



This experiment was carried out to evaluate different levels of peppermint (Mentha piperita) plant powder usage on feed conversion ratio (FCR), body weight (BW), feed intake (FI), body temperature, carcass parts (breast and thigh) and internal organs (liver, heart, gizzard) weights in broiler chicken. A total of 192 broiler chicken were randomly divided into 4 experimental treatments with 4 replicates (12 birds per replicate) arranged in a completely randomized design. Experimental diets consisted of: (1) basal diet (control); (2) basal diet + 1% peppermint powder; (3) basal diet + 2% peppermint powder and (4) basal diet + 300 mg of vitamin E per kilogram. Heat stress performed by setting room temperature on 34 °C for 8 hours/day from 35 to 42 days of age. Results showed peppermint powder supplement in all levels significantly affected the FCR at 21 days of age and BW at 42 days of age (P<0.05). Birds treated by basal diet plus vitamin E and control diet showed the highest and lowest FCR values, respectively, at 21days of age. Body weight and feed consumption were significantly reduced in birds in the heat stress group. Peppermint powder supplementation at the level of 1% reduced body temperature compared with the control group during heat stress period (P<0.05). Significant differences were observed between dietary treatments for the relative weights of carcass, breast and thigh at 35 days of age and breast, gizzard and liver relative weights at 42 days of age (P<0.05). Birds fed basal diet plus vitamin E had higher carcass weight than the control groups on 35 days. In general, the results of this study revealed that peppermint powder as a natural antioxidant has beneficial effects on chicken growth performance, body temperature regulation and carcass and internal organ weights.

KEY WORDS broiler, carcass traits, heat stress, peppermint, performance.

INTRODUCTION

Medicinal plants have been used for many years in the treatment of various diseases of animals and humans. Much research has been done on the role of medicinal plants in the treatment of diseases of birds, which some of these reported beneficial effects of medicinal plants (Cross *et al.* 2007; Hernandez *et al.* 2004). Peppermint (Mentha) is a member of the Labiatae family and one of the oldest me-

dicinal plants in the world which is a cross between two species namely *M. aquatic* and *M. spicata*. These are endemic to Europe and America, India, China and the Soviet Union. In fact, they are used in tradition medicine of eastern and western nations (Aflatuni, 2005; Schuhmacher *et al.* 2003; Pavela, 2005; Aridogan *et al.* 2002). Different species of mint are of particular importance because of their abundant essential oils that include menthol, carvone, limonene, beta pinene, Mentone, alpha-pinene and geraniol, and effective pharmacological compounds. However, the main flavonoid component of mint is menthol (Schuhmacher et al. 2003; Dorman et al. 2003). Peppermint, in addition to the essential oils, also contains tannins, glycosides, saponins and other bioactive components (Edris et al. 2003). Studies show that medicinal plants due to its antioxidant and flavonoid compounds could play an important role in improving cardiovascular health and liver diseases (Pouramir et al. 2006). Peppermint is widely used as a herbal medicine, and it is believed to improve the response of the immune system, fight secondary infections, treat effectively irritable bowel syndrome and inflammation of the gallbladder. It also has antiseptic and antispasmodic effects. Its effect on endophytic fungi muucihas been well examined and its role has been proven (Mimica et al. 2003). It has been proved that essential oils of this plant inhibits some types of salmonella infection (Tssou et al. 1995) and act against Candida ablicans (Ezzat, 2001). As an aromatic plant, it has been traditionally used as medicine. It extends the shelf life of food, inhibit bacteria and fungi growth (Jamroz et al. 2003; Menezes et al. 2004).

Peppermint oil use as a sedative has expanded in the tropics. It has been applied on the affected area to reduce pain and to improve blood flow. Moderate antibacterial effect against both gram-positive and gram-negative has been reported for this plant (Schuhmacher *et al.* 2003; Jamroz *et al.* 2003). Peppermint oil or peppermint tea is often used to treat gas and indigestion, and it may also increase the flow of bile from the gallbladder (Mimica Dukic *et al.* 2003; Forster, 1996). Also Mentha species can be used in reducing gas and cramping, alcoholic beverages, rheumatism and toothache (Shah and Mello, 2004).

Heat stress refers to the high ambient temperature and the heat resulting from metabolism, which increases body temperature (Aengwanich and Chinrasri, 2002). This is one of the most challenging environmental conditions affecting commercial poultry. Compared to other species of domestic animals, broiler chickens are more sensitive to high ambient temperatures. In spite of a rapid metabolism and high body temperature, broiler chickens have no sweat glands. Furthermore, fast-growing lean broilers generate more heat than their free-living counterparts living in the wild (Geraert et al. 1993). These physiological characteristics, in combination with confined housing, make it difficult for broilers to regulate their body temperature. As environmental temperature rises, food consumption, growth rate, feeding efficiency, carcass quality, egg shell quality and survivability all decline (Hashemi et al. 2007; Mashaly et al. 2004; Borges et al. 2004). The most dangerous heat illness is heat shock which may cause mortality. When the environmental temperature is above the thermal comfort zone, birds will suffer from heat stress and changes in blood acidity and metabolites occur. Cardiovascular system is a system that is involved in the regulation of body temperature. When the birds are housed under heat stress, cardiovascular system changes occur including the balance of acid-base, blood pH, respiratory alkalosis and decreased levels of blood viscosity, hematocrit and plasma protein concentration. And long-term heat stress may lead to damage to the lymphatic organs thus susceptible to bacterial diseases, viral and parasitic increases (Pardue and Thaxton, 1996). In order to reduce the harmful effects of heat stress, it is recommended that the birds be fed in the cool hours during the day and night, or to use food supplements, such as herbal medicine, along with the basal diet to reduce body temperature. These would result in increased feed consumption, feeding efficiency and bird's survival (Hayashi et al. 2004). Peppermint to have antimicrobial properties so prevent the growth of harmful bacteria in your digestive system thus improves digestion and absorption and increase the body weight (Aridogan et al. 2002). Also, Narimani-Rad et al. (2011) reported that dietary supplementation of medicinal plants mixture (1% Oregano, 0.5% Ziziphora and 0.5% Peppermint) caused performance and carcass quality improvement via more weight gain increase in carcass yield and then decreases abdominal fat deposition. And, they investigated 0.3 % ethanolic extract of peppermint to drinking water seem to have a positive influence on broiler performance productive via more carcass yield and decrease abdominal fat deposition. And Hernandez et al. (2004) reported supplementation of poultry diets with aromatic plants have a stimulating effects on digestive system of the animals through the increasing the production of digestive enzymes and by improving the utilization of digestive products through enhanced liver function. So that Lee et al. (2003) determined an increase in relative liver weight for birds given thymol, but this was seen only at the age of 21 d and not at 40 days that led increases of body weight. Also Galib and Al-Kassie (2010) showed the effect of peppermint on liver weight. They also reported that liver weight of control group was higher than those of the other groups. On the other hand Abbas (2010) presented that feeding of 3 g/kg of fenugreek, parsley and basil seeds had not significantly affected liver, carcass and abdominal fat. The aim of this study was to evaluate the effect of peppermint powder on growth performance of broilers, body temperature, body weight and the weight of internal organs.

MATERIALS AND METHODS

Plant preparation

Peppermint plant used in this experiment was collected in summer season when the plant was in vegetative stage, from the research farm $(36^{\circ}00'-16'')$ north latitude and

59°00'-36" east longitude; altitude:985 m) of Mashhad University of Ferdowsi, Mashhad, Iran. Collected leaves were shadow dried and grounded with a laboratory hammer mill (Iran khodsaz gristmill, ELS 300 C, Iran).

The total values of phenolic compounds were measured colorimetrically, using Folin-Ciocalteu method (Guo *et al.* 2000).

Chickens, diets and experimental design

A total, 192 one-day-old broiler chickens (Ross, 308) were purchased from a local commercial hatchery and raised over a 42-day experimental period. The chicks were placed in an environmentally controlled poultry house with wood shavings as litter at the research farm of Animal Faculty, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Golestan province, Iran. The temperature was set at 32 °C at 1 day of age and then decreased by 1 °C every 2 days until a permanent temperature of 24 °C was reached at 35 day of experiment. The heat stress was applied once daily (from 0800 to 1600 h=8 h/d) during the last experimental week by increasing room temperature to reach 34 °C. From 1600 to 0800 h, the environmental temperature was reduced to 21 °C. The lighting schedule provided 23 hrs of light per day.

A 2-phase feeding program was used, with a starter diet until 21 day and a finisher diet until 42 day of age. The compositions of the basal diets are shown in Table 1. Diets for each period were prepared with the same batch of ingredients, and all diets within a period had the same compositions. Diets were formulated to meet or exceed requirements by the NRC, (1994) for broilers of these ages.

The experiment was performed as completely randomized design in a 4×2 factorial arrangement, with 4 replicates of 12 chicks per replicate.

The dietary treatments included: basal diet (control), basal diet supplemented with 1 and 2 percent peppermint powder and basal diet supplemented with vitamin E. Broilers were provided with unlimited water and food *ad libitum*.

Measuring body temperature

For the measurement of body temperature on days 35 and 42 of the test, from each unit two chickens were randomly selected. The body temperature was measured using a digital thermometer in the time before and the peak of heat stress.

The relative weights of carcass and internal organs

In order to measure the relative weights of carcass and internal organs, at days 35 and 42 two chicks near the average weight of each experimental unit were selected and slaughtered. After slaughtering, carcass and internal organs weights were measured by a digital scale.

 Table 1
 Ingredients and calculated analyses of the basal diets¹

Ingredient (%)	0 to 21 d	22 to 42 d
Corn grain	56.5	60.56
Soybean meal	37.27	32.33
Soybean oil	2.38	3.69
Dicalcium phosphate	1.44	1.09
Calcium carbonate	1.28	1.38
Vitamin premix ²	0.25	0.25
Mineral premix ³	0.25	0.25
DL-methionine	0.15	0.07
Salt	0.43	0.33
Calculated analysis		
Metabolizable energy (kcal/kg)	2950	3100
Crude protein (%)	21.2	19.38
Calcium (%)	0.92	0.87
P (available) (%)	0.41	0.34
Sodium (%)	0.18	0.15
Lysine (%)	1.15	0.03
Methionine (%)	0.48	0.37
Cysteine (%)	0.83	0.69
Threonine (%)	0.81	0.73

¹ Calculated composition was according to NRC (1994).

 2 Each kg of vitamin premix contained: vitamin A: 3600000 IU; vitamin D₃: 800000 IU; vitamin E: 9000 IU; vitamin K₃: 1600 mg; vitamin B₁: 720 mg; vitamin B₂: 3300 mg; vitamin B₃: 4000 mg; vitamin B₅: 15000 mg; vitamin B₆:

150 mg; vitamin B_9 : 500 mg; vitamin B_{12} : 600 mg and Biotin: 2000 mg. ³ Each kg of mineral premix 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 performed as completely randomized design in a 4×2 factorial arrangement. A GLM procedure was performed (SAS, 2003) and the difference among the mean values was tested using the Duncan multiple range test at (P<0.05). Mean values and SEM are reported.

RESULTS AND DISCUSSION

Total phenol, flavonoids and antioxidants

The determined amounts of total phenol, flavonoids and antioxidants of alcoholic extract peppermint leaf are shown in Table 2.

 Table 2
 Total phenol, flavonoids and antioxidant of alcoholic extract peppermint (as dry weight)

Compounds	(mg/g)
Total phenol	2.7
Flavonoids	1.8
Antioxidants	72.0 %

Growth performance

The effects of peppermint powder on growth performance of broilers are shown in Table 3.

Tractment		0 to 21		0 to 42		
Treatment	BWG	FI	FCR	BWG	FI	FCR
Control	643.36	956.65	1.48 ^b	2005.02 ^{ab}	4209.73	2.10
1% peppermint	619.48	951.57	1.53 ^{ab}	1992.17 ^b	4210.64	2.11
2% peppermint	650.32	975.12	1.49 ^{ab}	2100.56 ^{ab}	4291.60	2.03
300 mg vitamin E	626.15	968.76	1.54 ^a	2121.36 ^a	4361.59	2.05
SEM	15.24	17.72	0.02	38.60	51.78	0.03
P-value	0.469	0.774	0.097	0.079	0.169	0.427

Table 3 The effects of peppermint on growth performance¹ of broiler chickens

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

BWG: body weight gain (g); FI: feed intake (g) and FCR: feed conversion ratio (g of feed/g of BWG). SEM: standard error of the means.

There were significant (P<0.05) differences among the treatments for feed conversion ratio (FCR) on day 21 and body weight (BW) gain on day 42 of experiment. Vitamin E supplemented group showed significantly (P<0.05) higher BW than the other treatments at 42 day of age. A significantly lower (P<0.05) FCR was observed in control group at 21 day of age.

Body temperature

The effect of dietary treatments on body temperature is shown in Table 4. Results showed significant differences between dietary treatments in body temperature in the peak time of heat stress on day 42. Body temperature was highest in the control group and lowest in 2 percent peppermint powder treated group (P<0.05).

The relative weights of carcass and internal organs

Data on the relative weights of carcass and internal organ are shown in Tables 5 and 6. Carcass, breast and thigh relative weights were significantly influenced by the dietary treatments at day 35 (P<0.05). The treatments did not show any significant effect on liver, heart and gizzard weights at day 35 (P>0.05). Peppermint powder did not affect on relative weights of carcass, breast and thigh on day 42 (P>0.05). The chicks fed diet supplemented with 1% peppermint powder had the highest gizzard and liver weights. Our results showed that peppermint powder ameliorated the stress effects on BW gain and FCR. Medicinal plant supplements are used commonly as dietary additives for humans.

They are chosen for their non toxic chemical composition, relatively low cost and easy availability. Also, over the past decades, medicinal plants and their extracts have been used in animal diets as feed additives in order to improve their performance, health and the quality of their products .Consistent with our findings, Nobakht and Aghdam Shahriar (2011) report 2% mixture of medicinal herbs (mint, camel thorn and mallow) improved daily gain and FCR than the control group, probably because of the antibacterial and antifungal effects of botanicals used in the experiment.

Data reported by Galib and Al-Kassi, (2010) shows an improvement in BWG and FCR under dietary treatment with peppermint powder. Al-Ankari et al. (2004) observed the positive effect of wild mint on performance of broiler chicks. In contrast, Toghyani et al. (2010) reported no effect for mint leaves on broiler performance measures, which may be due to the amounts or combinations of the active ingredients. Amasaib et al. (2013) revealed no significant effect for Mentha spicata on feed intak. The insignificant effect of addition of spearmint to the basal diet may be due to the fact that, the diets were isocaloric and it is expected that the feed consumption could be similar or may be due to the similar environmental during this period. On the other hand Ocak et al. (2008) reported that adding 0.2 percent mint to basal diet help increase growth and reduce mortality in chickens. Medicinal plants with an impact on microbial population, enhance metabolizable energy. In addition, the mint with antimicrobial properties prevent the growth of harmful bacteria in the digestive tract, thereby improving the digestion and absorption as well as body weight gain (Cross et al. 2007). Results of this study are in agreement with some previous research that indicated herbs, plant extracts, essential oil and / or the main components of essential oil did affect body weight, feed intake and feed efficiency in broilers because they have appetizing and digestion-stimulating properties and antimicrobial effects (Abbasi and Samadi, 2014). Most studies about medicinal herbs have been carried out their essential oils. However, the vast majority of studies on dietary essential oil supplementation did not find any stimulating or depressive effect of oils on voluntary feed intake of broiler chickens (Hernandez et al. 2004). The findings about body temperature of this study are in agreement with some preceding studies. Heat stress results from a negative balance between the net amount of energy flowing from the animal's body to its surrounding environment and the amount of heat energy produced by the animal. This imbalance may be caused by variations of a combination of environmental factors (e.g., sunlight, thermal irradiation and air temperature, humidity and movement) and characteristics of the animal e.g., species, metabolism rate and thermoregulatory mechanisms)

(Nienaber and Hahn, 2007; Renaudeau et al. 2012).

Treatment		35 d	42 d		
	Before heat stress	During peak heat stress	Before heat stress	During peak heat stress	
Control	41.62	42.11	41.50	42.28 ^a	
1% peppermint	41.58	41.87	41.38	42.05 ^{ab}	
2% peppermint	41.60	41.90	41.45	42.07 ^{ab}	
300 mg vitamin E	41.59	41.93	41.43	42.07 ^{ab}	
SEM	0.97	0.16	0.96	0.14	
P-value	0.08	0.07	0.07	0.07	

Table 4 The effects of peppermint on body temperature (°C) of broiler chickens

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 The effects of peppermint on organs weight (% of body weight) of broiler chickens at 35 d

Treatment	Carcass	Thigh	Breast	Heart	Gizzard	Liver
Control	54.65 ^b	16.70 ^b	17.05 ^b	0.58	1.87	2.71
1% peppermint	54.65 ^b	16.58 ^b	17.52 ^b	0.60	1.97	2.86
2% peppermint	55.20 ^b	17.06 ^b	18.00 ^b	0.60	1.96	2.76
300 mg vitamin E	57.67 ^a	18.09 ^a	20.19 ^a	0.65	1.91	2.62
SEM	1.05	0.40	0.90	0.03	0.13	0.24
Gender						
Male	56.40	17.41	18.46	0.60	1.94	2.62
Female	54.90	16.94	18.04	0.62	1.92	2.83
SEM	1.02	0.32	0.93	0.05	0.14	0.22
P-value						
Diet	0.03	0.008	0.01	0.39	0.86	0.79
Gender	0.07	0.09	0.07	0.30	0.90	0.32

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 6	The effects o	f peppermint	on organs	weight (%	% of body	v weight) of broiler chickens at 42 d
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Treatment	Carcass	Thigh	Breast	Heart	Gizzard	Liver
Control	58.42	18.73	19.57 ^{ab}	0.61	1.62 ^{ab}	2.25 ^b
1% peppermint	57.30	18.31	18.69 ^b	0.68	1.86 ^a	2.71 ^a
2% peppermint	58.70	19.09	19.68 ^{ab}	0.61	1.71 ^{ab}	2.41 ^{ab}
300 mg vitamin E	59.29	18.86	21.27ª	0.62	1.66 ^{ab}	2.53 ^{ab}
SEM	1.20	0.28	0.85	0.04	0.10	0.20
Gender						
Male	58.95	19.28 ^a	20.14	0.62	1.60	2.39
Female	58.26	18.31 ^b	19.96	0.64	1.71	2.46
SEM	0.90	0.36	0.96	0.06	0.09	0.17
P-value						
Diet	0.08	0.52	0.02	0.42	0.04	0.004
Gender	0.07	0.001	0.08	0.32	0.5	0.06

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.

Stresses such as heat stress leads to excessive production of free radicals, which reduces antioxidant capacity (Robert *et al.* 2003). In fact, heat stress affects the sympathetic nerves and causes catecholamine release thereby leading to an increase in free radicals in the blood and tissues of the body. Free radicals attack the structure of unsaturated fat hence damaging cell membranes (Curi *et al.* 2003). Free radicals give rise to peroxidation in cells and thereby increase lipoperoxide concentrations in the tissues. Lipoperoxide surplus leads to reduced enzyme activity of glutathione peroxidase, superoxide dismutase and catalase (Du *et al.* 2000). In these conditions, the plasma levels of certain vitamins and minerals involved in the antioxidant system decreases and the amount of active oxygen radicals (ROS) increases. In order to prevent free radical damage to the body, the use of antioxidants such as herbs, vitamins etc and reduce heat stress are beneficial as these donate electrons to free radicals, converting them into harmless com-

pounds and thus protects cells from oxidative damages (Aryaeian *et al.* 2011). Also, researchers have noted that heat stress causes an increase in body temperature, which consequently bring about alkalosis. Yet, heat stress, by increasing blood albumin, controls, up to some extent, the alkalosis (Aengwanich and Chinrasri, 2002). Mirsalimi *et al.* (1996) reported a male bird's body temperature is higher than female because ofhigher metabolism. Hashemi *et al.* (2007) argued that on day 38, the male bird's body temperature is significantly higher than the degree of female body temperature.

Under conditions of high temperatures, birds change their behavior and physiological homeostasis, or by taking some herbs, regulate body temperature and thus reduce their body temperature. This study has showed that peppermint can reduce body temperature under heat stress, which may be due to the presence of menthol in peppermint.

Wang *et al.* (1998) found that complex bioactive components in natural medicines, with their function not clearly identified, along with nutrients and bioactive compounds have antimicrobial activity, increase immunity and reduce stress such as heat stress.

The results of this study suggest the important role of peppermint in controlling the weight of carcass and internal organ. Such findings are consistent with the results Narimani Rad et al. (2011) who reported that the complementary mix of medicinal herbs (1% Oregano, 0.5% Ziziphora and 0.5% Mint) improves the performance and carcass quality. In fact, medicinal plants, by reducing harmful bacteria in the digestive tract, slow down the decomposition rate of amino acids and proteins in the digestive juices which results in more uptake, improved carcass quality and reduced protein conversion to fat (Szewczyk et al. 2006; Lee et al. 2004). In accordance with this Alcicek et al. (2003) observed an improvement on carcass yield of broilers when supplemented with an essential oil combination in a broiler diet, in addition Chand et al. (2011) also used different levels of medicinal plants in broiler chicks and found significant improvement in leg weight. Toghyani et al. (2010) and Ocak et al. (2008) evaluted that, use of peppermint had not any significant effect on relative weight of organs and body parts. Also Hernandez et al. (2004) reported that, use of antibiotic or mixtures of plant extracts had not any significant effect on carcass traits of broilers. And, Gurbuz and Ismael (2015) said peppermint and basil as feed additive weren't significant effect on the carcass, carcass yield and abdominal fat.

Kusandi and Djulardi (2011) showed that high temperature reduces weight of the bursa of fabricius, liver and spleen. This reduction may be due to rising temperatures and loss of appetite. Khaligh *et al.* (2011) observed no significant differences in liver weight of chicks fed with herb mixtures. In contrast, Guo *et al.* (2000) reported that the use of medicinal plants has led to the increased weight of the lymphoid organs such as thymus, spleen and bursa of fabricius in broiler chickens, this difference may be due to different levels of medicinal plants. Various research has been carried out on the use of medicinal plants on the weight of the carcass. The use of medicinal plants in the production of broiler chickens significantly improve the gizzard weight (Chand *et al.* 2011; Khan and Durrani, 2007).

CONCLUSION

In general, results indicated that supplementation of peppermint powder in the diet improve oxidative stability, performance, body temperature in heat stress, carcass and internal organ weights.

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