Poultry Science Journal

ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir

Effects of Various Levels of Oxidized Oil on Performance, Egg Quality, and Some Blood Metabolites in Laying Hens

Saki AA, Aliarabi H, Cheraghi P, Mirzaie Goudarzi S & Ahmadi A

Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran

Poultry Science Journal 2016, 4 (1): 13-18

Keywords
Laying hen
Oxidized oil
Performance
Blood parameter

Corresponding author
Ali Asghar Saki
dralisaki@yahoo.com

Article history
Received: December 21, 2015
Revised: February 29, 2016
Accepted: April 27, 2016

Abstract

The influence of dietary oxidized oil was studied on laying hen performance, egg quality and blood metabolites. Experiment was conducted on 160 laying hens (Hy-Line W-36, 54-61 wk of age) in a completely randomized design with five treatments and four replicated cages containing eight birds per cage. Dietary treatments replaced fresh soybean oil in the control diet (3% fresh soybean oil, 15.25% crude protein, and 2858 Kcal/kg metabolizable energy) with 0, 25, 50, 75 and 100% oxidized oil. Egg production and egg weight were recorded daily and feed intake, feed conversion ratio, and egg mass were calculated weekly. Egg quality traits were recorded on a biweekly basis. Hen's body weight was measured individually at the beginning and end of the experiment. Serum metabolites were determined at the end of the experiment. There was a significant difference between diets with different oxidized oil levels in egg weight, egg mass, Egg production, and feed conversion ratio ($P < 0.05$). Feed intake was not affected by dietary treatments. There was no significant difference between oxidized oil levels on blood serum triglyceride, total cholesterol, high-density lipoprotein, low-density lipoprotein and very low-density lipoprotein. Malondialdehyde of the liver was not affected by oxidized oils. The results of this study have clearly demonstrated that maximum 25% oxidized oil could be replaced by fresh oil in the diets without any adverse effect on the performance of laying hens.

Introduction

Dietary oils are often used in poultry nutrition to protect birds from heat stress, especially in summer due to lowered body temperature. Vegetable oils that contain high levels of unsaturated fatty acids are commonly used in poultry diets (Yue *et al*, 2011). Storage and processing of vegetable oils causes lipid peroxidation and increases reactive oxygen species production. So, the levels of antioxidants in food must be increased when consuming these oils (Warnants *et al*, 1996). Oxidation products including lipid peroxides have detrimental biological impacts, and are related to a number of chronic and degenerative diseases (Esterbauer *et al*., 1991; Guardiola *et al*., 2002). Therefore, excessive consumption of oxidized oils can produce reactive oxygen species overwhelm the antioxidant system, and potentiate oxidative stress in the animal (Urso and Clarkson, 2003).

Oxidized oils reduce feed intake (FI) and growth rate in animals (Alexander, 1981). Fatty liver syndrome – one of the most important metabolic disorders in laying hens during the

Please cite this article as: Saki AA, Aliarabi H, Cheraghi P, Mirzaie Goudarzi S & Ahmadi A. 2016. Effect of various levels of oxidized oils on performance, egg quality, and some blood metabolites in laying hens. Poult. Sci. J. 4 (1): 13-18.

© 2016 PSJ. All Rights Reserved

period of high production – is a disease whose incidence may be reduced by dietary oils. The disease is especially common in birds confined in cages in summers and are fed energetic diets. The negative effects of fat and oil consumption are acid and/or heat- damage, which have a tendency to develop chemical methods for determination of these (Poling *et al.*, 1962). Therefore, the aim of this study was to determine the influence of oxidized oil supplementation on productive performance, egg quality, blood parameters and malondialdehyde (MDA) in the liver of Hy-Line W-36 laying hens.

Materials and Methods

All procedures during this study were approved by Animal Care Committee of Bu-Ali Sina University, Hamedan, Iran.

Oil samples and analysis

Oxidized oil was created from thermal heating of fresh soybean oil obtained from a poultry feed sales office (methods modified from Seppanen and Csallany, 2004; 2006). Briefly, the fresh oil was heated and kept at 185ºC for 6 hrs with constant blending. The heated oil was then cooled at room temperature and stored unstirred for 24 hrs. This oil was reheated to 185ºC for 3 hrs with constant blending and air supplementation. Finally, the chemical composition of the experimental oils (fresh and oxidized oil) including moisture (ISO 662), peroxide value (ISO 3960), and fatty acid composition were determined (Table 1). Gross energy was determined by a calorimetric bomb in the laboratory of agricultural engineering and livestock services. The metabolizable energy was calculated by multiply gross energy in coefficient 0.82.

Birds, diets and experimental design

In total, 160 laying hens (Hy-Line W-36) at 54 wks of age were used in a completely randomized design (CRD) over eight weeks with 5 treatments and 4 replicates of 8 birds in each treatment. To prepare experimental treatments, a control diet containing 3% fresh oil was formulated. Then four levels of oxidized oil (25, 50, 75 and 100%) were replacaced by fresh oil in the control diet. The experimental diets were in mash form and water was provided *ad libitum*. The feeding program was offered from 54 to 61 wk of age. Compositions of the experimental diets are given in Table 2. Light was provided 16 hrs daily and house temperature was maintained at 18-23ºC throughout the experimental period. All birds were maintained under similar management conditions throughout the experimental period.

Table 1. Chemical composition of the experimental oils

	Fresh oil	Oxidized oil
Appearance	Yellow, light	Dark brown
Gross energy (kcal/kg)	10079	9699
Metabolizable energy(kcal/kg)	8265	8175
Moisture (%)	4.0	5.8
Peroxide value (mEq of O_2/kg)	4.0	99.3
Fatty acid composition (mg/g of total analyzed fatty acids)		
C18:0	38.7	43.2
C18:2 n-6	480.3	398.2
C18:3 n-3	58.5	47.9
C16:0	105.2	112.6

All samples were tested three times.

Performance and egg quality

Body weight of the hens was measured individually at 54 and 61 wk of age to coincide with the beginning and end of the experiment. Eggs were collected daily. Egg production (EP) and egg weight (EW) were recorded daily, and feed intake (FI) was measured bi-weekly. This information was used to calculate, hen-day EP, egg mass (EM), ADFI, and feed conversion ratio (FCR). Egg mass was calculated by multiplying percentage of EP with EW for each replicate. Egg quality was measured in two eggs per replicate that were produced on the last day of each week, and the average value for each period was used for further analysis. The eggs were individually weighed and the external and internal quality were determined. To measuring shell weight

(SW), the shell was separated from the yolk and albumen, then dried over night at 60ºC as indicated by Grobas *et al*. (2001). Shell thickness (ST) was measured using a digital micrometer (Echometer 1061, Robotmation Company, Tokyo, Japan). Specific gravity (SG) was determined based on methods from Yannakopoulos and Tserveni-Gousi (1986) and Paganelli *et al*. (1974). Haugh units (HU) was calculated using the formula, HU = 100 Log (AH + 7.57– 1.7 $EW^{0.37}$) where HU is haugh unit, AH is albumen height (mm) and EW is egg weight (g) (Haugh, 1937).

Table 2. Ingredients and chemical analysis of the experimental diets (%, as fed basis)

	Levels of oxidized oil that replaced fresh oil				
Ingredients	0	25	50	75	100
Yellow corn	46.10	46.09	46.06	46.01	46.00
Wheat	20.00	20.00	20.00	20.00	20.00
Soybean meal (42% CP)	13.90	13.95	14.02	14.09	14.14
Corn gluten meal (60% CP)	4.83	4.79	4.75	4.72	4.68
Soybean oil (fresh)	0	0.75	1.5	2.25	3
Soybean oil (oxidized)	3	2.25	1.5	0.75	0
Oyster shell	9.84	9.84	9.84	9.84	9.84
Di-calcium phosphate	1.37	1.37	1.37	1.37	1.37
Sodium Chloride	0.40	0.40	0.40	0.40	0.40
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.03	0.03	0.03	0.04	0.04
L-Lysine HCL	0.03	0.03	0.03	0.03	0.03
Calculated analysis					
ME (kcal/kg)	2858	2858	2858	2858	2858
Crude protein (%)	15.25	15.25	15.25	15.25	15.25
Lysine (%)	0.7	0.7	0.7	0.7	0.7
Methionine (%)	0.3	0.3	0.3	0.3	0.3
Methionine + Cystine (%)	0.6	0.6	0.6	0.6	0.6
Calcium (%)	4.1	4.1	4.1	4.1	4.1
Available phosphorus (%)	0.37	0.37	0.37	0.37	0.37

[1] Vitamin premix supplied per kg of diet; Vit A 8800IU, Vit D_3 2500IU, Vit E 11IU, Vit B_1 1.5 mg, Vit B_2, 4.0 mg, Vit B_3 (Calcium panthotenate) 8 mg, Vit B_5 (Niacin) 35 mg, Vit B_6 2.5 mg, Vit B_{12} 0.01 mg, Biotin, 0.15 mg, Folic Acid 0.48 mg, Choline Chloride 400 mg, Vit K_3 2.2 mg.

[2] Mineral premix supplied per kg of diet; Manganese 75 mg, Iron 75 mg, Zinc 64.8 mg, Copper 6.0 mg, Iodine 0.87 mg, Selenium 0.2 mg.

Serum metabolites

At the end of the experiment, two hens were randomly selected from each replicate (n=4) for blood parameters measurement. Blood samples were collected from the left jugular vein. Serum was isolated by centrifugation at 1861 × g for 15 min then stored at -20°C until analysis. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) of serum were measured using commercial kits (Pars- Azmoon, Co., Tehran, Iran) and calorimetrically using a spectrophotometer. The very low-density lipoprotein cholesterol (VLDL-C) concentration was determined with an ELISA kit (Pars-Azmoon, Co., Tehran, Iran)

Liver Sampling

After slaughter (8 birds from each treatment), the left hepatic lobe was sampled and quickly frozen in liquid nitrogen. Liver samples were stored at -20°C until analysis. MDA of the liver was measured as described by Cherian *et al*. (1996). Briefly, tissue samples (2 g) were weighed into a test tube with 50 mL of 3.86% perchloric acid. 50 mL of butylated hydroxyanisole (BHA) solution was added before homogenizing at a high speed for 30 sec. In the samples, homogenized solution was filtered by Whatman 1 filter paper to 25 mL glass tube. The filtrate (2 mL) was mixed with 2 mL of 20 mM thiobarbituric acid (TBA) in distilled water and incubated in a boiling water

bath for 30 min. The blank sample lacked liver tissue. TBA concentration was used to determine standard preparation. After cooling, the absorbance of the filtrate was determined at 531 nm. TBA concentrations were expressed as milligrams of MDA per kilogram of tissue.

Statistical analysis

The experiment was conducted as a completely randomized design with 5 treatments and 4 replicates employing one-way analysis of variance (SAS, 2008). Treatment means were compared with Duncan's multiple range tests (Duncan, 1955). All differences were considered significant when $P < 0.05$. The normal distribution of data was evaluated using Shapiro-Wilk test.

Results and Discussion

The appearance, gross and metabolizable energy, moisture, peroxide value, and fatty acid composition of the experimental oils (fresh and oxidized) are shown in Table 1. Compared to oxidized oil, fresh oil had higher gross energy (10079 *vs.* 8265 Kcal/kg) and metabolizable energy (9699 *vs.* 8175 Kcal/kg), but lower moisture (4 *vs.* 5.8 %) and peroxide value (4 *vs.* 99.3 mEq of O_2)/kg.

Table 3. Influence of oxidized oils levels of the diet on productive performance of laying hens from 54 to 61 wk of age

Levels of oxidized oil (%)	Egg production (%)	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	FCR[1]
0	86.83[a]	60.07[ab]	52.18[a]	97.88	1.88[c]
25	83.36[b]	60.56[a]	50.48[ab]	97.87	1.94[bc]
50	81.13[bc]	59.70[abc]	48.45[bc]	98.68	2.04[ab]
75	79.01[c]	59.04[c]	46.63[c]	97.49	2.12[a]
100	79.68[c]	59.43[bc]	47.41[c]	97.57	2.08[a]
SEM	1.12	0.32	0.79	0.28	0.04
P-value	<0.0001	0.0124	<0.0001	0.8157	<0.0001

The means with a different letter in each column are significantly different ($P < 0.05$).
SEM: Standard error of the means.
[1] Feed conversion ratio.

Results regarding the productive performance of laying hens are shown in Table 3. Egg weight, EP, EM and FCR, but not feed intake, were significantly affected by dietary treatments ($P < 0.05$). Egg weight was significantly higher with 25% oxidized oil in comparison to 75 and 100% oxidized oil ($P < 0.05$). All concentrations of oxidized oil measured reduced egg production relative to the control ($P < 0.05$). Feed conversion ratio was significantly lower in control diet compared to 50, 75 and 100% oxidized oil but no significant difference was shown with 25% oxidized oil. Oxidized oil levels above 25% reduced laying performance but only higher concentrations (75 and 100%) significantly reduced EW, EP, and EM ($P < 0.05$). Some studies have reported a decline in performance with dietary oxidized oils (Sanchez-Muniz *et al*., 1998; Yue *et al*, 2011), whereas others have found no negative effects (Suomela *et al*, 2004; Bou *et al*, 2005; Lewis-McCrea and Lall, 2006).

Table 4. Effects of oxidized oils levels on egg quality parameters

parameter	Levels of oxidized oil (%)					*SEM* [1]	*P*-value
	0	25	50	75	100		
SG [2]	1.08	1.08	1.08	1.08	1.08	0.0003	0.5377
SW [3] (g)	5.78	5.59	5.66	5.56	5.46	0.0950	0.2619
ST [4] (g)	0.36	0.35	0.36	0.36	0.35	0.0027	0.6553
HU [5] (%)	94.68[a]	94.38[a]	92.93[a]	92.72[a]	94.17[a]	0.5552	0.0058
EC [6] (g)	48.06[a]	45.03[b]	43.30[bc]	41.87[c]	43.30[bc]	0.9264	<0.0001

The means with a different letter in each row are significantly different ($P < 0.05$).
[1] Standard error of the means.
[2] Specific gravity, [3] Shell weight, [4] Shell thickness, [5] Haugh unit, [6] Egg content.

Effect of oxidized oils on egg quality parameters are shown in Table 4. Some of these parameters including SG, SW, and ST were not affected significantly by oxidized oils, while

Haugh unit was significantly higher in the control and 25% oxidized oil diet compared with 100% oxidized oil ($P < 0.05$).

There were no significant differences in the concentrations of serum TG, TC, HDL-C, LDL-C, and VLDL-C (Table 5). Though there was a progressive decrease in serum TG as levels of oxidized oil increased, it was not significant. In contrast, oxidized oil significantly reduced serum TG in the rat (Eder, 1999) and in laying hen (Yue *et al.*, 2011). Consistent with our findings, Ammouche *et al.* (2002) also found no significant effect of dietary oxidized oil on serum TC. In contrast, Koch *et al* (2007) observed a reduction in plasma TC concentrations in rats fed oxidized sunflower oil. The slight decrease in serum VLDL-C by increasing levels of oxidized oil is dissimilar with results obtained by Yue *et al.* (2011).

Table 5. The effect of oxidized oil levels on blood parameters of laying hens at 61 wk of age

Levels of oxidized oil (%)	TG [1] (mg/dL)	TC [2] (mg/dL)	VLDL-C [3] (mg/dL)	HDL-C [4] (mg/dL)	LDL-C [5] (mg/dL)
0	1552.7	152.00	309.10	9.00	167.53
25	1548.3	181.50	309.65	15.33	162.95
50	1473.8	139.75	294.75	10.00	165.00
75	1297.8	157.50	259.55	10.25	112.30
100	1248.3	117.25	249.65	9.50	141.90
SEM	114.794	33.718	22.958	1.686	15.012
P-value	0.3434	0.7439	0.3434	0.4139	0.2027

The means with a different letters in each column are significantly different ($P < 0.05$).
SEM: Standard error of the means.
[1] Triglyceride, [2] Total cholesterols, [3] Very low-density lipoprotein cholesterol, [4] High-density lipoprotein cholesterol, [5] Low-density lipoprotein cholesterol.

Table 6. Effects of oxidized oil levels on liver Malondialdehyde concentration of laying hens at 61 wk

Levels of oxidized oil (%)	Malondialdehyde (Nano mol/gram tissue)
0	2.64
25	3.61
50	2.83
75	2.18
100	3.33
SEM	0.522
P-value	0.422

SEM: Standard error of the means.

MDA concentration in the liver was not affected by dietary oxidized oils (Table 6, $P > 0.05$). Similar results were obtained by Zalejska-Fiolka *et al.* (2012) in the rat. It seems liver has little ability to control tissue MDA levels and its function will fail when the levels of MDA enhanced.

Conclusion

The results of this study showed that dietary oxidized oils decreased laying hen's performance. Although, the 25% dietary oxidized oil level had no adverse effects on laying hens performance, but the upper levels severely reduced EW, EP, and EM.

References

Alexander JC. 1981. Chemical and biological properties related to toxicity of heated fats. Journal of Toxicology and Environmental Health, Part A Current Issues, 7: 125–138. [Link]

Ammouche A, Rouaki F, Bitam A & Bellal MM. 2002. Effect of ingestion of thermally oxidized sunflower oil on the fatty acid composition and antioxidant enzymes of rat liver and brain in development. Annals of Nutrition and Metabolism, 46: 268–275. [Link]

Bou R, Tres A, Baucells MD & Guardiola F. 2005. Increase of geometrical and positional fatty acid isomers in dark meat from broilers fed heated oils. Poultry Science, 84: 1942–1954. [Link]

Cherian G, Wolfe F & Sim J. 1996. Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids, and oxidative stability. Poultry Science, 75: 423-431. [Link]

Duncan DB. 1955. Multiple range and F-tests. Biometrics, 11: 1-42. [Link]

Eder K. 1999. The effects of dietary oxidized oil on lipid metabolism in rats. Lipids, 34: 717–725. [Link]

Esterbauer H, Schaur RF & Zollner H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related

aldehydes. Free Radical Biological Medicine, 11: 81-128. [Link]

Grobas S, Mendez J, Lazaro R, de Blas C & Mateos GG. 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Poultry Science, 80: 1171-1179. [link]

Guardiola F, Dutta PC, Codony R & Savage GP. 2002. Cholesterol and phytosterol oxidation products: analysis, occurrence, and biological effects. AOCS Press: Champaign, IL, 2002. [Link]

Haugh RR. 1937. The Haugh unit for measuring egg quality. U.S. Egg and Poultry Magazine, 43, 552 - 555 and 572.

Hy-Line International. 2003. Commercial Management Guide for Hy-Line W-36. [Link]

Koch A, Konig B, Spielmann J, Leitner A, Stangl GI & Eder K. 2007. Thermally oxidized oil increases the expression of insulin-induced genes and inhibits activation of sterol regulatory element-binding protein-2 in rat liver. Journal of Nutrition, 137: 2018–2023. [Link]

Lewis-McCrea LM & Lall SP. 2006. Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnormalities in juvenile Atlantic halibut (*hippoglossus hippoglossus*). Aquaculture, 262: 142–155. [Link]

Paganelli CV, Olszowka A & Ar A. 1974. The avian egg surface area, volume, and density. Condor, 319-325. [Link]

Poling CE, Warner WD, Mone PE & Rice EE. 1962. The influence of temperature, heating time and aeration upon the nutritive value of fats. Journal of the American Oil Chemists Society, 39: 315-320. [Link]

SAS (Statistical Analysis System). 2008. SAS/STAT® 9.2. User's Guide. SAS Institute Inc. Cary, North Carolina. [Link]

Sanchez-Muniz FJ, Varela LS, Polonio GCM & Cuesta C. 1998. Dietary effect on growth, liver peroxides and serum and lipoprotein lipids in rats fed a thermoxidized and polymerized sunflower oil. Journal of the Science of Food and Agriculture, 76: 364-372. [Link]

Seppanen CM & Csallany AS. 2004. Incorporation of the toxic aldehyde 4-hydroxy-2-trans-nonenal into food fried in thermally oxidized soybean oil. Journal of the American Oil Chemists' Society, 87: 1137–1141. [Link]

Seppanen CM & Csallany AS. 2006. The effect of intermittent and continuous heating of soybean oil at frying temperature on the formation of 4-hydroxy-2-trans-nonenal and other α-, β-unsaturated hydroxyaldehydes. Journal of the American Oil Chemists' Society, 83: 121–127. [Link]

Suomela JP, Ahotupa M, Sjovall O, Kurvinen JP & Kallio H. 2004. Diet and lipoprotein oxidation: Analysis of oxidized triacylglycerols in pig lipoproteins. Lipids, 39: 639–647. [Link]

Urso ML & Clarkson PM. 2003. Oxidative stress, exercise and antioxidant supplementation. Toxicology, 189: 41–54. [Link]

Warnants N, Van Oeckel MJ, & Boucque CV. 1996. Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. Meat Science, 44: 125–144. [Link]

Yannakopoulos A & Tserveni-Gousi A. 1986. Quality characteristics of quail eggs. British Poultry Science, 27: 171-176. [Link]

Yue HY, Wang J, Qi XL, Ji F, Liu MF, Wu SG, Zhang HJ & Qi GH. 2011. Effects of dietary oxidized oil on laying performance, lipid metabolism, and apolipoprotein gene expression in laying hens. Poultry Science, 90: 1728–1736. [Link]

Zalejska-Fiolka J, Wielkoszynski T, Kasperczyk S, Kasperczyk A & Birkner E. 2012. Effect of Oxidized Cooking Oil and α-lipoic acid on blood antioxidants: Enzyme activities and lipid peroxidation in rat fed a high-fat diet. Biological Trace Element Research, 145: 217-221. [Link]