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Effect of higher dietary vitamin E concentrations on physical and biochemical characteristics of semen in Kadaknath cockerels

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Abstract 1. This experiment was to investigate the effects of increasing dietary vitamin E on physical and biochemical characteristics of semen in Indian reared Kadaknath (KN) cockerels. DL-α-Tocopherol acetate was used as the source of vitamin E.
2. A total of 135 one-day-old male KN chicks were randomly selected and divided into 9 groups with 15 chicks in each group (3 dietary treatments × 3 replicates).
3. The basal diet contained 15 IU (10 mg) vitamin E/kg and the two experimental diets were supplemented with 150 IU (100 mg) and 300 IU (200 mg) vitamin E/kg (diets T_2 and T_3, respectively).
4. Physical characteristics in terms of semen volume, sperm concentration, sperm motility and percentage live sperm did not differ significantly, whereas proportion of abnormal and dead spermatozoa were significantly lower and fertility higher in the T_2 group.
5. Biochemical characteristics in term of quantities of protein and nitric oxide (NO) did not differ significantly, whereas the quantity of glucose, acid phosphatase (ACP) and vitamin E were significantly higher in the T_2 group.
6. In contrast, the quantities of alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were significantly lower in T_2 group and higher in the T_1 (control) group.
7. From this study it can be concluded that moderate supplementation of dietary vitamin E may be beneficial for physical and biochemical characteristics of semen in Indian reared KN cock.

INTRODUCTION

Kadaknath (KN) is an Indian reared poultry breed which is well known for poor egg production (80–120 eggs/year), slow growth rate, frequent broodiness, smaller body size (1.2–1.5 kg) as well as late sexual maturity (25–26 week). Despite the importation of high yielding strains from across the world the local breed still retains preference in its native environment mainly due to its special capabilities e.g., good foraging, efficient mothers and lower cost. KN is the preferred bird over exotic counterparts for landless labourers and marginal farmers because of their greater adaptability to extreme climatic conditions and resistance to protozoans and ecto-parasites. They are comparatively hardy and need minimum health care compared to other breeds. The meat from KN fowl has significantly higher linoleic acid, (24%) as against 21% in commercial table chickens and is widely preferred especially because of pigmentation, taste and leanness. It is enigmatic that the concentration of cholesterol 184.75 mg/100 g in KN meat was significantly lower than that of commercial table chicken meat 218.12 mg/100 g, in spite of significantly higher blood cholesterol 352.37 mg/dl in KN than in routine table chickens 253.12 mg/dl. It is of immense interest to choose foods which are richer in adequate proteins, low in cholesterol and their fat possesses higher degree of unsaturation in terms of linoleic acid, which possibly postpone the hazards of atherosclerosis (Kawalkar and Bhambal, 1996).

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Vitamin E is the major lipid soluble antioxidant present in the cell membrane and plays an important role in avian reproduction (Freisleben and Packer, 1993). It is a natural antioxidant and enhances semen quality and fertilising ability of chicken when it is provided at a level some 500 times greater than the NRC (1994) recommendation (15 IU/kg diet). In the chicken, vitamin E prevents lipid peroxidation in spermatozoa (Surai *et al*., 1997), but deficiency of vitamin E decreased the testosterone synthesis in Leydig cells (Bensoussan *et al*., 1998). In recent years, vitamin E supplements have been widely used in poultry diets and the levels for enhancing production and reproductive performance have been increased (Surai, 1999; Biswas *et al.*, 2007). Vitamin E increased sperm count and motility, reduced the percentage of dead sperm and enhanced the antioxidative status of seminal plasma in chicken (Eid *et al*., 2006).

Several studies have been conducted to investigate the effects of higher levels of vitamin E on reproductive performance in exotic breeds of chicken (Surai, 1999; Lin *et al*., 2005; Eid *et al*., 2006) and quails (Biswas *et al*., 2007) but studies on KN fowls are lacking. So, the study described here attempted to enhance the physical and biochemical characteristics of semen by supplementing the diet of KN cock with vitamin E.

MATERIALS AND METHODS

Housing and rearing of birds

A total of 135 one-day-old male KN chicks were randomly divided into 9 groups, each of 15 chicks (3 treatments × 3 replicates). The experiment had a randomised design (Snedecor and Cochran, 1994). Chicks were reared under uniform husbandry condition (14 h light/d and 25–32°C). The same technician provided feed, water and collected data from the birds during the course of the experiment. The experiment followed the guidelines of 'Institutional Animal Ethics Committee (IAEC, CARI, Izatnagar)'.

Formulation of experimental diets

The basal diet (T_1) contained 210 g crude protein (CP) and 12.2 MJ ME per kg, 30 g/kg total calcium and 5 g/kg total phosphorus (Table 1). Two experimental diets, T_2 and T_3, were formulated to contain an additional 150 IU (100 mg) and 300 IU (200 mg) vitamin E/kg diet respectively. DL-α-Tocopherol acetate was used as the source of vitamin E (1 IU = 0.67 mg DL-α-tocopherol acetate).

Collection of semen

The birds were maintained on the experimental diets for 30 weeks. Semen collected during the final 3 weeks of the trial was used for the measurement. Semen was collected two times a week by using the method described by Kelso *et al*. (1996). Each adult male was gently picked up with one person holding the legs apart and another collecting the semen discharge after gently massaging the lumbar region of the bird 3–4 times with the hand. Special care was taken to minimise contamination by faeces and watery fluids from the cloacal region.

Table 1. *Composition of the basal diet*

Ingredients	Composition (g/kg)	Calculated composition
Maize	590	Crude protein (g/kg) – 210
Soybean	200	Crude fibre (g/kg) – 23·5
DORB	75	Total calcium (g/kg) – 30·0
Fish meal	60	Total phosphorus (g/kg) – 5·0
Limestone	25	ME (MJ/kg) – 11·45
Oyster shell	15	Vitamin E (IU/kg) – 15·0
Marble chips	20	Selenium (mg/kg) – 0·20
Dicalcium phosphate	10	
Sodium chloride	05	
	Amount (g/kg)	
DL-Methionine	0·150	
Choline chloride	0·300	
Mineral mixture (Premix-1)	0·125	
Vitamin A, B_2, D_3, K (Premix-2)	0·040	
Vitamin B complex (Premix-3	0·050	

DORB, de-oiled rice bran.

Premix-1: Each g of mineral mixture contained: 200 mg of $FeSo_4 \cdot 7H_2O$, 20 mg of $CuSo_4 \cdot 5H_2O$, 200 mg of $MnSo_4 \cdot H_2O$, 150 mg of $ZnSo_4 \cdot 7H_2O$, 1 mg of KI.

Premix-2: Each g of vitamin A, B_2, D_3, K (Spectromix, Ranbaxy) provided: vitamin A (retinol) – 540 mg, vitamin B_2 (riboflavin) – 50 mg, vitamin D_3 (cholecalciferol) – 400 mg, vitamin K (menadione) – 10 mg.

Premix-3: Each g of B-Complex provided: vitamin B_1 (thiamine)–2 mg, folic acid – 10 mg, pyridoxine HCl – 4 mg, cyanocobalamin – 10 µg, nicotinamide – 12 mg.

Semen Characteristics

Physical

Immediately after collection of semen the volume was determined using a graduated collection tube and sperm concentration was determined using a haemocytometer (American Optical Company, New York, USA). The percentage of motile spermatozoa was determined by compound microscope at $10\times$ magnification after placing a cover slip over 2–3 mm drop of semen on a warmed microscope slide. The percentage of live, dead and abnormal spermatozoa was determined using the method described by Yousef *et al.* (2003) with an eosin-nigrosin stained smear and bright field microscopy at $100\times$ magnification. Stained spermatozoa were counted as dead. The percentage of abnormal spermatozoa was evaluated in the same sample by examining the morphology of a total count of 100 spermatozoa.

Biochemical

After collection of semen, seminal plasma was prepared for biochemical estimation. Protein, acid phosphatase (ACP) and alkaline phosphatase (ALP) were estimated by the Lowry *et al.* (1951) method, Andersch and Szcypinski (1947) method and Bessay and Lowry (1946) method respectively, while glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were estimated by Reitman and Frankel (1957) method, Nitric oxide (NO) was estimated by the method described by Sastry *et al.* (2002) and glucose was estimated using the GOD (Glucose oxidase)–POD (peroxidase) method.

Determination of α-tocopherol

α-Tocopherol was determined by the method described by Gaal *et al.* (1995). The semen samples were saponified with ethanolic KOH in the presence of pyrogallol. Then α-tocopherol was extracted from the mixture with petroleum ether. The extract was dried under nitrogen and then redissolved in methanol and injected on to a Rheodyne manual loop injector with reverse phase High performance liquid chromatography (HPLC) column (Particle size 5 µm: 4.6×250 mm, Phenomenex). Chromatography was performed using a flow rate of 2.00 ml/min. Fluorescent detection and quantification of α-tocopherol was performed by monitoring the eluent at excitation and emission wavelengths of 296 and 330, respectively. The calibration curve was obtained by using a standard solution of α-tocopherol in methanol.

Determination of fertility

Healthy egg laying KN females (135) of similar age to the males were randomly selected and housed with male in the ratio of 1:1 in each cage. After two weeks of adaptation, the eggs were collected daily for 7 d and labelled. After collection, eggs (a total of 350) were stored in the egg holding room at the hatchery. After fumigation, the eggs were set and incubated for 9 d in the hatchery, when they were broken open to confirm they were fertile with the presence of developing embryo. Fertility was determined as the ratio of number of fertile eggs to the number of total eggs set.

Statistical analysis

The data were analysed using statistical software package developed at the computer centre of the Institute following standard procedure for ANOVA (Snedecor and Cochran, 1994) and Duncan's multiple range tests (Duncan, 1955) by comparing means for significant differences.

RESULTS

A summary of the values for physical and biochemical characteristics of semen are presented in Tables 2 and 3.

Table 2. *Effect of vitamin E on the physical parameters of semen in KN cockerels (Mean ± SEM,* N = *15)*

Dietary treatment	Vitamin E added IU/kg[1]	Volume (ml)	Concentration (sperm count/ $mm^3\times10^{-9}$)	Motility (%)	Live (%)	Dead (%)	Abnormal (%)	Fertility (%)
T_1	0	0·18 ± 0·04	4·12 ± 0·53	82·4 ± 9·22	89·14 ± 3·08	6·23 ± 0·21[b]	4·73 ± 0·24[b]	81·24 ± 2·12[a]
T_2	150	0·20 ± 0·04	4·24 ± 0·74	89·2 ± 11·35	92·20 ± 2·76	4·02 ± 0·23[a]	3·78 ± 0·12[a]	93·45 ± 3·89[b]
T_3	300	0·17 ± 0·05	4·02 ± 0·44	84·8 ± 8·42	88·52 ± 2·02	6·36 ± 0·14[b]	5·12 ± 0·08[b]	85·37 ± 2·35[a]
		NS	NS	NS	NS			

[1]As DL-α-tocopherol acetate; 1 IU = 0·67 mg DL-α-tocopherol acetate.
[a,b]Mean values, within a column, not bearing a common superscript differ significantly ($P<0·05$).
NS, non significant.

Table 3. *Effect of vitamin E on the biochemical parameters of seminal plasma in KN cockerels (Mean ± SEM,* N = *15)*

Dietary treatment	Vitamin E added IU/kg[1]	Protein (g/100 ml)	Glucose (mg/100 ml)	ACP (μ mol/min/ mg protein)	ALP (μ mol/min/ mg protein)	GOT (n mol/min/ mg protein)	GPT (n mol/min/ mg protein)	NO (μg/ml)	Vitamin E (seminal plasma) (ng/ml)	Vitamin E (spermatozoa) (ng/10^9 cells)
T_1	0	0·83 ± 0·04	51·36 ± 1·92[a]	120·84 ± 5·40[a]	13·13 ± 0·23[b]	52·16 ± 3·90[b]	4·52 ± 0·08[b]	41·27 ± 1·64	182·28 ± 2·32[a]	89·23 ± 0·35[a]
T_2	150	0·99 ± 0·06	59·24 ± 1·73[b]	147·04 ± 7·53[b]	9·23 ± 0·15[a]	46·46 ± 2·23[a]	3·99 ± 0·07[a]	44·53 ± 2·72	306·18 ± 4·25[b]	142·56 ± 1·20[b]
T_3	300	0·81 ± 0·07	50·26 ± 2·02[a]	124·52 ± 6·12[a]	11·25 ± 0·16[ab]	49·17 ± 2·38[ab]	4·17 ± 0·03[ab]	43·30 ± 3·12	303·29 ± 3·75[b]	139·54 ± 1·07[b]
		NS						NS		

[1]As DL-α-tocopherol acetate; 1 IU = 0·67 mg DL-α-tocopherol acetate·

[a,b]Mean values, within a column, not bearing a common superscript differ significantly ($P < 0·05$).

NS, non significant.

Physical characteristics

Physical characteristics (Table 2) of semen in terms of volume, sperm concentration, sperm motility and percentage of live spermatozoa did not differ statistically between the different dietary treatment groups. Numbers of dead and abnormal spermatozoa were significantly ($P<0.05$) lower in group T_2. However, fertility rate (%) was significantly higher in T_2 ($P<0.05$) than in the other two groups.

Biochemical characteristics

There were no significant differences ($P>0.05$) in seminal plasma protein and nitric oxide (NO) quantities in any of the treatment groups (Table 3), whereas the concentration of glucose and acid phosphatase were significantly higher ($P<0.05$) in the T_2 group compared with the other groups at all times examined. Alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were significantly lower ($P<0.05$) in the T_2 group and significantly higher ($P<0.05$) in T_1 (control) group.

The concentration of α-tocopherol in seminal plasma and spermatozoa displayed significant ($P<0.05$) increases with dietary enhancement. Increasing the dietary supplementation with vitamin E from 15 to 150 IU/kg diet produced around a two-fold increase in the concentration of the vitamin in the seminal plasma and spermatozoa.

DISCUSSION

Physical characteristics

In the present study, no significant differences were observed in semen volume, sperm concentration, sperm motility or percentage of live spermatozoa. Vitamin E is naturally present in chicken sperm, where it helps to maintain membrane integrity and sperm motility (Donoghue and Donoghue, 1997), and a variation in sperm concentration is reflected in the degree of motility of spermatozoa (Latshaw and Osman, 1974). In our study, there was no significant difference in sperm motility and sperm concentration between groups. However, percentages of dead and abnormal spermatozoa were significantly lower in the T_2 group. Our results are in agreement with the findings of Eid *et al.* (2006), who reported that vitamin E reduced the percentage of dead spermatozoa and increase the percentage of live spermatozoa in chicken semen. To our knowledge, the present study is the first one on this topic in the KN breed. Fertility was significantly higher after adding 150 IU vitamin E/kg diet. In the chicken, fertility was lower when basal diets were provided but increased when vitamin E was added (Lin *et al.*, 2005). Vitamin E deficiency adversely affects the fertility of male Japanese quail (Biswas *et al.*, 2007).

Biochemical characteristics

The biochemical evaluation of seminal plasma in vitamin E treated and control group of birds have been presented in Table 3. No significant differences were observed among the three treated groups in relation to protein and nitric oxide. As compared to other groups, glucose concentration was significantly higher in the T_2 group. This was reflected by the higher presence of transparent fluid in semen at the time of collection. Mohan and Moudgal (1996) reported that chicken semen has a small quantity of glucose which comes mostly from the transparent fluid of the cloacal gland at the time of semen collection. So, it may be surmised that the transparent fluid concentration in semen is directly proportional to glucose content in seminal plasma. The phosphatase activity in semen is influenced by the dietary vitamin E as evidenced by significantly higher of acid phosphatase in the T_2 group. In contrast, an opposite trend was noticed in relation to alkaline phosphatase activity. This suggested that a reverse relation exists between acid phosphatase and alkaline phosphatase activities. Our results suggested many fold higher activity of acid phosphatase than alkaline phosphatase in seminal plasma. This is an agreement with the work of various workers (Datta *et al.*, 1980; Yousef *et al.*, 2003). The transaminase activity in seminal plasma is considered as an index of sperm cell membrane instability (Kundu and Panda, 1991). The release of transaminase into seminal plasma after the processing of semen is a useful indicator of injury to chicken spermatozoa (Matsumoto *et al.*, 1985). In the present study, the tranaminase activity was significantly higher in the T_1 group reflected the poor quality of semen as compared to T_2 group. This may be due to greater leakage of the enzyme from the sperm in control group. The results from the present study indicated that supplementation of vitamin E reduced the production of free radicals and the profile of transaminase activity in seminal plasma can be considered as an index of semen quality of KN cock.

In the case of vitamin E concentration, increasing the amount of supplementation to 150 IU vitamin E/kg diet resulted in a marked increase in the concentration of vitamin E in seminal plasma and spermatozoa compared to control group. However, when the diet was supplemented with 300 IU vitamin E/kg diet, the response was less than with 150 IU vitamin

E/kg diet. A relatively minor increase in the concentration of the vitamin in semen was achieved by increasing the dietary provision from 150 to 300 IU vitamin E/kg diet. The result suggesting that the saturation level is attained at a fairly low concentration of α-tocopherol in the spermatozoa and seminal plasma. Our results are in agreement with Surai *et al*. (1997), who reported that the concentration of α-tocopherol was approximately twice as high when supplementation were 200 mg/kg diet compared with control (20 mg/kg). However supplementation of 1000 mg/kg diet did not achieve any further increase in the concentration of α-tocopherol in the seminal plasma and spermatozoa.

CONCLUSION

It is concluded from the foregoing discussion that a moderate level of vitamin E (150 IU/kg) supplementation is beneficial for semen characteristics, i.e., physical and biochemical of Indian reared KN cock. α-Tocopherol concentrations were also significantly higher in seminal plasma and spermatozoa when supplementing the diet with a moderate level of vitamin E. However, further work is essential to confirm and extend these findings.

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