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Dietary inclusion of thyme essential oil alleviative effects of heat stress on growth performance and immune system of broiler chicks

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Ali OLFATI[1*], Ali MOJTAHEDIN[2]

[1]*Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, IRAN*
[2]*Assistant Professor of Physiology, University of Mohaghegh Ardabili, Ardabil, IRAN*
Corresponding author: A.olfati65@gmail.com; Tel: +989195966273;

Abstract. Heat stress (HS) is known to have adverse effects on growth performance and immune system. Medicinal plants are utilized as growth and immunity promoter. Thus, 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 submitted to HS. Broilers were divided into five groups; 1) the chicks fed with basal diet and reared under thermoneutral condition; 2) the chicks fed with basal diet and submitted to HS condition; 3, 4 and 5) the broilers fed with basal diet containing 100, 150 and 200 mg TEO/kg of diet and exposed to HS. Broiler chicks were evaluated for antibody titre against sheep red blood cell (SRBC) on 35 and 42 days. Birds were also bled (42 d) to evaluate the blood biochemical variables. HS increased feed conversion ratio and mortality and decreased body weight gain and feed intake (P<0.05). Dietary inclusion of TEO at levels of 150 and 200 mg/kg alleviated negative effects of HS on growth performance (P<0.05). HS increased the serum concentrations of cholesterol, triglycerides, corticosterone, malondialdehyde and heterophil count (P<0.05) and also reduced antibody titre against SRBC, lymphocyte count and lymphoid organs weight (P<0.05). Dietary inclusion of TEO at levels of 150 and 200 mg/kg alleviated negative effects of HS on blood biochemical variables and immune responses (P<0.05). TEO at levels of 150 and 200 mg can be advised for improving the growth performance and immune responses of broiler chicks in hot regions.
Keyword*:* broiler chicks, heat stress, humoral immunity, lymphoid organs.

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.,* 2009 a; b, GROZEA et al., 2017].

High environmental temperatures enhance the plasma concentration of corticosterone [QUINTEIRO–FILHO *et al.,* 2012] and heterophil to lymphocyte ratio [YALCIN *et al.,* 2003]. Other studies have reported that HS decreases relative weight of lymphoid organs [NIU *et al.,* 2009a. PENTEA et al., 2016, BUTU *et al.,* 2014c. SAMFIRA *et al.,* 2015, BUTNARIU *et al.,* 2015b; BUTU *et al.,* 2014b] and it also has negative effects on some blood biochemical variables in heat–stressed broiler chicks [HABIBIAN *et al.,* 2014].

HS could disturb a balance between the production of reactive oxygen species (ROS) and the antioxidant systems, resulting in the 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 [MUJAHIDD *et al.,* 2007, VARDANIAN *et al.,* 2018; STOLERU *et al.,* 2018, CAUNII *et al.,* 2015; IANCULOV *et al.,* 2004].

Poultry breeders need suitable strategies to alleviate the negative effects of HS on immune system. The use of plants and their derivatives may be suitable strategy for alleviating negative effects of HS. Essential oils are complex and variable chemical compounds [SIMITZIS *et al.,* 2017, SAMFIRA *et al.,* 2014; BUTNARIU; 2012; BUTU *et al.,* 2014a, STOLERU *et al.,* 2016] which are consisted of terpenes and phenylpropenes. *Thymus vulgaris* is a medicinal herb belonging to *Lamiaceae* family which is mainly cultivated for culinary uses, cosmetic perennial and medical in worldwide.

Thyme contains high amount polyphenols which are responsible for antioxidant activity in essential oils [DAHAL and FARRAN, 2011]. It is shown that flavonoids and polyphenolic substances have

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several pharmacological effects, such as antioxidant activity, preventing histamine release and arachidonic acid metabolism [AMRESH *et al.*, 2007, DIMITRIU *et al.*, 2016; GEORGIEVA *et al.*, 2018; BUTNARIU and CAUNII, 2013].

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, BUTNARIU *et al.*, 2012].

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, BAGIU *et al.*, *2012*; BUTNARIU and CORADINI, 2012]. Thyme products are known to have hypochelestolemic and antihyperlipdemic activities in broiler chickens [ABDULKARIMI *et al.*, 2011; DAHAL and FARRAN, 2011]. On the other hand, plant derivatives are usually utilized in animal feeding as growth promoter, because of their antioxidant, antimicrobial and digestion properties [ABDULKARIMI *et al.*, 2011; ASSIRI *et al.*, 2016, PUTNOKY *et al.*, 2013, BUTNARIU *et al.*, 2014; BUTNARIU and GIUCHICI, 2011]. Attia and collab. have reported that dietary inclusion of TEO improved growth performance in broiler chicks [ATTIA *et al.*, 2017]. Ragga and collab. have also shown that dietary inclusion of TEO increased weight gain and immune system in broiler chicks [RAGGA *et al.*, 2016, PETRACHE *et al.*, 2014, BUTNARIU *et al.*, 2014, BARBAT 2013, BUTU *et al.*, 2015].

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.

Material 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 ± 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) and each replicate 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.

Table 1.

Composition of the experimental diets (g/kg)

Diet composition (g/kg)	Starter (1–21d)	Grower (22–42d)	*Calculated chemical composition* Diet composition (g/kg)	Starter (1–21d)	Grower (22–42d)
			Metabolizable energy (MJ/kg)	12.13	12.89
Corn (8.5% CP)	647.10	699.50	Crude protein (g/kg)	210.50	190.0
Soybean Meal (48% CP)	312.00	251.90	Calcium (g/kg)	10.10	9.60
Soybean oil	0.00	5.00	Available phosphorus (g/kg)	5.00	4.80
Calcium carbonate	13.60	13.0	Lysine (g/kg)	11.60	13.30
Dicalcium phosphate	16.00	15.70	Methionine+ Cysteine (g/kg)	8.50	7.60
Salt	4.60	4.00	Na (g/kg)	2.00	1.50
Mineral and Vitamin Premix[a]	5.00	5.00	K (g/kg)	8.50	7.80
HCL–Lysine	0.00	4.40	Cl (g/kg)	2.50	2.30
DL–Methionine	1.70	1.50	Na+K−Cl (meq/kg)	221.00	203.00

a Mineral–vitamin premix provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D 3, 2,100 IU; vitamin E, 30 mg; nicotinic acid, 30 mg; vitamin B 12, 0.12 mg; calcium pantothenate,10 mg; vitamin K 3, 5 mg; thiamin, 1.1 mg; riboflavin, 4.5 mg; vitamin 6, 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; monensin, 100 mg.

The birds in chamber A were exposed to HS (23.9–38 °C daily) and broiler chicks in chamber B were reared under thermoneutral condition (TN; 23.9 °C) from 22 to 42 d of age. 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 [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 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 [NIU *et al.*, 2009b; AKBARI and TORKI, 2014] 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 and the data are presented in Table 2 [JULIANO 2000].

Table 2.

Composition of thyme essential oil

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

Growth performance. The broiler chicks were weighed at 1 and 42 days of age, and the body weight gain or BWG (g/bird) per replicate was calculated. Feed intake (FI) was recorded for each replicate (g/bird) as well as feed conversion ratio (FCR, g feed/g gain) was calculated. Mortality rate was considered to calculate the growth performance parameters during experiment.

Determination of blood biochemical variables. On d 42 and after 12–h 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.

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 De Jong and collab. [DE JONG *et al.*, 2001]. Malondialdehyde (MDA) was evaluated, as lipid peroxidation index, as described by Mj and collab. [MJ *et al.*, 1992]. The MDA is one of the thiobarbituric acid reactive substances (TBARS) substances. Mixture of these substances is in biological specimens and it is an index for oxidative stress.

Immune system variables. The sheep red blood cells (SRBC) were used, as an antigen test, to detect the specific antibody responses. 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 SRBC. At d 28, two birds per replicate were intravenously (i.v.) treated with 1 mL of 7 % SRBC suspension administrated to right wing [HABIBIAN *et al.*, 2014]. 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 1,500 × *g* at 4 °C for 20 minutes and the obtained sera were stored in –20 °C for further analysis. On day 35, 1 mL from 7 % SRBC suspension were i.e. 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, PETRACHE *et al.*, 2014, BUTNARIU *et al.*, 2014, BARBAT 2013, BUTU *et al.*, 2015].

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 µL 2.5 % SRBC suspension was administrated 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, BUTNARIU *et al.*, 2014, BARBAT 2013], 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 [GROSS and SIEGEL, 1983, BUTU *et al.*, 2015]. 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 software (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 ± standard deviation (±SD). The $\log_2$ 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.

Table 3.

Effects of dietary inclusion of thyme essential oil (TEO) on feed intake (g/chick), body weight gain (g/chick), feed conversion ratio (kg feed/kg BWG) and mortality (%) of broilers

Groups	Feed intake	Body weight gain	Feed conversion ratio	Mortality
Control–TN	4717.00±75.28^{a}	2608.00±91.74^{a}	1.80±0.04^{c}	0.00±0.00^{b}
Control–HS	3900.00±141.40^{c}	1775.00±82.16^{d}	2.24±0.09^{a}	6.67±5.16^{a}
100 TEO–HS	3942.00±128.10^{c}	1758.00±58.45^{d}	2.28±0.09^{a}	5.00±5.47^{a}
150 TEO–HS	4217.00±75.28^{b}	2108.00±111.40^{c}	2.00±0.08^{b}	0.00±0.00^{b}
200 TEO–HS	4292.00±66.46^{b}	2367.00±40.82^{b}	1.81±0.03^{c}	0.00±0.00^{b}
P	0.0001	0.0001	0.0001	0.0001
SEM	57.23	63.05	0.039	0.78

SEM: Standard Error of Means. The data were presented as means ± standard deviation (±SD). Superscripts (a–d) show significant differences among groups per column.

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 ($P<0.05$).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.

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

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

SEM: Standard Error of Means. The data were presented as means ± standard deviation (±SD). Superscripts (a–d) show significant differences among groups per column.

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 was 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).

Table 5.

Effects of dietary inclusion of thyme essential oil (TEO) on the anti–SRBC antibody response (log_2) of broiler chicks

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

SEM: Standard Error of Means. The data were presented as means ± standard deviation (±SD). Superscripts (a–d) show significant differences among groups per column.

The highest antibody titres against SRBC was observed in birds treated with 200 mg/kg of TEO (P<0.01). However, 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 group in comparison to control–TN group (P<0.0001; Tables 6–7).

Effects of dietary inclusion of thyme essential oil (TEO) on the relative weight of lymphoid organs of 42–day–old broilers. Our results showed that HS supressed growth performance but dietary inclusion of high levels of TEO improved growth performance. Olfati and collab. [OLFATI *et al.*, 2018] have reported that HS supressed 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

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crypt–depth ratio [DENG *et al.*, 2012]. Thus, HS by increasing corticosterone decreases growth performance which was confirmed by our findings.

Table 6.

Effects of dietary inclusion of thyme essential oil (TEO) on count of heterophil, lymphocyte or their ratio (H/L; in 100 cells) of 35–day–old heat–stressed broilers

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

SEM: Standard Error of Means. The data were presented as means ± standard deviation (±SD). Superscripts (a–d) show significant differences among groups per column

Results showed that corticosterone concentration was significantly higher in control–HS in comparison to control–TN. With regards to dietary inclusion of TEO, Pournazari and collab. [POURNAZARI *et al.*, 2017] have reported that dietary inclusion of TEO and probiotic, singly form, increased FI. Similar to our findings, Attia and collab. [ATTIA *et al.*, 2017] have reported that dietary inclusion of TEO improved growth performance in broiler chicks during summer season. Ragga and collab. [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.*, 2004]. TEO not only helps to absorb amino acids but also increases secretion the digestive enzymes which improves growth [LEE *et al.*, 2004. BUTU *et al.*, 2015]. 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.

Table 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).

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

SEM: Standard Error of Means. The data were presented as means ± standard deviation (±SD). Superscripts (a–d) show significant differences among groups per column

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, BUTU *et al.*, 2015]. 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 and collab. [ABDULKARIMI *et al.*, 2011].

The TEO could reduce cholesterol content through its effect on hepatic 3–hydroxy–3–methyl glutaryl coenzyme A reductase, the limiting enzyme in cholesterol biosynthesis [LEE *et al.*, 2004]. RAHIM and collab. [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. 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, BUTNARIU and SAMFIRA, 2012] 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 HS–control and TN–control 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 [QUINTEIRO–FILHO *et al.*, 2012, IANCULOV *et al.*, 2005, STOLERU *et al.*, 2018]. The TEO alleviated negative effects of HS on antibody titre and mortality. Similarly, Ragga and collab. have reported that dietary inclusion of TEO improved immune system in broiler chicks [RAGGA *et al.*, 2016]. Talazadeh and Mayahi have reported that water inclusion of thyme extract improved immune response in broiler chickens. In contrast to these findings [TALAZADEH and MAYAHI, 2017], 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 and collab. reported that diet supplementing with thymol+carvacrol increased the cellular and humoral immune responses in broilers [HASHEMIPOUR *et al.*, 2013]. Flavonoids and other phenolic components, present in essential oils, increased activity of vitamin C as immunostimulator [COOK and SAMMAN, 1996; MANACH *et al.*, 1996].

Amresh and collab. showed flavonoids and polyphenolic compounds help immune system through their antioxidant activity [AMRESH *et al.*, 2007]. 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 immunity 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, STOLERU *et al.*, 2018] 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, 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 [YALCIN *et al.*, 2003] and reduces relative weights of lymphoid organs of birds [NIU *et al.*, 2009a]. Glucocorticoid hormones reduce lymphocytes count [DHABHAR, 2002], since circulating lymphocytes in response to glucocorticoids join to the endothelial cells and subsequently emigrates from circulation into other tissues [DHABHAR, 2002].

Similarly, corticosterone decreases food consumption and it may reduce relative weight of lymphoid organs [NIU *et al.*, 2009a] 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 [PARVAR *et al.*, 2013] 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.

Conclusions

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 and 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 and 200 mg/kg, help to improve the immune system in tropical regions and summer season.

Declaration of conflict of interests. The authors report that they have no other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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