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DOI 10.1007/s11356-015-5852-1

Link to the publisher: https://link.springer.com/article/10.1007/s11356-015-5852-1#:~:text=In%20broilers%2C%20the%20antioxidant%20effect,metabolism%20of%20polyphenols%20mainly%20occurs.

An extra-virgin olive oil rich in polyphenolic compounds has antioxidant effects in meat-type broiler chickens

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Abstract

The aim of this study was to extend the knowledge on the antioxidant effect of extra-virgin olive oil (EVOO) in liver of broiler chickens not subjected to any form of insult. A total of 120 male broiler chickens (Hubbard strain) were divided into three groups and fed ad libitum three isoenergetic diets from hatching until slaughter age (49 days) on a completely randomized design. The dietary treatments consisted of 2.5% added oil or fat from three sources as follows: diet containing sunflower oil (SFO); diet containing lard (LRD), and diet containing extra-virgin olive oil (EVOO). The activity of the main antioxidative enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GS-Px) and glutathione S-transferase (GST), and lipid peroxidation as thiobarbituric acid-reactive substances (TBARS) content, was measured in the liver of chickens. The susceptibility to undergo lipid peroxidation was assessed by exposing liver homogenate to 30°C or to an ascorbate/iron mixture as pro-oxidant system. Dietary oil or fat type improved significantly ($P < 0.05$) body weight and gain as well as feed efficiency in birds fed EVOO compared to those fed the other treatments. Supplementing EVOO in diet significantly ($P < 0.05$) reduced lipid peroxidation by increasing antioxidant defense system. These findings, besides adding more results on antioxidant effect of extra-virgin olive oil on liver of other experimental model other than rats and humans, could be significant for animal welfare, with consequent benefits for both producers and consumers.

Keywords: Extra-virgin olive oil; Diet; Liver; Antioxidative status; Chickens.

Introduction

The reactive oxygen species (ROS), are continuously formed as a result of normal metabolic processes, however when not effectively and safely removed by endogenous antioxidant system, they can oxidise and damage cellular macromolecules, possibly leading to oxidative stress

(Kadenbach et al., 2009). Oxidative stress is believed to play an important role in the regulation of the metabolic activity of some organs and productivity in farm animals. It may impair health both directly, by peroxidative damage to lipids and macromolecules, and indirectly by changes in cellular membranes or modifying some metabolic pathways, resulting in altered physiology and possibly pathology (Celi et al., 2014). Exogenous antioxidants are important because of they have at twofold function: to prevent food oxidation, in particular lipid oxidation, and at the same time to increase the amount of antioxidant agents present in the organism, protecting against metabolic disorders.

Extra-virgin olive oil (EVOO), the major dietary fat component in the Mediterranean diet, has shown anti-inflammatory, immunomodulatory, antiproliferative and anti-apoptotic effects (Sánchez-Fidalgo et al., 2013). EVOO is rich in phenolic compounds which have been shown to delay in vitro metal-induced and radical-dependent low density lipoprotein oxidation (Owen et al., 2001). Polyphenolic compounds in EVOO help reduce pathogenic microorganisms that might potentially spread in birds' digestive organs, while also preventing the formation of toxins in feed and increasing the digestive enzymes activity. The EVOO oleuropein-derivatives, especially hydroxytyrosol, have been shown to have protective effects against markers associated with the atherogenic process (Oliveras-López et al., 2008). Scientific evidence supports the potential use of nutraceuticals focused in polyphenols constituents as agents capable to prevent or accelerate healing of cardiovascular disease (Romano et al., 2012).

However, in some respects, there are conflicting results between human and experimental models. Indeed, about the capacity antiatherogenic and cardioprotective effect of HT, no Increase in nitric oxide production in human endothelial cells was found, indicating HT may not exerts its action directly on endothelial nitric oxide synthase (Schmitt, Handler et al., 2007). This finding contrasts with the observations by González-Correa et al. (2008) which indicated that HT raised plasmatic

and aortic levels of nitric oxide in rats. Therefore, for better understanding of the several beneficial mechanisms of HT, more animal and human studies are needed.

Domestic birds like chickens are believed to be particularly sensitive to oxidative stress as a likely result of genetic selection towards larger pectoral muscles, total weight gain, and the faster growth rate (Fellenberg and Speisky, 2006; Sihvo et al., 2013). Moreover, during their production cycle, poultry may be exposed to environmental stressors, resulting in oxidative stress. According to the existing literature, heat and diet are the most remarkable means of oxidative stress in domestic birds that may lead to biological damage, serious health disorders, lower growth rates, and, hence, economic losses. The extent and, hence, the negative consequences of the oxidative stress in the living birds muscles can be modulated by livestock handling and by dietary means. Dietary fats are known to influence cell membrane, tissues lipid composition and plasma lipoprotein concentrations depending on their constructive fatty acid content. Moreover, it is well documented that chronic diseases such as coronary arterial disease, hypertension, diabetes, arthritis, other inflammatory and auto immune disorders as well as cancer can be prevented via the intake of olive oil (Bermudez et al., 2011), in fact its beneficial properties are due to in part to the high MUFA content.

In this way, the role different dietary fat sources on antioxidant system in liver of poultry has never been investigated. Therefore, the best of our knowledge and based on previous considerations, the present study aimed to evaluate the effects of dietary fats supplementation on growth traits and antioxidant status and lipid peroxidation in the liver of broiler chickens.

Materials and methods

Reagents

All chemicals and biochemicals used were of the highest quality and purchased from Sigma Chemical Co. Chelex 100 ion exchange resin (Bio-Rad Laboratories) was used to remove

contaminating metals from all reagents. Organic solvents used were of analytical grade. All solutions were prepared in double-distilled water.

Experimental design, animals, rearing system and diets

The experiment was conducted in a poultry research facility situated at the University of Bari "Aldo Moro", Valenzano, Bari, Italy. The trial lasted 49 d, and pens were randomly assigned to three dietary treatments with each having four replicates of 10 birds (each bird initially occupied 0.095 m^2 of floor space). In preparation to trial, the poultry facility was accurately cleaned and rinsed using pressurized water in order to disinfect the environment. The hall was gasified after drinker and feeder installation and 24 h before broilers allocation. A total of 120 d-old male chicks (Hubbard strain), from a commercial hatchery, were raised in a conventional environment. The study was conducted in a completely randomized design with three dietary treatments. Each diet (treatment) was replicated four times, with each replicate comprising one pen of 10 birds. Chicks were vaccinated following a standard vaccination schedule, and in order to reduce the stress caused by vaccination to birds, 24 h before and after vaccination, a multi-electrolyte solution was added in drinking water. Poultry facilities had standard thermostatically controlled curtains and cross-ventilation as well as lighting program. Pens were equipped with a pan feeder, a manual drinker and wood shavings. Drinkers were regularly washed to prevent faecal and microbial contaminations. A single-phase feeding program was used in the study. Up to slaughtering age (49 d), birds were fed three diets containing different oil or fat sources formulated to meet or exceed broiler nutrient requirements (NRC, 1994). The dietary treatments consisted of 2.5% added oil or fat from three different sources as follows: SFO, diet containing 2.5% sunflower oil; LRD, diet containing 2.5% lard; and EVOO, diet containing 2.5% extra-virgin olive oil.

Lard was heated to a liquid state and then added to the feed and mixed. The oils were kept in cold room at 4°C prior mixing and the diets were prepared weekly and kept in cold room in air-tight

containers. The extra-virgin olive oil (from *Coratina* variety) used for experimental diet had a high total polyphenols concentration equal to 253.58 mg/kg referred 42.98 mg/kg of hydroxytyrosol (Laudadio et al., 2015). The sunflower oil used for control diet had very low levels of total polyphenols (De Leonardis et al., 2005). Ingredients and chemical composition of the basal diet are shown in Table 1. Diet samples were ground in a hammer mill with a 1 mm screen and analysed in triplicate for dry matter (DM, method 945.15), crude protein (Kjeldahl N×6.25, method 990.03), ether extract (method 945.16) and ash (method 967.05) according to AOAC (2000). Feed (mash form) and water were provided ad libitum throughout the whole trial. Body weight (g) and feed intake by replicate were weekly assessed for all birds. Average daily gain (g/d), feed intake (g/d) and feed efficiency (g/g) were then calculated. At the end of the feeding trial (49 d of age), after a feed withdrawal period of 12 h, a total of 12 broilers per treatment (three from each replicate) were selected according to average weight and slaughtered by cervical dislocation to collect the blood and liver samples.

Sample collection and analysis

At the day 49 of the experiment, the pooled livers were homogenized using an electrically driven Teflon pestle in a weight/buffer (1:10) ratio. A 100 mM phosphate buffer, pH 7.4, containing 1 mM EDTA, 0.15 M KCl, 0.1 mM PMSF, and 1 mM DTT was used. The homogenate was centrifuged at 800g for 10 min to discard unbroken cells, and the resulting supernatant used for lipid peroxidation. For the determination of antioxidant enzyme activities, supernatant obtained as above was further centrifuged at 6,000 g × 20 min. The pellet of the latter centrifugation, consisting mainly of mitochondria, was eliminated, while the supernatant was immediately used for analysis.

Enzyme assay

Superoxide dismutase (SOD, EC1.15.1.1) was examined by method of Misra (1985). The activity was determined from its ability to inhibit the autoxidation of epinephrine. Stimulation of epinephrine autoxidation by traces of heavy metals present as contaminants in the reagents or by the other metals under investigation was prevented by adding 10^{-4}M EDTA in the buffer to chelate these ions. One unit of SOD is defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50%. The enzyme activity was expressed as U/mg protein.

Catalase (CAT, EC 1.11.1.6) activity was assayed by the method of Clairborne (1985) by following the decrease in absorbance of H_2O_2 at 240 nm (ε 40 $M^{-1}cm^{-1}$). One unit of enzyme activity is defined as the amount of enzyme required to degrade 1 micromole of H_2O_2 in 1 min and is expressed as U/mg protein.

Glutathione peroxidase (GS-Px, EC 1.11.1.9.) activity was measured by method of Gunzler and Flohe (1986). The reaction measured the rate of GSH oxidation by tert-butyl hydroperoxide, catalyzed by GPx. GSH was maintained at constant concentration by the addition of exogenous GR and NADPH, which converted the GSSG to GSH. The rate of GSSG formation was then measured by the change in the absorbance of NADPH at 340 nm. ($\varepsilon_{340\ nm}$ 6.2 mM^{-1} cm^{-1}) and activity expressed as nmoles of NADPH oxidized/min/mg protein.

Glutathione S-transferase (GST, EC 2.5.1.18) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate (Habig and Jakoby, 1981). This assay was based on the rate of increase in conjugate formation between GSH and CDNB. The enzyme activity was determined by monitoring the changes in absorbance at 340 nm. The enzymatic activity was expressed as nmoles of CDNB–GSH conjugates formed/min/mg protein.

Determination of lipid peroxidation

The thiobarbituric acid-reactive substances (TBARS) assay quantifies oxidative stress by measuring the peroxidative damage to lipids that occurs with free radical generation. Free radical damage to

lipids results in the production of malonaldehyde (MDA), which reacts with TBA under conditions of high temperature and acidity generating a chromogen that can be measured spectrophotometrically at 535 nm. The TBARS assay, as follows, was used to quantify the oxidative damage (lipid peroxidation). Briefly, aliquot of liver homogenate was added to the reaction mixture containing TCA–HCl–TBA as reported by Buege and Aust (1987). Butylated hydroxytoluene (0.03 %, final concentration) was added prior to heating the mixture in 80°C water bath for 15 min .to avoid any artifactual oxidation due to heating. After cooling, reaction mixture was centrifuged to precipitate the denaturated proteins and the supernatant was used to measure the absorbance at 535 nm. The concentration of lipid peroxides was expressed as nmoles TBARS per mg protein, using tetramethoxypropane as an external standard.

Alternatively, to test the susceptibility to undergo lipid peroxidation, an in vitro experiment was developed in which an oxidative stress was elicited by incubating aliquots of homogenate either at 37°C for 20 min in a medium consisting of 0,175 M KCl and 25 mM Tris–HCl pH 7.4, or at 30°C for 1 h. In both cases, the reaction was stopped on ice and TBARS levels were evaluated as described above.

Protein concentration

Protein concentrations were determined by the Bradford assay procedure using bovine serum albumin as the standard (Bredford, 1976).

Statistical analysis

The results are expressed as mean ± standard error of mean (SEM). The statistical significance of data was evaluated by one-way analysis of variance (ANOVA), using Statistical Package for Social Sciences (SPSS) Software Version 9.0 for Windows. Data were analyzed by post-hoc analysis with least square difference to compare the means. Differences were considered significant at $P < 0.05$.

Results

The effect of dietary treatments on growth performance of broiler chickens are presented in Table 2. The average final BW tended to increase when birds fed dietary EVOO and it was significantly higher ($P < 0.05$) than those in the LRD and SFO groups, respectively. Chickens from EVOO and LRD groups were characterized by a significantly higher ($P < 0.05$) daily weight gain compared to SFO treatment. Conversely, broilers fed dietary sunflower oil significantly exhibited a higher ($P < 0.05$) feed intake compared to the other groups. After 49 days of feeding period, including EVOO in diet led to a positive effect on chickens' feed efficiency, resulting significantly lower ($P < 0.05$) compared to LRD and SFO (2.56 vs. 2.65 and 2.88, respectively). There were no significant differences ($P > 0.05$) between dietary treatments regarding mortality rate that was generally low, averaging 1 % over the whole experiment.

The oxidative status of broilers of the three dietary groups, measured as TBARS production, is illustrated in Fig. 1. As you can see, the amount of TBARS in blood of animals fed with EVOO is lower than animals fed with LRD and SFO by 32.8 and 20.1% respectively. A similar situation is observed in liver, where TBARS production in EVOO group (0.41 nmoles/mg protein) is lower if compared to LRD group (0.63 nmoles/g protein) as well as to SFO group (1.05 nmoles/mg protein). Furthermore, the susceptibility to undergo lipid peroxidation was assessed in each group by exposing liver homogenate to an ascorbate/iron mixture, as pro-oxidant system. As shown in Fig. 2, although this treatment enhances TBARS production within the same experimental group, however such increase is only three times compared to endogenous content of peroxides in EVOO group. In animals of LRD group the prooxidant mixture induces a twenty-eight times increase in TBARS production compared to endogenous content, while in animals of SFO group, increase of peroxides induced by the same mixture is twenty-four times.

The results of analysis of antioxidant enzymes involved in the metabolism of ROS, SOD and CAT, are shown in Fig. 3. It can be noted that SOD activity in EVOO group is greater than LRD group (144.3 vs. 103.4 U/mg protein), as well as CAT whose activity is of 114.6 vs. 67.07 U/mg protein in EVOO and LRD groups, respectively. On the contrary, the above activities are lower compared to SFO group, as SOD (169.8 U/mg protein) and CAT (143.6 U/mg protein) activities are higher than other two groups.

Regarding enzymes involved in glutathione metabolism, Fig. 4 shows that activity of GS-Px is lower in EVOO than LRD group (94.88 vs. 153.86 μmoles/min /mg protein), but higher than SFO fed broilers (73.19 μmoles/min/mg protein). The opposite situation occurs for GST activity, the enzymatic activity of chickens fed with EVOO is higher compared to the animals of the LRD group (158.74 vs. 116.26 μmoles/min mg protein) but lower than animals fed with SFO (210.7 μmoles min/mg protein).

Discussion

There is a plethora of studies on the positive effect of olive oil, or its isolated components, among which especially hydroxytyrosol, in living organisms (Casalino et al., 2002; Jemai et al., 2008; Hamden et al., 2009) or of isolated cells (Manna et al., 2002; Zhang et al., 2009; Rodríguez-Ramiro, Martín et al., 2011) or in vitro systems (Gutierrez et al., 2001; Casalino et al., 2002; Hamden et al., 2009) subjected to various types of stress. The aim of this study was to examine the effects of extra-virgin olive oil diet on oxidative status and some antioxidant enzymes in liver of broilers not subjected to any form of insult. We observed a lower level of lipid peroxidation in blood and liver of broiler fed to EVOO compared to those fed LRD and SFO diets. In our opinion, this result could be due to the high amount of monounsaturated fatty acids in EVOO, compared to LRD and SFO, which are less susceptible to lipid peroxidation than PUFA, highly present in SFO. When thermal or chemical stress has been simulated in vitro, by exposing the liver of chickens at temperature of

30°C or to a prooxidant mixture consisting of Asc/Fe, we observed that lipid peroxidation was strongly stimulated, giving rise to elevated amount TBARS within the same experimental group, and again the process is less evident in EVOO group < LRD < SFO. Our results are in agreement with those reported by Oliveras-López et al. (2008) showing that dietary supplementation with EVOO protected liver and pancreatic islets incubated with 10 μmol/L hydrogen peroxide against cell membrane lipid peroxidation compared to control and SFO fed rats. Furthermore, Maraschiello et al. (1999) have shown that cooking increases the amount of TBARS in the muscle of chickens. Accordingly to our results, in cooked meat from chickens fed with sunflower oil, TBARS values were significantly higher than meat from chickens fed with lard and olive oil. The lesser amount of TBARS we observed in liver of chickens from EVOO group can be attributed, in addition to the high ratio MUFA/PUFA, also to the presence of phenolics antioxidants in EVOO, which generally improved nutritional status (El-Kholy et al., 2014)

The hydroxytyrosol and other phenolic compounds have multiple biological actions commonly related to activity as scavenging of free radicals (Rice-Evans, 1995), but the current evidence strongly supports that natural biophenols may also provide indirect protection by increasing endogenous defence systems.

When considering enzymes involved in ROS metabolism, our results show that SOD and CAT in broilers fed to EVOO exhibit higher activity compared to animals in LRD group. Among components of EVOO, the hydroxytyrosol is an activator of phase II enzymes to reduce oxidative stress. Bayram et al. (2012) examined the Nrf2 protein, a key regulator of the phase II defence system and found that hydroxytyrosol could increase Nrf2 expression and nuclear translocation in the hearth of mice. In the nucleus Nrf2, together with a cofactor, stimulates the transcription of antioxidant and detoxifying enzymes (Martín et al., 2010). Furthermore, recent results reported by Zheng et al. (2015) showed that prenatal stress induced decrease of SOD2 expression and the total SOD activity was significantly improved after HT supplement by activating FOXO3, a known

regulator of SOD. Finally, antioxidant efficiency of olive oil could be accounted for a mechanism acting upstream of ROS production. It has been previously shown that production of ROS in mitochondria can be controlled by some uncoupling proteins present in many organisms, which act as antioxidants (Skulachev, 1998). Olive oil feeding up-regulates uncoupling protein genes in rat brown adipose tissue and skeletal muscle (Rodriguez et al., 2002). Mujahid et al. (2007) have shown that the protein of avian uncoupling (avUCP) is inhibited in acute stress by exposure to heat, and that this inhibition stimulates mitochondrial ROS production and consequent oxidative damage. Therefore, we can assume that in our case, an up-regulation of this protein would be responsible for the improvement of the oxidation state of the broilers fed olive oil.

Regarding activities of peroxide-removing enzymes, namely GSPx and GST, an apparent reduction of GSPx activity in broiler of EVOO group, compared to LRD group, was observed. Similar results have obtained by Maraschiello et al. (1999) in muscle of chickens and they suggested that the higher enzyme activity in muscle from LRD and SFO fed broilers is due to higher oxidative stress in these groups. Further, in our experiments, broilers fed to SFO showed higher GST activity. The enzyme, known for its ability to catalyze conjugation of xenobiotic to the reduced form of glutathione (GSH), also has peroxidase activity. Given the high amount of TBARS found in SFO group, despite the increase in the activities of SOD and catalase, it is reasonable to assume that rising of GST will serve to counteract peroxides present, while GSPx is depressed by homeostatic compensation.

Many experimental data show that cellular systems treated with polyphenols exhibit increased GSH and related enzymes; interestingly ,the observed effects are strongly dependent on the chemical structure of the polyphenol and the cellular system used. and therefore it seems conceivable that there is a sort of selectivity of action for the polyphenols which appear to act differently on different enzymatic activities. The phenol composition in olive oil depends on variety, climate, area of growth, latitude, and ripeness of the olive (Vissers et al., 2004). In addition, the first requirement for

293 a dietary compound to be a potential in vivo antioxidant in organism is that it enters the blood
294 circulation. Animal and human studies show that olive oil phenols are well absorbed. The
295 absorption of HT takes place in the small intestine and colon with absorption rate that varies
296 according to the species of animal, for instance, different rates are found in rats compared to
297 humans because of the absence of a gallbladder in these rodents (Visioli et al. 2003). Once absorbed,
298 polyphenols are subjected to the conjugation process that mainly includes methylation, sulfation,
299 and glucuronidation. Owing this, olive oil phenolics bioactivity it is likely to be derived mainly
300 from its biological metabolites. The relative importance of these three types of conjugation appears
301 to vary according to the nature of the substrate and the dose ingested. The balance between sulfation
302 and glucuronidation of polyphenols also seems to be affected by species and sex (D'Archivio et al.,
303 2007).
304 In parallel to the positive effect of extra-virgin olive oil on oxidative status, our results showed a
305 marked improvement in the growth performances in broilers fed extra-virgin olive oil than other
306 dietary fat sources. The favorable results of EVOO diet on growth performance of chickens could
307 be explained by the positive impact of this oil on the reduced passage rate of the digesta through the
308 gastrointestinal trait, allowing for better nutrient absorption and utilization (Latshaw, 2008).
309 Indeed, during the gastric digestion the low pH stimulates dietary lipid and protein oxidation
310 (Larsson et al., 2012); the consequence of lipid oxidation is concerning the loss of essential fatty
311 acids and vitamins. On the other hand, in addition to essential amino acids degradation, food protein
312 oxidation also induces a further nutritional loss as oxidized food proteins may not be fully degraded
313 by digestive enzymes such as trypsin (Estévez, 2015). It has been previously reported that dietary
314 supplementation with vegetable or animal fats did not affect feed passage rate through the digestive
315 tract in chickens (Latshaw, 2008). However, the dissimilarity of this statement with our results
316 could be attributed to the differences in dietary fat source and especially to the birds age.
317

Conclusion

The antioxidant effect of extra-virgin olive oil and its phenolic compounds, mainly hydroxytyrosol and oleuropein, has been amply demonstrated in various organs of human and animal models, nevertheless interspecies variability makes the comparison between the model species, complex and sometimes controversial. In broilers, the antioxidant effect of olive oil has been studied mainly in the muscle, as it has been found that supplementation with antioxidants is an effective means to increase the oxidative stability of raw meat and after cooking, and less in liver where metabolism of polyphenols mainly occurs. This work extends the study of the antioxidant effect of extra-virgin olive oil in liver of broilers, an organ responsible for the homeostasis of the whole organism and easily subjected to oxidative stress. Furthermore, this is a new approach, in which the activity of antioxidant enzymes was measured in healthy animals, fed virgin EVOO as part of their diet, not previously exposed to any oxidizing agent. These findings, besides adding more results on antioxidant effect of olive oil on an additional experimental model other than rats and humans, could be significant for animal welfare, and hence for poultry products, with consequent benefits for both producers and consumers. However, our study is limited in some aspects because of it does not examine the individual contribution of the various components of olive oil and their interactions in this experimental feeding model. Therefore, further studies investigating the possible mechanism of olive oil are in progress.

Acknowledgments

The Authors are thankful to “Azienda Agricola Dr. Giuseppe Colia; Olearia AIPO Puglia Società Cooperativa Agricola, Andria (BT), Italy” for the supply of the Coratina cultivar extra-virgin olive oil to carry out this work.

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449

450 **Table 1**. Ingredients and chemical analysis of the basal diet fed to
451 broiler chickens.

Ingredients	Diet
	g/kg as-fed basis
Corn	541.0
Soybean meal (48% CP)	175.0
Corn gluten meal (60% CP)	45.0
Dicalcium phosphate	17.0
Oil or Fat[1]	25.0
Calcium carbonate	90.0
L-Lys HCl	2.0
DL-Met	2.0
Vitamin-mineral premix[2]	2.5
Sodium chloride	2.5
L-Thr	1.0
Yeast	1.5
Sodium bicarbonate	2.5
Chemical analysis, %	
Dry matter	88.13
Crude protein	19.00
Crude fibre	2.80
Crude fat	5.35
Starch	42.87
Ash	5.59
Calculated analysis	
ME (kcal/kg of diet)	3,050
Lys, %	0.98
Ca, %	1.01
Met, %	0.44
Na, %	0.17
Met + Cys, %	0.65
Thr, %	0.64
Available P, %	0.42

452 [1]Each diet contained one of the following sources: extra-virgin olive oil
453 (EVOO), lard (LRD) or sunflower oil (SFO), respectively.
454 [2]Supplied per kilogram of diet: vitamin A 12,000 IU; vitamin E, 10 mg;
455 vitamin D 2,200 IU; niacin 35.0 mg; D-pantothenic acid 12 mg;
456 riboflavin 3.63 mg; pyridoxine 3.5 mg; thiamine 2.4 mg; folic acid 1.4
457 mg; biotin 0.15 mg; vitamin B 0.03 mg; Mn 60 mg; Zn 40 mg; Fe 1,280
458 mg; Cu 8 mg; I 0.3 mg; Se 0.2 mg.
459

460 **Table 2.** Growth performance traits of broilers fed the experimental diets.

	Diet[1]			
Item	EVOO	LRD	SFO	SEM
Body weight, g/bird[1]	2,643[a]	2,570[c]	2,424[b]	21.05
Body weight gain, g/d	53.9[a]	52.6[a]	49.7[b]	0.33
Feed intake, g/bird/d	138[b]	139[b]	143[a]	0.71
Feed efficiency, g/g	2.56[a]	2.65[b]	2.88[c]	0.09
Mortality, %	1.2	1.2	1.1	-

461

462 [1]Each diet contained one of the following oil or fat sources at 2.5% of inclusion level: extra-virgin

463 olive oil (EVOO), lard (LRD) and sunflower oil (SFO), respectively.

464 [2]Body weight at 49 days of age.

465 [a-c] Means within each row with no common superscript differ significantly ($P < 0.05$).

466 SEM, standard error of the means.

467

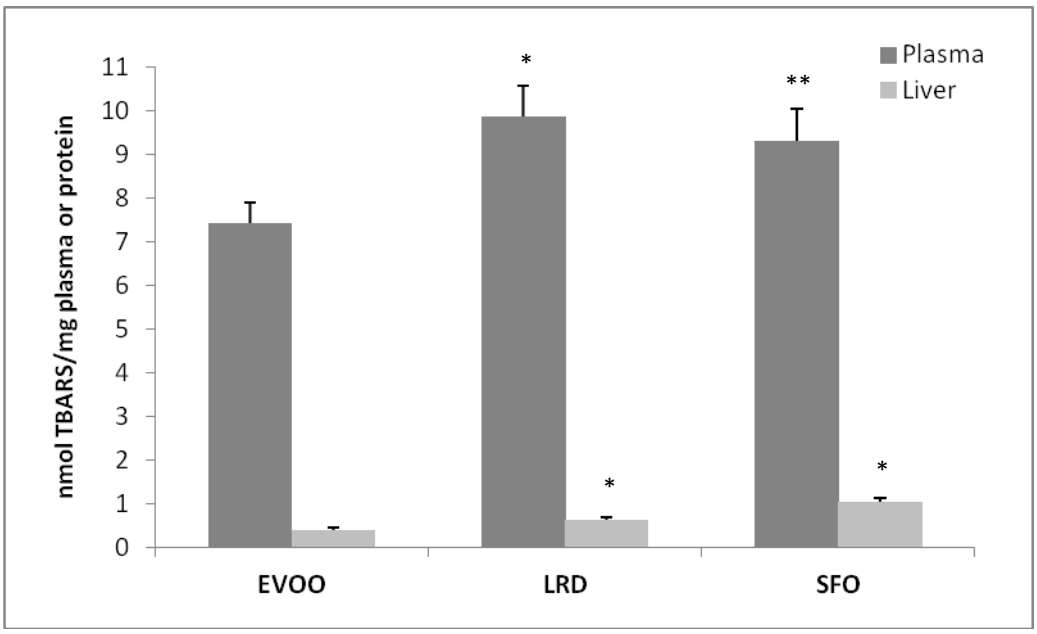

Figure 1. Lipid peroxidation (TBARS) in plasma and liver homogenate of chickens fed extra-virgin olive oil (EVOO), lard (LRD) or sunflower oil (SFO)-supplemented diet. The results represent the mean ± SD of fifteen determinations per diet. For each determination, a pool of three bird was used. Each determination was performed in triplicate.

** $P < 0.05$ vs. EVOO; * $P < 0.01$ vs. EVOO

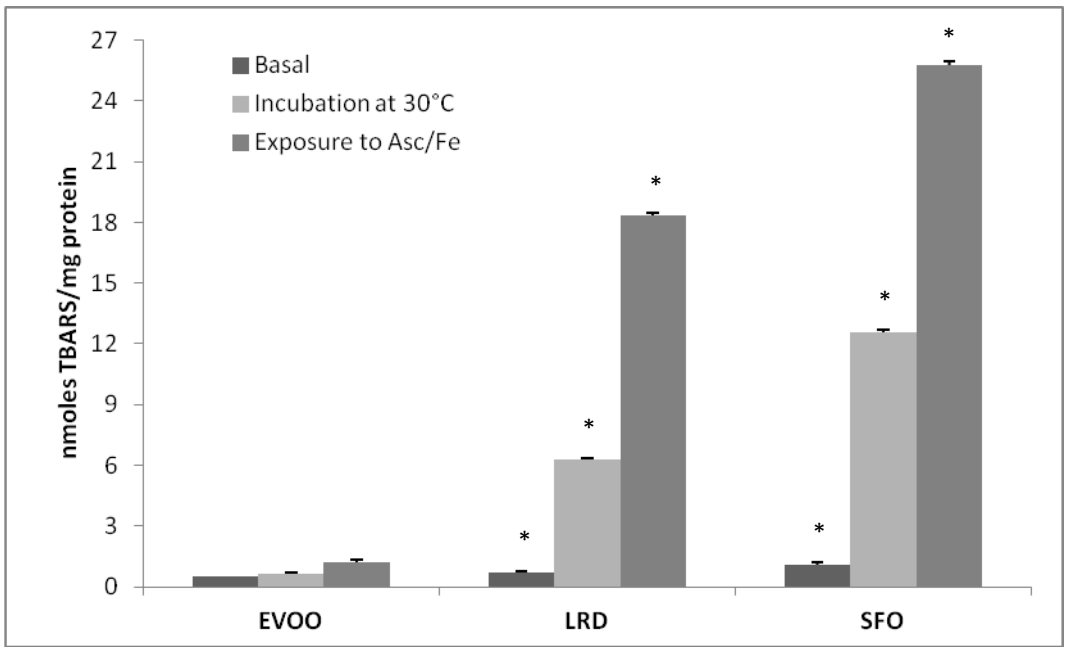

Figure 2. Induction of lipid peroxidation in liver homogenate of chickens fed extra-virgin olive oil (EVOO), lard (LRD) or sunflower oil (SFO)-supplemented diet. The results represent the mean ± SD of fifteen determinations per diet. For each determination, a pool of three bird was used. Each determination was performed in triplicate; * $P < 0.01$ vs. EVOO.

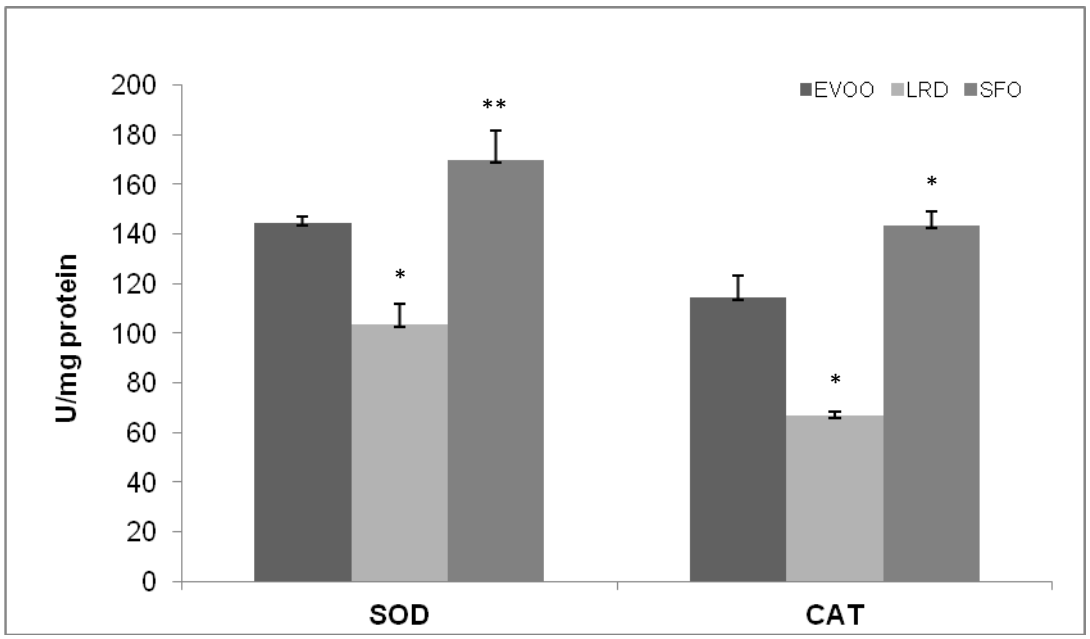

Figure 3. Superoxide dismutase (SOD) and catalase (CAT) activity in liver of chickens fed extra-virgin olive oil (EVOO), lard (LRD) or sunflower oil (SFO)-supplemented diet. The results represent the mean ± SD of fifteen determinations per diet. For each determination, a pool of three bird was used. Each determination was performed in triplicate;

** $P < 0.05$ vs. EVOO; * $P < 0.01$ vs. EVOO

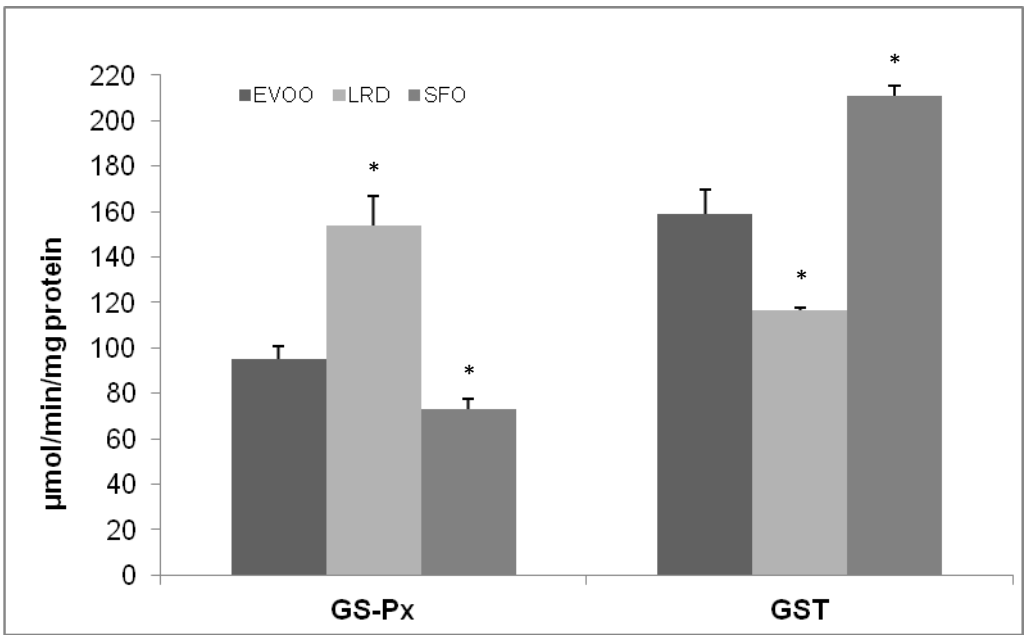

Figure 4. Glutathione peroxidase (GS-Px) and glutathione S-transferase (GST) activity in liver of chickens fed extra-virgin olive oil (EVOO), lard (LRD) or sunflower oil (SFO)-supplemented diet. The results represent the mean ± SD of fifteen determinations per diet. For each determination, a pool of three bird was used. Each determination was performed in triplicate.

* $P < 0.01$ vs. EVOO