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The Effect of Thyme Essential Oil Added to Quail Diets on Antioxidative Metabolism in Breast Meat*

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Summary: This study was conducted to determine the effect of diets containing different levels of thyme essential oil (*Thymus vulgaris*) on antioxidative metabolism in breast meat of quails. A total of 200 sixteen-day-old Japanese quails *(Coturnix coturnix japonica)* were used in this study. Animals were divided into four groups with five replicates of 10 animals each; control group was fed only basal diet but 150, 300 and 450 mg/kg thyme essential oil were added to diet of groups thyme 1, thyme 2 and thyme 3, respectively. At the end of the study, ten quails were selected randomly from every group and decapitated. The analyses were made on their breast meat. In thyme groups, glutathione peroxidase (GSH-Px) activity was significantly higher than the control group (P<0.05). However, the superoxide dismutase (SOD) activity was significantly higher only in group thyme 2 (P<0.05). It was determined that the level of malondialdehyde (MDA) decreased in the thyme 1 and thyme 3 groups (P<0.05). Among all groups, catalase (CAT) activity did not differ. In conclusion, thyme essential oil had a significant effect on the antioxidant metabolism in breast meat.

Key words: Antioxidant metabolism, breast meat, quail, thyme essential oil

Bıldırcın Rasyonlarına Katılan Kekik Esansiyel Yağının Göğüs Etinde Antioksidan Metabolizma Üzerine Etkisi
Özet: Bu çalışmada, bıldırcın rasyonuna katılan farklı miktarlardaki kekik esansiyel yağının göğüs etinde antioksidan metabolizma üzerine etkisi araştırıldı. Çalışmada toplam 200 adet 16 günlük yaşta Japon bıldırcını *(Coturnix coturnix japonica)* kullanıldı. Hayvanlar, kontrol, kekik 1, kekik 2 ve kekik 3 olmak üzere dört gruba, her grup ta her birinde 10 hayvan olacak şekilde beş alt gruba ayrıldı. Gruplara sırasıyla bazal rasyona ilave olarak 0, 150, 300 ve 450 mg/kg kekik esansiyel yağı katıldı. Çalışmanın sonunda her gruptan rastgele seçilen 10 adet bıldırcın kesilerek göğüs etinde analiz yapıldı. Kekik esansiyel yağı uygulanan gruplarda Glutatyon peroksidaz (GSH-Px) aktivitesi kontrol grubuna göre önemli oranda yüksek bulundu (P<0.05). Süperoksit dismutaz (SOD) aktivitesinin sadece kekik 2 grubunda yüksek olduğu belirlendi (P<0.05). Malondialdehit (MDA) seviyesinin kekik 1 ve kekik 3 gruplarında önemli oranda azaldığı belirlendi (P<0.05). Gruplar arasında katalaz (CAT) aktivitesi yönünden fark bulunmadı. Sonuç olarak kekik esansiyel yağının göğüs etinde antioksidan metabolizma üzerine önemli etkisinin olduğu görüldü.

Anahtar kelimeler: Antioksidan metabolizma, bıldırcın, göğüs eti, kekik esansiyel yağı

Introduction

Upon surfacing the fact that synthetic additives used as feed preservation have harmful effects, natural additives have gained popularity in recent years (13). It was discovered that the essential oils and extracts of the plants prevent the microorganism that cause diseases from residing in the digestion systems in addition to increasing the effects of the digestive enzymes and strengthening the antioxidant metabolism (19). One of the above mentioned natural/herbal additives is thyme plant which and whose products can be used as feed additives in feeding animals (9,19).

Thymus vulgaris belongs to the *Lamiaceae* family and has plenty of phenolic components primarily thymol and carvacrol with antioxidant activities (8). It was revealed that these components manifest their antioxidant effect by eliminating free radicals and preventing the for-

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mation of chelates formed by metal ions (14). The study carried out showed that thyme essential oil significantly decreases lipid oxidation in meat (24). Thyme was also found out to have various biological effects such as antibacterial, anti-inflammatory and immunity booster in addition to its antioxidant effects (6).

In this study, the effects of thyme essential oil which is added to quail ration at different amounts upon the malondialdehyde (MDA) levels, a lipid peroxidation product, as well as upon catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), the antioxidant metabolism enzymes were investigated.

Table 1. Formulation and analysis of the basal diet (%)

Contents	Groups			
	Control	Thyme1	Thyme2	Thyme3
Maize	58.740	58.740	58.740	58.740
Maize gluten	20.000	20.000	20.000	20.000
Wheat bran	7.000	6.985	6.970	6.955
Soybean oil	3.720	3.720	3.720	3.720
Soybean meal	7.140	7.140	7.140	7.140
Calcium carbonate	1.230	1.230	1.230	1.230
Dicalcium phosphate	0.910	0.910	0.910	0.910
L-lysine	0.420	0.420	0.420	0.420
Sodium chloride	0.270	0.270	0.270	0.270
Vitamin-mineral premix**	0.200	0.200	0.200	0.200
Toxin binder	0.100	0.100	0.100	0.100
Coccidiostat	0.100	0.100	0.100	0.100
Sodium bicarbonate	0.090	0.090	0.090	0.090
DL-Methionine	0.050	0.050	0.050	0.050
Phyzyme XP TPT	0.030	0.030	0.030	0.030
Thyme oil*	-	0.015	0.030	0.045
Calculated nutrient contents				
Metabolisable energy (MJ/kg)	13.400	13.400	13.400	13.400
Crude protein %	21.000	21.000	21.000	21.000

*the thyme essential oil was added in place of wheat bran.
**the vitamin-mineral premix provides the following (per kg): all-rac-α-tocopherol acetate 1.25 mg; all-transretinyl acetate 1.8 mg; thiamine (thiamine mononitrate) 1.1 mg; menadione sodium bisulphate 1.1 mg; Ca-pantothenate 10 mg; vitamin B12 0.02 mg; choline chloride 175 mg; niacin 35 mg; manganese (from manganese oxide) 40 mg; vitamin B6 2.2 mg; folic acid 0.55 mg; iron (from iron sulphate) 12.5 mg; iodine (from potassium Iodide) 0.3 mg; copper (from copper sulphate) 3.5 mg; riboflavin 4.4 mg; d-biotin 0.1 mg; zinc (from zinc oxide) 25 mg; selenium (from sodium selenite) 0.15 mg.

Material and Method

Animals, trial design and feed

This study was carried out with the permission of Cumhuriyet University, Animal Experiments Local Ethics Board numbered 15 and dated 13.02.2015. The animals were hosted in Cumhuriyet University Faculty of Veterinary Medicine Department of Research and Implementation throughout their feeding trial period which lasted 35 days. Two hundred Japanese quail *(Coturnix coturnix japonica)* of 16 days old were used as the materials of the study. The study was designed to consist of four groups as control, thyme 1, thyme 2 and thyme 3. In addition to basal ration, the groups were given 0,150,300 and 450 mg/kg thyme essential oil respectively (Thyme oil®, Çiftçizade Herbal Products Ltd. Antalya/Turkey) (Table 1).

Each group was divided into five subgroups (with 10 quails in each subgroup). Each subgroup was hosted in cages with 100×45×20 cm sizes. Feed and water were provided *ad libitum*. Throughout the study animals were provided with thermal comfort (24°C) and 23 hours of

lightness and 1 hour of darkness daily. The rations of the animals were formulized in compliance with the national research council (16) recommendations and chemical analysis were carried out in line with official methods of analysis (3).

Biochemical analysis

At the end of the experiment (age 51 days), 10 randomly selected quails from each group were cut in the appropriate medium and the breast meats was removed. The breast meats were homogenized using liquid nitrogen and homogenization buffer was added after the liquid nitrogen flow was expected. The samples were homogenized via a tissue homogenizer (Bioprep24, Allsheng). The homogenized samples were centrifuged prior to 2500 g for 10 min +4°C and then for 12 min at 20.000 g +4°C of supernatant. The supernatant was then stored at -80°C until the day of the biochemical analysis.

1. Determination of lipid peroxidation (LP) by MDA

For measuring MDA levels, the method put forward as described (25) was used. Dilutions in amount of 10,5,2.5,1.25 and 0.625 µmol/L were prepared out of 20 µmol/L standard solution. Samples amount of 0.5 mL were put into test tubes while 0.5 standard dilutions were put into standard tubes. Amount of 3 mL of TCA of 20% was added into blank tube while for other tubes this amount was 2.5 mL. Upon adding 1 ml of TBA into each tubes, the tubes were closed and incubated for 30 minutes in water baths of 95°C. After the incubation all the tubes were cooled in ice bath and then centrifuged at 3000 rpm for 10 minutes with 4 ml n-butanol added into each. N-butanol layer absorbance was seen in 535 nm.

2. Determination of SOD activity

The method developed as described was used for the determination of superoxide dismutase (SOD) activity in the meats taken from the quails (23). The principle of the method, is to compose formazan by reducing the colorants, such as nitro blue tetrazolium (NBT), that are formed during the oxidation of xanthine oxidase (XO) with xanthine. At the presence of SOD, which allows the dismutation of superoxide anion's to H_2O_2 by consuming the O_2 produced by XO reaction, the amount of O_2 that would react with NBT will decrease and the formation of formazan would be prevented. SOD activity of a reaction catalyzed by XO was measured depending on the degree of inhibition of NBT reduction. The formed NBT is based on the spectrophotometric measure of the absorbance of the color at 560 nm.

3. Determination of CAT activity

Catalase activity was calculated using method as described (1). Amount of 50 mM phosphate buffer with pH 7.0 was prepared; also 30 mM hydrogen peroxide solution was prepared. And then 1mL H_2O_2 was added to 2 mL sample whereas 1mL phosphate buffer was added to the formed solution and measurements were made at 230 nm.

4. Determination of GSH-Px activity

Glutathione is a chemical substance with tripeptide structure and it is mostly found in animals. Glutathione is an important molecule that acts as an electron donor for enzyme glutathione peroxidase, which is used in the detoxification of harmful molecules in biochemical metabolism. Catalytic activity detection was performed according to the method (17). The decrease of NADPH's absorbance was monitored at 340 nm and determined kinetically.

Statistical analysis

The output data were evaluated by using SPSS 20 statistical package program (22). In order to identify whether there is a statistical difference or not between antioxidant parameter data, one-way analysis of variance (ANOVA) was made. For paired comparisons Duncan's multiple range test was applied ($P<0.05$).

Results

The analyses were made on their breast meat. In thyme groups, GSH-Px activity was significantly higher than the group of control ($P<0.05$). However, the SOD activity was significantly higher in only group thyme 2 ($P<0.05$). It was determined that the level of MDA decreased in the thyme 1 and thyme 3 groups ($P<0.05$). The all groups did not differ for CAT activity (Table 2).

Discussion and Conclusion

So far synthetic antioxidants have been used as preservers in feed industry; yet with the recent developments showing the harmful effects of those, natural agents/products have started to appear on shelves. It was stated that there is a positive relation between the phenolic compounds within the plants used for this purpose and antioxidant activity and that polyphenol compounds that thyme plant includes prevent the formation of free radicals (24).

Superoxide radical is one of the free radicals mostly responsible from the oxidative damage

Table 2. The effects of thyme essential oil in quail ration on antioxidant metabolism in breast meat

Parameters	Groups				p Value
	Control	**Thyme 1**	**Thyme 2**	**Thyme 3**	
SOD, U/g pro	69.309±2.71^{b}	73.182±1.24^{ab}	77.227±1.82^{a}	72.346±1.84^{ab}	0.032
GSH-Px, U/g pro	40.318±11.76^{b}	120.079±25.67^{a}	161.111±33.22^{a}	171.27±33.23^{a}	0.021
CAT, U/mg pro	0.066±0.01	0.059±0.02	0.040±0.01	0.058±0.02	0.642
MDA nmol/g	12.69±2.27^{a}	6.90±0.68^{b}	8.22±1.97^{ab}	5.87±0.82^{b}	0.030

All values are given as mean ± standard error of mean (SEM), ns: not significant (p>0.05).
$^{a, b}$: A letter in the same line means significantly different (*: p<0.05),
Control: basal ration alone, thyme1: basal ration+150 mg/kg of thyme essential oil, thyme 2: basal ration+300 mg/kg of thyme essential oil, thyme 3: basal ration+450 mg/kg of thyme essential oil. CAT; catalase, SOD; superoxide dismutase, GSH-Px; glutathione peroxidase, MDA; malondialdehyde

and SOD enzyme catalyzes this radical to convert into hydrogen peroxide and molecular oxygen (7). It was highlighted that under various stress conditions radical formation is accelerated and thus SOD activity might be more effective in fending these radicals off the environment (4,21). This study found out that the thyme essential oil of 300 mg/kg added to the ration increased SOD activity, while other dosages did not have any such effect. Similarly, it was also stated that thymol+carvacrol in 60, 100 and 200 mg/kg dosages did not have any effect upon the SOD activity in thigh and breast meat (12). Gümüş et al. (10) reported that essential oil at doses of 100, 300 and 450 mg/kg increased the activity of SOD in quail the liver, but not for serum levels (10). In this study, only 300 mg/kg dose significantly increased SOD activity and but doses of 150 and 450 mg/kg because of like low dose of thyme essential oil to high level of essential oil did not have the positive effect.

Glutathione, consisting an important part of the antioxidant defense system is the substrate of the GSH-Px enzyme and the changes in GSH level under oxidative stress were suggested to have an effect upon this enzyme (11). The studies carried out on broiler revealed that thymol and carvacrol positively affect the GSH-Px activity in serum under oxidative stress (20). This study also put forward that 150, 300 and 450 mg/kg thyme essential oil significantly increased the activity of GSH-Px enzyme in breast meat when compared to the control group. In addition to these results, 200 and 400 mg/kg thymol added onto broiler rations were discovered to raise GSH levels both in thigh and breast meats significantly (26). In their studies on broiler Hashemipour et al. (12) also reported that thymol+carvacrol added into ration increased GSH-Px activity in thigh meat yet had no effect upon breast meat (12).

Lipid peroxidation is a reaction process which starts with the oxidation of poly unsaturated fatty acids with radicals and continues with autocatalytic chain reactions causing overall damage in most biological structures (5). One of the most outstanding results of this study is that in thyme1 and thyme2 groups intervened with thyme essential oil, MDA level which is a marker of lipid peroxidation significantly decreased when compared with the control group. Gümüş et al (9) reported that oregano essential oil at doses of 200 and 400 mg/kg significantly reduced the level of LPO in the muscles but only lowered the level of LPO 200 mg/kg in serum levels and that 400 mg/ kg dose didn't differ of LPO. Similar to these results, in this study it can be said that statistically not affecting the MDA level of 300 mg/kg thyme essential oil dose although in a numerical lower. It may be due to the fact that there is no linear ratio between the effect and doses of thyme essential oil applied. Another study (26) affirmed that 200 and 400 mg/kg thymol added to the ration decreased the MDA levels significantly in breast and thigh meats. Similarly, thyme powder of 0.5% dosage added to the broiler ration was found to decrease the MDA level in thigh meat significantly (18). On the other hand, it was also found out that thymol+carvacrol of 60,100 and 200mg/kg added to the broiler ration did not have any effect upon the MDA levels in thigh and breast meats (12); for rabbits 3% of thyme powder added to the ration did not have any effect upon the MDA levels in plasma (15).

Catalase is an enzyme which can be found in all live cell types at different concentrations with antioxidant effect and it breaks the hydrogen peroxide of free radicals down into molecular oxygen and water (2). Studies carried out on

lambs proved that oregano essential oil added to the ration significantly increased CAT activity in liver and meat tissues (9). Contrary to these findings, this study found no effect of thyme essential oil added to the ration upon CAT enzyme activity.

To conclude, thyme essential oil added to the quail ration was shown to have a clear effect upon GSH-Px enzyme which has an active role on the metabolism and to have a limited effect upon SOD while having no effect upon CAT activity at all. Thyme essential oil was also seen to have effects upon antioxidant enzymes and significantly decreased MDA level, marker of the oxidation in the meats. The results of this study can contribute to further studies in showing that thyme essential oil has clear effects upon antioxidant metabolism.

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