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Antioxidant Enzyme Activity and Meat Quality of Meat Type Ducks Fed with Dried Oregano (*Origanum vulgare* L.) Powder

J. H. Park, S. N. Kang[1], D. Shin[2], and K. S. Shim[3,*]
Department of Animal Resource and Science, Dankook University, Cheonan 330-714, Korea

ABSTRACT: One-day-old Cherry valley meat-strain ducks were used to investigate the effect of supplemental dried oregano powder (DOP) in feed on the productivity, antioxidant enzyme activity, and breast meat quality. One hundred sixty five ducks were assigned to 5 dietary treatments for 42 days. The dietary treatment groups were control group (CON; no antibiotic, no DOP), antibiotic group (ANT; CON+0.1% Patrol), 0.1% DOP (CON+0.1% DOP), 0.5% DOP (CON+0.5% DOP), and 1.0% DOP (CON+1.0% DOP). Upon feeding, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of oregano extracts was higher than that of tocopherol, although it was less than that of ascorbic acid. As a result of *in vivo* study, DOP in the diet showed no effects on final body weight, feed intake, or feed conversion ratio. However, dietary 0.5% and 1% DOP supplementation caused a significant increase in the serum enzyme activity of superoxide dismutase (SOD) compared with CON and ANT, while glutathione peroxidase (GPx) in tissue was increased as compared to ANT ($p<0.05$). Cooking loss from ducks fed with DOP decreased compared with the control ducks. Thiobarbituric acid reactive substance (TBARS) values of duck breast meat at 5 d post slaughter was found to be significantly reduced in ducks whose diets were supplemented with 0.5% and 1% DOP ($p<0.05$). These results suggest that diets containing 0.5% and 1% DOP may beneficially affect antioxidant enzyme activity of GPx and SOD, improve meat cooking loss, and reduce TBARS values in breast meat at 5 d of storage in ducks. (**Key Words:** Antioxidant Enzyme Activity, Duck, Meat Quality, Oregano, Phenolic Compound)

INTRODUCTION

In recent years, there has been a growing interest in isolating antioxidants from plant ingredients and using them in animal nutrition with the intention of replacing antibiotics. Many studies have been exploring the additive effects of herb materials such as oregano, rosemary, thyme, sage, basil, and mint as growth promoters, antimicrobial agents, or natural antioxidants in poultry production (Botsoglou et al., 2003; Guo et al., 2004; Hernandez et al., 2004). And these plant extracts have shown potential as possible alternatives to antibiotics, as well as showing growth-promoting effects which are on par with antibiotics (Hernandez et al., 2004; Cross et al., 2007; Windisch et al., 2008). Many herbal products are already utilized in commercial applications. Oregano (*Origanum vulgare* L.) is one of the many herb extracts is used as an additive to poultry diets. Oregano is an aromatic plant, containing more than 30 mainly phenolic antioxidants constituents, with anti-microbial and anti-inflammatory activity (Alma et al., 2003). Oregano supplementation has been shown to have a beneficial effect on productivity, mortality, modulation of gastrointestinal microflola, pathogen inhibition, and immune system stimulation in poultry.

Although, modern strains of ducks have been intensively selected for growth and feed efficiency, modern ducks exhibit excessive body fat. Moreover, duck meat, characterized by a high proportion of unsaturated fat content, is particularly susceptible to oxidation causing rancidity and deterioration in flavor and color (Suryanti et al., 2014). That is one of the main problems encountered by

* Corresponding Author: K. S. Shim. Tel: +82-63-270-2609, Fax: +82-63-270-2614, E-mail: ksshim@jbnu.ac.kr
[1] Department of Animal Resources, Daegu University, Gyeongsan 712-714, Korea.
[2] Department of Food and Nutrition, Hallym University, Chunchoen 200-702, Korea.
[3] Department of Animal Biotechnology, Chonbuk National University, Jeonju 561-756, Korea.
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both duck producers and consumers today, because it can cause great economic loss because of shortened storage life of meats. In order to improve the stability of meat-derived product, oregano must be considered an important candidate with well-known biological and antioxidant effects. Considering that, until now, there are few studies on ducks, the influence of dietary oregano on live performance, oxidative lipid stability, and on the quality of breast meat was investigated in ducks.

MATERIAL AND METHODS

Extraction of samples

For 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, phenolic and flavonoid content in dried oregano powder (DOP), 40 g of dried sample were extracted with 400 mL of distilled water at 85℃ in reflux for 3 h to obtain an initial extract (fraction I). The residues were extracted with 400 mL of distilled water at 85℃ for 2 h to obtain fraction II. After cooling to room temperature and then filtering (Whatman No. 2 filter paper), the two fractions were combined and dried under vacuum below 40℃. The extracts were completely dried in a freeze-drier and stored at –20℃ until further use.

Measurement of total phenolic content

Total phenolic contents of the extract were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). A 150 mL of sample at a concentration of 1 mg/mL, 2.4 L of deionized water, and 150 mL of 0.25 N Folin-Ciocalteu reagents were combined in a plastic vial and then 300 mL of 1 N Na_2CO_3 solution was added. The solution was incubated in a dark place for 2 h and the absorbance was measured at 725 nm using a spectrophotometer (Hewlett Packard 8452A, Diode Array, Santa Clara, CA, USA). Total phenolic content of the extract were expressed as microgram catechin equivalents (CE)/mg.

Measurement of total flavonoids

Total flavonoid content was determined by using the modified method described by Meda et al. (2005). In brief, 0.25 mL of sample (1 mg/mL) was added to a tube containing 1 mL of double-distilled water. Next, 0.075 mL of 5% $NaNO_2$, 0.075 mL of 10% $AlCl_3$ and 0.5 mL of 1 M NaOH were added sequentially. Finally, the volume of the reacting solution was adjusted to 2.5 mL with double-distilled water. The absorbance of the solution at a wavelength of 410 nm was detected using the Ultrospec 2100 pro UV-visible spectrophotometer. Quercetin was used as standard to quantify the total flavonoid content of the extract. Results were expressed in microgram quercetin equivalents (QE)/mg.

Measurement of free radical scavenging activity on 1,1-diphenyl-2-picryl-hydrazyl assay

The DPPH free radical scavenging activity of samples was determined according to the modified method of Brand-Williams et al. (1995). L-ascorbic acid and tocopherol were used as a positive control, and then diluted to obtain five different concentrations. A quantity of each sample (10 μL) and standards were mixed with DPPH solution (100 μL, 0.2 mM DPPH in dimethyl sulfoxide. The mixture was then incubated at 37℃ for 30 min. The absorbance was measured by a spectrophotometer (Ultrospec 2100 pro UV-visible spectrophotometer; Amersham Pharmacia Biotech Co., Piscataway, NJ, USA) at 517 nm. The inhibition percentage was calculated from the following equation: Inhibition % = ([absorbance of control – absorbance of sample])/absorbance of control) ×100.

Preparation of oregano powder

For feeding the ducks, Oregano (*Origanum vulgare* L.), which was cultivated in Korea, was obtained from Namwon Herb Food Cluster Business Association (Namwon, Korea). Leaves and stems were air dried at room temperature in the shade for a month, and then were powdered with a mill (IKA M 20, IKA, Staufen, Germany). The powdered oregano was stored at 4℃ until further use.

Experimental design

All animal managements and experimental procedures were approved by the University Institutional Animal Care and Use Committee.

A feeding study with 165 one-day-old Cherry Valley meat-strain ducks (body weight 288±0.5 g) was conducted in floor fens for 42 days. The ducks were individually weighed and were assigned to 5 treatments (33 ducks/treatment) with 3 replicates (11 ducks/pen) each based on body weights. Environmental temperature was gradually reduced from 35℃ on day 1, to 22℃ until the end of experiment and 24 h consistent light was provided. During the entire trial, the experimental diets and fresh water were offered *ad libitum* daily. The experimental diet was typically of corn-soybean meal, and the feeding program consisted of two phases. During the first 21 d, ducks were fed the crumble starter diet [metabolizable energy (ME), 2,950 kcal/kg; crude protein (CP), 20%] and fed a finisher diet from 22 to 42 d (ME, 3,100 kcal/kg; (CP), 17%) pelleted with a 4-mm die. The calculated nutrient concentrations of the experimental diet and analyzed nutrients concentrations of oregano are listed in Table 1 and 2, respectively. Dietary treatments in the experiment

Table 1. Calculated chemical composition of experimental diet (as-fed basis)

	Starter[1]	Finisher
Calculated composition		
ME (kcal/kg)	2,950	3,100
CP (%)	20.0	17.0
Methionine (%)	0.40	0.30
Lysine (%)	0.90	0.65
Ca (%)	0.8	0.8
Total P (%)	1.0	1.0
Crude fat (%)	2.5	2.5
Crude fiber (%)	5.5	5.5
Ash (%)	8.0	8.0

ME, metabolizable energy; CP, crude protein.
[1] Stater diet (Crumble) provided during d 1 to 21; finisher diet (pellet) provided during d 22 to 42.

included an unsupplemented diet (CON; no antibiotic, no DOP), a diet supplemented with 0.1% Patrol (ANT; antibiotic), and 3 DOP treatment groups, with varying amounts of DOP. The DOP treatment groups consisted of 0.1% DOP, 0.5% DOP, and 1.0% DOP-fed groups, respectively.

Chemical composition of dried oregano powder

Moisture, crude protein, crude fat, crude ash, and crude fiber contents of DOP were determined according to AOAC (1995) methods. Calcium, phosphorus, and iron contents were determined by inductively coupled plasma (ICP)-atomic emission spectroscopy (ICPS-7510, Shimadzu, Japan). Gross energy was measured using a bomb calorimeter (PARR 1351, USA).

Growth performance and sampling

Body weight and feed intake per pen were recorded at the end of the experiment (d 42), and feed conversion ratio was calculated. At the end of the feeding period, blood samples of 9 ducks from each treatment (3 ducks/pen) were randomly collected from the wing vein, and breast meat samples of same ducks were also taken to determine the activities of 2 antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx). Blood samples for enzyme determinations were placed in serum separator tubes (*BD Vacutainer*) and were centrifuged at 3,000 rpm for 15 min. The serum was stored at –20℃ until used. Breast muscles were washed in phosphate buffer, pH 7.4. Then, the tissue (400 mg) was homogenized in 1 mL/gm cold phosphate-buffered saline (PBS)/ ethylenediaminetetraacetic acid (EDTA) buffer (PBS, 100 mg EDTA, pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min at 4℃. The supernatant was used for the estimation of antioxidant enzymes.

Table 2. Chemical composition of dried oregano powder

Chemical composition	Dried oregano powder
Moisture (%)	10.5 ±0.28
Gross energy (kcal/kg)	4,192 ±62.23
Crude protein (%)	15.2 ±0.42
Crude fat (%)	2.0 ±0.14
Crude fiber (%)	14.4 ±0.42
Ca (mg/kg)	8,028 ±69.30
P (mg/kg)	2,772 ±11.31
Fe (mg/kg)	113.3 ±4.53

Values are represented as mean±standard deviation (n = 3).

Superoxide dismutase, glutathione peroxidase, and protein assay

The activities of SOD and GPx in breast muscle and serum were measured using a commercial kit from Enzo Life Sciences Company (Ann Arbor MI, USA) according to the instructions of the manufacturer. The protein concentrations in breast muscle were quantitated by the bicinchonibate (BCA) method, using a protein assay kit (Bio-Rad, Hercules, CA, USA).

Physicochemical properties of raw duck breast meat

At the end of the experiment, 9 ducks (3 ducks/pen) of similar body weight from each group were killed, and breast meat samples of each individual duck was taken, after completion of bleeding from the jugular vein. The physiochemical qualities of the breast meat were analyzed. The pH values of raw breast meat were measured 24 h post-slaughter, using a digital pH meter (8603, Metrohm, Swiss) after blending 10 g of finely homogenized sample (3 mm) with 90 mL of double-distilled water. For cooking loss estimation, raw meat samples (3×5×8 cm) were placed inside plastic bag after weighing and were then cooked in a water bath at 80℃ for 1 h. The samples were cooled at 4℃ temperature for 2 h and weighed again. Cooking losses were calculated based on the difference in weight between the initial raw and cooked samples. The shear force of breast meats was estimated with an Instron 3343 (US/MX50, A&D Co., Norwood, MA, USA) attached to a Warner Bratzler shearing device. Cores (2×2×1 cm) of each meat were analyzed at room temperature with a crosshead speed of 100 mm/min. The average shear force value was calculated for each treatment and was expressed as kg/cm^2. For measurement of 2-thiobarbituric acid reactive substances (TBARS), breast meats were sliced in 1.5 cm thickness, and packed in a polyethylene tray covered with low-density polyethylene film. The amount of TBARS was measured by the modified method of Buege and Aust (1978). Five grams of sample was homogenized with 15 mL of distilled water using a homogenizer (IKA model T-25 Basic, Selangor, Malaysia) for 10 s, after which 2 mL of homogenate was placed in a test tube, and 2 mL of the thiobarbituric acid (TBA)/trichloroacetic acid (TCA)

Table 3. Total phenolic and flavonoid content in water extracts of oregano

Oregano extract	Contents
Total phenolic content (μg CE/mg extract)	21.29±0.84
Total flavonoid content (μg QE/mg extract)	730.36±2.35

CE, catechin equivalents; QE, quercetin equivalents.
Values are represented as mean±standard deviation (n = 6).

reagent (0.72% TBA, 37.5% TCA) was added and mixed with the homogenate. The mixture was then incubated in a 95℃ water bath for 15 min, and then centrifuged for 10 min at 3,000 rpm. The absorbance values of the each solution and reagent blank were measured at 531 nm. The TBARS of the samples were determined at 1, 5, and 10 days of storage (4℃), respectively.

Statistical analysis

The pen was the experimental unit for growth performance; whereas, each duck was the experimental unit for antioxidant enzyme activity, and physicochemical properties. Data were statistically analyzed using the general linear model procedure of the SAS program (SAS, 2002). Tukey's test was performed to detect the significance of differences among groups, and the difference of TBARS values was determined every storage days. The statistical difference of the values was expressed at $p<0.05$ and results are expressed as means±standard error of the mean.

RESULT

The DOP contained around 4,192±62.23 kcal/kg of gross energy. Moisture, crude protein, crude fat, crude fiber, Ca, P, and Fe contents in DOP were 10.5±0.28%, 15.2±0.42%, 2.0±0.14%, 14.4±0.42%, 8,028±69.30 mg/kg, 2,772±11.31 mg/kg and 113.3±4.53 mg/kg, respectively (Table 2).

The total phenolic and flavonoids contents of the oregano extract were determined as 21.29±0.84 μg CE/mg and 730.06±2.35 μg QE/mg extract, respectively (Table 3).

The DPPH radical scavenging activity of oregano extract at the concentration of 1,000 μg/mL was 28.68±2.32%, which was lower than that of ascorbic acid (95.27±1.57%), but was higher than that of tocopherol (17.19±0.44) (Table 4).

Table 4. DPPH radical scavenging activities (%) of dried oregano extracts

Concentration (μg/mL)	Oregano	Ascorbic acid	Tocopherol
10	6.65±0.52	94.61±1.21	-
50	5.52±0.88	95.19±1.04	-
100	9.52±0.97	95.21±0.89	-
500	17.21±1.58	94.80±1.08	2.86±0.15
1,000	28.68±2.32	95.27±1.57	17.19±0.44

DPPH, 1,1-diphenyl-2-picryl-hydrazyl.
Values are represented as mean±standard deviation (n = 6).

Ducks fed diets supplemented with DOP for 42 days showed no significant difference in weight gain, feed intake, and feed conversion compared with the control groups (Table 5).

As shown in Table 6, ducks fed with 0.5% and 1% DOP showed higher GPx activity in breast tissue than that of ANT treatment. In addition, the addition of 0.5% and 1% DOP increased the serum SOD activity compared with CON and ANT treatments.

The pH and shear force values were not different among treatments, but cooking loss was lower in ducks fed with DOP diets compared to ducks fed with control diets. Furthermore, the TBARS values at 5 d post slaughter were found to be decreased in 0.5% and 1% DOP treatments compared with control groups (Table 7).

DISCUSSION

Herb plants are rich source of a many biologically active substances and medicinal components (Park et al., 2014). Among these substances, phenolic acids and flavonoids particularly, have been shown to have various physiological and biochemical functions in the body. We confirmed that oregano also contained total phenolic and flavonoid concentration of 21.39 μg CE/mg extract and 730.36 μg QE/mg extract (Table 2). Phenolic compounds,

Table 5. Effect of dietary dried oregano powder (DOP) supplementation on growth performance in meat ducks[1]

Treatment	CON[2]	ANT[2]	0.1% DOP[2]	0.5% DOP[2]	1% DOP[2]	SEM	p-value
Initial BW (g)	285	287	288	289	290	2.92	0.987
Final BW (g)	2,994	3,084	2,973	3,032	2,964	26.71	0.585
Weight gain (g)	2,715	2,796	2,682	2,746	2,678	26.92	0.597
Feed intake (g)	7,558	7,422	7,200	7,071	6,644	164.0	0.497
Feed conversion ratio	2.79	2.66	2.71	2.59	2.54	0.07	0.881

BW, body weight; SEM, standard error of means.
[1] Each mean was calculated 3 pens per treatment (n = 3).
[2] CON, basal diet (no antibiotic, no dried oregano powder); ANT, CON+0.1% antibiotic (Patrol) diet; 0.1% DOP, CON+0.1% dried oregano powder; 0.5% DOP, CON+0.5% dried oregano powder; 0.1% DOP, CON+1% dried oregano powder.

Table 6. Effect of dietary dried oregano powder (DOP) supplementation on superoxide dismutase (SOD) and glutathione peroxidase (GPx) in meat ducks[1]

Treatment	CON[2]	ANT[2]	0.1% DOP[2]	0.5% DOP[2]	1% DOP[2]	SEM	p-value
GPx							
Tissue (U/mg of protein)	1.47[ab]	1.28[b]	1.46[ab]	1.62[a]	1.74[a]	0.05	0.022
Serum (U/mL)	6.26	7.16	6.51	7.68	7.51	0.28	0.451
SOD							
Tissue (10^3 U/mg of protein)	7.13	7.19	13.05	6.97	11.94	1.01	0.202
Serum (10^8 U/mL)	1.27[b]	1.38[b]	2.68[b]	3.84[a]	3.52[a]	0.26	0.001

SEM, standard error of means.
[1] Each mean represented by 9 ducks per treatment (n = 9).
[2] CON, basal diet (no antibiotic, no dried oregano powder); ANT, CON+0.1% antibiotic (Patrol) diet; 0.1% DOP, CON+0.1% dried oregano powder; 0.5% DOP, CON+0.5% dried oregano powder; 0.1% DOP, CON+1% dried oregano powder.

including flavonoids, are important plant constituents because the phenolic content of plants may relate directly to the antioxidant activities (Kahkonen et al., 1999) and herb extracts have confirmed to possess a high linear relationship between the phenol concentration and antioxidant activity (Zheng and Wang, 2001; Cai et al., 2004; Kim et al., 2011). The DPPH radical scavenging activity assay is one of the most widely used method for rapid screening the antioxidant activity of plant extract. Sokmen et al. (2004) reported that DPPH radical scavenging activity of oregano extracts are on par with those of antioxidant butylated hydroxytoluene. In our study, oregano extracted exhibits more potent DPPH radical scavenging activities than tocopherol, though the values were less than that of ascorbic acid. Therefore, we hypothesize that the supplementation of DOP into duck diets would result in positive effects in growth performance, antioxidant enzyme activity, and physicochemical properties of the breast meat of meat ducks.

However, in our *in vivo* study, the growth performance of ducks fed the DOP did not reveal a significant difference, and previous reports investigating the effect of oregano on the performance parameters are inconsistent among researchers (Lewis et al., 2003; Hernandez et al., 2004; Bampidis et al., 2005; Cross et al., 2007). Despite the existence of some phytochemicals as growth promoters in herb plant, several authors reported no positive effects on performance parameters in poultry (Lewis et al., 2003; Cross et al., 2007). On the other hand, several authors found that herbs and essential oil extracted from herbs did exhibit significant effects on the performance of poultry (Hernandez et al., 2004; Bampidis et al., 2005). These contradictory results can be explained because healthy and well nourished birds reared in clean, ideal environmental condition, often may not respond to growth promoting supplements. This was confirmed by broilers that were challenged with coccidiosis which showed improved growth performance while being fed herb extracts (Arczewska-Wlosek and Swiatkiewicz, 2013). In the present study, the ducks were raised in clean condition; growth promoting agents such as oregano might have more effect when animals are housed under poor environmental or infectious conditions.

Antioxidant enzymes such as SOD and GPx are the first line defense antioxidants (Ray and Husain, 2002). In the present study, an increase in these GPx and SOD enzymes were observed in DOP treatments. Changes in these enzymes could be attributed to the presence of phenolic compounds, rich in terpenoids, such as carvacrol, thymol, and rosmarinic acid, in the oregano plant (Shan et al., 2005). The substances have strong antioxidant properties, which could protect organisms against oxidative stress. In the present study, similar to the *in vitro* DPPH radical scavenging activity of oregano extract, *in vivo* study

Table 7. Effect of dietary dried oregano powder (DOP) supplementation on meat quality in meat ducks[1]

Treatment	CON[2]	ANT[2]	0.1% DOP[2]	0.5% DOP[2]	1% DOP[2]	SEM	p-value
pH	5.96	5.90	5.92	5.95	5.96	0.01	0.309
Cooking loss (%)	15.17[a]	9.79[b]	7.71[b]	7.76[b]	8.35[b]	0.57	0.001
Shear force (kg/cm^2)	2.82	2.79	2.37	2.54	2.60	0.05	0.063
TBARS (mg/kg)							
1 d	0.12	0.12	0.13	0.11	0.11	0.01	0.479
5 d	0.29[a]	0.29[ab]	0.29[ab]	0.27[b]	0.22[b]	0.01	0.025
10 d	0.31	0.23	0.24	0.14	0.20	0.02	0.224

SEM, standard error of means; TBARS, thiobarbituric acid reactive substance.
[1] Each mean represented by 9 ducks per treatment (n = 9).
[2] CON, basal diet (no antibiotic, no dried oregano powder); ANT, CON+0.1% antibiotic (Patrol) diet; 0.1% DOP, CON+0.1% dried oregano powder; 0.5% DOP, CON+0.5% dried oregano powder; 0.1% DOP, CON+1% dried oregano powder.

showed that DOP also possess a significant radical scavenging activity and have a potent antioxidant capacity. The finding of the present study is in agreement with those of other studies (Song et al., 2010; Yao et al., 2010), which have reported a significant relationship between phenolic content and antioxidant enzyme activity. Therefore, the higher concentrations of SOD and GPx, due to the addition of DOP, may provide a more efficient scavenging of free reactive radicals in ducks.

Cooking loss is known to be one of a main factor affecting meat quality, because some nutrients may be lost in the exudates by water loss, and affect juiciness and tenderness. In our study, the decrease in the cooking loss of breast meat of ducks fed with the DOP was statistically significant, though the detailed reasons for this are unknown. Therefore, further study is needed, to determine the systemic estimation of the association between DOP and the meat quality of ducks. However, Symeon et al. (2009) reported that dietary supplementation of oregano essential oil decreased the cooking loss of broilers, and Kołodziej-Skalska et al. (2011) reported that dietary plant extracts mixture (5.4% (wt/wt) *Origanum* spp.+3.2% *Cinnamomum* spp.+2.2% *Capsicum annum*) decreased the cooking loss and increased the water holding capacity of pork. The findings of our study support these studies, which determined that plant extract could affect meat quality by decreasing the cooking loss of meats.

Some plant extracts including oregano have been shown to inhibit peroxidation of polyunsaturated fatty acids (Bhale et al., 2007). Furthermore, *Helichrysum italicum* (curry plant) extract was found to inhibit or delay the formation of oxygen free radicals, particularly superoxide ions and hydroxyl radical (Facino et al., 1990). Loperz-Bote et al. (1998) also suggested that the breast meat of broilers fed diets containing herbal extracts had lower concentrations of lipid oxidation products than those of the control group. Jang et al. (2008) found that broiler muscles fed plant extract showed higher total phenolic content than those fed the control diet, and that the DPPH radical scavenging ability of muscle was greater and that TBARS value were decreased in the treatment groups. The present study also observed that the DOP addition decreased the breast meat TBARS level of ducks relative to that of the control. The results from the present study show that DOP has the potential to inhibit the production of lipids in ducks thereby improving the storage stability of the meat after slaughter. Similarly, oregano has been shown to improve the duration of meat storage after slaughter in poultry (Botsoglou et al., 2003). Therefore, we believe that antioxidant substances in DOP are delivered to the muscle of ducks, and, the antioxidant defense system counteracts the action of prooxidants.

In conclusion, the present study shows that DOP is a valuable natural feed ingredient for meat ducks, particularly in terms of greater SOD and GPx enzyme activities, lower meat cooking loss, and lower TBARS values.

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