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Yolk Color and Lipid Oxidation of the Eggs of Commercial White Layers Fed Diets Supplemented with Vegetable Oils

■Author(s)

Faitarone ABG[I]
Garcia EA[II]
Roça RO[III]
Andrade EN[IV]
Vercese F[V]
Pelícia K[VI]

[I] Post-doctoral, Department of Agribusiness Management and Technology, FCA, Unesp, Botucatu, SP, Brazil.
[II] Prof. Head Professor, Department of Animal Production, FMVZ, Unesp, Botucatu, SP, Brazil.
[III] Prof. Associate Professor, Department of Agribusiness Management and Technology, FCA, Unesp, Botucatu, SP, Brazil.
[IV] Ph.D. in Animal Science, FMVZ, Unesp, Botucatu, SP, Brazil.
[V] Ph.D. in Animal Science, FMVZ, Unesp, Botucatu, SP, Brazil.
[VI] Professor, Department of Animal Science, UNIFENAS, Alfenas, MG, Brazil.

■Mail Address

Corresponding author e-mail address
Ana Beatriz Garcia Faitarone
Rua Professor Antônio Ruete, 81 – Nova Jaboticabal – Jaboticabal – SP – Brasil – CEP 14887-032
E-mail: abiazinha2003@yahoo.com.br

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Polyunsaturated fatty acids, yolk color, canola oil, linseed oil, soybean oil, TBARs.

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ABSTRACT

The objective of this study was to evaluate the influence of the supplementation of vegetable oils rich in polyunsaturated fatty acids to the diet of Leghorn layers on yolk color and on yolk lipid oxidation of eggs stored at room temperature for 10 days. Sixty eggs laid by commercial white layers (Lohmann LSL) fed diets supplemented with different vegetable oils were used. Hens were fed one of the following treatment diets: conventional diet with no oil inclusion (T1); T1 diet with 2.5% linseed oil inclusion (T2); T1 diet with 2.5% canola oil (T3); T1 diet with 2.5% soybean oil (T4); T1 with 5.0% linseed oil (T5); T1 diet with 5.0% canola oil (T6); T1 diet with 5.0% soybean oil (T7); T1 diet with 2.5% linseed oil + 2.5% soybean oil (T8); T1 diet with 2.5% canola oil + 2.5% soybean oil (T9); and T1 diet with 2.5% linseed oil + 2.5% canola oil (T10). Eggs were evaluated as to yolk lipid peroxidation (TBARS values) and yolk color, as determined by colorimetry and subjective sensorial analysis. Data were submitted to analysis of variance and means were compared by the test of Tukey at 5% significance level. It was concluded that the inclusion of vegetable oils in commercial white layer diets does not significantly change egg yolk pigmentation, as colorimetrically evaluated. However, when subjectively assessed, the yolks of the eggs laid by hens fed diets supplemented with vegetable oils tend to be paler. The yolks of the eggs laid by layers fed diets containing sources of polyunsaturated fatty acids presented high lipid oxidation, particularly when compared with those derived from layers fed the diet with no oil supplementation.

INTRODUCTION

Egg quality have different meanings, according to egg producers', consumers', and processors' perspectives. The main egg quality aspects considered by egg producers are egg weight and eggshell quality, whereas consumers are interested in shelf life, external appearance, and sensorial qualities, such as eggshell and yolk color. On the other hand, processors take into account easy eggshell removal and separation of the yolk from the albumen, as well as egg functional properties (Alleoni & Antunes, 2001).

The contents of egg components maybe changed by the diet, and the inclusion of specific ingredients in layer feeds have been used to change the yolk lipid profile and to improve yolk quality. The use of nutritional strategies to improve the quality and change the composition of animal products used for human consumption links animal production with food technology and nutrition (Szymozyk & Pisulewiski, 2003).

When added to layer diets, lipids increase dietary energy density; improve diet palatability; reduce heat increment, increasing energy metabolism efficiency; and change egg yolk composition (Braga & Baião, 2001).

Some of the fatty-acid sources added to layer diets are linseed oil (used for the production of ω-3 enriched oil), soybean oil and sunflower, which are used as sources of mono- and polyunsaturated fatty acids (Baucells *et al*., 2000; Schreiner *et al*., 2004).

The use of linseed oil in layer diets enriches the egg as it increases the egg content of unsaturated fatty acids, particularly of linolenic acid, as well as by the incorporation of small amounts of eicosapentaenoic acid (EPA) and doxosahexaenoic acid (DHA) in the yolk.

Most of egg lipids are in the form of yolk lipoproteins (4 g of its average weight), with the lipids associated with the proteins vitelline and vitellinin. The lipid profile of the egg yolk is influenced by genetics, egg size, feed composition, and type of fat added to feed (Barreto *et al*., 2006; Cherian, 2008).

The main yolk fatty acids are oleic acid (38%), palmitic acid (23%), and linoleic acid (16%), according to Grosch (1997). Botsoglou *et al*.(1998) reported that chicken eggs contain 33.84% saturated fatty acids and 45.26% monounsaturated fatty acids, and 17.63% and 2.34% polyunsaturated fatty acids of the series ω-6 and ω-3, respectively.

Among yolk sensorial attributes, its color is considered as a quality indicator, and plays an important role of egg acceptance by the consumers. Higher yolk color intensity increases the egg acceptance by the consumers, who associated more intense yolk pigmentation with higher egg nutritional value (Silva *et al*., Albino & Godói, 2000; Tocchini & Mercadante, 2001).

Yolk color intensity is determined by the incorporation of xanthophylls present in corn, particularly of lutein and zeaxanthin, and depends on the inclusion levels of yellow corn in layer diets. However, other feedstuffs may change yolk color, depending on their inclusion level (Silva *et al*., Albino & Godói, 2000).

Oliveira (2008) studied the influence of dietary lipid sources on yolk color in layers, and reported more intense color when hens were fed diets with sunflower oil compared with those fed a diet that did not contain vegetable oil.

Lee *et al*. (2001) did not find any effect of corn oil addition to layer diets on yolk color, and concluded that changes in yolk color may be observed when supplemental carotenoid sources are added to the diet, as carotenoid pigments are fat soluble and therefore, absorbed in the intestine together with lipids.

Another important egg quality item is lipid stability, as the yolk fatty acids may suffer lipid oxidation during storage. Lipid oxidation affects food quality, particularly its aroma, taste, and nutritional value, in addition of producing toxic compounds. Fatty acids, particularly unsaturated fatty acids, are the compounds most susceptible to oxidation (Fennema, 2000). Consequently, the inclusion of polyunsaturated fatty acids in layer diets may increase the susceptibility of eggs to lipid oxidation (Cherian *et al*., 2007). Antioxidants, such as tocopherol, may be added to layer diets to protect fatty acids from oxidation and to enrich eggs with vitamin E (Pita *et al*., 2006).

Xavier *et al*. (2008) reported that, when stored at room temperature, the shelf life of chicken eggs ranges between four to 15 days after lay, with no impairment of their internal quality.

Despite its limitations, the typical method used to assess lipid oxidation in fatty acid-rich foods is the thiobarbituric acid (TBA) test because it is simple and fast. This test quantifies the level of malonaldehyde (MDA), which is one of the main products of the breakdown of hydroperoxides produced during the oxidation of polyunsaturated fatty acids (Osawa *et al*., 2005).

Foods appropriate for consumption should present lipid oxidation values below 3 mg MDA/kg of sample, with an upper limit of 7-8 mg MDA/kg (Cadun *et al*., Cakli & Kisla, 2005).

Giampietro *et al*. (2008) evaluated the degree of lipid oxidation using TBARS in the yolks of eggs laid by brown layers and stored for 0, 7, 14, and 21 days at room temperature (25ºC). The authors detected yolk loss quality, as determined by progressive lipid oxidation up to 14 days of storage, and concluded that storing eggs at room temperature does not preserve egg internal quality.

Therefore, this study aimed at evaluating the influence of the supplementation of white layer diets with vegetable oils rich in polyunsaturated fatty acids on the sensorial egg characteristics and on yolk lipid oxidation of eggs stored at room temperature for 10 days.

MATERIAL AND METHODS

A total of 480 Lohmann LSL layers, with 33 weeks of age at the beginning of the experiment were used. Hens were housed in 84 metal battery cages designed

for layers in a masonry house. Cages were equipped with individual trough feeders placed in front of the cage, and nipple drinkers. A 17-h of light lighting program was adopted.

The following treatments were applied: conventional diet with no oil (T1); T1 diet with 2.5% linseed oil inclusion (T2); T1 diet with 2.5% canola oil (T3); T1 diet with 2.5% soybean oil (T4); T1 with 5.0% linseed oil (T5); T1 diet with 5.0% canola oil (T6); T1 diet with 5.0% soybean oil (T7); T1 diet with 2.5% linseed oil + 2.5% soybean oil (T8); T1 diet with 2.5% canola oil + 2.5% soybean oil (T9); and T1 diet with 2.5% linseed oil + 2.5% canola oil (T10).

Birds were offered feed and water *ad libitum* during the entire experimental period. All diets were based on corn, soybean meal, and wheat middlings. The diets were formulated to contain identical energy and protein levels, and to supply the hens' nutritional requirements, according to Rostagno *et al*. (2005). The only difference was the oil source included. The ingredient and calculated nutritional composition of the experimental diets are shown in Table 1.

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
Linseed oil	-	2.5	-	-	5.00	-	-	2.50	-	2.50
Soybean oil	-	-	-	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
S alt	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
Adsorbent	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidante (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
Calculated 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 IU; Vitamin D3: 2,000 IU; 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.

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).

Fatty acids	Canola oil	Linseed oil	Soybean oil
		%	
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 (ω3)	3.3264	50.1580	5.2094

On day 60 of the experimental period, egg yolks were analyzed for TBARS levels (degree of lipid oxidation). Six eggs per treatment were collected and stored in paper pulp egg trays at environmental temperature (19 ± 2ºC) for 10 days. At the end of the storage periods, eggs were broken, and their yolks were separated and frozen. When frozen, yolks were freeze-dried by removing water and other solvents by sublimations, that is, solid-state water was directly transformed into steam, and did not pass by the liquid state. Dehydrated yolks were then analyzed for lipid oxidation (TBARS), according to the methodology described by Vyncke (1970) and modified by Ramanathan & Das (1992).

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

Egg yolk color sensorial assessment was performed at the end of the experimental period using the Multiple Comparison test. Yolk color was also measured using a colorimeter (Minolta CR-400), according to Honikel (1998).

The multiple comparison sensorial test for the determination of yolk color was performed according to Roça *et al*. (1988), with 23 trained and selected tasters (Roça & Bonassi, 1985). A structured scale with nine scores, ranging from 1 = extremely less intense than the standard to 9 = extremely more intense than the standard, was used. Eggs were cooked for 10 min at 96ºC, and then longitudinally cut to expose the yolks for assessment. Sensorial egg yolk color assessment was analyzed according to a completely randomized experimental design with 10 treatments of 23 replicates (panelists) each.

For egg yolk color instrumental measurements, eight eggs per treatment were duly identified, cooked for 10 min at 96ºC, and longitudinally cut to expose the yolks. Yolk color was measured using a colorimeter (Minolta CR-400) according to the methodology proposed by Honikel (1998). The colorimeter was previously calibrated against a white ceramic surface according to the standards established by Bible & Singha (1997).The CIE (Commission Internationale de l'Éclairage) color evaluation system (L*, a*, and b*) was applied. The L* values correspond to luminosity, with maximum value of -100 corresponding to perfect diffuse reflection and the minimum value of 0 representing black. The a* value corresponds to redness, and ranges from red (+a*) to green (-a*), and the b* value indicates yellowness, and ranges from yellow (+b*) to blue (-b*). The a* and b* values to not have specific numerical limits. Yolk color was determined as the average of five readings in the center of the yolk of each egg. Instrumental egg color results were analyzed according to a completely randomized experimental design, with ten treatments with eight replicates (eggs) each. Data were submitted to analysis of variance (ANOVA).

The GLM (General Linear Models) procedure of SAS® statistical software (version 9.0 for Windows®; SAS, 2002) was used. Treatment means were compared by the test of Tukey at 5% significance level.

RESULTS AND DISCUSSION

Table 3 shows the yolk lipid oxidation results of the different treatments, as measured by TBARS.

Table 3 – Lipid oxidation (TBARS values) of the yolks of eggs laid by white layers fed diets supplemented with different vegetable oils.

Treatment	TBARS (mgTMP/kg)
1 (control)	0.1700c
2 (2.5% linseed oil)	0.2583abc
3 (2.5 % canola oil)	0.2150abc
4 (2.5% soybean oil)	0.1467c
5 (5% linseed oil)	0.3000a
6 (5% canola oil)	0.2000abc
7 (5% soybean oil)	0.2217abc
8 (2.5% linseed oil + 2.5% soybean oil)	0.1817bc
9 (2.5% canola oil + 2.5% soybean oil)	0.2917ab
10 (2.5% linseed oil + 2.5% canola oil)	0.1867abc
Probability	p<0.05
CV (%)	27.32

Means followed by the same letters in the same column are not statistically different by the test of Tukey (p<0.05).

The analysis of variance showed significant yolk lipid oxidation differences among treatments, as shown by TBARS values. After 10 days of storage at room temperature, the eggs laid by hens fed the diet with no oil supplementation or supplemented with 2.5% soybean oil presented lower yolk lipid oxidation degree than those of hens fed diets supplemented with 5% linseed oil and with 2.5% canola oil + 2.5% soybean

oil, but were not significantly different from the other treatments. According to Giampietro *et al* (2008), egg yolk lipid oxidation increase as egg age. These authors detected TBA values of 0.1343 in fresh eggs, which increased to 0.1698 in eggs stored for seven days, and to 0.2138 in eggs stored for 14 days.

Gómez (2003) stated that because polyunsaturated have several double bonds, they are very susceptible to oxidation. Therefore, the egg yolks enriched with these oils are more susceptible to lipid deterioration. This is supported by the results of the present experiment, which detected that the eggs enriched with polyunsaturated fatty acids, laid by hens fed diets supplemented with soybean, canola, and linseed oils, presented high degree of lipid oxidation. In addition, the yolks of the eggs laid by hens fed 5% linseed oil presented the highest degree of lipid oxidation. This result is consistent with the findings of Gómez (2003), who obtained higher lipid oxidation values in the eggs of layers fed diets with 5% linseed oil compared with those of hens fed diets with no oil supplementation. That author suggests the higher degree of unsaturation of linolenic acid (ω3) would account for the higher degree of yolk lipid oxidation. Aymond and Van Elswyk (1995) carried out a study to determine TBARS values in the yolk of PUFA ω-3 enriched eggs produced by layers fed diets supplemented with 5 or 15% intact or ground linseed for five weeks, and did not detect any TBARS differences between linseed treatments and the control (diet with no linseed inclusion). However, in the present experiment, significant differences between the treatment with no oil addition and that with 5% linseed oil supplementation, indicating that lipid oxidation occurred due the presence of a higher concentration of polyunsaturated fatty acids in the yolk. It is possible that if the antioxidant (BHT) was no included in the experimental diets, a more expressive yolk lipid oxidation would be detected.

Table 4 shows the yolk color results obtained by comparative sensorial assessment and by objective measurement (L*, a*, and b* values) of the eggs laid by white layers fed diets supplemented with different vegetable oils.

Both colorimetry and sensorial assessment revealed differences among treatments, with egg yolks from hens fed diets supplemented with 5% canola oil, 5% soybean oil, and 2.5% linseed oil + 2.5% canola oil presented similar yolk pigmentation as that of the eggs of the control treatment (Table 4). However, the pigmentation of the yolks of the eggs laid by hens fed the diet with 5% soybean oil supplementation were similar only to that of the control treatment and tended to be slightly more intense.

Both colorimetry and sensorial assessment revealed significant differences among treatments, with egg yolks from hens fed diets supplemented with 5% canola oil, 5% soybean oil, and 2.5% linseed oil + 2.5% canola oil presented similar yolk pigmentation as that of the eggs of the control treatment (no oil). However, the pigmentation of the yolks of the eggs laid by hens fed the diet with 5% soybean oil supplementation were similar only to that of the control treatment and tended to be slightly more intense.

The analysis of variance showed significant differences among treatments as to redness values. The hens fed diets supplemented with 2.5% linseed oil + 2.5% soybean oil produced yolks with lower redness values compared with those produced by hens fed the diets with no oil inclusion or supplemented with 2.5%

Table 4 – Average yolk color values, as determined by sensorial assessment and instrumental measurement, of the eggs laid by white layers fed diets supplemented with different vegetable oils.

Treatments	L* (luminosity)	a* (redness)	b* (yellowness)	Sensorial attribute Yolk color
T1 no oil addition	86.29	-2.41cd	42.45a	5.00cd
T2 2.5% linseed oil	85.55	-2.48bcd	37.68ab	2.86ab
T3 2.5 % canola oil	85.36	-2.24d	41.20a	2.60ab
T4 2.5% soybean oil	86.33	-3.06ab	39.26ab	2.17a
T5 5% linseed oil	86.31	-2.87abcd	35.89ab	3.39ab
T6 5% canola oil	86.06	-2.63bcd	34.94ab	3.86bc
T7 5% soybean oil	86.52	-2.53bcd	39.67ab	5.60d
T8 2.5% linseed oil + 2.5% soybean oil	86.55	-3.39a	41.97a	2.95ab
T9 2.5% canola oil + 2.5% soybean oil	86.26	-2.85abcd	37.83ab	3.13ab
T10 2.5% linseed oil + 2.5% canola oil	86.28	-2.92abc	32.38b	3.78bc
Probabilidade	p>0.05	p<0.05	p<0.05	p<0.05
CV (%)	1.32	14.49	12.05	38.69

Means followed by the same letters in the same column are not statistically different by the test of Tukey (p<0.05).

linseed oil, 2.5% canola oil, 5% canola oil, and 5% soybean oil. Moreover, the yolks of the eggs laid by layers supplemented with 2.5% canola oil tended to present higher redness values compared with those of hens fed diets supplemented with 2.5% soybean oil, 2.5% linseed oil + 2.5% soybean oil, and 2.5% linseed oil + 2.5% canola oil.

Yellowness (b* values) were also significantly different among treatments. The egg yolks of layers fed diet with no oil supplementation or supplemented with 2.5% canola oil, 2.5% linseed oil + 2.5% soybean oil presented higher b* values than those of hens supplemented with 2.5% linseed oil + 2.5% canola oil, but were not significantly different from the other treatments. Higher yellowness values were detected in the eggs of layers fed the diet with no oil supplementation, which may be explained by the higher inclusion of corn, which has natural pigments, in this diet. Perhaps a higher number of yolks sampled may have yielded statistical differences. Xanthophylls are red or yellow carotenoid pigments responsible for yolk color and are present in some plants such as corn, which contains approximately 20mg kg^{-1} (Cheeke, 1999).

No luminosity differences ($p>0.05$) were detected among treatments.

Carbó (1987) mentioned that the inclusion of fats in the diet aid the transfer of feed carotenoids to the egg yolk because dietary carotenoids and xanthophylls are fat soluble, that is, the intestinal absorption of these pigments is optimized when lipids are added to the diet. The results of the present study seem to suggest that the applied lipid sources did not contain pigments capable of affecting yolk color intensity. Carbó (1987), on the other hand, observed that the inclusion of antioxidants in diets rich in unsaturated fatty acids, which are susceptible to oxidation, improves yolk pigmentation. In addition, that author mentions that, when the dietary lipids produce peroxides, yolk pigmentation maybe negatively affected due to the oxidation of carotenoids.

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CONCLUSIONS

It was concluded that the supplementation of commercial white layer diets with vegetable oils does not considerably change egg yolk pigmentation, as colorimetrically evaluated. However, when subjectively assessed, the yolks of the eggs laid by hens fed diets supplemented with vegetable oils tend to be paler.

The yolks of the eggs laid by layers fed the diet with 5% linseed oil (rich in omega-3 polyunsaturated fatty acids) presented high lipid oxidation, particularly when compared with those derived from layers fed the diet with no oil supplementation.

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