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Cholesterol Levels and Nutritional Composition of Commercial Layers Eggs Fed Diets with Different Vegetable Oils

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ABSTRACT

This experiment aimed at evaluating the effects of the supplementation of different vegetable oils at different levels to the diet of commercial layers on egg cholesterol levels and nutritional composition (proteins, total solids, lipids, and ashes) for 112 days. Birds were distributed according to a completely randomized experimental design with 10 treatments (T1 – control; T2 – inclusion of 2.5% rapeseed oil; T3 – inclusion of 2.5% canola oil; T4 – inclusion of 2.5% soybean oil; T5 – inclusion of 5% rapeseed oil; T6 – inclusion of 5% canola oil; T7 – inclusion of 5% soybean oil; T8 – inclusion of 2.5% rapeseed oil + 2.5% soybean oil; T9 – inclusion 2.5% canola oil + 2.5% soybean oil; T10 – inclusion of 2.5% rapeseed oil + 2.5% canola oil) of six replicates of eight birds each, totaling 480 birds. Yolk cholesterol levels and nutritional composition were determined on days 20, 60 and 112 days of the experimental period. Data obtained during the experimental period were submitted to analysis of variance. Egg yolks produced by layer fed oils presented lower cholesterol levels after 20 days of inclusion in the experimental diets. On days 60 and 112, cholesterol levels were higher. It was concluded that supplementing layer diets with vegetable oils rich in polyunsaturated fatty acids does not change the nutritional composition of egg yolks. The supply of diets containing oils rich in polyunsaturated fatty acids does not reduce yolk cholesterol content.

INTRODUCTION

The enrichment of eggs with polyunsaturated fatty acids (PUFA), particularly with omega-3 fatty acids has attracted the attention of both researchers and the food industry, because these fatty acids are essential for normal body development and play an important role in the prevention of heart diseases, diabetes, arthritis, inflammatory and auto-immune conditions, and cancer (Simopoulos, 2000).

Most egg lipids are concentrated in the yolk, and consist of lipoproteins, phospholipids, triacylglycerols, and cholesterol. The lipid fraction of the yolk is composed of 8.7g saturated fatty acids, 13.2g monounsaturated fatty acids, 3.4 g polyunsaturated fatty acids and 1.120 mg cholesterol per 100 g of fresh yolk (Holland *et al*., 1997).

Some researchers (Baucells *et al*., 2000; Grobas *et al*., 2001; Gómez, 2003; Mazalli *et al*., 2004) demonstrated the possibility of changing the fatty acid profile of the lipid fraction of eggs by reducing the dietary concentration of some fatty acids (lauric acid and short-chain saturated fatty acids) in exchange of long-chain fatty acids, such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), which present more than 18 carbons in their chemical structure.

Cholesterol deposition in the egg yolk can also be affected by nutrition (Hargis *et al*., 1991).The inclusion of specific feedstuffs in commercial layer diets, such as vegetable oils rich in unsaturated fatty acids used to change egg lipid profile and to reduce egg cholesterol content. Literature results on the possible effect of the diet on egg and blood cholesterol levels are contractor. According to Holland *et al*. (1980) and Mori (2001), the addition of PUFA-rich oils in in the diet reduces blood and egg cholesterol concentrations. However, other studies showed that yolk cholesterol content cannot be changed because it seems to be constant, independently of dietary factors (Bertechini, 2003).

This study aimed at evaluating the effects of the supplementation of rapeseed, soybean, and canola oil in commercial layer diets on yolk cholesterol levels and composition in terms of total solids, protein, lipids, and minerals.

Table 1 – Ingredients and calculated nutritional composition of the experimental diets.

	Treatments									
Ingredients	Control	2	3	4	5	6	7	8	9	10
Ground corn	64.20	52.66	53.05	53.10	41.17	41.62	41.75	41.40	41.80	41.40
Soybean meal	25.60	24.02	24.10	24.10	22.31	22.45	22.48	22.39	22.47	22.32
Wheat midds	-	10.67	10.22	10.16	21.44	20.85	20.69	21.12	20.66	21.19
Canola oil	-	-	2.50	-	-	5.00	-	-	2.50	2.50
Rapeseed oil	-	2.5	-	-	5.00	-	-	2.50	-	2.50
Soybean oil1	-	-	-	2.5	-	-	5.0	2.5	2.5	-
Limestone	7.98	8.05	8.03	8.02	8.08	8.07	8.07	8.08	8.07	8.08
Dicalcium phosphate	1.36	1.25	1.25	1.27	1.14	1.15	1.15	1.15	1.15	1.15
salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
DL-Methionine	0.20	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.20
Mineral supplement[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin supplement[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mycotoxin adsorbent	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant (BHT)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutritional composition										
Crude protein (%)	17.50	17.50	17.50	17.50	17.50	17.50	17.50	17.50	17.50	17.50
ME (kcal/kg feed)	2.750	2.750	2.750	2.750	2.750	2.750	2.750	2.750	2.750	2.750
Calcium (%)	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Available phosphorus	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Avail. lysine (%)	0.78	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.76	0.76
Avail. methionine (%) (%)	0.44	0.43	0.42	0.42	0.43	0.43	0.43	0.43	0.43	0.43
Avail. Met+Cys (%)	0.50	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48

[1] Supplied per kg feed: copper: 8 mg; iron: 50 mg; manganese: 70 mg; zinc: 50 mg; iodine: 1.2 mg; selenium: 0.2 mg.

[2] Supplied per kg feed: Vitamin A: 7.000 UI; Vitamin D3: 2.000 UI; Vitamin E: 5 mg; Vitamin K3: 1.6 mg; Vitamin B2: 3 mg; Vitamin B12: 8 mcg; Niacin: 20 mg: pantothenic acid: 5 mg; Antioxidant: 15 mg; vehicle QSP: 1 g.

MATERIALS AND METHODS

In this trial, 480 commercial white Lohman LSL layers, with 3 weeks of age at the beginning of the experiment, were fed for 112 days with diets supplemented with vegetable oils rich in polyunsaturated fatty acids, particularly in omega-3 and omega-6 fatty acids, according to the following experimental treatments: Conventional feed with no oil addition (T1); inclusion of 2.5% rapeseed oil (T2); inclusion of 2.5% canola oil (T3); inclusion of 2.5% soybean oil (T4); inclusion of 5% rapeseed oil (T5); inclusion of 5% canola oil (T6); inclusion of 5% soybean oil (T7); inclusion of 2.5% rapeseed oil + 2.5% soybean oil (T8); inclusion 2.5% canola oil + 2.5% soybean oil (T9); and inclusion of 2.5% rapeseed oil + 2.5% canola oil (T10).

Table 1 shows the ingredients and the calculated nutritional composition of the experimental diets.

Birds were housed in a brick house with 84 metal cages specific for egg production. Each cage was divided in two compartments with capacity of housing four birds each, housing a total of eight hens per cage. Independent feeders were placed in front of the cage, as well as nipple drinkers. A 17-h of light lighting program was adopted.

Feed intake and egg weight were weekly measured.

Yolk chemical composition (protein, total solids, lipid, and ash contents) and yolk cholesterol levels were determined on days 20, 60, and 112 of the experimental period.

Yolk protein content was determined according to the micro-Kjeldahl method, n. 39.1.19, of the AOAC (2007) for total nitrogen determination. The methodology proposed by Silva & Queiroz (2002) was applied to determine the percentage of total solids. Yolk ether extract content was determined according to the methodology described by the AOAC (2007), item 39.1.05. Ash content was determined according to the method described by Silva & Queiroz (2002).

A completely randomized experimental design, with ten treatments with three replicates of six eggs each was applied.

The analyses to determine egg cholesterol content were carried out according to the methodology proposed by Bragagnolo & Rodriguez-Amaya (2003) adapted as suggested by Mazalli *et al*. (2003), and cholesterol was determined using a commercial enzyme kit.

The option for using the enzymatic method for cholesterol determination was based on the work of Nogueira & Bragagnolo (2002) and Mazzali *et al*. (2003), who found that there were no cholesterol quantification differences between the enzymatic method, routinely used to determine serum cholesterol levels, and the chromatographic method, routinely used to determine cholesterol content in foods.

Data were submitted to analysis of variance of SAS statistical package (2000), and means were compared by the test of Tukey at 5% significance level.

Yolk cholesterol was analyzed according to a completely randomized experimental design in a 10 x 3 factorial arrangement (10 treatment and three periods), with four replicates per treatment.

RESULTS AND DISCUSSION

Table 2 shows the fatty acid composition of the vegetable oils used in the experimental diets.

Table 2 – Analyzed fatty acid composition of the vegetable oils included in the experimental diets (% of total fatty acids).

	Canola oil	Rapeseed oil	Soybean oil
Fatty acids		%	
C16:0 – palmitic acid	12.3751	6.3720	13.0608
C18:0 – stearic acid	2.4502	3.9842	2.2366
C18:1 – oleic acid	45.2735	24.8235	23.0449
C18:2 – linoleic acid (ω6)	36.5747	14.6624	56.4484
C18:3 – linolenic acid (ω6)	3.3264	50.1580	5.2094

Table 3 – feed intake (FI) and egg weight (EW) of white commercial layers fed diets containing different vegetable oils.

	FI (g/hen/day)	EW (g)
Treatments		
Control	120.35c	64.13
2.5% Rapeseed oil	122.10abc	64.91
2.5% canola oil	125.24abc	64.04
2.5% soybean oil	126.89a	64.96
5% rapeseed oil	123.03abc	63.77
5% canola oil	123.14abc	64.36
5% soybean oil	122.79abc	65.10
2.5% rapeseed oil + 2.5% soybean oil	126.43a	65.04
2.5% canola oil + 2.5% soybean oil	121.89bc	65.35
2.5% rapeseed oil + 2.5% canola oil	121.08c	64.70
Probability	$p \leq 0.01$	$p \leq 0.05$
CV (%)	2.08	1.36

Means followed by different letters in the same column are statistically different by the test of Tukey ($p \leq 0.05$)

Table 4 shows the yolk nutritional composition of the eggs of commercial layers fed diets containing different vegetable oils.

The analysis of variance indicated significant treatment effects on the lipid and mineral composition of the egg yolk, but there was no influence of treatments on yolk total solids and protein contents.

Seibel *et al*. (2005) did not find any differences in albumen or yolk protein content in the eggs of Japanese quails fed diets containing 2.7% fish oil or 5 and 10% of the solid fraction of fish chemical silage for 30 days.

The results obtained in the present study are consistent with the findings of Naber (1979), who asserted that egg protein characteristics are not influenced by the diet. Cobos *et al*. (1995), working with lipid supplementation of layer diets also did not find any differences in egg protein content.

Several authors reported the influence of diet on yolk vitamin (Surai & Sparks, 2001), ashes (Cobos *et al*., 1995), and mineral (Manson *et al*., 1993) composition.

According to Naber (1979), the main egg component (lipids) may be easily changed by dietary manipulation. Hall & Mckay (1993) found that egg lipid content is influenced by age in domestic fowl. According to Chwalibog (1992), as birds age, egg lipid content increases while protein content decreases.

The results obtained in the present study relative to yolk lipid percentage are consistent with those reported by Fennema (1993), who argues that variations in total yolk lipid content is more influenced by bird genetic strain than diet.

Sotelo & Gonzáles (2000), analyzing the percentage composition of egg with low cholesterol levels, found that yolk lipid percentage varied between 29.37 and 30%, which is consistent with the values obtained in the present study.

Relative to mineral composition, yolks from egg produced by hens fed the control treatment (no oil) were significantly different only from those derived from hens fed the diet supplemented with 2.5% canola oil + 2.5% soybean oil, which present lower mineral content.

Yolk mineral content in the present study ranged between 1.76 and 2.05%, which is consistent with the 1.92% determined by Sotelo & Gonzáles (2000) when evaluating the composition of low-cholesterol eggs.

The obtained differences in yolk lipid and mineral contents may be attributed to individual differences inherent to each bird and tis metabolism than to the diet fed, as the yolk composition of eggs produced by layers supplemented or not with vegetable oils were similar. Therefore, it is unlikely that vegetable oil type may have influenced yolk mineral or lipid deposition.

Table 4 – Percentage composition in the fresh matter of total solids, proteins, lipids, and minerals in the egg yolk of commercial layers fed diets containing different vegetable oils.

	Total solids (%)	Proteins (%) (%)	Lipids (%)	Minerals (%)
Treatments				
Control (no oil)	51.49	16.99	30.14ab	2.05b
2.5% rapeseed oil	51.21	16.81	27.94a	2.05b
2.5% canola oil	51.20	17.07	32.21b	2.03b
2.5% soybean oil	51.29	16.82	29.45ab	1.96ab
5% rapeseed oil	51.54	17.18	27.93a	1.93ab
5% canola oil	51.31	16.69	27.63a	2.05b
5% soybean oil	51.03	16.88	29.75a	2.00ab
2.5% rapeseed oil + 2.5% soybean oil	52.00	16.57	28.46a	1.91ab
2.5% canola oil + 2.5% soybean oil	51.64	16.73	28.72a	1.76a
2.5% rapeseed oil + 2.5% canola oil	51.78	16.82	28.97a	1.81ab
Probability	$p\leq0.05$	$p\leq0.05$	$p\leq0.01$	$p\leq0.05$
CV (%)	1.41	3.15	7.83	10.19

Means followed by different letters in the same column are statistically different by the test of Tukey ($p\leq0.05$)

There was a significant interaction between treatments and periods relative to yolk cholesterol content, as shown in Tables 5 and 6.

Table 5 – Yolk cholesterol content (mg/100g) of the eggs of commercial layers fed diets containing different vegetable oils.

Treatments	Cholesterol (mg/100g)
Control (no oil)	1078.81
2.5% rapeseed oil	1049.83
2.5% canola oil	1023.41
2.5% soybean oil	1066.43
5% rapeseed oil	1114.11
5% canola oil	1138.12
5% soybean oil	1096.14
2.5% rapeseed oil + 2.5% soybean oil	1090.15
2.5% canola oil + 2.5% soybean oil	1055.68
2.5% rapeseed oil + 2.5% canola oil	987.53
Probability	p≤0.01
Period	
20	792.04
60	1356.56
112	1061.47
Probability	p≤0.01
Treatment x Period	p≤0.01
CV (%)	8.74

There was a significant interaction between treatment and period for yolk cholesterol content in eggs from layers submitted to all experimental treatments. The highest cholesterol levels in the egg yolk of layers fed the diet with no oil were obtained on days 60 and 112 of the experimental period.

Egg yolks produced by layer fed oils presented lower cholesterol levels after 20 days of inclusion in the experimental diets; however, on day 60, cholesterol levels increased, and were intermediate compared with those recorded on day 112 of the experimental period. It was observed that egg cholesterol content tended to increase as birds aged. These results are consistent with those reported by Beyer & Jensen (1989), who asserted that egg cholesterol level is positively correlated with bird genetics and age, egg weight and yolk weight, and negatively correlated with lay percentage and dietary protein levels.

The high yolk cholesterol content in the eggs of hens fed 5% vegetable oil may be explained by the study of Vargas & Naber (1984), who correlated yolk cholesterol content with dietary energy balance and argued that excessive energy intake, beyond maintenance and production requirements, increases body weight and cholesterol synthesis. Therefore, excessive cholesterol would be transferred to the egg yolk.

Studies on layer dietary manipulation to reduce egg cholesterol content have shown conflicting results. Some report that the polyunsaturated fatty acids that compose the vegetable oils supplemented in the diet reduce both egg and blood cholesterol levels (Harris & Wilcox, 1963; Holland *et al*., 1980; Mori *et al*., 1999). On the other hand, these results were not obtained by other authors (Bartov *et al*., 1971; Wasburn & Nix, 1974), or in the present study.

Santos (1998) found that the addition of soybean (2 and 4%), canola (2 and 4%), or polyunsaturated marine (0.1 and 0.2%) to the diet of commercial layers did not affect egg yolk cholesterol levels.

Table 6 – Details of the interaction between periods of experimental diet supply and experimental treatments relative to yolk cholesterol content of the eggs of commercial layers fed diets containing different vegetable oils.

		Treatment									
	Period (days)	No oil	2.5%R	2.5%C	2.5%S	5%L	5%C	5%S	2.5%R 2.5S	2.5%C 2.5S	2.5%R 2.5%C
Cholesterol (mg/100g)	20	844.37Ab	834.092ABc	797.59ABCc	827.71ABc	836.05ABc	824.57ABc	823.56ABc	745.72ABCc	680.22Cc	706.55BCc
	60	1233.28Ca	1324.95ABCa	1306.53ABCa	1271.42BCa	1373.62ABa	1448.43Aa	1390.34ABa	1432.64Aa	1374.75ABa	1409.65Aa
	112	1158.80Aa	990.47Bb	966.12BCb	1100.18ABb	1132.67Ab	1141.39Ab	1074.51ABb	1092.12ABb	1112.09ABb	846.42Cb

Means followed by the same capital letter in the same row and small letter in the same column are not significantly different by the test of Tukey (P≤ 0.05).
No oil = Control; 2.5%R= 2.5% rapeseed oil; 2.5%C= 2.5% canola oil; 2.5%S= 2.5% soybean oil; 5%L=5.0% rapeseed oil; 5%C= 5.0% canola oil; 5%S= 5.0% soybean oil; 2.5%R+2.5%S=2.5% rapeseed oil + 2.5% soybean oil; 2.5%C+2.5%S= 2.5% canola oil + 2.5% soybean oil; 2.5%R+2.5%C=2.5% rapeseed oil + 2.5% canola oil.

Consistent results were obtained by Grobas *et al*. (1997), who did not observe any differences in egg cholesterol control when comparing a wheat- and soybean-based diet with no fat supplementation (control) with the same diet supplemented with 7.5% tallow, olive oil, soybean oil, rapeseed oil, or fish oil.

According to Bertechini (2003), the chicken is capable of producing 10 times more cholesterol per kg of liver than humans. Therefore, manipulating layer diets to reduce egg cholesterol levels is not very effective, as chickens are able to maintain the egg cholesterol levels that are considered essential for egg composition, as observed in the present study.

Yolk cholesterol concentration is very resistant to changes because there is a required yolk cholesterol level to ensure embryo development (Shafey & Cham, 1994). However, hens are able to change yolk polyunsaturated fatty acid content in response to dietary lipid source. The reason is that, as opposite to mammals, poultry absorb dietary fat through the portal system as portomicrons, which are directly absorbed into the blood and transported to the liver, the main lipogenesis location, thereby allowing direct fat absorption by the liver (Van-Elswyk *et al*., 1994).

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CONCLUSIONS

Based on the obtained results, it is concluded that the supplementation of different vegetable oils to commercial white layer diets does not change egg nutritional composition (total solids, protein, lipids, and minerals).

The supply of diets containing oils rich in polyunsaturated fatty acids does not reduce yolk cholesterol content. However, egg cholesterol content tends to increase as birds age.

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