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RESEARCH ARTICLE

Use of sage *(Salvia triloba L.)* and laurel *(Laurus nobilis L.)* oils in quail diets II. The effect on the oxidative status of serum and breast meat and on some serum biochemical parameters

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Bıldırcın rasyonlarında adaçayı (*Salvia triloba L.*) ve defne yağının (*Laurus nobilis L.*) kullanımı II. Serum ve göğüs eti oksidatif durum ile bazı serum biyokimyasal parametreler üzerine etkisi

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Öz

Amaç: Bu araştırmada adaçayı ve defne yağlarının farklı düzeylerde ayrı ayrı ve birlikte bıldırcın rasyonlarına ilavesinin serum ve göğüs eti oksidan-antioksidan denge ile bazı serum parametreleri üzerine etkisinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Araştırmada toplam 800 adet karışık cinsiyette Japon bıldırcını *(Coturnix coturnix japonica)* kullanıldı. Bıldırcınlar her biri 80 bıldırcından oluşturulan 10 gruba ayrıldı. Kontrol grubu temel rasyonla beslendi. Deneme grupları ise temel rasyona sırasıyla 100, 200 ve 400 mg/kg adaçayı yağı; 100, 200 ve 400 mg/kg defne yağı ile 100, 200 ve 400 mg/kg adaçayı yağı+defne yağı karışımı ilavesiyle oluşturuldu. Deneme 5 hafta sürdürüldü.

Bulgular: Serum ve göğüs eti malondialdehid düzeyinin 400 mg/kg defne grubunda azaldığı, aynı zamanda defne yağının serum ve göğüs eti malondialdehid düzeyi ile serum antioksidan aktivite üzerine lineer etki oluşturduğu görüldü. Serum trigliserit, total protein, kolesterol, alkalin fosfotaz, alanin aminotransferaz ve aspartat aminotransferaz düzeylerinin deneme gruplarında değişmediği belirlendi (P>0.05).

Öneri: Bıldırcın rasyonlarına defne yağı katılmasının serumda antioksidan etkinliği artırarak oksidatif stresi baskılayabileceği ifade edilebilir.

Anahtar kelimeler: Adaçayı yağı, defne yağı, göğüs eti, oksidatif stres, antioksidan etkinlik

Abstract

Aim: The aim of this research was to determine the effects of separate and combination use of sage and laurel oils in quail diets at different levels on serum and breast meat oxidant-antioxidant balance and on some serum biochemical parameters.

Materials and Methods: A total of 800 mixed gender Japanese quails *(Coturnix coturnix japonica)* were used in the study. The quail were divided into 10 groups each containing of 80 quails. The control group was fed with basic diet. Experimental groups were formed by adding 100, 200 and 400 mg/kg of sage oil; 100, 200 and 400 mg/kg of laurel oil, and 100, 200 and 400 mg/kg of sage oil+laurel oil mixture into the basic diet, respectively. The experiment was lasted for 5 weeks.

Results: It was observed that serum and breast meat malondialdehyde level decreased in 400 mg/kg of laurel group, and laurel oil caused a linear effect on serum and breast meat malondialdehyde level and serum antioxidant activity. Serum triglycerides, total protein, cholesterol, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase levels were determined not to change in the experimental groups (P>0.05).

Conclusion: It may be stated that the addition of laurel oil in quail diets suppressed oxidative stress by increasing serum oxidant activity.

Keywords: Sage oil, laurel oil, breast meat, oxidative stress, antioxidant activity

Introduction

Being important particularly for essential oils, aromatic plants have many families and subtypes (Bernath 2009). These plants contain rich compounds of especially polyphenols, quinones, flavonoids, alkaloids and polypeptides in their structures (Christaki et al 2012). Aromatic plants have antimicrobial (Hellander et al 1998, Dorman and Deans 2000), anticoccidial (Giannenas et al 2003), antifungal (Shin and Lim 2004), antioxidant (Montoro et al 2005, Karadaş et al 2014), antilipidemic (Craig 1999), hypocholesterolemic (Case et al 1995, Konjufca et al 1997), the digestive system stimulant (Hernandez et al 2004), performance enhancer (Çabuk et al 2006, Cross et al 2007, Biricik et al 2012) effects of which efficiency vary owing to the properties of the active substances they contain. Moreover, these plants' extracts having antioxidative effect are added to the diets of the poultry which are rich in polyunsaturated fatty acids in order to reduce oxidative disintegration (Florou-Paneri et al 2005). Such kind of an impact may vary according to the type, level and active substance compounds of the extracts as well as the plant harvest time (Economou et al 1991, Özcan and Arslan 2011, Boulanouar et al 2013). Essential oils have been reported to alter antioxidative status in some serum parameters (Botsoglou et al 2002, Bülbül et al 2012, 2014,) and have a positive influence on meat expendability, shelf life and quality by preventing lipid oxidation (Lopez-Bote et al 1998, Boutsoglou et al 2002, 2003). Among the plants, which grow in Turkey that has a lush greenfinch of aromatic plants, sage *(Salvia triloba L.)* of Lamiaceae family and laurel *(Laurus nobilis L.)* of Lauraceae family are valuable essential oil resources (Bernath 2009). These plants have been reported to have appetizing, digestive stimulating, antiseptic, antibacterial and antioxidant properties depending on the type and the level of their active substance contents (Baratta et al 1998, Christaki et al 2012). The addition of sage and laurel oils in quail diets at levels mentioned in our previous study proved not having any adverse effects on the growth performance (Bulbul et al 2015). Besides, no literature regarding the effects particularly on oxidative status resulting from separate or combination use of these oils in quails has been found. Within this context, this study was designed to evaluate the effects of separate and combination use of oils of sage and laurel, which widely grow in regions where Mediterranean climate dominate through, at different levels in quail diets on oxidant-antioxidant balance and some serum biochemical parameters.

Table 1. Chemical composition of the basal diet.

Ingredients	%
Corn	43.1
Wheat	3
Soybean meal (48%)	33
Full fat soybean	12.6
Meat and bone meal (38%)	2
Vegetable oil	4
Limestone	1
Salt	0.25
Dicalcium phosphate	0.8
Vitamin-mineral premix[1]	0.25
Chemical composition (analyzed)	
Crude protein (%)	24.28
Metabolizable energy (kcal/kg)	2934
Calcium (%)	0.85
Total phosphorus (%)	0.[3]

[1]Composition per 2.5 kg: 12.000.000 IU vitamin A, 2.400.000 IU vitamin D3, 30 g vitamin E, 2.5 g vitamin K3, 2.5 g vitamin B1, 6 g vitamin B2, 4 g vitamin B6, 20 mg vitamin B12, 25 g niacin, 8 g calcium-D-panthotenate, 1 g folic acid, 50 g vitamin C, 50 mg D-biotin, 400 g choline chloride, 1.5 g canthaxanthin, 80 g Mn, 60 g Zn, 60 g Fe, 5 g Cu, 1 g I, 0.5 g Co, 0.15 g Se.

Materials and Methods

Animals and diets

The experimental design of the study has been reported by Bulbul et al (2015). A total number of 800 mixed gender 3-day-old Japanese quails *(Coturnix coturnix japonica)* were used. They were divided into 10 groups each containing 80 chicks. Each group was divided into 4 replicates as subgroups, each comprising 20 chicks. This study was conducted at the Animal Research Center of Afyon Kocatepe University (AKU, Turkey), following the approval by AKU the Ethics Committee (AKUHADYEK-303-13). In the research, group feeding was applied to the quail grown in a cage system, and feed and water were given as ad libitum for daily consumption. During the study, the quail were exposed to 24 hours of lightening by the day light and fluorescent lamps at night time. Air conditioning was achieved through windows and fans as to provide 22-24°C of heat during the experiment. The experimental period lasted 5 weeks.

Feed raw ingredients used in the study were obtained from Tınaztepe Flour and Feed Factory (Afyonkarahisar, Turkey). The diets with corn, wheat, soybean meal, full fat soybean and meat-bone meal were prepared according to energy and nutrient requirements suggested by NRC (1994) (Table 1). The active substances and their levels in dietary sage *(Salvia triloba L.)* and laurel *(Laurus nobilis L.)* oils were determined via GC/MS method in West-Mediterranean Agricultural Research Institute, Food Medicinal and Aromatic Plants Research Laboratory provided from Talya Herbal Products Trade and Industry Limited Company (Antalya, Turkey). The research was carried out with 10 groups involving the control group with the basic diet, experimental groups with addition

Table 2. Chemical composition of sage and laurel oils.

Sage *(Salvia triloba L.)* oil	%	Laurel *(Laurus nobilis L.)* oil	%
α-Pinene	61.59	1,8-cineole	58.99
Camphene	1.32	α-pinene	2.94
Beta pinene	8.49	Beta-pinene	2.71
3-Carene	0.76	Sabinene	6.65
Beta myrcene	1.20	Limonene	1.08
Limonene	3.00	Gama-terpinene	1.02
1,8-Cineole	18.96	Cymene	3.20
Thujone	1.10	4-terpineol	2.50
Camphor	1.25	Terpinyl acetate	1.08
Caryophyllene	1.46	α-terpineol	1.39
α-Terpineol	0.87	α-terpinyl acetate	6.00
		Phellandral	1.53
		Myrtenol	1.28
		Spathulenol	2.56
		Others	5.20
		Unidentified	1.87

Table 3. Effects of sage and laurel oils on serum (nmol/L) and breast meat (mg/kg samples) MDA and serum AOA (nmol/L).

	Control	S100	S200	S400	L100	L200	L400	SL100	SL200	SL400	SEM	P
S-MDA	2.28^{ab}	2.19^{ab}	2.12^{abc}	2.25^{ab}	2.24^{ab}	1.94^{bc}	1.83^{c}	2.32^{a}	2.12^{abc}	2.01^{abc}	0.038	0.040
BM-MDA	6.16^{ab}	6.84^{a}	5.59^{ab}	6.69^{a}	5.67^{abc}	4.67^{bc}	4.44^{c}	6.41^{a}	5.44^{abc}	5.35^{abc}	0.168	0.009
S-AOA	7.043	6.41	6.72	7.51	7.26	8.26	8.53	7.23	7.27	7.76	0.166	0.058

S-MDA: Serum MDA, BM-MDA: Breast meat MDA, S-AOA: Serum AOA, $^{a, b, c}$: Different letters in the same line are statistically significant (P<0.05).

Table 4 . Effect of sage and laurel oils on some serum biochemical parameters.

	Control	S100	S200	S400	L100	L200	L400	SL100	SL200	SL400	SEM	P
ALP (IU/L)	975	930	744	746	823	857	948	797	852	734	24.03	0.207
ALT (IU/L)	7.88	6.82	6.87	6.96	6.59	6.65	7.08	6.18	6.19	7.49	0.247	0.914
AST (IU/L)	230	230	229	198	237	232	221	205	223	207	6.26	0.917
TP (g/dL)	2.96	2.77	3.09	3.52	3.11	3.28	2.95	3.27	2.66	3.05	0.076	0.378
TRG (mg/dL)	175	145	176	168	168	170	148	175	163	174	3.93	0.619
CHL (mg/dL)	325	318	335	302	343	338	344	347	328	339	4.28	0.395

ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TP: Total protein, TRG: Triglyceride, CHL: Cholesterol.

of 100, 200 and 400 mg/kg of sage oil, and 100, 200 and 400 mg/kg of laurel oil, and 50, 100 and 200 mg/kg of sage and laurel oil mixture into the basic diet.

Measurements, sample collection and laboratory analysis

The nutrient content of the basic diet used in the groups was determined according to the AOAC (2000) analysis methods.

In order to calculate metabolizable energy (ME) level, the formula by Carpenter and Clegg (Leeson and Summers 2001) was used. At the end of the research, 8 quails from each group (2 from each replicate) were slaughtered and their blood samples were kept in heparin free tubes at +4°C for 24 hours. Then, the blood serum was obtained by centrifuging at 3000 rpm for 15 minutes.

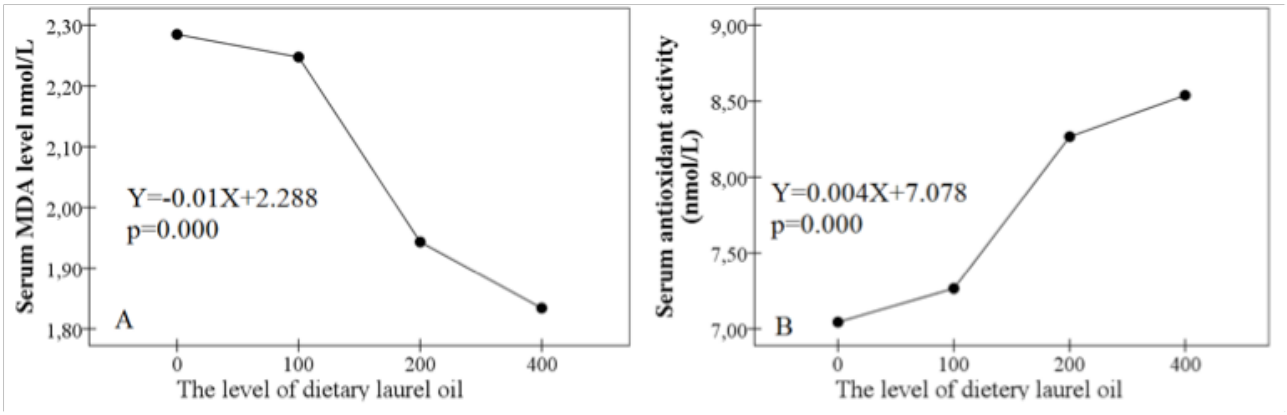

Figure 1. Linear effect of dietary sage and laurel oils on serum MDA (Figure 1A) and AOA (Figure 1B).

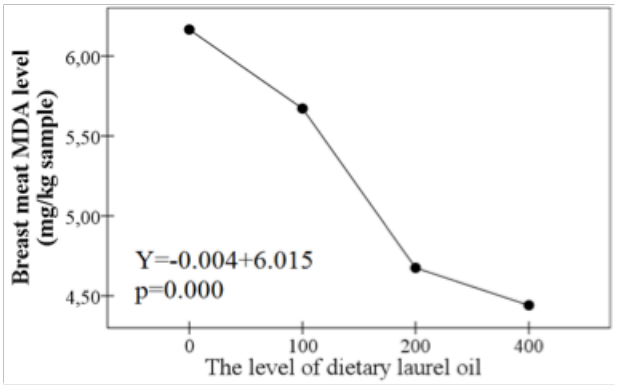

Figure 2. Linear effect of dietary sage and laurel oils on breast meat MDA.

Serums were stored in opaque eppendorfs at -18°C in order to determine triglyceride, total protein, cholesterol, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehit (MDA) and antioxidant activity (AOA) levels. At the same time, by harvesting quail serum and breast meat, serum and MDA level of serum and these tissues were determined.

Serum MDA level was determined by Draper and Hadley's (1990) double boiling method for MDA originating from free radicals. According to the method, MDA reacting with thiobarbituric acid (TBA) is measured calorimetrically at a wavelength of 532 nm. 0.5 mL of serum was mixed with 2.5 mL of 10% trichloroacetic acid in a clean screw-cap test tube and boiled at 95°C for 15 minutes. Then, it was cooled and centrifuged at 5000 rpm for 10 minutes.1 mL of resulting supernatant was isolated and by adding 0.5 mL of TBA at 0.67% it was boiled for 15 minutes and cooled down immediately. Consequently, the value of absorbance to water was determined in ELISA Reader at a wavelength of 532 nm.

The MDA level in breast meat was determined via the method by Botsoglouet al (2002). Accordingly, 1 g of the isolated sample was centrifuged in homogenizer subsequent to homogenization with 8 ml of trichloroacetic acid and 5 mL of butylated hydroxytoluene solutions. After centrifugation the supernatants were discarded and in a capped tube, 2.5 mL from lower sections were topped with 1.5 ml of TBA solution and rested in a hot bath at 100°C for 30 minutes. Having immediately been cooled, the absorbance values at 530 nm were read in a spectrophotometer. Then, standard MDA and standard curve were determined and MDA values were calculated according to these curve values.

Serum AOA level was measured calorimetrically via the method by Koracevic et al (2001). For each sample its own Fe-EDTA mixed control group was formed and H202 was added. For each set of the analysis, a negative control group was formed. For measurement, standards containing of 1 mmol/L of uric acid were used. They were incubated in water bath at 100°C for 10 minutes. After being cooled, they were read at 540 nm in ELISA Reader.

Measurement of serum triglyceride, total protein, ALP, ALT and AST levels was achieved by using commercial kits (Instrumentation Laboratory Company, Milan, Italy) in an autoanalyzer (ILab 300 Plus, Instrumentation Laboratory Company, Milan, Italy) in Selcuk University, Faculty of Veterinary Medicine, Pharmacology Department.

Statistical analyzes

The analysis of data was done by GLM procedure of the SPSS 13.0 for window version. The related values were subjected to ANOVA procedures appropriate for a completely randomized design. When differences ($P<0.05$) among means were found, means were separated using the Duncan test. The effects of increasing dietary concentrations of supplemental sage and laurel oils were partitioned into linear and nonlinear components using orthogonal polynomial contrasts.

Results

The chemical composition of sage and laurel oil used in the study are shown in Table 2. According to that, main active compound of sage *(Salvia triloba L.)* oil was found to be

α-pinene (61.59%), and 1.8 cineole (58.99%) was seen in laurel (Laurus nobilis L.) oil. Serum and breast meat MDA level decreased in the group with 400 mg/kg of dietary laurel oil (Table 3). There was no difference among the groups in terms of serum AOA, whereas the addition of laurel oil in the diet had a linear effect on serum (Figure1A) and breast meat (Figure 2) MDA levels, and serum AOA (Figure 1B). In the study, no significant differences were seen in serum cholesterol, triglycerides, total protein, ALP, ALT and AST levels occurred in any of the experimental groups which dietary sage, laurel and sage + laurel oils were used (P>0.05, Table 4).

Discussion

This study was carried out to determine the effects of separate and combination use of sage *(Salvia triloba L.)* and laurel *(Laurus nobilis L.)* oils at different levels in quail diet on serum and breast meat oxidant-antioxidant balance (MDA, AOA), as well as some serum parameters. It was seen in the study that the sage used was rich in α-pinene, and laurel oil was rich in 1.8–cineole (Table 2). Lipid oxidation is one of the most important factors in the decay of poultry feed and products. Particularly, being rich in polyunsaturated fatty acids poultry meat is vulnerable to lipid oxidation (Cortinas et al 2005). Lipid oxidation results in deterioration in organoleptic characteristics of meat, loss of nutrient value and shortening in shelf life; due to excessive oxidative degradation, whole product quality suffers and short chain aldehydes, ketones and other oxidative compounds emerge (Botsoglou et al 2002, 2003). Many studies have suggested that lipid oxidation in poultry meat (Biricik et al 2012, Yesilbag et al 2012) can be prevented by adding aromatic plant oils or extracts in diets. Thus, it was observed in this study that 400 mg/kg of laurel oil addition to the diet reduced MDA level in breast meat as well as in serum. On the other hand, it was determined that breast meat MDA level did not change in the groups with sage and sage + laurel oil. Botsoglou et al (2002) observed that oregano essential oil at levels of 50 mg/kg and 100 mg/kg did not affect broilers' performance; therefore, oregano oil did not have growth enhancing influence, yet, antioxidant effect increased depending on the rise in levels. Within this context, it has been suggested that in order to enhance antioxidant efficiency of sage and the mixture of sage and laurel oils, they ought to be added in the diet at higher levels. Furthermore, the fact that some of the findings of this research have differentiated from some literature data may spring from factors such as the race, gender, and age of the animals used, the type of the feed ingredients in diets, the composition and the levels of the plant extracts in diets. Also, bigger number of groups formed for this study might have made it difficult to notice the differences. In this research, it was determined that serum ALP, ALT and AST levels (Scholtz et al 2009), which are acknowledged as the indicator of liver damage, were not affected by separate or combination use of sage and laurel oil (P>0.05, Table 4). In their study on the quail, Bulbul et al (2014) reported that the addition of myrtle oil at 500-5000 mg/kg levels in diet did not cause a change in serum ALP, ALT and AST levels. In broilers, the addition of garlic (Dieumou et al 2009) and nigella sativa essential oil (Al-Homidan et al 2002) in the diet was reported not to affect serum ALT and AST levels. However, Kocaoglu Guclu et al (2010) reported that the use of garlic powder in the quail (2% and 4%) raised serum AST and (4%) ALT values, and that increase might have resulted from garlic powder's enhancing liver metabolism. The fact that ALP, ALT and AST values in serum have not changed in this study proves that the essential oils used in the study do not have any reverse impacts on the liver metabolism.

The finding of the study regarding total protein level which was set as another serum parameter collides with several other researchers' findings (Al-Homidan et al 2002, Kaya et al 2003) suggesting that the addition of herbal extracts in the ration has not affected total protein level. On the contrary, Kocaoglu Guclu et al (2010) reported that garlic powder at the rates of 1%, 2% and 4% increased serum total protein level. Serum total protein level was also noted to have decreased by 0.75 g/kg of herb mixture (Köksal and Küçükersan 2012) and 2% and 4% of black seed (Shewita and Taha 2011) addition to the broiler diets.

Considering the other serum parameters; cholesterol and triglycerides examined in the study, it was determined that none of the herbal extracts used at any level did not change those parameters. This finding corresponds with the other literature findings obtained from studies which various essential oils used in the quail (Tonbak and Çiftçi 2013) and broilers (Horton et al 1991, Lee et al 2010, Köksal and Küçükersan 2012) did not change serum cholesterol and triglyceride values. Furthermore, it was also acknowledged by the literature that 200 mg/kg of thymol and carvacrol (Lee et al 2003), black seed (Al-Homidan et al 2002,) and 0.1% of garlic powder (Kocaoğlu Güçlü et al 2010) addition to the diet did not influence plasma total cholesterol level in broilers.

On the other hand, many other studies have reported that the active substances in the plants lower serum cholesterol level in the quail (Kaya et al 2003, Khaksar et al 2012, Bulbul et al 2014), broilers (Konjufca et al 1997, El-Hüsseiny et al 2008) and laying chicks (Case et al 1995). Active compounds found in essential oils used in poultry nutrition shows cholesterol lowering effect. This effect is composed by the inhibition of 3-hydroxy-3metilglutaril coenzyme A (HMG-CoA) reductase which is the regulatory enzyme in the cholesterol synthesis of these compounds (Yu et al 1994, Elson 1995, Crowell, 1999, Lee et al 2010). There are also some studies reporting that 500 mg/kg of thyme oil (Bölükbaşı and Erhan 2007) addition to broiler diets have raised serum cholesterol, and thyme oil (Basmacıoglu et al 2004) at 200 ppm levels have increased both cholesterol and triglyceride values in serum.

Conclusion

The results of this study indicate that dietary laurel oil at the level of 100-400 mg/kg suppressed oxidative stress by increasing AOA in serum, whereas sage oil and the mixture of sage and laurel oils used at the same levels did not create a similar impact. It is considered to be beneficial to reassess the impacts of higher levels particularly of sage oil on similar parameters.

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