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

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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 (Kohler 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 intake (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 hypocholesterolemic and anti-dermic 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 malondialdehyde 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 carvacrol 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

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>% In grower diet</th>
<th>% In finisher diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>62.77</td>
<td>66.23</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>28.11</td>
<td>27.69</td>
</tr>
<tr>
<td>Corn gluten</td>
<td>3.34</td>
<td>0.00</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Methionin</td>
<td>0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>Lysin</td>
<td>0.17</td>
<td>0.10</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Dried coffee pulp (DCP)</td>
<td>1.63</td>
<td>0.90</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>1.03</td>
<td>1.05</td>
</tr>
<tr>
<td>Salt</td>
<td>0.26</td>
<td>0.30</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin premix¹</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Calculated nutrient

- AME (kcal/kg): 3000
- Crude total protein (TP) %: 20.6
- Lysine (SID) %: 0.97
- Methionine (SID) %: 0.43
- Cystine (SID) %: 0.30
- Meth + Syc (SID) %: 0.73
- Threonine (SID) %: 0.61
- Tryptophan (SID) %: 0.21
- Arginine (SID) %: 1.08
- Ca %: 0.90
- P %: 0.45
- Na %: 0.20
- CI %: 0.23
- DCAB meq/kg: 186
- Linoleic acid %: 1.50
- Fiber %: 4.33

¹ Each kg of vitamin and trace mineral premix provided: vitamin A: 13500 IU; vitamin D₃: 2000 IU; vitamin E: 30 mg; vitamin K₃: 2 mg; vitamin B₁: 1 mg; vitamin B₂: 6 mg; vitamin B₆: 3 mg; vitamin B₁₂: 10 μg; Niacin: 30 mg; Pan- tothenic 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 so- dium: 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-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 secretory 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 α 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters</th>
<th>Treatment</th>
<th>Parameters</th>
<th>Treatment</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BWG (kg)</td>
<td>FI (kg)</td>
<td>FCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.88</td>
<td>2.20</td>
<td>1.79</td>
<td>T. vulgaris extract</td>
<td>NS</td>
</tr>
<tr>
<td>T. vulgaris extract 1%</td>
<td>3.58</td>
<td>2.03</td>
<td>1.79</td>
<td>S. khuzestanica extract 1%</td>
<td>NS</td>
</tr>
<tr>
<td>T. vulgaris extract 1% + S. khuzestanica extract 1%</td>
<td>3.84</td>
<td>2.09</td>
<td>1.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. khuzestanica extract 2%</td>
<td>3.83</td>
<td>2.26</td>
<td>1.73</td>
<td>T. vulgaris extract 1% + S. khuzestanica extract 2%</td>
<td>3.71</td>
</tr>
<tr>
<td>SEM</td>
<td>0.28</td>
<td>0.18</td>
<td>0.063</td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. vulgaris extract</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. khuzestanica extract</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>T. vulgaris extract × S. khuzestanica extract</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 antioxidant 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).

### Table 3

Effects of dietary treatments on broilers’ blood biochemical parameters (mg/dL)

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

**ANOVA**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. vulgaris extract</td>
<td>NS</td>
</tr>
<tr>
<td>S. khuzestanica extract</td>
<td>NS</td>
</tr>
<tr>
<td>T. vulgaris extract × S. khuzestanica extract</td>
<td>NS</td>
</tr>
</tbody>
</table>

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.

**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).
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>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>C</th>
<th>T 1%</th>
<th>S 1%</th>
<th>T 1% + S 1%</th>
<th>S 2%</th>
<th>T 1% + S 2%</th>
<th>SEM</th>
<th>P-value</th>
<th>T</th>
<th>S</th>
<th>T × S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast meat</td>
<td>pH-45 min&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6.61</td>
<td>6.38</td>
<td>6.48</td>
<td>6.67</td>
<td>6.38</td>
<td>6.60</td>
<td>0.10</td>
<td>0.240</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>pH-24 h&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.93</td>
<td>5.96</td>
<td>5.99</td>
<td>5.81</td>
<td>5.86</td>
<td>5.87</td>
<td>0.08</td>
<td>0.590</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>ΔpH&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.10</td>
<td>0.036</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Drip loss %</td>
<td>1.80</td>
<td>2.14</td>
<td>2.76</td>
<td>2.39</td>
<td>2.16</td>
<td>2.69</td>
<td>0.31</td>
<td>0.330</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Moisture %</td>
<td>74.19</td>
<td>72.53</td>
<td>74.91</td>
<td>74.63</td>
<td>74.78</td>
<td>74.77</td>
<td>0.88</td>
<td>0.180</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

| 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<sup>1</sup> | 6.65<sup>ab</sup> | 6.55<sup>a</sup> | 6.62<sup>ab</sup> | 6.75<sup>a</sup> | 6.57<sup>a</sup> | 6.46<sup>ab</sup> | 0.08 | 0.025 | NS | NS | * |
| pH-24 h | 6.60<sup>1</sup> | 6.44<sup>ab</sup> | 6.43<sup>ab</sup> | 6.44<sup>a</sup> | 6.53<sup>b</sup> | 6.27<sup>a</sup> | 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<sup>1</sup> | 3.90<sup>b</sup> | 3.56<sup>b</sup> | 6.01<sup>ab</sup> | 5.13<sup>b</sup> | 5.36<sup>ab</sup> | 0.93 | 0.02 | NS | NS | * |
| b* | 5.06<sup>1</sup> | 2.44<sup>ab</sup> | 3.07<sup>b</sup> | 3.84<sup>ab</sup> | 3.20<sup>b</sup> | 3.56<sup>ab</sup> | 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 increase broilers’ white blood cell population and their immunity.

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