The influence of inclusions of vitamin E and corn oil on semen traits of Japanese quail (*Coturnix coturnix japonica*)

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Abstract

Reported was an investigation of the effect of vitamin E (Vit.E) and corn oil on semen traits of male Japanese quail (*Coturnix coturnix japonica*). From 8 to 20 wk of age, birds were raised on corn-based diets supplemented with corn oil (0 and 3%) and Vit.E (National Research Council (NRC) recommended 25 mg/kg/day/dry matter and 150 mg/kg/day/dry matter) in a 2 × 2 factorial manner. The diet was supplemented with corn oil and Vit.E (E2C2) which provided additional *n*-6 polyunsaturated fatty acids in the form of 20:4*n*-6 and 22:4*n*-6 in spermatozoa phospholipid. The left testes weights were increased ($P < 0.01$) in groups that received Vit.E in the diet (3.95 and 4.12 g, respectively) ($P = 0.03$) and combined testes weight was the greatest in E2C2 group (7.57 g) ($P = 0.02$). Semen volume increased throughout the experiment in the E2C2 group. E2C1 and E2C2 birds had the greatest (90.05% and 92.1%, respectively) live sperm percent by comparison with other groups. The susceptibility of semen to lipid peroxidation *in vitro* was increased in quail fed E1C1 and E1C2, but was reduced when 150 mg Vit.E kg/day/dry matter feed was provided in the diet. The amount of Vit.E in the seminal plasma of E1C1 and E1C2 groups was ($P < 0.01$) less than that in the other two groups (E2C1 and E2C2). From this study, it may be concluded that increasing diet *n*-6/*n*-3 ratio can be beneficial for semen traits, however, this application increased sperm peroxidation sensitivity but it can be controlled by inclusion of antioxidant such as Vit.E (150 mg/kg/day/dry matter) to diet.

Keywords: Cloacal gland; Foam; Semen traits; Polyunsaturates; Japanese quail

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1. Introduction

In all species, phospholipids are the major lipid component of spermatozoan membranes. In addition, they contain large amounts of polyunsaturated fatty acids (PUFAs). PUFAs of the *n*-3 and *n*-6 series are essential fatty acids, because they cannot be synthesized in vertebrates and must be provided in the diet (Parks and Lynch, 1992).

Since the 19th century, many researchers have reported that lipids are a basic component of semen, contributing to the membrane structure of spermatozoa, the metabolism of the sperm cells, and their ability to capacitate and fertilize the female gamete. In birds, the lipid composition of spermatozoa has an influence on fertility (Ansah and Buckland, 1982).

Spermatozoa are rich in phospholipids (about 80% of total lipids) and may be quite sensitive to the availability of dietary PUFAs (Anderson and Conner, 1994). Different oil sources have been supplied to male chicken breeders with the aim to improve sperm production and fertility through sperm enrichment in *n*-6 or *n*-3 polyunsaturates (Cerolini et al., 2006).

Physiologically, the greater proportions of PUFA in avian sperm are integral for maintaining membrane fluidity and flexibility during the fertilization process (Surai et al., 2001). Numerous studies have demonstrated a direct link between compromised poultry sperm function after *in vitro* storage and lipid peroxidation (Cecil and Bakst, 1993). Indeed, peroxidative damage to spermatozoa is believed to be a major cause of male subfertility in human (Jones et al., 1979] and [Selley et al., 1991]) and turkey (Cecil and Bakst, 1993).

Vitamin E (Vit.E) is the major antioxidant in biological membranes and prevents free radical-induced oxidative damage by trapping reactive oxygen radicals (Cerolini et al., 2006). The amount of PUFA present in chicken sperm is dependent by the amount of Vit.E bound in the plasma membrane that in turn is dependent on dietary supplementation (Surai et al., 1997).

Damaging effects of lipid peroxidation on poultry sperm include morphological defects, reduced motility, and poor fertilizing ability (Long and Kramer, 2003). Research with mammalian spermatozoa has documented other deleterious effects of lipid peroxidation, such as loss of membrane fluidity, decreased acrosomal function, damage to sperm chromatin, and reduced sperm oocyte fusion, which also may be involved in the compromised fertility of stored poultry semen (Long and Kramer, 2003). Vit.E also plays a general stabilizing effect on membranes, which can be independent by its antioxidant properties (Cerolini et al., 2006).

The above findings indicated possible beneficial effects of Vit.E and PUFAs in male fertility. Therefore this experiment was designed with several objectives: (1) to evaluate the effects of feeding corn oil on the lipid and fatty acid composition of quail

spermatozoa; (2) to evaluate the ability of Vit.E to reduce lipid peroxidation; and (3) to evaluate the effects of different amounts of dietary Vit.E and corn oil supplementation on reproductive parameters of quail breeders (*Coturnix coturnix japonica*).

2. Materials and methods

2.1. Diets and birds

Male Japanese quail (*n* = 112) with similar mean weight were housed in wire cages equipped with nipple drinkers under uniform environmental conditions in East Azarbaijan (Bonab) Animal Science Research Centre from Feb to Apr 2008. Male Japanese quail were randomly allotted to four treatment groups of similar mean ± sd weight each of which included 28 Japanese quail cock breeders (4 respective replicates of 7 birds each) from 8 to 20 wk of age. Two concentrations of Vit.E (National Research Council (NRC) recommended 25 mg/kg/day/dry matter and 150 mg/kg/day dry matter, respectively) and corn oil (0% and 3%, respectively) were tested in a completely randomized design with a 2 × 2 factorial arrangement of treatments. The following four dietary treatments were used, the group E1C1 received the control diet which contained NRC recommended (25 mg/kg) Vit.E without corn oil; group E1C2 received the control diets supplemented with 3% corn oil; group E2C1 received the 150 mg/kg Vit.E without corn oil and group E2C2 received the 150 mg/kg Vit.E with 3% corn oil. Before the experimental diet formulation, feed ingredients were analyzed for their CP, CF, crude fat and total sugar according to the methods of the Association of Official Analytical Chemists (1984). The experimental diets were formulated to meet minimum nutrient requirements of the Japanese quail breeder, as established by the National Research Council (NRC, 1994). All experimental groups received a quail breeder diet (19.31% CP, 2800 kcal of ME/kg, 2.41% total calcium and 0.34% available phosphorus) from 8 to 20 wk of age. The experimental diets in mash form and drinking water were provided *ad libitum*. Quails were kept at 22–24 °C and with a light regimen of 16L:8D photoperiod until the end of the experiment. The ambient relative humidity was 50–60%.

2.2. Sample collection and laboratory analyses

Live body weights were recorded at the beginning and at the end of the study. Feed consumption was measured weekly. The foam produced by each bird was collected in separate airtight glass bottles to prevent evaporation (Mohan et al., 2002). Quantitative measurements on cloacal gland foam production were conducted immediately using an electronic analytical balance.

The cloacal gland of each bird was measured to the nearest 0.01 mm. Measurements were done on width (lateral) and height (dorsoventral) using callipers (500–161 U, Mitutoyo, Japan). The product of these two measurements (height and width; mm^2) was used as an index for gland size, as described by Siopes and Wilson (1975) and Biswas et al. (2007).

Semen was collected twice-weekly (after 14 wk of age) via the method described by Burrows and Quinn (1937) and evaluated for semen volume, sperm concentration, sperm viability, abnormal sperm, and sperm motility. Immediately after collection of semen, the volume was determined using a graded-level collection tube, and sperm concentration was determined using a haemocytometer procedure (American Optical Company, New York, USA) ([Bakst and Cecil, 1997] and [Golzar Adabi et al., 2007]). The percentage of motile spermatozoa was determined by compound Olympus-BX50 light microscope (Olympus Optical Co, Ltd., Tokyo, Japan) at 10× magnification. For this reason a 2–3 mm drop of semen was placed on a warmed (+40 °C) microscopic slide and covered with a cover glass (Biswas et al., 2007). At least six microscopic fields were examined. Each slide was evaluated twice. The percentage of live, dead and abnormal spermatozoa was evaluated after nigrosin–eosin staining (Golzar Adabi et al., 2007) and bright field microscopy at 100× magnification. Dead sperm will absorb the stain and appear the same colour as the background and live sperm will resist the stain and appear clear, therefore, unstained spermatozoa were regarded as live whereas stained or partially stained spermatozoa were counted as dead. The percentage of abnormal spermatozoa was evaluated from the same by examining the morphology of a total count of 100 spermatozoa (Golzar Adabi et al., 2007).

Blood samples (1 ml) were drawn from the wing vein using a 25-gauge needle (0.5 mm × 16 mm) and 2.5 ml syringe. Blood samples were immediately centrifuged in a micro-liter centrifuge at 3000 × *g* for 10 min (D-78532, Hettich Zentrifugen, Tuttlingen, Germany) and the serum was separated for hormone assay. Testosterone was assayed in each sample by a spectrophotometer using standard commercial testosterone kits (Kavoshyar-Iran Co kit®; 8386681, Tehran, Iran). At the end of the experiment, three birds from each pen were euthanized by cervical dislocation. The abdominal cavity was opened and testes were dissected out. The weight of each testis was measured using an electronic analytical scale. At 20 wk of age, the semen samples of five male Japanese quails in each experimental group were collected to determine lipid peroxidation of the semen; consequently three replications were obtained per experimental group (El-ansary et al., 2004).

Lipid peroxidation of the semen was determined by measuring malonaldehyde (MAL), which was the primary stable by-product of lipid peroxidation using a procedure of Cecil and Bakst (1993) and Wang et al. (1997). The MAL concentration of the sample was calculated by comparing it with the optical density produced by MAL standard (T-9889, Sigma Chemical Co.).

The total fat of spermatozoa was extracted according to Folch et al. (1957) and methylated with 5% boron trifluoride methanol complex in methanolic solution. The lipid profile was determined by means of gas chromatography equipped with a BPX70 capillary column (SGE capillary column; length, 30 m; I.D., 0.22 mm; film thickness 0.25 μm; 70% cyanopropyl polysilphenylene-siloxane stationary phase), film and a flame ionization detector. The operating conditions of the gas chromatograph were as follows: the initial temperature was 140 °C for 6 min. It was increased to 180 °C at the rate of 40 °C per a minute and remained at this temperature for 8 min. Thereafter, the

temperature increased to 190 °C at the rate of 20 °C per a minute. It remained stable at final temperature for 26 min. The injection temperature was 240 °C and helium was used as the carrier gas. The fatty acid percentage was integrated and then calculated by means of direct normalization of the peak areas. Each fatty acid was identified in the form of a methyl ester by comparing the retention times with the standard acquired at Sigma Interlab A.S.[1]

For the assessment of the Vit.E concentrations in seminal plasma, a high performance liquid chromatography (HPLC) method was used. Measurement of Vit.E was done according to Lee et al. (1992) method. Briefly, an aliquot of the seminal plasma was placed into a sample preparation vial, which was filled with a reagent (20 mg sodium sulphate) for precipitation. A stabilizing reagent (butanol ethyl acetate: 1-1, v/v) was added. The solid component of seminal plasma being precipitated was removed by centrifugation. Finally, 20 μL of the supernatant was injected into the HPLC system. Detection was determined by fluorescence at 286 nm.

2.3. Statistical analysis

All percentage data were subjected to arcsin square root transformation (Steel and Torrie, 1960). The data were analyzed by general linear model procedure of SAS software (SAS Institute, 1986, SAS Institute Inc., Cary, NC, USA). When necessary mean separation was accomplished by using Duncan's multiple-range test (Duncan, 1955) a probability value of less than 0.01 and 0.05 was considered significant, unless otherwise noted. The statistical model used was:

$y_{ijk}=\mu+a_i+b_j+(a\times b)_{ij}+e_{ijk}$ where y_{ijk} is any observation; μ is the overall mean; a_i is the effect of the corn oil (control, inoculated); b_j is the effect of the addition of the Vit.E (control, inoculated); $(a \times b)_{ij}$ is the interactions; and e_{ijk} = error term.

2.4. Ethical and permissions statement

The experiment was performed in accordance with ethical commissions for animal welfare of Iran Agricultural Ministry.

3. Results and discussion

The fatty acid composition of the diets (Table 1) clearly reflected the origin of the added corn oil. As expected, the corn oil diet contained a greater proportion of linoleic acid (18:2*n*-6) which was the major fatty acid (50.8%) and the only one of the *n*-6 series. The ratio between *n*-6 and *n*-3 fatty acids were 21.10 and 38.77 in the basal and corn oil diet, respectively (Table 1). Fatty acid compositions of spermatozoa are presented in Table 2. Analysis of spermatozoa fatty acid (collected after 14 wk old) elucidates the effects of varying the fatty acid profile of the feed on the composition of sperm lipids (Table 2).

In the present experiment dietary *n*-6 PUFAs were successfully transferred into spermatozoa and E2C2 group had the greatest amount of C20:4*n*-6 and C22:4*n*-6. Surai et al. (1997) showed that supplemented diet with 200 mg/kg/day/dry matter Vit.E resulted in 53.3% and 31.94% increases in the proportions of the 20:4*n*-6 and 22:4*n*-6 components of the spermatozoa, respectively compared to the control group.

Table 1. Fatty acid composition of the experimental diet (as % of total fatty acids).

Fatty acid	Basal diet	Corn oil diet
Saturated		
16:0	23.12	13.41
18:0	2.1	1.75
20:0	4.1	2.8
Total	29.71	18.36
Monounsaturated		
16:1*n*-9	nd	0.3
18:1*n*-9	27.31	27.45
Total	27.31	27.75
PUFA *n*-3		
18:3*n*-3	1.86	1.31
Total	1.86	1.31
PUFA *n*-6		
18:2*n*-6	39.25	50.8
Total PUFAs	41.11	52.11
n-6/*n*-3	21.10	38.77

nd, not detected.

PUFAs = polyunsaturated fatty acids.

Table 2. Fatty acid composition (values are means ± SE) of the spermatozoa of male Japanese quail breeders (as % of total fatty acids).

Fatty acid	E1C1	E1C2	E2C1	E2C2	*P*-value
Saturated					
14:0	1 ± 0.21	0.9 ± 0.19	0.8 ± 0.23	0.9 ± 0.18	0.09
16:0	15.1 ± 0.62	14.9 ± 0.37	15.6 ± 0.43	15.3 ± 0.33	0.07
18:0	21.3^{a} ± 0.44	22.4^{a} ± 0.46	20.2^{b} ± 0.52	20.9^{b} ± 0.49	0.03
Total	37.4^{ab} ± 1.06	38.2^{a} ± 1.08	36.6^{b} ± 10.6	37.1^{ab} ± 10.9	0.02
Monounsaturated					
16:1n-9	0.8 ± 0.09	0.75 ± 0.07	0.81 ± 0.07	1 ± 0.03	0.07
18:1n-9	13.2^{a} ± 0.30	13.2^{a} ± 0.32	12.6^{b} ± 0.44	11.4^{c} ± 0.65	0.002
20:1n-9	1.8 ± 0.07	1.2 ± 0.08	1.7 ± 0.06	1.9 ± 0.08	0.1
Total	15.8 ± 0.82	15.15 ± 0.87	15.11 ± 0.80	14.3 ± 0.83	0.3
PUFA n-3					
20:5n-3	0.44^{b} ± 0.03	0.51^{a} ± 0.05	0.45^{b} ± 0.02	0.2^{c} ± 0.01	0.04
22:5n-3	1.75^{b} ± 0.1	1.83^{a} ± 0.1	1.58^{b} ± 0.1	1.4^{c} ± 0.1	0.02
22:6n-3	0.19^{ab} ± 0.03	0.25^{a} ± 0.04	0.2^{ab} ± 0.02	0.16^{b} ± 0.02	0.001
Total	2.38^{ab} ± 0.31	2.59^{a} ± 0.35	2.23^{ab} ± 0.31	1.76^{b} ± 0.32	0.03
PUFA n-6					
18:2n-6	2.6^{b} ± 0.57	1.9^{c} ± 0.52	2.8^{b} ± 0.51	3.1^{a} ± 0.56	0.02
20:4n-6	9^{b} ± 0.60	9.6^{b} ± 0.60	9.7^{b} ± 0.60	10.2^{a} ± 0.60	0.04
22:4n-6	28.7^{b} ± 1.65	27.3^{c} ± 1.45	28.2^{b} ± 1.58	30.4^{a} ± 1.82	0.03
Total	40.3^{b} ± 2.30	38.8^{c} ± 2.19	40.7^{b} ± 2.41	43.7^{a} ± 2.56	0.009
Total PUFAs	42.68^{b} ± 2.18	41.39^{c} ± 2.20	42.93^{b} ± 2.37	45.46^{a} ± 2.25	0.03
n-6/n-3	16.93	14.98	18.25	24.83	

[abc]For comparison among birds on different diets, values within a row that do not share a common letter are significantly different ($P < 0.05$).

This increase in 20:4*n*-6 and 22:4*n*-6 in sperm phospholipid was accompanied by a decrease in the proportion of 18:2*n*-6. The characteristic polyunsaturate of avian spermatozoa (22:4*n*-6), in chickens is normally derived from 18:2*n*-6 by sequential desaturation and elongation steps (Walzem, 1996). C22:4*n*-6 is functionally, not only quantitatively, but also the most important sperm PUFA and has been suggested as a marker of the reproductive efficiency of the male chicken (Cerolini et al., 2006). Oleic acid (18:1*n*-9) was the main monounsaturated fatty acid in the diets as in the spermatozoa. 16:1*n*-9 was not detected in basal diets and was negligible in the corn oil diet (0.3%). It was also present in low proportions in spermatozoa (max. 1% in E2C2 group). Wathes et al. (2007) reported that altering the PUFA sources in the diet resulted in concomitant changes in the *n*-6 and *n*-3 composition of sperm (e.g., boar, cockerel).

In consideration, the amount of linoleic acid (18:2*n*-6) was low in spermatozoa because that was replaced by arachidonic (20:4*n*-6) and docosatetraenoic (22:4*n*-6) acids.

The 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3 fatty acids were detected in all sperm samples. Sperm enriched in C22:5*n*-3, C22:6*n*-3 or C22:4*n*-6 results in greater ($P < 0.01$) fertility values following artificial insemination compared to control sperm, and such an effect is clearly age dependent being present in young and not in old birds (Cerolini et al., 2006). However PUFAs of the *n*-3 series were represented to a greater extent in the birds fed the E1C2 diet (2.59%) than in those fed the E2C2 diet (1.76%). Consequently, the corresponding *n*-6/*n*-3 ratios were greater in the spermatozoa of males fed the E2C2 diet (24.83%) as opposed to the E1C2 diet (14.98%) ($P < 0.05$; Table 2). Total PUFAs were greater with the E2C2 (45.46%) diet by comparison with other groups.

Mean body weight (bwt) and feed intake of the birds in the different treatment groups showed no significant differences. There was no interaction of Vit.E and corn oil on body weight and feed intake (Table 3).

Foam weight was not affected by corn oil, but varied significantly according to the Vit.E and it increased in E2C1 and E2C2 group ($P = 0.002$). There was no significant change of foam weight between corn oil and Vit.E (Table 3). A reduction of foam weight was observed due to birds fed the E1C1 and E1C2 diet. A similar trend was noted for size of the cloacal gland scores. A positive correlation was evident for the size of cloacal gland with frequency of foam discharge and foam production (Biswas et al., 2007). Fujihara and Koga (1991) reported that fertilizing ability of quail spermatozoa was increased when a foamy substance was mixed with freshly ejaculated spermatozoa.

There were no differences in plasma testosterone concentration in any of the treatment groups (Table 3). Dietary supplementation in Taiwan native cockerels of Vit.E at 0, 20, 40, 80 and 160 mg/kg, did not significantly affect plasma testosterone concentration, although there was a trend towards an increase with 80 and 160 mg/kg (Lin et al., 2005). Testosterone concentrations ranged from 1.017 to 1.102 (ng/ml; Table 3).

The weights of the left testes of E2C1 and E2C2 groups were greater ($P < 0.01$) than that of all other groups; additionally, there were no significant differences among mean weights of the other treatment groups. Right testicular weights, however, were similar in all groups. The combined testes weights of groups E2C2 were 15.4% and 20.5% greater compared with the control (E1C1) and E1C2 groups, respectively (Table 3; $P < 0.01$). There was no interaction between Vit.E and corn oil for any of the left and combined testes weights (Table 3). In agreement with these findings, Surai and Ionov (1992) reported increased testes weight when ganders were fed with 20–40 IU Vit.E.

Table 3. The effects of dietary Vit.E and corn oil supplementation on bwt, feed intake, testes weight, foam weight, cloacal gland index and testosterone concentration of male Japanese quail breeders.

Parameters	E1C1	E1C2	E2C1	E2C2	SEM	Probabilities		
						E	C	E × C
Initial live bwt (g)	195.19	191.45	197.57	200.24	3.24	0.12	0.15	0.23
Ultimate live bwt[‡] (g)	196.31	194.28	199.41	198.87	3.60	0.14	0.17	0.20
Feed intake[†] (g/quail per d)	21	22	20	21	0.32	0.16	0.13	0.14
Testes weight[2]								
Left (g)	3.18^{b}	3.21^{b}	3.95^{a}	4.12^{a}	0.23	0.03	0.12	0.1
Right (g)	3.41	3.07	3.18	3.45	0.24	0.13	0.10	0.13
Combined (g)	6.56^{b}	6.28^{b}	6.77^{ab}	7.57^{a}	0.12	0.02	0.09	0.06
Foam weight[‡] (mg/bird)	21.12^{b}	25.46^{b}	30.78^{a}	30.89^{a}	0.04	0.002	0.23	0.18
Cloacal gland index (mm^2)	321.98^{b}	319.55^{b}	342.21^{a}	378.68^{a}	0.24	0.0001	0.08	0.12
Testosterone (ng/ml)	1.017	1.098	1.101	1.102	0.005	0.10	0.16	0.21

[ab]For comparison among birds on different diets, values within a row that do not share a common letter are significantly different ($P < 0.05$).

[†] Values represent the means of 15 observations (15 birds/dietary treatment).
[‡] Values represent the means of 9 birds per dietary treatment at 20 wk of feeding the diets.

The decreased sperm concentration in E1C1 and E1C2 groups may be related to the decreased weight of the testes. It is well known that amount of spermatozoa is dependent upon testes weight (Danikowski et al., 2002).

Sperm characteristics *in vitro* are presented in Table 4. Semen volume and sperm concentration was ($P < 0.01$) affected by treatments. Most notably, the volume of semen per ejaculate obtained from the birds fed with corn oil and Vit.E was greater (25.8% and 20.2%, respectively, for E2C1 and E2C2 group compared to the control diet) than observed in the birds fed with the other treatments.

Table 4. The effects of dietary Vit.E and corn oil supplementation on semen characteristics, malondialdehyde and sperm Vit.E status of male Japanese quail breeders[†].

Parameters	E1C1	E1C2	E2C1	E2C2	SEM	Probabilities		
						E	C	E × C
Sperm motility (%)	72.61^{b}	70.26^{b}	80.24^{a}	80.89^{a}	0.068	0.004	0.09	0.19
Semen volume (µl)	15.02^{c}	16.51^{c}	17.78^{b}	19.25^{a}	0.008	0.002	0.02	0.07
Sperm concentration (million/mm^{3})	2.41^{b}	2.35^{b}	3.02^{ab}	3.25^{a}	0.012	0.006	0.09	0.2
Dead sperm (%)	6.48^{a}	7.89^{a}	4.92^{b}	4.04^{b}	0.0035	0.03	0.014	0.13
Live sperm (%)	86.82^{b}	84.9^{c}	90.05^{a}	92.1^{a}	0.079	0.004	0.003	0.08
Abnormal sperm (%)	6.7^{a}	7.21^{a}	4.03^{b}	3.86^{b}	0.0019	0.001	0.28	0.06
Malondialdehyde formed (µg/ml initial semen)	1.8^{b}	2.3^{a}	0.6^{c}	0.5^{c}	0.001	0.004	0.007	0.11
Vit.E (µg g^{-1})	1.2^{c}	0.8^{d}	2.0^{a}	1.6^{b}	0.024	0.001	0.004	0.009

^{abc}For comparison among birds on different diets, values within a row that do not share a common letter are significantly different ($P < 0.05$).

[†] Values represent the means of 72 observations (36 birds/dietary treatment × twice per wk).

Sperm concentration in E1C2 was numerically less than E1C1. There was no interaction of Vit.E and corn oil on semen volume and semen concentration (Table 4). This finding is in contrast to that Blesbois et al. (1997), who found no improvements in

the mean concentration of spermatozoa, the volumes of the ejaculates, or consequently number of spermatozoa per ejaculate, however, Cerolini et al. (2006), reported both *n*-3 and *n*-6 rich diets affect the semen production. In spite of the fact that highly variable results have been reported for the effect of *n*-6 rich diets on spermatozoa production, a positive effect on semen volume and total sperm number (Surai et al., 2000) has been reported.

There were no effects of corn oil and corn oil × Vit.E interaction on sperm motility, but Vit.E ($P = 0.004$) increased sperm motility so, the greatest motility (forward motion) of spermatozoa was in groups E2C1 and E2C2 (80.2% and 80.9%, respectively) and differed significantly from the control group (72.6%) and group E1C2 (70.3%) ($P < 0.01$; Table 4).

Blesbois et al. (1993) showed that variation in sperm concentration is reflected in the degree of motility of spermatozoa. Both *n*-3 and *n*-6 polyunsaturate rich diets improve the progressive movement of the male gametes, the fundamental action of the spermatozoon within the oviduct if it is to reach the place of fertilization. Such a positive effect is further emphasized if the polyunsaturates are fed in combination with a greater amount of Vit.E (Cerolini et al., 2003).

The percentage of dead sperm was less in the E2C1 and E2C2 treatments compared to other treatments (Table 4; $P < 0.05$), but the effect of Vit.E × corn oil interaction was not significant for surviving sperm. The percent of abnormal sperm per ejaculate was also decreased by Vit.E supplementation ($P = 0.001$), although there was no effect of corn oil and Vit.E × corn oil interaction (Table 4).

Natural antioxidants, such as Vit.E are present in chicken and turkey sperm where it helps to maintain membrane integrity and sperm motility (Donoghue and Donoghue, 1997). Wu et al. (1973) reported that the spermatozoa from rats on the basal diet with the addition of both Vit.E (60 ppm) and selenium (0.1 ppm) showed normal morphology and excellent motility. Supplementing the diet of quail with Vit.E and corn oil changed sperm characteristics in the present study favorably, increasing viability, and the sperm concentration with progressive motility and normal morphology. In an earlier study with turkey semen, supplemental Vit.E improved sperm motility and membrane integrity (Donoghue and Donoghue, 1997), whereas Long and Kramer (2003), data do not reflect improvements in either sperm motility or viability after 24 h in the presence of Vit.E. Beneficial effects of Vit.E (8 μg/ml diluent) on motility and fertility of stored rooster sperm have been reported, although the benefits occurred only early in production for birds 30–40 wk old (Blesbois et al., 1993). Latshaw and Osman (1974) reported that, in the chicken, fertility was less when basal diets were provided but increased when Vit.E was added. On the basis of previous reports, Vit.E deficiency adversely affects fertility of male Japanese quail and an adequate amount of Vit.E is needed in the breeding quail diet for achievement of normal reproduction ([Price, 1968] and [Golzar Adabi and Davoudi, 2005]) consistent with findings in the present study.

The MAL was detected in fresh ejaculates from quail throughout the 20 wk production period (Table 4). Semen from birds fed without Vit.E displayed an increased susceptibility to lipid peroxidation *in vitro* (Table 4), as expected, corn oil supplementation without Vit.E has the greatest impact ($P < 0.01$) on enhancing MAL production. However, the dietary combination of corn oil with Vit.E at 150 mg/kg markedly ($P < 0.01$) decreased this susceptibility and increased semen quality. According to Klasing (1998) highly unsaturated acids in the diet increase Vit.E requirements, to prevent oxidation of unsaturated lipid materials within the cells. Both the proportion of abnormal sperm and the amount of MAL followed a similar trend: the greatest values were recorded in the E1C1 and E1C2 groups, and the least and most desirable values in the 150 mg/kg/day/dry matter Vit.E supplemented groups (Table 4). Landat et al. (2002) showed that MAL of sperm was greater in infertile men when compared to fertile men.

Measurements of the products of lipid peroxidation, such as MAL and 4-hydroxyalkenal, correlate negatively with semen quality in both man and domestic animals (Kasimanickam et al., 2006). Semen from birds fed without Vit.E displayed an increased susceptibility to lipid peroxidation. This information corroborates previous studies (Surai et al., 2001) where dietary combination of arasco and tuna orbital oils with Vit.E at 200 mg/kg markedly decreased this susceptibility below that observed for semen from birds on the maize oil diet.

The effect of the increased dietary provision of Vit.E on sperm Vit.E content is reported in Table 4. There was a reduction in Vit.E content of the seminal plasma when the birds were fed on the E1C1 and E1C2 ($P < 0.01$; Table 4). In spite of the attenuated response of the content of Vit.E in semen to dietary manipulation, the 40% and 33.3% increase that was achieved in the concentration of this vitamin in semen (groups E2C1 and E2C2, respectively, in comparison to the control group) did result in an improvement in the resistance of the samples to lipid peroxidation (Table 4) but feeding birds with the corn oil diet without supplementation with excess Vit.E resulted in a marked depletion of Vit.E from the sperm, similar to previous reports (Surai et al., 2000) where a noticeable reduction of Vit.E was found in tissues. According to Surai et al. (1998), Vit.E supplementation in the diet resulted in an increase in the α-tocopherol concentrations of sperm and testicles. These effects were associated with a decrease in susceptibility to lipid peroxidatoin. Experiments on chickens have shown that feeding more PUFAs in the diet reduced the antioxidant status and quality of the semen (sperm concentration, semen volume). The importance of lipid peroxidation in this context was suggested by the ability of Vit.E, a chain breaking antioxidant, to reverse the negative impact of PUFA supplementation ([Zanini et al., 2003] and [Cerolini et al., 2005]).

However in research on human Nouri et al. (2008) showed that the amount of vitamins E and C in seminal plasma of normozoospermic were greater than in asthenoteratozoospermic males ($P < 0.01$) and the amount of these vitamins in the spermatozoa of normozoospermic was less than in asthenoteratozoospermic males ($P < 0.01$).

Considering the conditions from the current study it can be concluded that dietary PUFAs enhance sperm quality by elevating the ratio of *n*-6 to *n*-3. It, however, increases sperm susceptibility to peroxidation so, inclusion of 150 mg/kg Vit.E to diet is necessary for attenuating the threat. However, further research is necessary to confirm these findings and explore other dietary oil sources supplemented by different amounts of Vit.E and other antioxidants.

Conflict of interest

There was no conflict of interest throughout the duration of the current investigation.

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