21.4^{b}
Finishing	4109.2 ± 55.2^{b}	4458.0 ± 21.2^{a}	4078.3 ± 25.4^{b}	4429.2 ± 52.6^{a}	4090.0 ± 11.5^{b}
			Female		
Starting	572.0 ± 8.1^{c}	715.0 ± 3.5^{a}	406.0 ± 2.9^{e}	605.7 ± 7.0^{b}	490.1 ± 5.3^{d}
Growing	1770.5 ± 27.7^{c}	2003.8 ± 10.5^{a}	1772.5 ± 10.3^{c}	1850.61 ± 15.8^{b}	1759.2 ± 11.0^{c}
Finishing	2801.9 ± 32.6^{b}	2937.5 ± 14.8^{a}	2772.5 ± 12.7^{b}	2747.5 ± 11.8^{b}	2640.0 ± 13.9^{b}

a-b Means within the same raw having different superscript are significantly different ($P < 0.05$). Treatments: Corn oil (0.1 ml); corn oil plus vitamin E (10 mg); saline (0.1 ml); and saline plus ascorbic acid (3 mg). Number of replicates is 4 of 8 to 10 ducklings depending upon hatch size.

Table 3. Feed conversion ratio (FCR) of male and female Muscovy ducklings hatched from eggs injected with vitamin E and ascorbic acid (mean ± SE).

Group Period	Control	Vitamin E	Corn oil	Ascorbic acid	Saline
			Male		
Starting	1.47 ± 0.04^{a}	1.47 ± 0.01^{a}	1.51 ± 0.02^{a}	1.49 ± 0.02^{a}	1.42 ± 0.05^{a}
Growing	1.85 ± 0.04^{c}	2.19 ± 0.01^{a}	1.82 ± 0.01^{c}	1.98 ± 0.02^{b}	1.78 ± 0.07^{c}
Finishing	2.21 ± 0.04^{c}	2.51 ± 0.03^{b}	2.24 ± 0.01^{c}	2.60 ± 0.04^{a}	2.23 ± 0.01^{c}
			Female		
Starting	1.40 ± 0.02^{a}	1.30 ± 0.01^{b}	1.38 ± 0.02^{a}	1.40 ± 0.01^{a}	1.41 ± 0.01^{a}
Growing	2.25 ± 0.04^{b}	2.35 ± 0.01^{a}	2.23 ± 0.04^{c}	2.25 ± 0.02^{b}	2.25 ± 0.01^{b}
Finishing	3.30 ± 0.06^{b}	3.75 ± 0.02^{a}	3.17 ± 0.01^{c}	3.66 ± 0.03^{a}	3.18 ± 0.01^{b}

a-b Means within the same raw having different superscript are significant different ($P < 0.05$).

Treatments: Corn oil (0.1 ml); corn oil plus vitamin E (10 mg); saline (0.1 ml); and saline plus ascorbic acid (3 mg). Number of replicates is 4 of 8 to 10 ducklings depending upon hatch size.

Carcass characteristics

There have been considerable studies looking at improving carcass traits. In-ovo injection of nutrients is interested possible method to meet this objective, but there is very little research looking at in-ovo supplementation with vitamins. In this study, in-ovo injection of vitamin E or ascorbic acid had no significant effect on dressing and total edible parts (as % of body weight) except the dressing percentage of females hatched from eggs injected with ascorbic acid, it significantly increased as compared to the uninjected control and saline injected group (Table 4). In male ducklings, in-ovo injection of either corn oil or saline significantly decreased ($P < 0.05$) dressing percentage compared to the uninjected control. Moreover, in-ovo injection of oil solution with or without vitamin E significantly decreased ($P < 0.05$) relative weights of liver and heart as compared to uninjected control. On the other hand, in-ovo injection of saline solution with or without ascorbic acid significantly decreased ($P < 0.05$) relative weight of liver and increased ($P < 0.05$) relative weight of heart compared to uninjected control. Further investigations are needed to highlight the effect of in-ovo injection of vitamin E and ascorbic on the development of digestive organs in Muscovy ducks.

Table 4. Carcass characteristics of male and female Muscovy ducklings after in-ovo injection with vitamin E and ascorbic acid (mean ± SE).

Group Item (as % of BW)	Control	Vitamin E	Corn oil	Ascorbic acid	Saline
			Male		
Dressing	82.50 ± 1.10[a]	80.50 ± 0.80[a]	71.60 ± 0.70[c]	82.20 ± 0.90[a]	75.60 ± 1.29[b]
Liver	2.25 ± 0.12[a]	1.90 ± 0.02[b]	1.75 ± 0.03[b]	2.07 ± 0.01[b]	2.00 ± 0.03[b]
Gizzard	2.52 ± 0.08[ab]	2.66 ± 0.09[a]	2.53 ± 0.04[ab]	2.56 ± 0.03[ab]	2.32 ± 0.11[b]
Heart	0.67 ± 0.01[b]	0.59 ± 0.03[c]	0.60 ± 0.01[c]	0.69 ± 0.01[ab]	0.74 ± 0.03[a]
Visible fat	2.30 ± 0.30[a]	2.53 ± 0.30[a]	1.95 ± 0.23[a]	2.52 ± 0.50[a]	2.30 ± 0.20[a]
Total edible parts	7.85 ± 0.54[a]	7.52 ± 0.28[a]	7.34 ± 0.20[a]	7.72 ± 0.10[a]	7.32 ± 0.19[a]
			Female		
Dressing	75.70 ± 0.10[b]	76.50 ± 0.80[b]	73.60 ± 0.80[b]	89.70 ± 0.80[a]	74.40 ± 0.70[b]
Liver	1.92 ± 0.02[ab]	1.86 ± 0.01[ab]	1.81 ± 0.02[b]	1.93 ± 0.02[a]	1.85 ± 0.04[ab]
Gizzard	2.63 ± 0.02[a]	2.53 ± 0.09[a]	2.50 ± 0.08[a]	2.56 ± 0.03[a]	2.50 ± 0.11[a]
Heart	0.77 ± 0.01[a]	0.83 ± 0.01[a]	0.76 ± 0.01[a]	0.80 ± 0.01[a]	0.79 ± 0.01[a]
Visible fat	2.64 ± 0.30[a]	3.13 ± 0.13[a]	2.45 ± 0.30[a]	2.26 ± 0.26[a]	2.74 ± 0.30[a]
Total edible parts	7.96 ± 0.12[a]	8.35 ± 0.04[a]	7.52 ± 0.01[a]	7.55 ± 0.06[a]	7.96 ± 0.26[a]

a_b Means bearing different letters in the same raw differ significantly ($P < 0.05$).

Treatments: Treatments: Corn oil (0.1 ml); corn oil plus vitamin E (10 mg); saline (0.1 ml); and saline plus ascorbic acid (3 mg). Number of replicates is 4.

Immune status

In-ovo injection of vitamin E caused no significant changes in the relative weights of thymus gland, bursa of fabricus, and spleen for males and females as compared to the uninjected control. The relative weights of lymphoid organs were comparatively higher in the vitamin E injected females group than the uninjected control (Table 5). These results are agree with the results obtained by Gore and Qureshi (1997) who found that turkey and chick embryos exposed to 10 IU vitamin E into the amnion 3 days prior to hatch had no differences in the bursa and spleen weights compared to the control group. In-ovo injection of ascorbic acid caused no significant changes in the relative weights of lymphoid organs of males and females groups except that's of bursa of females significantly increased as compared to the uninjected control group. The relative weights of these lymphoid tissues were comparatively higher in ascorbic acid injected males and females group than the uninjected control group (Table 5). Comparative researches looking at in-ovo injection of ascorbic acid are unavailable.

Table 5. Relative weight of lymphoid organs (as % of body weight) of male and female Muscovy ducklings hatched from eggs injected with vitamin E and ascorbic acid (mean ± SE).

Group Item	Control	Vitamin E	Corn oil	Ascorbic acid	Saline
			Male		
Thymus	0.545 ± 0.006[a]	0.545 ± 0.006[a]	0.540 ± 0.008[a]	0.580 ± 0.012[a]	0.541 ± 0.006[a]
Bursa	0.131 ± 0.004[ab]	0.136 ± 0.001[a]	0.120 ± 0.008[b]	0.137 ± 0.001[a]	0.119 ± 0.007[b]
Spleen	0.054 ± 0.001[a]	0.055 ± 0.006[a]	0.053 ± 0.004[a]	0.054 ±0.004[a]	0.054 ± 0.003[a]
			Female		
Thymus	0.620 ± 0.016[a]	0.665 ± 0.006[a]	0.621 ± 0.014[a]	0.660 ± 0.008[a]	0.658 ± 0.006[a]
Bursa	0.118 ± 0.007[b]	0.127 ± 0.006[b]	0.160 ± 0.016[b]	0.225 ± 0.038[a]	0.142 ± 0.017[b]
Spleen	0.050 ± 0.005[a]	0.052 ± 0.004[a]	0.050 ± 0.004[a]	0.055 ± 0.006[a]	0.050 ± 0.001[a]

a-b Means within the same raw having different superscript are significant different ($P < 0.05$).

Treatments: Corn oil (0.1 ml); corn oil plus vitamin E (10 mg); saline (0.1 ml); and saline plus ascorbic acid (3 mg). Number of replicates = 4

Table 6. Serum antibody titers (HI titer log–2) of male and female Muscovy ducks after in-ovo injection with vitamin E and ascorbic acid (mean ± SE).

Group Item	Control	Vitamin E	Corn oil	Ascorbic acid	Saline
		Male			
1st antibody titer	3.5 ± 0.2^{b}	4.5 ± 0.2^{a}	3.0 ± 0.4^{b}	4.5 ± 0.2^{a}	3.5 ± 0.2^{b}
2nd antibody titer	3.0 ± 0.4^{ab}	4.0 ± 0.4^{a}	2.5 ± 0.2^{b}	3.5 ± 0.2^{ab}	2.5 ± 0.2^{b}
		Female			
1st antibody titer	3.5 ± 0.2^{c}	5.5 ± 0.2^{a}	3.5 ± 0.2^{c}	4.5 ± 0.2^{b}	3.5 ± 0.2^{c}
2nd antibody titer	3.5 ± 0.2^{b}	4.5 ± 0.2^{a}	3.5 ± 0.2^{b}	4.5 ± 0.2^{a}	3.5 ± 0.2^{b}

Means bearing different letters in the same raw differ significantly ($P < 0.05$).

Treatments: Corn oil (0.1 ml); corn oil plus vitamin E (10 mg); saline (0.1 ml); and saline plus ascorbic acid (3 mg). Number of replicates = 4

In-ovo injection of vitamin E or ascorbic acid resulted in significant increase in the geometric mean of the 1st estimate for antibodies titers of males and the 1st and 2nd estimate for antibodies titers of females as compared to the uninjected control and sham groups (Table 6). These results were in agreement with those obtained by Gore and Qureshi (1997) who reported that the hatched turkeys and chicks from the 10 IU vitamin E exposed embryos were high responders compared to the control group for antibody production, and Hossain et al. (1998) observed that the antibody titer to killed Newcastle disease vaccine were increased significantly by the level of vitamin E supplements in the diets or its direct injection into eggs.

Conclusion

It could be concluded that early supplementation of nutrients through in-ovo injection such as vitamin E can be regarded as a possible method to improve hatchability, body weight at hatch, marketing weights, and immune status of Muscovy ducks. Further investigations are needed to highlight the effect of in-ovo injection of Vitamin E and ascorbic acid on the humoral, cell mediated immunity, and development of digestive organs in Muscovy ducks.

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