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Effects of Basil (*Ocimum basilicum*) Leaf Extract (BLE) on Performance, Blood Biochemistry, Antioxidant Status and Oxidative Stability of the Meat of Broiler Chickens

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Abstract

The present study was conducted to evaluate the effects of basil (*Ocimum basilicum*) leaf extract (BLE) in drinking water on growth performance, hepatic and serum-antioxidant related parameters, enzyme activity and biochemical parameters of serum, and breast muscle lipid peroxidation of (after storage at 2-3°C, for 1, 7 and 14 d**).** A total of 450 one-day-old male broiler chickens of Ross 308 were randomly divided to three groups, with five replicates of 30 birds and allocated to the following treatments including control (only drinking water), or drinking water supplemented with either 300 or 600 ppm of BLE. At the end of the experiment (day 42), Two birds were randomly selected from each cage and decapitated. There were no significant effects of dietary treatments on body weight gain, feed intake and feed conversion ratio of the broilers. Basil leaf extract significantly increased the hepatic catalase, superoxide dismutase, and glutathione peroxidase activities and decreased lipid peroxidation in the liver and serum. Serum-enzyme activity (aspartate aminotransferase and lactate dehydrogenase) and biochemical parameters (low density lipoprotein and cholesterol) were significantly decreased by basil leaf extract. Moreover, BLE significantly reduced the malondialdehyde formation in breast muscle during storage period. In conclusion, supplementation of basil leaf extract significantly increased the antioxidant capacity of broilers and consequently increased the oxidative stability of frozen meat.

Introduction

In recent years, the inclusion of antibiotics in the diet of livestock has been severely limited, and a considerable research interest in developing new natural products, such as medicinal plants supplements and edible extracts, has also been generated. These plant extracts have been shown to have anti-inflammatory, antifungal, antimicrobial and antioxidant effects. Moreover, they reduce the oxidation of meat lipids by exerting antioxidant effects, thereby increasing the oxidative stability of meat and meat products (Fathi *et al*., 2016). There are also some reports that the use of extracts and essential oils in animal and poultry feed improves growth performance, stimulates blood circulation, enhances the production of digestive secretions and imporves immune status (Vahdatpour *et al*., 2011; Yakhkeshi *et al*., 2011).

Basil (*Ocimum basilicum*) also known as sweet and garden basil, is a member of the Lamiaceae family commonly cultivated throughout the Mediterranean region (Abbas, 2010). Flowering tops and leaves of sweet basil are used in human and animal nutrition and health as an antispasmodic and carminative in folk medicine. Therefore, the use of basil in the form of extracts, essential oils, etc., especially as antimicrobial and antioxidant agents has been studied recently. Several different compounds

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have been reported in basil products that give basil its medicinal properties. However, methyl chavicol, linalool, methyl cinnamate, methyl eugenol, eugenol, and geraniol are significant components of the oils of different chemotypes of *O. basilicum*, Which have beneficial effects on the body of consumers (Sajjadi, 2006). Therefore, the main purpose of this study was to investigate the effects of basil leaf extract (BLE) on antioxidant status, performance and oxidative stability of broiler chicken meat.

Materials and Methods

Management and measurements

A total of 450 one-day-old male broiler chickens (Ross 308) were applied in a completely randomized experimental design with three treatments (0 and 300 and 600 ppm of BLE in drinking water) with five replicates of thirty birds. Birds had free access to feed and water, with 23-hour light per day throughout the experimental period.

Prepare Basil Extract

To prepare the hydroalcoholic extract, the prepared fresh basil leaf (*Ocimum basilicum*) is divided into small pieces and ground using powder, these parts are ground and 30 grams of this powder in a sterile Erlenmeyer flask containing 500 ml ethanol 80% for 48 hours was placed in laboratory conditions. Then, using a shaker, the Erlenmeyer contents were thoroughly mixed again for 5 minutes. for final purification and removal of existing solvents, it was dewatered in a vacuum at a temperature of 55 °C for 30 minutes by a rotary evaporator (IKA-Rotary, made in Germany). The prepared extract was then kept in a dark container in the refrigerator for 24 hours (Dasgupta *et al*., 2004).

Experimental Measurements

The body weight gain, feed intake and feed conversion ratio were measured every two weeks. In addition, the mentioned performance parameters were calculated for the whole period of the experiment. Mortality was recorded daily. At 42 days, two birds from each group with a body weight reflecting the average within the cage were slaughtered, eviscerated, and defeathered. The pectoral and breast muscle samples were collected in order to evaluation of lipid oxidation during storage under freezing conditions.

Biochemical parameters sampling

At 42 days, four birds per cage were randomly selected and blood samples collected from the wing vein with a 25-G needle. Two blood samples from each bird were obtained. The first sample was used to collect serum within 30 min after sampling (centrifuge at 839 g, 10 min)). Serum was collected and stored at −20 ^{0}C until the measurement of the biochemical analysis (Fathi *et al*., 2016). The second blood samples (2 mL/bird) were collected into tubes containing heparin, then transferred to the laboratory for analysis within two hours of collection and finally centrifuged (3000 *g*, for 10 min at room temperature).

Determination of antioxidant parameters in serum and liver

A commercially available kit was used to measure glutathione peroxidase (GSH-Px) activity kit (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK). Commercial kits available were also used to measure the activity of superoxide dismutase (SOD) and catalase (CAT) enzymes (Ransod, RANDOX/SD-125 supplied by Randox Laboratories). To measure the total antioxidant capacity of serum, a commercial kit produced by Pars Azmoun Company (Randox, Pars Azmoon Co. Tehran, Iran), and also an autoanalyzer (Alcyon 300, USA) were used (Fathi *et al*, 2016). The MDA content of serum samples was determined as a measure of lipid oxidative susceptibility. The determination of MDA was based on colorimetric assay of thiobarbituric acid reactive substances as described by Fathi et al. (2016).

The activity of CAT enzyme was measured by synthesis of H_2O_2 at a wavelength of 240 nm (Aebi,1984). In this protocol, 0.01 mL of ethanol was added to a certain amount of tissue extract and incubated on ice for 30 minutes. Later, 10% Triton X-100 with the final concentration of 1% was added to the mixture. The reaction was initiated by adding 0.05 mL H_2O_2 (30 mmol) to an appropriate amount of the tissue extract in 50 mM sodium phosphate buffer with a pH of 7. The resulting solution was used to determine enzyme activity. Finally, the adsorption rate was read after 3 minutes at a wavelength of 240 nm. Then the activity of catalase was estimated in terms of specific activity in which one unit of CAT activity was equal to 1 mM of H_2O_2 decomposed in 1 min.

Determination of Lipid Peroxidationin Breast Muscle

Lipid peroxidation of birds' breast muscle (*pectoralis superficialis*) was determinedby thiobarbituric acid - assay (McDonald and Hultin, 1987) on days 1, 7 and 14 after storage under cold conditions (2-3 °C). In brief, the raw pectoral muscle was grounded in a meat grinder, homogenized, and 0.5 g of the material was taken and mixed with 2 mL of 10% trichloroacetic acid and the suspension was centrifuged for 10 min at 4000 *g* (Sigma 3K30 Polygen). In next step, 2 mL of 0.02 M thiobarbituric acid was added to 2 mL of the collected supernatant and stirred vigorously to form a perfectly homogeneous mixture.

Homogeneous samples were incubated for 40 minutes at 95 to 100 °C in a water bath (Julabo

EcoTemp TW 12). It was then cooled under tap water for 20 minutes and the absorption rate against distilled water (distilled water) at λ = 530 nm (Evolution 160 UV-VIS) was read. Finally, the results were calculated using a standard calibration curve based on the concentration of malondialdehyde (MDA) and expressed as mg MDA per kg of meat. This procedure was performed in three replications.

Chemical analysis of serum enzymes activity and biochemical parameters

The activity of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of Burtis and Ashwood (1998) and using the Pars-Azmoon Diagnostics Infinity AST reagent kit. Automatic autoanalyzer (Alcyon 300, Abbott Park, USA) and Pars Azmoun kits were used to measure total cholesterol, HDL, LDL, and triglyceride parameters in serum.

Table 1. The composition of experimental diets

Ingredients (%)	Starter(1 to 10 d)	Grower (11 to 24 d)	Finisher (25 to 42 d)
Corn	56.64	57.05	61.15
Soybean meal (44% protein)	36.74	35.12	31.21
Soybean oil	0.95	3.20	3.22
Dicalcium phosphate	1.89	1.65	1.53
Ostyer shell	1.35	1.12	1.08
Sodium bicarbonate	0.26	0.26	0.26
Vitamin and mineral premix[1]	0.50	0.50	0.50
Salt	0.23	0.23	0.23
DL-Methionine	0.42	0.26	0.25
L-Lysine	0.38	0.11	0.12
L-Threonine	0.64	0.50	0.45
Analyzed chemical composition			
Metabolizable energy (kcal/kg)	2850	3000	3050
Crude protein (%)	22. 14	20.95	19.54
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.5	0.45	0.42
Sodium (%)	0.18	0.18	0.18
Potassium (%)	0.90	0.87	0.81
Chlorine (%)	0.17	0.17	0.17
Methionine + cystine (%)	1.07	0.90	0.86

[1]Supplied per Kg of diet: vitamin A, 11000 IU; vitamin D3, 5000 IU; vitamin E, 40 IU; vitamin K, 4 mg; riboflavin, 5 mg; vitamin B6,4 mg; vitamin B12, 0.011mg; niacin, 50 mg; biotin, 0.01 mg; thiamine, 3 mg; zinc 80 mg; manganese oxide, 100 mg; selenium , 10 mg; iron sulfate 80 mg.

Statistical analysis

Data were analyzed using SAS program (SAS, 2005) based on a completely randomized design (CRD) with four treatments and five replications per treatment and one-way analysis of variance using a general linear model (GLM). Also, means were compared using the Tukey test (Tukey, 1949) with a level of significancy of 5%.

Results

Growth performance

Effects of BLE on growth performance parameters including body weight gain, feed intake and feed conversion ratio of birds are given in Table 2. There were no significant difference in either body weight gain, feed intake and feed conversion ratio of treated broiler chickens ($P > 0.05$).

Serum antioxidant parameters

Our findings showed that serum antioxidant parameters, except SOD, were significantly affected by BLE administration in broiler chickens (Table 3). In other words, birds recived basal extract showed enhanced glutathione peroxidase (GSH Px), superoxide dismutase (SOD) activity, total antioxidant capability (T-AOC), but declined declined the serum MDA concentration ($P < 0.01$) compared to control groups.

Hepatic antioxidant parameters

The hepatic antioxidant enzyme activities of birds are shown in Table 4. Those birds exposed to BLE revealed upper CAT, SOD, and GPx activities in the liver than the control ones. Furthermore, administration of BLE significantly reduced MDA consecration in serum.

Table 2. Influence of basil leaf extract (BLE) on growth performance of broilers at different ages

Performance parameters	Groups			*SEM*	*P*-value
	Control	Control + 300 ppm of BLE	Control + 600 ppm of BLE		
Body weight gain (g/d/bird)					
1–14 days	24.55	22.45	23.50	2.50	0.70
15–28 days	49.30	47.30	46.54	1.50	0.68
29–42 days	66.54	65.60	66.70	2.25	0.59
1–42 days	46.79	45.13	45.25	3.58	0.78
Daily feed intake (g/d/bird)					
1–14 days	33.55	32.50	31.45	1.75	0.85
15–28 days	75.19	73.35	74.20	2.50	0.46
29–42 days	135.50	134.59	133.55	4.25	0.56
1–42 days	81.41	80.14	79.73	3.75	0.27
Feed convertion ration (g/g)					
1–14 days	1.37	1.45	1.39	0.15	0.56
15–28 days	1.52	1.55	1.63	0.20	0.69
29–42 days	2.04	2.05	2.00	0.09	0.73
1–42 days	1.74	1.78	1.76	0.05	0.35

[a,b,c] Means within a row without a common superscript letter differ significantly ($P < 0.05$).

Table 3. Influence of basil leaf extract (BLE) on serum antioxidants indices of broiler chickens

Groups	T-AOC[1]	GSH-Px[2]	SOD[3]	MDA[4]
Control	2.55 [c]	30.25 [c]	982.25[b]	1.30 [a]
Control + 300 ppm of BLE	2.63 [b]	35.25 [b]	1150.20[a]	1.04 [b]
Control + 600 ppm of BLE	2.95 [a]	39.25 [a]	1159.42[a]	0.90 [c]
SEM[5]	0.11	25.07	25.07	0.13
P-value	0.021	0.001	0.01	0.003

[a,b,c] Means within a column without a common superscript letter differ significantly ($P < 0.05$). [1]Total antioxidant capability (U/mL). [2]Glutathione peroxidase (mole/mL) [3]Superoxide dismutase(mole/mL). [4]Malondialdehyde(mole/mL). [5] Standard error of mean

Table 4. Influence of different doses of basil leaf extract (BLE) on broiler chickens hepatic-antioxidant parameters

Groups	GSH-Px [1]	SOD[2]	CAT[3]	MDA[4]
Control	3.05 [c]	10.07 [b]	62.32 [c]	0.88 [a]
Control + 300 ppm of BLE	5.63 [ab]	14.53[a]	85.17 [ab]	0.73 [b]
Control + 600 ppm of BLE	5.95 [a]	16.50 [a]	89.50 [a]	0.69 [bc]
SEM[4]	0.11	2.07	4.25	0.13
P-value	0.011	0.001	0.001	0.003

[a,b,c] Means within a column without a common superscript differ significantly ($P < 0.05$). **[1]** Glutathione peroxidase (mole/mg protein). **[2]** Superoxide dismutase (mole/mg protein). **[3]** Catalase (mole /mg protein). **[4]** Malondialdehyde (mole /mg protein)
[5] Standard error of mean

Serum Enzyme activities

The effects of BLE on serum Enzyme activities, including LDH, AST and ALT of the study groups are given in Table 5. The biochemical parameters examined were affected by the administration. The lowest serum Enzyme (LDH, AST and ALT) activities were observed in the lead supplemented groups ($P < 0.05$).

Table 5. Influence of different doses of basil leaf extract (BLE) on serum-enzyme activities of broiler chickens

Groups	LDH[1]	AST[2]	ALT[4]
Control	2975[a]	240.50[a]	3.55 [a]
Control + 300 ppm of BLE	2863 [b]	223.25 [b]	2.34 [b]
Control + 600 ppm of BLE	2795 [c]	201.45 [c]	2.05 [bc]
SEM[4]	20.11	15.07	0.13
P-value	0.021	0.001	0.003

[a,b,c] Means within a column without a common superscript differ significantly ($P < 0.05$). [1]Lactate dehydrogenase (U/L). [2]Aspartate aminotransferase (U/L). [3]Alanine aminotransferase (U/L). [4] Standard error of mean

Serum lipid parameters

The effects of BLE on Serum lipid parameters of broiler chickens are shown in Table 6. Even though dietary supplementation with BLE did not impact serum HDL and triglyceride levels, diets significantly decreased LDL and cholesterol level of serum in broiler chickens ($P < 0.05$).

Table 6. Influence of different doses of basil leaf extract (BLE) on serum lipid parameters in broiler chickens at day 42

Groups	LDL[1]	HDL[2]	CHO[4]	TG[4]
Control	26.55 [a]	85.25	150.55 [a]	91.55
Control + 300 ppm of BLE	21.63 [b]	87.25	125.34 [bc]	89.34
Control + 600 ppm of BLE	17.95 [c]	91.25	118.05 [c]	90.05
SEM[5]	0.11	4.07	0.13	2.07
P-value	0. 001	0.11	0.003	0.21

[a,b,c] Means within a column without a common superscript differ significantly ($P < 0.05$). [1]Low-density lipoprotein /mg/100mL. [2]High-density lipoprotein /mg/100mL. [3]Cholesterol/mg/100mL. [4]Triglycerides /mg/100mL. [5] Standard error of mean

MDA concentration in the breast muscle

Lipid oxidation in breast muscle (concentration of MDA) after 1,7 and 14 d of chilled storage was affected by dietary BLE in relation to control group (Table 7). Administration of the BLE in drinking water clearly reduced MDA in breast tissue ($P < 0.05$).

Table 7. Influence of different doses of basil leaf (BLE) extract on malondialdehyde content (mg/kg) in breast muscle stored at 2-3 °C for 1 ,7 and 14 d

Groups	Day of storage		
	1	7	14
Control	0.38[a]	0.67[a]	0.82[a]
Control + 300 ppm of BLE	0.35[b]	0.57[b]	0.72[b]
Control + 600 ppm of BLE	0.26[c]	0.52[c]	065[c]
SEM[2]	0.005	0.009	0.007
P-value	0.001	0.001	0.001

[a,b,c] Means within a column without a common superscript differ significantly ($P < 0.05$).

[2] Standard error of mean

Discussion

Ocimum basilicum is a widely used medicinal plant in many parts of the world that has been included in a number of herbal medicines for treatment of various diseases (Dasgupta *et al*., 2004). Many forms supplementaion of basil as ethanolic extract, flavonoids, seed oil, phenolic compounds, root extract, leaf extract, aqueous extract and leaf in the diet have been studied and shown positive results on heuman health (Ganasoundri *et al*. 1998; Prashar *et al*. 1994; Godhwani *et al*. 1998).

In addition to human reports, the results of some studies show that the use of basil essential oils in the diet of broilers improved performance similar to that of growth-promoting antibiotics (Gunal *et al*. (2006). Furthermore, Abbas (2010) and Osman *et al*. (2010) reported that the addition of basil leaf and seed to the diet improved growth performance. The results of the present study were consistent with the findings of Akbarian *et al*. (2013) who found no significant positive effects on the performance of broilers when basil extract was supplemented in diets.

Concerning antioxidant enzyme status in the liver and serum, almost all the antioxidant-related enzymes, including total antioxidant capability (T-AOC), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were establish to be higher above the control values. Additionally, the MDA concentration in serum and liver & breast tissues of both levels of BLE added groups were lower compared to the control group.

Superoxide dismutase (SOD) plays a very important role in the antioxidant enzyme defense system in serum and tissues. SOD can convert superoxide radicals into hydrogen peroxide. Since supplementation of BLE in broiler chickens increases the activity of superoxide dismutase enzyme, subsequently inhibits the production of reactive oxygen species, and the deformation of superoxide radicals may be accelerated by the catalytic role of SOD, and the dismutation of superoxide radicals may well be accelerated by the catalyzing role of SOD. Catalase is another antioxidant enzyme that significantly increased in activity under the influence of basil extract., services in eliminating the hydrogen peroxide formed by the action of SOD. According to our findings, BLE had a positive effect on oxidative stress and decreased MDA level in serum, liver and breast tissues (Li *et al*. 2000).

Possible hypothesis for the use of herbal supplements and other herbal supplements in the diet of poultry or other livestock assumes the transfer of compounds with antioxidant properties to meat (Botsoglou *et al*., 2002). Jang *et al.* (2008), showed a significant increase in phenolic compounds in the breast muscles of chickens fed a supplemental diet with *Morus alba* L., *Lonicera flos, Coptis chinensis* extract mixture. This hypothesis may be partly established by the results of this study, since the extensive effect of BLE on oxidation intensity in frozen breast muscles was identified. The absorption of anthocyanins of herbal extract from gastrointestinal tract has been also showed, as supported by the presence of these compounds in the blood and tissues of the eye of rabbits (Matsumoto *et al*., 2006) and the urine of rabbits and human (Nielsen *et al*., 2003).

Faiz *et al.* (2017) showed lower MDA values and greater activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, connected with the dose-dependent improvement in total phenolic compound recognized in meat of chickens receiving various levels of citrus waste.

Results obtained from the present study show that BLE direction to broilers significantly reduced the serum activity of liver enzymes such as LDH and AST. This decrease in serum enzymatic activity is probably due to the cytoprotective effect exerted by BLE (Dasgupta *et al.*, 2004). The present investigation has confirmed obviously that BLE protects against oxidative stress via the promotion of antioxidative defense enzyme, though significantly dropping the specific activity of LDH, AST & ALT and the level of lipid peroxidation (Dasgupta *et al*., 2004).

In this study addition of BLE to diet reduced serum cholesterol and low-density lipoproteins in broilers. These findings concur with the data of Abbas (2010), who indicated that the supplementation of feeding dietary basil seed significantly reduced the serum cholesterol level in broilers**.** According to the results of this study, the effects of reducing BLE on serum LDL and CHO is associated with antioxidant effects of basil extract and prevention of serum lipid oxidation. (Yousef *et al*., 2006; Ismail *et al*., 2010). Furthermore, it is speculated that increased glutathione peroxidase activity may lower adult LDL and cholesterol levels (Luoma *et al*., 1990).

Conclusion

The results of this study showed that BLE can be an effective source of antioxidants in broiler chickens feeding that can increase the antioxidant status and oxidative stability of frozen meat. However, not all antioxidant aspects of polyphenol compounds are fully understood and require further research.

Ethical Statement

All animal experiments were performed in accordance with the protocol of the Animal Use Committee of the Iranian Ministry of Science, Research and Technology were approved by the Animal Care Committee of the Department of Animal Science of Payam Noor University. All efforts were made to minimize animal suffering.

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