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Effects of dietary chicken grill oil and sunflower seed oil on blood and liver oxidant/antioxidant status and liver function tests in laying Japanese quails *(Coturnix coturnix japonica)*

[Yumurtlayan Japon bıldırcın *(Coturnix coturnix japonica)* karma yemlerine tavuk çevirme yağı ve ayçekirdeği yağı ilavesinin kan ve karaciğer oksidan/antioksidan denge ve karaciğer fonksiyon testleri üzerine etkileri]

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ABSTRACT

Objective: To investigate influence of dietary chicken grill oil (CGO) on blood and liver oxidant/antioxidant status and parameters of liver function in laying Japanese quails (n=192; 13-wk old) (*Coturnix coturnix japonica*).

Methods: Four groups were fed with experimental diets contained, 5% sunflower seed oil (SO_1), 5% chicken grill oil (CGO_1), 7.5% sunflower seed oil (SO_2) and 7.5% chicken grill oil (CGO_2), respectively. Experimental diets and water were provided *ad libitum* throughout the nine weeks of experiment.

Results: The added CGO supplementation lowered erythrocyte and liver superoxide dismutase (SOD) activity, but increased erythrocyte glutathione peroxidase (GSH-Px) activity. However, the supplemented CGO did not affect to serum and liver malondialdehyde (MDA) levels. But liver MDA level of the group added 7.5% SO (SO_2) significantly increased compared to other groups ($p<0.01$). Vitamin A and β-carotene concentrations in plasma of laying quails significantly decreased due to increased rates (from 5% to 7.5%) of both CGO and SO. However, plasma vitamin E concentrations in quails fed with diets supplemented with 7.5% SO and CGO were significantly higher ($p<0.05$) than in birds fed with the 5% SO and CGO diets. In the analysis of liver function, serum alanine transaminase (ALT) activity in birds fed diets supplemented with CGO (CGO_1 and CGO_2) increased. Also, serum aspartate transaminase (AST) activity showed a significant increase ($p<0.01$) in quails fed with 7.5% SO or CGO.

Conclusion: The results suggest that the antioxidant effect of CGO is not less effective than that SO. Both SO and CGO added to quail diets in 7.5 % ratio are not the positive effect on the oxidant/antioxidant status and liver function tests. However, the oxidant effect of dietary 7.5% CGO is lower than that SO. Therefore CGO obtained as described in the Material-Methods section up to 5% may be added to quail diets.

Key Words: Chicken grill oil, antioxidant status, liver function, quail

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: Bu çalışmada 13 haftalık yumurtlayan Japon bıldırcınlarının (n=192) (*Coturnix coturnix japonica*) karma yemlerine eklenen tavuk çevirme yağının (CGO) kan ve karaciğer oksidan/antioksidan denge ve karaciğer fonksiyon testleri üzerine etkisi araştırılmıştır.

Metod: Dört gruba ayrılan bıldırcınlar sırasıyla, %5 ayçekirdeği yağı (SO_1), %5 tavuk çevirme yağı (CGO_1), %7.5 ayçekirdeği yağı (SO_2) and %7.5 tavuk çevirme yağı (CGO_2) içeren karma yemlerle beslenmişlerdir. Yem ve su dokuz haftalık deneme boyunca *ad libitum* olarak verilmiştir.

Bulgular: Karma yemlere ilave edilen CGO'nun eritrosit ve karaciğer süperoksit dismütaz (SOD) aktivitesini azalttığı, fakat eritrosit glutatyon peroksidaz (GSH-Px) aktivitesini arttırdığı tespit edilmiştir. Bununla birlikte, karma yemlere ilave edilen CGO'nun serum ve karaciğer malondialdehit (MDA) düzeyi üzerine herhangi bir etkisi saptanmamıştır. Fakat %7,5 SO ilave edilen grubun (SO_2) karaciğer MDA düzeyi diğer gruplara göre önemli ölçüde ($p<0.01$) artmıştır. Plazma vitamin A ve β-karoten konsantrasyonları ise karma yemlere ilave edilen hem ayçekirdeği hem de tavuk çevirme yağının %5'den %7.5 oranına çıkarılması durumunda azalmıştır. Aksine plazma vitamin E konsantrasyonları %7.5 SO ve CGO ilave edilen karma yemlerle beslenen bıldırcınlarda, %5 SO ve CGO ilave edilen gruplara göre önemli ölçüde yüksek ($p<0.05$) bulunmuştur. Serum alanin transaminaz (ALT) aktivitesi CGO ilave edilen gruplarda (CGO_1 ve CGO_2) artmıştır. Ayrıca, serum aspartat transaminaz (AST) aktivitesi %7.5 oranında SO ya da CGO ilave edilen karma yemlerle beslenen bıldırcınlarda önemli ölçüde ($p<0.01$) artış göstermiştir.

Sonuç: Mevcut çalışmada, CGO'nun antioksidan etkisinin SO'dan daha az olmadığı sonucu ortaya çıkmaktadır. Her iki yağın da %7.5 oranın oksidan/antioksidan denge ve karaciğer fonksiyon testleri üzerinde olumlu etkisi bulunmamaktadır. Bununla birlikte, dietlere eklenen %7.5 CGO'nun oksidan etkisinin %7.5 SO'dan daha az olduğu görülmektedir. Bu nedenle %5 oranına kadar gereç ve yöntemler bölümünde belirtilen şekilde hazırlanmış olan CGO bıldırcın dietlerine eklenebilir.

Anahtar Kelimeler: Tavuk çevirme yağı, antioksidan denge, karaciğer fonksiyonu, bıldırcın

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Oils, which contain high levels of unsaturated fatty acids, are commonly used in poultry diets [1]. Various fats and oils are used in diets in order to improve the nutritional value of eggs, including an increase of their polyunsaturated fatty acid (L-PUFA n-3) content [2]. However, oils rich in (n-3) fatty acids are susceptible to oxidation because of their high degree of unsaturation. Radical species can attack the double bonds of unsaturated lipids and initiate chain reactions leading to end products such as aldehydes, dialdehydes (e.g. malondialdehyde) and short-chain hydrocarbons [3]. This situation may raise the requirement for nutritional antioxidants (vitamin E, β-carotene and vitamin A), and the intracellular defensive enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) [4].

Biological organisms generate harmful reactive oxygen species (ROS) and various free radicals in the course of normal metabolic activities in tissues such as brain, heart, lung and muscle. ROS and free radicals are generated and scavenged continuously and concentrations of ROS and free radicals maintain homeostasis. The normal concentrations of ROS and free radicals do not harm tissues or cells but exert unique physiological functions including the induction of antioxidant enzymes. Antioxidant enzymes such as GSH-Px, SOD, and CAT play an important role in the antioxidant protective system. Excessive free radicals are captured by SOD, GSH-Px and CAT. First, SOD converts the superoxide anion to hydrogen peroxide. Thereafter, GSH-Px and CAT independently detoxify the hydrogen peroxide produced [5].

In a previous study, it was found that the most common marker for lipid peroxidation, liver-thiobarbituric-acid reactive substances (TBARS) were highly influenced by the intake of thermoxidised compounds [6]. Also, dietary antioxidants such as vitamin E and carotenoids are beneficial in preventing the detrimental system, reducing lipid peroxidation. Vitamin E and A were measured as biomarkers for oxidative stress that could be associated with consumption of diets rich in PUFA [7,8].

Fat released from chicken during the grilling process as waste is used as a dietary fat in cooking by people on low incomes in some developing countries. The alteration in the levels of some physico-chemical properties of fat released from chicken during the grilling process and the effect of this fat on body weight, liver function, chromosomal aberrations and micronucleus formation in male rats have been studied [9]. The results indicates that grilled fat induces damage of liver cells and increases activities of serum aspartate transaminase (AST) [9]. There are no studies on the use of chicken grill oil (CGO) as an additive in poultry feed and its effects.

The aim of the present study was to investigate the effects of using two different dietary oils (sunflower seed oil, chicken grill oil) in diets on erythrocyte and liver activity of antioxidant enzymes GSH-Px, SOD, CAT, and serum non-enzymic antioxidants (vitamin A, β-carotene, vitamin E) and the level of malondialdehyde (MDA) and liver function parameters (AST, ALT) in Japanese quail.

Material and Methods

Preparation of Chicken Grill Oil

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 separation of oil fraction from other liquids. Thereafter, CGO was filtered by using a small poor filter for separation of any foreign matters and stored in a cool, dark environment for 10 days until the beginning of the experiment. CGO and the other trial raw materials were mixed with the standart feed mixer for 4 minute. In this experiment, the ready-mixed feed or basal feed were not used. To test the homogeneity of the prepared feed, the crude fat analysis (ether extract, %) was performed in the samples taken from three different parts of the feed mixtures (Table 1).

Animals and Experimental Design

The Research Ethics Committee of Veterinary Faculty of the Mehmet Akif Ersoy University reviewed the study proposal at a meeting held on 12 December 2007 and approval for the proposal was granted. Laying Japanese quails (n=192; 13-wk old; *Coturnix coturnix japonica*) were obtained from a local supplier. The birds were randomly assigned to four treatment groups. The groups, 1, 2, 3 and 4, were fed with diets containing 5% sunflower oil (SO_1), 5% chicken grill oil (CGO_1), 7.5% sunflower oil (SO_2) and 7.5% chicken grill oil (CGO_2), respectively (Table 1). Each of the groups was formed by 48 laying quails and groups were futher divided into 6 sub-groups containing 8 laying quails. Experimental diets and water were provided *ad libitum* throughout the nine weeks.

Ration Composition and Calculations

The experimental diets were formulated as isonitrogenous and isocaloric. The nutrient composition of diets was determined according to the AOAC [10]. In this study the levels of dry matter (DM), crude protein (CP), ether extracts (EE) and ash in feed samples were determined by Weende Analyses methods [10] and crude fiber (CF) quantities by Crampton and Maynard's method [11]. Formulation and calculation of the diets were prepared using the ration programme [12]. The ingredients and chemical composition of the diets and fatty acid composition of the experimental oils are presented in Tables 1 and 2, respectively.

Collection and Preparation of Blood and Liver Samples

At the end of the experiment, 12 birds per treatment (two per replicate) were randomly selected and sacrifice to col-

Table 1. Ingredients and chemical composition of mixed feeds used in the experiment (%)

Ingredients	Group 1 (SO_1[1])	Group 2 (CGO_1[2])	Group 3 (SO_2[3])	Group 4 (CGO_2[4])
Barley	12.50	12.50	35.50	35.50
Corn	39.90	39.90	15.50	15.50
Soybean meal	34.50	34.50	33.40	33.40
Dicalcium phosphate	1.10	1.10	1.00	1.00
Dl-methionine	0.10	0.10	0.10	0.10
Limestone	6.50	6.50	6.60	6.60
Salt	0.30	0.30	0.30	0.30
Vitamin-mineral premix	0.10	0.10	0.10	0.10
Oil	5.00	5.00	7.50	7.50
Analysed				
Metabolizable energy*, (kcal/kg)	2903	2903	2900	2900
Dry matter, (%)	89.98	89.82	90.26	90.59
Crude protein, (%)	19.35	19.20	19.17	19.12
Ash, (%)	11.62	11.32	9.62	9.60
Ether extract, (%)	8.16	8.151	11.10	10.80
Crude fibre, (%)	2.97	3.19	4.95	4.25
Ca, (%)*	2.45	2.45	2.45	2.45
P, (%)*	0.57	0.57	0.58	0.58
P(available), (%)*	0.33	0.33	0.34	0.34
Methionine + cystine, (%)*	0.77	0.77	0.75	0.75

[1]SO_1: 5% sunflower oil, [2]CGO_1: 5% oil released from chicken during grilling process, [3]SO_2: 7.5% sunflower oil, [4]CGO_2: 7.5% oil released from chicken during grilling process. *Calculated [12]

lect blood and liver samples. Blood samples were drawn into anticoagulant tubes containing sodium EDTA and into anticoagulant-free tubes. The anticoagulated blood was separated into plasma and erythrocytes by centrifugation at 1,500 x 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 the serum as described earlier. Liver tissue was removed and immediately rinsed with 0.9% ice-cold NaCl. The washed erythrocyte, liver samples, serum and plasma were stored at -20°C until analyses were performed.

Determination of Lipid Peroxidation (LP) [Malonyl Dialdehyde (MDA)] Levels in Serum and Liver

Lipid peroxidation in the serum were determined by the methods of Satoh [13] and Yagi [14] with minor modifications using 1, 1, 3, 3 - tetraethoxypropane (TEP) as standart. The method is based on the reaction between MDA (an aldehyde lipid peroxidation product) and thiobarbituric acid (TBA). MDA forms a pink-coloured complex with TBA. The absorbance of the solution containing the complex was measured at 532 nm in a spectrophotometer (UV-1201, Shimadzu, Japan). The values of LP in the serum were expressed in terms of MDA, nmol/ml serum. For determination of MDA level in liver, frozen liver was homogenised in a ratio of 1g of wet tissue to nine volumes of 1.15%KCl. Homogenates were then centrifuged at 2,500 x g, for 15 min, at 4°C to remove particulate material. Lipid peroxidation in the supernatant fraction was measured according to the method of Ohkawa et al. [15]. The LP levels in liver were defined as MDA, nmol/g protein.

Determination of Antioxidant Enzyme Activites in Erythrocyte and Liver

Catalase (CAT) activity

The washed erythrocyte and liver CAT (EC 1.11.1.6) activities were determined according to the method of Aebi [3], which measures absorbance of H_2O_2 at 240 nm. CAT activity in liver was expressed as *k/g* protein, where k is

Table 2. Fatty acid composition of chicken grill oil (CGO) and sunflower oil (SO) (% of total fatty acids)

Fatty acid	CGO	SO
Myristic (C14:0)	0.46	0.1**
Palmitic (C16:0)	20.29	3-6*
Palmitoleic (C16:1) (n-7)	1.95	0.1**
Stearic (C18:0)	6.63	1-3*
Oleic (C18:1) (n- 9)	30.63	14-43*
Vaccenic (C18:1) (n-7)	1.68	1.4**
Linoleic (C18:2) (n-6)	35.11	44-75*
Linolenic (C18:3) (n-3)	2.81	<0.7*
Arachidonic (C20:4) (n-6)	–	0.6- 4*

*Swern [37]

**Shingfield et al. [38]

Table 3. The effects of chicken grill oil (CGO) and sunflower oil (SO) on erythrocyte antioxidant enzyme activities and serum lipid peroxidation levels of laying quails

	Group 1 (SO_1)	Group 2 (CGO_1)	Group 3 (SO_2)	Group 4 (CGO_2)	*P*
Serum MDA (nmol/ml)	9.2517	9.6350	10.2400	10.2250	NS
Erythrocyte GSH-Px(U/gHb)	6.2900[b]	8.9767[a]	5.9617[b]	6.1783[b]	*
Erythrocyte TSOD (U/ml)	18.8800[a]	7.9450[b]	11.9217[ab]	10.4683[ab]	0.086
Erythrocyte CAT (k/gHb)	ND	ND	ND	ND	

ND, not detected. NS, not significant.

*p<0.05

Different superscripts a,b in the same row indicate significant differences between groups.

the first-order rate constant. Erythrocyte CAT activity was expressed as *k/g* haemoglobin (Hb).

Glutathione Peroxidase (GSH-Px) Activity

Liver and erythrocyte GSH-Px (EC 1.11.1.9) activities were assayed according to Paglia and Valentine [16]. In this method, GSH-Px catalyses the oxidation of glutathione in the presence of *ter*-butyl hydroperoxide. Oxidised glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidised to NADP. The reduction in absorbance of NADPH at 340 nm was measured. The liver GSH-Px activity was expressed as U/g protein. Erythrocyte GSH-Px activity was expressed as U/gHb.

Total Superoxide Dismutase (TSOD) Activity

Liver and erythrocyte TSOD (EC.1.15.1.1) activities were determined using a commercially available kit (Fluka, SOD Assay Kit-WST, Switzerland). Liver homogenate and erythrocyte TSOD activities were expressed as U/ml.

Determination of Serum Non-Enzymic Antioxidants

Vitamin E (α-tocopherol) was estimated by the method of Baker and Frank [17] and serum β-carotene and vitamin A (retinol) levels were measured according to the method of Suzuki and Katoh [18]. Results were expressed as μmol/L.

Liver Function Tests

Activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Reitman and Frankel [19]. Activities were expressed as IU/L.

Other Analyses

The haemoglobin content of erythrocyte hemolysates was determined using Drabkin's reagent [20]. The protein content of liver homogenate samples was determined according to the Biüret reaction [21] using bovine serum albumin as the standard. Fatty acid profiles of experimental oils were determined using GC/MS (QP-5050, Shimadzu, Japan). The oil extracted from each sample was methylated, and the fatty acids were separated and identified using a Cp Wax 52 CB 50m, 0.32 mm, 1.2 μm. The temperature of injector and detector was 250°C. Helium was used as the carrier gas.

Statistical Analysis

The data were analysed by using an SPSS-10 programme designed for Windows. Differences between obtained values were carried out by analysis of variance (ANOVA) and the Duncan's test was used for determining the significance level of at least $p<0.05$.

Results

Erythrocyte Antioxidant Enzyme Activities and Serum Lipid Peroxidation Levels

Laying quail erythrocyte CAT activity was negligible, as determined by the methods used in the present study. Erythrocyte GSH-Px activity was higher ($p<0.05$) in birds fed with the 5% CGO diet (group 2) compared with those fed the 5% and 7.5% SO or 7.5% CGO diets (groups 1, 3 and 4, respectively). Quails fed with the 5% CGO diet (group 2) exhibited lower ($p=0.086$) erythrocyte TSOD activity than those fed with 5% SO diet (group 1). Among the groups, serum MDA concentrations were not significantly influenced by dietary fat intake level (Table 3).

Activities of Antioxidant Enzymes and Lipid Peroxidation Levels in Liver

Table 4 shows that the effects of CGO on laying quail liver CAT activity were negligible. GSH-Px activity of liver was not affected ($p>0.05$) by dietary fat. Liver TSOD activity was significantly decreased ($p<0.05$) in quails fed with the 7.5% CGO diet (group 4) when compared with other groups. Also, MDA values in liver of quails fed with the 7.5% SO diet (group 3) were significantly increased compared with other groups (groups 1, 2 and 4) (Table 4).

Activities of Plasma Non-Enzymic Antioxidants

As shown in Table 5, vitamin A and β-carotene concentration in plasma of quails fed with the 5% CGO and SO diets (groups 1 and 2) were markedly higher ($p<0.01$ and

Table 4. The effects of chicken grill oil (CGO) and sunflower oil (SO) on liver antioxidant enzyme activities and lipid peroxidation levels of laying quails

	Group 1 (SO_1)	Group 2 (CGO_1)	Group 3 (SO_2)	Group 4 (CGO_2)	*P*
Liver MDA (nmol/g protein)	49.3500[b]	63.6667[b]	148.9667[a]	64.4000[b]	**
Liver GSH-Px (U/g protein)	41.8933	59.3760	44.7767	40.7350	NS
Liver TSOD (U/ml)	17.8783[a]	14.2320[a]	14.0800[a]	8.1433[a]	*
Liver CAT (k/g protein)	ND	ND	ND	ND	

ND, not detected. NS, not significant.

*p<0.05, **p<0.01

Different superscripts [a,b,c] in the same row indicate significant differences between groups

Table 5. The effects of chicken grill oil (CGO) and sunflower oil (SO) on plasma non-enzymic antioxidants of laying quails

	Group 1 (SO_1)	Group 2 (CGO_1)	Group 3 (SO_2)	Group 4 (CGO_2)	*P*
Vitamin A (µmol/L)	9.18[a]	8.77[ab]	6.38[c]	7.27b[c]	**
β-carotene (µmol/L)	1.47[a]	1.48[a]	1.01[b]	1.09[b]	*
Vitamin E (µmol/L)	19.62[b]	20.20[b]	21.63[ab]	23.03[a]	*

*p< 0.05, **p< 0.01

Different superscripts [a,b,c] in the same row indicate significant differences between groups

Table 6. The effects of chicken grill oil (CGO) and sunflower oil (SO) on serum ALT and AST levels of laying quails

	Group 1 (SO_1)	Group 2 (CGO_1)	Group 3 (SO_2)	Group 4 (CGO_2)	*P*
Serum ALT (IU/L)	38.72[ab]	42.64[a]	34.72[b]	40.44[a]	*
Serum AST (IU/L)	412.8[b]	444.8[b]	553.6[a]	524.8[a]	***

*p< 0.05, ***p<0.001

Different superscripts [a,b,c] in the same row indicate significant differences between groups

$p<0.05$, respectively) than those in quails fed with the 7.5% CGO and SO diets (groups 3 and 4). In brief, vitamin A and β-carotene concentrations in plasma of laying quails significantly decreased diets suplemented with 7.5% CGO and 7.5% SO. However, plasma vitamin E concentrations in quails fed with diets supplemented with 7.5% SO and CGO (groups 3 and 4) were significantly higher ($p<0.05$) than those fed with 5% SO and CGO diet (groups 1 and 2).

Analysis of Liver Function

Serum ALT concentration in quails fed with diets supplemented with CGO, both 5% and 7.5% (groups 2 and 4), were increased compared with those in birds fed with the 5% SO (group 1) or 7.5% SO (group 3) ($p<0.05$). Also, serum AST activities were not significantly ($p<0.001$) influenced by either SO or CGO dietary oil. However, addition of both 7.5% CGO and 7.5% SO to quail diets resulted in a significant increase in serum activities of AST ($p<0.001$) (Table 6).

Discussion

Sunflower oil have commonly been used as energy source in poultry diets because it contains the higher level of linoleic acid (omega 6). Linoleic acid improves the laying performance in poultry [22,23]. Thus, in the present study, we compare the effect of dietary SO and CGO on the oxidant/antioxidant status of specific tissues in the Japanese quail.

Experimental data indicate that birds, when compared with mammals, have greater resistance to oxidative damage, significantly lower levels of generation of ROS and greater antioxidant activity in mitochondrial membranes [24]. Cetingul et al. [25] investigated the effects of hazelnut oil (HO) and sunflower oil (SO) added to the diet on lipid peroxidation and haematological parameters (PCV, HB, RBC, WBC values) of laying hens. As a result, it was reported that the source and level of the dietary oil did not affect to lipid peroxidation or haematological values.

Male Japanese quail were fed with commercial poultry diets containing two levels of beef tallow (6.0 or 12.0 % w/w) for nine weeks. CAT, GSH-Px, SOD activities of erythrocyte, heart and aortae were not affected by dietary treatment. Hepatic SOD activities were lower (p=0.002) in birds fed diets containing high saturated fatty acid. Lipid peroxidation was lower (p<0.05) in heart and liver tissues from birds fed with atherogenic diets [26].

In our study, it was seen that the linoleic acid content of SO was higher than that of CGO. Also, CGO include a higher saturated fatty acid (Table 2). Thus, although serum MDA levels were not influenced by supplementation with CGO or SO, liver MDA level increased in quails fed with the 7.5% SO diet (group 3). Also, while erythrocyte SOD activity insignificantly decreased or unchanged with added high concentrations (7.5%) of both SO and CGO to diets, liver SOD activity significantly decreased (p<0.05) in quail fed with the 7.5% CGO diet. This reduction may be associated with the unsaturated fatty acid content of the CGO because linolenic acid (n-3) content of CGO is higher and arachidonic acid (n-6) content is negligible compared with SO.

Antioxidant enzyme activity profiles in red cells of man, rabbit, quail, pig and rat have been investigated and quail red cells were found to contain negligible CAT activity [26,27] but the highest levels of GSH-Px of all the species examined [27]. Yuan et al. [28] reported that the tissue antioxidant enzymes in the Japanese quail were minimally affected by dietary treatment. In the present study, we also failed to detect CAT activity in quail red cells and liver tissue because of the low level of CAT activity in this species [27,28].

In fact, an increased requirement for antioxidants when highly PUFAs are consumed has been demonstrated. GSH-Px activity increased in red cells of soybean oil-fed (n-6 PUFAs) quail; the activity of this enzyme in heart tissue reduced in these same animals. The association between altered tissue antioxidant enzyme activity and dietary PUFA is probably tissue -and species- specific and dependent on the specific target tissue. It was reported that increased incorporation of n-6 fatty acids into red cell membranes increased the intracellular GSH-Px activity in soybean oil-fed birds [28,29].

Similarly, in this study erythrocyte GSH-Px activity in CGO-fed quails increased (Table 3), whereas liver activity of this enzyme did not change in these same animals (Table 4). These results could relate to the fact that CGO contains higher linolenic acid (2.81%) than SO (<0.7%) (Table 2).

Vitamin A, vitamin E and β-carotene antioxidant vitamins have been suggested to protect cells against cancer and inflammatory disease, perhaps due to their antioxidant properties [30,31]. Broiler hens were fed with diets containing fresh or oxidised 9% rapeseed oil and 2% soybean oil and their antioxidant status was evaluated. Compared to the fresh oil, the concentrations of linoleic and linolenic acid were slightly lower in oxidised oil. The hens fed with oxidized fat showed significantly higher plasma concentrations of lipid peroxidation (p<0.01), and lower concentrations of tocopherols, lutein, β -carotene and retinol in plasma and tissues [32].

In the present study, plasma vitamin A and β-carotene concentrations significantly decreased in both 7.5% CGO and 7.5% SO although vitamin E concentrations in plasma were higher in quails fed with diets supplemented with 7.5% SO and particularly 7.5% CGO (p<0.05). It appears that n-3 fatty acids and vitamin E have opposite effects on the metabolic regulation of absorption and/or conversion of β-carotene to retinol in plasma and platelets [33].

ALT and AST are intracellular transaminases which are used as reliable markers for liver injury. These markers are released into the circulation in proportion to the extent of cellular damage and most are elevated in the acute phase of cellular necrosis [34].

Lea et al. [35] did not demonstrate significant differences in liver weight, serum AST or serum ALT activity in turkeys fed with diets containing oxidised fish oil. Hassan et al. [9] investigated alterations in levels of some physicochemical properties of fat released from chicken during the grilling process and the effect of grilled fat on body weight, liver function in male rats at the end of a two-month period. The results showed that AST levels increased significantly in rats fed with a diet containing 5% grilled fat compared to control rats fed with 5% corn oil. However, ALT levels were not affected by feding with a grilled fat diet. Our results indicate that the increase of lipid peroxidation (MDA) in the liver of 7.5% SO (group 3)- treated animals promoted damage to liver cells, which led to an increase of AST activity in the serum. These results suggest that 7.5% SO supplementation can promote damage to the liver.

In the same experimental groups, we evaluated the effects of CGO on performance presented in another article [36]. Specifically, dietary CGO had no adverse effect on egg weight, feed conversion ratio, cholesterol and fatty acid composition of egg yolk. Also, the egg production of groups fed with the diet containing CGO was higher than those of the SO groups (p<0.001).

Conclusion

There are few studies that investigated the effects of dietary fats on antioxidant components in the Japanese quail. The mechanisms for the association between altered tissue antioxidant enzyme activities and dietary PUFA are probably complex and remain to be elucidated. As a result of this study, dietary CGO obtained as described in the Materials-Methods section may be used up

to 5% ratio in laying quails without showing a negative effect on oxidant/antioxidant status. Further study will be required to elucidate the mechanism(s) of the possible relationship between effects on the antioxidant system and performance of CGO added to poultry diets.

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Conflict of Interest

There are no conflicts of interest among the authors.

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