IJAS Iranian Journal of Applied Animal Science

Effects of Encapsulated Nano- and Microparticles of Peppermint (*Mentha piperita*) Alcoholic Extract on the Growth Performance, Blood Parameters and Immune Function of Broilers under Heat Stress Condition

Research Article

S. Yarmohammadi Barbarestani[1], F. Samadi[1*], S. Hassani[1] and G. Asadi[2]

[1] Department of Animal and Poultry Physiology, Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran
[2] Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Received on: 15 Jan 2017
Revised on: 20 Apr 2017
Accepted on: 30 Apr 2017
Online Published on: Dec 2017

*Correspondence E-mail: f.samadi@gau.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

This study was aimed to investigate the effects of encapsulated nano- and microparticles of peppermint (*Mentha piperita*) extract on the growth performance, blood parameters and immune system of broilers under heat stress conditions. In total, 320 one-day-old broilers (Ross, 308) were assigned into 4 experimental treatments: the control, peppermint alcoholic extract (PE, 200 ppm/kg body weight (BW)), encapsulated nanoparticles of peppermint alcoholic extract (ENPE, 200 ppm/kg BW), and encapsulated microparticles of peppermint alcoholic extract (EMPE, 200 ppm/kg BW). Each treatment consisted of 4 replicates with 20 chicks. Results showed PE, ENPE and EMPE treatments significantly improved feed conversion ratio (FCR). The birds administered by PE, ENPE and EMPE had significantly higher blood serum, total protein, albumin and globulin. Administration of PE, ENPE and EMPE resulted in lower total cholesterol, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), LDL:HDL ratio and triglyceride, and higher levels of high-density lipoprotein (HDL). The birds treated by peppermint had higher bursa relative weight, in particular, in those that received ENPE and EMPE than control. Treated birds with PE, ENPE and EMPE showed significantly lower H:L ratio. The serum antibody titers against sheep red blood cells (SRBC) were significantly higher in birds administrated by PE, ENPE and EMPE. Birds receiving ENPE and EMPE treatments had significantly higher titers of IgG for primary and secondary responses than that of those receiving the other treatments. In general, this study indicated that encapsulated nano- and microparticles of peppermint alcoholic extract have potential to improve the immunity and growth performance of broilers under heat stress conditions.

KEY WORDS broilers, encapsulated nano- and microparticles, heat stress, peppermint extract.

INTRODUCTION

Stressors are the most challenging environmental conditions affecting broilers because they are more sensitive to stressors than other domestic species (Geraertet *et al*. 1993; Mashaly *et al*. 2004; Yu *et al*. 2008). Stress is associated with reactive oxygen species (ROS) production. Excess generation of ROS can cause oxidative damage to macromolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis (Khan and Sultana, 2009). Stress-induced immune suppression is frequently reported (Puvadolpirod and Thaxton, 2000; Post *et al*. 2003; Mumma *et al*. 2006; Shini *et al*. 2008). The protective function of immune cells depends on the fluidity of cell membranes.

Lipid peroxidation of cell membrane through free radicals results in lower membrane fluidity, which adversely affects immune response (Bendich, 1993).

Among different types of stress, heat stress is of great concern in poultry industry. Immune function and production parameters including feed efficiency, growth rate, mortality, and other important traits governing productivity are adversely affected by heat stress (Shini *et al*. 2010). Immunosupression is the most reported effect of stress, which adversely affects immune organs and immune cells (Gross and Siegel, 1983; Puvadolpirod and Thaxton, 2000; Post *et al*. 2003; Mumma *et al*. 2006; Shini *et al*. 2008). Additionally, it has been demonstrated that immune system responds to stress conditions by the redistribution of circulatory leukocytes between lymphoid and non-lymphoid tissues, moving the cells required for the nonspecific response (i.e., heterophils) into the circulation and lymphocytes into the lymphoid organs and resulting in higher heterophil:lymphocyte ratio. It seems corticosterone is an end product when the hypothalamic-pituitary-adrenal axis is involved (Cohen, 1972).

With the rapid development of the poultry industry and the fact that chickens are continually subjected to some type of stress, it is of urgent necessity to use immune modulator to enhance immune function or ameliorate the adverse effects of stressors. There is growing interest to the traditional herbal medicines that are claimed to have a role in the amelioration of the adverse effects of oxidative stresses due to their free radical scavenging capability (Sharma *et al*. 2006; Singh and Gupta, 2011), which are 20-fold superior to other well-known antioxidants. Peppermint (*Mentha piperita*) is traditionally used as an antiseptic, antispasmodic, carminative, mild tonic, antimicrobial, and for the treatment of irritable bowel syndrome, inflammatory bowel disease, disorders of the biliary system and liver problems (Taylor, 1984; Boukra *et al*. 2005; Witkoswa and Sowinska, 2013; Ghasemi-Pirbaluti *et al*. 2017). Studies have demonstrated that the antioxidant and inflammatory properties of peppermint contributes to the prevention and treatment of diseases associated with oxidative stress, through removing free radicals and neutralizing ferryl ion-induced peroxidation (Sharma *et al*. 2006; Singh and Gupta, 2011; Ghasemi-Pirbaluti *et al*. 2017).

Peppermint has also been documented for compounds like eugenol, caffeic acid, rosmarinic acid, flavonoids and α-tocopherol shaping its antioxidant and anti-peroxidant properties. There is evidence that flavonoid compounds exhibit antioxidant and antitumor acticities (Knekt *et al*. 2002; Ghasemi-Pirbaluti *et al*. 2017). These biological functions are attributed to the radical-scavenging properties of flavonoids (Wang and Huang, 2004). Regarding the stimulatory effects of peppermint on the immune system, Awaad *et al*. (2010) found that peppermint activates intracellular innate immune and humoral immune response of chickens. Inclusion of peppermint plant powder in broiler diet fortifies the immune system which belongs to its active compounds (Fallah *et al*. 2013). In addition, anti-inflammatory function of peppermint has been reported by its capability in the reduction of tissue proinflammatory factors (Ghsemi-Pirbaluti *et al*. 2017).

Recent investigations have shown that the use of the active compounds in form of encapsulated fine particles provide better performance in the gastrointestinal tract (Kosaraju *et al*. 2006; Gouin, 2004). Similarly, Majeed *et al*. (2015) reported that encapsulation of extracts increased their stability, durability and bioavailability. Additionally, Liang *et al*. (2012) reported that the nano-encapsulation of plant extracts and essential oils of peppermint increased their stability and durability as well as bioavailability.

Hafeez *et al*. (2015) reported an improved performance for encapsulated essential oils than its powder form in broilers. The present work aimed to study the immune modulatory function of encapsulated nano- and microparticles of peppermint extract in broilers under heat stress conditions.

MATERIALS AND METHODS

Experimental medicinal plant, extraction and encapsulation

Peppermint plant used in this experiment was collected in summer season when the plant was in vegetative stage, from a research farm (36˚00'–16" north latitude and 59˚00'–36" east longitude; altitude: 985 m) of Ferdowsi University of Mashhad, Mashhad, Iran. Collected peppermint was shadow dried and ground with a laboratory hammer mill (Iran Khodsaz gristmill, ELS 300C, Iran). Total phenolic compounds were measured colorimetric, using folin-ciocalteu method (Guo *et al*. 2000). To prepare the peppermint extract, 200 g of dried plant powder was mixed with 1L ethanol 80% with the ratio of 2:10. Then it was shaken for 24 hrs to be completely mixed, thereafter passed through a filter paper and the resulting alcohol was removed by distillation under vacuum. The colorimetric measurements of total phenol, flavonoids and antioxidants of peppermint alcoholic extract were 1.21 (mg/g), 1.03 (mg/g) and 72.53%, respectively.

Encapsulated nano- and microparticles of peppermint alcoholic extract were synthesized by the principle involving cation induced controlled gelification of alginate. Briefly, calcium chloride (1.5%) was added to sodium alginate solution (1%) containing peppermint alcoholic extract (2 mL). Then tween 80 was added to the solution to improve wettability.

Followed by stirring for 30 min, the mixture was kept at room temperature overnight. Peppermint alcoholic extract loaded nanoparticles were recovered by centrifugation at 1000 rpm for about 35 min and washed thrice with distilled water to obtain the final pellet (Krishna Sailaja *et al*. 2011; You and Peng, 2005).

An encapsulated nanoparticles of peppermint extract image was captured by transmission electron microscope (LEO-ZEISS, Cambridge, England). Dynamic light scattering was used to measure the size and distribution of average particle size. Shape and size of nanoparticles were determined using scanning electron microscopy (CARL-ZEISS, Oberkoshen, Germany) and transmission electron microscopy (LEO-ZEISS, Cambridge, England).

Experimental birds, diets and design

A total of 320, one-day-old, male and female broiler chicks (Ross, 308) were purchased from a local commercial hatchery.

Birds were raised over a 42-d experimental period in an environmentally controlled room with wood shavings as litter at the research farm of Animal Science Faculty, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Golestan, Iran. The ambient temperature was maintained at 32 ± 1 ˚C at the start of experiment and then decreased by 1 ˚C every 2 days until a permanent temperature of 24 ˚C was reached at 35 d of experiment. The heat stress was applied once daily (from 09:00 to 15:00 h=6 hrs/d) during the last week of the experiment by increasing temperature to reach 34 ˚C. From 18:00 to 09:00 hrs, the poultry house temperature was reduced to 21 ˚C. The lighting schedule provided 23 hrs of light per day. The experiment was performed in two-phases, including a starter phase from d 1 to 21 and a finisher phase from d 22 to 42. All diets were fed in mash form and were based on corn-soybean meal (Table 1). All essential nutrients provided in the diets met or slightly exceeded NRC recommendations (NRC, 1994).

The experiment was performed in a completely randomized design. Birds were randomly divided into 4 experimental treatments: the control, peppermint alcoholic extract (PE), encapsulated nanoparticles of peppermint alcoholic extract (ENPE), and encapsulated microparticles of peppermint alcoholic extract (EMPE). Each treatment consisted of 4 replicates with 20 chickens. The birds of control group were administered 1 mL of saline; the chickens of PE group were administered with 200 ppm/kg body weight of PE solution daily by oral gavage; the chickens of ENPE group were administered with 200 ppm/kg body weight of ENPE solution daily by oral gavage, and the chickens of EMPE group were administered with 200 ppm/kg body weight of EMPE solution daily by oral gavage.

Treatments were applied from 22 d of experiment. Birds were provided with free access to feed and water throughout the trial. All experimental protocols were approved by the Animal Care and Use Committee of the Faculty of Animal Science of Gorgan University of Agricultural Science and Natural Resources (Gorgan, Golestan, Iran).

Growth performance

On d 21 and 42, birds were weighed by pen, and body weight gain (BWG) and feed intake (FI) were recorded after 12 hrs fasting. Feed conversion ratio (FCR) was calculated as FI divided by BWG. Dead birds were weighed for adjustment of feed utilization.

Biochemical measurements

At d 42, two birds per pen were selected at random and humanely killed by cervical dislocation. Blood samples were collected (5 mL) into a 10-mL non-heparinized tubes and then centrifuged at 3000 × g for 10 min at 4 ˚C to obtain serum. The serum samples were stored at −20 ˚C until biochemical analysis. Serum samples were analyzed for total protein, albumin, triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL), using commercial kits (Pars-Azmoon Co., Tehran, Iran).

Immunological measurements

Immune organ weights were obtained from 2 birds per pen. Birds were weighed and killed. The bursa, spleen, and thymus were dissected and weighed immediately. Organ weights were expressed as a percentage of BW.

At 42 d of age, 2 birds per pen were selected and their blood samples were collected using heparin containing syringes to avoid blood clot formation for hematological analysis. Blood smears were prepared on slides and painted by giemsa method. One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope, and then heterophil to lymphocyte ratio (H/L) was measured (Gross and Siegel, 1983).

Sheep red blood cells (SRBC) were used as non-pathogenic cellular antigen to quantify humoral immune response, as an indicator of immune competence in birds (Martin *et al*. 2006). At d 28, eight birds per treatment were randomly selected.

A 0.1 mL of 25% SRBC was injected into the breast muscle of the birds. To determine the antibody response against SRBC, blood samples were taken from the wing vein at 7 and 14 d after injection. The serum from each sample was collected, heat inactivated at 56 ˚C for 30 min, then analyzed for total and IgG anti-SRBC antibodies. All antibody titers were reported as log of the reciprocal of the last dilution in which agglutination was observed (Cheema *et al*. 2003).

Table 1 Composition and calculated analyses of the basal diets[1]

Ingredients (%)	Growth phases	
	0 to 21 d	22 to 42 d
Corn	565.5	605.6
Soybean meal	372.7	323.3
Soybean oil	23.8	36.9
Dicalcium phosphate	14.4	10.9
Calcium carbonate	12.8	13.8
Vitamin premix[2]	2.5	2.5
Mineral premix[3]	2.5	2.5
DL-methionine	1.5	1.2
Salt	4.3	3.3
Calculated analysis (%)		
Metabolizable energy (kcal/kg)	2950	3100
Crude protein	212.5	193.8
Calcium	9.2	8.7
Phosphorus (available)	4.1	3.4
Sodium	1.8	1.5
Lysine	11.5	10.3
Methionine	4.8	3.7
Cysteine	8.3	6.9
Theronine	8.1	7.3

[1] Claculated composition was according to NRC (1994).
[2] Vitamin premix (each kg contained): vitamin A: 3600000 IU; vitamin D_3: 800000 IU; vitamin E: 9000 IU; vitamin K_3: 1600 mg; vitamin B_1: 720 mg; vitamin B_2: 3300 mg; vitamin B_3: 4000 mg; vitamin B_5: 15000 mg; vitamin B_6: 150 mg; vitamin B_9: 500 mg; vitamin B_{12}: 600 mg and Biotin: 2000 mg.
[3] Mineral premix (each kg contained): Mn: 50000 mg; Fe: 25000 mg; Zn: 50000 mg; Cu: 5000 mg; Iodine: 500 mg and Choline chloride: 134000 mg.

Statistical analysis

The experiment was carried out as a completely randomized design with 4 treatments and 4 replicates each. Data were analyzed statistically using GLM procedure of SAS software (SAS, 2003).

Differences among treatment means were determined using the Duncan's test. The $\log_2$ transformations were done on antibody titers before statistical analysis. Probability values less than 0.05 were considered statistically significant differences ($P<0.05$).

RESULTS AND DISCUSSION

Growth performance

The effects of experimental treatments on growth performance of broilers under heat stress are shown in Table 2. No significant differences were observed in BWG and FI between treatments throughout the trial, whereas FCR was significantly affected at the end of trial. Peppermint in the forms of alcoholic extract and encapsulated nano- and microparticles improved FCR compared with the control.

Blood biochemical parameters

The effects of experimental treatments on the blood serum biochemical parameters in broilers are shown in Tables 3 and 4. The birds treated with peppermint extract in the forms of alcoholic extract, encapsulated nano- and microparticles had significantly higher serum levels of total protein, albumin and globulin than those in control group, suggesting hepatoprotective effect of peppermint on broilers subjected to heat stress. The administration of peppermint extract in the forms of alcoholic extract and encapsulated nano- and microparticles significantly decreased (P=0.0001) serum concentrations of total cholesterol, VLDL, LDL and triglyceride, whereas increased (P=0.001) serum HDL compared with control, suggesting positive effects of peppermint on liver function. LDL:HDL ratio significantly decreased (P=0.0001) in birds treated with peppermint. Interestingly, the positive effect of encapsulated nano- and microparticles of peppermint alcoholic extract was significant than alcoholic form of extract.

Lymphoid organs and immune response

The effects of experimental treatments on the heterophil, lymphocytes, heterophil: lymphocyte ratio and relative weights of lymphoid organs and liver are shown in Table 5. Treated birds with PE, ENPE and EMPE treatments showed significantly lower (P=0.0001) circulating heterophil and higher lymphocytes percentage and consequently lower (P=0.001) H:L ratio compared with control birds, indicating immune protective effect of peppermint. The relative weights of spleen, thymus and liver were not affected by treatments. However, birds treated by peppermint had higher bursa relative weight, in particular, in those that administrated with the ENPE and EMPE treatments (P=0.001).

Table 2 Effects of dietary treatments on growth performance of broilers chickens

Treatments	Body weight gain (g)			Feed intake (g)			Feed conversion ratio (g/g)		
	0-21 d	21-42 d	0-42 d	0-21 d	21-42 d	0-42 d	0-21 d	21-42 d	0-42 d
Control	466.1	1432.8	1898.7	1103.2	2586.0	3691.0	2.36	1.80^{a}	1.94^{a}
PE	464.9	1489.4	1954.0	1098.2	2550.7	3694.5	2.36	1.71^{b}	1.86^{b}
ENPE	463.0	1412.3	1875.3	1095.0	2416.5	3514.2	2.36	1.70^{b}	1.87^{b}
EMPE	464.2	1503.5	1967.7	1102.0	2565.5	3668.2	2.37	1.70^{b}	1.86^{b}
SEM	2.07	38.53	39.17	4.72	60.06	63.94	0.01	0.02	0.01
P-valve	0.26	0.45	0.65	0.32	0.44	0.19	0.52	0.04	0.03

PE: peppermint alcoholic extract; ENPE: encapsulated nanoparticles of peppermint alcoholic extract and EMPE: encapsulated microparticles of peppermint alcoholic extract.
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 3 Effects of dietary treatments on blood biochemical parameters of broilers at 42 d

Treatments	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Albumin:globulin
Control	3.56^{b}	1.51^{c}	1.43^{b}	1.06^{c}
PE	3.95^{a}	2.02^{b}	1.81^{a}	1.12^{ab}
ENPE	3.96^{a}	2.30^{a}	1.83^{a}	1.22^{ab}
EMPE	4.15^{a}	2.32^{a}	1.90^{a}	1.26^{a}
SEM	0.092	0.095	0.078	0.061
P-valve	0.0001	0.0001	0.0001	0.008

PE: peppermint alcoholic extract; ENPE: encapsulated nanoparticles of peppermint alcoholic extract and EMPE: encapsulated microparticles of peppermint alcoholic extract.
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 the treatments on blood biochemical parameters of broilers at 42 d

Treatments	Blood biochemical parameters					
	Cholesterol (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)	LDL:HDL
Control	178.43^{a}	17.48^{a}	112.63^{a}	48.31^{c}	87.39^{a}	2.33^{a}
PE	163.14^{b}	15.09^{b}	86.07^{b}	61.97^{b}	75.47^{b}	1.39^{b}
ENPE	151.13^{c}	13.11^{c}	65.91^{c}	72.10^{a}	65.56^{c}	0.91^{c}
EMPE	149.52^{c}	12.48^{c}	64.81^{c}	72.22^{a}	62.43^{c}	0.89^{c}
SEM	2.45	0.40	2.99	1.40	2.00	0.07
P-valve	0.0001	0.0001	0.0001	0.001	0.0001	0.0001

PE: peppermint alcoholic extract; ENPE: encapsulated nanoparticles of peppermint alcoholic extract and EMPE: encapsulated microparticles of peppermint alcoholic extract.
VLDL: very low-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol and HDL: high-density lipoprotein cholesterol.
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 Effect of dietary treatments on heterophil (H), lymphocytes (L), H:L ratio, weight of lymphoid organs and liver of broilers at 42 d

Treatments	Heterophil (%)	Lymphocytes (%)	H:L	Bursa	Thymus	Spleen	Liver
				(% of body weight)			
Control	26.12^{a}	70.87^{b}	0.36^{a}	0.09^{c}	0.19	0.12	2.46
PE	23.25^{b}	74.62^{a}	0.31^{b}	0.13^{b}	0.18	0.11	2.44
ENPE	22.73^{b}	75.62^{a}	0.29^{b}	0.18^{a}	0.19	0.10	2.47
EMPE	22.37^{b}	74.75^{a}	0.29^{b}	0.21^{a}	0.21	0.11	2.33
SEM	0.65	0.56	0.008	0.01	0.03	0.01	0.11
P-valve	0.0001	0.0001	0.001	0.001	0.89	0.50	0.59

PE: peppermint alcoholic extract; ENPE: encapsulated nanoparticles of peppermint alcoholic extract and EMPE: encapsulated microparticles of peppermint alcoholic extract.
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.

The primary and second responses of immune system against SRBC are summarized in Table 6. The serum antibody titers against SRBC were significantly higher in birds treated with PE, ENPE and EMPE than birds of control, suggesting immune protective effect of peppermint in heat stress condition. The birds treated by EMPE had significantly higher titer of total antibody than others for primary response (P=0.0001).

Titer of total antibody for the secondary response significantly increased with administrating ENPE and EMPE (P=0.0001). Birds receiving ENPE and EMPE treatments had significantly higher titers of IgG for primary (P=0.0001) and secondary (P=0.001) responses than that of those receiving the other treatments. Stressor conditions, as unavoidable parts of poultry farms, are associated by failures in vaccination, lowered immune function, decreased

growth performance and increased morbidity and mortality. There is an increasing interest in the use of herbs and medicinal plants in poultry feeding to overcome these problems. Medicinal plants, due to having secondary metabolites, are claimed to have positive effects on growth performance as a consequence of immune function enhancement. Administration of herbal extracts improve gastrointestinal function by improving hepatic and pancreatic functions (Madrid *et al*. 2003), increasing villi length, crypt depth and the number of goblet cells (Reisinger *et al*. 2011), and healing inflammatory bowel diseases (Ghasemi-Pirbaluti *et al*. 2017). These positive effects have been attributed to active substances such as carvacrol, flavonoids and menthol. It has been documented that flavonoid compounds exhibit antioxidant and antitumor properties (Knekt *et al*. 2002; Ghasemi-Pirbaluti *et al*. 2017), through its radical-scavenging capability (Wang and Huang, 2004).

In current study, growth performance in terms of BWG and FI were not significantly influenced by experimental treatments. These results were in agreement with Khodadust *et al*. (2015) who reported no significant effect on growth performance of broilers administrated by 2 mL/kg alcoholic extract of peppermint, whereas Al-Kassi and Witwit, (2010) and Akbari and Torki, (2014) reported an improved growth performance in broilers treated by peppermint extract under heat condition. A better feed conversion of 42-d-old broilers treated by peppermint (in the forms of PE, ENPE and EMPE) than that of control, suggesting a better metabolic utilization of nutrients due to improved hepatic and pancreatic functions (Madrid *et al*. 2003).

It has been reported that supplementation of broilers diet by medicinal by-products improved the function of gastrointestinal tract by increasing intestinal mucus secretion (Jamroz *et al*. 2003), decreasing harmful gut microorganisms (Witkoswa and Sowinska, 2013), influencing gut morphology (Reisinger *et al*. 2011; Shang *et al*. 2015), enhancing activity of digestive enzymes (Lee *et al*. 2003; Jang *et al*. 2004), stimulating bile secretion (Platel and Srinivasan, 2000; Williams and Losa, 2001) and possessing anti-inflammatory property (Ghasemi-Pirbaluti *et al*. 2017). Previously, an antimicrobial function of peppermint has been also reported (Helander *et al*. 1998). In this connection, it is believed that peppermint improves growth performance through changing intestinal environment in favour to useful microorganisms (Lovkova *et al*. 2001).

The results of this study suggest the positive effect of peppermint extract on the liver function by the elevation of circulatory total protein and albumin concentrations. An increased albumin: globulin ratio in current study is believed to be due to improved liver function as a consequence of hepatoprotective function of peppermint. Similarly, Akbari and Torki (2014) and Fallah *et al*. (2013) reported elevated serum levels of protein and albumin in broilers treated by peppermint. Hepatoprotective function of peppermint extract against CCL_4-induced stress oxidative was been reported on broilers (Khodadust *et al*. 2015) and rats (Patil and Mall, 2012; Jain *et al*. 2012). It is believed that some active components of peppermint including tocopherol, menthol and menthone have potential to support hepatocytes function (Escop, 2003; Mansoub, 2011).

The antioxidant function of the flavonoid compounds has been attributed to its radical-scavenging capability (Wang and Huang, 2004). In agreement to the results of the current study, Akbari and Torki (2014) reported that peppermint has positive effect on lipid metabolism by increasing serum concentrations of HDL can decreasing total cholesterol and triglycerides. Similarly, Fallah *et al*. (2013) reported increased circulatory level of HDL can decrease levels of total cholesterol, triglycerides and LDL in broilers treated by peppermint extract. The hypocholesterolaemic effects of medicinal plants have been reported (Pish-Jang, 2011; Yalcın *et al*. 2012).

The cholesterol-lowering effects of spice products are proposed to be associated with the reduction in the function of HMG-CoA reductase enzyme in hepatocytes (El-Dakhakhny *et al*. 2000). The lower of de novo cholesterol synthesis also enhances the expression of low-density lipoprotein receptors on hepatocytes, leading to higher LDL uptake by the hepatocytes and ultimately lower the blood LDLc levels (Fukushima and Nakano, 1996).

The relative lymphoid organ weights, circulatory H:L ratio and SRBC antibody titres measured in the current study can show the overall health and immune status of the birds. Generally, an excellent immune status can be reached in broilers, when the relative weight of bursa is at least 0.2% of the total BW (Sellaoui *et al*. 2012). Our results showed a higher relative weight of bursa of fabricius for birds administered by PMEP and a closer one for those treated with PNEP to the this index, suggesting the protective effect of peppermint in the forms of encapsulated particles against heat stress-induced immune suppression. In birds, the H:L ratio is a reliable index of stress response (Gross and Siegel, 1983) and the reduction in H:L ratio in birds received PE, PNPE and PMEP treatments might be due to immune stimulatory effect of peppermint. The immune protective properties of medical plants have been reported (Tajodini *et al*. 2014; Efati *et al*. 2013).

The function of immune cells depends on the fluidity of cell membranes which is adversely affected by free radicals (Bendich, 1993). The function of free radicals in lipid peroxidation is banned by flavonoid compounds (Wang and Huang, 2004).

Table 6 Effect of the treatments on the antibody titers against SRBC (log_2) of broilers at 42 d

Treatments	Total antibody		IgM		IgG	
	35 d	42 d	35 d	42 d	35 d	42 d
Control	2.62^{c}	2.12^{c}	1.37^{c}	1.00^{b}	1.25^{c}	1.12^{b}
PE	3.75^{b}	3.12^{b}	1.87^{bc}	1.87^{a}	1.87^{b}	1.37^{b}
ENPE	3.75^{b}	4.62^{a}	2.12^{a}	1.75^{a}	2.62^{a}	1.87^{a}
EMPE	4.62^{a}	4.00^{a}	2.25^{a}	2.00^{a}	2.37^{a}	2.00^{a}
SEM	0.31	0.22	0.31	0.32	0.23	0.22
P-valve	0.0001	0.0001	0.04	0.02	0.0001	0.001

PE: peppermint alcoholic extract; ENPE: encapsulated nanoparticles of peppermint alcoholic extract and EMPE: encapsulated microparticles of peppermint alcoholic extract.
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.

Corticosterone, end product of the hypothalamic-pituitary-adrenal axis, is responsible for the suppression of immune organs (thymus, spleen and bursa) and immune cells (Post *et al*. 2003; Mumma *et al*. 2006; Shini *et al*. 2008). Thus the higher relative weight of bursa and a lower H:L ratio in birds treated by PE, ENPE and EMPE, suggesting protective effect of peppermint against heat stress-induced immunosuppression. In general, the number of heterophil increases while the number of lymphocytes decreases in response to stressors (Gross and Siegel, 1983). There were low levels of heterophils in birds treated by peppermint extracts, indicating the modulatory effects of peppermint to alleviate potential detrimental effects of heat stress on broilers.

Both primary and secondary antibody responses were significantly increased when birds treated by peppermint extracts under heat stress conditions, suggesting its immune protective activity. Stress-induced immunosuppression has been frequently reported (Shini *et al*. 2010; Efati *et al*. 2013).

The lymphocyte cells (T and B) are highly sensitive to oxidative stress (Von Schantz *et al*. 1999). Positive effects of flavonoid compounds on oxidative stress-induced immunosuppression are attributed to scavenging free-radicals, interfering with free-radical producing mechanisms and increasing the function of endogenous antioxidants (Nijveldt *et al*. 2001).

Additionally, flavonoids have potential to regenerate other antioxidants with known immune-enhancing activity, such as vitamin E (Zhu *et al*. 2000) and carotenoids (Pietta and Simonetti, 1998).

CONCLUSION

In conclusion, the results of the present study suggest that peppermint extract, in particular, in the form of encapsulated nano- and microparticles have potential to improve immunity, which may beneficially affect health and performance of broiler chickens under heat stress conditions.

ACKNOWLEDGEMENT

The authors are grateful to Gorgan University of Agricultural Science and Natural Resources, Iran, for providing the experimental facilities and financial support.

REFERENCES

Akbari M. and Torki M. (2014). Effects of dietary chromium picolinate and peppermint essential oil on growth performance and blood biochemical parameters of broiler chicks reared under heat stress conditions. *Int. J. Biometeorol.* **58,** 1383-1391.

Al-Kassi G.A. and Witwit N.M. (2010). A comparative study on diet supplementation with a mixture of herbal plants and dandelion as a source of probiotics on the performance of broilers. *Pakistan J. Nutr.* **9,** 67-71.

Awaad M.H.H., Abdel-Alim G.A., Sayed Kawkab K.S.S., Ahmed A., Nada A.A., Metwalli A.S.Z. and Alkhalaf A.N. (2010). Immunostimulant effects of essential oils of peppermint and eucalyptus in chickens. *Pakistan Vet. J.* **30,** 61-62.

Bendich A. (1993). Physiological role of antioxidants in the immune system. *J. Dairy. Sci.* **76,** 2789-2794.

Boukra C., Achouri M., Idrissi H.L.M. and Hamamovchi M. (2005). Chemical composition and antifungal activity of essential oils of seven Movoccan Labiatae against *Botrytis cinerea*. *J. Ethnopharmacol.* **89,** 165-169.

Cheema M.A., Qureshi M.A. and Havenstein G.B. (2003). A Comparison of the immune response of a 2001 commercial broiler with a 1957 random bred broiler strain when fed representative 1957 and 2001 broiler diets. *Poult. Sci.* **9,** 1591-1529.

Cohen J.J. (1972). Thymus-derived lymphocytes sequestered in the bone marrow of hydrocortisone-treated mice. *J. Immunol.* **108,** 841-844.

Efati M., Samadi F., Dastar B., Azari M.A. and Hadhemi S.R. (2013). Effects of different levels of artichoke (*Cynara scolymus*) on growth performance and immune responses of broilers under heat stress. *Iranian J. Appl. Anim. Sci*. **4,** 399-403.

El-Dakhakhny M., Mady N.I. and Halim M.A. (2000). Nigella sativa oil protects against induced hepatotoxicity and improves serum lipid profile in rats. *Arznei. Forschung. Drug. Res.* **50,** 832-836.

Escop. (2003). European Scientific Cooperative on Phytotherapy (ESCOP) Monographs. The Scientific Foundation for Medicinal Products, Thieme, United Kingdom.

Fallah R., Kiani A. and Azarfar A. (2013). Effect of artichoke leaves meal and menthe extract (*Mentha piperita*) on immune cells and blood biochemical parameters of broilers. *Global. Vet.* **10,** 99-102.

Fukushima M. and Nakano M. (1996). Effects of a mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat and cholesterol- enriched diet. *Br. J. Nutr.* **76,** 857-867.

Geraert P.A., Guillaumin S. and Leclercq B. (1993). Are genetically lean broilers more resistant to hot climate? *Br. Poult. Sci.* **34,** 643-653.

Ghasemi-Pirbaluti M., Mostaghi E. and Bozorgi H. (2017). The effect of menthol on acute experimental colitis in rats. *European J. Pharmacol*. **805,** 101-107.

Gouin S. (2004). Microencapsulation industrial appraisal of existing technologies and trends. *Trends. Food Sci. Technol.* **15,** 330-347.

Gross W.B. and Siegel H.S. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis.* **27,** 972-979.

Guo F.C., Savelkoul H.F.J., Kwakkel R.P., Williams B.A. and Verstegen M.W.A. (2000). Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. *World's Poult. Sci.* **59,** 427-440.

Hafeez A., Manner K., Schieder C. and Zentek J. (2015). Effect of supplementation of phytogenic feed additives (powdered *vs*. encapsulated) on performance and nutrient digestibility in broiler chickens. *Poult. Sci* . **95(3),** 622-629.

Helander I.M., Alakomi H., Latva-Kala K., Mattila-Sandholm T., Pol I., Smid E.J., Gorris L.G.M. and Von Wright A. (1998). Characterization of the action of selected essentialoil components on gram-negative bacteria. *J. Agric. Food. Chem.* **46,** 3590-3595.

Jain S., Jain D.K. and Balekar N. (2012). *In vivo* antioxidant activity of ethanolic extract of *Menthapulegium* leaf against CCl_4 induced toxicity in rat. *Asian Pac. J. Trop. Biomed.* **2,** 737-740.

Jamroz D., Or da J., Kamel C., Wiliczkiewicz A., Wertelecki T. and Skorupinska J. (2003). The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. *J. Anim. Feed Sci. Technol.* **12,** 583-596.

Jang I.S., Ko Y.H., Yang H.Y., Ha J.S., Kim J.Y., Kim J.Y., Kang S.Y., Yoo D.H., Nam D.S., Kim D.H. and Lee C.Y. (2004). Influence of essential oil components on growth performance and the functional activity of the pancreas and small intestine in broiler chickens. *Asian-Australasian J. Anim. Sci.* **17,** 394-400.

Khan T.H. and Sultana S. (2009). Antioxidant and hepatoprotective potential of Aeglemarmelos Correa against CCl_4 induced oxidative stress and early tumor events. *J. Enzyme Inhibit. Med. Chem.* **24,** 320-327.

Khodadust M.R., Samadi F., Ganji F., Jafari Ahangarani Y. and Asadi G.A. (2015). Effects of peppermint (*Mentha piperita*) alcoholic extract on carbon tetrachloride-induced hepatotoxicity in broiler chickens under heat stress condition. *Poult. Sci. J.* **3,** 1-16.

Knekt P., Kumpulainen J., Järvinen R., Rissanen H., Heliövaara M., Reunanen A., Hakulinen T. and Aromaa A. (2002). Flavonoid intake and risk of chronic diseases. *American J. Clin. Nutr.* **76,** 560-568.

Kosaraju S.L., Dath L. and Lawrence A. (2006). Preparation and characterisation of chitosan microspheres for antioxidant delivery. *Carbohyd. Polym.* **64,** 163-167.

Krishna Sailaja A., Amareshwar P. and Chakravarty P. (2011). Different techniques used for the preparation of nanoparticles using natural polymers and their application. *Int. J. Pharm. Pharm. Sci.* **3,** 45-50.

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.

Liang R., Xu S., Shoemaker C., Li Y., Zhong F. and Hung Q. (2012). Physical and antimicrobial properties of peppermint oil nanoemulsions. *J. Agric. Food Chem.* **60,** 7548-7555.

Lovkova M.Y., Buzuk G.N., Sokolova S.M. and Kliment'eva N.I. (2001). Chemical features of medicinal plants (Review). *Appl. Biochem. Microbiol.* **37,** 229-237.

Madrid J., Herandez F., Garcia V., Orengo J., Megias M.D. and Sevilla V. (2003). Effects of plant extracts on ileal apparent digestibility and carcass yield in broilers at level of farm. Pp. 187-188 in Proc. 14th European Symp. Poult. Nutr. Lillehammer, Norway.

Majeed H., Bian Y., Ali B., Jamil A., Majeed U., Farid Khan Q., Lqbalk H.J., Shoemaker C.F. and Zhong F. (2015). Essential oil encapsulations: uses, procedures, and trends. *J. Chem. Soci.* **5,** 58449-58463.

Mansoub N.H. (2011). The evaluation of different levels of Menthe pulagum on performance, and blood parameters of broilers. *J. American Sci*. **7,** 388-391.

Martin L.B., Weil Z.M. and Nelson R.J. (2006). Refining approaches and diversifying directions in ecoimmunology. *Integr. Comp. Biol.* **46,** 1030-1039.

Mashaly M.M., Hendricks G.L., Kalama M.A., Gehad A.E., Abbas A.O and PattersonP.H. (2004).Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poult. Sci*. **83,** 889-894.

Mumma J.O., Thaxton J.P., Vizzier-Thaxton Y. and Dodson W.L. (2006). Physiological stress in laying hens. *Poult. Sci.* **85,** 761-769.

Nijveldt R.J., Van Nood E., Van Hoorn D.E.C., Boelens P.G., Van Norren K. and Van Leeuwen P.A. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *American J. Clin. Nut*. **74,** 418-425.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Patil K. and Mall A. (2012). Hepatoprotective activity of *Mentha arvensis* leaves againts CCl_4 induced liver damage in rats. *Asian Pac. J. Trop. Dis*. **2,** 223-226.

Pietta P. and Simonetti P. (1998). Dietary flavonoids and interaction with endogenous antioxidants. *Biochem. Mol. Biol. Int.*

44, 1069-1074.

Pish-Jang J. (2011). Comparison of effect of cuminumcyminum and probiotic on performance and serum composition of broiler chickens. *Ann. Biol. Res*. **2,** 630-634.

Platel K. and Srinivasan K. (2000). Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Nahrung*. **44,** 42-46.

Post J., Rebel J.M. and Huurne A.A. (2003). Physiological effect of elevated plasma corticosterone concentrations in broiler chickens. An alternative means by which to assess the physiological effects of stress. *Poult. Sci*. **82,** 1313-1318.

Puvadolpirod S. and Thaxton J.P. (2000). Model of physiological stress in chickens. 1. Response parameters. *Poult. Sci*. **79,** 363-369.

Reisinger N., Steiner T., Nitsch S. and Schatzmayr G.A. (2011). Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. *J. Appl. Poult. Res*. **20,** 272-283.

SAS Institute. (2003). SAS®/STAT Software, Release 9. SAS Institute, Inc., Cary, NC. USA.

Sellaoui S., Alloui N. and Mehenaoui Sand Djaaba S. (2012). Evaluation of immune status of the chicken using morphometry and histology of the bursa of fabricius. *J. Vet. Adv*. **2,** 440-443.

Shang Y., Regassa A., Kim J.H. and Kim W.K. (2015). The effect of dietary fructooligosaccharide supplementation on growth performance, intestinal morphology, and immune responses in broiler chickens challenged with *Salmonella enteritidis* lipopolysaccharides. *Poult. Sci*. 94, 2887-2897.

Sharma A., Sharma M.K. and Kumar M. (2006). Protective effect of *Mentha piperit* against arsenic-induced toxicity in liver of swiss albino mice. *Basic Clin. Pharmacol. Toxicol.* **100,** 249-257.

Shini S., Huff G.R., Shini A. and Kaiser P. (2010). Understanding stress-induced immunosuppression: exploration of cytokine and chemokine gene profiles in chicken peripheral leukocytes. *Poult. Sci*. **89,** 841-851.

Shini S., Kaiser P., Shini A. and Bryden W.L. (2008). Biological response of chickens exposed to corticosterone and a bacterial endotoxin. *Comp. Biochem. Physiol. Biochem. Mol. Biol*. **149,** 324-333.

Singh D. and Gupta R.S. (2011). Hepatoprotective activity of methanol extract of Tecomella undulate against alcohol and paracetamol induced hepatotoxicity in rats. *Life Sci. Med. Res*. **26,** 1-6.

Tajodini M., Samadi F., Hashemi S.R., Hassani S. and Shams-Shargh M. (2014). Effect of different levels of Artichoke (Cynara scolymus) powder on the performance and immune response of broiler chickens. *Int. J. Agric. Sci*. **4,** 66-73.

Taylor B. (1984). The mechanism of the inhibitory action of menthol on gut smoothmuscle. *British J. Surg*. **71,** 902-908.

Von Schantz T., Bensch S., Grahn M., Hasselquist D. and Wittzell H. (1999). Good genes, oxidative stress and condition-dependent sexual signals. *Proc. Royal Soc. London B.* **266,** 1-12.

Wang S.P. and Huang K.J. (2004). Determination of flavonoids by high performance liquid chromatography and capillary electrophoresis. *J. Chromatogr. A*. **1032,** 273-279.

Williams P. and Losa R. (2001). The use of essential oils and their compounds in poultry nutrition. *World Poult*. **17,** 14-15.

Witkoswa D. and Sowinska J. (2013). The effectiveness of peppermint and thyme essential oil mist in reducing bacterial contamination in broiler houses. *Poult. Sci*. **92,** 2834-2843.

Yalcın S., Yalcın S., Uzunoglu K., Duyum H.M. and Eltan O. (2012). Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) and black cumin seed (*Nigella sativa*) on performance, egg traits, some blood characteristics and antibody production of laying hens. *Livest. Sci.* **145,** 13-20.

You J.O. and Peng C.A. (2005). Calcium-alginate nanoparticles for nonviral gene delivery. *NSTI. Nanotech*. **1,** 270-273.

Yu J., Bao E.D., Yan J.Y. and Lei L. (2008). Expression and localization of HSPs in the heart and blood vessel of heat-stressed broilers. *Cell. Stress. Chaperon.* **13,** 327-335.

Zhu Q.Y., Huang Y. and Chen Z.Y. (2000). Interaction between flavonoids and α-tocopherol in human low density lipoprotein. *J. Nutr. Biochem.* **11,** 14-21.