African Journal of Biotechnology Vol. 11(7), pp. 1800-1805, 24 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2605
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of various levels of rosemary and oregano volatile oil mixture on oxidative stress parameters in quails

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Accepted 23 November, 2011

The aim of this study was to determine the possible effects of various levels of rosemary and oregano volatile oil mixture dietary supplementation on oxidative status parameters in the blood and various organs of quails. A total of 880 one-day-old Pharaoh quails (*Coturnix coturnix* Pharaoh) including both males and females, were divided into four groups containing 220 quails and treated as follows: a control group with 0 mg volatile oil (VO)/kg of diet; group I, with 100 mg/kg rosemary VO (RVO) plus 100 mg/kg oregano VO (OVO, 50:50%); group II, 60 mg/kg rosemary VO plus 140 mg/kg oregano VO (30:70%); and group III, 140 mg/kg rosemary VO plus 60 mg/kg oregano VO (70:30%). The diets were prepared fresh for each treatment. The experiment was carried out for 42 days. The quails were euthanized and then serum, erythrocyte, heart, liver and spleen were obtained. Serum malondialdehyde (MDA), nitric oxide (NO), antioxidant activity (AOA), glutathione (GSH), vitamin A (VA), vitamin C (VC), erythrocyte superoxide dismutase (SOD), as well as MDA, NO and SOD levels from the heart, liver and spleen were determined by enzyme-linked immunosorbent assay (ELISA). The results of the study show that whereas the lowest serum MDA and serum NO values were observed in group III ($p< 0.05$), the highest serum MDA and serum NO values were in group I ($p<0.05$). Moreover, the highest NO and SOD levels in group I were found in the liver and spleen, respectively ($p<0.05$). In conclusion, supplementation with a rosemary and oregano VO mixture to the diets of quails may alter the antioxidant activity depending on the diets, and the most effective doses of rosemary and oregano VO mixture were 70 and 30%, respectively.

Key words: Rosemary, oregano, oxidative status, quail.

INTRODUCTION

During leukocytes and mitochondrial respiration chain, free radicals derived from oxygen are continuously produced in living organisms. These radicals are reactive substances that contain non-shared electrons in their orbit and are prone to break down surrounding configurations. The most commonly produced oxygen- derived radicals are singlet oxygen, superoxide radicals, hydroxyl radicals, hydrogen peroxide and nitric oxide (NO) (Cuzzocrea et al., 2001; Berger and Chiolero, 2007; Bulbul et al., 2008). Under normal conditions, there is a balance between generated free oxygen radicals (FOR) and enzymatic or non-enzymatic antioxidants. Superoxide dismutase (SOD) is an enzymatic antioxidant, whereas glutathione (GSH), vitamin A (VA) and vitamin C (VC) are classified as non-enzymatic antioxidants (Urso and Clarkson, 2003; Lee et al., 2009). Antioxidant activity (AOA) represents totally non-proteinaceous and

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non-enzymatic antioxidants (e.g. uric acid, and albumin) in serum and tissues (Collins, 2005; Mancini et al., 2010). Lipid peroxidation develops as a result of oxidative damage caused by excessive FOR production or a lack of antioxidant capacity. Several products are generated following oxidative damage; however, it is rather difficult to measure them. Malondialdehyde (MDA), an end-product of lipid peroxidation is considered a golden rule for oxidative damage and commonly used parameter (Urso and Clarkson, 2003; Berger and Chiolero, 2007).

Synthetic or natural antioxidants are supplied in the diet of poultry to have optimal performance. Nevertheless, some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) may be dangerous for animals (Attmann et al., 1986; Powell et al., 1986). Natural herbs and spices such as rosemary (*Rosmarinus officinalis*) and oregano (*Origanum vulgare*) are well-known antioxidative substances and therefore they have been widely used as a dietary supplement in poultry nutrition. *R. officinalis* is one of the most important sources of natural antioxidants. The antioxidative activities of rosemary extracts have been reported in many studies. Moreover, it has been demonstrated that antioxidant efficiency of rosemary extracts effectively inhibited hydroperoxide formation (Frankel et al., 1996) due to high content of phenolic compounds (Leung and Foster, 1996) such as mono-terpenes (eteric olis), diterpene phenols (carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol and methyl carnosate), phenolic acids (rosmarinic acid), flavonols, and triterpene acids (ursolic acid, oleanolic acid and butilinic acid).

On the other hand, oregano (*O. vulgare* L.) is an important Mediterranean herb rich in phenolic compounds with antioxidant and antimicrobial activities (Chun et al., 2005). Origanum plants belonging to different species and ecotypes are widely used in agriculture and animal nutrition as a natural antioxidant substance. A number of studies on antioxidant activities of essential oils from various aromatic plants have reported that the oregano essential oil rich in thymol and carvacrol had a considerable antioxidant effect on the process of lard oxidation (Lagouri et al., 1993). Thus, this study was designed to determine the effects of supplementation of rosemary and oregano volatile oils in the diets on the oxidative status of blood and various organs (that is, heart, liver and spleen) in quails.

MATERIALS AND METHODS

Animals, housing and experimental treatments

A total of 880 day-old Pharaoh (*Coturnix coturnix Pharaoh*) quails, males and females, were obtained from the Uludag University Animal Health and Production, Research and Application Center of quail breeding (Bursa, Turkey). The study protocol was approved by the Ethic Committee of Uludag University. The quails were randomly allocated into one control group and three treatment groups, each containing 220 quails. Each group was randomly divided into five replicates as subgroups comprising 44 quails each. Newly hatched chicks in all groups were reared under the same growing conditions in brooding cages (colony type) in an open-sided house with mechanical ventilation. Quails were transferred randomly at the 4th week of age from the growing cages to laying cages (100 cm wide, 45 cm deep, 21 cm high in front, and 17 cm high in the rear, 112.5 cm^2 per quail) and housed until the end of the research. All chicks were brooded and reared at 28°C for the 1st week, 27°C for the 2nd week, 24°C for the 3rd week, and 18 to 21°C from the 28th day until 42 days of age.

The quails received a basal diet [maize and soya bean based; 246.9 g/kg crude protein; 12.26 MJ/kg metabolisable energy (ME)], formulated to meet the NRC (1994) requirements of all nutrients including vitamins and minerals though without the addition of antibiotics, coccidiostats or growth promoters. The content of the basal diet is presented in Table 1. Group feeding was applied in all replications. The volatile oil dosages added to the diet were chosen on the basis of information from the literatures and benefits from the effective dosage of a previous study. There were 4 treatments: control with 0 mg volatile oil/kg of diet; group I, 100 mg/kg rosemary oil plus 100 mg/kg oregano volatile oil (50: 50%); group II, 60 mg/kg rosemary volatile oil plus 140 mg/kg oregano volatile oil (30:70%); and group III, 140 mg/kg rosemary volatile oil plus 60 mg/kg oregano oil (70:30%). The diet in the form of mash and water was provided *ad libitum* during the entire experimental period (42 d). A 24-h constant lighting program was also maintained throughout the experimental period.

Determination of volatile oils in rosemary and oregano

The pure volatile oil of oregano (density: 0.959 at 20°C, gas chromatography–mass spectrometry (GC-MS) tested, origin Alanya/Turkey, NBT company, steam distillation extraction) and rosemary (density: 0.895 at 20°C, GC-MS tested, origin Mersin/Turkey, Semi Eterik Company, hydrodistillation extraction) was obtained from a commercial volatile oil company. Gas chromatography analysis was carried out on an MS-Thermo Polaris Q GC-Thermo Trace GC (Thermo Fisher Scientific, MA, USA) ultra fitted with a fused HP5-MS capillary column (Thermo Fisher Scientific, MA, USA) (30 × 0.25 × film thickness 0.5 µM). The temperature was programmed to rise from 95 to 240°C at 4°C/min. The injection was performed at 250°C in the split mode. Helium gas was used as a carrier at 1.3610 atm. The detection was performed by flame ionization detector (FID) at 250°C, and the injection volume for all samples was 0.1 µL. Chromatograms were determined using MS (mass spectrometer) or MS/MS. Data were calculated using internal standards (Pala-Paul et al., 2004).

Blood sampling and erythrocyte's hemolysate extraction

At the 42nd day of the study, 16 quails from each subgroup were randomly selected and decapitated. During the euthanasia, blood samples were collected into heparinized and non-heparinized tubes. Serum was obtained from non-heparinized tubes by centrifugation at 3000 rpm for 10 min at 4°C. Blood samples placed in heparinized tubes were centrifuged at 3000 rpm for 10 min and the supernatant plasma was removed. Saline solution was added to the remaining pellet of erythrocyte and re-centrifuged at 3000 rpm for 10 min. The supernatant was removed. This process was repeated three times. After the last application, the remaining

Table 1. Ingredients and chemical composition of the basal diet.

Ingredient	
Corn, grain	443.0
Soybean meal	360.0
Wheat	80.0
Corn gluten	50.0
Vegetable oil	30.0
$CaCO_3$	16.0
Dicalcium phosphate	6.20
Salt	3.0
L Lysine	3.50
DL Methionine	4.0
L Threonine	0.80
Vitamin mineral premix[a]	3.50
Metabolisable energy[b] (MJ/kg)	12.26
Crude protein	246.9
Crude fiber	36.0
Ether extract	50.7
Ash	73.6
Dry matter	880.3

[a]Supplied the following per kilogram of diet: 3.000.000 IU vitamin A, 1.200.000 IU vitamin D3, 0.36 g vitamin E, 1 mg vitamin K, 3 mg vitamin B1, 4 mg vitamin B2, 3 mg vitamin B6, 0.003 mg vitamin B12, 10 mg pantethonic acid, 20 mg niacin, 40 mg folic acid, 1 g choline, 0.3 mg biotin, 6 mg Cu, 300 mg I, 100 mg Fe, 0.2 mg Se, 60 mg Mn, 50 mg Zn. [b]Metabolisable energy content of diets was estimated using the equation of Carpenter and Clegg (Leeson and Summers, 2001).

erythrocyte fraction was stored at -20°C until further analysis. During the study, hemolysis was induced by the addition of 50 times of distilled water.

Homogenization of heart liver and spleen

The liver, heart and spleen were removed from quails following euthanasia and homogenized by 100 mmol/L phosphate buffer containing sodium acid in a homogenize (Ultra-Turrax T25, Germany) in an ice bath for 1 min. 10% of the obtained homogenize were sonicated for 30 s in an ice bath (Bandelin Sonoplus UW 2070, Germany). Samples were further centrifuged at 3000 rpm for 10 min at 4°C in order to obtain a supernatant. Protein content of the supernatant was determined by the method of Lowry et al. (1951).

Concentrations of serum MDA (Draper et al., 1986), NO (Miranda et al., 2001), AOA (Koracevic et al., 2001), GSH (Beutler et al., 1986), VA (Suzuki and Katoh, 1990) and VC (Kyaw, 1978), SOD in erythrocyte (Sun et al., 1988), NO (Miranda et al., 2001), SOD (Sun et al., 1988) in the heart, liver, and spleen were determined with ELISA and a spectrophotometric reader (MWGt Lambda Scan 200, Bio-Tek Instruments, USA).

Statistical analyses

Statistical analysis was performed using the SPSS (1997) software package for Windows (SPSS Inc., Chicago, IL, USA). One way analysis of variance (ANOVA) was used to evaluate the effects of essential oil combinations. Tukey's test was used as a post-hoc test. The significance level was set at $p<0.05$.

RESULTS

Chemical composition

13 components were identified in the oil of *R. officinalis* and 8 components were detected in the oil of *O. vulgare*. The most significant components of *R. officinalis* identified were 1.8–cineole (43.96%), α-pinene (25.33%) and camphene (11.09%), whereas it was carvacrol (71.2%), p-cymene (9.8%) and γ-terpinene (8.2%) for *O. vulgare* (Table 2).

Oxidative status of serum, erythrocyte packet and tissue samples

The serum MDA level was lower in the group III ($p<0.05$), whereas NO level was increased in the group I in comparison to those in control and group III ($p<0.05$). No differences were observed among the groups in terms of

Table 2. Components of the rosemary (*Rosmarinus officinalis*) and oregano (*Origanum vulgare*) volatile oils.

Component	Percentage	Component	Percentage
α-Pinene	25.33	Thymol	0.4
Camphene	11.09	Carvacrol	71.2
β-pinene	1.40	p-cymene	9.8
Limonene	1.77	γ-terpinene	8.2
D-limonene	2.5	α-Pinene	1.7
1,8-cineole (Eucalyptol)	43.96	α-terpinene	1.8
3-carene	10.7	β-Caryophyllene	1.2
Camphor	0.73	Myrecene α-phellnadrene	1.0
Ocimen	1.68		
Borneol	0.03		
Isoborneol	0.02		
Caryophyllene	0.02		
Bornyl acetate	0.04		

Table 3. MDA (nmol/ml), NO (µmol/L), AOA (nmol/ml), GSH (µmol/L), VA (µg/dl), VC (µg/dl) levels in the serum and SOD (IU/ml) level in erythrocyte in groups (mean ± standard error (SE), n:10).

	Control	Group I	Group II	Group III
Rosemary VO mg/kg	**0**	**100**	**60**	**140**
Oregano VO mg/kg	**0**	**100**	**140**	**60**
MDA	2.85 ± 0.11^{a}	2.82 ± 0.13^{a}	2.47 ± 0.09^{ab}	2.33 ± 0.12^{b}
NO	5.75 ± 0.44^{b}	8.45 ± 1.17^{a}	6.11 ± 0.45^{ab}	5.45 ± 0.43^{b}
AOA	5.56 ± 0.21	6.10 ± 0.25	5.41 ± 0.29	5.43 ± 0.24
SOD	188 ± 16.1	169 ± 15.7	220 ± 16.6	164 ± 18.6
GSH	20.8 ± 1.47	22.0 ± 2.03	20.9 ± 1.14	20.9 ± 1.55
VA	54.1 ± 9.01	46.9 ± 3.76	72.9 ± 8.17	70.6 ± 9.36
VC	1.41 ± 0.07	1.46 ± 0.08	1.37 ± 0.08	1.43 ± 0.09

MDA, Malondialdehyde; NO, nitric oxide; AOA, antioxidant activity; SOD, superoxide dismutase, GSH, glutathione (reduced form); VA, vitamin A; VC, vitamin C. a, b in the same line indicate significant differences between different letters (Tukey's test, $p<0.05$).

concentrations of AOA, GSH, VA and VC in serum and SOD in erythrocytes. The NO level of the liver in the group III and the SOD level of the spleen in the group I and group III were found to be high ($p<0.05$). Again, no differences were noted among the groups with respect to the MDA, NO and SOD levels in the liver and spleen.

DISCUSSION

The aim of the present study was to evaluate the effects of supplementation of various levels of rosemary and oregano VO mixtures to the diets of quails on the oxidative status of blood and various organs (heart, liver and spleen). The major components of rosemary and oregano oils were determined to be 1,8-cineole (43.96%), α-pinene (25.33%) and camphene (11.90%) versus carvacrol (71.2%), p-cymene (9.8%) and γ-terpinene (8.2%), respectively. Our results are consistent with other studies investigating the major components of either rosemary (Wang et al., 2008; Pintore et al., 2009) or oregano (Başer et al., 1993).

In the present study, the lowest serum MDA concentration, the end product and the most common indicator of lipid peroxidation, was seen in group III having the highest rosemary VO (140 mg/kg RVO + 60 mg/kg OVO; $p<0.05$; Table 3). Rosemary herb may exert protective effects by exhibiting antioxidant activity in the liver (Sotelo-Felix et al., 2002) and brain (Posados et al., 2009) as observed in this study. Oxidative stability may be accomplished in meat products derived from turkeys in which their diets were supplemented with rosemary leaves (Botsoglou et al., 2007). Sotelo-Felix et al. (2002) stated that this protective activity might be due to carnosol contained in the rosemary herb. Similarly, in rats it was observed that either 1,8-cineole *in vivo* or 1,8-cineole, α-pinene and β-pinene *in vitro* showed strong antioxidant activity (Wang et al., 2008). In this study, despite of the lack of carnosol in the volatile oil of *R. officinalis,* 1,8-cineole (43.96%), α-Pinene (25.33%) and β-pinene (1.40%) were present. Thus, the reason for the lowest MDA level seen in the highest rosemary VO ratio

Table 4. MDA (nmol/g protein), NO (µmol/g protein) and SOD (U/g protein) levels in the heart, liver and spleen.

	Control	Group I	Group II	Group III
Rosemary VO mg/kg	**0**	**100**	**60**	**140**
Oregano VO mg/kg	**0**	**100**	**140**	**60**
MDA				
Heart	1.69 ± 0.02	1.77 ± 0.04	1.78 ± 0.05	1.73 ± 0.04
Liver	0.45 ± 0.03	0.43 ± 0.01	0.37 ± 0.01	0.43 ± 0.02
Spleen	0.32 ± 0.01	0.31 ± 0.02	0.32 ± 0.01	0.30 ± 0.01
NO				
Heart	0.21 ± 0.01	0.22 ± 0.02	0.22 ± 0.01	0.22 ± 0.01
Liver	0.41 ± 0.01^{b}	0.53 ± 0.02^{a}	0.51 ± 0.04^{ab}	0.47 ± 0.02^{ab}
Spleen	0.30 ± 0.02	0.35 ± 0.02	0.36 ± 0.02	0.37 ± 0.01
SOD				
Heart	0.18 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
Liver	0.62 ± 0.04	0.73 ± 0.03	0.63 ± 0.03	0.64 ± 0.02
Spleen	0.22 ± 0.02^{b}	0.38 ± 0.02^{a}	0.32 ± 0.01^{ab}	0.35 ± 0.04^{a}

MDA, Malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase; a, b in the same line indicate significant differences between different letters (Tukey's test, $p<0.05$).

group may be attributed to 1,8-cineole, α-pinene and β-pinene. In other applications in this study, the MDA value was insignificant compared to the control group ($p>0.05$; Table 3).

Although oregano extracts had an antioxidant activity and could be used as a food supplement or for medical purposes (Celik et al., 2010; Lahucky et al., 2010), it showed no antioxidant activity when evaluating the MDA level in the serum and tissues such as liver, kidney and brain. In a previous study, quails given oregano (*O. majorana* × *O. vulgare*) leaves orally did not produce antioxidant activity in eggs and the liver (Handl et al., 2008). Moreover, it was stated that the antioxidant capacity of these types of herbs may show variation de0pending on the harvesting season (Ozkan et al., 2010). However, we do not agree with this statement because in our study of carvacrol, the most salient content of oregano herb showing antioxidant activity was 70%, indicating that this ineffectiveness may not be associated with the harvesting season.

NO mediates a number of physiological processes as an intracellular messenger in mammals (Bulbul et al., 2007) and poultry (Leo et al., 2008), but because it contains unoccupied/coupled electrons in its molecular orbits, it is also known as a free radical. Therefore, it can yield hazardous effects (Das and Vasudevan, 2007). The highest NO level in the serum was observed in the 100 mg/kg RVO + 100 mg/kg OVO group ($p<0.05$; Table 3). In this particular group, the undiminished MDA level may be caused by the increased NO level. Also, in this study, there were no differences in the antioxidant levels of AOA, (enzymatic and non-enzymatic) in the serum ($p>0.05$), whereas a statistically significant difference was observed in the MDA level in the serum ($p<0.05$; Table 3). Increased MDA levels with unaltered AOA levels have been reported previously (Urso and Clarkson, 2003). In the current study, the observation of no changes in the levels of antioxidants was an expected result because lipid peroxidation did not develop.

In the current study, there were significant changes in the spleen SOD activity ($p<0.05$) (Table 4), but no changes in the MDA levels were seen in the spleen and other organs studied. In studies directed to evaluate the parameters for antioxidant capacity in which only antioxidants are considered (enzymatic and non enzymatic), the results were discordant. Therefore, it is feasible to evaluate the MDA levels, which are a marker for lipid peroxidation (Konyalioglu et al., 2007; Er et al., 2010a, 2010b; Yazar et al., 2010). For this reason, by considering only SOD activity, it is difficult to comment on the antioxidant capacity of tissues. However, the observation of no significant difference in the MDA levels in tissues ($p>0.05$) may indicate that SOD activity could be in the normal range (Table 4).

Conclusion

It was concluded that supplementation of rosemary and oregano VO mixture to diets of quails may alter the antioxidant activity depending on the diets, and the most effective doses of rosemary and oregano VO mixture were found to be 70 and 30%, respectively.

ACKNOWLEDGEMENT

This study was edited by American Journal Experts (AJE).

REFERENCES

Attmann HJ, Grunov W, Mohr U, Richterreichhelm MB, Wester PW (1986). Effects of BHA and related phenols on the forestomach of rats. Food Chem. Toxicol. 24: 1183-1188.

Başer KHC, Özek T, Tümen G, Sezik E (1993). Composition essential oil of Turkish Origanum species with commercial importance. J. Essent. Oil. Res. 5: 619-623.

Berger MM, Chiolero RL (2007). Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. Crit. Care Med. 35: 584-590.

Beutler E, Gelbart T, Pegelow C (1986). Erythrocyte glutathione synthetase deficiency leads not only to glutathione but also to glutathione-S-transferase deficiency. J. Clin. Invest. 77: 38-41.

Botsoglou NA, Govaris A, Giannenas I, Botsoglou E, Papageorgiou G (2007). The incorporotion of dehydrated rosemary leaves in the rations of turkeys and their impact on the oxidative stability of the produced raw and cooked meet. Int. J. Food Sci. Nutr. 58: 312-320.

Bulbul A, Yagci A, Altunbas K, Sevimli A, Celik HA, Karadeniz A, Akdag E (2007). The role of nitric oxide in the effects of ovarian steroids on spontaneous myometrial contractility in rats, Theriogenology, 68: 1156-1168.

Bulbul A, Celik HA, Sireli M, Avci G, Civelek T (2008). Blood nitric oxide and ovarian streoids levels during the cycle stages in Brown Swiss cows. Vet. J. Ankara Univ. 55: 155-159

Celik A, Nur Herken E, Arslan I, Zafer Ozel M, Mercan N (2010). Screening of the constituents, antimicrobial and antioxidant activity of endemic *Origanum hypericifolium*. Nat. Prod. Res. 24: 1568-1577.

Chun SS, Dhiraj A, Vattem YL, Kalidas S (2005). Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. Proccess Biochem. 40: 809-816

Collins AR (2005). Assays for oxidative stres and antioxidant status: applications to research into the biological effectiveness of polyphenols. Am. J. Clin. Nutr. 81: 261-267.

Cuzzocrea S, Riley DP, Caputi AP, Salvemini D (2001). Antioxidant therapy: A new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol. Rev. 53: 135-159.

Das SK, Vasudevan DM (2007). Alcohol-induced oxidative stress. Life Sci. 81: 177-187.

Draper HH, Mcgirr LG, Hadley M (1986). The metabolism of malondialdehyde. Lipids, 21: 305-307.

Er A, Altan F, Cetin G, Uney K, Tras B, Elmas M, Yazar E (2010a). Effects of enrofloxacin, flunixin and dexamethasone on indicators of oxidative and organ damage in lipopolysaccharide-induced endotoxemia. J. Anim. Vet. Adv. 9: 1495-1500.

Er A, Ulutas E, Altan F, Cetin G, Bulbul A, Elmas M, Yazar E (2010b). Tulathromycin disturbs blood oxidative and coagulation status. Afr. J. Biotechnol. 10: 3243-3247

Frankel EN, Huang SW, Aeschbach R, Prior E (1996). Antioxidant activity of rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in water emulsion. J. Agric. Food Chem. 44: 131-135.

Handl S, Hellweg P, Khol-Parisini A, Rossmann B, Thurner K, Luf W, Novak J, Zentek J (2008). Effect of oregano (O. majorana x O. vulgare) on performance and antioxidative capacity of quails fed a diet rich in omega3 fatty acids. J. Anim. Physiol. Anim. Nut. 92: 242-245.

Konyalioglu S, Er A, Uney A, Elmas M, Yazar E (2007). Effect of flunixin meglumin on the antioxidant status in endotoxoemia. Acta. Vet. Beograd. 57: 241-246.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001). Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol. 54: 356-361.

Kyaw A (1978). A simple colorimetric method for ascorbic acid determination in blood plasma. Clin. Chim. Acta. 86: 153-157.

Lagouri V, Blekas G, Tsimidou M, Kokkini S, Boskou D (1993). Composition and antioxidant activity of essential oil from Oregano plants grown in Greece. Lebensmitt Unters. Forsch. 197: 20-23.

Lahucky R, Nuernberg K, Kovac L, Bucko O, Nuernberg G (2010). Assessment of the antioxidant potential of selected plant extracts – *in vitro* and *in vivo* experiments on pork. Meat Sci. 85: 779-784.

Lee HP, Casadesus G, Zhu X, Lee HG, Perry G, Smith MA, Gustaw-Rothenberg K, Lerner A (2009). All-trans retinoic acid as a novel therapeutic strategy for Alzheimer's disease. Expert. Rev. Neurother. 9: 1615-1621.

Leo MDM, Siddegowda YKB, Kumar D, Tandan SK, Sastry KVH, Prakash VR, Mishra SK (2008). Role of nitric oxide and carbon monoxide in N-Nitro-L-arginine methyl ester-resistant acetylcholine-induced relaxation in chicken carotid artery. Eur. J. Pharmacol. 596: 111-117.

Leung AY, Foster S (1996). Encyclopedia of common natural ingredients used in foods, drugs and cosmetics. 2nd ed. New York: JohnWiley & Sons. pp. 446-448.

Lowry OH, Rosenbrough NJ, Fair AL, Randall RJ (1951). Protein measurement with the Folin-phenol reagents. J. Biol. Chem. 193: 265-275,.

Mancini A, Festa R, Di Donna V, Leone E, Littarru GP, Silvestrini A, Meucci E, Pontecorvi A (2010). Hormones and antioxidant systems: role of pituitary and pituitary-dependent axes. J. Endocrinol. Invest. 33: 422-433.

Miranda KM, Espey MG, Wink DA (2001). A rapid, simple spectrophotometric method for detection of nitrate and nitrite. Nitric Oxide, 5: 62-71.

NRC National Research Council (1994). Nutrient requirements of poultry.9 th ed. Washinhton (DC): National Academy Press.

Ozkan G, Baydar H, Erbas S (2010). The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of Turkish oregano (*Origanum onites* L.). J. Sci. Food Agric. 90: 205-209.

Pala-Paul J, García-Jiménez R, Pérez-Alonso MJ, Velasco-Negueruela A, Sanz J (2004). Essential oil composition of the leaves and stems of Meum athamanticum Jacq., from Spain. J. Chrom. A. 1036: 245-247.

Pintore G, Marchetti M, Chessa M, Sechi B, Scanu N, Mangano G, Tirillini B (2009). *Rosmarinus officinalis* L.: chemical modifications of the essential oil and evaluation of antioxidant and antimicrobial activity. Nat. Prod. Commun. 4: 1685-1690.

Posadas SJ, Caz V, Largo C, De la Gándara B, Matallanas B, Reglero G, De Miguel E (2009). Protective effect of supercritical fluid rosemary extract, *Rosmarinus officinalis*, on antioxidants of major organs of aged rats. Exp. Gerontol. 44: 383-389

Powell CJ, Connelly JC, Jones SM, Grasso P, Bridges JW (1986). Hepatic responses to the administration of high doses of BHT to the rat: their relevance to hepatocarcinogenicity. Food Chem. Toxicol. 24: 1131-1143.

Sotelo-Felix JI, Martinez-Fong D, Muriel De la Torre P (2002). Protective effect of carnosol on CCl(4)-induced acute liver damage in rats. Eur. J. Gastroenterol. Hepatol. 14: 1001-1006.

Sun Y, Oberley LW, Li Y (1988). A simple method for clinical assay of superoxide dismutase. Clin. Chem. 34: 497-500.

Suzuki J, Katoh N (1990). A simple and cheap methods for measuring serum vitamin A in cattle using only a spectrophotometer. Nippon Juigaku Zasshi. 52: 1281-1283.

Urso ML, Clarkson PM (2003). Oxidative stress, exercise, and antioxidant supplementation, Toxicology, 189: 41-54.

Wang W, Wu N, Zu YG, Fu YJ (2008). Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem. 108: 1019-1022

Yazar E, Er A, Uney K, Bulbul A, Avcı GE, Elmas M, Tras B (2010). Effects of drugs used in endotoxic shock on oxidative stress and organ damage markers. Free Radic. Res. 44: 397-402.