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A blend of thyme and rosemary powders with poultry by-product meal can be used as a natural antioxidant in broilers

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ABSTRACT. A total of 500, 14-d old male Ross 308 broilers were allocated into five treatments (4 replicates each) including: a negative (NC) and positive control diet (PC) which supplemented without and with 7% poultry by-product meal (PBPM), respectively and three levels of additive supplementation of thyme and rosemary powders in the basal diets: 0.750% rosemary powder (PCR); 0.375% thyme powder + 0.375% rosemary powder (PCRT), and 0.750% thyme powder (PCT) rosemary powder. The PCRT diet improved ($p < 0.05$) average daily gain and feed conversion ratio by 5.62 and 10.37% compared to PC, respectively. The serum concentration of lipids (triglycerides, cholesterol, high-density lipoprotein, and low-density lipoprotein) was decreased while the ileal villus height and ratio of villus height to crypt depth as well as ileal microbiota population were improved ($p < 0.05$) in response to inclusion PCRT diet compared to PC diet. The blood serum concentration of malondialdehyde was statistically decreased ($p < 0.05$) by supplementation of PCRT in broiler diets. Our results suggested that supplementation of a blend of thyme and rosemary powders at the level of 0.375% of each into broiler diet during d 14-42 has merit to be used as a natural antioxidant in diets formulated by PBPM.

Keywords: broilers; thyme; rosemary; growth performance; antioxidants activities; gut health.

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Introduction

Feeds correspond to the two-thirds of the cost of poultry production and among the ingredients, protein supplements covered the most expensive per unit of cost (Hassanabadi, Amanloo, & Zamanian, 2008). Poultry by-product meal (PBPM), which is generally made from viscera, heads, and feet of poultry by conventional dry-rendering methods can be used to reduce the cost of poultry feeds (Samli, Senkoylu, Ozduven, Akyurek, & Agma, 2006). The chemical composition of PBPM can vary greatly depending on the raw material sources and processing methods (Robbins & Firman, 2006), but generally has a proper profile of available essential amino acids and is rich in minerals and vitamins, and also, has high biological value (National Research Council [NRC], 1994). Senkoylu, Samli, Akyurek, Agma, & Yasar (2005), and Samli et al. (2006) reported that supplementation of PBPM up to the level of 5 and 10%, respectively in laying hen diets, did not any adverse effect on performance and egg quality ($p < 0.05$).

The susceptibility of PBPM to lipid oxidation during the post-slaughter processing of carcasses and storage of PBPM is of further concern as novel feeding strategies have been adopted to cease the lipid oxidation of PBPM (Bakhshalinejad, Akbari Moghaddam Kakhki, & Zoidis, 2018). Lipid oxidation in foods is of major importance because it related to the development of cardiovascular diseases and other diseases. Antioxidants are natural or synthetic substances used to prevent lipid oxidation (Lobo, Patil, Phatak, & Chandra, 2010). During recent years, medicinal plants have been used widely as natural antioxidants because of the affordability and profitability factors of them (Nameghi, Edalatian, & Bakhshalinejad, 2019).

Among aromatic plants, thyme and rosemary from *Lamiaceae* family, in particular, reportedly reduced lipid oxidation in foods and food model systems (Thanissery, Kathariou, & Smith, 2014). Thyme powder obtained by drying leaves and flowers of *Thymus vulgaris* L. plants is also well known for its antimicrobial and antioxidant properties (Nameghi et al., 2019). Major components principally responsible for these

properties are thymol, carvacrol, whereas other minor constituents are *γ*-terpinene and *p*-cymene (Lee, Umano, Shibamoto, & Lee, 2005). Rosemary powder obtained by drying leaves of *Rosmarinus officinalis* L. plants contains a wide range of different phenolic compounds with varying antioxidative activity (Galobart, Barroeta, Baucells, Codony, & Ternes, 2001). Carnosic acid, carnosol, rosmanol, epirosmanol, rosmarinic acid are the major phenolic constituent present in rosemary leaves (Botsoglou et al., 2005).

The growth-promoting effects of thyme have been demonstrated in broiler chickens by Hassan and Awad (2017) who reported that supplemented thyme powders (5%), in broilers diets improved growth performance and immune status ($p < 0.05$). Petricevic et al. (2018) reported that supplemented rosemary leaves powder at the level of 0.4% in broilers diets improved growth performance, fat deposition and cecal microbiological composition ($p < 0.05$).

Since there has not been yet any report dealing with the antioxidant effect of dietary supplemented thyme and rosemary leaves on PBPM, the objective of this study was to evaluate the use of natural antioxidants thyme and rosemary powders added to feed mixes which supplemented by 7% PBPM and fed to broiler chickens from d 14 to d 42 of life, and then evaluate growth performance, carcass characteristics, blood parameters, antioxidants activities and ileal morphology and microbiology of broilers.

Materials and methods

The experiment protocol used in this study was conducted under the guide for the use of animals of Research and Education Center of Khorasan Razavi Agricultural and Natural Resource, Mashhad, Iran.

Birds and housing

A total of 500 fourteen-d-old male Ross 308 chicks were randomly distributed to 5 dietary treatments using 4 floor pens per treatment and 25 chicks per pen (2.0 m × 1.5 m). Each floor pen equipped with five nipple drinkers and a tube feeder. Water and feed were offered *ad libitum*. The chicks were housed in a room with controlled temperature, ventilation, and lighting (23h/d).

Experimental design and diets

Grower (14 to 24 d), and finisher (25 to 42 d) diets were formulated to meet or exceed the nutrient requirements according to Ross 308 guidelines (Aviagen, 2014; Table 1). All experimental diets were produced in pelleted form and they all are iso nutritive. The ingredient composition and nutrient content of the experimental diets are shown in Table 1.

Poultry by-product meal, thyme and rosemary powders were ground through a 1-mm screen and then stored for further analysis. The nutritional composition of PBPM, thyme and rosemary powders are shown in Table 2. Nutritional compositions of PBPM, thyme and rosemary powders were determined for dry matter, ether extract, total ash, crude protein (Kjeldahl N × 6.25), crude fiber, calcium and total phosphorus (Association of Official Agricultural Chemists [AOAC], 2005). The gross energy contents of the PBPM, thyme, and rosemary were measured using an adiabatic bomb calorimeter (model C 4000; IKA, Heitersheim, Germany). Nitrogen-corrected apparent metabolizable energy (AMEn) estimated from the chemical composition (WPSA, 1984). All analyses were performed in triplicate.

Five experimental diets include of a negative control diet (NC; without supplementation of PBPM), a positive control diet (PC; with supplementation of 7% PBPM), and three levels of additive supplementation of thyme and rosemary powders in the basal diets: 0.750% rosemary powder (PCR); 0.375% thyme powder + 0.375% rosemary powder (PCRT), and 0.750% thyme powder (PCT) rosemary powder.

Measurements

Bodyweight and feed consumption by pen were recorded weekly to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR; feed/gain). Mortality was recorded daily.

At the end of the experiment, two chicks from each pen (8 birds per treatment) were randomly selected and then slaughtered by cervical dislocation for carcass evaluation. Each individual organ was weighed, recorded, and were presented as percentage of live body weight; breast and thigh muscles, liver, heart, spleen, thymus, bursa of Fabricius and abdominal fat (Kakhki, Bakhshalinejad, & Shafiee, 2016).

Table 1. Ingredient and nutrient composition of the experimental diets (as-is basis).

Item	Grower (14-24 d)		Finisher (25-42 d)	
Ingredient, g kg^{-1}				
Corn grain (78 g crude protein)	583.0	582.0	653.7	641.5
Soybean meal (440 g crude protein)	349.0	292.4	294.0	248.0
Soybean oil	23.5	20.4	10.9	9.3
Dicalcium phosphate	18.1	10.8	17.4	9.4
Limestone	12.3	10.5	11.5	9.8
Sodium chloride	3.5	3.5	3.0	3.0
Vitamin premix[1]	2.5	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5	2.5
$_{DL}$-Methionine	2.1	2.0	2.2	1.9
$_{L}$-Lysine-HCL	2.3	2.3	1.6	1.4
$_{L}$-Threonine	1.2	1.1	0.7	0.7
Animal byproduct meal	-	70	-	70
Total	1000	1000	1000	1000
Nutritive value of mixtures, calculated, per kg				
Metabolizable energy, kcal (MJ)	2980 (12.47)	2980 (12.47)	3000 (12.55)	3000 (12.55)
Crude protein, g	210	210	190	190
Calcium, g	9.6	9.6	9.0	9.0
Available phosphorus, g	4.5	4.5	4.2	4.2
Digestible lysine, g	11.5	11.0	10.2	10.2
Digestible methionine, g	5.1	5.8	4.2	4.2
Digestible methionine + cystine, g	8.5	8.7	7.8	7.8
Digestible threonine, g	6.8	6.8	7.2	7.2
Nutritive value of mixtures, analyzed, per kg				
Dry matter, g	890.2	889.1	889.1	888.2
Crude protein, g	202.1	201.7	184.9	185.8
Ether extract, g	52.5	59.1	41.9	49.7
Crude ash, g	62.8	63.1	58.2	58.6
Calcium, g	9.4	9.4	8.8	8.8
Total phosphorus, g	6.8	6.6	6.5	6.2

[1]Provides in kg of diet: vitamin A (retinol), 12,000 IU; vitamin D_3 (cholecalciferol), 5,000 IU; vitamin E ($_{DL}$-α-tocopheryl acetate), 65.0 IU; vitamin K_3 (menadione), 3.2 mg; vitamin B_1 (thiamin), 3.2 mg; vitamin B_2 (riboflavin), 8.6 mg; vitamin B_3 (niacin), 57.5 mg; vitamin B_5 (pantothenic acid), 15.0 mg; vitamin B_6 (pyridoxine), 5.0 mg; vitamin B_9 (folic acid), 2.0 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; vitamin H_2 (biotin), 0.25 mg; choline chloride, 600 mg. [2]Provides (mg kg^{-1} of diet): Mn (manganese oxide), 110.6; Zn (zinc oxide), 100.0; Fe (iron carbonate), 25.0; Cu (copper sulfate), 15.5; I (calcium iodate), 1.1; choline chloride, 500.0 mg.

Table 2. Analyzed chemical composition of poultry by-product meal, and thyme and rosemary powders.

Item, per kg	Poultry by-product meal	Thyme powder	Rosemary powder
Dry matter, g	885.8	930.8	920.9
Crude protein, g	552.2	91.3	141.4
Crude fiber, g	152.5	181.6	201.4
Crude ash, g	172.2	115.2	65.1
Ether extract, g	152.3	32.4	123.2
Calcium, g	35.2	10.7	8.3
Phosphorus, g	21.4	5.8	2.6
Gross energy, kcal (MJ)	3632 (15.2)	4278 (17.9)	4350 (18.2)
AMEn, kcal (MJ)	2461 (10.3)	2940 (12.3)	2987 (12.5)

All analyses were performed in triplicate. Nitrogen-corrected apparent metabolizable energy (AMEn) estimated from chemical composition (WPSA, 1984).

After 8h feed withdrawal at 24 and 42 d of age, two birds per pen were randomly selected, and then their blood samples were taken from the brachial vein. The blood samples were spin at 2,000 × *g* for 5 min. at 4°C and frozen at -20°C for future analysis. Concentrations of serum glucose (Glu), triglycerides (TG), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assessed using an automatic biochemical analyzer (Hitachi 717, Boehringer Mannheim, Ingelheim am Rhein, Germany) with commercial assay kits according to the manufacturer's instructions (Pars Azmoon Co. kits, Tehran, Iran). The blood samples were analyzed by a spectrophotometer (UV-2100, Unico Instruments Co., Shanghai, China) to measure superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities and concentration of malondialdehyde (MDA) as described by Bakhshalinejad et al. (2018).

Besides, two chicks per pen were randomly selected and then killed by cervical dislocation for further determination of ileal morphology. Ileum segment (determined at 1 inch above from ileocaecal junction) was removed and fixed in 10% neutral buffered formalin solution and embedded in paraffin wax. Samples (5-cm section of the midpoint) were stained by hematoxylin and eosin and examined by Olympus BX41 microscope (Olympus Cooperation, Tokyo, Japan) at 100× magnification. The villus length and crypt depth were measured with digital micrometer, and the villous length to crypt depth ratio was calculated. The average 20 values from 8 chicks were represented as mean villus length and crypt depth for a treatment group (Nameghi et al., 2019).

The ileal contents of euthanized broilers were immediately collected, mixed, placed in a microtube and stored at -70°C until the microbial assay. Microbial populations were determined according to the method described by Nameghi et al. (2019). A specific agar was used to culture bacteria as follows: Rogosa SL agar and MacConkey agar (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) for *Lactobacillus* and *E. coli*, respectively.

Statistical analysis

A completely randomized design was used, and the data were tested for normality with UNIVARIATE plot normal procedure and then analyzed by the GLM procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA). The Tukey test was performed for comparison of the means (Tukey, 1991). The pen replicate was the experimental unit and differences were considered significant when $p < 0.05$. All the numerical data in the tables were presented as average values and accompanied by SEM (standard error of the means) values.

Animal welfare statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, were approved by the Animal Ethics Committee of the Research and Education Center of Khorasan Razavi Agricultural and Natural Resource (Mashhad, Iran).

Results

The supplementation of PBPM in broilers diets decreased ADG in the PC group compared to the NC group, at the periods of d 14 to 21, and 14 to 42 ($p < 0.05$; Table 3). The FCR was increased by 0.06 units in the broilers fed 7% PBPM (PC group) compared to broilers fed basal diet (NC group) at period of d 14 to 21 ($p < 0.05$). The inclusion of thyme and rosemary powders combination (PCRT group) in broilers diets increased ($p = 0.001$) ADG at the period of d 14 to 21, and improved ($p < 0.05$) FCR at periods of d 14 to 21, and 14 to 42 compared to broilers fed PBPM (PC group). However, ADG and FCR were not affected during the periods of d 22 to 28, 29 to 35, and 36 to 42 ($p > 0.05$). There were no differences ($p > 0.05$) in ADFI among treatments in each phase and the entire experimental period. Also, the mortality rate was not affected ($p > 0.05$) by the treatments during the experiment (data are not shown).

Table 3. Effect of experimental treatments on growth performances of broilers at 14-42 d.[1]

Item	Treatments[2]					SEM[3]	p-value
	NC	PC	PCR	PCRT	PCT		
ADG, g/b/d	63.07[b]	60.96[c]	63.66[ab]	64.39[a]	61.78[c]	0.378	0.001
ADFI, g/b/d	116.90	119.35	120.07	114.17	117.65	2.146	0.365
FCR, g/g	1.853[ab]	1.958[a]	1.886[ab]	1.774[b]	1.905[a]	0.036	0.033

[a-b] Different letters within the same row indicate significant differences among treatment groups ($p < 0.05$). [1] Growth performance data are means of 4 pens with 25 chickens per each. [2] NC, negative control diet; PC, positive control diet; PCR, positive control diet supplemented with rosemary powder (7.50 g kg^{-1}); PCRT, positive control diet supplemented with rosemary (3.75 g kg^{-1}) and thyme powder (3.75 g kg^{-1}); PCT, positive control diet supplemented with thyme powder (7.50 g kg^{-1}). [3] ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio. [3] SEM, standard error of the mean.

The thyme and rosemary powders alone or in combination supplementation in broiler diets did not affect the carcass, breast muscle, heart, spleen and thymus yields ($p > 0.05$; Table 4). The inclusion of thyme and rosemary powders combination (PCRT group) in broiler diets increased thigh muscle ($p = 0.008$) and decreased ($p < 0.05$) liver, bursa of Fabricius and abdominal fat compared to broilers fed PBPM (PC group).

Ileal villus height and villus height to crypt depth ratio were lower (p <0.05) in the PC group compared to NC group at d 21 and 42 (Table 5). Birds of PCRT had higher (p <0.05) ileal villus height than PC birds on d 21 and 42. Furthermore, birds of PCT at d 21, and PCR and PCT at 42 d of age had higher (p <0.05) ileal crypt depth than PC birds.

The effects of different dietary treatments on the population of ileal selected bacteria at 21 and 42 d of age are shown in Table 5. The ileal *E. coli* count was lower in PCRT (P = 0.001) compared to PC at 21 and 42 d of age. Moreover, broilers fed thyme and rosemary powders combination diet (PCRT group) had a higher (p =0.001) number of ileal *Lactobacillus* than the PC birds at d 21 and 42.

Table 4. Effect of experimental treatments on carcass characteristics of 42-d-old broilers.[1]

Item, %	Treatments[2]					SEM[3]	p-value
	NC	PC	PCR	PCRT	PCT		
Carcass	66.10	65.85	66.17	67.61	66.22	0.507	0.166
Breast muscle	30.35	30.17	30.52	31.45	30.72	0.454	0.357
Thigh muscle	18.48^{c}	18.80^{bc}	19.03^{ab}	19.50^{a}	19.21^{ab}	0.172	0.008
Liver	3.182^{a}	3.263^{a}	2.885^{ab}	2.724^{b}	2.933^{ab}	0.069	0.003
Heart	0.562	0.590	0.522	0.505	0.543	0.026	0.227
Spleen	0.135	0.143	0.112	0.106	0.123	0.012	0.247
Bursa of Fabricius	0.128^{a}	0.077^{b}	0.110^{ab}	0.137^{a}	0.117^{ab}	0.009	0.009
Thymus	0.402	0.391	0.417	0.445	0.432	0.030	0.720
Abdominal fat	1.727^{ab}	1.875^{a}	1.378^{b}	1.275^{c}	1.582^{b}	0.051	0.001

$^{a-c}$ Different letters within the same row indicate significant differences among treatment groups (p < 0.05). [1] Each value represents the mean of 4 replicates with 2 birds per replicate. [2] NC, negative control diet; PC, positive control diet; PCR, positive control diet supplemented with rosemary powder (7.50 g kg^{-1}); PCRT, positive control diet supplemented with rosemary (3.75 g kg^{-1}) and thyme powder (3.75 g kg^{-1}); PCT, positive control diet supplemented with thyme powder (7.50 g kg^{-1}). [3] SEM, standard error of the mean.

Table 5. Effect of different experimental treatments on the ileal morphology and microbiota content of broilers.[1]

Item	Treatments[2]					SEM[3]	p-value
	NC	PC	PCR	PCRT	PCT		
24 d							
Villus height, µm	872.7^{b}	809.5^{c}	902.0^{ab}	921.7^{a}	904.5^{ab}	13.463	0.002
Crypt depth, µm	163.2^{c}	172.7^{bc}	181.5^{b}	161.5^{c}	196.7^{a}	3.786	0.001
Villus height: crypt depth	5.32^{b}	4.70^{cd}	4.97^{c}	5.72^{a}	4.60^{d}	0.103	0.001
E. coli, CFU log g^{-1}	3.59^{a}	4.87^{a}	3.55^{ab}	3.36^{b}	3.47^{ab}	0.134	0.001
Lactobacillus, CFU log g^{-1}	6.15^{c}	5.88^{c}	7.40^{b}	7.95^{a}	7.27^{b}	0.109	0.001
42 d							
Villus height, µm	1106.2^{b}	973.5^{c}	1116.2^{ab}	1131.5^{a}	1120.5^{ab}	13.423	0.001
Crypt depth, *µm*	189.7^{b}	192.5^{b}	205.7^{a}	199.0^{ab}	208.7^{a}	3.233	0.003
Villus height: crypt depth	5.85^{a}	5.05^{c}	5.42^{bc}	5.70^{ab}	5.37^{bc}	0.121	0.003
E. coli, CFU log g^{-1}	6.38^{b}	7.27^{a}	5.40^{cd}	5.14^{d}	5.55^{c}	0.105	0.001
Lactobacillus, CFU log g^{-1}	6.55^{c}	5.40^{c}	6.57^{b}	7.50^{a}	6.72^{b}	0.158	0.001

$^{a-b}$ Different letters within the same row indicate significant differences among treatment groups (p < 0.05). [1] Data are means of duplicated analysis of 8 samples per each treatment. [2] NC, negative control diet; PC, positive control diet; PCR, positive control diet supplemented with rosemary powder (7.50 g kg^{-1}); PCRT, positive control diet supplemented with rosemary (3.75 g kg^{-1}) and thyme powder (3.75 g kg^{-1}); PCT, positive control diet supplemented with thyme powder (7.50 g kg^{-1}). [3] SEM, standard error of the mean.

The concentrations of Glu, TG, CHOL, HDL, LDL, ALT, AST, and ALP in blood serum are shown in Table 6. There were no differences (p >0.05) between the treatments for sera Glu at d 21 and 42, and also, CHOL, ALT, AST, and ALP at 42 d of age. Serum TG, CHOL, HDL, and LDL concentrations were lower (p <0.05) in PCRT compared to PC at d 21 and 42. The supplementation of PBPM in broiler diets increased the serum concentration of ALT, AST, and ALP in the PC group compared to the NC group at d 21 (p =0.001). Moreover, the hepatic concentration of ALT, AST, and ALP in PCRT birds were lower (p =0.001) compared the PC at d 21.

The effects of the experimental treatments on the activities of superoxide dismutase, glutathione peroxidase, catalase, and concentration of malondialdehyde in the blood serum of broiler chickens on d 24 and 42 d are presented in Table 7. The activities of superoxide dismutase, glutathione peroxidase and catalase in the blood serum were not affected on d 24 and 42 (p >0.05). The serum concentration of malondialdehyde decreased (p <0.05) in PCRT birds compared to the PC group at 21 and 42 d of age.

Table 6. Effect of different experimental treatments on the blood serum parameters of broilers.[1]

Item[3]	Treatments[2]					SEM[4]	p-value
	NC	PC	PCR	PCRT	PCT		
24 d							
Glu, mg dL^{-1}	257.02	263.51	253.24	250.25	256.00	4.601	0.367
TG, mg dL^{-1}	110.60^{a}	111.12^{a}	92.23^{b}	83.30^{c}	97.23^{b}	3.284	0.001
CHOL, mg dL^{-1}	149.75^{a}	163.72^{a}	138.50^{b}	131.72^{c}	140.22^{b}	4.947	0.004
HDL, mg dL^{-1}	74.00^{a}	77.00^{a}	71.25^{ab}	53.72^{b}	67.24^{ab}	1.950	0.001
LDL, mg dL^{-1}	24.75^{a}	25.52^{a}	23.21^{a}	17.25^{b}	22.51^{a}	1.185	0.002
AST, U L^{-1}	296.23^{b}	315.25^{a}	288.21^{bc}	276.00^{c}	278.22^{bc}	4.338	0.001
ALT, U L^{-1}	22.25^{b}	26.75^{a}	18.28^{bc}	18.00^{c}	18.22^{bc}	0.722	0.001
ALP, U L^{-1}	271.90^{b}	307.95^{a}	239.10^{bc}	223.25^{c}	233.42^{bc}	6.915	0.001
42 d							
Glu, mg dL^{-1}	269.73	272.25	265.74	265.02	264.00	2.751	0.221
TG, mg dL^{-1}	112.17^{a}	126.70^{a}	96.32^{b}	91.82^{c}	99.45^{b}	2.696	0.001
CHOL, mg dL^{-1}	153.00^{a}	159.52^{a}	145.06^{b}	134.74^{c}	149.53^{b}	5.386	0.044
HDL, mg dL^{-1}	81.00^{a}	82.75^{a}	79.00^{ab}	57.25^{b}	77.23^{ab}	3.342	0.001
LDL, mg dL^{-1}	21.50^{a}	23.52^{a}	19.75^{bc}	17.03^{c}	19.00^{bc}	1.146	0.012
AST, U L^{-1}	337.50	339.56	331.75	288.21	328.52	7.698	0.125
ALT, U L^{-1}	14.37	14.75	13.75	12.50	13.37	0.568	0.097
ALP, U L^{-1}	136.97	141.45	134.52	125.65	129.82	6.164	0.353

[a-b] Different letters within the same row indicate significant differences among treatment groups ($p < 0.05$). [1] Data are means of duplicated analysis of 8 samples per each treatment. [2] NC, negative control diet; PC, positive control diet; PCR, positive control diet supplemented with rosemary powder (7.50 g kg^{-1}); PCRT, positive control diet supplemented with rosemary (3.75 g kg^{-1}) and thyme powder (3.75 g kg^{-1}); PCT, positive control diet supplemented with thyme powder (7.50 g kg^{-1}). [3] Glu, glucose; TG, triglycerides; CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. [4] SEM, standard error of the mean.

Table 7. Effect of different experimental treatments on the activities of superoxide dismutase, glutathione peroxidase and catalase, and concentration of malondialdehyde in the blood serum of broilers.[1]

Item[3]	Treatments[2]					SEM[4]	p-value
	NC	PC	PCR	PCRT	PCT		
24 d							
SOD, U mL^{-1}	135.25	134.74	137.00	141.50	135.52	1.973	0.153
GSH-Px, U mL^{-1}	151.22	149.25	153.01	157.23	147.25	5.726	0.774
CAT, nmol mL^{-1}	14.25	13.01	15.37	16.51	15.00	1.151	0.320
MDA, nmol mL^{-1}	7.12^{a}	7.92^{a}	6.92^{ab}	6.47^{b}	7.10^{ab}	0.212	0.004
42 d							
SOD, U mL^{-1}	144.50	140.53	150.25	152.04	150.75	5.009	0.458
GSH-Px, U mL^{-1}	167.25	162.51	172.75	181.53	176.02	6.549	0.321
CAT, nmol mL^{-1}	15.70	14.02	15.03	15.75	15.00	0.677	0.379
MDA, nmol mL^{-1}	7.00^{ab}	8.47^{a}	6.90^{b}	5.72^{c}	6.55^{b}	0.270	0.001

[a-b] Different letters within the same row indicate significant differences among treatment groups ($p < 0.05$). [1] Data are means of duplicated analysis of 8 samples per each treatment. [2] NC, negative control diet; PC, positive control diet; PCR, positive control diet supplemented with rosemary powder (7.50 g kg^{-1}); PCRT, positive control diet supplemented with rosemary (3.75 g kg^{-1}) and thyme powder (3.75 g kg^{-1}); PCT, positive control diet supplemented with thyme powder (7.50 g kg^{-1}). [3] SOD, superoxide dismutase activity; GSH-Px, glutathione peroxidase activity; CAT, catalase; MDA, malondialdehyde. [4] SEM, standard error of the mean.

Discussion

The addition of thyme and rosemary combination in broiler positive control diets (PCRT) improved ADG and FCR compared with the PC. The ADFI was statistically non-significantly differed in the thyme- and rosemary-treated groups compared to control. The results are confirmed by the study done by Nameghi et al. (2019). Feizi and Bijanzad (2010) reported that the addition of 200 ppm thyme extract in drinking water of broilers had a beneficial effect on ADG and FCR compared with control birds (NC). A similar response was reported by Al-Kassie (2009), in which birds received 1% rosemary herb supplementation in the diet, had higher ADG and lower FCR compared with control birds (NC). The major components of thyme and rosemary are thymol, carvacrol, ρ-cymene, 1,8-cineol, carnosic acid, carnosol, rosmanol (Botsoglou et al., 2005; Nameghi et al., 2019). The beneficial effects of thyme and rosemary on ADG and FCR may be due to these active components by the improvement of digestibility of feed and the absorption of the nutrients as described by Nameghi et al. (2019).

This study found that supplementation of thyme and rosemary in broiler diets does not affect carcass yield, but could, to some extent, improve carcass quality. The relative weight of abdominal fat was decreased statistically by supplementation of thyme and rosemary combination compared to PC birds which were in line with the results of Al-Kassie (2009), who reported lower abdominal fat weight in broiler chickens fed diet

supplemented by 200 ppm thyme essential oil. Similar slaughter results with the use of rosemary powder in chicken diets were obtained by Ghazalah and Ali (2008), who argued that carcass yields and heart shares did not differ significantly between groups, although a lower proportion of abdominal fat was determined in chickens fed mixtures with the addition of 0.5% rosemary compared to the control. This can be explained by the effect of active components of thyme and rosemary on the CHOL metabolism, because of a significant correlation obtained between the serum CHOL and relative weights of abdominal fat (Zhang et al., 2009).

However, the inclusion of thyme and rosemary powders in broiler diet did not alter ($p > 0.05$) breast muscle, heart, spleen and thymus yields at 42 d of age which is in agreement with the results of Rahimi, Teymori Zadeh, Torshizi, Omidbaigi, and Rokni (2011) and Nameghi et al. (2019). In the current study, our findings demonstrated that the relative weight of bursa of Fabricius was increased in response to thyme and rosemary blend supplementation, which was consistent with the findings of Nameghi et al. (2019). Similar positive results for bursa of Fabricius were observed by Osman, Yakout, Motawe, and El-Arab (2010), who reported an increase of bursa of Fabricius relative weight at 1.0% rosemary supplementation. Thus, these studies suggested that the thyme and rosemary can positively affect the development of lymphoid organs and consequently a putative effect on immunity modulation; however, more researches are necessary to determine the potential interactions of thyme and rosemary with other bioactive compounds.

The inclusion of thyme and rosemary powders in broiler diets change ileal microflora toward increasing *Lactobacillus* and decreasing *E. coli*. These results are in agreement with previous works (Rahimi et al., 2011; Norouzi, Qotbi, Seidavi, Schiavone, & Marín, 2015). Saki et al. (2014) showed that birds received 200 ppm thyme essential oil in drinking water, had higher ($p < 0.05$) ileal *Lactobacillus* and lower ($p < 0.05$) *E. coli* enumeration compared to the control birds. Moreover, Mathlouthi et al. (2012) found that rosemary essential oil was *in vitro* effective against *E. coli*, although its antimicrobial activity was lower than that of oregano oil. Herb derivatives may affect an increase in the production of lactic acid bacteria, thus increasing the population of beneficial bacteria and reducing the presence of pathogen bacteria (Nameghi et al., 2019). Active compounds of thyme and rosemary can reduce the various intestinal infections as a result of its high activity to resist pathogenic bacteria, as well as the increase of mature enterocytes of which increases the villus height, decreases the depth of the crypt, and increase their absorption efficiency (Zeng, Zhang, Wang, & Piao, 2015). The result of our study was supported previously by Nameghi et al. (2019). Although, Menati, Ali, and Abidelhuseen (2018) reported no significant difference in villus height and crypt depth in birds fed by a diet supplemented an extract of thyme.

For sera Glu concentration after 24 and 42 days, we found no variations of significance between all treatments. Our results regarding the serum lipid profile showed that supplementation of thyme and rosemary powders in broiler diets decreased the total serum CHOL, TG, HDL and LDL concentrations which were in agreement with other studies (Abdulkarimi, Daneshyar, & Aghazadeh, 2011; Nameghi et al., 2019). The reduction in serum CHOL level can be attributed to the down-regulatory effect of phenol and flavonoids in thyme and rosemary on 3 hydroxy-3-methyl-glutaryl-CoA reductases, the rate-limiting enzyme of CHOL synthesis as described previously by Crowell (1999).

Liver cells contain a high concentration of ALT, AST and ALP enzymes. Destruction of the liver cells leads to the leakage of these enzymes into the bloodstream and an increase in their concentration. The results of this study confirmed the important role of thyme and rosemary in controlling the liver function which is consistent with the results of Nameghi et al. (2019) who reported a significant role of thyme essential oil to suppress ($p < 0.05$) liver enzymes activity in broilers.

Lipid peroxidation leads to the formation of various products as the MDA. Therefore, blood MDA level is often determined in some studies as an indicator of lipid peroxidation in the body (Kakhki et al., 2016). In a biological system, many oxidation reactions are essential for our survival. Sometimes, inside the normal cells, oxidation reactions release uncontrolled reactions and produce unstable oxygen molecules, which called free radicals. These produced compounds will react with many different vital molecules in vital organs like lipid, protein, and DNA forming a new compound that damages DNA (Kakhki et al., 2016). Antioxidants are the first line of defense against free radical damage and are critical for maintaining optimum health (Lobo et al., 2010). Furthermore, herbs that are rich in such flavonoids as thymol, carvacrol, carnosic acid, and carnosol extend the activity of vitamin C, act as antioxidants and may enhance the immune function (Nameghi et al., 2019). The data of the present study revealed that the supplementation of thyme and rosemary combination to the diet of broiler chickens reduced the sera MDA concentration, which indicates a decreased lipid peroxidation. This result came following with that

obtained in the study of Abd El-Hack and Alagawany (2015) and Yildirim, Tunc, Gül, Yildirim, and Yıldız (2018) where they recognized a reduction in serum MDA of birds fed 0.9% thyme powder and 0.2% rosemary extract, respectively.

Conclusion

It can be concluded that the supplementation of the blend of thyme and rosemary powders in broiler diets at a level of 0.75 g kg^{-1} could promote growth performance, carcass yield, and antioxidant activities in broiler chickens. Thus, they have the potential to be used as a natural antioxidant to broiler diets formulated by poultry by-product meal.

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