

## Dietary Inclusion of Thyme Essential Oil Alleviative Effects of Heat Stress on Growth Performance and Immune System of Broiler Chicks

### Research Article

K. Rafat Khafar<sup>1</sup>, A. Mojtahedin<sup>2</sup>, N. Rastegar<sup>3</sup>, M. Kalvani Neytali<sup>4</sup> and A. Olfati<sup>5\*</sup>

<sup>1</sup> Department of Animal Physiology, Sulaimani Technical Institute, Sulaimani Polytechnic University, Sulaymaniyah, Iraq

<sup>2</sup> Department of Physiology, Faculty of Agriculture and Natural Resources Moghan, University of Mohaghegh Ardabili, Ardabil, Iran

<sup>3</sup> Department of Animal Science, Abouraihan Campus, University of Tehran, Tehran, Iran

<sup>4</sup> Department of Animal Science, Gorgan University of Agriculture Science and Natural Resources, Gorgan, Iran

<sup>5</sup> Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Science, Tabriz, Iran

Received on: 6 Aug 2018

Revised on: 10 Oct 2018

Accepted on: 30 Oct 2018

Online Published on: Sep 2019

\*Correspondence E-mail: [aajbs@casrp.co.uk](mailto:aajbs@casrp.co.uk)

© 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran

Online version is available on: [www.ijas.ir](http://www.ijas.ir)

### ABSTRACT

This study was conducted to evaluate the effects of dietary inclusion of thyme essential oil (TEO) on growth performance and immune responses of broiler chicks subjected to heat stress (HS). Broilers were divided into five groups; group 1) chicks fed with basal diet and reared under thermoneutral condition; group 2) chicks fed with basal diet and subjected to HS condition; and groups 3, 4 and 5) chicks exposed to HS and fed with basal diet containing 100, 150 and 200 mg TEO/kg of diet, respectively. Broiler chicks were evaluated for antibody titre against sheep red blood cell (SRBC) on 35 and 42 days. Broiler chicks were bled at the end of the experiment (day 42) for blood metabolites measurements. Heat stress increased feed conversion ratio and mortality and decreased body weight gain and feed intake ( $P < 0.05$ ). Dietary inclusion of TEO at 150 and 200 mg/kg of diet alleviated negative effects of HS on growth performance ( $P < 0.05$ ). Heat stress increased the serum concentrations of cholesterol, triglycerides, corticosterone, malondialdehyde and heterophil count ( $P < 0.05$ ) and reduced antibody titre against SRBC, lymphocyte count and lymphoid organs weight ( $P < 0.05$ ). Dietary inclusion of TEO at 150 and 200 mg/kg of diet reversed negative effects of HS on blood biochemical metabolites and immune responses ( $P < 0.05$ ). In conclusion, thyme essential oil supplementation at 150 and 200 mg/kg of diet can be advised for improving the growth performance and immune responses of broiler chicks in heat stress condition.

**KEY WORDS** blood metabolites, broiler chicks, heat stress, immunity, performance.

### INTRODUCTION

Heat stress (HS) is one of major problems in the poultry industry, especially in hot regions, because of its adverse effects on economic components (Niu *et al.* 2009b). Studies have shown that HS reduces growth performance and antibody production in young chickens (Niu *et al.* 2009b; Niu *et al.* 2009a). High environmental temperatures enhance the plasma concentration of corticosterone and heterophil to

lymphocyte ratio (Yalçın *et al.* 2003). Heat stress also decreases relative weight of lymphoid organs (Niu *et al.* 2009b) and has negative effects on some blood metabolites in broiler chicks (Habibian *et al.* 2014). Heat stress could disturb the balance between the production of reactive oxygen species (ROS) and the antioxidant systems, resulting in increased production of ROS (Feng *et al.* 2008). The produced ROS can cause oxidative changes such as lipid peroxidation and oxidative damages to proteins and DNA

(Mujahid *et al.* 2007). Therefore, poultry production systems need to adopt suitable strategies to alleviate the negative effects of HS on immune system. The use of plants and their derivatives may be a good choice to achieve this goal. Essential oils are complex compounds with variable chemical composition and concentration (Simitzis, 2017).

*Thymus vulgaris* is a medicinal herb belonging to Lamiaceae family which mainly cultivated worldwide as culinary uses, cosmetic perennial and medical herb. Thyme contains high amount of polyphenols which are responsible for antioxidant activity in its essential oils (Dahal and Farran, 2011).

Numerous studies have shown that flavonoids and polyphenolic substances have several pharmacological effects, such as antioxidant activity, preventing histamine release and arachidonic acid metabolism (Amresh *et al.* 2007). Studies have also shown that the use of antioxidant plants in diet can prevent the oxidative changes created by free radicals and other reactive species (Soler-Rivas *et al.* 2000). Antioxidants may also prevent oxidation of low-density lipoproteins (Aoudi *et al.* 2014). Dietary inclusion of thyme essential oil (TEO) and peppermint essential oil, combined form, reduced the serum concentration of cholesterol in laying hens submitted to cold stress condition (Akbari *et al.* 2015).

Thyme products are known to have hypocholesterolemic and antihyperlipidemic activities in broiler chickens (Abdulkarimi *et al.* 2011; Dahal and Farran, 2011). On the other hand, plant derivatives are utilized in animal feeding as growth promoter, because of their antioxidant, antimicrobial and digestion properties (Abdulkarimi *et al.* 2011; Assiri *et al.* 2016). Attia *et al.* (2017) have reported that dietary inclusion of TEO improved growth performance in broiler chicks.

Raggar *et al.* (2016) have also shown that dietary inclusion of TEO increased weight gain and immune system in broiler chicks. It was hypothesized that TEO may alleviate adverse effects of HS on growth performance, immune responses and blood biochemical variables. Thus, this study was conducted to evaluate the effects of dietary inclusion of TEO on growth performance, blood biochemical variables and immune responses of heat-stressed broilers.

## MATERIALS AND METHODS

### Chickens and diets

All the used procedures were approved by standard ethical committee of Tabriz University (Tabriz-Iran: No. T1101) for care and treatment of animals. Three hundred, one-day-old, male broiler chicks, Ross-308, initial body weight of  $42 \pm 3.0$  g, were purchased from a commercial hatchery.

One-d-old broiler chicks were randomly allocated in five treatments with six cages (6 replicates) each with 10 birds.

They were allocated in two chambers; chamber A (4 treatments) and chamber B (1 treatment). The recommended brooding temperatures were applied until 21 days of experiment, i.e., the temperature was gradually decreased from 33 to 23.9 °C during 1 to 21 d of age in the both chambers. From 22 to 42 d of age, the birds in chamber A were exposed to HS (23.9-38 °C daily) and those in chamber B were reared under thermoneutral condition (TN; 23.9 °C).

The heat-stressed birds (chamber A) were fed with basal diet (control-HS), 100 mg TEO/kg of diet (100 TEO-HS), 150 mg TEO/kg of diet (150 TEO-HS) and 200 mg TEO/kg of diet (200 TEO-HS), from 1 to 42 days. The birds in chamber B were fed with basal diet (control-TN).

Feed, in mash form, and water were provided *ad libitum* during the experiment. Two iso-caloric and iso-nitrogenous experimental diets were formulated to meet the nutrient requirements for starter and grower periods as recommended by NRC (1994) (Table 1).

The calculated amounts of TEO were firstly mixed with small amounts of the basal diet, as a small batch. The small batch was subsequently mixed with larger amount of the basal diet to obtain a homogenized mixture. The lighting schedule was 23 h of light/1 h dark cycle with an average light intensity of 15 lx which maintained until the end of the experiment.

### Temperature and treatments

After 3 weeks of age, the birds in stress group were daily exposed to temperatures (regulatory) as follows: 12 h of 23.9 °C, 3 h of 23.9 to 38 °C, 5 h of 38 °C, and 4 h of 38 to 23.9 °C [2 and 29] and other birds (TN group) were reared in constant temperature (23.9 °C). The relative humidity was maintained in 50-55%.

### Component analysis of thyme essential oil

TEO was prepared from Gareban Company (Kermanshah-Iran). Components of essential oil were analysed by using gas chromatography as described by Juliano *et al.* (2000) and the data are presented in Table 2.

### Growth performance

The broiler chicks were weighed at 1, 21 and 42 days of age, and the body weight gain (BWG, g/bird) per replicate was calculated.

Feed intake (FI) was recorded for each replicate (g/bird) as well as feed conversion ratio (FCR) was calculated. Mortality rate was considered to calculate the growth performance parameters during experiment.

**Table 1** Composition of the experimental diets (g/kg)

Diet composition (g/kg)	Starter (1-21 days)	Grower (22-42 days)
Corn (8.5% CP)	647.10	699.50
Soybean meal (48% CP)	312.00	251.90
Soybean oil	0.00	5.00
Calcium carbonate	13.60	13.0
Dicalcium phosphate	16.00	15.70
Salt	4.60	4.00
Mineral and vitamin premix <sup>1</sup>	5.00	5.00
HCL-lysine	0.00	4.40
DL-methionine	1.70	1.50
<b>Calculated chemical composition</b>		
Metabolizable energy (MJ/kg)	12.13	12.89
Crude protein (g/kg)	210.50	190.0
Calcium (g/kg)	10.10	9.60
Available phosphorus (g/kg)	5.00	4.80
Lysine (g/kg)	11.60	13.30
Methionine + cysteine (g/kg)	8.50	7.60
Na (g/kg)	2.00	1.50
K (g/kg)	8.50	7.80
Cl (g/kg)	2.50	2.30
Na + K - Cl (meq/kg)	221.00	203.00

<sup>1</sup> Mineral-vitamin premix provided the following per kilogram of diet: vitamin A: 9000 IU; vitamin D<sub>3</sub>: 2100 IU; vitamin E: 30 mg; Nicotinic acid: 30 mg; vitamin B<sub>12</sub>: 0.12 mg; Calcium pantothenate: 10 mg; vitamin K<sub>3</sub>: 5 mg; Thiamin: 1.1 mg; Riboflavin: 4.5 mg; vitamin B<sub>6</sub>: 2.0 mg; Folic acid: 0.5 mg; Biotin: 0.5 mg; Fe: 50 mg; Cu: 10 mg; Mn: 70 mg; Zn: 50 mg; Co: 0.2 mg; I: 1.0 mg; Se: 0.3 mg; Butylated hydroxytoluene (BHT): 150 mg and Monensin: 100 mg.

#### Determination of blood biochemical variables

On d 42 and after 12-hours fasting, blood samples were collected in non heparinised tubes from two birds per each cage (3 mL per bird) via brachial vein and centrifuged at 2500 × g for 15 min (SIGMA 4-15 Lab Centrifuge, Germany) and serum samples were obtained. The serum samples were individually analysed for cholesterol, triglyceride using Pars Azmoon commercial kit package (Pars Azmun, Tehran, Iran).

Corticosterone concentration was evaluated as explained by manufacture protocol kit. Malondialdehyde (MDA) was evaluated, as lipid peroxidation index, as described by Richards *et al.* (1992). The MDA is one of the thiobarbituric acid reactive substances (TBARS). Mixture of these substances is in biological specimens and it is an index for oxidative stress.

**Table 2** Composition of thyme essential oil

Components	%
Thymol	45.00-50.00
γ-Terpinen	18.00-22.00
p-Cymene	10.00-14.50
Myrcene	4.00-6.50
α-Terpinene	3.00-5.20
α-Pinene	3.50-4.20
Limonene	3.50-4.10
Carvacrol	3.00-5.10
Linalool	3.00-5.00
Eugenol	Max 0.50

#### Immune system variables

On day 26, three ml blood was taken of two birds per replicate to pre-challenge antibody titre analysis which followed to determine the presence of antibodies prior to challenge with sheep red blood cells (SRBC). At d 28, two birds per replicate were intravenously treated with 1ml of 7% SRBC suspension administrated to right wing (Habibian *et al.* 2015). On d 35, the same birds were bled through brachial venipuncture, and 3 mL blood was taken for primary antibody response. Blood samples were centrifuged at 2500 × g at 4 °C for 15 minutes and the obtained sera were stored in -20 °C for further analysis. On day 35, 1 mL from 7% SRBC suspension were intravenously administrated to same birds and blood samples (3 mL per bird) were collected for secondary responses on day 42. Blood samples were centrifuged and stored as explained for primary responses. Samples were investigated for IgM and IgG by the 2-mercaptoethanol (ME) method as previously explained by Lepage *et al.* (1996).

Summary, sera samples were inactivated at 56 °C by water bath and 50 µL phosphate buffer saline (PBS) was then added in the first row from wells in a 96-well V-bottom microtitration plate. Subsequently, 50 µL of serum was added to same wells and those were sealed and incubated in 37 °C to 30 min. The plates were subsequently ejected from incubator and other wells, 11 remaining same row, were treated with 50 µL PBS.

Other wells were serially diluted with a 2-fold dilution and 50  $\mu$ L 2.5% SRBC suspension was administered to each well, and plates were again sealed and incubated for 0.5 h. Titres were expressed by holding plates on a lighted mirror to see wells for agglutination. Antibody titres were reported as log 2 of the reciprocal of the last dilution in which agglutination was seen.

On d 35, blood samples (2 drops per bird) were taken from 2 birds per replicate and smeared on glass slides (one drop on per slide). The prepared smears were then stained by May–Grünwald-Giesma stains (Lucas and Jamroz, 1961), about 3 h after methyl alcohol fixation. Heterophil (H) count, lymphocyte (L) count and heterophil/lymphocyte ratio (H/L) was estimated. The data were presented as mean of two slides.

On d 42, 2 birds per replicate were weighed, killed and lymphoid organs including bursa, spleen and thymus were weighted. Organ weights were expressed as a percentage of body weight.

### Statistical analysis

The data were analysed using the ANOVA procedure from SAS (2001) to assign the significant differences among groups. Means were subsequently compared using Duncan's least significance multiple-range test. All data were expressed as means  $\pm$  standard deviation ( $\pm$ SD). The log<sub>2</sub> transformations were done on antibody titres before statistical analysis.

## RESULTS AND DISCUSSION

### Growth performance

Effects of dietary inclusion of TEO on growth performance are shown in Table 3. Results showed that HS significantly increased FCR and mortality and decreased BWG and FI (control-HS vs. control-TN) ( $P < 0.05$ ). However, dietary inclusion of TEO (150 and 200 mg/kg) alleviated negative effects of HS on growth performance (FI, BWG and FCR) ( $P < 0.05$ ). Lower levels of TEO (100 mg/kg) could not alleviate adverse effects of HS on growth performance ( $P > 0.05$ ).

### Blood biochemical variables

Effects of HS and dietary inclusion of TEO on the blood biochemical variables of broiler chickens are presented in Table 4. HS increased the serum contents of triglycerides, cholesterol, corticosterone and malondialdehyde ( $P < 0.05$ ) when compared with TN condition (control-HS vs. control-TN). The broiler chicks treated with TEO (150 and 200 mg/kg) showed lower the serum concentrations of cholesterol, triglycerides, corticosterone and MDA when compared with other birds ( $P < 0.05$ ; Table 4).

It was not observed significant difference between 200 TEO-HS and control-TN for the serum concentrations of cholesterol and triglycerides ( $P > 0.05$ ).

### Immune system variables

The data for effects of HS and dietary inclusion of TEO on immune system are displayed in Tables 5 to 7. Sera samples for pre-challenge antibody titre were negative. Comparing control-HS and control-TN shows that heat stress suppressed immune responses ( $P < 0.001$ ); but the both primary and secondary immune response against SRBC were affected by TEO treatments ( $P < 0.01$ ). The highest antibody titres against SRBC was observed in birds treated with 200 mg/kg of TEO ( $P < 0.01$ ). However, broiler chicks at 100 TEO-HS group was similar to antibody titre compared with control-HS; broiler chicks at 150 TEO-HS group had higher antibody titre compared with control-HS ( $P < 0.01$ ; Table 5). Heterophil count and H/L ratio were increased, while lymphocyte count and relative weight of lymphoid organs were decreased in control-HS and 100 TEO-HS group in comparison to control-TN group ( $P < 0.0001$ ; Tables 6-7). Dietary inclusion of TEO (150 and 200 mg/kg) reduced heterophil count and H/L ratio and increased lymphocyte count and relative weight of lymphoid organs in comparison to control-HS ( $P < 0.001$ ).

Our results showed that HS suppressed growth performance but dietary inclusion of high levels of TEO improved growth performance. Olfati *et al.* (2018) have reported that HS suppressed growth performance in broiler chicks. Thermal stress influences the productive performance of poultry by affecting nutrient metabolism and digestibility (Zhang *et al.* 2012) while increasing corticosterone blood levels (Sahin *et al.* 2002). Corticosterone reduces nutrient utilization and digestibility and finally decreases performance. In addition, HS-exposed birds showed a reduced villus-height to crypt-depth ratio (Deng *et al.* 2012). Thus, HS by increasing corticosterone decreases growth performance which was confirmed by our findings. Results showed that corticosterone concentration was significantly higher in control-HS in comparison to control-TN. With regards to dietary inclusion of TEO, Pournazari *et al.* (2017) have reported that dietary inclusion of TEO and probiotic, separately, increased FI. Similar to our findings, Attia *et al.* (2017) have reported that dietary inclusion of TEO improved growth performance in broiler chicks during summer season.

Ragga *et al.* (2016) also showed that dietary inclusion of TEO increased BWG in broiler chicks. Improved growth performance in high levels of TEO can be due to antioxidant properties and phenolic components of EOs that reduces effects of pathogens on intestinal system and help to absorb the amino acids (Lee *et al.* 2003).

**Table 3** Effects of dietary inclusion of thyme essential oil (TEO) on feed intake (g/chick), body weight gain (g/chick), feed conversion ratio and mortality (%) of broilers<sup>1</sup>

Groups	Feed intake	Body weight gain	Feed conversion ratio	Mortality
Control	4717.00±75.28 <sup>a</sup>	2608.00±91.74 <sup>a</sup>	1.80±0.04 <sup>c</sup>	0.00±0.00 <sup>b</sup>
Control-HS	3900.00±141.40 <sup>c</sup>	1775.00±82.16 <sup>d</sup>	2.24±0.09 <sup>a</sup>	6.67±5.16 <sup>a</sup>
100 TEO-HS	3942.00±128.10 <sup>c</sup>	1758.00±58.45 <sup>d</sup>	2.28±0.09 <sup>a</sup>	5.00±5.07 <sup>a</sup>
150 TEO-HS	4217.00±75.28 <sup>b</sup>	2108.00±111.40 <sup>c</sup>	2.00±0.08 <sup>b</sup>	0.00±0.00 <sup>b</sup>
200 TEO-HS	4292.00±66.46 <sup>b</sup>	2007.00±40.82 <sup>b</sup>	1.81±0.03 <sup>c</sup>	0.00±0.00 <sup>b</sup>
P-value	0.0001	0.0001	0.0001	0.0001
SEM	57.23	63.05	0.039	0.78

<sup>1</sup> The data were presented as means ± standard deviation (Mean±SD).

HS: heat stress.

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.

**Table 4** Effects of dietary inclusion of thyme essential oil (TEO) on the serum contents of triglycerides (mg/dL), cholesterol (mg/dL), corticosterone (mol/L), and MDA (mmol/mL) of broilers<sup>1</sup>

Groups	Triglycerides	Cholesterol	Corticosterone	MDA
Control	110.00±8.74 <sup>c</sup>	111.00±3.74 <sup>c</sup>	1.48±0.15 <sup>d</sup>	1.43±0.18 <sup>d</sup>
Control-HS	136.00±3.14 <sup>a</sup>	132.00±3.44 <sup>a</sup>	2.53±0.12 <sup>a</sup>	2.59±0.14 <sup>a</sup>
100 TEO-HS	135.00±2.66 <sup>a</sup>	129.00±4.55 <sup>a</sup>	2.58±0.20 <sup>a</sup>	2.63±0.19 <sup>a</sup>
150 TEO-HS	125.00±3.10 <sup>b</sup>	117.00±4.23 <sup>b</sup>	2.10±0.21 <sup>b</sup>	2.15±0.18 <sup>b</sup>
200 TEO-HS	112.00±5.43 <sup>c</sup>	109.00±3.58 <sup>c</sup>	1.85±0.22 <sup>c</sup>	1.83±0.23 <sup>c</sup>
P-value	0.005	0.021	0.002	0.003
SEM	1.65	1.26	0.05	0.06

<sup>1</sup> The data were presented as means ± standard deviation (Mean±SD).

HS: heat stress.

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.

**Table 5** Effects of dietary inclusion of thyme essential oil (TEO) on the anti-sheep red blood cells (SRBC) antibody response (log<sub>2</sub>) of broiler chicks<sup>1</sup>

Groups	Primary (IgG)	Secondary (IgG)	Primary IgM	Secondary IgM
Control	2.20±0.16 <sup>a</sup>	3.80±0.18 <sup>a</sup>	3.14±0.07 <sup>a</sup>	2.11±0.04 <sup>a</sup>
Control-HS	1.28±0.05 <sup>d</sup>	2.52±0.13 <sup>d</sup>	2.20±0.09 <sup>d</sup>	1.35±0.10 <sup>d</sup>
100 TEO-HS	1.28±0.07 <sup>d</sup>	2.64±0.15 <sup>d</sup>	2.34±0.14 <sup>d</sup>	1.30±0.10 <sup>d</sup>
150 TEO-HS	1.67±0.11 <sup>c</sup>	2.86±0.10 <sup>c</sup>	2.56±0.12 <sup>c</sup>	1.61±0.13 <sup>c</sup>
200 TEO-HS	1.96±0.06 <sup>b</sup>	3.05±0.14 <sup>b</sup>	2.83±0.11 <sup>b</sup>	1.83±0.13 <sup>b</sup>
P-value	0.007	0.002	< 0.0001	< 0.0001
SEM	0.05	0.06	0.047	0.03

<sup>1</sup> The data were presented as means ± standard deviation (Mean±SD).

HS: heat stress.

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.

TEO not only helps to absorb amino acids but also increases secretion the digestive enzymes which improves growth (Lee *et al.* 2003). Growth performance can also be suppressed because of increased corticosterone. Our findings also indicated high levels of TEO decreased the levels of corticosterone. Thus, TEO can improve growth performance by decreasing corticosterone levels under HS condition.

Results also showed that HS increased cholesterol, triglycerides, corticosterone and malondialdehyde concentrations. Previous studies have reported that HS increases the serum concentrations of MDA (Tawfeek *et al.* 2014), cholesterol and total lipids (Nawalany *et al.* 2010) in broiler chicks. Stress enhances free radicals production and formation of ROS, thus it increases lipid peroxidation and subsequently MDA levels in blood and tissues (Ates *et al.* 2006).

HS reduces food consumption in birds and they compensate their need to energy through lipolysis (Rashidi *et al.* 2010).

It can be concluded that HS increases lipolysis through increased corticosterone secretion, production of free radicals and formation of ROS and decreased feed intake. Dietary inclusion of TEO at high levels, 150 and 200 mg/kg, reduced the serum concentration of corticosterone, triglycerides, and cholesterol. Results are in agreement with those reported by Abdulkarimi *et al.* (2011). The TEO could reduce cholesterol content through its effect on hepatic 3-hydroxy-3-methyl glutaryl CoA reductase, the limiting enzyme in cholesterol biosynthesis (Lee *et al.* 2003). Rahim *et al.* (2011) reported that the TEO, as an antioxidant, increases the synthesis of nitric oxide, a vasodilator, which may prevent excess of cholesterol in the blood vessels.

**Table 6** Effects of dietary inclusion of thyme essential oil (TEO) on count of heterophil, lymphocyte or their ratio (in 100 cells) of 35-day-old heat-stressed broilers<sup>1</sup>

Groups	Heterophil (%)	Lymphocyte (%)	Heterophil/lymphocyte
Control	18.33±1.21 <sup>d</sup>	75.50±1.18 <sup>a</sup>	0.24±0.02 <sup>d</sup>
Control-HS	37.16±1.50 <sup>a</sup>	55.16±1.89 <sup>d</sup>	0.67±0.04 <sup>a</sup>
100 TEO-HS	36.00±1.35 <sup>a</sup>	55.50±2.19 <sup>d</sup>	0.64±0.03 <sup>a</sup>
150 TEO-HS	31.66±2.00 <sup>b</sup>	61.81±1.54 <sup>c</sup>	0.51±0.03 <sup>b</sup>
200 TEO-HS	26.16±1.66 <sup>c</sup>	67.83±1.94 <sup>b</sup>	0.38±0.02 <sup>c</sup>
P-value	< 0.0001	< 0.0001	< 0.0001
SEM	0.91	1.11	0.02

<sup>1</sup> The data were presented as means ± standard deviation (Mean±SD).

HS: heat stress.

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.

**Table 7** Effects of dietary inclusion of thyme essential oil (TEO) on the relative weight of lymphoid organs of 42-day-old broilers<sup>1</sup>

Groups	Spleen	Bursa of fabricius	Thymus
Control	0.19±0.005 <sup>a</sup>	0.13±0.001 <sup>a</sup>	0.29±0.013 <sup>a</sup>
Control-HS	0.11±0.009 <sup>d</sup>	0.08±0.014 <sup>d</sup>	0.22±0.010 <sup>c</sup>
100 TEO-HS	0.12±0.014 <sup>d</sup>	0.08±0.005 <sup>d</sup>	0.22±0.010 <sup>c</sup>
150 TEO-HS	0.16±0.007 <sup>c</sup>	0.11±0.002 <sup>c</sup>	0.27±0.009 <sup>b</sup>
200 TEO-HS	0.17±0.010 <sup>b</sup>	0.12±0.001 <sup>b</sup>	0.27±0.009 <sup>b</sup>
P-value	< 0.0001	< 0.0001	< 0.0001
SEM	0.007	0.002	0.004

<sup>1</sup> The data were presented as means ± standard deviation (Mean±SD).

HS: heat stress.

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.

On the other hand, the decreased cholesterol synthesis by TEO can be responsible for decreased corticosterone synthesis because cholesterol is precursor for corticosteroid hormones. MDA, end product of lipid peroxidation, is index for level of ROS-induced biological damage (Popova and Popov, 2002) which is isolated from urine, blood, and tissues. In the present study, the decreased triglycerides concentration is paralleled with decreased MDA content. This phenomenon can be explained by antioxidant theory. In this study, the decreased MDA concentration confirms antioxidant activity of TEO. Antioxidant theory states that the decreased antioxidant vitamins increase lipid peroxidation. On the other hand, HS excretes antioxidant minerals and vitamins. The TEO, as an antioxidant, may compensate antioxidant deficiencies and prevent the lipid oxidation under HS condition. This idea was confirmed by other researchers who indicated that essential oils prevented oxidative changes produced by ROS production (Soler-Rivas *et al.* 2000) and inhibited oxidation of low-density lipoproteins (Aoudi *et al.* 2014). It is possible that the TEO, at high levels, reduces lipid peroxidation by decreasing corticosterone concentration because corticosterone increases lipid peroxidation.

Comparing control-HS and control-TN showed that HS reduces antibody titres against SRBC and increases mortality. HS suppressed anti-SRBC antibody responses in broiler chicks (Bartlett and Smith, 2003; Niu *et al.* 2009b). Studies have shown that HS suppresses immune system through increasing inflammatory cytokines (Ogle *et al.* 1997), and

increases corticosterone concentrations (Trout and Mashaly, 1994). Some researchers have shown that HS activates the hypothalamic-pituitary-adrenal axis and increases the plasma concentration of corticosterone which enhances ROS production, thus it suppresses the immune system (Manach *et al.* 1996). The TEO alleviated negative effects of HS on antibody titre and mortality. Similarly, Ragga *et al.* (2016) have reported that dietary inclusion of TEO improved immune system in broiler chicks. Takazadeh and Mayahi (2017) have reported that water inclusion of thyme extract improved immune response in broiler chickens. In contrast to these findings, other researchers reported that TEO, alone and in combination with other feed additives, had no significant effect on antibody titre in poultry (Ozek *et al.* 2011; Hosseini *et al.* 2013). Hashemipour *et al.* (2013) reported that diet supplementing with thymol + carvacrol increased the cellular and humoral immune responses in broilers. Flavonoids and other phenolic components, present in essential oils, increased activity of vitamin C as immunostimulator (Manach *et al.* 1996). Amresh *et al.* (2007) showed flavonoids and polyphenolic compounds help immune system through their antioxidant activity. As mentioned, HS increases ROS which subsequently causes injuries in cells (Flanagan *et al.* 1998), or induces cytotoxicity (Mujahid *et al.* 2005). ROS also damages immunity organs in broilers (Pamok *et al.* 2009) which finally suppress immune system. It seems that the TEO, as an antioxidant, alleviates negative effects of HS on immune system through blocking or preventing ROS production.

The idea is confirmed by other researchers who showed that essential oils, or antioxidants, prevent ROS production through interaction with peroxide radicals (Yanishlieva *et al.* 1999) and prevents oxidative injuries to immune cells (Nickels, 1996). In addition, HS provides opportunities for infectious factors which dominate on immune system (Deying *et al.* 2005), but essential oils balance gut microbial ecosystem (Williams and Losa, 2001). Thus, TEO, at high levels (150 and 200 mg/kg), may help immune responses by intestine microbial balance.

HS increased heterophil count and H/L ratio and it reduced L count and relative weight of lymphoid organs (control HS vs. control TN). These findings were confirmed by other researchers who showed HS disturbs leucocytes count and H/L ratio (Yalçın *et al.* 2003) and reduces relative weights of lymphoid organs of birds (Niu *et al.* 2009b).

Glucocorticoid hormones reduce lymphocytes count, since circulating lymphocytes in response to glucocorticoids join to the endothelial cells and subsequently emigrate from circulation into other tissues (Dhabhar, 2002). Similarly, corticosterone decreases food consumption and it may reduce relative weight of lymphoid organs (Niu *et al.* 2009b) because of organs need to more feed for the proper development.

TEO at high levels, 150 and 200 mg/kg, improved leucocytes count and increased relative weight of lymphoid organs. Diet supplementing with TEO and other essential oils improved leucocytes count, but they had not significant effect on relative weight of lymphoid organs (Parvar *et al.* 2013). As mentioned, HS influences lymphocyte count and relative weight of lymphoid organs through glucocorticoid hormones. Results showed that TEO at high levels, 150 and 200 mg/kg, reduced negative effects of HS on corticosterone concentration. Thus, it is reasonable that TEO improves leucocytes count and relative weight of lymphoid organs.

## CONCLUSION

Results indicated that HS has negative effects on growth performance, the serum concentrations of lipid profile, antioxidant system and immunity parameters. Dietary inclusion of TEO at levels of 150 or 200 mg/kg alleviated negative effects of HS on growth performance, blood biochemical variables and immune responses. On the basis findings, it can be advised dietary inclusion of TEO in broiler chicks, at levels of 150 or 200 mg/kg, help to improve the immune system in tropical regions and summer season.

## ACKNOWLEDGEMENT

The authors would like to acknowledge from Immunology Research Center, Tabriz University of Medical Science wh-

ich provided excellent assistance with the preparation of this research. We would like also to thank all of the members of our laboratories (especially Dr Azad Abdollahzadeh (DVM)) for their scientific contributions during these years.

## REFERENCES

- Abdulkarimi R., Daneshyar M. and Aghazadeh A. (2011). Thyme (*Thymus vulgaris*) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. *Italian J. Anim. Sci.* **10**, 101-105.
- Akbari M., Torki M. and Kaviani K. (2015). Single and combined effects of peppermint and thyme essential oils on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition (6.8±3 °C). *Int. J. Biometeorol.* **60**(3), 447-454.
- Amresh G., Reddy G., Rao C.V. and Singh P. (2007). Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. *J. Ethnopharmacol.* **110**, 526-531.
- Aoudi D., Luciano G., Vasta V., Nasri S., Brogna D.M.R., Abidi S., Priol A. and Ben Salem H. (2014). The antioxidant status and oxidative stability of muscle from lambs receiving oral administration of *Artemisia herba alba* and *Rosmarinus officinalis* essential oils. *Meat Sci.* **97**, 237-243.
- Assiri A.M.A., Elbanna K., Abulreesh H.H. and Ramadan M.F. (2016). Bioactive Compounds of cold-pressed Thyme (*Thymus vulgaris*) oil with antioxidant and antimicrobial properties. *J. Oleo Sci.* **65**, 629-640.
- Ates B., Dogru M.I., Gul M., Erdogan A., Dogru A.K., Yilmaz I., Yurekli M. and Esrefoglu M. (2006). Protective role of caffeic acid phenethyl ester in the liver of rats exposed to cold stress. *Fundam. Clin. Pharmacol.* **20**, 283-289.
- Attia Y.A., Bakhshwain A.A. and Bertu N.K. (2017). Thyme oil (*Thymus vulgaris*) as a natural growth promoter for broiler chickens reared under hot climate. *Italian J. Anim. Sci.* **16**, 275-282.
- Bartlett J.R. and Smith M.O. (2003). Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. *Poult. Sci.* **82**, 1580-1588.
- Dahal I. and Farran M. (2011). Effect of dried medicinal crops on the performance and carcass flavour of broilers. *Int. J. Poult. Sci.* **10**, 152-156.
- Deng W., Dong X.F., Tong J.M. and Zhang Q. (2012). The probiotic *Bacillus licheniformis* ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. *Poult. Sci.* **91**, 575-582.
- Deying M.A., Anshan-Shan A., Zhihui-Chen C., Juan-Du H., Kaii-Song L., Jianping-Li K. and Qiyu X. (2005). Effect of *Ligustrum lucidum* and *Schisandra chinensis* on the egg production, antioxidant status and immunity of laying hens during heat stress. *Arch. Anim. Nutr.* **59**, 439-447.
- Dhabhar F.S. (2002). A hassle a day may keep the doctor away: stress and the augmentation of immune function. *Integr. Com. Biol.* **42**, 556-564.
- Feng J.H., Zhang M.H., Zheng S.S., Xie P. and Ma A.P. (2008). Effects of high temperature on multiple parameters of broilers *in vitro* and *in vivo*. *Poult. Sci.* **87**, 2133-2139.

- Flanagan S.W., Moseley P.L. and Buettner G.R. (1998). Increased flux of free radicals in cells subjected to hyperthermia: Detection by electron paramagnetic resonances spin trapping. *FEBS Lett.* **431**, 285-286.
- Habibian M., Ghazi S., Moeini M. and Abdolmohammadi A.R. (2014). Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermo neutral or heat stress conditions. *Int. J. Biometeorol.* **58**, 741-752.
- Hashemipour H., Kermanshahi H., Golian A. and Veldkamp T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poult. Sci.* **92**, 2059-2069.
- Hosseini S.A., Meimandipour A., Alami F., Mahdavi A., Mohiti-Asli M., Lotfollahian H. and Cross D. (2013). Effects of ground thyme and probiotic supplements in diets on broiler performance, blood biochemistry and immunological response to sheep red blood cells. *Italian J. Anim. Sci.* **12(1)**, 116-120.
- Juliano C., Mattana A. and Usai M. (2000). Composition and *in vitro* antimicrobial activity of the essential oil of *Thymus herba-barona* Loisel growing wild in Sardinia. *J. Essent. Oil Res.* **12**, 516-522.
- Lee K.W., Everts H., Kappert H.J., Frehner M., Losa R. and Beynen A.C. (2003). Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *Br. Poult. Sci.* **44**, 450-457.
- Lepage K.T., Bloom S.E. and Taylor Jr R.L. (1996). Antibody response to sheep red blood cells in a major histocompatibility (B) complex aneuploid line of chickens. *Poult. Sci.* **75**, 346-350.
- Lucas A.M. and Jamroz C. (1961). Atlas of Avian Haematology. Washington D.C., USA.
- Manach F., Regeat F. and Texier O. (1996). Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. *Neutral Res.* **16**, 517-544.
- Mujahid A., Pumford N.R., Bottje W., Nakagawa K., Miyazawa T., Akiba Y. and Toyomizu M. (2007). Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. *J. Poult. Sci.* **44**, 439-445.
- Mujahid A., Yoshiki Y., Akiba Y. and Toyomizu M. (2005). Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poult. Sci.* **84**, 307-314.
- Nawalany G., Bieda W. and Radoń J. (2010). Effect of floor heating and cooling of bedding on thermal conditions in the living area of broiler chickens. *Arch. Geflugelkd.* **74**, 98-101.
- Nickels C.H.F. (1996). Antioxidants improve cattle immunity following stress. *Anim. Feed Sci. Technol.* **62**, 59-68.
- Niu Z.Y., Liu F.Z., Yan Q.L. and Li L. (2009a). Effects of different levels of selenium on growth performance and immunocompetence of broilers under heat stress. *Arch. Anim. Nutr.* **63**, 56-65.
- Niu Z.Y., Liu F.Z., Yan Q.L. and Li W.C. (2009b). Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. *Poult. Sci.* **88**, 2101-2107.
- NRC. (1994). Nutrient Requirements of Poultry, 9<sup>th</sup> Rev. Ed. National Academy Press, Washington, DC., USA.
- Ogle C.K., Valente J.F., Guo X., Li B.G., Ogle J.D. and Alexander J.W. (1997). Thermal injury induces the development of inflammatory macrophages from nonadherent bone marrow cells. *Inflammation.* **21**, 569-582.
- Olfati A., Mojtahedin M., Sadeghi T., Akbari M. and Pastor F.M. (2018). Comparison of growth performance and immune responses of broiler chicks reared under heat stress, cold stress and thermoneutral conditions. *Spanish J. Agric. Res.* **16(2)**, e0505.
- Ozek K., Wellmann K.T., Ertekin B. and Tarım B. (2011). Effects of dietary herbal essential oil mixture and organic acid preparation on laying traits, gastrointestinal tract characteristics, blood parameters and immune response of laying hens in a hot summer season. *J. Anim. Feed. Sci. Technol.* **20**, 575-586.
- Pamok S., Aengwanich W. and Komutrin T. (2009). Adaptation to oxidative stress and impact of chronic oxidative stress on immunity in heat-stressed broilers. *J. Thermal. Biol.* **34**, 353-357.
- Parvar R., khosravinia H. and Azarfar A. (2013). Effect of supplementation of Satureja essential oils in drinking water on immune performance of broiler chickens reared under heat stress. *J. Cell. Anim. Biol.* **10**, 121-124.
- Popova M. and Popov C. (2002). Damage to subcellular structures evoked by lipid peroxidation. *Z. Naturforsch C.* **57(3)**, 361-365.
- Pournazari M., Qotbi A.A.A., Seidavi A.R. and Corazzin M. (2017). Prebiotics, probiotics and thyme (*Thymus vulgaris*) for broilers: Performance, carcass traits and blood variables. *Rev. Colombiana Cienc. Pecuaris.* **30**, 3-10.
- Ragga N.M., Kornay R.S. and Mohmad F. (2016). Effects of thyme / or formic acid dietary supplementation on broiler performance and immunity. *Agric. Sci. Proc.* **10**, 270-279.
- Rahim A., Mohsen D. and Alimirza A. (2011). Thyme (*Thymus vulgaris*) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. *Italian J. Anim. Sci.* **10(2)**, 101-105.
- Rashidi A.A., Gofrani Ivvari Y., Khatibjoo A. and Vakili R. (2010). Effects of dietary fat, vitamin E and zinc on immune response and blood parameters of broiler reared under heat stress. *Res. J. Poult. Sci.* **3**, 32-38.
- Richard M.J., Portal B., Meo J. and Jamroz C. (1992). Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. *Clin. Chem.* **38**, 704-709.
- Sahin K., Sahin N. and Yarlioglu S. (2002). Effects of vitamin C and vitamin E on lipid per-oxidation, blood serum metabolites and mineral concentrations of laying hens reared at high ambient temperature. *Biol. Trace. Elem. Res.* **85**, 35-45.
- SAS Institute. (2001). SAS<sup>®</sup>/STAT Software, Release 9.2. SAS Institute, Inc., Cary, NC. USA.
- Simitzis P.E. (2017). Enrichment of animal diets with essential oils-agreat perspective on improving animal performance and quality characteristics of the derived products. *Medicines.* **4**, 35-52.
- Soler-Rivas C., Espin J.C. and Wichers H.J. (2000). Oleuropein



- and related compounds. *J. Agric. Food Sci.* **80**, 1013-1023.
- Takazadeh F. and Mayahi M. (2017). Immune response of broiler chickens supplemented with pediatric cough syrup including thyme extract in drinking water against influenza vaccine. *J. Herbmед. Pharmacol.* **6(1)**, 33-36.
- Tawfeek S.S., AbdAlla Hassanin K.M. and Ibrahim Youssef I.M. (2014). The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions. *J. World's. Poult. Res.* **4(1)**, 10-19.
- Trout J.M. and Mashaly M.M. (1994). The effect of adrenocorticotrophic hormone and heat stress on the distribution of lymphocyte populations in immature male chickens. *Poult. Sci.* **73**, 1694-1698.
- Williams P. and Losa R. (2001). The use of essential oils and their compounds in poultry nutrition. *World's Poult. Sci. J.* **17**, 14-15.
- Yalçın S., Özkan S., Çabuk M.C. and Siegel P.B. (2003). Criteria for evaluating husbandry practices to alleviate heat stress in broilers. *J. Appl. Poult. Res.* **12**, 382-388.
- Yanishlieva N.V., Marinova E.M., Gordon M.H. and Raneva V.G. (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem.* **64**, 59-66.
- Zhang Z.Y., Jia G.Q., Zuo J.J., Zhang Y., Lei J., Ren L. and Feng D.Y. (2012). Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poult. Sci.* **91**, 2931-2937.
-