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Effect of *Thymus vulgaris* and *Satureja khuzestanica* Ethanolic Extracts on Broiler Chickens' Performance and Immune Response

Research Article

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ABSTRACT

An experiment was conducted to investigate the effects of thyme (*Thymus vulgaris*) and satureja (*Satureja khuzestanica*) ethanolic extracts on the performance, blood metabolites and immune response of broiler chickens. 300 day-old Ross chicks were assigned to six dietary treatments in a randomized 2 × 3 factorial block design. Each treatment was given to five replicates of ten birds. Variables were *T. vulgaris* extract (0% or 1%) and *S. khuzestanica* extract (0%, 1% or 2%) in drinking water. Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) was recorded at the end of the experiment. Serum glucose, total protein (TP), triglycerides (TG), low-density lipoprotein cholesterol (LDL-ch) and high-density lipoprotein cholesterol (HDL-ch) were measured after blood sampling at 42 days of age. Specific IgG and IgM against sheep red blood cells (SRBC) were quantified six days after the injection of SRBC into breast muscle on day 23 and day 30. The plant extracts did not affect BW, FI or FCR, or the relative weights of the cloacal bursa, spleen or thymus gland (P>0.05). *S. khuzestanica* extract increased TG, total cholesterol and HDL-ch (P<0.05). The plant extracts did not affect the humoral immune response against SRBC (P>0.05). However, 2% *S. khuzestanica* extract alone, or 1% *T. vulgaris* extract alone or in combination with 1% *S. khuzestanica* extract increased the heterophil percentage (H) and heterophil:lymphocyte ratio (H/L) (P>0.05), while it diminished the lymphocyte percentage (L) (P>0.05). Breast meat pH, redness (a*), yellowness (b*) and lightness (L*) were not affected by dietary treatments. However 2% and 1% *S. khuzestanica* extract respectively decreased thigh meat's pH 24 h postmortem and its a* and b* values (P<0.05). 1% *T. vulgaris* extract, and 1% and 2% *S. khuzestanica* extract, increased pH in breast meat (P<0.05). It was concluded that under these research conditions, low levels of these extracts decreased H and H/L and may be beneficial to broiler chickens' immunity.

KEY WORDS broiler, immunity, performance, *Satureja khuzestanica* extract, *Thymus vulgaris* extract.

INTRODUCTION

The use of chemical compounds such as antibiotics has been broadly studied in the poultry industry (Mansoub, 2011). To find a suitable substitute to antibiotics and other banned chemotherapeutic drugs, feed additives have been developed using medicinal plants. These plants' important derivatives are secondary metabolic components of low molecular weight such as glucosides, alkaloids, phenolic compounds, terpenoides and essential oils (Al-Shami *et al.* 2011). In contrast to antibiotics, most active components of medical plants are readily absorbed and metabolized in con-

junction with glucoronate and excreted to the urine. Due to short half-life, the risk of tissue accumulation is probably minimal (Kohlert *et al.* 2000). Research on the use of herbal mixtures to substitute for antibiotics in broiler diets has produced inconsistent results (Fritz *et al.* 1993). Some authors reported positive effects on performance (Ertas *et al.* 2005; Peric *et al.* 2008) but others established no effects on body weight gain (BWG), feed intakc (FI) or feed conversion ratio (FCR) (Mikaili *et al.* 2010; Ocak *et al.* 2008).

Thyme (*T. vulgaris*) was used traditionally to treat respiratory disease, and for its anti-microbial and anti-nociceptive properties (Demir *et al.* 2008). Thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-1-2-isopropyl phenol) are the main antibacterial active substances in *T. vulgaris*. Consequently this plant might be used instead of commercial antibiotics. Give its antimicrobial properties (Dorman and Deans, 2000; Rahimi *et al.* 2011) this plant either alone or in combination with other agents might promote the growth of broiler chickens (Khan *et al.* 2012; Mansoub, 2011). The addition of 1 g/kg *T. vulgaris* to broilers' diet increased BWG and feed conversion efficiency (FCE) (Mansoub, 2011). 100 ppm and 200 ppm thyme oil (Al-kassi, 2009) or 2% *T. vulgaris* plant (El-Ghousein and Al-Beitawi, 2009) increased FI, BWG and FCE as well as the dressing percentage and the weights of liver, heart and gizzard and those treatments decreased abdominal fat. Other authors suggested that an absence of effect of thyme on birds' performance may be related to the composition of the diet and ingredients, altering the gut microflora due to the unavailability of substrate, leading to reduced antimicrobial effects of the plant extracts (Lee *et al.* 2003a). A 0.1% dose of thyme for 42 days improved the antibody response in poultry (Rahimi *et al.* 2011). On the other hand, 5 g/kg or 10 g/kg of thyme powder in broilers' diet had no effect on antibody titers against Newcastle and influenza viruses or sheep red blood cells (SRBC) (Toghyani *et al.* 2010).

Thyme products have hypochelestolemic and antilipidemic effects on broiler chickens (Abdulkarimi *et al.* 2011; Al-Kassie, 2009; Dahal and Farran, 2011; El-Ghousein and Al-Beitawi, 2009). Some authors found no effect of thyme on the cholesterol level in birds (Ghasemi *et al.* 2010; Sengül *et al.* 2008). For example, plasma total cholesterol, high-density lipoprotein cholesterol (HDL-ch) and low-density lipoprotein cholesterol (LDL-ch) were not changed by feeding 0.5% or 1% thyme to laying hens.

S. khuzestanica is prevalent in Iran. Its main medicinal component is carvacrol, which has been shown to decrease glucose and malondealdehyde in diabetic patients' serum (Abdollahi *et al.* 2003). This plant's uses as an analgesic and antiseptic in folk medicine resulted from its essential oil. Other constituents being identified in this plant are flavones, triterpenoids, steroids and tannins (Moghaddam *et al.* 2007). Both carvacerol and flavonoids have been found to have antioxidant properties. Oral administration of *S. khuzestanica* essential oil (SKEO) to rats induced antioxidative effects without toxicity or unwanted effects. The human and animal studies of *S. khuzestanica* illustrate this plant's antioxidative potential.

A considerable decrease in the normal lipid peroxidation and an increase in body antioxidant power were reported while the cholesterol level did not change in hyperlipidemic rats (Abdollahi *et al.* 2003).

It has been suggested that acute pre-slaughter stress results in an acceleration of muscle metabolism that continues when the animal is slaughtered. This acceleration leads to a fast decline in muscle pH postmortem while carcass temperatures are still high, which results in protein denaturation (Vosmerova *et al.* 2010). Protein denaturation can result in pale meat with poor water-holding capacity and poor texture. Pre-slaughter stressed animals have unusually high temperatures, rapid glycolysis (falling pH) and early onset of rigor mortis. Although the postmortem changes are rapid, some degree of antemortem muscle temperature rise, lactic acid buildup, and depletion of ATP also occurs. Muscles from pre-slaughter stressed birds usually become pale, soft, and exudative (PSE) after a normal 18-24 h chilling period.

This condition most often results in lower processing yields, increased cooking losses and reduced juiciness (Froning and Uijttenboogaart, 1988). Antemortem stress, including heat-stress struggle before slaughter, has been shown to accelerate glycogen depletion and increase the rate of pH decline, and possibly to result in tough meat (Papinaho *et al.* 1995). The aim of this study was evaluate the effects of *T. vulgaris* and *S. khuzestanica* ethanolic extracts in drinking water on the performance, immune responses and meat quality of broiler chickens.

MATERIALS AND METHODS

Birds and housing management

300 day-old Ross chicks were randomly allocated to a four-floor battery cage. A four-phase feeding program was used: super-starter (1-7 days), starter (8-14 days), grower (15-28 days) and finisher (29-42 days). Corn-soybean based diets were formulated according to standardized ileal digestible (SID) amino acids (Table 1). At 14 days of age, broilers were weighed, grouped with the same average body weight, then assigned to six dietary treatments in a randomized 2×3 factorial block design. Each treatment was given to five replicates of ten birds. Variables were *T. vulgaris* ethanolic extract (0% or 1%) and *S. khuzestanica* ethanolic extract (0%, 1% or 2%) in drinking water.

Table 1 Ingredients and nutrient composition of experimental diets

Ingredient (%)	% In grower diet	% In finisher diet
Corn	62.77	66.23
Soybean meal	28.11	27.69
Corn gluten	3.34	0.00
Vegetable oil	1.00	1.00
Fish meal	2.00	2.00
Methionin	0.16	0.13
Lysin	0.17	0.10
Threonin	0.00	0.00
Dried coffee pulp (DCP)	1.63	0.90
Oyster shell	1.03	1.05
Salt	0.26	0.30
$NaHCO_3$	0.1	0.05
Vitamin premix[1]	0.25	0.25
Mineral premix	0.25	0.25
Calculated nutrient		
AME (kcal/kg)	3000	3070
Crude total protein (TP) %	20.6	18.00
Lysine (SID) %	0.97	0.90
Methionine (SID) %	0.43	0.40
Cystine (SID) %	0.30	0.26
Meth + Syc (SID) %	0.73	0.66
Threonine (SID) %	0.61	0.61
Trytptophan (SID) %	0.21	0.19
Arginine (SID) %	1.08	1.13
Ca %	0.90	0.86
P %	0.45	0.52
Na %	0.20	0.20
Cl %	0.23	0.23
DCAB meq/kg	186	204
Linoleic acid %	1.50	1.50
Fiber %	4.33	4.35

[1] Each kg of vitamin and trace mineral premix provided: vitamin A: 13500 IU; vitamin D_3: 2000 IU; vitamin E: 30 mg; vitamin K_3: 2 mg; vitamin B_1: 1 mg; vitamin B_2: 6 mg; vitamin B_6: 3 mg; vitamin B_{12}: 10 µg; Niacin: 30 mg; Pantothenic acid: 12 mg; Biotin: 0.1 mg; Choline chloride: 500 mg; Fe: 50 mg; Cu: 8 mg; Mn: 80 mg; Zn: 60 mg; I: 0.5: mg; Co: 0.2 mg; Se: 0.15 mg; Monensin sodium: 100 mg and Flavophospholipol: 3 mg.
SID: standardized ileal digestibility and AME: apparent metabolizable energy.

The birds were kept under conventional conditions for vaccination, temperature, ventilation and lighting based on catalogue recommendations, applying standard management practices of commercial broiler production (Ross, 2009). They were fed experimental diets from 15 to 42 days of age. The diets were formulated based on SID amino acids (Hoehler *et al.* 2005) and other requirements following catalogue recommendations (Ross, 2009). Water and feed were supplied *ad libitum* throughout the experiment. Body weight (BW) and FI were recorded every two weeks during the experiment, then feed conversion ratio (FCR) was calculated. At 42 days of age, five birds from each treatment with average BW for that treatment were selected and blood was sampled. Heterophil percentage (H), lymphocyte percentage (L) and heterophil:lymphocyte ratio (H/L) were calculated. Birds were killed by cervical dislocation and the weights of lymphoid organs (cloacal bursa, spleen and thymus gland) were calculated as percentages of BW.

Antibody response to SRBC

In order to investigate humoral immunity, SRBC was used as a T cell-dependent antigen. Two birds from each replicate were injected intramuscularly with SRBC (2.5% suspension in PBS, 1 mL/bird) at 23 days of age, followed by a booster injection eight days later. Blood samples were collected seven days after each injection. The serum from each sample was separated, heat inactivated at 56 ˚C for 30 min and then analyzed for total specific Ig, mercaptoethanol-6-sensitive (MES) specific IgM and mercaptoethanol-resistant specific IgG (Delhanty and Solomon, 1966; Qureshi and Havenstein, 1994). Briefly, 50 µL serum was added to 50 µL PBS (to measure total specific IgG) or to 50 µL 0.01 M mercaptoethanol in PBS (to measure MES IgM) in the first column of a 96-well V-shaped bottom plate. A 1:2 serial dilution was made before adding 50 µL of 2% SRBC suspension to each well. Plates were incubated for 30 min at 37 ˚C. The well immediately preceding a well with a distinct button (agglutinated SRBC) was considered as the endpoint titer for agglutination. The difference between the total Ig response and the specific IgG response was considered to be equal to the IgM antibody response (Cheema *et al.* 2003).

Biochemical parameters detection

Serum glucose, cholesterol, total protein (TP), LDL-ch and HDL-ch were measured in each blood sample.

Transport stress and meat quality parameters

At 43 days of age, five birds from each treatment with average BW for that treatment were selected. They were crated, put in baskets, transported for 2 h at 25 ˚C then slaughtered. After slaughtering, blood samples were recollected and blood metabolites and cell concentration were analyzed. Then one broiler from each replicate was hung by the legs for 10 min to bleed out. Thereafter standard processing was performed and breast and thigh meat samples were collected. Meat pH (Jeacocke, 1977), color (CIE, 1978) and drip loss (Kannan *et al.* 1997) were recorded.

Statistical analysis

Data were analyzed by two-way ANOVA using GLM (SAS, 2001) with *T. vulgaris* and *S. khuzestanica* extracts as main effects. Duncan's multiple range test was used to compare means ($P<0.05$).

RESULTS AND DISCUSSION

Performance

Measurements of broilers' performance are shown in Table 2. The addition of *T. vulgaris* and *S. khuzestanica* ethanolic extracts did not affect BW, FI or FCR across the whole experiment ($P>0.05$). However, *T. vulgaris* extract in-

creased FCR during the grower and finisher stages (14-42 days of age; 1.78 *vs*. 1.84; P<0.05). Previously, supplementation of the basal diet with antibiotics or essential oil was not shown to affect BW, FI or FCR (Jang *et al*. 2007). Other authors (Lee *et al*. 2003a; Sengül *et al*. 2008) reported no differences in live weight between treatment groups, but FI differed (P<0.05) at 0-5 weeks of age of Japanese quails and broiler chickens which received thyme oil or water-soluble extract respectively. The authors suggested that the reduction in FI may have resulted from the bitter taste of the phenolic compounds.

In laying hens, feeding thyme powder did not affect FI and body weight gain (BWG) from 60-70 weeks of age, but the best FCR, the highest weight of eggs and the highest percentage egg production were seen in the group receiving 2% thyme powder (Mansoub, 2011). Others reported that feeding thyme as an antioxidant to laying hens did not affect FI and FCR but the addition of thyme decreased BWG (Ali *et al*. 2007).

Thyme extracts in broiler diets decreased FCR but FI and BWG were not affected (Rahimi *et al*. 2011). The level of thyme powder or extract used can affect results so that a low dosage (5 g/kg) has been shown to affect BW and FCR, while a high dosage (10 g/kg) did not. Improved FCR in thyme-treated groups could have been due to this plant's antibacterial and antifungal effects which can decrease populations of harmful microbes in the digestive system, improving birds' immunity and performance (Toghyani *et al*. 2010).

Harmful microbes in the digestive system cause increased degradation of proteins and amino acids, decreased activity of these molecules and rapid decomposition of these molecules due to bacterial secretary substances such as urease. Plant-extracted oils had no effect on broilers' growth performance during the first six weeks of age, while BW, daily BWG, daily FI and FCR were unaffected (Stef *et al*. 2009). Properdine is a euglobulin in the ß and γ globulin fraction of blood serum which, together with lysozyme, is important in non-specific immunity. Oils extracted from medicinal plants had a stimulatory effect on the levels of lysozyme and properdine in blood serum (Stef *et al*. 2009). *S. khuzestanica* contains vitamin A and unknown beneficial factors that may improve chickens' health as well as protecting the birds, resulting in better BW and FCR (Zamani Moghaddam *et al*. 2007). It has been postulated that because of the antioxidant, antifungal and antiseptic activities of *S. khuzestanica* it may protect chickens' feed from oxidation and preserve dietary vitamins. *S. khuzestanica* can protect the feed from damage by mycotoxins. At the same time, the antiseptic activities of this herb may reduce the number of harmful intestinal bacteria and improve feed absorption.

Table 2 Effects of dietary treatments on broilers' performance from 1-42 days of age

Treatment	Parameters		
	BWG (kg)	FI (kg)	FCR
Control	3.88	2.20	1.79
T. vulgaris extract 1%	3.58	2.03	1.79
S. khuzestanica extract 1%	3.80	2.14	1.81
T. vulgaris extract 1% + *S. khuzestanica* extract 1%	3.84	2.09	1.86
S. khuzestanica extract 2%	3.83	2.26	1.73
T. vulgaris extract 1% + *S. khuzestanica* extract 2%	3.71	2.02	1.87
SEM	0.28	0.18	0.063
P-value	0.53	0.52	0.09
ANOVA	P-value		
T. vulgaris extract	NS	NS	*
S. khuzestanica extract	NS	NS	NS
T. vulgaris extract × *S. khuzestanica* extract	NS	NS	NS

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
BWG: body weight gain; FCR: feed conversion ratio and FI: feed intake.
* (P<0.05).
NS: non significant.

Blood metabolites

Measurements of serum biochemistry are shown in Table 3. Serum biochemistry is a labile system which can reflect the condition of the organism and changes happening under the influence of internal and external factors. Glucose, total cholesterol, HDL-ch and LDL-ch were not affected by dietary treatments. *T. vulgaris* extract increased triglycerides (TG; 51.58 *vs*. 61.01; P<0.05) whereas *S. khuzestanica* extract increased total cholesterol (91.52 *vs*. 64.09; P<0.05) and LDL-ch (73.94 *vs*. 50.24; P<0.05). The effect of a low dose of *S. khuzestanica* extract was stronger than the effect of a high dose on total cholesterol (data not shown). The main constituents of *S. khuzestanica* are isopropanoids such as carvacrol, thymol and flavonoids.

SKEO therapy did not influence blood glucose but it decreased hepatic phosphoenol pyruvate carboxykinase activity by 26% and increased hepatic glycogen phosphorylase by 24% (Saadat *et al*. 2004). Disturbance of hepatic glucose metabolism was proposed as a mechanism of the anti-diabetic action of SKEO, which might be related to the antioxidative effect of *S. khuzestanica*. Thus any medicine to alter hepatic gluconeogenesis or glycogenolysis might affect glucose homeostasis. Decreases in fasting blood glucose and TG were reported when SKEO was given to diabetic and hyperlipidemic rats. A decline in normal lipid peroxidation and an increase in body antioxidant power were reported while the cholesterol level did not change in hyperlipidemic rats (Abdollahi *et al*. 2003).

Reductions in TG and cholesterol noticed with thyme in animal studies were attributed to the lowering effect of thymol or carvacrol on 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme

of cholesterol synthesis (Case *et al.* 1995; Lee *et al.* 2003b). However, more in agreement with our results, (Lee *et al.* 2003b) reported that dietary carvacrol and not thymol, reduces plasma TG and phospholipids and suggested that carvacrol may have more impact on lipogenesis than on cholesterol biosynthesis. Thyme extracts decreased low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) but did not affect hematocrit or hemoglobin percentage (Rahimi *et al.* 2011). In agreement with our results (Sengül *et al.* 2008) reported no changes in plasma cholesterol, TG, HDL, LDL or alkaline phosphates of Japanese quails receiving thyme oil or water-soluble thyme extract. In a previous study, it was reported that *S. khuzestanica* decreased fasting blood glucose and TG in diabetic and hyperlipidemic rats whereas in other research it was reported that *S. khuzestanica* extract. Related to the effects of plant extracts on blood biochemical parameters (Case *et al.* 1995) found that feeding of 150 ppm thymol to Leghorn chickens for 21 days reduced serum cholesterol by 9% and dietary carvacrol lowered plasma TG and phospholipids by 12 and 7%, respectively.

These results indicated that dietary carvacrol, but not thymol, may have had more impact on de-novo lipogenesis than on cholesterol biosynthesis in their study (Lee *et al.* 2003a).

However, most of essential oil is known to alter lipid metabolism. Previous studies have shown that hyperlipidemia increases the plasma levels of oxygen free radicals (Prasad and Kalra, 1993) and produces oxidized compounds such as malondialdehyde. From previous discussion it may be concluded that the decreasing of plasma lipids by thyme and anise may be the reason of increasing the plasma antioxidant capacity of hens fed those diets. It has been shown that thymol and carvacrol decreased serum cholesterol levels as they increased microsomal geranyl pyrophosphate pyrophosphatase activity by 2-fold (Vosough-Ghanbari *et al.* 2010).

Supplementation of broiler feed with another *Satureja* species, *S. hortensis*, cannot significantly alter the carcass, abdominal fat, and breast and thigh muscle percentages. It is postulated that inclusion of *S. hortensis* did not affect abdominal fat metabolism (Zamani Moghaddam *et al.* 2007).

Immunity

White blood cell counts and lymphatic organ weights are shown in Table 4. *T. vulgaris* extract decreased L (10.16 *vs.* 11.58; P<0.05) and increased H (75.58 *vs.* 72.91; P<0.05) and H/L (0.12 *vs.* 0.1; P<0.05) whereas *S. khuzestanica* extract did not affect those parameters. 1% *T. vulgaris* extract alone or on combination with 1% *S. khuzestanica* extract increased H and H/L and decreased L (P>0.05) although 1% *T. vulgaris* extract in combination with 2% *S. khuzestanica* extract had the opposite effect on these parameters (P>0.05). On the other hand, 2% *S. khuzestanica* extract in combination with *T. vulgaris* extract significantly decreased heterophil and H/L (P>0.05). None of the dietary treatments affected the weights of cloacal bursa, spleen or thymus gland. In agreement with our results (Al-Kassie, 2009) showed that groups fed diets with oil derived from thyme and cinnamon had lower cholesterol and H/L, and higher RBC (P<0.05). It is probably due to the levels of additives applied in our study. Immune responses to SRBC are shown in Table 5. Those responses were not affected by dietary treatments, except that *S. khuzestanica* extract raised the first IgM measurement (1.56 *vs.* 1.18; P<0.05; data not shown). In poultry production, it is very important to improve immunity in order to prevent infectious diseases. A variety of factors such as vaccination failure, infection by immunosuppressive diseases, and abuse of antibiotics can induce immunodeficiency. Utilization of immune stimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious disease (Chen *et al.* 2003).

Table 3 Effects of dietary treatments on broilers' blood biochemical parameters (mg/dL)

Treatment	Parameters				
	Glucose	Cholesterol	TG	LDL-ch	HDL-ch
Control	140.59	63.44	41.90	51.06	4.00
T. vulgaris extract 1%	160.98	64.76	60.61	49.43	3.20
S. khuzestanica extract 1%	155.13	79.74	47.77	69.14	1.04
T. vulgaris extract 1% + *S. khuzestanica* extract 1%	177.41	97.35	68.44	78.74	10.88
S. khuzestanica extract 2%	154.81	74.23	65.08	53.61	7.60
T. vulgaris extract 1% + *S. khuzestanica* extract 2%	157.43	84.58	68.99	62.94	7.84
Standard error of the mean (SEM)	15.19	9.09	7.19	8.78	3.12
P-value	0.77	0.48	0.46	0.77	0.20
ANOVA	P-value				
T. vulgaris extract	NS	NS	*	NS	NS
S. khuzestanica extract	NS	*	NS	*	NS
T. vulgaris extract × *S. khuzestanica* extract	NS	NS	NS	NS	NS

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
TG: triglycerides; HDL-ch: high-density lipoprotein and LDL-ch: low-density lipoprotein cholesterol.
* (P<0.05).
NS: non significant.

Table 4 Effects of dietary treatments on broilers' blood parameters and immune organs

Treatment	Parameters					
	H %	L %	H/L	Cloacal bursa as % of BW	Spleen as % of BW	Thymus gland as % of BW
Control	9.75^{bc}	77.00^{a}	0.12^{b}	0.11	0.14	0.14
T. vulgaris extract 1%	13.00^{a}	70.75^{b}	0.18^{a}	0.11	0.12	0.14
S. khuzestanica extract 1%	8.75^{c}	77.00^{a}	0.11^{b}	0.10	0.11	0.18
T. vulgaris extract 1% + *S. khuzestanica* extract 1%	13.00^{a}	72.00^{b}	0.18^{a}	0.08	0.12	0.13
S. khuzestanica extract 2%	12.00^{ab}	72.75^{b}	0.16^{a}	0.08	0.14	0.15
T. vulgaris extract 1% + *S. khuzestanica* extract 2%	8.75^{c}	76.00^{a}	0.11^{b}	0.07	0.12	0.16
SEM	0.78	0.98	0.01	0.05	0.034	0.027
P-value	< 0.01	< 0.01	< 0.01	0.84	0.7	0.007
ANOVA	P-value					
T. vulgaris extract	*	*	*	NS	NS	NS
S. khuzestanica extract	NS	NS	NS	NS	NS	NS
T. vulgaris extract × *S. khuzestanica* extract	*	*	*	NS	NS	NS

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
H: heterophil; L: lymphocyte and BW: body weight.
SEM: standard error of the means.
* (P<0.05).
NS: non significant.

Table 5 Effect of treatments on broilers' humoral immunity against sheep red blood cells[1]

Treatment	Parameters			
	First IgG	First IgM	Second IgG	Second IgM
Control	1.50	2.00	1.12	1.87
T. vulgaris extract 1%	1.62	2.38	1.25	1.75
S. khuzestanica extract 1%	1.88	2.25	1.62	2.00
T. vulgaris extract 1% + *S. khuzestanica* extract %	1.75	1.75	1.50	1.88
S. khuzestanica extract 2%	1.88	2.00	1.13	2.00
T. vulgaris extract 1% + *S. khuzestanica* extract 2%	1.50	2.12	1.25	2.12
SEM	1.00	0.96	1.01	1.02
P-value	0.56	0.66	0.22	0.58
ANOVA	P-value			
T. vulgaris extract	NS	NS	NS	NS
S. khuzestanica extract	NS	*	NS	NS
T. vulgaris extract × *S. khuzestanica* extract	NS	NS	NS	NS

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] The data represent mean ± standard errors of Log2 of the reciprocal of the last dilution exhibiting agglutination.
SEM: standard error of the means.
* (P<0.05).
NS: non significant.

Absence of positive effect of thyme oil and some extracts in some experiments may be due to using a smaller dose which was insufficient to produce its effect on poultry. Cachectins are produced by extravascular effector cells such as macrophages in response to invasive stimuli. Diversion of the host's nutrient pool as a consequence of cachectin release may lead to retarded growth (Grimble, 1994). Intraperitoneal stimulation of chickens with IL-1 and SRBC led to reduced growth and FI, probably due to the cachectin activities of IL-1, IL-6, and TNF-α (Klasing *et al.* 1987). These cytokines are the earliest mediators secreted by the host in response to antigens and other injurious stimuli (Van Miert, 1995). When three herbal extracts were fed to broilers, thyme extract did not affect the anti- SRBC immune response (Rahimi *et al.* 2011). Herbs rich in flavonoids such as *T. vulgaris* extend the activity of vitamin C, act as antioxidants and may therefore enhance immune function (Cook and Samman, 1996; Manach *et al.* 1996).

Using an aldehyde/carboxylic acid assay demonstrated that carvacrol and thymol (5 ppm) can inhibit oxidation almost completely for 30 days.

The primary aromatic compounds in thyme include 1,8-cineole, thymol, carvacrol, and α-terpineol (Lee *et al.* 2005).

Given that thymol is the most effective antioxidative component and also one of the primary aromatic compounds in thyme, extracts of this herb would be likely to impart unwanted flavors to foods unless other antioxidative but nonaromatic components can be separated from the extract (Brewer, 2011).

The addition of 0.3% *S. hortensis* to broilers'diet raised chickens' Newcastle disease titers because high levels of vitamin A and vitamin E in this herb play a positive role in antibody production, increasing serum antibody levels and the phagocytic activity of immune cells (Tampieri *et al.* 2005). Flavonoids and polyphenolic compounds show sev-

eral pharmacological effects, including antioxidant activity, inhibition of histamine release from mast cells and inhibition of arachidonic acid metabolism (Amresh *et al.* 2007). Essential oil extracted from *S. hortensis* reversed oxidative damage to rat lymphocytes induced by hydrogen peroxide (Hajhashemi *et al.* 2011).

Meat quality

The effects of transportation on postmortem pH and color changes in breast and thigh meat are shown in Table 6. The pH and color of breast meat were not influenced by dietary treatments, but those parameters were affected in thigh meat.

The combination of 1% *T. vulgaris* extract and 2% *S. khuzestanica* extract resulted in the lowest pH 45 min and 24 h postmortem (P<0.05). 1% *S. khuzestanica* extract resulted in the lowest redness (a*) and yellowness (b*) values in thigh meat. Dietary treatments did not affect the drip loss and moisture percentage of breast or thigh meat after transport stress.

The pH change between 45 min and 24 h postmortem (ΔpH) is an indicator of lactate production and glycogen breakdown during this period. 1% *T. vulgaris* extract, or *S. khuzestanica* extract at 1% or 2%, or a combination of these plant extracts, increased ΔpH in breast meat. The postmortem decline in muscle pH is due to an accumulation of lactic acid as a result of glycolysis.

Lactic acid production is dependent upon glycogen, as it is the substrate in glycolysis (Lawrie, 1998). Anaerobic glycolysis changes glycogen, the major energy reserve in muscle, into lactate (Choe *et al.* 2008). Previously, researchers have found that transportation stresses animals (poultry and swine), as indicated by increases in plasma corticosterone and cortisol concentrations (Kannan *et al.* 1997). Glucocorticoids, corticosterone, and cortisol stimulate epinephrine release which then causes an increase in glycogenolysis in the muscle. Color is often used to distinguish pale, soft and exudative (PSE) meat from dark, firm, and dry (DFD) meat. Lightness (L*) is negatively correlated with muscle pH (Barbut, 1997a; Barbut, 1997b). Muscle with high pH generally holds a large proportion of water as intracellular water rather than extracellular water, resulting in greater absorption (less scattering) of light in the muscle and thus lower L* (Lawrie, 1998; Swatland, 1993). Water-holding capacity has been correlated with muscle pH, indicating that lower cooking losses are associated with higher muscle pH and better protein functionality (Barbut, 1997a). Our results indicate that thymol and carvacerol in *T. vulgaris* and *S. khuzestanica* extracts may increase glycogen breakdown. In other words due to a lack of oxygen, lactate in breast muscle resulted in pH reduction as compared with using thymol and carvacerol individually. Consequently, pH reduction led to a better quality of breast meat.

Table 6 Effect of transport stress on broilers' breast and thigh meat quality

Item	Treatment								ANOVA		
	C	T 1%	S 1%	T 1% + S 1%	S 2%	T 1% + S 2%	SEM	P-value	T	S	T × S
	Breast meat								P-value		
pH-45 min[1]	6.61	6.38	6.48	6.67	6.38	6.60	0.10	0.240	NS	NS	NS
pH-24 h[2]	5.93	5.96	5.99	5.81	5.86	5.87	0.08	0.590	NS	NS	NS
ΔpH[3]	0.67^{ab}	0.48^{b}	0.48^{b}	0.85^{a}	0.51^{b}	0.73^{ab}	0.10	0.036	NS	NS	*
Drip loss%	1.80	2.14	2.76	2.39	2.16	2.69	0.31	0.330	NS	NS	NS
Moisture%	74.19	72.53	74.91	74.63	73.78	74.77	0.88	0.180	NS	NS	NS
	Meat color										
L*	50.29	50.20	49.46	52.99	50.09	48.62	1.31	0.15	NS	NS	NS
a*	5.35	5.86	4.79	5.37	5.06	4.40	0.81	0.69	NS	NS	NS
b*	4.98	5.07	4.78	4.57	3.85	3.44	0.67	0.92	NS	NS	NS
	Thigh meat										
pH-45 min	6.65^{ab}	6.55^{ab}	6.62^{ab}	6.75^{a}	6.57^{ab}	6.46^{b}	0.08	0.025	NS	NS	*
pH-24 h	6.60^{a}	6.44^{ab}	6.43^{ab}	6.44^{ab}	6.53^{ab}	6.27^{b}	0.10	0.015	NS	NS	*
ΔpH	0.05	0.11	0.19	0.31	0.04	0.20	0.10	0.96	NS	NS	NS
Drip loss %	1.24	1.38	1.38	1.39	1.30	1.46	0.17	0.89	NS	NS	NS
Moisture %	72.87	73.37	74.41	74.96	74.16	72.39	1.86	0.77	NS	NS	NS
	Meat color										
L*	52.25	50.73	49.96	55.83	50.68	49.25	2.00	0.11	NS	NS	NS
a*	6.65^{a}	3.90^{ab}	3.56^{b}	6.01^{ab}	5.13^{ab}	5.36^{ab}	0.93	0.02	NS	NS	*
b*	5.06^{a}	2.44^{b}	3.07^{b}	3.84^{ab}	3.20^{ab}	3.56^{ab}	0.62	0.01	NS	NS	*

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
C: control; T: *T. vulgaris* extract and S: *S. khuzestanica* extract.
[1] pH at 45 min postmortem.
[2] pH at 24 h postmortem.
[3] pH at 45 min postmortem - pH at 24 h postmortem.
L*: lightness; a*: redness and b*: yellowness.
SEM: standard error of the means.
* (P<0.05).
NS: non significant.

CONCLUSION

From this study it is concluded that *S. khuzestanica* or *T. vulgaris* ethanolic extract alone or in combination in drinking water, did not influence the performance, blood cholesterol or triglycerides of broiler chickens while *T. vulgaris* extract decreased the heterophil percentage and heterophil:lymphocyte ratio. The levels of extracts given to broilers were very low, and they did not ameliorate transport effects on breast and thigh muscle. It is probable that these extracts can be used to imcrease broilers' white blood cell population and their immunity.

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