Effects of dietary fats and vitamin E on the fatty acid composition of egg yolk of fresh and refrigerated table eggs

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The aim of the present experiment was to investigate how different types of dietary fats and levels of vitamin E affect the fatty acid profile of egg yolk during refrigerated storage of table eggs. ISA Brown hens (n=90; 28 weeks old) were randomly assigned to five experimental diets. The dietary treatments included: (1) control diet without fat supplementation and added vitamin E (2) 5% Alifet supplement (rich in saturated fatty acids) and 20 IU/kg vitamin E (3) 5% Alifet and 40 IU/kg vitamin E (4) 5% pumpkin seed oil (rich in n-6 polyunsaturated fatty acids) and 20 IU/kg vitamin E (5) 5% pumpkin seed oil and 40 IU/kg vitamin E. The diets were fed for two weeks. After two weeks, 20 eggs per dietary group were collected Fatty acid composition of egg yolk was analyzed by means of gas chromatography from fresh samples (n=10 per treatment) and eggs stored for 4 weeks at 5 °C (n=10 per treatment). Feeding of diets supplemented with Alifet increased the proportion of total monounsaturated fatty acids (MUFA), whereas decreased the content of n-6 and total polyunsaturated fatty acids (PUFA) in the fresh egg yolk in comparison to the other groups (P<0.05). In the pumpkin seed oil groups, the contents of MUFA and n-3 PUFA were lower and the content of n-6 and total PUFA and the ratio of n-6 to n-3 fatty acids were higher than in the other groups (P<0.05). The effect of dietary vitamin E on the fatty acid composition of fresh egg yolk was influenced by the type of dietary fat. In the two Alifet treatments, significantly higher saturated fatty acid and MUFA content, and lower n-3, n-6 and total PUFA were measured in the egg yolks from hens fed 40 IU/kg vitamin E compared to 20 IU/kg vitamin E. In contrast, different levels of vitamin E supplementation did not influence these parameters in the pumpkin seed oil groups (P>0.05). In comparison to fresh samples, refrigerated storage of eggs for 4 weeks decreased the the proportion of n-3, n-6 and total PUFA and increased the ratio of n-6 to n-3 PUFA in the egg yolk regardless of dietary fat type when 20 IU/kg vitamin E was fed. Although vitamin E at the level of 40 IU/kg was able to prevent the significant changes of these parameters in the pumpkin seed oil group, the proportion of n-3 PUFA decreased, whereas the ratio of n-6 to n-3 increased during storage in the egg yolk of hens fed 5% Alifet and 40 IU/kg vitamin E (P<0.05).

Keywords: dietary fat; vitamin E; egg yolk; fatty acid composition

Introduction

Animal fats, vegetable and marine oils have been widely used as sources of energy and essential fatty acids in poultry feeds. Their fatty acid composition affects the fatty acid profile of poultry products and human health aspects must be considered when dietary fat supplements are used. The effects of dietary fat supplements rich in saturated (SFA), monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA) on the fatty acid composition of egg yolk can be evaluated according to changes in the ratio of saturated to unsaturated fatty acids and n-6 to n-3 PUFA, total amount of n-3 PUFA and oxidative stability of egg yolk lipids during storage (Noble, 1987).

In our experiment two types of fat supplements were investigated that can help meet the energy demands of laying hens. The fat supplement Alifet^{TM} (Alifet USA, Cincinatti, OH) is a type of partially hydrogenated tallow and well known as a ruminally inert specialty fat for high-producing dairy cows (Palmquist et al., 1989). The total lipid content of Alifet is about 90 % and 60-70 % of its fatty acids are saturated (Palmquist et al., 1989). Pumpkin seed oil is produced for human nutrition purposes and its main fatty acid is the n-6 type polyunsaturated linoleic acid (18:2n-6) like in corn and sunflower oil (Pál et al., 2002). In order to compare these supplements combined with two levels of added vitamin E in the feed, the fatty acid composition of fresh egg yolk samples and changes of fatty acid profile during refrigerated storage of eggs were determined in this study.

Materials and methods

Five groups of 18 ISA Brown hens (n=90) at 28 weeks of age were randomly assigned to each of five dietary teratments. The folowing five dietary treatments were used: (1) control diet without fat supplementation and added vitamin E (2) 5% Alifet supplement (Alifet^{TM}, Alifet, USA, Cincinatti, OH) and 20 IU/kg vitamin E (3) 5% Alifet and 40 IU/kg vitamin E (4) 5% pumpkin seed oil (rich in n-6 polyunsaturated fatty acids) and 20 IU/kg vitamin E (5) 5% pumpkin seed oil and 40 IU/kg vitamin E. The control diet was formulated to meet the daily nutrient requirements of hens according to the breeder company (Hubbard ISA S.A.) with the exception of vitamin E. The composition and calculated nutrient content of experimental diets are shown in Table 1. The fatty acid composition of fat supplements is presented in Table 2.

Table 1. Composition and calculated nutrient content of experimental diets

Ingredients and composition	Dietary treatments				
	Control diet	Alifet Treatment [1]		Pumpkin seed oil treatment [1]	
		+ 20 IU/kg vit. E	+ 40 IU/kg vit. E	+ 20 IU/kg vit. E	+ 40 IU/kg vit. E
Corn	52.30	28.80		28.80	
Wheat	10.00	27.60		27.60	
Extracted soybean meal (44%)	9.40	27.10		27.10	
Full fat soybean	8.10	-		-	
Corn gluten meal (60%)	8.30	-		-	
Alifet	-	5.00		-	
Pumpkin seed oil	-	-		5.00	
Monocalcium phosphate	1.30	1.10		1.10	
Limestone	9.60	9.50		9.50	
L-Lysine	0.30	-		-	
DL-Methionine	-	0.10		0.10	
Salt	0.30	0.30		0.30	
Premix without vitamin E [2]	0.50	0.50		0.50	
Calculated nutrient content					
AME_n (MJ/kg)	11.90	11.91		11.91	
Crude protein	17.00	17.00		17.00	
Crude fibre	3.10	3.10		3.10	
Calcium	3.70	3.70		3.70	
Phosphorus (available)	0.37	0.37		0.37	
Lysine	0.95	0.95		0.95	
Methionine	0.38	0.38		0.38	
Methionine + cystine	0.71	0.72		0.72	

[1] 20 or 40 IU/kg vitamin E in the form of dl-α-tocopheyl acetate was added to the diets

Table 2. Fatty acid composition of supplemental fat sources (wt % of total fatty acids)

Fatty acids	Alifet™	Pumpkin seed oil
C14:0	2.16	0.13
C16:0	19.84	10.04
C18:0	63.36	3.51
C16:1n-7	0.24	1.51
C18:1n-9	4.00	30.69
C20:1n-9	ND[1]	0.09
C18:2n-6	0.09	53.65
C20:4n-6	0.48	0.19
C20:5n-3	0.23	ND

[1] ND = not detected

The experimental diets were fed for two weeks and 20 eggs per dietary group were collected at day 14. Fatty acid composition of egg yolk was analyzed by means of gas chromatography from fresh samples (n=10 per treatment) and eggs stored for 4 weeks at 5 °C (n=10 per treatment). Total fat content of egg yolk was extracted according to Folch et al. (1957). Total lipid extracts were converted to fatty acid methyl esters by using BF_3-methanol. The fatty acid methyl esters were separated and analyzed by means of gas chromatography. A Carlo Erba HRGC 5300 Mega Series chromatograph (Carlo Erba Strumentazione SA, Italy), equipped with an Omegavax 320 capillary column (30 m length x 0.32 mm I.D., 0.25 μm film – catalogue Nr. 24152; Supelco, Bellefonte, USA), was used to determine the fatty acid composition of egg yolk. A standard mixture of fatty acid methyl esters (PUFA-2 – catalogue Nr.4-7015-U; Supelco, Bellefonte, USA) was used to identify individual fatty acids. Statistical analysis was carried out by analysis of variance using dietary treatments and refrigerated storage as main effects. Significant differences were tested by the Duncan test. All statistical analysis were conducted using the Statistica 5.0 statistical package (Statsoft, USA).

Results and discussion

Fatty acid composition of egg yolk as influenced by dietary treatments and refrigerated storage is presented in Table 3.

Table 3. Fatty acid composition of egg yolk (wt % of total fatty acids)

Fatty acids [1]	Type of sample	Control diet	Alifet treatment [1]		Pumpkin seed oil treatment [1]	
			+ 20 IU/kg vit. E	+ 40 IU/kg vit. E	+ 20 IU/kg vit. E	+ 40 IU/kg vit. E
SFA	Fresh	35.24^{bB}	34.52^{B}	37.07^{bA}	34.12^{B}	33.53^{B}
	Stored	37.41^{aB}	35.46^{C}	39.06^{aA}	35.17^{C}	33.89^{D}
MUFA	Fresh	40.82^{C}	43.80^{bB}	46.86^{aA}	37.01^{bD}	38.02^{D}
	Stored	40.90^{C}	47.05^{aA}	43.29^{bB}	38.39^{aD}	37.19^{E}
n-3 PUFA	Fresh	1.64^{A}	1.53^{aA}	1.14^{aB}	0.82^{aC}	0.64^{C}
	Stored	1.51^{A}	1.01^{bB}	0.74^{bC}	0.53^{bD}	0.69^{C}
n-6 PUFA	Fresh	17.94^{bB}	16.15^{aC}	13.00^{bD}	25.06^{aA}	24.12^{A}
	Stored	19.32^{aC}	14.42^{bD}	15.01^{aD}	22.99^{bB}	24.44^{A}
Total PUFA	Fresh	19.59^{B}	17.68^{aC}	14.14^{bD}	25.89^{aA}	24.76^{A}
	Stored	20.84^{C}	15.43^{bD}	15.75^{aD}	23.52^{bB}	25.13^{A}
n-6/n-3 ratio	Fresh	11.56^{C}	10.77^{bC}	11.91^{bC}	30.51^{bB}	38.28^{A}
	Stored	13.03^{D}	14.81^{aD}	20.27^{aC}	43.58^{aA}	37.18^{B}

[1]SAT = saturated fatty acids (C14:0+C16:0+C18:0); MUFA = monounsaturated fatty acids (C16:1n-7+C18:1n-9+C18:1n-7+C20:1n-9); n-6 PUFA = n-6 polyunsaturated fatty acids (C18:2n-6+C18:3n-6+C20:4n-6+C22:4n-6); n-3 = n-3 polyunsaturated fatty acids (C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3). $^{a-b}$ Different letters indicate significant differences of means between fresh and stored samples ($P<0.05$) $^{A-B}$ Different letters indicate significant differences of means between dietary treatments in a row ($P<0.05$)

Animal fats are rich sources of SFA and MUFA and contain a relatively low level of PUFA. Alifet supplement of diets containing high level of SFA with 20 IU/kg vitamin E did not change the SFA content of fresh egg yolk significantly ($P>0.05$). These results support the observation of early studies that the effects of dietary changes on MUFA and PUFA content of egg yolk are substantial while dietary manipulation of SFA level in the egg yolk is less effective (Cruickshank, 1934; Summers et al, 1966). The predominant saturated fatty acid in Alifet is the saturated stearic acid (C18:0). Along with palmitic acid (C16:0), stearic acid is the major substrate for the enzyme stearoyl-CoA desaturase, which catalyzes the conversion of stearate to the monounsaturated oleate (C18:1n-9), the preferred substrate for the synthesis of triacylglycerols and other complex lipids in the liver of hens. Also this process could contribute to the observation that the proportion of MUFA was higher ($P<0.05$), while the content of n-6 and total PUFA were lower in the fresh egg yolk after feeding diets supplemented with 5% Alifet in comparison to the other groups. Previous experiments reported similar changes of egg yolk fatty acid profile with dietary supplementation of tallow (Grobas et al., 2001), lard and poultry fat (Latour et al., 1998). Feeding diets supplemented with 5% pumkin seed oil rich in n-6 PUFA resulted in lower proportion of MUFA, n-3 PUFA and higher proportion of n-6 and total PUFA and the ratio of n-6 to n-3 fatty acids as compared to the control and Alifet treatments ($P<0.05$). These are in agreement with the results obtained by other authors using n-6 PUFA-rich oils like corn (Latour et al., 1998) and sunflower oil (Galobart et al., 2001). The ratio of n-6 to n-3 PUFA was 11.5 in the control fresh eggs which nearly meets the 6:1 ratio suggested for a healthy diet (British Nutrition Foundation, 1992). The Alifet supplement did not increase this ratio significantly but the pumkin seed oil inclusion negatively affected the parameter.

In contrast to the results obtained with inclusion of 20 IU/kg vitamin E, feeding diets containing 5% Alifet supplement increased the content of SFA of egg yolk when 40 IU/kg vitamin E was added to the diet. Furthermore, significantly higher MUFA content, and lower n-3, n-6 and total PUFA were measured in the egg yolks from hens fed 40 IU/kg vitamin E compared to 20 IU/kg vitamin E in the two Alifet treatments. However, different levels of vitamin E supplementation did not influence levels of SFA, MUFA and PUFA in the pumpkin seed oil group ($P>0.05$). Generally, data concerning the relationship between dietary fatty acids and vitamin E metabolism are still controversial.The higher level of added vitamin E with the Alifet treatment could promote intestinal absorption and/or transport of SFA and led to a significantly higher content of SFA in the egg yolk of fresh eggs. Husvéth et al. (2000) reported that SFA level in the liver lipid of chicks fed the diet containing fish oil and palmitic acid (C16:0) level in those fed fish oil and beef tallow diets were significantly increased by vitamin E supplementation (100 IU/kg). Meluzzi et al. (2000) observed significantly higher content of the saturated stearic acid (C18:0) in the egg yolk of hens using lard and fish oil treatment with vitamin E inclusion at the level of 50 IU/kg than in the treatment groups with no added vitamin E after two weeks experimental feeding. Animal fat and fish oil used in both studies contain higher amount of SFA than pumpkin seed oil so dietary vitamin E may be more effective in increasing SFA level of egg yolk. The higher level of added vitamin E reduced the amount of n-6 and n-3 PUFA in the egg yolk and this effect was significant in the Alifet treatment group ($P<0.05$). This result can be explained by the competition between vitamin E and PUFA either at the level of intestinal absorption or hepatic incorporation into lipoproteins. Higher levels of vitamin E are suggested to associate with lower levels of n-3 PUFA in the egg yolk (Meluzzi et al., 1999; Galobart et al., 2001).

Lipid peroxidation is one of the the main factors that can influence fatty acid composition of egg yolk lipids during storage. PUFA are the most susceptible to oxidative damage due to high number of double bounds in the molecules. In comparison to fresh samples, refrigerated storage of eggs for 4 weeks decreased the proportion of n-3, n-6 and total PUFA and increased the ratio of n-6 to n-3 PUFA in the egg yolk regardless of dietary fat type when 20 IU/kg vitamin E was fed. The simultaneous increase of MUFA was observed in both fat groups.Vitamin E can protect the fatty acids from oxidative deterioration and enhance the oxidative stability of eggs rich in PUFA, but its effectiveness varies and depends on inclusion level, amount and type of PUFA (Galobart et al., 2001; Cherian et al.,1996). Furthermore, prooxidant effects can be attributed to vitamin E at high supplementary levels (200 IU/kg; Gebert et al., 1998). Inclusion of vitamin E at the level of 40 IU/kg was able to prevent the significant decrease of n-3 PUFA and increase of n-6 to n-3 PUFA ratio during storage when it was

added to the diet together with pumpkin seed oil, but failed to hinder these unfavourable changes of fatty acid composition in the Alifet treatment group.

In conclusion, the Alifet supplement rich in SFA at the level of 5% can increase the SFA level of egg yolk. It does not affect the ratio of n-6 to n-3 PUFA in the fresh egg yolk, but the proportion of n-3 fatty acids can decrease during refrigerated storage and vitamin E at the levels of 20 and 40 IU/kg is not protective against this process. Feeding pumpkin seed oil diet leads to undesirable changes in the ratio of n-6 to n-3 PUFA of fresh egg yolk.

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