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Effects of dietary chicken grill and sunflower seed oils on performance, egg yolk cholesterol level, biochemical parameters, and oxidant/antioxidant status of laying hens

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Abstract: The objective of this study was to investigate the effects of dietary chicken grill oil (CGO) and sunflower seed oil (SO) on performance, some serum and egg yolk biochemical parameters, and blood and liver oxidant/antioxidant status of laying hens. Fifty-four 19-week-old Lohmann laying hens were randomly assigned to three groups. The groups were fed experimental diets containing 5% SO, 5% CGO1, or 7.5% CGO2, respectively. Diets and water were provided ad libitum over 17 weeks. The feed efficiency ratios and cholesterol content in egg yolks with cholesterol and the triglyceride levels in serum were not affected by the diets. However, egg yolk triglyceride levels were affected by diets ($P < 0.05$). Among the egg quality parameters egg yolk color was affected by the diets ($P < 0.01$). The blood biochemical parameters did not vary with CGO supplementation. Serum ALT activity was not influenced by the dietary CGO treatment, whereas serum AST activity was significantly different for the three diets ($P < 0.01$). The dietary CGO did not affect blood and liver MDA levels with antioxidant enzyme activities. Therefore, up to 7.5% of CGO can be used in diets of laying hens without adverse effects on performance, egg yolk cholesterol, and oxidant/antioxidant status.

Key words: Laying hen, chicken grill oil, performance, biochemical parameters, oxidant/antioxidant status

1. Introduction

The metabolic rates and energy requirements of poultry are very high. Therefore, poultry diets must be rich in energy. Oils, which are concentrated sources of energy, are an important part of poultry diets (1). Use of fats or oil in animal feed has many benefits. Fats and oils are essential nutrients in both human and animal diets. Of any feedstuff, they provide the most concentrated source of energy, supply essential fatty acids (which are precursors for important hormones, such as prostaglandins), and contribute significantly to the feeling of satiety after eating. Moreover, they are carriers of fat-soluble vitamins, and they make foods more palatable (2).

Oil released from chicken during the grilling process, which is taken from chicken carcasses during cooking on a rotating system, is obtained through a collection reservoir. Chicken grill oil (CGO) is different from restaurant waste oil. Restaurant waste oil is exposed to a high temperature and is used repeatedly for frying (3).

The degree of unsaturation of dietary fats has been reported to influence susceptibility to oxidation. Moreover, the cellular antioxidant defense system varies significantly with dietary fatty acids. Febel et al. (4) reported that antioxidant protective mechanisms are more efficient in broilers fed a diet high in n-3 polyunsaturated fatty acid (PUFA; linseed oil), which counteracts oxidative injury. Dobrzanski et al. (5) reported that feeding oils rich in n-3 PUFA (linseed and fish oils) does not affect the total antioxidant status (TAS) levels of laying hens; however, glutathione peroxidase (GPx) activity is increased. CGO has a fatty acid composition rich in linolenic acid (C18:3, n-3). The use of CGO in layer quail diets affects egg quality and layer performance (3).

The objective of this study was to investigate the effects of using CGO instead of sunflower seed oil (SO) on feed intake, feed conversion ratio (FCR), fatty acid (oil and egg yolk) and cholesterol contents, oxidant/antioxidant status, and selected biochemical parameters of serum and egg yolks of laying hens.

2. Materials and methods

2.1. Preparation of chicken grill oil

CGO was collected from a local chicken grill shop. The liquid was held in cool and dark conditions for 12 h for separating the oil fraction from other liquids. Thereafter, the CGO was filtered using a small-pore filter to remove

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any foreign matter and was then stored in a cool, dark environment for 10 days until the beginning of the experiment. The filtered CGO and the other trial raw materials were mixed for 4 min using a standard feed mixer. In this experiment, ready mixed feed or basal feed was not used. To test the homogeneity of the prepared feed, crude fat analysis (ether extract, %) was performed on samples taken from three different parts of the feed mixtures (Table 1).

2.2. Animals and diets

The Research Ethics Committee of the Veterinary Faculty of Mehmet Akif Ersoy University reviewed the study proposal at a meeting held on 12 December 2007 and approval for the proposal was granted.

Table 1. Ingredients and chemical composition of feeds used in the experiment.

Ingredients	Diets, g/kg		
	SO[1]	CGO_1[2]	CGO_2[3]
Barley	71	71	248
Corn	440	440	235
Soybean meal	272	272	250
Sunflower meal	45	45	70
Dicalcium phosphate	16	16	16
DL-Methionine	1	1	1
Limestone	100	100	100
Salt	3	3	3
Vitamin premix[4]	1	1	1
Mineral premix[5]	1	1	1
Oil (SO, CGO)	50	50	75
Total	1000	1000	1000
Calculated			
Crude protein, g/kg	180	180	180
Metabolizable energy, MJ/kg	11.75	11.75	11.76
Analyzed			
Dry matter, g/kg	914.9	916.9	924.6
Crude protein, g/kg	180.6	180.1	181.5
Ash, g/kg	126.3	128.1	127.8
Ether extract, g/kg	72.2	72.0	77.5
Crude fiber, g/kg	33.7	34.1	39.9

[1]SO: 5% sunflower oil; [2]CGO_1: 5% oil released from chicken during grilling process; [3]CGO_2: 7.5% oil released from chicken during grilling process.

[4]1 kg vitamin premix: vitamin A 12,000,000 IU; vitamin D3 2,400,000 IU; vitamin E 30,000 mg, vitamin K3 (menadione) 2500 mg; vitamin B1 (thiamine) 3000 mg; vitamin B2 (riboflavin) 7000 mg; vitamin B6 (pyridoxine) 4000 mg; vitamin B12 (cyanocobalamin) 15 mg; nicotinamide 10,000 mg; Ca D-pantothenate 8000 mg; D-biotin 45 mg; folic acid 1000 mg; vitamin C (ascorbic acid) 50,000 mg; choline chloride 125,000 mg; canthaxanthin 1500 mg; apo-carotenoic acid ester 500 mg.

[5]Mineral premix: manganese 80,000 mg; iron 40,000 mg; zinc 60,000 mg; copper 5000 mg; iodine 400 mg; selenium 150 mg; cobalt 100 mg.

In this study, Lohmann laying hens (n = 54; 19 weeks old) were divided into three groups. The groups were fed diets containing 5% SO, 5% chicken grill oil (CGO_1), or 7.5% chicken grill oil (CGO_2; Table 1). Each group included 18 laying hens, and the groups were further divided into 3 subgroups of 6 laying hens. The experimental diets and water were provided ad libitum over 17 weeks.

2.3. Ration composition and calculations

The experimental diets were formulated to be isonitrogenous and isocaloric. The nutrient composition of the diets was analyzed using AOAC methods (6). In this study, dry matter (DM), crude protein (CP), ether extract (EE), and ash levels in the feed samples were determined using the Weende method (6). Furthermore, the crude fiber (CF) quantities were determined using the method proposed by Crampton and Maynard (7). The diets were prepared in accordance with the ration program (8). The ingredients and chemical composition of the diets are listed in Table 1.

2.4. Sample collection

Eggs were collected each morning, and egg production was recorded daily. Feed intakes were recorded biweekly, and FCRs were calculated for one dozen eggs and 1 kg of eggs. Egg quality (egg weight and shape index) and egg yolk color (with Roche color scale) were also determined.

At the end of the experiment, six laying hens were randomly selected per treatment (two per replicate) and sacrificed to collect blood and liver samples. Blood samples were drawn into anticoagulant tubes containing sodium EDTA and anticoagulant-free tubes. The anticoagulated blood was separated into plasma and erythrocytes by centrifugation at 1500 × *g* for 10 min. The erythrocyte samples were washed three times in NaCl (0.9%, v/w). The anticoagulant-free blood was centrifuged to obtain serum as described earlier. Liver tissue was removed and immediately rinsed with ice-cold 0.9% NaCl. The washed erythrocyte and liver samples and sera were stored at –20 °C until analyses were performed.

2.5. Lipid peroxidation (malonyldialdehyde) levels in serum and liver

Serum lipid peroxidation (LP) was determined using the method proposed by Satoh (9) and Yagi (10) but with 1,1,3,3-tetraethoxypropane as the standard. This method is based on the reaction between malonyldialdehyde (MDA, an aldehyde lipid peroxidation product) and thiobarbituric acid (TBA). MDA forms a pink-colored complex with TBA. The absorbance of solution containing the complex was measured at 532 nm using a spectrophotometer (UV-1201, Shimadzu, Japan). The serum LP values were expressed in terms of MDA as nmol/mL serum. To determine the MDA level in liver, frozen liver was homogenized in a ratio of 1 g of wet tissue to 9 volumes of 1.15% KCl. The homogenates were then centrifuged at 2500 × *g* for 15 min at 4 °C to remove particulate materials. Lipid peroxidation levels in the supernatant fraction were measured using the method of Ohkawa et al. (11). The LP levels in liver were defined as MDA in nmol/g protein.

2.6. Determination of catalase, glutathione peroxidase, and total superoxide dismutase enzyme activity in erythrocytes and liver

The catalase (CAT) activities of the washed erythrocyte and liver samples (EC 1.11.1.6) were determined using the method of Aebi (12) by measuring the absorbance of H_2O_2 at 240 nm. Catalase activity in liver was expressed as k/g protein, where k is a first-order rate constant. Erythrocyte CAT activity was expressed as k/g hemoglobin (Hb). Liver and erythrocyte glutathione peroxidase (GSH-Px; EC 1.11.1.9) activities were assayed using the method of Paglia and Valentine (13). In this method, GSH-Px catalyzes the oxidation of glutathione in the presence of tert-butyl hydroperoxide. Oxidized glutathione is then reduced in the presence of glutathione reductase and NADPH, and NADPH is oxidized to NADP. A reduction in absorbance of NADPH was found at 340 nm. The liver and erythrocyte GSH-Px activities were expressed as U/g protein and U/g Hb, respectively. Liver and erythrocyte total superoxide dismutase (TSOD; EC 1.15.1.1) activities were determined using a commercially available kit (SOD Assay Kit-WST, Fluka, Switzerland) and expressed as U/mL.

2.7. Determination of serum nonenzymatic antioxidants

Vitamin E (α-tocopherol) was estimated using the method of Baker and Frank (14) that is based on reduction of ferric ions to ferrous ions by α-tocopherol; thus, a red colored complex is formed with 2,2'-dipyridyl at 520 nm. The calculated serum vitamin E levels were expressed as mg/dL. Serum β-carotene and vitamin A (retinol) levels were measured using the method of Suzuki and Katoh (15). Briefly, 1.0 mL of serum was mixed with 1.0 mL of butylated hydroxytoluene containing 3 mL of n-hexane, and the mixture was then shaken for 10 min. After centrifugation, the absorbance of the n-hexane (top) layer was measured at 453 nm and 325 nm for β-carotene and retinol, respectively, and expressed as μmol/L.

2.8. Biochemical parameters

The serum and egg yolk total cholesterol and triglyceride levels were measured with a spectrophotometer (UV-1601, Shimadzu, Japan) using commercially available kits (Chema Diagnostica, Italy). Egg yolk cholesterol analysis was performed every 3 weeks from the beginning of the trial. Egg yolk cholesterol was determined according to the method proposed by Küçükersan (16). Serum and/or liver homogenate protein and serum glucose levels were determined using the methods of Gornall et al. (17) and Feteris (18), respectively. Hemoglobin (19) and hematocrit

(microhematocrit centrifuge) levels were also determined. Activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed using the method proposed by Reitman and Frankel (20) and were expressed as IU/L.

2.9. Other analyses

The egg yolk fatty acid profile was analyzed at the end of the experiment. Each group of egg samples contained of a pool of six egg yolks. The fatty acid profiles of experimental oil and egg yolks were analyzed using GC/MS (QP-5050, Shimadzu, Japan) at the Oil and Fats Laboratory of Süleyman Demirel University. The fat extracted from each sample was methylated, and fatty acids were separated and identified using a CP Wax 52 CB 50 m × 0.32 mm, 1.2 µm capillary column. The injector and detector were at a temperature of 250 °C, and helium was used as the carrier gas.

2.10. Statistical analysis

The data were analyzed using SPSS 10 for Windows. The differences in obtained values were analyzed using the analysis of variance (ANOVA) method and Duncan's test (21) was used for determining a significance level of at least $P < 0.05$.

3. Results

At the end of the 17-week experimental period, no difference was found between the body weight gain of chickens fed SO and CGO diets ($P > 0.05$; Table 2). Dietary CGO supplementation did not significantly affect egg production, feed intake, FCR, and egg parameters except egg weight and egg yolk color ($P < 0.05$ and $P < 0.01$, respectively; Table 2). The egg yolk and serum cholesterol levels did not differ among the groups (Table 3). Egg yolk triglycerides decreased significantly ($P < 0.05$), whereas serum triglyceride levels decreased slightly ($P > 0.05$) in the 7.5% CGO_2 dietary group. Moreover, dietary CGO did not affect blood and liver MDA levels, antioxidant enzyme (TSOD, GPx, and CAT) activities, and serum nonenzymatic antioxidants (vitamins E and A and β-carotene; Table 4). Some blood biochemical parameters including hemoglobin, hematocrit, glucose, cholesterol, and protein did not vary with dietary CGO supplementation. Serum ALT activity was not affected by SO or CGO dietary treatment. However, serum AST activity was significantly lower for the dietary CGO group ($P < 0.01$; Table 5). Fatty acid compositions of egg yolks and chicken grill and sunflower oils are shown in Table 6.

Table 2. Effects of chicken grill and sunflower oils on performance and selected egg quality parameters in laying hens, mean ± SD.

Parameters	SO[1]	CGO_1[2]	CGO_2[3]	P-value
Daily feed consumption, g (feed/chick)	112.28 ± 2.72	110.30 ± 4.08	107.43 ± 1.01	0.199
Initial body weight, g (19 weeks old)	1328.56 ± 132.91	1277.00 ± 153.39	1275.44 ± 129.44	0.433
Final body weight, g (36 weeks old)	1652.61 ± 93.32	1613.44 ± 127.82	1609.76 ± 105.09	0.441
Feed conversion ratio (FCR1), kg feed/kg eggs	1.98 ± 0.18	1.93 ± 0.07	1.87 ± 0.03	0.530
Feed conversion ratio (FCR2), kg feed/dozen eggs	1.24 ± 0.11	1.19 ± 0.04	1.15 ± 0.02	0.320
Egg production, %	95.91 ± 4.61	97.37 ± 1.64	98.05 ± 1.30	0.677
Egg weight, g	61.06[a] ± 2.13	57.67[b] ± 3.34	59.05[ab] ± 1.24	0.021
Egg length, mm	57.49 ± 0.88	56.59 ± 1.27	57.19 ± 1.05	0.216
Egg width, mm	43.40 ± 0.55	42.78 ± 0.82	42.78 ± 0.40	0.067
Shape index,%	75.56 ± 0.78	75.71 ± 0.81	74.89 ± 1.67	0.306
Egg yolk color[4]	8.57[a] ± 0.43	8.44[a] ± 0.27	7.86[b] ± 0.44	0.01
Egg yolk diameter, mm	40.56 ± 1.19	39.76 ± 0.79	39.57 ± 1.10	0.121
Egg white length, mm	94.84 ± 4.03	95.17 ± 4.22	95.38 ± 4.01	0.960

[a, b]Mean values differ significantly within the same row.
$P < 0.05$; $P < 0.01$.
[1]SO: 5% sunflower oil; [2]CGO_1: 5% oil released from chicken during grilling process;
[3]CGO_2: 7.5% oil released from chicken during grilling process.
[4]Measured with the Roche egg yolk color scale.

Table 3. Effects of chicken grill and sunflower oils on egg yolk cholesterol level in laying hens, mean ± SD.

Parameters	SO[1]	CGO_1[2]	CGO_2[3]	P-value
Egg weight, g	60.01 ± 2.35	59.85 ± 3.94	59.35 ± 2.88	0.896
Boiled egg yolk weight, g	14.60 ± 0.47	14.85 ± 1.59	14.29 ± 0.67	0.520
Egg yolk cholesterol, mg/dL	88.99 ± 7.96	91.77 ± 3.15	93.72 ± 4.19	0.207
Egg yolk cholesterol, mg/g egg yolk	36.78 ± 1.31	36.71 ± 1.26	37.49 ± 1.67	0.452
Egg yolk triglyceride, mg /dL	337.69[a] ± 20.13	331.45[a] ± 30.19	301.53[b] ± 31.70	0.025

$P < 0.05$.
[a, b]Mean values differ significantly within the same row.
[1]SO: 5% sunflower oil; [2]CGO_1: 5% oil released from chicken during grilling process;
[3]CGO_2: 7.5% oil released from chicken during grilling process.

Table 4. Effects of chicken grill and sunflower oils on antioxidants and lipid peroxidation in serum, erythrocytes, and livers of laying hens, mean ± SD.

Parameters	SO[1]	CGO_1[2]	CGO_2[3]	P-value
Serum				
MDA, nmol/mL	3.91 ± 0.90	5.82 ± 3.67	5.22 ± 0.87	0.347
Vit A, µmol/L	5.17 ± 1.28	5.47 ± 1.38	5.44 ± 1.17	0.905
β-carotene, µmol/L	1.25 ± 0.27	1.24 ± 0.36	0.93 ± 0.18	0.122
Plasma vit E, µmol/L	137.81 ± 23.10	144.08 ± 24.15	135.06 ± 16.91	0.784
Erythrocytes				
GSH-Px, U/g Hb	0.64 ± 0.22	0.74 ± 0.12	0.86 ± 0.17	0.118
TSOD, U/mL	12.30 ± 8.79	14.52 ± 13.79	16.36 ± 9.22	0.813
CAT, k/g Hb	8.66 ± 1.78	9.59 ± 4.26	10.37 ± 9.81	0.895
Liver				
MDA, nmol/g protein	12.28 ± 5.66	10.85 ± 4.17	9.13 ± 1.53	0.441
CAT, k/g protein	1.88 ± 2.69	2.28 ± 2.23	1.24 ± 0.63	0.680
GSH-Px, U/g protein	3.66 ± 2.41	3.69 ± 0.93	3.34 ± 0.85	0.912
TSOD, U/mL	13.01 ± 3.94	12.69 ± 6.79	11.64 ± 2.60	0.875

[1]SO: 5% sunflower oil; [2]CGO_1: 5% oil released from chicken during grilling process;
[3]CGO_2: 7.5% oil released from chicken during grilling process.

4. Discussion

In this study, no significant differences were observed in daily feed consumption, FCR, and average egg production among the groups. These findings are similar to those reported by Çetingül and İnal (22) and Balevi and Coskun (23). Çetingül and İnal (22) reported that addition of SO (1.5%) and hazelnut oil (1.5%–3%) to the ration did not affect egg productivity. Balevi and Coskun (23) reported that supplementation of nine different types of oil sources (sunflower, cotton, corn, flax, soybean, olive, fish, tallow, and rendering oils) at 2.5% concentration did not significantly affect egg production parameters.

In our study, egg yolk cholesterol and serum cholesterol and triglyceride levels were not affected by the diets. However, egg yolk triglyceride levels decreased significantly in hens fed 7.5% CGO (CGO_2)-supplemented diets when compared with those fed 5% SO or CGO ($P < 0.05$). No significant differences were found in serum total cholesterol and triglyceride levels between the experimental groups. Karakaş Oğuz et al. (2) reported similar results for laying quails that were fed SO or CGO. None of the egg quality parameters including length, width, and shape index of eggs were affected by the diets. However, the egg yolk color of the group fed 7.5% dietary

Table 5. Effects of chicken grill and sunflower oils on selected biochemical parameters in sera and blood of laying hens, mean ± SD.

Parameters	SO[1]	CGO_1[2]	CGO_2[3]	P-value
Hemoglobin, mmol/L	12.78 ± 1.19	13.13 ± 1.32	12.39 ± 1.34	0.622
Hematocrit, %	32.33 ± 1.97	31.33 ± 1.63	30.33 ± 2.07	0.222
Glucose, mmol/L	10.82 ± 0.61	10.49 ± 0.93	10.33 ± 0.91	0.581
Total cholesterol, mmol/L	3.16 ± 0.96	3.39 ± 0.99	3.24 ± 0.88	0.915
Triglycerides, mmol/L	11.65 ± 4.18	12.09 ± 3.59	11.16 ± 3.76	0.915
Total protein, g/L	44.4 ± 3.63	45.3 ± 4.03	46.83 ± 4.58	0.594
ALT, IU/L	20.76 ± 4.21	23.92 ± 6.69	21.44 ± 2.20	0.494
AST, IU/L	361.60[a] ± 51.40	309.33[b] ± 35.66	254.67[c] ± 16.33	0.01

$P < 0.01$.
[a, b, c]Different superscripts in the same row indicate significant differences among groups.
[1]SO: 5% sunflower oil; [2]CGO_1: 5% oil released from chicken during grilling process;
[3]CGO_2: 7.5% oil released from chicken during grilling process.

Table 6 . Fatty acid composition of egg yolks and chicken grill and sunflower oils.

Fatty acid	Oils		Egg yolk		
	CGO	SO*	SO[1]	CGO_1[2]	CGO_2[3]
Myristic (C14:0)	0.46	0.1	-	-	-
Palmitic (C16:0)	20.29	3–6	26.99	27.15	27.77
Palmitoleic (C16:1; n-7)	1.95	0.1	0.29	0.89	1.06
Stearic (C18:0)	6.63	1–3	17.15	15.08	15.08
Oleic (C18:1; n-9)	30.63	14–43	25.52	32.69	30.84
Vaccenic (C18:1; n-7)	1.68	1.4	0.71	1.61	1.58
Linoleic (C18:2; n-6)	35.11	44–75	23.34	17.86	18.98
Linolenic (C18:3; n-3)	2.81	<0.7	-	-	-
Arachidonic (C20:4; n-6)	-	0.6–4	5.52	4.44	4.51
Total MUFA	34.26	15.1–44.5	26.52	35.19	33.48
Total PUFA	37.92	45.3–79.7	28.86	22.30	23.49
Total SFA	27.38	4.1–9.1	44.14	42.23	42.85
Total omega 3	2.81	<0.7	-	-	-
Total omega 6	35.11	44.6–79	28.86	22.30	23.49

*Based on Grompone (29) and Shingfield et al. (30).
[1]SO: 5% sunflower oil; [2]CGO_1: 5% oil released from chicken during grilling process;
[3]CGO_2: 7.5% oil released from chicken during grilling process.

CGO (CGO_2; $P < 0.01$) was reduced significantly, possibly because of the lower corn seed level (23.5%) in this group when compared with the other groups (44%).

Küçükersan et al. (24) reported that supplementation with different oils sources (3% sunflower, fish, soybean, and hazelnut oils) in laying hen rations had no effect on egg quality parameters and egg cholesterol levels. Ceylan et al. (25) reported that egg performance parameters including egg production, egg weight, feed intake, feed conversion, live weight, and cholesterol content of egg yolks were not significantly affected by diets containing 1.5% and 3.0% sunflower, fish, linseed, and rapeseed oils. Similarly, in our study, the three supplemental diets did not significantly affect egg quality parameters and egg yolk cholesterol levels of laying hens.

Kralik et al. (26) studied the effect of oil rich in omega-3 fatty acids on production and quality of eggs of 9-month-old laying hens over a period of 28 days. No differences were found with respect to production characteristics (weight of laying hens, food consumption, and laying intensity) and quality of eggs (egg weight, albumen, yolk, shell, shell thickness, albumen:yolk ratio, and relative portion of main parts to egg) among the investigated groups. Our results also indicate that egg performance parameters and egg yolk cholesterol content of the groups were not significantly affected.

Febel et al. (4) found no significant difference in blood glucose concentration, plasma triglyceride level, the activity of GSH-Px, and the level of GSH in the plasma, erythrocytes, and livers of broilers fed diets containing lard and sunflower, soybean, and linseed oils. These findings are consistent with our results. In the same study, higher PUFA content in the diet led to increased MDA levels in the erythrocytes and liver; however, plasma total cholesterol concentration was reduced. Schumann et al. (27) reported that supplementation with diets containing 100 g/kg ground flaxseeds, 40 g/kg flax oil, or 100 g/kg Dry n-3® did not significantly affect hepatic MDA levels in laying hens; however, hepatic fat content was significantly reduced when compared with those of hens fed a control diet containing animal and vegetable oils. Similarly, no significant differences were found in serum and liver MDA levels among the groups in our study, possibly because CGO contains higher linolenic acid and lower arachidonic acid contents than SO (Table 6). Dobrzariski et al. (5) studied the effects of experimental diets containing fish and linseed oil on the oxidation potential of blood of laying hens. Different oils did not significantly affect the TAS level; however, GPx activity increased significantly in the experimental groups. Moreover, dietary CGO did not significantly affect antioxidant enzyme (TSOD, GPx, CAT) activities of erythrocytes and livers or serum nonenzymatic antioxidants (vitamin E, vitamin A, and β-carotene). Additionally, n-3 PUFAs that react with free radicals can serve as free radical scavengers rather than proliferators in conjunction with coupling systems such as vitamins and glutathione. In our study, moreover, CGO has a fatty acid composition rich in linolenic acid (C18:3, n-3; Table 6). Therefore, dietary CGO does not negatively affect oxidant/antioxidant balance. Febel et al. (4) reported that AST activity was highest in the SO group whereas ALT values were lowest in plasma of broilers fed a linseed oil diet.

Blas et al. (28) used recycled oil in rabbit feed, and this supplementation did not affect nutrient digestibility, growth performance, and serum hepatic (GGT, AST, ALT, ALP) and renal (urea and creatinine) markers ($P > 0.10$), thus indicating that recycled oil can be used in rabbit diets.

In the current study, significantly lower AST values were found in the sera of laying hens fed CGO diets ($CGO_2 < CGO_1$) when compared with those fed the 5% SO diet ($P < 0.01$). However, the ALT levels were not affected by CGO diet. Therefore, CGO, rich in linolenic acid (C18:3, n-3), does not negatively affect liver function in laying hens (Table 6).

Supplementation of 5% and 7.5% dietary CGO for laying hens does not negatively affect performance when compared with dietary SO. Therefore, dietary CGO (at 5% and 7.5% levels) can be added to mixed feed without affecting layer performance, egg yolk cholesterol level, biochemical parameters, and oxidant/antioxidant status of laying hens.

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