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Effects of Thymol and Carvacrol on Productive Performance, Antioxidant Enzyme Activity and Certain Blood Metabolites in Heat Stressed Broilers

Research Article

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ABSTRACT

The present study was carried out to evaluate the effects of thymol and carvacrol supplementation on performance, immune response, antioxidant enzyme activity and blood parameters of heat stressed broilers. Broilers were fed with commercial diet till 25 d of age, then they were allocated to a completely randomized design with a 3 × 2 factorial arrangement with 6 treatments including 3 levels of carvacrol (0, 300 and 500 mg/kg of diet) and 2 levels of thymol (0 and 250 mg/kg of diet) in 4 replicates of 9 birds each. To induce heat stress, diurnal cyclic temperature at 35 ˚C for 8 h from 09:00 h to 17:00 h, were used from 26 day of age until the end of the experiment. Dietary carvacrol did not affect performance parameters of broilers but feeding 250 mg/kg thymol increased body weight gain and decreased feed conversion ratio by 6 and 4%, respectively ($P<0.05$). Red and white blood cells, hematocrit and hemoglobin were not influenced by treatments, but heterophile and heterophile to lymphocyte ratio decreased in the birds fed on thymol- and carvacrol- added diets ($P<0.05$). Carvacrol and thymol supplementation decreased ($P<0.05$) serum cholesterol level but had no effect on triglyceride, low and high density lipoproteins and glucose. Both thymol and carvacrol elevated ($P<0.05$) serum glutathione peroxidase activity compared to the control group. Carcass and breast percentage were increased ($P<0.05$) in the birds received thymol-added diet ($P<0.05$). No carvacrol and thymol effect were observed on relative weight of internal organs in broilers. It was concluded that dietary thymol at an inclusion rate of 250 mg/kg diet might positively modulate the negative effect of heat stress in broiler chickens through improved feed conversion ratio (FCR), increased water intake and antioxidative potential of blood tissue.

KEY WORDS essential oil, glutathione peroxidase, heterophil, lymphocyte, stress.

INTRODUCTION

High ambient temperature imposes severe stress on birds and leads to important economic losses in poultry industry. The thermo neutral zone for optimum performance has been verified at 18 to 22 ˚C for growing broilers (Lin *et al.* 2006). When ambient temperature exceeds thermo neutral temperature, heat stress occurs with detrimental consequences including decreased feed consumption, lower body weight gain (Quinteiro-Filho *et al.* 2010), immune suppression, lipid peroxidation (Sohail *et al.* 2011) and increased mortality in broiler chicken. In heat-stressed broilers with decreased feed intake, metabolism energy expenditure increases to drive out extra body heat through evaporation, therefore less energy is used for growth, leading to the retarded weight gain (Lei and Slinger, 1970). In addition, exposure to high ambient temperature modifies certain components of immune system such as T-cell counts, cyto-

kine secretion, antibody production, lymphocyte proliferation, and serum immunoglobulin concentrations (Zulkifli *et al*. 2000). Heat stress increases lipid per oxidation as a consequence of increased free radical generation, a condition that enhances the formation of reactive oxygen species (ROS) and induces oxidative stress in many tissues. Therefore, antioxidant enzymes (catalase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)) increase to protect more susceptible cells and tissues from harmful effects of ROS (Altan *et al*. 2003). Gene expression regulation for these enzymes and increased demand for complementary endogenous as well exogenous (dietary antioxidants) elements helping them to overcome the important physiological responses, in terms of animal response to stress conditions.

Many herbs, botanicals and phyto-derivations, including essential oils have shown to exert antioxidative properties based mainly on their phenolic compounds (Wallace *et al*. 2010). Many research works reported that natural polyphenolic additives could recover the weakened immune response in heat stressed birds through scavenging free radicals and peroxides in immune cells (Bub *et al*. 2003).

Moreover, phylogenic essential oils are potentially able to influence gastrointestinal microflora and positively affect nutrients amelioration leading to improved growth performance (Windisch *et al*. 2008). Thymol, a major component of thyme essential oil, has been widely studied for its antimicrobial and growth promoter abilities (Cross *et al*. 2007). Carvacrol, an isomer of thymol, is found in essential oils isolated from oregano, thyme, marjoram, summer savory (Guenther, 1949) and particularly in Satureja khuzistanica (Khosravinia, 2013). Administration of natural plant bioactive chemicals and also blends of these phytochemicals as supplements to combat disease and stress in growing chickens are developing as a significant area of interest in the world poultry industry (Cross *et al*. 2003).

Phytogenic feed additives may have multifaceted mode of action, including modifying feed color and flavor, stimulating the secretion of digestive enzymes, increasing gastric and intestinal motility, endocrine stimulation, antimicrobial activity, coccidiostat activity, immune stimulation, anti-inflammatory and anti-oxidative activity (Lee *et al*. 2003).

Like other feed additives, the use of herbs and phytoextracts in broiler diets or into drinking water for birds raised under standard production conditions including thermo neutral circumstances were investigated frequently. However, the efficacy of such products in broilers kept in stress conditions, including presence of unfavorable environmental temperatures, compromised health and / or low nutrient content of the diet have not been characterized in details.

There are few reports dealing with single and combined effects of thymol and carvacrol on performance and immune response of heat-stressed chicks in literature warranting further examination of the subject. Therefore, this study was conducted to study the effects of dietary thymol and carvacrol related to stress in diets on growth performance, immunity, antioxidant enzyme activity, blood parameters, carcass and gastrointestinal characteristics in heat stressed broiler chicken.

MATERIALS AND METHODS

Broilers, housing and feeding

A total of two hundred sixteen one-day old-male Ross-308 broiler chicks were obtained from Mahan hatchery (Kerman, Iran) and allocated in 24 floor pens (furnished with wood shavings) of 9 birds each. House temperature was kept at 33 ˚C during the first 3 day of age. Thereafter, temperature was decreased by 3 ˚C per week to reach 24 ˚C at 21 days of age. After that, they were maintained at approximately 24 ˚C until 25 days of age, thereafter, diurnal cyclic temperature at 35 ˚C (with 50-60% relative humidity) for 8 h daily from 09:00 h to 17:00 h and 24 ˚C from 17:00 h to 07:00 h (the temperature was gradually increased from 24 ˚C to 35 ˚C during 2 hours) were used starting from day 26 until the end of the experiment. A 23:1 lighting to darkness regimen was provided to the birds throughout the experimental period.

Broilers were fed with a commercial basal diet through day 5 of age and then were given six experimental diets arranged in a 3 × 2 factorial fashion with 3 levels of carvacrol; 0, 300 and 500 mg/kg and 2 levels of thymol; 0 and 250 mg/kg. Each diet was randomly fed to 4 groups of chicks. Diets were formulated to meet the nutrient requirements according to Ross-308 rearing guideline (Aviagen, 2007).

Diets in mash form and water were provided *ad libitum* throughout the experimental period. The ingredients and chemical composition of the basal diets are shown in Table 1.

Growth performance

Considering pen as experimental unit, body weight gain (BWG) and feed intake (FI) were measured at day 26 and 42 of age. Feed consumption was calculated by subtracting residual feed from the offered feed. Data for feed consumption and BWG were used to calculate the feed conversion ratio (FCR). The FCR was adjusted for mortality.

Serum biochemical analysis

After 2 h starvation, two birds per replicate were randomly selected and 2 mL of blood samples were collected from the wing vein by a syringe on day 42 of age. Blood samples were kept in labeled sterile test tubes and centrifuged at 3000 × g for 10 min for serum collection.

Serum triglyceride (mg/dL), total cholesterol (mg/dL), high density lipoprotein (HDL, mg/dL), low density lipoprotein (LDL, mg/dL) and glucose (mg/dL) concentrations and serum glutathione peroxidase (GSH-Px, U/mL) activity were measured using an autoanalyzer (HITACHI 912, Japan).

The analyzer employed enzymatic procedures using SEPPIM Diagnostic Kits (SEPPIM SAS., Zone Industrielle, 61500, SEES, France) in two replicates, at 25 ºC, based on the methods described by Elliott and Dover (1985).

Table 1 The ingredients and calculated nutrient contents of the basal diets

Ingredient (%)	Starter (1 to 10 d)	Grower (11 to 25 d)	Finisher (26 to 42 d)
Corn	53.38	54.81	60.63
Soybean meal	38.45	36.23	30.85
Vegetable oil	3.49	5.00	4.85
Limestone	1.54	1.35	1.30
Dicalcium phosphate	1.64	1.29	1.21
Common salt	0.29	0.29	0.29
DL-methionine	0.29	0.29	0.25
L-lysine	0.23	0.23	0.13
Vitamin and Mineral premix[1]	0.50	0.50	0.50
Calculated nutrient contents			
Metabolisable Energy (kcal/kg)	3025	3150	3200
Crude protein (%)	22.0	21.0	19.0
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42
Lysine (%)	1.43	1.30	1.09
Methionine + cystine (%)	1.07	0.95	0.86

[1] Supplied per kg of diet: vitamin A: 10000 IU; vitamin D_3: 3500 IU; vitamin E: 60 mg; vitamin K_3: 3 mg; vitamin B_{12}: 0.1 mg; Thiamine: 3 mg; Riboflavin: 6 mg; niacin, 40 mg; Pyridoxine: 5 mg; Pantothenic acid: 11 mg; Folic acid: 1 mg; Biotin: 0.15 mg; Cholin chloride: 500 mg; Etoxycoin: 150 mg; Fe: 60 mg; Zn: 60 mg; Mn:100 mg; Cu: 10 mg; I: 1.6 mg and Se: 0.15 mg.

Size of different organs

At Day 42 of experiment, two broilers per replicate whose body weights were closest to the mean weight of the pen were selected and killed by severing the jugular vein and carotid artery, plucked and eviscerated. Whole carcass, breast, thigh, abdominal fat, gizzard, liver, pancreas, bursa of fabricius and spleen percentage were measured individually.

Hematological measurements

At 42 day of age, 2 broilers per replicate were selected and 0.5 mL of blood samples was collected in heparin containing tubes to avoid blood clot formation for hematological analysis. Blood smears were prepared on slides and stained by Gimsa stain. One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under a light microscope and then heterophile to lymphocyte (H/L) ratio was calculated. The white blood cells (WBC) and red blood cells (RBC) counts were determined by an improved Neubauer hemocytometer method (Jain, 1986). The hematocrit (HCT) and hemoglobin (Hb) values were measured by microhematocrit and colorimetric cyanomethemoglobin methods, respectively (Baker and Silverton, 1985).

Statistical analysis

Collected data were subjected to ANOVA using GLM procedure of SAS (SAS, 2001). Analysis of variance was performed using a completely randomized design with a factorial arrangement of treatments. All percentages data were transferred to arc-sin before statistical analysis. Data were statistically tested for main effects of dietary thymol and carvacrol levels and their interactions. Means were partitioned for significant differences using the Tukey multiple range test. Statements of significance are given based on $P < 0.05$ for all variables.

RESULTS AND DISCUSSION

Growth performance

The effect of thymol and carvacrol on growth performance of male broilers subjected to heat stress from 26 to 42 day of age is shown in Table 2.

Table 2 Effects of thymol and carvacrol on growth performance of broilers subjected to heat stress from 26 to 42 d of age

Effects	Feed intake (g/bird)	Body weight gain (g/bird)	FCR[1]
Carvacrol (mg/kg of diet)			
0	2277.15	1365.00	1.67
300	2283.47	1352.92	1.68
500	2305.90	1365.56	1.69
SE	8.26	8.10	0.01
Thymol (mg/kg of diet)			
0	2285.88	1328.01[b]	1.72[a]
250	2291.81	1394.30[a]	1.64[b]
SE	6.74	6.61	0.01
P-value			
Carvacrol	0.06	0.47	0.43
Thymol	0.54	< 0.0001	< 0.0001
Carvacrol × thymol	0.053	0.42	0.15

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
FCR: feed conversion ratio.
SE: standard error.

Dietary carvacrol did not affect BWG, FI and FCR in broiler chickens during days 26 to 42 of age. Supplementation of diet with 250 mg thymol/kg increased BWG and decreased FCR (P<0.05) by 6 and 4%, respectively. There was no thymol × carvacrol interaction on performance parameters, however minute improvement in BWG and FCR was observed in the birds fed on diets containing 500 mg

carvacrol/kg concomitant with 250 mg thymol/kg. The effects of dietary thymol and carvacrol on feed and water intake 09:00 h to 17:00 h (heat stress hours) in male broilers from 26 to 42 day of age are shown in Table 3. No change in FI for thymol or carvacrol receiving birds was observed but 250 mg/kg thymol increased water intake in heat temperature hours (P<0.05).

Table 3 Effect of thymol and carvacrol on feed and water intake of broilers between 09:00 h to 17:00 h (heat stress hours)

Effects	Feed intake (g/bird/d)	Water intake (mL/bird/d)
Carvacrol (mg/kg of diet)		
0	57.59	196.95
300	54.89	199.48
500	58.71	200.54
SE	2.20	3.39
Thymol (mg/kg of diet)		
0	55.92	193.49[b]
250	58.21	204.48[a]
SE	1.80	3.59
P-value		
Carvacrol	0.46	0.84
Thymol	0.38	0.04
Carvacrol × thymol	0.47	0.08

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

Carcass characteristics

The effects of dietary levels of thymol and carvacrol on carcass characteristics in male broiler chicken exposed to high ambient temperatures at 42 day of age are presented in Table 4. No treatments effects were observed on carcass characteristics and organ weights with the exceptions of carcass and breast percentage. Carcass and breast relative weights were increased (P<0.05) in the birds fed on diets supplemented with 250 mg thymol/kg.

Hematological analysis

The effect of thymol and carvacrol on immunological parameters of male broilers chickens exposed to heat stress at 42 day of age is shown in Table 5. Red blood cells and WBC count, HCT and Hb, were not influenced by dietary treatments. Heterophile percentage and H/L ratio were decreased by the inclusion of thymol and carvacrol and lymphocyte percentage was increased in birds fed on diets with thymol (P<0.05). There was no interaction between dietary thymol and carvacrol on immunological parameters.

Serum biochemical analysis

The effects of dietary thymol and carvacrol on certain serum metabolites concentration in male broilers subjected to heat stress at 42 day of age are shown in Table 6. Addition of thymol and carvacrol to the diets decreased (P<0.05) serum cholesterol level while had no effect on serum triglyceride, LDL, HDL and glucose concentrations. Supplementation of diet with thymol and carvacrol elevated (P<0.05) serum GSH-P_X activity compared to the control broilers. There was no significant dietary thymol into carvacrol interaction effect from serum metabolites concentrations.

In this study, dietary carvacrol exhibit no promising effects on BWG and FCR of the broiler chicken in days 26 to 42 of age when birds maintained under high ambient temperature. It has been shown that supplementation of drinking water with high doses of a carvacrol riched essential oil (ranging from 500 to 2500 mg/L) adversely affected production parameters in broilers in 1 to 28 days of age (Khosravinia, 2013). The same researchers found that lowering the carvacrol in water to 200, 300 or 400 mg/L, compared to the treated birds with 500 mg carvacrol/L could effectively compensate their weight gain during 29 to 42 days of age. These findings are in consistent with results of Lee *et al*. (2003), who reported 2 percent increase in average daily gain of broiler chicken by inclusion of 0.2 g carvacrol/kg in diet. Addition of thymol, an isomer of carvacrol, at the same dose caused 3% decrease in daily weight gain (DWG), a results which disagree with the effects exerted by dietary thymol in the current study. Lee *et al*. (2003) did not report water intake of the treated birds. However, the differences in the results could be endorsed by physiological status of the birds where in the current study birds were not raised in standard conditions. In agreement with the findings of the current study, Basmacioglu *et al*. (2004) reported that dietary oregano extract (a natural product rich in carvacrol) at 0.15 g/kg decreased DWG in broiler chicken by 2 percent compared to control group. With doubling the dietary dose (0.3 g/kg), however, the same researchers observed +2% difference in the same parameter compared to control group.

The unchanged FI for the both carvacrol- and thymol-treated birds agreed the findings of Lee *et al*. (2003) but disagreed the results reported by Basmacioglu *et al*. (2004) who reported addition of 0.2 g carvacrol and 0.15 g oregano extract in each kg of diets caused +2 and -6% difference in FI of treated birds compared with the relevant control groups.

The results on FCR in the current study were disagreed with findings of almost all other reports. In this study, carvacrol caused no change but thymol improved FCR by 4% compared with control birds. These results are to some extent in discord with the findings of Lee *et al*. (2003) and Basmacioglu *et al*. (2004) who found decreased FCR for the carvacrol-received birds. Such difference may reason by differences in managerial practices applied and physiological state of the birds.

Table 4 Effect of thymol and carvacrol on relative weights (% of live BW) of carcass parts of broilers subjected to heat stress at 42 d of age

Effects	Carcass	Breast	Thigh	Abdominal fat	Gizzard	Liver	Pancreas	Burs	Spleen
Carvacrol (mg/kg of diet)									
0	88.40	29.97	22.58	1.59	2.42	1.99	0.23	0.09	0.10
300	88.25	31.17	23.43	1.51	2.36	2.09	0.24	0.11	0.11
500	88.90	32.07	24.02	1.50	2.44	2.06	0.24	0.11	0.11
SE	0.59	0.83	0.67	0.93	0.12	0.04	0.006	0.006	.004
Thymol (mg/kg of diet)									
0	87.68^{b}	29.27^{b}	22.47	1.63	2.43	2.01	0.23	0.10	0.10
250	89.35^{a}	32.84^{a}	24.22	1.44	2.37	2.09	0.25	0.11	0.11
SE	0.48	0.68	0.69	0.07	0.10	0.06	0.009	0.005	0.006
P-value									
Carvacrol	0.73	0.24	0.51	0.73	0.83	0.26	0.91	0.25	0.90
Thymol	0.03	0.001	0.09	0.10	0.64	0.08	0.11	0.42	0.21
Carvacrol × thymol	0.56	0.55	0.63	0.81	0.74	0.18	0.96	0.23	0.93

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

Table 5 Effect of thymol and carvacrol on immunological parameters of broilers subjected to heat stress at 42 d of age

Effects	**RBC (10^6/µL)**	**WBC (10^3/µL)**	**HCT (%)**	**Hb (g/dL)**	**H**	**L**	**H:L**
Carvacrol (mg/kg of diet)							
0	2.52	236.51	33.74	8.97	18.00^{a}	83.25	0.22^{a}
300	2.60	238.08	33.88	8.71	17.00^{a}	83.75	020^{b}
500	2.60	239.39	33.04	8.66	16.63^{b}	83.75	0.19^{b}
SE	0.05	6.17	0.62	0.10	0.30	0.38	0.004
Thymol (mg/kg of diet)							
0	2.55	236.96	33.86	8.87	17.66^{a}	82.75^{b}	0.21^{a}
250	2.60	239.02	33.21	8.69	16.75^{b}	84.42^{a}	0.19^{b}
SE	0.04	5.04	0.51	0.08	0.25	0.31	0.01
P-value							
Carvacrol	0.40	0.95	0.63	0.10	0.01	0.58	0.009
Thymol	0.38	0.77	0.39	0.14	0.02	0.001	0.002
Carvacrol × thymol	0.82	0.94	0.96	0.28	0.36	0.052	0.24

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.
RBC: red blood cell; WBC: white blood cell; HCT: hematocrit; Hb: hemoglobin; H: heterophile; L: lymphocyte and H:L: heterophile to lymphocyte ratio.

Table 6 Effect of thymol and carvacrol on serum parameters of broilers subjected to heat stress at 42 d of age

Effects	**Triglycerid (mg/dL)**	**Cholesterol (mg/dL)**	**HDL (mg/dL)**	**LDL (mg/dL)**	**Glucose (mg/dL)**	**GSH-Px[1] (U/mL)**
Carvacrol (mg/kg of diet)						
0	107.13	117.90^{a}	86.38	17.75	221.00	175.93^{b}
300	104.00	111.44^{ab}	84.63	17.76	218.00	182.62^{a}
500	103.00	107.96^{b}	85.75	16.88	212.75	184.26^{a}
SE	2.69	2.39	4.37	0.68	8.11	4.74
Thymol (mg/kg of diet)						
0	105.33	115.44^{a}	84.50	17.43	219.17	177.95^{b}
250	104.08	109.43^{b}	86.67	16.88	215.33	183.92^{a}
SE	2.2	1.95	3.57	0.56	6.62	1.16
P-value						
Carvacrol	0.54	0.03	0.96	0.87	0.77	0.001
Thymol	0.69	0.04	0.67	0.50	0.68	0.002
Carvacrol × thymol	0.94	0.65	0.94	0.93	0.97	0.06

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.
HDL: high density lipoprotein; LDL: low density lipoprotein and GSH-Px: glutathione peroxidase activity.

Deyoe *et al.* (1962) showed that the flavor of chickens' diets can stimulate or depress feed intake. Generally, an improvement in gain: feed ratio (or FCR) in broilers when feeding phytogenic has been evidenced in the majority of the studies recently reviewed by Brenes and Roura (2010), who concluded that in most studies the improvement in FCR comes as a result of a reduced FI at a largely unchanged BWG. In this study, the improved FCR was concomitant not only with decreasing FI but also with the increasing BWG with the thymol-added diet.

Case *et al*. (1995) reported that dietary carvacrol and thymol, at 150 mg/kg, did not influence BWG of cockerels with initial weights of 126 g in a 21 d feeding trial. In an experiment with female broilers, Lee *et al*. (2003) also found lack of difference in growth performance and digestive enzyme activity of broiler chicken fed on diets containing 100 mg thymol/kg for a period of 6 wk compared with corresponding control birds. No beneficial effects for growth performance were seen when oregano essential oil was added at 50 and 100 mg/kg of a wheat soybean meal based diet (Botsoglou *et al*. 2002) or when thymol, cinnamaldehyde and a commercial phytogenic preparation were included at 100 mg/kg of a maize soybean meal based diet (Lee *et al*. 2003) or when wheat-maize-soybean meal based diet were supplemented with two commercial products containing a blend of three phytogenic compounds at 200 mg/kg and 500 mg/kg levels (Hernandez *et al*. 2004). Thymol increased water intake, which agreed with the earlier work (Lee *et al*. 2003). However, carvacrol did not affect water intake.

In the present study, Thymol increased water consumption, which agreed with the earlier work (Lee *et al*. 2003). However, carvacrol did not affect water consumption. Water is involved in every aspect of broiler metabolism with playing important roles in regulating body temperature, digesting food and eliminating body wastes. At normal temperatures, poultry consume at least twice as much water as feed. When heat stress occurs, water intake will increase.

Some researchers verified that the bitter-tasting essential oils adversely affect water intake in broiler flocks (Lee *et al*. 2004a; Lee *et al*. 2004b). In many other studies the quality of drinking water, but not with special reference to additives, have been considered (Barton, 1996; Grizzle *et al*. 1997). Unfortunately, effects of flavors on chickens performance has not been investigated in details since based on early indications most researchers believe that broiler may not actually respond to flavor when compared to mammals (Moran, 1982). Recently it has been shown that dietary administration of carvacrol to broiler chicks reduced feed intake by modulating the birds' appetite (Lee *et al*. 2003).

Heterophile to lymphocyte ratio was significantly depressed in broilers fed thymol and carvacrol. This is in agreement with Khaksar *et al*. (2012a), who showed lower H/L ratio in broilers fed a wheat-based diet contained thyme. Heterophile to lymphocyte ratio has been widely used as a physiological indicator for different forms of stress (Maxwell, 1993). The reduced H/L ratio for thymol-received birds in the present study demonstrated positive effect of thymol in diminishing stress in broilers which may exert through modified gastrointestinal microflora and enhanced immune system which justified better immune responses.

No change in hematological parameters was found in the birds fed on diets supplemented with thymol, carvacrol or both compounds. In contrary to our results, chicks fed on thyme containing essential oils showed lower heterophil and higher lymphocyte counts compared to the birds received control diet (Najafi and Torki, 2010). Al-Kassie (2009) showed that feeding diets supplemented with oil extract derived from thyme and cinnamon to broilers significantly increased WBC, RBC, HCT and Hb values compared to control group.

In this study broiler chickens fed on diets supplemented with carvacrol and thymol showed lowered serum cholesterol concentration. Isoprenoids such as thymol, carvacrol and α-ionone have been shown to lower plasma cholesterol concentration in chickens (Case *et al*. 1995). The hypocholesterolemic effect of thymol and carvacrol has been ascribed to inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate controlling enzyme of the cholesterol synthetic pathway (Elson, 1995).

However, Khosravinia (2013) proposed the possibility of testosterone-coupled hypolipidemic properties for carvacrol in broiler chicken. The same report demonstrates that carvacrol may modify the lipids metabolism in broiler chicken through altered steroids metabolism.

During oxidative stress, changes in the level of GSH could alter the activity of GSH-Px, ultimately changing the removal of free radicals and hydroperoxides. Sahin *et al*. (2003) found that heat stress reduced SOD, catalase and GSH-Px activity in broiler chicken. In the present study, dietary treatments prevented the decrease of GSH-Px activity during the oxidative stress indicating that excessive free radicals may be removed by dietary thymol and carvacrol, due to the presence of phenolic OH groups which act as hydrogen donors to the proxy radicals produced during the first step in lipid oxidation, thus reducing the hydroxyl peroxide formation (Yanishlieva *et al*. 1999). The serum and liver SOD and GSH-P_X activities results obtained in a study of Hashemipour *et al*. (2013) imply that the active substances of the phytogenic product may improve the antioxidative status of broilers due to the antioxidant property of thymol and carvacrol by elevating the activity of antioxidant enzymes. In line with our results, Youdim and Deans (1999) reported that dietary supply of thyme oil or thymol to ageing rats showed a beneficial effect on the antioxidative enzymes SOD and GSH-Px as well as on polyunsaturated fatty acid composition in various tissues. Animals receiving these supplements had higher enzyme levels and higher concentrations of polyunsaturated fatty acids in phospholipids of the brain than the untreated control (Youdim and Deans, 2000). Oregano essential oil added in doses of 50-100 mg/kg to the diet of chickens exerted an antioxidant effect in the animal tissues (Botsoglou *et al*.

2002). Such antioxidant effects would be expected to improve the health of poultry livestock.

Carcass and breast percentage were increased by dietary inclusion of thymol. These results agreed finding of Khaksar *et al.* (2012b) who reported supplementation of thyme essential oil in Japanese quail diet resulted in greater carcass and breast percentages, but no change in other carcass characteristics such as relative weight of thighs, spleen, liver and abdominal fat were observed by the same feed additive.

The relative weights of pancreas, spleen, liver and heart were not affected by thyme powder or essential oil (Hernandez *et al.* 2004; Basmacioglu *et al.* 2010). However, Lee *et al.* (2003) determined an increase in relative weight of liver in the birds given thymol, but this was seen only at the age of 21 day and not at 40 day. Thyme leaves increased the abdominal fat pad at 42 day of age (Ocak *et al.* 2008). The relative weights of lymphoid organs were not affected in the birds fed on diets with thyme and carvacrol, these results were in line with findings of Rahimi *et al.* (2011) who showed no differences in the relative weight of spleen and bursa in broilers fed diet containing thyme compared to control birds.

CONCLUSION

Results of this study showed dietary thymol at an inclusion rate of 250 mg/kg diet might counteract the negative effect of heat stress in broiler chickens as shown by improved BWG and FCR, and increased water intake. The lower H/L ratio observed in broilers fed on diets containing thymol and carvacrol implies the positive influence of these natural products on alleviating stress in broilers through improved anti oxidative status of blood tissue.

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