Research Article

Effects of Heat Stress on Some Productive and Histological Traits in Broiler Feed 3% and 5% Mint Powder Supplement

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Abstract | Thermal stress is considered the greatest important environmental challenge due to the gradual and fast increase in mean global temperature. The current study was designed to estimate the therapeutic role of dried mint leaves powder in the diet of broiler chicken exposed to thermal stress on production and histological parameters. Broiler chickens (n= 160, Ross 308) were divided randomly into four groups first one was the negative control group which kept without exposure to heat stress or mint treatment, while groups 2-4 kept under heat stress. Group 2 kept under heat stress but without mint treatment, while the groups 3 and 4 exposed to heat stress accompanied with treatments of 3% and 5% peppermint leaves powder respectively in the diet. The experiment lasted for 35 days. Results revealed that the negative control group, as well as both the 3% and 5% mint-supplemented groups, exhibited significantly ($P<0.05$) higher feed intake, body weight, ALT, and AST levels compared to the positive control group, which showed significantly ($P<0.05$) lower levels. In contrast, the positive control group showed a significantly ($P<0.05$) higher cortisol hormone level compared to all other treated and negative control groups. In the histological examination of the liver and small intestine, all treated groups exhibited normal organs without adverse gross or microscopic changes, while the positive control group that exposed to heat stress without mint treatment, showed macroscopic and microscopic deleterious alterations, especially in the livers. The livers displayed swelling, congestion, paleness, edema, and enlargement with rounded edges in a gross examination. The small intestine showed congestion of blood vessels with petechial hemorrhages and appeared pale to whitish in color. Based on the current results, it can be concluded that using dried peppermint powder in the diet of broiler chickens exposed to thermal stress has positive effects in preventing the various deleterious effects of heat stress, especially when used at a 5% mint leaves percentage.

Keywords | Thermal, Calamite, Poultry, Mentha Longifolia, Microbiome, Medicinal Plant, Mint.

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INTRODUCTION

It is worth mentioning that 2023 was the warmest year since global records began in 1850, and climate change will have a major impact on Middle Eastern countries, especially Iraq, with temperatures expected to increase by 1-2 °C by 2030-2050 (IPCC. Climate Change, 2013). Thermal stress is a vital issue of animal production in tropical and sub-tropical regions which has numerous adverse effects on poultry as well as livestock. Several researchers showed that the adverse effects of thermal stress are principally associated with oxidative stress and free radical production, the disorders of hormone synthesis, cell apoptosis, and abnormal cell functions (Chen et al., 2021; Dalolio et al.,2021). Thermal stress is considered a significant problem in handling poultry industries in hot weather regions, causing important economic losses in the poultry trade and industry (Diarra and Tabuaciri, 2014) High climate temperature prompts oxidative impairment in the liver, and soft tissues of chicken, which promotes catabolism and disturbs lipid metabolism (Emami et al., 2020). Several unfavorable outcomes, such as low fertility, dehydration, low live-ability, high morbidity, altered meat quality characterized by augmented adiposity and reduced skeletal muscle mass associated with poor production were recorded in birds persistently kept at >30°C (Emami et al., 2020). The most observable stress behaviors include elevation of respiratory frequency, panting, reduced appetite, and adverse change in metabolism pathways (Raza et al., 2021). It is well known that thermal stress causes a rise in reactive oxygen free radical production, which in turn causes oxidative impairment and a reduction in oxidative capacity (Song et al., 2018).

Many therapeutic and physiological methods were used to decrease the deleterious effects of thermal stress like the use of medicinal plants. Mint plant has high antioxidant and free-radical scavenging activity related to *Mentha longifolia* species. Its extract has a potent ability to protect against crucial cell damage due to its markedly high free-radical scavenging activity (Ocak et al., 2008). The composition of essential oils from mint exhibits strong antibacterial and antioxidant activities. Several therapeutic properties are associated with the stems and leaves of mint. The leaves contain essential oils that are beneficial to digestion (Mikaili 2013). Mint is positively associated with broilers' growth in an earlier study (Ocak et al., 2008). It also supports a healthy microbiome in poultry guts (Asadi, 2017). So, this experiment aimed to investigate the effects of dried mint leaves inclusion in feed on some physiological parameters, production performance and histological alterations in broiler chickens exposed to heat stress.

MATERIAL AND METHODS

Design of the experiment

The present work conducted to estimate the effects of mint leaf powder as feed additives in diets of broiler chickens exposed to thermal stress. The study was carried out in accordance with the animal ethics protocol and was done in an animal farm of the College of Veterinary Medicine - Baghdad University –Iraq, during a period from 1/5/2023 to 15/6/2023. A total of one hundred sixty (160) broiler chicken (Ross 308 strain) with an average initial body weight of 50 gm were used. The birds were obtained from local market, and randomly divided into 4 treatment groups (40 to each) in cages with 2 replicate/group (20/cage). The diet was formulated each twice weeks (Table 1). The first group supplied with standard diet and was regarded as the negative control group, the second group fed the similar basal diet and kept as positive control, while the third and fourth groups supplied with same basal diet including with 3% and 5% shadow dried mint leaves respectively. All groups provided with diet and water ad libitum. The chickens of the negative control group maintained at suitable and normal climate conditions (23 ± 2°C with a 70% humidity). While the other groups including positive control and 3% and 5% mint groups were exposed to 33 ± 3°C for 10 hours per day (8:00–18:00 each day).

Table 1: Composition and ingredients of diets used in the experiment.

Item	Starter diet %	Finisher diet %
Maize	50	53
Soya bean meal	28	25
Wheat	7.5	7.5
Animal sources Protein (50%)	10	10
Premix	3	3
Common Salt , NaCl	0.3	0.3
Limestone	1.2	1.2
Total	100	100
Nutrient ingredients		
Crude protein %	22.50	20.00
Moisture %	6.54	6.20
Total ash %	8.15	9.20
Calcium %	0.975	1.025
Phosphate %	0.61	0.78
Methionine	0.53	0.55

(NCR, 1994 ; Olusegun et al, 2019)

Mint powder preparation

The fresh mint leaves bought from local market were shadow-dried for a couple of days then ground by an electric machine grinder, and powder was stored in plastic bags until directly mixed with a diet of chicken according to mentioned percentages.

Sampling and analysis

Feed consumption and body weight were calculated and analyzed weekly. At 35 day age, 3 birds from each group were sacrificed and specimens of liver tissue (about 1 cubic cm) and small intestine (duodenum, about 1 inch) were cut and prepared for histological examination. Additionally blood was collected and used to separate serum from the whole blood using centrifugation (12,000 rpm, 10 min at 4°C). For serum biochemical analyses, enzyme linkage immuno-sorbent assay (ELISA) kits were obtained from commercial source (Monobind Inc. Lake Forest, CA92630, USA) and used following the procedure mentioned in the kit to analyze the alanine transaminase (ALT), aspartate transaminase (AST) enzymes as well as cortisol hormone.

Histological examination

The tissues of liver and small intestine were fixed with 4% paraformaldehyde for 48 hours, dehydrated by ethanol, and

Table 2: Effect of 3%, and 5% mint supplementation in diet of broiler under heat stress on body weight (gram).

Groups*	7days	14 days	21 days	28 days	35days
C-	164.17±1.86C	473.24±3.52aC	813.07±21.76aB	1322.73±12.18aA	2341.73±32.28aA
C+	159.23±1.20C	309.05±2.38bC	647.74±120.68cB	1058.90±63.16cA	1942.90±18.66cA
3%	163.76±1.34C	429.47±6.71aC	760.71±80.12bB	1156.12±41.26bA	2150.12±83.96bA
5%	164.16±0.99C	435.31±0.28aC	797.34±43.63aB	1158.01±31.38bA	2198.01±91.88bA
LSD			141.91		

a-c: different lowercase letters in a column refer to present significant difference at (P<0.05)
A-C: different uppercase letters in a row refer to present significant difference at (P<0.05)
* C-: negative control group, without exposure to heat stress or mint treatment; C+: positive control group, heat stressed but without mint treatment; 3%: heat stressed, and supplemented 3% mint leaf powder in the diet; 5%: heat stressed, and supplemented 5% mint leaf powder in the diet.

Table 3: Effect of 3%, and 5% mint supplementation in diet of broiler under heat stress on feed intake/bird /day (gram).

Groups*	7days	14 days	21 days	28 days	35days
C-	125.54±5.00aD	230.51±2.00aC	308.76±0.54aB	397.15±1.25aA	510.25±1.25aA
C+	115.20±4.44cD	215.32±4.08bC	267.59±3.76dB	345.77±6.21cA	435.17±6.21cA
3%	122.55±2.15bcD	235.54±5.97aC	289.86±4.86cB	389.77±3.97bA	475.77±3.97bA
5%	129.54±3.99abD	233.65±3.28aC	310.04±3.63aB	403.48±9.88aA	495.48±9.88aA
LSD			8.43		

a-c: different lowercase letters in a column refer to present significant difference at (P<0.05)
A-C: different uppercase letters in a row refer to present significant difference at (P<0.05)
* C-: negative control group, without exposure to heat stress or mint treatment; C+: positive control group, heat stressed but without mint treatment; 3%: heat stressed, and supplemented 3% mint leaf powder in the diet; 5%: heat stressed, and supplemented 5% mint leaf powder in the diet.

finally embedded in paraffin. Then sections were cut into 5 µm slices. Sections were seen under a microscope (Olympus, Tokoyo, Japan) after staining with hematoxylin and eosin stain. Five to 10 fields of each section and at least two sections per animal.were examined using the microscope.

Statistical analysis

Data were analysed using Statistical Analysis System (SAS) by employing ANOVA test (SAS, 2012). The least significant differences (LSD) and P value were calculated and presented in tables. For significance of results (P < 0.05) was considered.

RESULTS

Body weight

The data of body weight illustrated in Table 2 revealed that negative control group which kept in normal comfortable climate condition without mint treatment exhibited significantly (P<0.05) high levels of body weight as compared to other groups. The positive control group that kept under heat stress exhibited a lower (P<0.05) body weight as compared to negative control group. Interestingly, mint treatment showed positive effect (P<0.05) on body weight and improved the weight of birds as compared with positive control group.

Feed intake

The feed consumption results presented in Table 3 showed that birds in positive control group that kept under heat stress significantly (P<0.05) reduced the feed intake as compared to the birds kept in normal comfortable climate condition (negative control). Mint treatment (both 3 and 5%) exhibited a raise (P<0.05) in the feed intake as compared to the birds given heat stress without mint treatment (positive control).

Cortisol hormone

The results showed that serum cortisol level of the positive control group was significantly (P<0.05) elevated than both mint treated groups as well as the negative control group which exhibited a significantly (P<0.05) lowest value. Moreover, there were insignificant differences between the 3% and 5% mint received groups (Table 4).

Liver enzymes

Data of liver enzymes presented in Table 5 and 6 revealed that the positive control group exhibited a significantly (P<0.05) higher levels of both AST and ALT in comparison with negative control and both mint treated groups. All groups exhibited a gradual increase in AST and ALT level started from the first test at 7 day age till to final one at 35 day age.

Table 4: Effects of adding 3% and 5% mint powder in diet of broiler, exposed to heat stress on blood cortisol level (nmol/L).

Groups*	Test at 28 day	Test at 35 day
C-	2.09±0.03b	2.16±0.01b
C+	2.97±0.24a	3.04±0.21a
3%	2.18±0.10b	2.30±0.09b
5%	2.38±0.11b	2.21±0.07b
Least Significant Differences	0.39	

a-c: different lowercase letters in a column refer to present significant difference at (P<0.05)

* C-: negative control group, without exposure to heat stress or mint treatment; C+: positive control group, heat stressed but without mint treatment; 3%: heat stressed, and supplemented 3% mint leaf powder in the diet; 5%: heat stressed, and supplemented 5% mint leaf powder in the diet.

Table 5: Effects of adding 3% and 5% mint powder in the diet of broiler, exposed to heat stress on blood ALT enzyme level (U/L).

Groups*	7days	14 days	21 days	28 days	35 days
C-	2.26±0.03b	2.31±0.05b	2.43±0.12b	2.23±0.20b	2.47±0.10b
C+	3.07±0.28aC	3.85±0.45aB	4.86±0.38aA	4.25±1.28aAB	4.45±0.38aAB
3%	2.83±0.29ab	2.77±0.33b	2.61±0.20b	2.61±1.25b	2.67±0.15b
5%	2.60±0.09ab	2.38±0.28b	2.09±0.31b	2.96±1.20b	2.30±0.10b
LSD			0.73		

a-c: different lowercase letters in a column refer to present significant difference at (P<0.05)

A-C: different uppercase letters in a row refer to present significant difference at (P<0.05)

* C-: negative control group, without exposure to heat stress or mint treatment; C+: positive control group, heat stressed but without mint treatment; 3%: heat stressed, and supplemented 3% mint leaf powder in the diet; 5%: heat stressed, and supplemented 5% mint leaf powder in the diet.

Table 6: Effects of adding 3% and 5% mint powder in the diet of broiler, exposed to heat stress on blood level of AST enzyme (U/L).

Groups*	7days	14 days	21 days	28 days	35days
C-	184.96±1.19b	194.97±1.32b	195.37±1.51b	188.13±1.30b	199.53±2.90b
C+	241.68±3.78aB	257.29±8.18aB	274.22±9.29aB	274.27±13.16aA	294.57±23.06aA
3%	188.55±2.18b	194.87±1.35b	192.58±2.41b	181.66±2.14b	192.66±1.04b
5%	191.47±2.97b	196.55±1.25b	198.38±3.37b	183.44±3.26b	196.74±2.96b
LSD			19.44		

a-c: different lowercase letters in a column refer to present significant difference at (P<0.05)

A-C: different uppercase letters in a row refer to present significant difference at (P<0.05)

* C-: negative control group, without exposure to heat stress or mint treatment; C+: positive control group, heat stressed but without mint treatment; 3%: heat stressed, and supplemented 3% mint leaf powder in the diet; 5%: heat stressed, and supplemented 5% mint leaf powder in the diet.

Histological alterations

As shown in Figure 1, heat stress significantly deteriorated the gross and histological structures of liver and intestine. Liver showed swelling and paleness at multiple spots with marked congestion, edematous and enlarged rounded edges. There was dilatation of liver sinusoids portal vein. The small intestine showed congestive blood vessels with petechial hemorrhages. Duodenum exhibited villi hyperplasia, proliferation of epithelial cells with destructive intestinal glands.

DISCUSSION

The current study exhibited that mint treatment have positive effects on body weight and feed intake of broilers reared under heat stress. These results are in close agreement with (Ramiah et al., 2019) who suggested that the avian species are greatly predisposed to thermal stress thus very sensitive to current global warming (Ramiah et al., 2020). On the same context, some researches determined that long exposure to heat stress has clear effects on the mass and quantity of breast and thigh muscle in broilers, and also showed a lower protein composition and high amount of fat in chicken meat exposed to thermal stress (Tafrahi et al., 2021; Hassan, 2023). Durrani et al. (2008) reported that the supplementation of peppermint leave powder in broiler diet improved the feed intake and weight gain compared to control group.

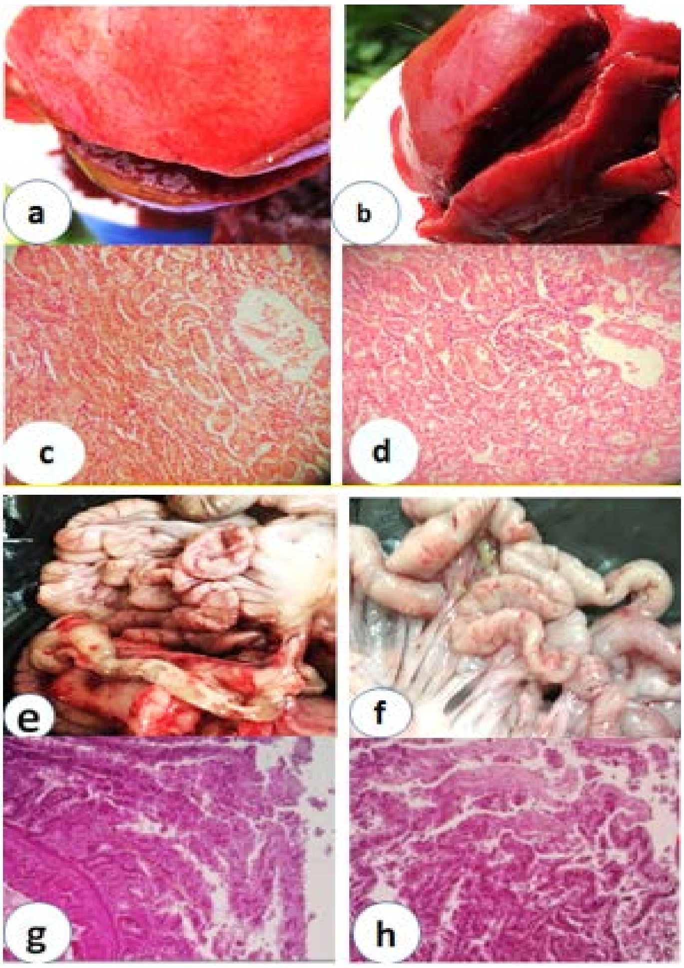

Figure 1: Gross and histological images of the liver and intestine of chickens reared under heat stress. **a**. Liver showed swelling and multiple spots of congestive pales to whitish areas. **b.** Liver showed marked congestion, edematous with enlarged rounded edge. **C**. Liver tissue with cloudy swelling of liver cells with dilatation of sinusoidal portal vein. **d**. Liver tissue showed dilated liver parenchyma, lymphocytic cuffing, and disorganization of the hepatic cord. **e**. Small intestine shows congestive blood vessels with petechial hemorrhages. **f**. Small intestine appeared congestive of blood vessel dilatation and empty lumen of the intestine. **g**. Small intestine (Duodenum) appears hyperplasia villi, proliferation of epithelial cells with, destructive some intestinal glands **h**. Small intestine (duodenum) with necrosis and destructive of the cell wall and proliferation of the villi with intestinal catarrhal discharge.

Furthermore, stress due to heat exposure might be led to endocrine hormones alterations and stimulate lipid accumulation by increasing lipogenesis and reducing lipolysis as well as increase amino acid degradation (Abdulabbas and Al- Saadi, 2022). Current work is consistent with Asadi et al. (2017) whom found that usage of peppermint in the feed of broiler showed an improvement in the ratio of beneficial to hazardous bacteria in cecum which reflected the satisfactory microbiological balance in the microbiota of gastrointestinal tract that probably led to improved health status and nutrient digestibility of birds. On the other side, these results disagreed with finding of Ocak et al. (2008) who reported that the feed supplemented with mint leaves powder doesn't have a considerable effect on body weight, feed intake and feed conversion ratio. It has been reported that the antioxidants found in mint leaves have ability modulate intestinal architecture by improving villi size that results the improve feed intake and weight gain of birds (Aguiar et al., 2023).

The current study exhibited an increase in cortisol hormone level of birds reared under heat stress and that's in agreement with (Ríus, 2019) who suggested that the increase may be caused due to increase in corticosteroid stimulation via the hypothalamic-pituitary-axis. This finding is also consistent with some investigators who reported that the elevated air temperature prompts the synthesis and secretion of corticosteroids, causing catabolic impacts (protein and lipid mobilization) via decreased growth rate and causing muscle atrophy (Safa et al., 2019). Interestingly, mint treatment reduced the cortisol level and kept it within the normal limit. Our results also exhibited that mint treatment regulated the AST and ALT levels in birds reared in heat stress. Furthermore, histological observations also indicated the profound effect of mint leaves on intestine and liver. This might be attributed to positive effects of flavonoid compounds present in mint powder which ameliorate the deleterious effects of free radicals resulted from the negative effects of heat stress (Wasserman, 2013).

It is well known that active compounds found in medicinal plants, like peppermint, enhance feed efficiency, which causes improvement in digestion and absorption of nutrients, and stimulates appetite in broilers (Mehranpoor,, 1995). Many studies have indicated that the antibacterial effects of such medicinal plants prevented harmful bacteria growth in the intestine leading to reduced mortality rate in flocks which might be attributed to the vital function of antioxidant substances (Zeinab et al., 2024). Based on the obtained results it might be assured that the biological activity of mint leaves which can fight oxidative stress and free radicals and act as an immune-stimulating factor against numerous diseases consequently improve the chicken health and production (Fabris et al., 2017).

CONCLUSION

There were significant improvements in some productive and physiological parameters of broilers supplied with 3% and 5% dried mint leaves powder and kept under heat stress. Therefore, powder of dried mint leaves in the diet as feed additives, especially at ratio of 5% may be used to avoid the adverse effects of thermal stress in broiler chicken.

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NOVELTY STATEMENT

The current work assures the positive role of adding the medicinal plant, mint leaf powder in feed to reduce the harmful effects of heat stress in broilers. It is one of the few important studies in Iraq which addressed an important economic and production issue of poultry sector in Iraq due to long summer with high temperatures that may reach 55 C°.

CONFLICT OF INTEREST

Authors declared that have no conflict of interest.

AUTHOR CONTRIBUTION

M.J.Al-Saadi designed idea and Jassim E.Q.Al-Musawi carried out the laboratory analysis. Both authors collectively write, and approved the final draft.

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