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Effects of Dietary Fat Type and Different Levels of Vitamin E on Performance and Some of Eggs Characters of Broiler Breeder

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Abstract: This study carried out to evaluate the effects of fat type and different levels of vitamin E on the performance and some of the egg characters of broiler breeding hens. Ninety broiler breeder hens (Ross 308 strain) at 27 weeks of age were fed in a 2×3 factorial trial (4% canola oil and tallow with 0. 75 and 150 mg kg^{-1} of vitamin E) in 8 weeks period. At the end of the experiment no significant differences were found in body weight, feed intake, feed conversion rate, number and weight of eggs, laying percentage and hatchability variables between fat type and vitamin E treatments. There were no significant differences in biochemical characters of eggs including cholesterol, triglyceride and MDA. The difference between levels of eggs vitamin E, linoleic and linolenic acids ($p<0.01$), total fat percentage and oleic acids ($p<0.05$) were significant, while in eggs arachidonic and stearic acid there were no significant difference. The results showed that eggs fatty acids profile were significantly influenced (mainly in the linoleic and linolenic acids) by fat type and vitamin E levels of diet. In a conclusion, it was found that the fat type and vitamin E levels could be effective in the egg fatty acids profile and vitamin E content and fat source do not limit vitamin E absorption, although they may increase its degradation in the gastrointestinal tract.

Key words: Fat type, vitamin E, fatty acid, egg, broiler breeder

INTRODUCTION

Broiler breeder diet influence subsequent egg production performance (Peebles *et al*., 2000a) and embryogenesis and hatchability of broiler eggs (Peebles *et al*., 1998, 2000b). Fats are frequently applied in poultry diets to increase the energy density (Sanz *et al*., 1999). Studies have shown that type of dietary lipids of the laying hen, can drastically alter the lipid profile of the egg-yolk (Yang *et al*., 2000; Grobas *et al*., 2001). Canola oil has been recognized as rich plant source of linolenic acid (C18:3). Linolenic acid can be converted to longer chain omega-3 fatty acids, such as Eicosapentaenoic Acid (EPA, C20:5), Docosapentaenoic Acid (DPA, C22:5) and Docosahexaenaic Acid (DHA, C22:6) in poultry through elongation and desaturation pathway, thus the egg yolk is enriched with omega-3 fatty acids (Yang *et al*., 2000).

A high concentration of n-6 and n-3 PUFA in the cell membranes increases the susceptibility to peroxidative degradation (McKay and King, 1980; Marshall *et al*., 1994) and increases the requirement for vitamin E. In avians, dietary supplementation of TOC reported in order to increasing the TOC content of eggs and increasing oxidative stability of hen tissues (Jiang *et al*., 1994; Cherian *et al*., 1996).

The oxidative damages of lipids can be prevented or limited by natural antioxidants such as tocopherols and carotenoids. Supplementation of hen's diets with α-tocopherol seems to be the most suitable choice to increase the content of this vitamin and to decrease the amount of the primary (lipid hydro peroxides) and secondary (Thiobarbituric Acid Reactive Substances; TBARS) oxidation products in eggs and egg products (Cherian *et al*., 1996; Galobart *et al*., 2001).

Fat soluble vitamins and fatty acids can interfere in their absorption from the intestinal lumen, hepatic metabolism and transport to the yolk (Surai, 1999).

Studies reported by several authors have shown a negative correlation between dietary PUFA content and uptake of fat soluble vitamins, especially vitamin E (Hollander, 1981). On the other hand, contradictory results reported concerning the effects of vitamin E supplementation on the incorporation of n-3 fatty acids in the yolk (Cherian *et al*., 1996; Galobart *et al*., 2001). Also some authors have studied the combined influence of dietary vitamin E and vegetable sources of n-3 fatty acids, such as flax seed, on sensory quality of the eggs (Leeson *et al*., 1998) and egg production (Scheideler and Froning, 1996). However, there is lack of information about the dietary use of vitamin E associated with canola

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oil and tallow. We have tried to fill this gap. In effect, the aim of the present research was to investigate the possibility of fortifying eggs both with n-3 fatty acids and vitamin E added to hen diets and to determine whether the vitamin E addition is dose related and is influenced by the type of lipid used (canola oil or tallow). Moreover, the effects of egg storage on fatty acid profile and vitamin E were evaluated as well as laying performance.

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 27-35.

The treatments diets of were isonitrogenous and isoenergetic and accompanied by a photoperiod change to a 15.5L: 8.5D cycle to initiate lay. Experimental diet containing (Table 1).

4% CO + 0 mg kg^{-1} vit E
4% CO + 75 mg kg^{-1} vit E
4% CO + 150 mg kg^{-1} vit E
4% TF + 0 mg kg^{-1} vit E
4% TF + 75 mg kg^{-1} vit E
4% TF + 150 mg kg^{-1} vit E

The vitamin E content and the fatty acid composition of the 6 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. After 8 week of experiment, three eggs per replicate were collected and individually weighed and their yolks pooled. The pooled yolks, homogenized and frozen stored at 70°C until they were analyzed for content of vitamin E, fatty acid composition and MDA. Also a batch of 12 eggs per group was stored at room temperature (20-25°C) for 4 d and then subjected to the same sampling procedure as above. The analysis of egg, Total Cholesterol (TCOL) and Triglyceride (TG) were measured on autoanalyzer by using commercially available kits.

Table 1: Composition and calculated analysis of the basal diet

Ingredients and analysis	(%)	(%)
Yellow corn	44.59	55.05
Soybean meal (44% CP)	21.27	21.86
Wheat	12	2
Soft wheat bran	6	4.98
Canola oil	4	-
Tallow	-	4
Oyster	3	3
Bone meal	3.9	3.9
Dicalcium phosphate	1.26	1.26
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
DL-methionine	0.12	0.08
Salt	0.36	0.37
Sand	3	3
Calculated analysis		
Metabolizable energy (kcal kg^{-1})	2760	2759
Crude protein	15.41	15.35
Lysine	0.71	0.71
Methionine	0.32	0.32
Methionine plus cystine	0.58	0.58
Calcium	2.8	2.8
Available phosphorus	0.35	0.35
Sodium	0.16	0.16
Linoleic acid	1.87	1.55

[1]Vitamin premix provided the following per kilogram of feed: vitamin A, 4800 IU (retinyl acetate); cholecalciferol, 880 IU; vitamin E, 10 mg (dl-α-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; [2] 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: Canoal oil and tallow profile (%)

Fat type	C18:0	C18:1, n-9	C18:2, n-6	C18:3, n-3
Canola oil	3.5	50.56	21.25	18.64
Tallow	5.66	18.05	16.52	7.77

Analysis of samples

Fatty acids: Total lipid was extracted from egg yolk according to the method of Folch *et al.* (1957). Approximately 0.5 g of yolk weighed into a test tube with 20 mL of (chloroform: Methanol = 2:1, vol/vol) and homogenized with a polytroon for 5-10 s at high speed. The BHA dissolved in 98% ethanol added prior to homogenization. The homogenate filtered through a Whatman filter paper into a 100 mL graduated cylinder and 5 mL of 0.88% sodium chloride solution added, stopper and mixed. After phase separation, the volume of lipid layer recorded and the top layer completely siphoned off. The total lipids converted to Fatty Acid Methyl Esters (FAME) using a mixture of boron-trifluoride, hexane and methanol (35:20:45, vol/vol/vol) (Metcalfe *et al.*, 1961). The FAME separated and quantified by an automated gas chromatography equipped with auto sampler and flame ionization detectors, using a 30 and 0.25 mm inside diameter fused silica capillary column, as described previously (Cherian and Sim, 1991). A Dany (Italy) chromatography model-1000 used to integrate peak areas.The calibration and identification of fatty acid peak carried out by comparison with retention times of known authentic standards. The fatty acid results expressed as weight percentages.

Vitamin E: One gram of egg yolk was extracted and saponified with 30 mL of ethanol: 50% KOH (1:1 vol/vol) and kept overnight in the dark under nitrogen gas at room temperature. Twenty mililiter of hexane plus butylated hydroxytoluene (1 g L^{-1}) and 20 mL of KH2PO4 added to the flask and mixed for 5 min. After 1 h, 5 mL of the upper organic solvent layer was drawn and evaporated with nitrogen gas. The dried material 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. Seven 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^{-1}. 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: All data were analyzed by ANOVA using the General Linear Model (GLM) procedures of the SAS Institute (2000). The amounts of vitamin E added (0.75and 150 mg kg^{-1}) and the type of lipid supplement (canola oil or tallow were fixed effects). When interactions occurred ($p<0.05$), interaction means were separated using Duncan multiple range test to compare different treatment means.

RESULTS AND DISCUSSION

Performance parameters: Performance of hens was not affected by vitamin E levels or fat types in diets (Table 3). These data were according with other researches (Meluzzi *et al*., 1997; Hargis *et al*., 1991). Egg weight could not significantly different between treatments. This result is agreement with (Farrell, 2002). His not observed significantly different about egg weight when canola oil was added to the diet. But is not agreement with Grobas *et al*. (2001) findings, his reported feeding soybean oil and flax oil increased egg weight. Also laying percentage and hatchability did not significant different ($p>0.05$) among groups. Egg cholesterol, TG and MDA concentration were not affected by fat types and vitamin E levels ($p>0.05$).

The total lipids% of yolk, between fat types and vitamin E levels were significantly ($p<0.05$). The egg yolk total lipid content ($p<0.05$) obtained from hens fed in tallow with 150 mg kg^{-1} Vit E in diet were higher than the canola oil diets. The yolk lipid content difference may related to the influence of n-3 PUFA on hepatic lipids metabolism, so that reducing hepatic lipid biosynthesis, secretion and plasma lipid levels (Van Elswyk *et al*., 1991). This result is agreement with Van Elswyk *et al*. (1994) his found between yolk and egg weight with using menhaden oil and animal-vegetable oils was significant differences ($p<0.05$). Scheideler and Froning (1996) showed egg yolk hens fed with 5 to 15% range of flax seed and 1.5% of fish oil decrease egg yolk weight and this condition was affected long chain fatty acids estrogen activity in hens. Whitehead *et al*. (1993) observed that decrease egg size was accompanied by a decrease of plasma estuarial concentration and postulated a nutritional control of the dietary long-chain fatty acids on the hormonal metabolism of the birds.

Fatty acid composition of total yolk lipids: The FA composition of total yolk lipids of the broiler breeder hens showed in Table 5. After feeding diets with canola oil in comparison with diets with tallow, observed content of n-3 fatty acids was significantly higher ($p<0.01$) and (LNA; C18:3n-3) content in yolk for diets with canola oil were 3 times higher than to diets with tallow. After feeding diets with canola oil, Linoleic Acid (LA; C18:2n-6) content was approximately 1.6 time increased than to diets with tallow. In the present study, ratio of n-6 to n-3 fatty acid in breeders egg yolk fed on diets with canola oil was 15.58% and in tallow groups 29.6%.

Result of breeders egg yolk fatty acids analysis showed the Monounsaturated Fatty Acid (MUFA) content in the yolk lipids was significantly ($p<0.05$) affected by fat types.

The arachidonic acid (C20:4, n-6) content was not affected among fat types and vitamin E. Result showed the highest level of vit E (150 mg kg^{-1}) significantly reduces the yolk total n-3 content in canola oil groups,

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

Treatments	Body weight (kg)	Egg weight (g egg^{-1})	FCR[1] (kg kg^{-1})	Egg production (% hen-day)	Hatchability (%)	Chick weight (g)
Fat Types (FT)	ns	ns	ns	ns	ns	ns
Canola oil	3.682±31	61.36±0.64	3.1±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	77.4±7.31	43.69±0.47
Vitamin E Dose(VD)	ns	ns	ns	ns	ns	ns
0	3.686±19	60.86±0.86	3.26±0.08	87.18±1.39	75.0±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
	FT * VD	ns	ns	ns	ns	ns

[1]FCR = Feed Conversion Rate

Table 4: Analysis of variance of fatty acid profile of egg yolk

	% of total					
Treatments	C18:0	C18:1, n-9	C18:2, n-6	C18:3, n-3	C20:4, n-6	Total lipid
Fat Types (FT)	ns	*	**	***	ns	*
Canola oil	9.47±0.55	47.07^{a}±0.77	13.25^{a}±0.59	0.85^{a}±0.10	1.63±0.09	28.65^{a}±1.22
Tallow	10.46±0.21	43.78^{b}±1.48	8.29^{b}±0.21	0.28^{b}±0.01	1.47±0.04	26.22^{b}±0.61
Vitamin E Dose(VD)	ns	ns	ns	*	ns	*
0	10.68±0.54	44.28±2.34	11.37±1.72	0.72^{a}±0.20	1.69±0.12	27.5^{ab}±0.85
75	9.22±0.60	46.83±1.19	10.14±0.94	0.54a^{b}±0.14	1.42±0.06	25.6^{b}±0.96
150	10.0±0.34	45.14±0.83	10.79±0.78	0.42^{b}±0.06	1.54±0.05	29.22^{a}±1.55
df	17	17	17	17	17	17
FT * VD	*	**	**	**	ns	*

Table 5: Analysis of variance of lipid and vitamin E of egg yolk

			MDA (ng g^{-1})		
Treatments	Cholesterol yolk (mg g^{-1})	TG yolk (mg g^{-1})	Final experiment	4d storage	Vitamin E (µg g^{-1})
Fat Types (FT)	ns	ns	ns	ns	**
Canola oil	273.9±9.3	4426.3±22	35.2±1.5	88.7±2.0	3.06^{a}±0.18
Tallow	262.0±10.6	4434.2±29	38.5±2.4	84.9±1.9	1.99^{b}±0.20
Vitamin E Dose(VD)	ns	ns	ns	ns	**
0	257.1±11.9	4425.3±30	40.3±2.0	89.67±2.6	2.34^{b}±0.03
75	273.1±15.8	4586.5±41	35.6±1.3	86.1±1.9	2.40^{b}±0.55
150	273.6±8.5	4279.0±24	34.6±3.4	85±2.9	2.83^{a}±0.13
df	17	17	17	17	17
FT * VD	ns	ns	ns	**	**

however, when FA composition was compared between the eggs from the unsupplemented treatments and those from all the α-TA supplemented treatments together, a reduction of LNA content was observed (Table 5). It has been suggested that α-tocopherol at high levels can interfere in the intestinal absorption of some long-chain FA (Meluzzi *et al*., 1999) or it can act as a prooxidant in eggs (Meluzzi *et al*., 2000). Atkinson *et al*. (1972) also observed a reduction in EPA and DHA contents in tissue associated with *á*-tocopherol treatment. In the stored eggs with the highest level of vitamin E, the yolk contents of total n-3 were reduced significantly (about 6%) (Table 4). This reduction could be ascribed to a pro-oxidant effect rather than an antioxidant effect of vitamin E when used at a very high concentration, as suggested by Fennema (1987). According to Leeson *et al*. (1998) the development of off-flavors from the eggs laid by hens fed high levels of vitamin E and n-3 fatty acids is due to the pro-oxidant effect of the vitamin.

Vitamin E: Vitamin E concentrations in egg yolk of breeders fed on diets containing different fat types and different levels of added sα- tocopheryl acetate are shown in Table 4. The type of the dietary fat supplement significantly influenced the vitamin E content. The yolk vitamin E content significantly had higher ($p<0.01$) values in the canola oil than the tallow group. We may conclude that high levels of dietary vitamin E associated with 4% canola oil reduce the total n-3 fatty acids deposition in the yolk, whereas high levels of dietary n-3 decrease the vitamin E deposition. Lynch (1994) reported that the presence of PUFA reduces tocopherol absorption from the intestine. Also Miller and Huang (1993) reported that breast and thigh vitamin E content is reduced by dietary fish oil.

The amount of vitamin E in the yolk was strictly related to the amount of α-tocopherol in the diet and increased linearly as dietary dl-α-tocopheryl acetate increased. The highest levels of 2.4 and 2.83 µg g^{-1} of egg for groups receiving 75 and 150 mg kg^{-1} of vit E per egg were recorded, respectively. Similar results were obtained by Jiang *et al*. (1994).

The effect of dietary fat types on MDA values was no significant ($p>0.05$). Dietary α-tocopheryl acetate and interaction of α-tocopheryl acetate and fat type ($p<0.01$) showed significant effect on the yolk MDA content after 4 day of storage (Table 4). The reason may be due to the fact that 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).

CONCLUSION

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^{-1} α-tocopheryl 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 in 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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