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Nutritional and physiological influences of dietary supplementation of garlic oil on alleviating heat stress impact in broiler chickens

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ABSTRACT

This study evaluated impact of dietary supplementation with garlic oil (GO) on growth performance and alleviation of heat stress (HS) impact in broiler chickens. A total of 240 one-day old Cobb broilers were randomly assigned to four treatments, each with six replicates of 10 birds. Treatment one (- Control) was offered a basal diet (BD) and raised under thermo-neutral temperature throughout the experiment (42 days), while Treatment 2 (+Cont) received BD throughout the experimental period, and raised under thermoneutral temperatures during the experiment weeks, but subjected to HS during the fifth week. Treatments 3 and 4 were exposed to the same HS during the fifth week and fed a BD supplemented with 5 g GO/kg, and 15g GO/kg throughout the experimental period. Compared to the thermoneutral control, the heat-stressed control displayed a lower growth performance, in terms of decreased feed intake, higher feed conversion ratio, higher mortality, altered serum metabolites, and lower antioxidative activity. Both GO treatments resulted in deteriorated growth performance compared to the thermoneutral control and relatively worse than the CHS control (P > 0.05). The GO treatments reduced serum triglycerides, cholesterol, and malondialdehyde concentrations, but enhanced glutathione peroxidase activity and total antioxidant capacity compared to HS control. The two GO treatments exhibited significantly higher villus length/crypt depth ratio than the thermoneutral control. In conclusion, GO treatments displayed poor growth performance results, but enhanced the serum antioxidant properties under the study conditions.

Introduction

The phenomenon of global warming, particularly in tropical and subtropical regions, gradually increases the length of summer season and elevates environmental temperatures. Commercial broilers, as well as all avian species, lack sweat glands in their skins; this hindering them from dissipating all heat they produce, especially under high environmental temperatures. They are often raised in houses providing optimum conditions; however, the elevated summer temperatures and increased humidity reduce the efficiency of cooling systems in poultry houses. Broiler chickens, therefore, experience severe oxidative stress, health disorders, increased mortality, and economic losses even raised in controlled houses (Saeeda and Neeraj, 2014). Heat stress disrupts the natural homeostsis in bird's body and impairs the balance between the innate antioxidative capacity and the level of reactive oxygen species, due to excessive generation of free radicals (Feng *et al*., 2008). The optimal house temperature for commercial broilers ranges from 21 to 25°C, and temperatures exceeding 30°C cause heat stress (Daghir, 2008). Broilers in tropical and subtropical regions, certainly, experience heat stress where environmental temperatures are often above 45°C throughout 5-7 months annually.

Several feed additives have been suggested to promote growth and to counteract deleterious impact of heat stress on broiler's performance. Synthetic anti-stressors, such as vitamin E and vitamin C, have been suggested for poultry challenging heat stress; however, they are costly and less efficient, and any overuse or excessive level can damage bird's liver and reduce performance (Bhatti *et al*., 2018). Great efforts, consequently, have been invested in evaluating feed additives from cheap natural origins. Phytogenic sources, mainly, are gaining increasing interest among animal nutritionists as potential feed additives that can serve as anti-stressors, antibiotic alternatives, antioxidatives, immunity enhancers, and growth promoters, particularly under stress conditions (Abdelli *et al*., 2021). Additionally, they are natural, residue-free, and have reasonable prices when compared to the synthesized sources (Ali *et al*., 2014).

Garlic is a promising phytogenic supplement for poultry especially under stress conditions. It has been seen as a medicinal herb that shows strong antioxidant properties and antiseptic roles, and improves intestinal activity, feed utilization, health, and body weight gain (Al-Shwilly, 2017; Liao *et al*., 2022). Garlic oil is of interest in the current study to assess its feasibility in improving growth and antioxidative status of broilers under thermo-neutral and heat-stress conditions. Several studies showed that garlic is a good source of phytochemicals such as flavonoids, saponin, alkaloid, and tannin (Indrasanti *et al*., 2017). Several researchers have reported that garlic bioactive substances enhance growth rate, immunity, and health status of animals and poultry, due to their antimicrobial and antioxidant properties (Lewis *et al*., 2003; Hanieh *et al*., 2010; Stanacev *et al*., 2010; Mansoub, 2011). In previous studies, the dietary inclusion level of garlic powder or extract ranged between 100 ppm/kg up to 3% of the diet (Ogbuewu *et al*., 2019), but levels beyond 4% in broiler diets displayed deleterious effects on growth performance variables (Choi *et al*., 2010; Stanacev *et al*., 2011). Under non-stress conditions, the dietary inclusion of garlic at 0.5% improved weight gain, feed conversion ratio, intestinal villus height and area, and reduced serum cholesterol level (Incharoen *et al*., 2010; Milosevic *et al*., 2013). The dietary inclusion of garlic at 2% in broilers diet improved packed cell volume by 6% (Ufele and Ogbumuo, 2018), reduced the serum cholesterol content by 18% (Qureshi *et al*., 1983), and decreased hepatic cholesterol content (Yeh and Liu, 2001). The objective of this manuscript was to evaluate effects of dietary inclusion of two levels of garlic oil on broilers growth performance under thermo-neutral conditions, and alleviating the heat stress impact on broilers performance, serum analyses, cecal bacterial count, and villus morphology.

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Materials and methods

This experiment was carried out at the Poultry Research Farm, Faculty of Agriculture, Assiut University, Assiut, Egypt. This study conformed to guidelines of scientific committee of research ethics, of Faculty of Agriculture, Assiut University, with the approval number of "2023/7/23/01". Additionally, an ethical approval "06/2023/0110" was obtained from the Scientific Research Committee of the Faculty of Veterinary Medicine, Assiut University, Egypt, based on the OIE standard for the use of experimental animals.

Management and experimental design

A total number of 240 one-day old, Cobb broiler chicks were purchased from a local commercial hatchery (Cairo, Egypt). All chicks were wing-banded, individually weighed, and randomly housed in 24 equal floor pen replicates (2x1.5 m) each of 10 chiks. The replicates were then assigned to four treatments each of 6 replicates (N = 60 chicks/ treatment) based on a Complete Randomized Design (CRD). The pen floors were covered with straw litter. Water and feed were provided *ad libitum*. The four dietary treatments were as follows: T1, received a basal diet (BD) and raised under thermoneutral conditions and was considered as the negative control (- CON); T2, received the BD and raised under thermoneutral conditions from 1 to 42 days of age, except the fifth week of age where they were exposed to cyclic heat stress (CHS), and was considered as the positive control (+ Cont); treatments 3, and 4, were also subjected to CHS during the fifth week of age and fed the same BD supplemented with 5 g/kg garlic oil, and 15g/kg garlic oil throughout the experiment (0-42 d of age), respectively. All birds were raised under thermoneutral temperature from 0 to 42 d of age, except that the CHS treatments were exposed to 36°C from 9 am to 5 pm, then to 24°C from 5 pm to 9 am during the fifth week of age. As shown in Table 1, the results of growth performance variables of T1 (-Cont) and T2 (+CONT) were merged during the first 28 days, where both controls were raised under the same temperatures and fed the same basal diet.

The basal diet was formulated to meet the nutrient requirements of broiler chicks according to NRC (1994). The birds were fed a soybean/ Maize based diet, contating 23% CP and 2950 kcal ME/kg between 1 and 21 days of age, and 21% CP, and 3100 kcal ME/kg between 22 and 42 days of age. The tested garlic oil was purchased from AB-Chem company, Mansoura Governorate, Egypt, and was mixed weekly with the basal diet and offered to the birds, in treatments 3 and 4, from 0 to 42 days of age. The birds were raised under continuous artificial light (24 hrs) throughout the experiment. The initial brooding temperature was 33-34°C for the first week of age, then it was gradually decreased until 23-24°C at the 4th week of age and thereafter till the end of the experiment except for the heat stress treatments. The relative humidity was monitored throughout the experimental period which ranged between 55-65%.

Analyses of total phenol content, total flavonoids, and total antioxidant capacity in garlic oil

Total phenolic content and total flavonoids in GO were assayed colorimetrically according to the methods described by Taga *et al*. (1984), and Dewanto *et al*. (2002). The total antioxidant capacity was determined by measuring the radical scavenging activity (IC50) using spectrophotometer (L7 Double Beam UV-VIS Spectrophotometer, USA) based on the reduction of a methanol solution of DPPH according to the methods of Blois (1958) and Zhang *et al*. (2010). The IC50 assay (µg/ml) showed 43.18; in this assay, the lower the IC50 value the higher the total antioxidant capacity of the tested oil. The total phenolic contents (mg GAE/ 100 ml oil), and total flavonoid content in garlic oil (mg QE/ ml oil) amount to 129.6 and 15.65, respectively.

Studied variables

Growth performance

The average daily feed intake (ADFI) was calculated on a per replicate basis. The initial body weight (IBW), final body weight (FBW), average daily gain (ADG), feed conversion ratio (g feed: g gain ratio) (FCR), and mortality (%) were calculated on a per replicate basis for the periods of 0-4 weeks of age, the 5th, and the 6th week of age.

Sampling

On day 42 of age, after 10 hours of feed withdrawal, blood samples were collected in 5 mL plain tubes, from 12 broilers per treatment (2/replicate), having the closest BW to the average of their treatments. Blood sample was centrifuged at 3000 x g for 20 min, the separated serum samples were pipetted and stored at -20ºC until analysis. The birds were slaughtered by approved methods for subsequent analyses.

Serum analyses

The serum samples were analyzed for total protein, albumin, total cholesterol, triglycerides, glucose, and malondialdehyde (MDA) contents, and activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and total antioxidant capacity (TAC). They were assayed using kits purchased from Biodiagnostics (Biodiagnostics, Cairo, Egypt) following the instructions of the manufacturer and using a spectrophotometer (L7 Double Beam UV-VIS Spectrophotometer, USA, of 3 ml sealed quartz-glass cuvettes with a path length of 1 cm).

Morphology of intestinal villi

For histological analysis, from each of the 48 slaughtered birds at 42 days of age (12/treatment), 3 cm tissue samples from the middle length of the jejunum were transected; ingesta was washed away using PBS and fixed in 10% formalin. Tissues were dehydrated by transferring through a series of alcohol concentrations (70, 80, 95, and 100%, respectively), then placed into xylol and embedded in paraffin. Five cuts of each sample were made by a microtome Leica RM 2235 (Leica Biosystems Nussloch GmbH, Germany) and stained with eosin and hematoxylin. For each slide, a photo was taken by a digital camera (ToupTek, Version x 64, 3. 7. 7892) and light microscopy (Olympus CX31, Olympus, Hicksville, New York, USA) at 4 x magnification. Morphometric analysis was done by image-J software to measure the villi height and crypt depth by examining 6 random villi and 6 crypt depth per sample. Besides, the ratio between villi height and crypt depth was calculated. The method was adapted from Rady *et al*. (2023)

Enumeration of intestinal bacteria

To determine the cecal bacterial enumeration, at 42 d of age, 48 birds (12 birds/treatment) were slaughtered without feed withdrawal. Cecal digesta of every two birds/replicate were pooled, placed into a 15-mL sterile Falcon tube, and kept in laboratory at 5°C until determining bacterial counts (Pope *et al*., 2003). From each pooled sample, one gram was mixed with 9 ml of peptone solution 0.9% (w/v). The resulting mixture was then serially diluted in sterile peptone solution (v/v) up to 1010. *E. coli* was enumerated on the plate count MUG agar and the eosin methylene blue agar counts. The inoculated eosin methylene blue and MUG plates were incubated aerobically for 24 h at 37°C. Lactobacilli populations were estimated using the MRS agar, where the inoculated MRS plates were incubated anaerobically for 48 hours. The obtained results were presented as the $\log_{10}$ of colony forming units. In this study, the agar media were purchased from HiMedia Pvt. Ltd. Company (23, Vadhani Ind., LBS Marg, Mumbai-40086, India).

Statistical analysis

The obtained data were tabulated and subjected to a One-way ANOVA following a completely randomized design by applying the General Linear Model Procedure of SAS software (SAS, version 9.4, Copyright (c) 2002–2012 by SAS Institute Inc., Cary, NC, USA). Duncan multiple range tests (Duncan, 1955) were used to determine significant differences among means of different treatments.

Results

Growth performance

The effects of dietary supplementation with garlic oil on broiler chickens' growth performance are shown in Table 1. The effects on growth performance were presented during three phases: Thermo-neutral phase (0–28 days of age), Cyclic heat exposure (29–35 days of age) and Recovery phase (36–42 days of age).

During the thermo-neutral phase, between days 1 and 28 of age, broilers in the 0.5% and 1.5% GO groups showed 20.35% and 28.14% reductions in body weight gains, respectively, compared to those in the control group ($P < 0.01$). The feed intake in 0.5% and 1.5% GO treatments was significantly decreased ($P < 0.05$) than that of the control; the reduction in 1.5% GO treatment was relatively higher than that in 0.5% GO treatment. Broilers fed on a diet supplemented with 1.5% GO had the poorest FCR, followed by those fed on a diet supplemented with 0.5% GO. No mortalities were observed in this phase.

During the cyclic heat stress phase, although not statistically different, the body weight gain of broilers fed on a basal diet and exposed to CHS was lower by 10.02% than that of the thermo-neutral control. Broilers fed on GO diets gained significantly less weight than did those in the negative control ($P < 0.05$). Compared to the thermo-neutral control group, broilers fed 0.5% and 1.5% GO diets showed reductions in body weight gain of 20.42% and 22.87%, respectively. Broilers in the thermo-neutral control consumed more feed amount than did those in the CHS treatments ($P < 0.05$). Broilers fed diets supplemented with GO consumed significantly less feed than birds in the two controls. The reduction in feed intake was more pronounced in the 1.5% GO than in the 0.5% GO group ($P < 0.05$). Birds in the thermo-neutral control had a better FCR compared to those in the cyclic heat stress treatments. The poorest FCR was observed in 0.5% GO, and the positive control and 1.5% GO treatments showed intermediate values. The heat-stressed control and 0.5% GO showed 10% mortality, the 1.5% GO showed 6% mortality, and no mortality was recorded in the thermo-neutral control. The 1.5% GO treatment showed lower mortality value ($P < 0.05$) than the positive control, estimating (6% vs. 10%).

During week 6, broilers in the CHS control showed lower body weight ($P < 0.05$), lower body weight gain ($P < 0.05$), lower feed intake ($P < 0.05$), and relatively higher FCR ($P > 0.05$) compared to those in the thermoneutral control. Growth variables of the 5.0% GO treatment was improved during the recovery phase; it showed a high body weight gain, which was similar to that in the thermoneutral control (82.51 vs. 82.42 g/d), but significantly higher ($P < 0.05$) than that in the CHS control; it also showed feed intake value relatively lower ($P > 0.05$) than that in the CHS control, and better FCR than those in both controls. The 1.5% GO treatment exhibited greater body weight gain than that in the CHS control, lower feed intake, and better FCR than those in both controls.

Serum analyses

Table 2 shows the blood serum biochemical responses of broiler chickens exposed to CHS during the fifth week of age and fed diets supplemented with two levels of GO. In broilers fed the basal diet, exposure to CHS led to increases in serum contents of glucose, triglycerides, cholesterol, and malondialdehyde and reduced TAC, total protein and globulin concentrations. Additionally, the cyclic heat stress reduced serum activity of GPx and SOD. The inclusion of any of the GO levels in broiler chicken diets decreased serum content of triglycerides, cholesterol, and MDA, and displayed higher serum activity of GPx and TAC compared to those in the CHS control.

Table 1. Growth performance of heat-stressed broilers fed diets containing garlic oil from 0 to 42 d of age.

Variables	(-) Ctrl		0.5% GO	1.5% GO	SEM	*P-value*
0-4 weeks of age						
Initial BW (g)	49.22		49.37	49.12	0.36	0.98
Body weight (g)	1076.43^{a}		943.00^{b}	820.75^{c}	31.10	<0.001
Body weight gain (g/day)	36.68^{a}		31.91^{b}	27.53^{b}	1.28	<0.001
Feed intake (g/d)	52.82^{a}		48.5^{b}	44.59^{b}	3.91	<0.001
FCR (g feed: g gain)	1.44^{b}		1.52^{ab}	1.62^{a}	0.12	0.05
	(-) Ctrl	Cyclic heat stress treatments (CHS)			SEM	*P-value*
		(+) Ctrl	0.5% GO	1.5% GO		
5th week						
Body weight (g)	1562.08^{a}	1490.57^{a}	1331.52^{c}	1194.40^{d}	39.51	<0.001
Body weight gain (g/day)	69.37^{a}	61.96^{ab}	55.5^{b}	53.37^{b}	2.97	0.00
Feed intake (g/d)	105.44^{a}	100.12^{b}	93.72^{c}	86.38^{d}	1.63	<0.0011
FCR (g feed: g gain)	1.52^{c}	1.61^{b}	1.68^{a}	1.61^{b}	0.08	0.04
Mortality rate (%)	0.00^{c}	10.00^{a}	10.00^{a}	6.00^{b}	0.72	0.05
6th week						
Body weight (g)	2139.39^{a}	1979.71^{b}	1908.62^{b}	1730.40^{c}	36.82	<0.001
Body weight gain (g/day)	82.42^{a}	69.87^{c}	82.51^{a}	76.57^{b}	0.79	<0.001
Feed intake (g/d)	154.34^{a}	140.70^{b}	134.54^{bc}	131.41^{c}	6.72	0.05
FCR (g feed: g gain)	1.87^{a}	2.01^{a}	1.63^{c}	1.7^{b}	0.03	0.01

Means within the same row without similar superscripts are significantly different ($P < 0.05$).
* Cyclic heat stress (CHS) was applied during the fifth week of age
(-) Ctrl: fed a basal diet (BD) + thermo-neutral temperatures; (+) Ctrl: CHS + BD; 0.5% GO: CHS + BD containing 0.5% garlic oil (GO); 1.5% GO: CHS + BD containing 1.5% garlic oil.

Morphology of jejunal villi

The effects of supplementing diets with garlic oil under thermoneutral and heat stress conditions on jejunal villi morphology at 42 days of age are shown in Table 3. The results showed that late exposure to cyclic high temperatures did not influence villus height, crypt depth, or villus/crypt ratio. Broilers fed a diet supplemented with 0.5% and 1.5% GO showed shorter (P < 0.05) crypt depth than those in both controls. The villus height/crypt depth values were higher (P < 0.05) in broilers fed diets supplemented with 1.5% GO than in those fed either a basal diet or those supplemented with 0.5% GO.

Caecal bacterial count

Table 4 shows the effects of feeding diets supplemented with garlic oil on caecal *Lactobacillus* and *E. coli* counts in broilers at six weeks of age. No significant differences in caecal bacterial counts were observed among different treatment groups. However, broilers fed a 0.5% GO diet tended to have lower *E. coli* in the caecum compared to those in the control groups.

Discussion

A meta-analysis of 40 studies has shown that garlic inclusion in the diets of broiler chickens has decreasing effects on FI and FCR during the grower and finisher periods (P < 0.01) but was not observed in the starter period (Rusli *et al*., 2022). The decrease in feed intake can be attributed to the pungent scent and the flavor of garlic can be off-putting to broiler chickens, particularly when incorporated in substantial amounts into their diet, which is directly reflected in reductions in body weight gain. This may result in a decreased appetite and reluctance to consume their feed with the same enthusiasm as they would in its absence. Choi *et al*. (2010) and Stanacev *et al*. (2011) found that the dietary inclusion of garlic at levels higher than 4% depressed growth performance variables of broiler chickens. Regarding its effect on feed intake, body weight gain, and feed conversion, the inclusion of garlic in broilers diets showed disparity of results among different studies (Javed *et al*., 2009; Kumar *et al*., 2010; Pourali *et al*., 2010), and this was attributed to the difference in dietary form of garlic, inclusion rate and duration of feeding (Ogbuewu *et al*., 2019). Heat stress affects the performance of broiler chickens and is responsible for a series of physiological and chemical reactions, including oxidative damage, acid-base imbalance, increased osmotic pressure, and increased loss of body water that can result in prostration and even death. The adverse effects of heat stress include decreased feed intake, impaired feed efficiencies, poorer growth rates, compromised health, and high mortality rates (Koh and Macleod, 1999; Deeb and Cahaner, 2001). Under high ambient temperatures, birds decrease feed intake in an attempt to reduce heat production (May and Lott, 1992). This reduces the nutrients available for normal growth. Although hyperventilation is an important mechanism by which birds lose heat; it is associated with increased energy expenditure and disruption of acid-base balance (Teeter and Belay, 1996). Diversion of blood flow away from internal organs to the periphery to facilitate heat dissipation increases the osmotic pressure on these organs (Cronje, 2007). In addition, hyperthermia is capable of

Table 2. Serum analyses of heat-stressed broilers fed diets containing garlic oil.

Variables	(-) Ctrl	Cyclic heat stress treatments (CHS)*			SEM	*P*
		(+) Ctrl	0.5% GO	1.5% GO		
Glucose (mg/dl)	168.56^{b}	211.61^{a}	200.37^{a}	201.28^{a}	4.22	<0.001
Triglyceride(mg/dl)	60.05^{b}	74.45^{a}	60.66^{b}	61.54^{b}	2.84	0.01
Cholesterol (mg/dl)	151.97^{b}	186.73^{a}	114.70^{c}	141.76^{b}	3.77	<0.001
Total antioxidant capacity (mM/L)	1.11^{a}	0.71^{c}	0.88^{b}	1.02^{a}	0.03	<0.001
Malondialdehyde (nmol/ml)	5.40^{b}	7.25^{a}	3.97^{c}	4.91^{bc}	0.36	<0.001
Glutathione peroxidase (mU/mL)	196.72^{a}	173.05^{c}	192.54^{a}	180.00^{b}	3	<0.001
Superoxide dismutase (U/ml)	182.48^{a}	105.09^{b}	110.89^{b}	114.03^{b}	3.88	<0.001

Means within the same row without similar superscripts are significantly different (P < 0.05).
* Cyclic heat stress (CHS) was applied during the fifth week of age.
(-) Ctrl: fed a basal diet (BD) + thermo-neutral temperatures; (+) Ctrl: CHS + BD; 0.5% GO: CHS + BD containing 0.5% garlic oil (GO); 1.5% GO: CHS + BD containing 1.5% garlic oil

Table 3. Morphology of jejunal villi of heat-stressed broilers fed diets containing garlic oil.

Variables (µm)	Treatments				SEM	*P - value*
	(-) Ctrl	Cyclic heat stress (CHS) treatments*				
		(+) ctrl	0.5% GO	1.5% GO		
Villus height	1130.43	1064.59	1068.67	1117.77	39.60	0.08
Crypt depth	234.31^{a}	240.71^{a}	211.03^{b}	191.77^{b}	8.02	<0.001
Villus/crypt	4.93^{c}	4.53^{c}	5.05^{bc}	5.89^{a}	0.21	<0.001

Means within the same row without similar superscripts are significantly different (P < 0.05).
* Cyclic heat stress (CHS) was applied during the fifth week of age
(-) Ctrl: fed a basal diet (BD) + thermo-neutral temperatures; (+) Ctrl: CHS + BD; 0.5% GO: CHS + BD containing 0.5% garlic oil (GO); 1.5% GO: CHS + BD containing 1.5% garlic oil

Table 4. Caecal bacterial count (Log10 of CFU) of heat-stressed broilers fed diets containing garlic oil.

Bacterial count (Log^{10} CFU/g)	Treatments				SEM	*P*
	(-) Ctrl	Cyclic heat stress (CHS) treatments*				
		(+) ctrl	0.5% GO	1.5% GO		
Lactobacilli	8.56	8.29	8.34	8.77	0.28	0.28
E. coli	7.71	7.89	6.66	7.79	0.43	0.18

*Cyclic heat stress (CHS) was applied during the fifth week of age
(-) Ctrl: fed a basal diet (BD) + thermo-neutral temperatures; (+) Ctrl: CHS + BD; 0.5% GO: CHS + BD containing 0.5% garlic oil (GO); 1.5% GO: CHS + BD containing 1.5% garlic oil

increasing the generation of free radicals and reactive oxygen species causing damage to body tissues (Mujahid *et al*., 2009). All these physiological and biochemical events may be responsible for the reduced body weight gain and poor FCR.

In the current study, broilers fed the basal diet and exposed to cyclic high temperatures showed increases in glucose, triglycerides, and cholesterol concentrations in the blood serum. Exposure to various stressors is associated with similar biochemical and metabolic changes. These metabolic changes include increases in serum glucose, cholesterol, triglyceride, and high-density lipoprotein concentrations (Puvadolpirod and Thaxton, 2000). Therefore, the biochemical changes in cholesterol and glucose are used as indicators of stress in domestic poultry (Mumma *et al*., 2006). Several studies have shown that exposure to stressful conditions can induce hyperglycemia and hypercholesterolemia in animals (Paré *et al*., 1973; Surwit *et al*., 1992; Zardooz *et al*., 2006). Mumma *et al*. (2006) suggested that as lipids are mobilized in response to increased glucocorticoid secretion, they are broken down into nonesterified fatty acids, which are converted to triglycerides in the liver. Then, triglycerides are transported by very low-density lipoproteins from the liver to adipose tissues and muscles, where they are hydrolyzed by lipoprotein lipase. During this process, the very low-density lipoproteins are converted to cholesterol-ester-rich low-density lipoproteins.

Compared to the positive control, adding garlic oil as a supplement to broiler diets reduced triglyceride and cholesterol concentrations in serum. These results indicate that adding oils to the di*et al*leviates some of the adverse effects of heat stress. Rusli *et al*. (2022) conducted a meta-analysis of 40 peer-reviewed papers to assess the effects of garlic supplementation on daily weight gain and blood lipid profile. The authors concluded that dietary supplementation with garlic or its derivatives improves blood lipid profile by decreasing cholesterol, triglyceride, and low-density lipoprotein and increasing high-density lipoprotein concentrations. Our results agree with previous reports (Elbaz *et al*., 2022) that supplementing broiler diets exposed to heat stress with garlic oil resulted in reductions in serum cholesterol and triglyceride levels. The authors attributed these cholesterol-lowering effects to the garlic content of organosulfur, primarily alliin derivatives. Garlic has active compounds that can effectively reduce the activity of certain enzymes in the liver. These enzymes include malic enzyme, fatty acid synthase, glucose-6-phosphatase dehydrogenase, and 3-hydroxy 3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, which are responsible for the production of cholesterol and lipids in the body (Qureshi *et al*., 1983; Chi *et al*., 1982). Triglycerides are released into the bloodstream by triglyceride-rich lipoproteins in the liver. Impaired hepatic lipogenesis decreases triglyceride levels in the blood plasma (Zhou *et al*., 2009).

High ambient temperatures are reported to increase the generation of free radicals and reactive oxygen species, which can cause a cascade of detrimental reactions within cellular membranes and to macromolecules, eventually leading to cell death (Sahin *et al*., 2010; Mujahid *et al*., 2009). The breakdown of polyunsaturated fatty acids in cellular membranes (lipid peroxidation) is the main consequence of free radical toxicity in biological cells. MDA is a main degradation product of lipid peroxidation and is widely used as a measure of the degree of lipid peroxidation (Sahin *et al*., 2010). In the current study, exposure to cyclic high temperatures during week 5 increased serum MDA concentrations in broilers fed the basal diet. In addition, serum TAC was decreased in heat-exposed broilers in the positive control, and 0.5% garlic groups compared to the negative control. However, both GO treatments had higher levels of serum TAC than those in the positive control group.

Garlic and its derivatives contain lots of compounds that possess powerful anti-oxidative properties against free radicals such as allyl cysteine, alliin, allicin, and allyl disulfide (Chung, 2006). Alliin is the chief constituent of garlic that via enzymatic reaction transforms into allicin. Allicin, a thio-2-propene-1-sulphinic acid S-allyl ester, and other sulfur-containing compounds have strong hepatoprotective effects due to their antioxidant properties (Shukry *et al*., 2020). Garlic powder supplementation in broiler chicken diets led to significant increases in TAC levels and reductions in serum MDA concentrations (Ismail *et al*., 2021). In another study, dietary garlic powder decreased MDA levels in intestinal tissues of broiler chickens challenged with lipopolysaccharide-induced oxidative stress (Zhang *et al*., 2022). In the current study, broilers fed on diets supplemented with GO showed the lowest serum MDA concentrations, indicating reduced lipid peroxidation and oxidation damage. In our study, the total phenolic content and total flavonoid content in the tested garlic oil estimated 129.6 mg GAE/ 100 ml oil and 15.65 mg QE/ ml oil, respectively. It showed 43.18 IC50 assay (µg/ml) total antioxidant capacity. Additionally, it also contains a substantial amount of carotenoids and sterols. We attribute the high values of total antioxidant capacity and glutathione peroxidase and the decreased malondialdehyde levels in the serum of broilers fed diets supplemented with GO to these bioactive molecules having potent antioxidant properties as suggested by Shukry *et al*. (2020) and Zhang *et al*. (2022). Broilers exposed to high temperatures generally experienced a decrease in GPx activity compared to the thermo-neutral group. However, adding 0.5% GO to the feed maintained the same GPx activity as the thermo-neutral control. The activity of GPx was decreased in the heat stressed control and 1.5% GO groups compared to that of the thermoneutral control, but 1.5%GO showed higher GPx activity in serum.

key indicator of intestinal health is the length of the villi and the depth of the crypts. Research has demonstrated that longer villi and shallower crypts significantly enhance nutrient absorption in the gastrointestinal tract. It is well known that heat stress diverts blood flow from internal organs to peripheries to increase heat dissipation (Wolfenson *et al*., 1981). This results in reduced blood supply to the gut and a decrease in nutrient and oxygen supply that might lead to hypoxia (Cronje, 2007). Hypoxia was reported to decrease the villi surface area in the duodenum of ascites susceptible broilers (Rocha-Santos *et al*., 2018). Based on the results obtained, it seems that the temperature regimen used in the study did not have a negative impact on the morphology of the intestine. From the findings, it can be inferred that the major effects observed in villi length and crypt depth were due to the level of garlic oil that was added to the diet. Souza *et al*. (2021) reported that broiler chickens fed diets supplemented with orange essential oil had longer villus heights compared to those of the control group. Señas-Cuesta *et al*. (2023) reported that heat stress had a negative impact ($p < 0.05$) on villus height, crypt depth, villus width, villus/crypt ratio, and villus surface area index in the duodenum and ileum when compared with those in thermo-neutral control.

Some reports reported that medicinal herbs and their extracts can enhance the growth of beneficial bacteria and suppress pathogenic bacteria in poultry guts. Friedman *et al*. (2002) used a microtiter plate bactericidal assay to evaluate the bactericidal effects of 96 essential oils against *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* obtained from food and clinical sources. The authors found that cinnamaldehyde, thymol, carvacrol, and eugenol were most active against *Escherichia coli* and *Salmonella*. In addition, these essential oils had a minimal effect on beneficial gut bacteria. In contrast, similar to our results with garlic oil here, others reported low antimicrobial activity of essential oils against pathogens. For instance, Kırkpınar *et al*. (2011) found that neither the dietary addition of garlic oil individually nor when combined with oregano essential oil affected the ileal count of total bacteria, *Streptococcus*, *Lactobacillus* spp., and Coliform counts compared to control. Lis-Balchin (2003) found that oregano oil exhibited a low level of antimicrobial activity against both *E. coli* and *Salmonella* Typhimurium. Similarly, Placha *et al*. (2014) found that thyme oil had a slight effect on bacteria in the caecum and the large intestine. Some factors, such as the composition and solubility of oil (Friedman *et al*., 2002), adsorption time, and interaction with other digesta components (Si *et al*., 2006), can interfere with the impact of essential oils on suppressing pathogenic bacterial growth.

Conclusion

Under the conditions of this study, supplementation of garlic oil (GO), especially the high level (15g/kg), led to impaired growth performance compared to both the thermoneutral and CHS controls. This outcome could be attributed to the strong pungent taste and odor of garlic oil, which may deter broilers from consuming their feed effectively. However, GO treatment remarkably enhanced the serum antioxidant properties of broiler chickens.

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Conflict of interest

The authors have no conflict of interest to declare.

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