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EFFECT OF OMEGA-3 SOURCES AND VITAMIN E SUPPLEMENTATION IN THE TURKEY TOMS DIET ON SEMEN CHARACTERISTICS AND FERTILIZING ABILITY.

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ABSTRACT

This study was carried out to investigate the effect of different oils (sunflower, fish and linseed oils) as sources of omega 3 and 6 with or without vitamin E (VE) supplementation, on semen quality and reproductive performance of turkey toms. Thirty six Bronze turkey toms at thirty-six weeks of age were used, birds were divided to equal homogenous three groups (n=12 toms). The 1st group was fed the basal diet containing 2% sunflower oil (SO, as control), while the 2nd and 3rd groups were fed the same diet with 2% fish oil (FO) or linseed oil (LO) instated of SO. All groups were divided into two subgroups (n=6 toms), the 1st subgroup was fed diet without vitamin E (VE) supplementation (the diet contain 50 mg as alpha-tocopheryl acetate/kg diet from premix, VE), while the 2nd subgroup was fed diet supplemented with 100 mg VE/kg diet as anti-oxidant (150 mg VE /kg diet as a total content). Results indicated that FO or LO in the diet of turkey toms at a level of 2% may affect slightly weight without any adverse effect on body weight at 48 wks of age or body weight gain (36-48 wks of age). Dietary supplementation of VE increased (P<0.05) body weight as compared to those fed diet without VE. Semen characteristics of toms were affected (P<0.05) by omega-3 sources and VE supplementation, except for ejaculate volume. Tomes fed 2% FO+150 mg VE/kg diet had higher (P<0.05) percentages of sperm motility (75.66%) and livability (82.61%), followed by those fed the same oil diet without VE (74.70 and 81.50%) and LO +150 mg VE/kg diet (74.24 and 81.38%) as compared to other treatment groups, respectively. All treatments increased (P<0.01) fertilizing ability of toms and hatchability rate of total eggs. Marked differences were noted among dietary treatments in the histological examination of testicular tissue and epididymis of toms. This study concluded that turkey tom diets containing 2% fish oil or linseed oil plus 150 mg VE/kg diet as a total content are needed to obtain the best reproductive performance, semen quality, fertility and hatchability.

Keywords: *Turkey toms, sunflower oil, fish oil, linseed oil, semen characteristics, fertility, hatchability.*

INTRODUCTION

Supplementations of fat and oil to poultry diets were used to increase the energy density, which represent an economic tool of producing rich energy diet. Fatty acids consist of carbon, oxygen and hydrogen and are classified to three types including, Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA), or Polyunsaturated Fatty Acids (PUFA). Fats consist of different fatty acids which changed according to source of animal or plant fats. Animal fats contain a long-chain saturated fatty acid (palmitic acid), except for fish oil, while vegetable oils contain a huge quantities of long chain unsaturated fatty acids Midilli *et al*., 2009). Lipids represent as a major component of semen (spermatozoa and seminal plasma) which may act various specific roles. Generally, sperm lipids were supposed to be an energy substrate for sperm metabolism, and involved in most functions, leading to higher fertilizing ability (Scott, 1973). Male diets significantly alter the fertilization process of fresh sperm. Structure of membrane, fluidity, or susceptibility to peroxidation of spermatozoa may change by altering specific phospholipids, fatty acids, and/or n-6: n-3 ratios (Blesbois *et al*., 1997). As controlled by these modifications, sperm viability and ability to interact with the female tract, or sperm binding to the egg, phospholipids structure of sperm membrane may be altered and consequently affect fertility. Therefore, dietary fats, especially PUFA, was reported to affect reproductive processes (Wathes *et al*. 2007), but impact of fat on fertility was conflicted (Santos *et al*. 2008). In comparing with other species, semen of turkey toms is characterized by specific fatty acid profile. Docosatrienoic acid (C22:3n-9) is the second most abundant LC-PUFA (Surai *et al*., 1998). The LC-PUFAs of n-3 and n-6 series cannot be synthesized by vertebrate thus they must be added to the diet as 18-carbon plant precursor (a-linolenic acid, C18:3n-3 or linoleic acid, C18:2n-6) or its long chain of animal sources (20–22 carbons; 4, 5 and 6 double bonds) (Cook, 1996).

Vitamin E (VE) is the major fat-soluble antioxidant, which reduces lipid peroxidation and depresses oxidative stress (Sahin *et al*., 2006). In fowl semen, VE is antioxidant inhibits lipid peroxidation in cell membranes by scavenged lipid peroxyl and alkoxyl radicals resulting in reducing oxidative damage (Cerolini *et al*., 2000). For cockerels, VE has traditionally been known as anti-sterility vitamin. This vitamin was first detected in turkey semen in 1981 later also confirmed in chicken semen. The importance of this vitamin can be seen from the fact that it constitutes 88% of chicken semen antioxidant capacity (Khan *et al*., 2012).

There is a little information about the impact of n-3 PUFA supplementation and vitamin E in the diet of turkey on semen quality and fertilizing ability and hatchability. The present work was planned to study the impact of n-3 PUFA and vitamin E dietary supplementation on semen quality and fertility of turkey toms.

MATERIALS AND METHODS

The current work was conducted at Experimental Station of Mehalet Mousa, Turkey branch,

Kafrelsheikh, Institute of Animal Production Researches, Agricultural Research Center, Ministry of Agriculture, Egypt.

Birds and feeding system:

Thirty six bronze male turkeys, aging 36 weeks and weighing 9088±79.22 g were used. Birds were divided to three homogenous groups (n=12 toms). The 1st group was fed the basal diet containing 2% sunflower oil (as control), while the 2nd and 3rd groups were fed the same diet with 2% fish oil and linseed oil instated of sunflower oil, respectively. All groups were divided into two subgroups, the 1st subgroup (n=6) was fed diet without vitamin E supplementation (the diet contain 50 mg as alpha-tocopheryl acetate/kg diet from premix, Vit. E), while the 2nd subgroup (n=6) was fed diet supplemented with 100 mg Vit. E/kg diet (150 mg Vit. E /kg diet is the total content) as anti-oxidant. Each experimental sub-group was maintained in floor pens (2x3 m).

Birds were fed *ad-libitum* and fresh water was available all the time, during the experimental period. The ingredients and the calculated chemical composition of the basal diet are shown in Table (1).

Experimental procedures:

Body weight (g) of birds in each experimental group was individually recorded at 36 and 48 weeks of age. Semen was collected once a week for 12 weeks as a collection period at 8-9 a.m. Semen was collected using the abdominal manual massage method as described by Al-Daraji (2007).

Volume of each ejaculation was measured using a graduated test tube. Sperm cell concentration was determined using the Hemocytometer method. Semen was evaluated for determination of sperm motility and livability percentages. The percentage of live and dead spermatozoa was determined from 10 µL semen mixed with 50 µL of eosin-nigrosin stain to make a thin smear. The smear was air-dried for 10 min. At least 200 spermatozoa were examined (400×) under emulsion oil to count differential morphology of spermatozoa as percentage of abnormality.

Table 1: Ingredients and calculated chemical analysis of the experimental diets.

Ingredient	Sunflower oil diet	Fish oil diet	Linseed oildiet
Yellow corn	63.4	62.4	62.9
Soybean meal (48% CP)	23.5	23.0	23.0
Wheat bran	6.0	7.5	7.0
DL Methionine	0.2	0.2	0.2
DL Lysine	0.5	0.5	0.5
Calcium carbonate	2.0	2.0	2.0
Dicalcium-phosphate	1.7	1.7	1.7
Oil (%)	2.0	2.0	2.0
Salt (Nacl)	0.4	0.4	0.4
Premix*	0.3	0.3	0.3
Calculated analysis			
Metabolizable energy, kcal/kg	2966	2954	2954
Crude protein (CP, %)	18	17. 4	17. 4
Fat (%)	2.11	2.14	2.14
Fiber (%)	4.17	4.20	4.21
Calcium (%)	1.26	1.26	1.26
Available-phosphate (%)	0.45	0.45	0.45
Lysine (%)	1.32	1.32	1.32
Methionine (%)	0.51	0.51	0.51
Methionine cystein (%)	0.79	0.79	0.79

*** Vitamins and minerals premix per kilogram diet contains: Vit. A, 5000.0 IU; Vit. D, 1100.0 IU; Vit. E, 50.0 mg; Vit. K_3, 2.0 mg; Vit. B_1, 2.0 mg; Vit. B_2, 4.0 mg; Vit. B_6, 4.5 mg; Vit. B_{12}, 30.0 µcg; Nicotinic acid, 50.0 mg; Biotin, 50.0 µcg; Folic acid, 10.0 mg; Choline chloride, 250.0 mg; Zinc, 50.0 mg; Manganese, 85.0 mg; Iron, 50.0 mg; Copper, 5.0 mg; Iodine, 0.2 mg; Selenium, 0.2 mg; Cobalt, 0.1 mg. [2]According to NRC (1994) for turkey requirement.**

Fertility trail:

Sixty untreated hens were used to study the fertilizing ability of toms and hatchability for each experimental group (10 hens/subgroup). The insemination was made as soon as possible after semen collection. The artificial insemination was done once a week. All hens were inseminated intra-vaginally. The insemination was performed by inserting 0.1 ml of the diluted semen with one millimeter tuberculin syringe into the vagina of each hen..

Fertility and hatchability rates (six hatches) of eggs were made every 2 weeks of the experimental period. The eggs were incubated in Petersime incubators. Incubators, hatcheries and eggs were fumigated for 30 min directly before setting eggs. Eggs were collected for 7 days and stored in a cooling room at 18.5 °C and 70% relative humidity. It was incubated at 99.5° F and 60% relative humidity during the first 25 days of incubation period. Eggs were turned automatically through an angle of 90° every hour until the 25th day of incubation period. Ventilation rate was automatically controlled during the incubation period. Incubated eggs were transferred from the incubator to a single hatchery at the 25th day of incubation period. The relative humidity was increased in each hatchery up to 75 to 80%, while temperature was decreased to 99° F until the end of hatching of poults in the 28th day of incubation period. Incubated eggs were individually candled using a hand candling ultraviolet lamp at 14 and 25 days of incubation period to detect the infertile eggs and embryonic mortality.

Fertility rate (FR) was calculated as the following: FR= number of fertile eggs/ total number of eggs set into the incubator. Hatchability rate (HR) was

calculated as the following: HR= number of healthy hatched chicks/total number of eggs or number of fertile eggs.

At 48 weeks of age, three toms from each treatment were randomly chosen and slaughtered to study some histological parameters of the testes and epididymis. Specimens of testes and epididymis were taken immediately after sacrifice of the toms and dissection was performed. Tissue samples from testes and epididymis were fixed by immersion in freshly prepared solution of Boun's fixative, then processed by standard technique and sections wave stained with Haematexylin and Eosin (Bancroft and Cook, 1994).

Statistical analysis:

Data were statistically analyzed by analysis of variance General Linear Model procedures (SAS, 2003). Percentage values were transformed to arcsine values before being statistically analyzed. Significant differences among means were detected by Duncan multiple range test (Duncan, 1955).

The following fixed model was used for parameters investigated as follows

$$Y_{ijk} = \mu + T_i + V_j + (TV)_{ij} + e_{ijk}$$

Where,

Y_{ijk} = Observed values

μ = Overall adjusted mean,

T_i = Fixed effect of i^{th} omega-3 source in the diet (oils, i = 1, 2 and 3);

V_j = Fixed effect of j^{th} vitamin E supplementation in the diet (j = 1 and 2);

TV_{ij} = Interaction effect between i^{th} omega-3 sources and j^{th} vitamin E supplementation;

e_{ijk} = Random error of the model, NID (0, σ2e).

RESULTS AND DISCUSSION

Composition of fatty acid of the tested oils:

Fatty acid composition of sunflower oil (SO), fish oil (FO) and linseed oil (LO) used in expremental diets as percentage of total fatty acids shown in Table (2) revealed that

FO contained higher levels of docosahexaenoic acid (C22:6n3) (10.43%), eicosenoic acid (C20:1n9) (11.18%), eicosapentaenoic acid (C20:5n3) (9.97%), erucic acid (C22:1n9) (7.78%) and palmitoleic acid (C16:1) (8.28%) than the plant oils while linoleic acid level (1.10%) was lower. The highest linolenic acid (C18:3n3) concentration was recorded in LO (56.62%). The highest linoleic acid (C18:2n6) concentrations were found in SO (62.95%). The highest levels of palmitic acid (C16:0) and total saturated fatty acids were measured in FO (14.72 and 23.21%) while LO contained the lowest level (5.85 and 9.27%). The highest total omega-3 fatty acid levels were shown in LO (57.33%), followed by FO (21.14%), whereas the highest total omega-6 fatty acid levels were shown in SO (63.15%,Table 2). Furthermore, the lowest ratio of total omega-6/total omega-3 fatty acids was recorded in FO and LO (0.094 and 0.222%), respectively. In general, these findings were nearly similar to the results of previous studies using the same oils (Güçlü *et al*., 2008; Al-Daraji *et al*., 2010).

Table 2. Fatty acid composition of sunflower oil, fish oil and linseed oil used in the experimental diets (% of total fatty acids).

Fatty acid	Sunflower oil	Fish oil	Linseed oil
SFA	10.82	23.21	9.27
C14:0	0.09	5.34	0.13
C16:0	6.31	14.72	5.85
C18:0	3.59	2.97	2.53
C20:0	0.26	0.15	0.51
C22:0	0.57	0.03	0.25
MUFA	24.216	52.59	21.15
C18:1n7	0.055	0.75	0.43
C16:1	0.09	8.28	0.42
C18:1n9	23.8	22.19	19.78
C20:1	0.26	11.18	0.28
C22:1	0.006	7.78	0.02
C24:1	0.005	2.41	0.22
n-3 PUFA	0.246	21.14	57.33
C18:3n-3	0.102	0.52	56.62
C22:6n-3	0.011	10.43	0.00
C20:5n-3	0.119	9.97	0.15
C22:5n-3	0.014	0.87	0.56
n-6 PUFA	63.15	1.99	12.74
C18:2n-6	62.95	1.10	11.9
C20:2n-6	0.145	0.15	0.04
C22:2n-6	0.09	0.34	0.0
C22:5n-6	0.33	0.40	0.8
Total PUFA	63.396	23.13	70.07
n-6/n-3 PUFA	256.71	0.094	0.222

Body weight and body weight gain of toms:

Tom body weight at 48 weeks of age and body weight gain were not affected significantly by dietary treatment through the entire experimental period (Table 3). similarly, Bozkurt *et al*. (2008) found that the dietary treatment of 1.5% fish oil to corn-soybean meal diet had no significant effect on male body weight of broiler breeder chicken at 22, 34, 46, and 58 wks of age. Also, Jankowski *et al*. (2012) reported insignificant differences in the body weights of turkeys until 63 days of age when diets were supplemented with oils from soybean, linseed or rapeseed. These findings showed that body weight gain were not adversely affected by omega-3 source and implied that these oils did not alter palatability or odour of the diet.

Disrespect to omega-3 source in the diet, toms fed diet supplemented with VE had significantly ($P<0.01$) higher body weight than those fed without VE (Table 3). These results are in agreement with Gorgy (2013), who found that toms fed diet supplemented with VE at a level of 250 mg/kg had significantly higher body weight and body weight gain at 40, 44 and 48 weeks of age than toms in the control group. In poultry, Lin *et al*. (2005) found that cockerels receiving supplements of 80 or 160 mg/kg VE had higher body weight gain than 0, 20 or 40 mg VE/kg diet. Vitamin E is necessary as an antioxidant as a regulator of the transcription and of the activity of enzymes.

Table 3. Body weight and body weight gain (gm) of turkey toms as affected by omega-3 source and vitamin E supplementation in the diet and their interaction.

Variable	Body weight at 36 weeks	Body weight at 48 weeks	Body weight Gain(gm/h)
Omega-3 source :			
Sunflower oil (control)	9134	10862	1727.91
Fish oil	9081	10794	1712.91
Linseed oil	9051	10776	1725.58
Stander error	79.22	81.51	17.53
Probability (P<)	0.75	0.73	0.81
*Vitamine E supplementation *:*			
Vit. E 50 mg/kg	9038.6	10699.2 [b]	1660.6 [b]
Vit. E 150 mg/kg	9139.1	10922.7 [a]	1783.6 [a]
Stander error	64.68	66.55	14.31
Probability (P<)	0.28	0.02	0.01
Omega-3 source x Vitamine E supplementation :			
Sunflower oil x Vit. E 50	9108.3	10750.8	1642.5
Sunflower oil x Vit. E 150*	9160.0	10973.3	1813.3
Fish oil x Vit. E 50	9055.8	10681.6	1625.8
Fish oil x Vit. E 150	9106.6	10906.6	1800.0
Linseed oil x Vit. E 50	8951.6	10665.3	1713.6
Linseed oil x Vit. E 150	9150.8	10888.3	1737.5
Stander error	112.04	115.2	24.79
Probability (P<)	0.75	0.74	0.06

*** Vit. E = 50 mg from premix and 100 mg supplementation/kg diet.**
[a, b, c] Means with different superscripts in the same column for each factor differed significantly (P<0.05).

The effect of interaction between omega-3 source and VE supplementation (Table 3).on body weight of toms at 48 wks of age and body weight gain from 36-48 wks of age studied was not significant.

Semen characteristics of toms

Regardless to the effect of VE supplementation in the diet, semen characteristics of turkey toms was significantly (P<0.05) affected by omega-3 sources in the diets, except for ejaculate volume. Toms fed diet supplemented with FO showed significantly (P<0.05) the best semen quality in terms of the highest percentages of sperm motility and livability, and sperm concentration as well as the lowest sperm abnormality percentage (Table 4). These results are in agreement with Olubowale *et al*. (2014), who found that different sources of dietary fatty acids (FO, SO and LO) had marked effects on some semen characteristics (total sperm output and sperm motility) in the cockerels tested. Also, Zaniboni *et al*. (2005) found that semen volume was not affected by dietary treatments (2% FO and control). Sperm cell concentration of turkey toms were 6.64, 6.83 and 6.87×10^9/ml in groups fed 2% SO, FO and LO in the diets, respectively (Table 4). These results are in according with Surai (2003), who found that type of the diet had particular effect on content of membrane PUFA. This content had a positive correlation with sperm number/ejaculate. Bongalhardo *et al*. (2009) also found that sperm concentration had

strong correlation with ejaculate volume in all diets containing different sources of lipids or control group. Lipid contents of head and membranes of sperm may be altered semen characteristics. In the present study, toms fed 2%FO in the diet had significantly ($P<0.01$) higher sperm motility (75.18%), live sperm cell (82.05 %) and lower ($P<0.01$) abnormal sperm cell percentages (20.27%), followed by birds fed LO (73.47, 81.11 and 21.52%, respectively) compared to those fed diets contained SO (71.27, 79.83 and 23.22%, respectively, Table 4).

These results agreed with the results obtained by Samadian *et al*. (2010), who found that semen quality in terms of sperm motility and semen concentration were significantly higher in the FO than control group. Dietary FO increased docosahexaenoic acid (DHA, C22:6 n-3) proportion in fatty acid composition of sperm. Also, Rooke *et al*. (2001) showed that sperm motility percentage in human, boar and chicken had a positive correlation with DHA. The improvements of semen quality can be discussed from the point that spermatozoa contain large amounts of PUFAs. Enriching diet with FO or LO as a source of n-3 PUFAs may be improve bird reproductive performance. Content of LC-PUFAs in semen is essential for all cell membranes (Sardesai, 1992).

Table 4. Semen characteristics of toms as affected by omega-3 sources, vitamin E supplementation in the turkey diet and their interaction.

Factors Viable	Ejaculate volume (ml)	Sperm concentration ($x10^9$cells/ml)	Sperm motility (%)	live sperm (%)	Abnormal sperm (%)
Omega-3 source :					
Sunflower oil	0.362	6.64 [b]	71.27 [c]	79.83 [c]	23.22 [a]
Fish oil	0.380	6.83[a]	75.18 [a]	82.05 [a]	20.27 [c]
Linseed oil	0.369	6.87 [a]	73.47 [b]	81.11 [b]	21.52 [b]
Stander error	0.01	0.06	0.49	0.17	0.23
Probability (P<)	0.18	0.02	0.01	0.01	0.01
Vitamine E supplementation :					
Vit. E 50 mg/kg	0.368	6.65 [b]	72.62 [b]	80.50 [b]	22.33 [a]
Vit E 150 mg/kg	0.373	6.91 [a]	74.00 [a]	81.50 [a]	21.01 [b]
Stander error	0.01	0.05	0.40	0.14	0.19
Probability (P<)	0.48	0.03	0.02	0.01	0.01
Omega-3 source x Vitamine E supplementation :					
Sunflower oil + Vit E 50	0.361	6.30	70.46	79.16	24.11
Sunflower oil + Vit E 150	0.364	6.99	72.08	80.50	22.33
Fish oil+ Vit E 50	0.374	6.67	74.70	81.50	21.00
Fish oil+ Vit E 150	0.386	6.99	75.66	82.61	19.55
Linseed oil+ Vit E 50	0.368	6.67	72.70	80.83	21.88
Linseed oil+ Vit E 150	0.370	7.08	74.24	81.38	21.16
Stander error	0.010	0.124	0.649	0.328	0.438
Probability (P<)	0.83	0.01	0.05	0.04	0.05

[*] **Vit. E =50mg from premix and 100mg supplementation/kg diet.**
[a,b,c] **Means with different superscripts in the same column for each factor differed significantly (P<0.05).**

Sperm have a great amount of n-3 LC-PUFAs (mainly DHA; C22:6, n-3) as in the brain and retina. The difference detected in phospholipids in their PUFA content may effect on the flexibility and compressibility of cell membranes. Furthermore, there is considerable evidence that the major determinant of sperm motility is the lipid composition of sperm membrane (Hammerstedt, 1993). In this respect, turkey fed diets with FO increased n-3 and n-6 PUFAs contents in sperm lipids, particularly DHA, and improved the semen quality in comparing with control (Zaniboni *et al*., 2005).

Regardless to the effect of omega-3 source in the diet, semen characteristics of turkey toms was significantly ($P<0.05$) affected by VE level as antioxidant in the diets (50 or 150 mg/kg diet), except for ejaculate volume (Table 4). Tomes fed diet supplemented with 100 mg VE/kg had significantly ($P<0.5$) higher sperm cell concentration ($6.91x10^9$/ml), sperm motility (74.00%), live sperm cell percentages(81.50%) and lower abnormal sperm cell percentage (21.01%) than those fed diet without supplementation ($6.65x10^9$/ml, 72.62%, 80.50% and 22.33 %, respectively).

Results indicated that addition of VE extra recommendation than in NRC improved semen traits studies. These results are parallel with those reported by Gorgy (2013), who found that toms fed diets supplemented with VE produced semen of higher ($P<0.05$) sperm concentrations, advanced motility and live sperm percentages than controls. Also, the present results are in agreement with a previous results report

by Lin *et al*. (2005), who found that cockerels receiving 40 to 160 mg VE/kg diet had higher sperm viability and motility after 39 weeks of age and those fed 80 mg VE/kg diet had higher sperm concentration at 39 weeks of age.

The positive effects of VE dietary supplementation on semen quality of toms is related to its role as a natural antioxidant in the semen and possibly other additional preventative mechanisms occur, such as increased levels of superoxide dismutase or glutathione (Surai *et al*., 2001). Avian semen was characterized by extremely high content of LC-PUFAs in the phospholipid fraction of sperm, which is a necessity for maintenance fluidity, integrity and flexibility of sperm. In addition, VE provides additional protection in case of fatty acid manipulation of the semen (Cerolini *et al*., 2005) and plays an important role in improving semen characteristics and fertility Khan *et al*. (2012).

Semen characteristics of Bronze turkey toms were significantly ($P< 0.05$) affected by interaction between omega-3 sources and vitamin E supplementation in the diets, except ejaculate volume (Table 4). Such effects reflected the best sperm characteristics in semen of toms fed diet contained 2% FO plus 150 mg VE/kg diet, flowed by those fed LO plus 150 mg VE/kg diet or FO plus 50 mg VE/kg diet. While, tomes fed SO without VE supplementation had the poorest semen quality. This trend may suggest importance of FO as a source of omega 3 fatty acids in comparing with LO. Also, VE supplementation as an antioxidant had beneficial effect on semen quality of toms.

The improvement in semen quality of toms fed 2% FO plus 150 mg VE/kg diet, followed by LO plus 150 mg VE/kg diet compared to SO may be due to the different rate of omega-6 to omega-3 in these diets and VE levels in the diets, which positive effects on lipids composition of semen. Because of their highly unsaturated lipids, spermatozoa are very susceptible to peroxidative damage resulting from the acts of free radicals and reactive oxygen species (Zaniboni *et al*., 2005). Oxidative stress is known to play a most important role in the etiology of defective sperm function via mechanisms involving the induction of peroxidative damage to the plasma membrane, a subsequent reduction of sperm motility and a decline in cell quality which results in insufficient records of viable spermatozoa and fertility (Boonsorn *et al*., 2010). Positive results in terms of improved sperm motility following the supplementation of n-3 and n-6 in diets with varying levels of Vitamin E have been reported by Cerolini *et al*. (2006).

Fertilizing ability of toms and hatchability of eggs:

Regardless to the effect of dietary VE supplementation, the fertilizing ability of toms and hatchability rate based on total number or fertile eggs were significantly ($P<0.05$) affected by different oils (Table 5). Toms fed 2% FO/kg diet had significantly ($P<0.05$) higher rates of fertility and hatchability based on total or fertile egg number (94.82, 87.04 and 91.78%), followed by those fed 2% LO (92.86, 82.74 and 89.12%) as compared to SO (89.43, 77.09 and 86.21%), respectively, but no significant difference was observed between FO and LO in hatchability rate of total or fertile eggs.

These results are in agreement with Olubowale *et al*. (2014), who found that dietary fatty acid supplementation from various sources (FO, SO and tallow) to have distinct effects on certain semen quality and reproductive performance of cockerels. Also, Blesbois *et al*. (2004) established that FO diet very effectively increased the percentage of n-3 fatty acids (22:5n-3 and 22:6n-3) in spermatozoa and correspondingly decreased the percentage of n-6 PUFAs (20:4-6 and 22:4n-6): the n-3/n-6 ratio in spermatozoa fatty acids were 0.04-0.07 with the standard diet and 0.32-0.4 with the fish oil diet.

The improvement in fertilizing ability of toms as affected by omega-3 supplementation can be due to the role of omega-3 in regulating reproductive function. There is considerable evidence that dietary PUFA supplementation can influence biosynthetic pathways involved in both prostaglandin synthesis and steroidogenesis that play multiple roles in the regulation of reproductive function. The PUFA composition of the cell membranes of the sperm and oocyte is important during fertilization (Aitken and Baker, 1995). In cockerels, the lipid composition of the diet modifies the fatty acid composition of the semen, plasma membrane integrity and fertilizing ability of spermatozoa (Zanini *et al*., 2003).

Results in Table (5) also revealed that toms fed 2% LO had higher fertility rate than SO. Dietary supplementation with linolenic acid (18:3n-3) significantly improved fertility (Kelso *et al*., 1997). Linseed oil contains a high concentration of this fatty acid. Also, the present study showed that FO significantly increased fertility rate as compared to LO. These results are in harmony with those reported by Bongalhardo *et al*. (2009), who found that dietary FO was more effective than flax in increasing n-3 PUFA in the phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol phospholipids in membranes from both the sperm head and body, consequently decreasing the n-6:n-3 ratio. In the phosphatidylinositol phospholipids, fish oil decreased arachidonic acid and increased distinct n-3 fatty acids in both sperm head and body membranes. Unique membrane responses were seen in the phosphatidylethanolamine phospholipids, with fish oil, followed by flaxseed oil, increasing the n-3 in the body membranes and decreasing n-6 PUFA in sperm head. Also, the obtained results in this study are in accordance with those reported by Blesbois *et al*. (2004), who found increase in hatching rates of nearly 2 points at 48-58 weeks for males fed 2% FO diet. These results may indicate that an increase in the dietary ratio of n-3/n-6 PUFAs is valuable to sustain the reproductive capacity of male turkeys especially when they are getting older. It is noticeable that there are limited data about fat and fatty acid component of breeder diets on fertility metabolism, although detailed reports were available on embryonic growth and hatchability

Table 5. Fertilizing ability and hatchability rate of total or fertile eggs of toms as affected by omega-3 source, vitamin E supplementation in the diet and their interaction.

Viable	Fertilizing ability (%)	Hatchability total egg (%)	Hatchability fertile egg (%)
Omega-3 source :			
Sunflower oil	89.43 [c]	77.09 [b]	86.21 [b]
Fish oil	94.82 [a]	87.04 [a]	91.78 [a]
Linseed oil	92.86 [b]	82.74 [a]	89.12 [ab]
Stander error	0.60	1.45	1.72
Probability (P<)	0.05	0.05	0.05
Vitamine E supplementation :			
Control (50 mg/kg diet)	90.86 [b]	80.00 [b]	88.05
150 mg Vit. E/kg diet *	93.87 [a]	84.59 [a]	90.03
Stander error	0.49	1.19	1.40
Probability (P<)	0.04	0.05	0.334
Omega-3 source x Vitamine E supplementation :			
Sunflower oil x Vit E 50	87.86	75.05	85.50
Sunflower oil xVit E 150*	90.99	79.14	86.92
Fish oil x Vit E 50	93.31	83.61	89.66
Fish oil x Vit E 150	96.33	90.46	93.91
Linseed oil x Vit E 50	91.41	81.33	88.99
Linseed oil x Vit E 150	94.13	84.61	89.25
Stander error	1.06	2.23	2.38
Probability (P<)	0.05	0.05	0.11

*** Vit. E = 50mg from premix and 100 mg supplementation/kg diet.**
[a, b, c] Means with different superscripts in the same column differed significantly (P<0.05).

Irrespective to the effect of omega-3 sources in the diet, addition of VE access in tom diet significantly ($P<0.05$) increased fertility rate from 90.86 to 93.87% and hatchability of total eggs from 80.00 to 84.59%, while Hatchability rate of fertile eggs was insignificantly affected by VE supplementation in the diet. These results are in accordance with Khan *et al*. (2012), who reported that VE plays an important role in improving semen quality and subsequent better fertility. The antioxidants are playing important roles in avian reproduction. Vitamin E is a natural antioxidant capable of enhancing semen quality and fertilizing ability of chickens, when it is provided at a level of 500 times greater than the NRC requirements (15 IU/kg diet) (Biswas *et al*., 2007). Vitamin E is mainly found in the hydrocarbon part of membrane lipid bilayer towards the membrane interface and in close proximity to oxidase enzymes which initiate the production of free radicals, protects cells and tissues from oxidative damage induced by free radicals. Vitamin E ameliorates oxidative stress in spermatozoa helping to maintain optimum fertilizing ability. Vitamin E supplements have been widely used in poultry diets to enhance productive and reproductive performances several folds (Surai, 1999). Natural antioxidants, such as VE, are present in poultry semen. Vitamin E is partitioned between the seminal plasma and spermatozoa, with higher concentrations contained within turkey sperm cells than in the seminal plasma (Surai *et al*., 2001).

The effect of interaction between omega-3 sources and VE on rates of fertility and hatchability of total eggs was significant ($P<0.05$), while it was insignificant on hatchability of fertile eggs (Table 5). Rates of fertility and hatchability of total eggs were almost higher in toms fed 2% FO with 150 mg VE/kg diet, followed by those fed 2% LO with 150 mg VE/kg or FO without VE supplementation versus the lowest values for toms fed SO with 150 mg VE/kg diets.

The observed enhancement in rates of fertility and hatchability of total eggs may be due to that VE supplementation as antioxidant and different ratio of n-3, n-6 and n-9 and PUFA in different oils used, which effect on PUFA components of spermatozoa and reflected that on reproductive capacity, in view of the fact that almost 60% of the total sperm lipids in turkey sperm cells are phospholipids (Cerolini *et al*., 1997). PUFA feeding increases IGF-I level in body fluids, which is suggested to have a positive correlation with semen function (Macpherson *et al*., 2002), and modulating IGF-I concentration in seminal plasma may improve semen fertility. PUFAs are known to contribute to membrane fluidity, flexibility, acrosome responsiveness and the packing of membrane-bound receptors (Strzezek *et al*., 2004). Also, Lenzi *et al*. (1996) stated that PUFAs are the precursors of prostaglandins and leukoterienes, which are essential components for sperm motility and inflammatory processes. The fatty acids of turkey spermatozoa were

mainly unsaturated fatty acids, with a predominance of PUFAs, particularly n-6 PUFAs and especially 22:4n-6, as in other domestic bird species (Douard *et al*., 2002). This may indicate a functional system of elongation/desaturation from the 18:2n-6 provided by the diet. However, as in other birds, highly unsaturated n-6 PUFAs such as 22:5n-6 are not detected in turkey spermatozoa whereas this PUFA is the major 22-carbon PUFA in other predominantly n-6 PUFA rich spermatozoa such as the rat (Rettersol *et al*., 1998). This may indicate limitation of the elongase and desaturase activity in birds or competition between n-3 and n-9 PUFAs for these enzymes, especially in turkeys which differ from the other bird species by their high content in 22:3n-9 PUFA. As the presence of long chain n-9 PUFAs is usually indicative of deficiency in essential fatty acids (Blesbois *et al*., 2004), the changes in turkey spermatozoa fatty acids in response to n-3 supplementation in the diet are particularly interesting. Also, they added that the n-3/n-6 proportion of spermatozoa PUFAs was very similar to that found in the diet, whereas a degree of resistance of spermatozoa to dietary manipulation of fatty acids has previously been reported in the chicken. This could mean that the fatty acid transfer from the diet to the spermatozoa is more efficient in the turkey than in the chicken. The increase in proportions of n-3 PUFAs in turkey spermatozoa when males were fed a fish oil diet was not accompanied by a decrease in n-9 PUFAs. This could mean that the high 22:3n-9 PUFA content in turkey spermatozoa does not originate from n-3 PUFA deficiency in turkeys, but is specific to this species. They suggested that the existence of an original regulation of elongase and desaturase enzymes necessary to PUFA production in turkeys. The preferences of these enzymes for n-6 and n-9 fatty acids in the reproductive tract must be explored.

Some histological parameters studies on genitalia organs of toms turkey Breeders

The histological examination revealed differences among dietary treatments in the histological structure of testicular and epididymal tissues of toms. Testes of toms fed 2% SO with 50 mg VE/kg diet contained several countable multinucleated giant cells (MCG), indicator of degeneration (Fig. 1 A). The MCG numbers in testes were lower in toms fed 2% SO and supplemented with 100 mg VE/kg diet over the requirement in NRC were lower than in toms fed SO with 50 mg VE/kg diet. Testicular MGC are composed mainly of aggregates of degenerated spermatocytes and spermatids and are often sloughed into the lumen of seminiferous tubules (Sur *et al*., 1997). The presence of few numbers of MCG in testes of toms fed 2% LO with 50 mg VE/kg diet was observed (Fig. 1 B). Less number of MCG was found in testes of toms fed 2% FO with 50 mg VE/kg diet. However, in testes of toms fed 2% FO or LO plus 150 mg VE/kg diet, MCG were absent with presence of large numbers of spermatozoa in the lumenae of seminiferous tubules (Fig. 1 C).

Few numbers of spermatozoa were found in the lumen of epididymis turkey toms fed 2% SO with 50 mg VE/kg diet, whereas, epididymis of turkey toms fed 2% SO with 150 mg VE/kg diet showed considerable numbers of spermatozoa in the lumen (Fig. 1 D). Epididymis of turkey toms fed 2% LO with 150 mg VE/kg diet showed great number of spermatozoa in the lumen than epididymis of toms fed the same oil with 50 mg VE/kg diet only (Fig. 1 E). Obvious great number of spermatozoa was seen in the lumen of epididymis of turkey toms fed 2% FO with 50 mg VE/kg diet. Huge number of spermatozoa was observed in the lumen of epididymis of turkey toms fed 2% FO with 150 mg VE/kg diet (Fig. 1 F).

Delay formation of multinucleated giant cells (degenerative cells) in the testes and huge numbers of spermatozoa in the lumen of epididymis of turkey toms fed 2% FO or LO plus 150 mg VE/kg diets may be due to n-3/n-6 ratio and VE as antioxidant. Neuman *et al*. (2002) observed that the antioxidant properties of ascorbic acid were associated with reduced formation of multinucleated giant cells in the testes of 65 wk old breeder toms.

Based on the foregoing results, it is concluded that turkey toms fed diet supplemented with 2% fish oil with 100 mg vitamin E/kg diet could improve semen quality and reproductive performance, and consequently fertility and hatchability rate.

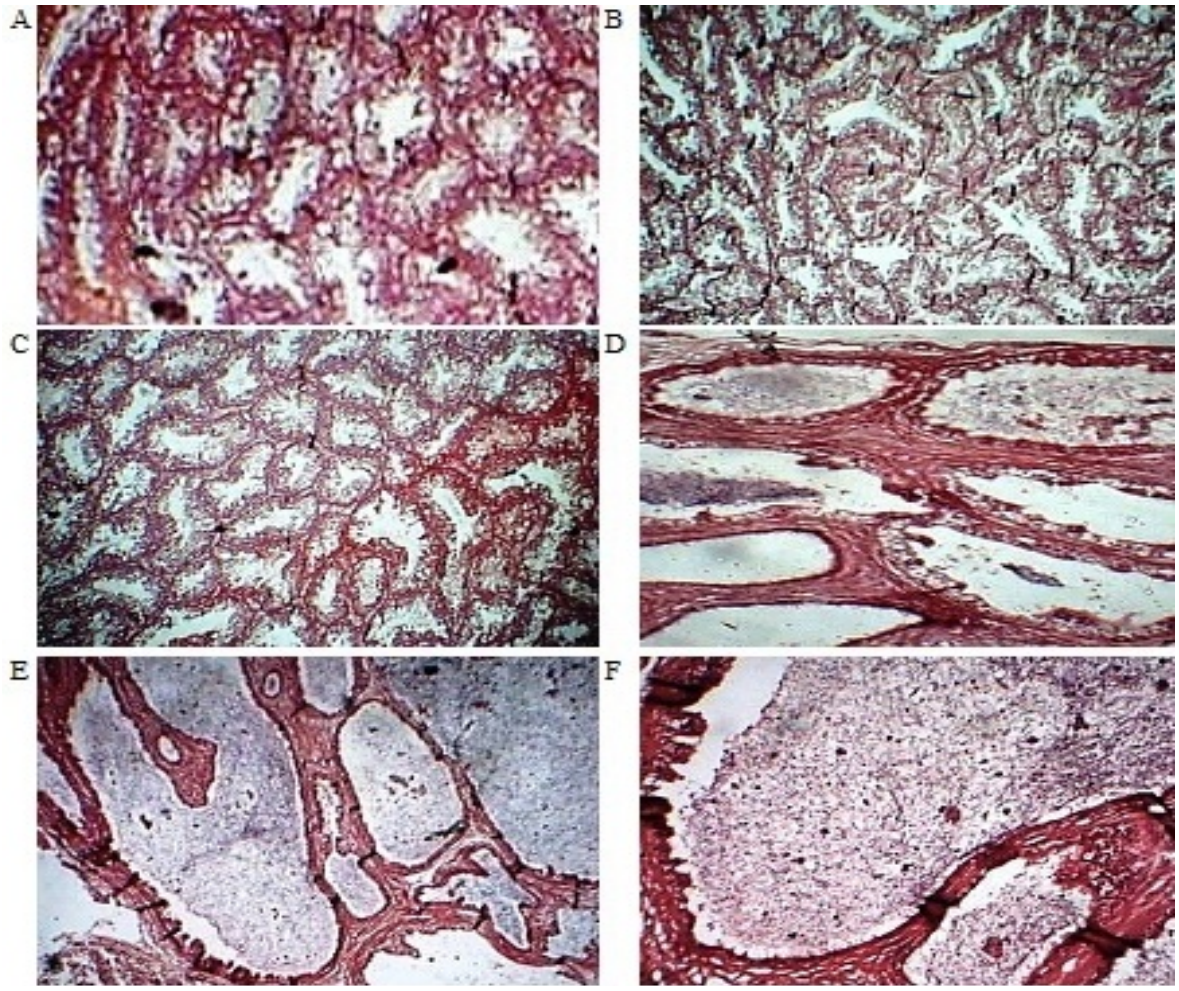

Fig. 1 A: Testis of turkey tom fed 2% SO with 50 mg VE/kg diet, showing atrophy of seminiferous tubules with present number of multinucleated giant cells (MGC), (H & E x 66). B: Testis of turkey tom fed 2% LO with 50 mg VE/kg diet, showing few numbers of MCG (H & E x 33). C: Testis of turkey tom fed 2% FO with 150 mg VE/kg diet, showing absence of MGC and presence of large numbers of spermatozoa in the lumen of seminiferous tubules (H & E x 33). D: Epididymis of turkey tom fed 2% SO with 150 mg VE/kg diet, showing considerable numbers of spermatozoa in the lumen (H & E x 66). E: Epididymis of turkey tom fed 2% LO with 150 mg VE/kg diet, showing great number of spermatozoa in the lumen (H & E x 66). F: Epididymis of turkey tom fed 2% FO with 150 mg VE/kg diet, showing huge number of spermatozoa in the lumen (H & E x 66).

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تأثير مصادر أوميجا-٣ وأضافه فيتامين هـ فى علائق ديوك الرومى على خصائص السائل المنوى و القدره على الأخصاب

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أجريت هذه الدراسة على عدد ٣٦ ديك رومي برونزى عمر ٣٦ أسبوع بهدف دراسة تأثير استخدام زيوت مختلفه (زيت عباد الشمس، زيت السمك و زيت الكتان) كمصدر للاوميجا ٣ و الاوميجا ٦ مع أو بدون أضافه فيتامين هـ علي الأداء الانتاجي والتناسلي في ذكور الرومي . تم تقسيم الديوك إلي ثلاث مجاميع متشابهه تقريبا بحيث تحتوي كل مجموعه علي ١٢ ذكر. غذيت المجموعة الأولى (مجموعة الكونترول)علي العليقه الضابطه تحتوي علي ٢ % زيت عباد شمس، بينما غذيت المجموعة الثانية و الثالثه علي نفس العليقه الضابطه مع أستبدال زيت عباد الشمس ب ٢% زيت سمك أو زيت بذرة الكتان. بعد ذلك قسمت كل مجموعه من المجاميع الثلاثه الى تحت مجموعتين (٦ ديوك)، غذيت تحت المجموعه الأولى على عليقه بدون أضافه فيتامين هـ (تحتوى على ٥٠ ملجرام فيتامين E من مسحوق البريمكس/كجم عليقه) بينما غذيت تحت المجموعه الثانيه على عليقه مضاف اليها ١٠٠ ملجرام فيتامين E (تحتوى أجماليا على ١٥٠ ملجرام فيتامين هـ/كجم عليقه) كمضاد أكسده.

أظهرت النتائج المتحصل عليها أن: ١-وجود ٢% زيت السمك أو زيت الكتان فى علائق ديوك الرومى ربما أثر قليلا و بدون تأثير ضار على وزن الجسم للذكور عند عمر ٤٨ أسبوع أو معدل التغير فى وزن الجسم (فى الفتره من ٣٦ الى ٤٨ أسبوع من العمر). ٢- أضافه فيتامين هـ الى علائق ديوك الرومى زادت معنويا (على مستوى ٥%) وزن الجسم مقارنه بالذكور التى غذيت على علائق بدون أضافه. ٣-تأثرت خصائص السائل المنوى للديوك معنويا بمصادر أومجا-٣ و أضافه فيتامين هـ الى العليقه، بأستثناء حجم القذفه المنويه. ٤-زادت النسبه المئويه لكل من الحركه التقدميه للحيوانات المنويه (٧٥,٦٦%) و الحيوانات المنويه الحيه (٨٢,٦١%) معنويا (على مستوى ٥%) فى الديوك التى غذيت على عليقه تحتوى على ٢% زيت سمك+١٥٠ ملجرام فيتامين هـ /كجم عليقه، يليها الطيور التى غذيت على نفس الزيت بدون أضافه فيتامين هـ (٧٤,٧٠ و ٨١,٥٠%) والتى غذيت على عليقه بها زيت الكتان+١٥٠ ملجرام فيتامين هـ/كجم غذاء (٧٤,٢٤ و ٨١,٣٨%) مقارنه مع مجاميع المعاملات الأخرى. ٥-لوحظ أرتفاع معنوى (على مستوى ٥%) لقدره الديوك على الأخصاب و نسبه الفقس للبيض الكلى راجعه الى تأثير المعاملات. ٦-وجد أختلافات فى التركيب النسيجى للخصيه والبربخ بين ديوك المعاملات الغذائيه المختلفه. وعلى ذلك توصى هذه الدراسة على أحتواء عليقه ديوك الرومى ٢ % زيت سمك أو كتان بالإضافة الي ١٥٠مجم فيتامين هـ/كجم عليقه للحصول على أفضل أداء تناسلي وكفاءة للديوك الرومى.