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## Effect of Dietary Fat Type and Different Levels of Vitamin E on Broiler Breeder Performance and Vitamin E Levels of Egg

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**Abstract:** An experiment was conducted to investigate the effects of dietary fat type and different levels of vitamin E on broiler breeder's performance and egg vitamin E contents. The experiment was carried out using a completely randomized design with factorial arrangement of 2×3 (0 and 4% of fat type and 0, 75 and 150 mg kg<sup>-1</sup> of vitamin E supplement). In this experiment fat type was Canola Oil (CO) and tallow (TF). Ninety broiler breeder hens (Ross 308) in 3 replicates of 5 broiler breeder hens per each were fed dietary treatments at 27 weeks in eight weeks period. Egg production and average of egg weight were recorded weekly. Eggshell quality was recorded at 4 weeks intervals. Results showed that Body weight, feed intake and feed conversion ratio of hens and males were not significantly affected by experimental diets. Also egg weight and daily egg production, laying percentage and hatchability were not significantly affected by experimental diets, but hens were fed diets with Canola oil than Tallow and added 75 mg kg<sup>-1</sup> vitamin E supplement had better performance. The levels of vitamin E in eggs (p<0.01), total fat percentage (p<0.05) were significant in 150 mg kg<sup>-1</sup> vitamin E supplement than other groups. MDA concentration of eggs in final of experiment was not significant between groups but after four day storage, interaction effects between fat type and vitamin E concentration were significant (p<0.01). These data were suggest that supplementation of 4% Canola oil with 75 mg kg<sup>-1</sup> of vitamin E to diets based on corn-soybean meal can influence egg vitamin E contents without any adverse effects on broiler breeder body weight and hatchability.

**Key words:** Fat type, vitamin E, performance, egg, broiler breeder

### INTRODUCTION

Broiler breeder diets influence subsequent egg production and performance and also embryogenesis and hatchability of broiler eggs (Peebles *et al.*, 1998, 2000). Fats are frequently included in broiler diets to increase the energy density. Several studies showed that an increase in energy concentration causes a decrease in feed intake but haven't negative affect on daily gain, resulting in an improvement in feed efficiency (Scaife *et al.*, 1994). The addition of 5% poultry fat to broiler breeder diets, without increasing Metabolizable Energy (ME) intake, has been reported to increase egg weight and egg production and the number of chicks produced per hen was maximized with 4% poultry fat included diet (Brake *et al.*, 1989).

Tallow, by-products obtained during slaughter processing have become more available to the poultry industry as a cheaper dietary fat source in some states. Deaton *et al.* (1981) reported the increased body fat related with amounts of tallow in diets (composed essentially of saturated fatty acids). Feed intake is an important factor in formulating the diets. Zollitsch *et al.* (1996) observed that unsaturated vegetable oils produce lower fecal energy losses and consequently, higher ME value than animals fats.

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Egg weight was influenced by increasing protein level (Liu *et al.*, 2005; Wu *et al.*, 2005a) and fat supplementation (Grobas *et al.*, 1999; Sohail *et al.*, 2003) and dietary energy (Bryant *et al.*, 2005) in broiler diets. However, there are inconsistent results in effect of supplemental fat or dietary energy on egg weight. Addition of fat in broiler breeder diets had no effect on egg production (Bohnsack *et al.*, 2002; Sohail *et al.*, 2003; Bryant *et al.*, 2005). Many egg producers do not use supplemental fat because of inadequate storing and mixing facilities (Sohail *et al.*, 2003). Using supplemental fat in diets would increase feed efficiency (Bryant *et al.*, 2005; Wu *et al.*, 2005b). There is also a lack of information about the effects of tallow and canola oil and vitamin E additive in diets through laying period on the production performance in broiler breeders.

Supplementation of poultry diets with antioxidant substances seems to be an efficient means for improving the oxidative stability of eggs. A high concentration of n-6 and n-3 PUFA in the cell membranes increases the susceptibility to peroxidative degradation (McKay and King, 1980) and increases the requirement for vitamin E. In the cellular membranes,  $\alpha$ -tocopherol ( $\alpha$ -TOC), the most important membrane-bound lipid-soluble antioxidant, has been reported to prevent oxidative injury to Long Chain Polyunsaturated Fatty Acids (LCPUFA). The need for antioxidant protection during late embryonic life may be attenuated by the LCPUFA composition of Yolk lipids. Therefore, knowledge of tissue tocopherol (TOC) concentration is important in providing protection against lipid peroxidation to increasing LCPUFA content in the developing chick embryo (Cherian and Sim, 1997). Supplementation of animal diets with tocopherols increases the content of this natural antioxidant in animal food products and prevents lipid oxidation in broiler meat (Ajuyah *et al.*, 1993) and eggs and egg products (Galobart *et al.*, 1999, 2001b). The transfer efficiency of  $\alpha$ -tocopherol from feed to egg has been studied by Grobas (1997) and Galobart *et al.* (2001a) found that the  $\alpha$ -tocopherol content of fresh egg increased in a dose dependent manner  $\alpha$ -tocopheryl acetate ( $\alpha$ -TA) supplementation, but transfer efficiency decreased, with increasing  $\alpha$ -tocopherol content in the diet (Cherian and Sim, 1997).

The objective of the present study was to determine the effect of dietary fat types with different levels of  $\alpha$ -tocopheryl acetate ( $\alpha$ -TA) on the broiler breeder performance and  $\alpha$ -tocopherol ability and efficiency for transfer to egg and the effect of this antioxidant in fresh and storage on lipid oxidation in eggs.

## MATERIALS AND METHODS

### Animals and Diets

Study was conducted in Shabestar university farm with ninety broiler breeder hens (Ross 308 strain) at 27 weeks of age in a 2×3 factorial trial were distributed to 3 replicates (5 hens and 1 cock per each pen). Dietary treatments began at week 27 to 35. The treatments diets were isonitrogenous and isoenergetic and accompanied by a photoperiod change to a 15.5L: 8.5D cycle to initiate lay. Experimental diets were isocaloric and isonitrogenous (2750 kcal kg<sup>-1</sup> ME and 15% crude protein). Experimental diet containing (Table 1):

- 4% CO + 0 mg kg<sup>-1</sup> vit E
- 4% CO + 75 mg kg<sup>-1</sup> vit E
- 4% CO + 150 mg kg<sup>-1</sup> vit E
- 4% TF + 0 mg kg<sup>-1</sup> vit E
- 4% TF + 75 mg kg<sup>-1</sup> vit E
- 4% TF + 150 mg kg<sup>-1</sup> vit E

Tallow was obtained from different local slaughterhouses. Hens were kept in an environmentally controlled house where daily house temperature was maintained at 21°C and body weight equal in initial experiment. Body weight estimated by weighting hens at the end of experiment. The vitamin E

Table 1: Composition and calculated analysis of the basal diet

Ingredients and analysis	Percentage	Percentage
Yellow corn	44.59	55.05
Soybean meal (44% CP)	21.27	21.86
Wheat	12.00	2.00
Soft wheat bran	6.00	4.98
Canola oil	4.00	-
Tallow	-	4.00
Oyster	3.00	3.00
Bone meal	3.90	3.90
Dicalcium phosphate	1.26	1.26
Vitamin premix <sup>1</sup>	0.25	0.25
Mineral premix <sup>2</sup>	0.25	0.25
DL-methionine	0.12	0.08
Salt	0.36	0.37
Sand	3.00	3.00
<b>Calculated analysis</b>		
Metabolizable energy (kcal kg <sup>-1</sup> )	2760.00	2759.00
Crude protein	15.41	15.35
Lysine	0.71	0.71
Methionine	0.32	0.32
Methionine plus cystine	0.58	0.58
Calcium	2.80	2.80
Available phosphorus	0.35	0.35
Sodium	0.16	0.16
Linoleic acid	1.87	1.55

<sup>1</sup>: Vitamin premix provided the following per kilogram of feed: vitamin A, 4800 IU (retinyl acetate); cholecalciferol, 880 IU; vitamin E, 10 mg (dl- $\alpha$ -tocopheryl acetate); vitamin K (menadione sodium bisulfate), 1.2 mg; thiamin, 0.8 mg; riboflavin, 2.4 mg; pantothenic acid, 12 mg; niacin, 3 mg; vitamin B12, 0.006 mg; biotin, 0.04 mg; pyridoxine, 0.8 mg; choline chloride 100 mg; anti oxidant 4 mg; <sup>2</sup>: Mineral premix provided the following per kilogram of feed: manganese, 40 mg; zinc, 24 mg; iron, 16 mg; copper, 2 mg; iodine, 0.4 mg; selenium, 0.08 mg; Ca, 280 mg and choline chloride 100 mg

Table 2: Fatty acid profile of the fat types (%)

Fat types	C18:0	C18:1, n-9	C18:2, n-6	C18:3, n-3
Canola oil	3.50	50.56	21.25	18.64
Tallow	5.66	18.05	16.52	7.77

content and the fatty acid composition of the six diets were regularly checked. Previously canola oil and tallow fatty acid content was determined (Table 2). Feed intake measured weekly, whereas egg production and weight recorded on a daily basis during the 8 week trial.

### Egg Production and Egg Quality

Egg were collected and recorded daily and egg productions were calculated weekly. Hatchable eggs were stored at 15-16°C until setting in the incubator. In 35 weeks of age eggs from all of cages usage for determine of egg average weight, also after 21 days incubation (JAMESWAY), the number and weights of live chicks were recorded. Hatchability of the eggs from each replicate pen were calculated and expressed in comparison with total of eggs set and fertile eggs.

### Analysis of Samples

After 8 week of experiment, for evaluation of MDA value and vitamin E concentration all eggs were collected from each pen and the pooled yolks, homogenized and frozen stored at -70°C until they were analyzed for content of vitamin E, fatty acid composition and MDA. The analysis of egg, Total Cholesterol (TCOL) and Triglyceride (TG) were measured on autoanalyzer (ALCYON 300) by using commercially available kits. Also a batch of 12 eggs per group was stored at room temperature (20 to 25°C) for 4 d and then subjected to the same sampling procedure as above. MDA measured by using spectrophotometer with 521.5 nm and vitamin E measured by using HPLC method.

### Vitamin E

One gram of egg yolk extracted and saponified with 30 mL of ethanol: 50% KOH (1:1 vol/vol) and was kept overnight in the dark under nitrogen gas at room temperature. Twenty milliliters of hexane plus butylated hydroxytoluene (1 g L<sup>-1</sup>) and 20 mL of KH<sub>2</sub>PO<sub>4</sub> were added to the flask and carefully mixed for 5 min. After standing 1 h, 5 mL of the upper organic solvent layer was drawn and evaporated with nitrogen gas. The dried material was recovered with 1 mL of ethanol and 10 µL was injected into a Hewlett Packard HPLC (series 1090), fitted with a Machery-Nagel (C18-5) column. Samples were eluted with a solution of methanol and water (97:3 vol/vol) and run isocratically at a flow of 1.5 mL min<sup>-1</sup>. α-tocopherol was read at a wavelength of 292 nm and was quantitatively measured using a solution of α-tocopherol as an external standard.

### Statistical Analysis

Data collected subjected to analysis of variance and significant differences observed in means subjected to Duncan's multiple range test. All data were analyzed by ANOVA using the General Linear Model (GLM) procedures of the SAS Institute (SAS, 2000).

## RESULTS AND DISCUSSION

The performance of the hens was not significantly affected by the vitamin different dose or fat supplementation with two type fat in diets. Also final body weight did not statistically difference between groups (Table 3).

Adding canola oil and tallow and different levels of vitamin E had no significantly effects on the feed intake and egg production. This result was according with the studies of Harms *et al.* (2000), Bryant *et al.* (2005) and Wu *et al.* (2005b), who reported that egg production was not affected by fat type or dietary energy. However, Grobas *et al.* (1999) reported that fat type could increase egg production from 38 to 61 week of age. In this experiment, there was no significantly difference in body weight among hens fed two fat type and different levels of vitamin E, which might lead to no response of egg weight. The difference in body weight probably related to place of research. Adding fat type and different levels of vitamin E had no significantly effects on egg production, egg weight and hatchability (Table 3) and treatment with canola oil and 75 mg kg<sup>-1</sup> of Vitamin E had the highest effect on this traits, but not significant (Table 3). The egg hatchability in 150 mg kg<sup>-1</sup> vitamin E was highest. This results was similar with Summers and Leeson (1983, 1993), who reported that egg weight was affected by body weight of laying hens, rather than fat type or dietary energy. However, several studies (Harms *et al.*, 2000; Bohnsack *et al.*, 2002; Sohail *et al.*, 2003; Bryant *et al.*, 2005) reported that increasing fat affected egg weight. The differences among result of researchers might be due to differences in strains, body weight of laying hens and composition of fat. Fat type and different levels of vitamin E in broiler breeder diet had no effect on chick weight and ratio of chick weight to egg weight.

Table 3: Analysis of variance of dietary effects on the performance of broiler breeder

Treatments	Body weight (kg)	Egg weight (g egg <sup>-1</sup> )	FCR <sup>1</sup> (kg kg <sup>-1</sup> )	Egg production (% hen-day)	Hatchability (%)	Chick weight (g)
<b>Fat Types (FT)</b>						
canola oil	3.682±31	61.36±0.64	3.10±0.04	89.64±0.73	78.36±3.59	44.61±0.57
Tallow	3.678±16	60.59±0.51	3.23±0.05	88.08±1.18	77.40±7.31	43.69±0.47
<b>Vitamin E dose (VD)</b>						
0	3.686±19	60.86±0.86	3.26±0.08	87.18±1.39	75.00±8.89	44.64±0.65
75	3.695±36	61.45±0.56	3.08±0.04	89.76±1.39	76.44±6.53	43.86±0.37
150	3.660±34	60.62±0.75	3.15±0.05	89.64±0.51	82.22±4.36	43.96±0.91
df	17	17	17	17	17	17
<b>FT*VD</b>						

<sup>1</sup>FCR = Feed Conversion Ratio, Statistically the values are not significant

Total of n-3 (linolenic acid) fatty acid contents in eggs were 0.85 and 0.28% for canola oil and tallow groups, respectively. These results are agreement with previous studies using linseed oil or flaxseed to obtain enriched n-3 fatty acids eggs (Cherian *et al.*, 1996; Baucells *et al.*, 2000; Galobart *et al.*, 2001b). There were significant differences in the FA composition of eggs due to application of  $\alpha$ -tocopherol. However, when fatty acids composition was compared between the eggs from unsupplemented treatment and those from all the  $\alpha$ -tocopherol supplemented treatments together, a reduction of LNA content was observed (Table 3). Present observations are in agreement with those reported by other authors using similar dietary doses of  $\alpha$ -tocopherol (Meluzzi *et al.*, 2000; Galobart *et al.*, 2001b). Meluzzi *et al.* (2000), reported that high levels of dietary vitamin E related with low levels of n-3 fatty acid reduce the total n-3 fatty acids deposition in the yolk, whereas high levels of dietary n-3 fatty acids could depress the vitamin E deposition.

The effects of tocopherol supplementation on the FA composition of eggs have scarcely been studied. It has been suggested that  $\alpha$ -tocopherol at high doses can interfere in the intestinal absorption of some long-chain FA (Meluzzi *et al.*, 1999) or that it can act as a prooxidant in eggs (Meluzzi *et al.*, 2000). However, as the effect seems to be very slight (Table 4).

Table 4 shows data on  $\alpha$ -tocopherol content of egg yolk. Vitamin E concentration in egg yolk of hens fed diets containing two fat type and different levels of  $\alpha$ -tocopherol acetate affect the vitamin E concentrations in eggs yolk. Hens fed canola oil and 150 mg kg<sup>-1</sup> of Vitamin E had significantly higher (p<0.01) values were obtained rather than other groups (Table 4). Significantly positive correlation observed between yolk vitamin E content and supplemental  $\alpha$ -tocopherol acetate in diet (R = 0.82, p<0.01). Similarly, difference in the yolk vitamin E concentrations between the hens fed supplemented diet by either 0, 75 or 150 mg kg<sup>-1</sup>  $\alpha$ -tocopherol acetate were significant (p<0.01) for canola oil and tallow groups. In our study, vitamin E deposition in egg yolk was affected by the type of supplemental fats in the groups receiving 0, 75 or 150 mg kg<sup>-1</sup> supplemental  $\alpha$ -tocopherol acetate in the diet. The addition of  $\alpha$ -tocopherol to breeder diets could increases contents of vitamin E in the egg yolk (Jiang *et al.*, 1994; Surai *et al.*, 1995; Meluzzi *et al.*, 2000).

The amount of vitamin E in the eggs yolk was strictly related with amount of  $\alpha$ -tocopherol in the diet and increment linearly as dietary dl-  $\alpha$ -tocopheryl acetate increased. The highest level of vitamin E for eggs yolk were 2.83 mg kg<sup>-1</sup> form group receiving 150 mg kg<sup>-1</sup> vitamin E in diet. Present observations are in agreement with those reported by other authors using similar dietary doses of dl- $\alpha$ -tocopheryl acetate (Meluzzi *et al.*, 2000; Galobart *et al.*, 2001b).

The  $\alpha$ -tocopherol transfer efficiency from feed to eggs was 48.3, 36.1 and 28.2%, with 0, 75 and 150 mg kg<sup>-1</sup>  $\alpha$ -tocopherol supplementation, respectively (Table 4). As noted by Grobas (1997), the highest transfer efficiency was obtained with the lowest dietary level of  $\alpha$ -tocopherol.

Table 4: Analysis of variance of lipid and vitamin E of egg yolk

Treatments	MDA (ng g <sup>-1</sup> )		C18:2, n-6 (%)	C18:3, n-3 (%)	Total lipid (%)	Vitamin E ( $\mu$ g g <sup>-1</sup> )
	Final experiment	4 day storage				
<b>Fat Types (FT)</b>			**	**	*	**
Canola oil	35.2±1.5	88.70±1.9	13.25a	0.85a	28.65±1.22 <sup>a</sup>	3.06±0.18 <sup>a</sup>
Tallow	38.5±2.4	84.90±2.0	8.29b	0.28b	26.22±0.61 <sup>b</sup>	1.99±0.20 <sup>b</sup>
<b>Vitamin E dose (VD)</b>				*	*	**
0	40.3±3.4	89.67±2.6	11.37	0.72a	27.50±0.85 <sup>ab</sup>	2.34±0.03 <sup>b</sup>
75	35.6±2.0	86.10±1.8	10.14	0.54ab	25.60±0.96 <sup>b</sup>	2.40±0.55 <sup>b</sup>
150	34.6±1.3	85.00±2.9	10.79	0.42b	29.22±1.55 <sup>a</sup>	2.83±0.13 <sup>a</sup>
df	17	17	17	17	17	17
FT*VD		**	**	**	**	**

Statistically the values are not significant, \*: Significant at p<0.05, \*\*: Highly significant at p<0.01

The independent effects of dietary fat types and dietary  $\alpha$ -tocopheryl acetate levels on MDA values was not significant ( $p>0.05$ ), but interaction of  $\alpha$ -tocopheryl acetate and fat type ( $p<0.01$ ) showed significant effect on the yolk MDA content after 4 day of storage (Table 4). In contrast to our results and those reported above, Gebert *et al.* (1998) found that addition of 100 or 200 mg kg<sup>-1</sup> of  $\alpha$ -TA to hen diets increased the TBA values of shell eggs stored for 6 month and attributed a prooxidant effect to  $\alpha$ -tocopherol at those doses. Similarly, Chen *et al.* (1998), using different levels of  $\alpha$ -TA in the hens diets, reported that  $\alpha$ -tocopherol was an antioxidant up to 50  $\mu$ g g<sup>-1</sup> egg yolk and a prooxidant at 75  $\mu$ g g<sup>-1</sup> egg yolk. In present case, no prooxidant effect was observed at any level of dietary supplementation.

Fresh egg is very stable to lipid oxidation; indeed the egg is a closed system, very resistant to lipid oxidation because of its natural antioxidant constituents such as vitamin E, avidin and phosphatine (Scheideler *et al.*, 1997) also as tocopherols, carotenoids and phosvitin and to the structure of the yolk Low Density Lipoproteins (LDL) (Temes and Leitsch, 1997).

In conclusion, the performance and production of the hens was not affected by the vitamin E level or by the fat types to the diet. The increment of doses of  $\alpha$ -TA in the diet could increase  $\alpha$ -tocopherol concentration in the egg but decreased  $\alpha$ -tocopherol transfer efficiency from feed to eggs. The Canola oil used in our experiment seems to be appropriate to increase the n-3 PUFA content of table egg. Dietary supplementation of 75 or 150 mg kg<sup>-1</sup>  $\alpha$ -tocopherol acetate results in higher yolk vitamin E content, but its effectiveness may be influenced by the type of dietary fat. Vitamin E enhances the oxidative stability of eggs rich with n-3 PUFA, however, it is ineffective at the levels used in this trial in reducing MDA values of broiler breeder eggs.

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