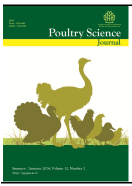

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Effect of Black Seed (*Nigella sativa*) on Antioxidant Status, Inflammatory Response, Biochemical Indices and Growth Performance in Broilers Subjected to Heat Stress

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Abstract

Heat stress has adverse effects on the health and performance of broilers. This study was conducted to investigate the protective effects of black seed (BS) supplementation on broilers subjected to heat stress (HS). A total of 500 (day-old) Ross-308 male broiler chicks were randomly assigned into five groups with five replicated pens (20 broilers per pen): thermoneutral (TN), heat stress (HS), and HS with a diet supplemented with three different levels of black seed (*Nigella sativa*) powder (BSP) at rates of 5g/kg (BSP-5), 10 g/kg (BSP-10), and 15 g/kg (BSP-15. Exposure to HS reduced feed intake, and weight gain and elevated feed conversion ratio (FCR), mortality ($P < 0.05$). Also, low antioxidant enzyme activity (such as glutathione peroxidase, superoxide dismutase, and catalase) and high malondialdehyde levels in serum, liver, and spleen were observed in the birds of the heat stress group compared to the TN treatment ($P < 0.05$). Moreover, HS elevated interleukin-6 and tumor necrosis factor-α and lowered interleukin-10 levels in serum, liver, and spleen ($P < 0.05$). In addition, heat stress causes an increase in ALT, AST, ALP, triglycerides, and cholesterol levels compared to the TV group ($P < 0.05$). Compared with the HS group, broilers in the BSP-10 group had a higher body weight gain, a lower feed conversion ratio, and mortality ($P < 0.05$). Broilers in the BSP-10 and BSP-10 groups showed higher levels of antioxidant enzyme activities and lower malondialdehyde in serum, liver, and spleen compared to the HS group. BSP supplementation at 10 and 15 mg/kg reduced TNF-α and interleukin-6 levels and enhanced interleukin-10 in serum, liver, and spleen compared to the HS group. Additionally, BSP supplementation at 15 mg/kg reduced the effect of the heat stress on ALT, AST, ALP, triglycerides, and cholesterol compared to the HS group. Obtained results showed that BSP in diet during heat stress may have a role in the reduction of the changes exerted by heat stress in broiler chickens.

Introduction

High ambient temperatures have become a serious problem in the poultry industry due to intense environmental stressors, causing heat stress. (Pawar *et al*., 2016). Heat stress has a negative impact on the global poultry industry, particularly in tropical regions such as the Middle East and Iran (Attia and Hassan, 2017; Israr *et al*., 2021). Broilers are more vulnerable to heat stress due to higher metabolic activity, lack of sweat glands, and feather covered (Liu *et al*., 2014). Previous studies (Lara and Rostagno, 2013) have shown that broilers' health, performance, and well-being are adversely impacted by heat stress. Moreover, heat stress in broilers induces oxidative stress (Lan *et al*., 2020) and pro-inflammatory cytokine production, leading to inflammation response and cell damage (Bouchama and Knochel, 2002; Yi *et al*., 2016; Zhang *et al*.,

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2018; Cheng *et al*., 2019a,b). It has been reported that exposure to heat stress stimulates the expression of pro-inflammatory cytokines mRNA expression (tumor necrosis factor-*α* (TNF-*α*), interleukin 1β (IL-1 β), and interleukin-6 (IL-6), and leads to inflammatory response (Jang *et al*., 2014; Ohtsu *et al*., 2015; Wang *et al*., 2018).

It is suggested that oxidative stress and inflammatory responses are closely related. Oxidative stress and inflammatory responses caused by heat stress have been demonstrated in previous studies (Jang *et al*., 2014; Song *et al*., 2017). Moreover, several reports show that oxidative stress and the resulting inflammatory responses can cause serious damage to the involved tissues. (Xia *et al*., 2014; Jiang *et al*., 2018). Therefore, it is important to suppress oxidative stress and inhibit inflammation response to prevent tissue damage under heat stress. However, research on reducing the effects of heat stress on the oxidative stress and inflammatory response of broilers is still limited.

Recently, it has been shown that one of the ways to eliminate or reduce the harmful effects of heat stress on the broiler industry is to use antioxidant supplements, especially plant-based ones such as polyphenols. In this regard, special attention has been paid to medicinal plants due to the high amount of natural antioxidant sources (Alishah *et al*., 2013; Nourozi *et al*., 2013; Surai, 2014; Khan *et al*., 2022; Haq *et al*., 2020; Chand *et al*., 2021; Shuib *et al*., 2021).

Black seed (*Nigella sativa*) has been used as a folk medicine due to its multilateral beneficial actions for more than 2000 years (Adam *et al*., 2016). Several important active ingredients of black seed have been identified that have medicinal and health properties, including thymoquinone, dithymoquinone, thymohydroquinone, nigilin, niglone, melanthin, niglamine, pinene, damascenone, and p-cymene. Black seed contains minerals such as calcium, magnesium, potassium, iron, phosphorus, manganese, zinc, cobalt, and multiple vitamins (Cheikh-Rouhou *et al*., 2007; Khan *et al*., 2012). Moreover, black seed is rich in proteins, essential oils, fixed alkaloids, saponins, flavonoids, and polyphenols.

In addition, numerous reports of analgesic, antioxidant, anti-inflammatory, anthelmintic, hypocholesterolemic, digestive stimulant and appetite, diuretic, anti-diarrheal, spasmolytic and bronchodilator effects, anti-ulcer, antimicrobial, antihypertensive, hepatoprotective, anti-cancer, anti-diabetic and renal protective activities and antioxidant properties have been reported from black seed (Adam *et al*., 2016). Previous research (Khan *et al*., 2012; Azeem *et al*., 2014) has shown that black seed has a wide spectrum of biological activities in broiler chicken, including immune-stimulating, growth-promoting, and antimicrobial effects. Moreover, Habeeb and El-Tarabany (2012), reported that Nigella can be used as an antioxidant agent as it inhibited the non-enzymatic peroxidation which may increase the immunity and may help the animals to tolerate the heat stress. The aim of this study was to determine how black seed supplementation affected the growth performance, antioxidant status, and inflammation response of broilers during heat stress.

Materials and Methods

Feeding and rearing management

A total of 500 (day-old) Ross-308 male broilers, weighing 42±2g, were purchased from Behshadafarin commercial hatchery in Gorgan, Iran. The chicks were separated into five groups, each having five replicates of 20 birds per pen. One group of 100 birds was raised in a thermoneutral environment (TN control), while the other four groups of 400 birds were exposed to heat stress (HS groups). HS broilers were initially raised in normal conditions until day 25, after which they were exposed to heat stress (34 ± 2◦C) for eight hours (0900 to 1700 h) from day 25 to day 42 (Bahrampour *et al*., 2021). The HS control group was fed a control diet without supplementation, while other HS groups (BSP-5, BSP-10, and BSP-15) were fed a basal diet supplemented with black seed (*Nigella sativa*) powder at 5, 10, and 15 g/kg, respectively.

The lighting program included continuous light for the first three days, followed by a cycle of 23 hours of light and 1 hour of darkness until the end of the trial. Each pen had feeders and drinkers and was sized 150 × 200 cm. Regular vaccination against infectious diseases, including New Castle disease, was administered to birds. Based on Ross 308 nutrient recommendations, a diet consisting of corn and soybean puree was formulated and used during the starter (1-10), grower (11-24), and finisher (25-42) periods (Table 1). Broilers were provided ad libitum access to feed and water throughout the study period.

Analysis of chemical composition and phytochemical constituents of *Nigella sativa* seeds

The approximate analysis of chemical compounds in black seed was done based on the protocol suggested by Mahmoud *et al*., (2021). To determine the content of dry matter, crude protein, and oil (as ether extract) compounds, the samples were dried in a forced draft oven at 105°C until they reached a constant weight (according to AOAC, 2000; method 934.01). Crude protein content was measured using the combustion method, as described in AOAC, 2000 (method 990.03) with a LECO FP-528 N analyzer from Leco Corp. (St. Joseph, MI). To extract oil using the ether extraction method (AOAC, 2000; method 945.16) in a Soxtec system (Foss Ltd., Warrington, UK), diethyl ether is utilized. The content of phenolic compounds was quantified, including chlorogenic acid, kaempferol, p-hydroxybenzoic acid, p-coumaric acid, catechin, ferulic acid, and sinapic acid, as previously described (Mahmoud *et al*., 2021) (Table 2).

Table 1. The ingredients and composition of the basal diet

Ingredients (%)	Starter (0-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Maize, 8% CP	47.52	51.63	57.56
Soybean meal, 44%CP	42.35	37.99	32.35
Soybean oil (9000 kcal/kg)	5.54	6.24	6.29
Limestone (38% Ca)	1.20	1.12	1.05
Di-calcium phosphate (21%Ca)	1.79	1.56	1.34
Vitamin premix	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25
NaCl	0.40	0.40	0.40
DL-Methionine (99%)	0.37	0.32	0.28
Lysine (78%)	0.28	0.22	0.22
Threonine (98.5%)	0.05	0.02	0.01
Calculated values			
Metabolizable energy (Kcal/kg)	2990	3082	3218
Crude protein, %	23	21.3	19.3
Calcium (Ca), %	0.96	0.87	0.79
Available phosphorus, %	0.456	0.409	0.361
Sodium (Na), %	0.16	0.16	0.16
Methionine, %	0.71	0.64	0.58
Methioninecysteine, %	1.07	0.89	0.89
Lysine, %	1.46	1.30	1.17
Arginine, %	1.56	1.45	1.30
Threonine, %	0.96	0.87	0.78
Tryptophan, %	0.35	0.32	0.29

Vitamin concentrations per kilogram of diet: retinol, 13.50 mg; cholecalciferol, 4.15 mg; tocopherol acetate, 32.00 mg; vitamin K3, 2 mg; thiamin, 2 mg; riboflavin, 6.00 mg; biotin, 0.1 mg; cobalamin, 0.015 mg; pyroxidine, 3 mg; niacin, 11.00 mg; d-pantothenic acid, 25.0; menadione sodium bisulphate, 1.10; folic acid, 1.02; choline chloride, 250 mg; nicotinamide, 5 mg; Mineral concentrations per kilogram of diet: calcium pantothenate, 25 mg; Fe (from ferrous sulphate), 35 mg; Cu (from copper sulphate), 3.5 mg; Mn (from manganese sulphate), 60 mg; Zn (from zinc sulphate), 35 mg; I (from calcium iodate), 0.6 mg; Se (from sodium selenite), 0.3 mg.

Table 2. The chemical composition and phytochemical constituents of *Nigella sativa*

Chemical composition	**Contents (%)**
Dry matter	93.40
Ether Extract	25.35
Crude protein	23.34
Phytochemical constituents	**Contents**
p-Hydroxybenzoic acid (μg/g)	69.685
Catechin (μg/g)	32.824
Chlorogenic acid (μg/g)	2.554
Ferulic acid (μg/g)	6.628
Sinapic acid (μg/g)	21.979
p-Coumaric (μg/g)	45.147
Kaempferol (μg/g)	1.277
Total phenols (mg GAE/g)	2.077
Total flavonoids (mg CE/g)	0.565

Measurements of Bird's performance traits

During the feeding trial, growth performance traits such as body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were assessed and recorded during days 1-42 of age. The mortality of birds during the study period was also recorded.

Blood Sampling for biochemistry *indices*

Two chicks from each cage were chosen by chance and about 2.5 mL of blood was taken via venipuncture on day 42. The serum was prepared by centrifuging at 2,500 g for 15 minutes at room temperature and then stored at -20 C for further biochemical analysis. Serum assessments of cholesterol and triglyceride were performed by Abbott ALCYON 300 autoanalyzer using laboratory kits (Pars Azmoon, Tehran, Iran).

Biochemical indices

The measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) serum levels were performed using laboratory kits from Pars Azmoon Company (Tehran, Iran). Enzyme activity for Glutathione peroxidase (GPx), Catalase (CAT), and Superoxide dismutase (SOD) was measured using enzyme kits. The Ransel kit (RANDOX/RS-504) from Randox Laboratories, Crumlin, UK, was used for the GPx assay. The commercially available enzyme kit prepared from Ransod (RANDOX/SD-125), also from Randox Laboratories, was used for CAT and SOD assay. The level of malondialdehyde (MDA) was measured in serum, liver, and spleen tissue to evaluate oxidative stress using the methodology explained previously (Fathi *et al*., 2022). The amount of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), as well as anti-inflammatory cytokines like interleukin-10 (IL-10), were evaluated in both serum and liver tissue. ELISA kits were used for the measurements, which were obtained from Pars Azmoon in Tehran, Iran, following the manufacturer's instructions.

Statistical Analysis

Values were averaged and presented as mean ± standard error of the mean (*SEM*). One-way ANOVA was performed by SAS Statistical Analysis Software and the significance level was set at $P < 0.05$. Pen served as an experimental unit.

Results

Growth performance traits and mortality of broilers

In the HS group, BWG and FI significantly reduced and FCR increased by heat stress ($P < 0.01$). Also, mortality was worthy of attention higher in HS group broilers compared to the TN group ($P < 0.01$). The highest value of feed consumption belongs to chickens of BSP-5 and BSP-15 and all three levels of BSP compared to CS broilers had the highest values of body weight and lowest FCR ($P < 0.01$). The birds in the BSP-10 group exhibited the best feed conversion ratio and lowest mortality compared to the other HS groups of birds ($P < 0.01$) (Table 3).

Table 3. Effects of black seed (*Nigella sativa*) powder supplementation on performance and mortality of broilers (1-42 d) reared in thermoneutral and exposed to heat stress

groups	FI (g)	BWG (g)	FCR	Mortality (%)	Mortality (%)
TN control	5670 ^{a}	3080^{a}	1.84^{b}	1.0^{c}	1.0^{c}
HS control	4832^{c}	2612 ^{b}	1.95 ^{a}	6.0^{a}	6.0^{a}
BSP-5	5336 ^{b}	2981 ^{a}	1.79 ^{c}	2.5^{b}	2.5^{b}
BSP-10	4849 ^{c}	3058 ^{a}	1.60 ^{d}	1.0^{c}	1.0^{c}
BSP-15	5345 ^{b}	2953 ^{a}	1.81 ^{b}	3.0^{b}	3.0^{b}
SEM	93.02	85.82	0.03	0.01	0.01
P-Value	0.001	0.010	0.001	0.001	0.001

a, b, c Mean values in the same row with different superscript letters were significantly different.

BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; TN control; Chicken were kept in the normal temperature environment and fed a basal diet, HS control; Chicken in these groups were kept in the hot environment and fed a basal diet, BSP-5, BSP-10, and BSP-15; Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black seed (*Nigella sativa*) powder respectively.

Table 4. Effects of black seed (*Nigella sativa*) powder supplementation on serum antioxidant capacity of broilers reared in thermoneutral and exposed to heat stress

groups	GPx (U/g Hb)	SOD (U/g Hb)	CAT (Nmol/min/mL)	MDA (Nmol/L)
TN control	2109.0^{a}	311.57^{a}	79.80^{a}	15.44^{c}
HS control	590.5^{d}	255.2^{d}	66.90^{b}	17.94^{a}
BSP-5	900.5^{c}	277.0^{c}	71.34^{b}	17.57^{ab}
BSP-10	1436.5^{b}	289.3^{b}	72.37^{b}	17.44^{b}
BSP-15	2086.1^{a}	312.5^{a}	77.00^{a}	17.29^{b}
SEM	184.50	7.51	1.23	0.08
P-Value	0.001	0.020	0.010	0.010

a, b, c Mean values in the same row with different superscript letters were significantly different.

GPx, glutathione peroxidase; SOD, Superoxide dismutase; CAT, catalase; MDA, Malondialdehyde;

TN control; Chicken was kept in the normal temperature environment and fed a basal diet, HS control; Chicken in these groups were kept in the hot environment and fed a basal diet; BSP-5, BSP-10, and BSP-15; Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black seed (*Nigella sativa*) powder respectively.

Table 5. Effects of black seed (*Nigella sativa*) powder supplementation on liver antioxidant capacity of broilers reared in thermoneutral and exposed to heat stress

groups	GPx (U/ mg protein)	SOD (U/ mg protein)	CAT (Nmol/min/mL)	MDA (Nmol/L)
TN control	1709.0^{a}	201.57^{a}	91.80^{a}	14.44^{c}
HS control	1218.5^{c}	151.2^{c}	82.25^{c}	16.56^{a}
BSP-5	1260.5^{c}	181.5^{b}	83.15^{bc}	16.52^{a}
BSP-10	1515.2^{b}	184.1^{b}	84.20^{bc}	16.34^{ab}
BSP-15	1579.1^{b}	184.1^{b}	86.68^{b}	16.15^{b}
SEM	66.91	5.50	1.57	0.05
P-Value	0.010	0.020	0.010	0.010

a, b, c Mean values in the same row with different superscript letters were significantly different.
GPx, glutathione peroxidase; SOD, Superoxide dismutase; CAT, catalase; MDA, Malondialdehyde;
TN control; Chicken were kept in the normal temperature environment and fed a basal diet, HS control; Chicken in these groups were kept in the hot environment and fed a basal diet; BSP-5, BSP-10, and BSP-15; Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black seed (*Nigella sativa*) powder respectively.

Table 6. Effects of black seed (*Nigella sativa*) powder supplementation on spleen antioxidant capacity of broilers reared in thermoneutral and exposed to heat stress

groups	GPx (U/mg protein)	SOD (U/mg protein)	CAT (Nmol/min/mL)	MDA (Nmol/L)
TN control	2409.0^{a}	281.57^{a}	62.80^{a}	15.44^{c}
HS control	1951.1^{d}	193.1^{c}	57.15^{b}	16.95^{a}
BSP-5	2018.2^{cd}	195.2^{c}	57.46^{b}	16.84^{ab}
BSP-10	2039.5^{c}	197.2^{c}	57.69^{b}	16.59^{b}
BSP-15	2170.1^{b}	214.2^{b}	58.23^{b}	16.50^{b}
SEM	45.11	3.94	0.89	0.20
P-Value	0.010	0.020	0.020	0.010

a, b, c Mean values in the same row with different superscript letters were significantly different.
GPx, glutathione peroxidase; SOD, Superoxide dismutase; CAT, catalase; MDA, Malondialdehyde;
TN control; Chicken were kept in the normal temperature environment and fed a basal diet, HS control; Chicken in these groups were kept in the hot environment and fed a basal diet; BSP-5, BSP-10, and BSP-15; Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black seed (*Nigella sativa*) powder respectively.

Serum and liver inflammatory cytokines of broilers

Birds under Heat stress (HS group) had significantly higher levels of cytokines, including *TNF*-α and *IL-6*, and reduced *IL-10* in both serum (Table 7) and liver (Table 8) compared to the TN group. In the BSP-10 and BSP-15 groups, lower serum levels of TNF-α and IL-6 were detected, and higher levels of IL-10 were found than in the HS group birds ($P < 0.01$). Meanwhile, supplementation with all levels of BSP did not significantly affect TNF-α and IL-6 levels in the liver. However, BSP-15 enhanced the level of IL-10 significantly.

Table 7. Effects of black seed (*Nigella sativa*) powder supplementation serum cytokines of broilers reared in thermoneutral and exposed to heat stress

groups	IL10 (ug/mL)	IL-6 (ug/mL)	TNF-*α* (ug/mL)
TN control	27.25^{a}	8.10^{d}	9.20^{d}
HS control	13.49^{d}	16.43^{a}	18.25^{a}
BSP-5	14.26^{cd}	16.52^{a}	17.92^{a}
BSP-10	15.95^{c}	14.25^{b}	15.25^{b}
BSP-15	19.30^{b}	11.32^{c}	12.30^{c}
SEM	1.01	0.95	1.15
P-Value	0.001	0.010	0.010

a, b, c Mean values in the same row with different superscript letters were significantly different.
IL-10, interleukin-10; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha
TN control; Chicken were kept in the normal temperature environment and fed a basal diet, HS control; Chicken in these groups were kept in the hot environment and fed a basal diet; BSP-5, BSP-10, and BSP-15; Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black seed (*Nigella sativa*) powder respectively.

Table 8. Effects of black seed (*Nigella sativa*) powder supplementation liver cytokines of broilers reared in thermoneutral and exposed to heat stress

groups	IL-10 (ug/mL)	IL-6 (ug/mL)	TNF-*α* (ug/mL)
TN control	19.21[a]	1.01[b]	10.51[b]
HS control	9.20[c]	1.90[a]	27.20[a]
BSP-5	10.10[c]	1.75[a]	29.20[a]
BSP-10	13.20[bc]	1.71[a]	28.50[a]
BSP-15	14.60[b]	1.72[a]	27.30[a]
SEM	2.35	0.17	3.20
P-Value	0.010	0.010	0.010

a, b, c Mean values in the same row with different superscript letters were significantly different. IL-10, interleukin-10; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.; TN control; Chicken were kept in the normal temperature environment and fed a basal diet, HS control; Chicken in these groups were kept in the hot environment and fed a basal diet; BSP-5, BSP-10, and BSP-15; Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black seed (*Nigella sativa*) powder respectively.

Serum biochemical metabolites of broilers

The serum enzyme levels of ALT, AST, ALP, TG, and cholesterol significantly increased due to heat stress in birds of the HS group compared to the TN group. However, BSP supplementation at all levels resulted in a significant decrease in these biochemical markers in serum (Table 9).

Table 9. Effects of black seed (*Nigella sativa*) powder supplementation serum biochemical parameters of broilers reared in thermoneutral and exposed to heat stress

groups	ALT (U/L)	AST (U/L)	ALP (U/L)	TG (mg/dl)	Cholesterol (mg/dl)
TN control	6.36[c]	98.8[c]	159.4[e]	52.25[d]	228.2[d]
HS control	27.12[a]	163.17[a]	261.00[a]	131.50[a]	571.50[a]
BSP-5	16.62[b]	125.40[b]	230.75[c]	110.50[b]	514.50[b]
BSP-10	16.23[b]	125.00[b]	251.10[b]	102.00[b]	496.00[b]
BSP-15	8.67[c]	107.50[c]	212.75[d]	82.50[c]	431.20[c]
SEM	2.19	6.13	5.71	6.34	21.41
P-Value	0.010	0.001	0.001	0.030	0.010

a, b, c Mean values in the same row with different superscript letters were significantly different.
ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; TG: Triglyceride; TN control; Chicken were kept in the normal temperature environment and fed a basal diet, HS control; Chicken in these groups were kept in the hot environment and fed a basal diet; BSP-5, BSP-10, and BSP-15; Chicken in these groups were kept in the hot environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black seed (*Nigella sativa*) powder respectively.

Discussion

Improving production performance, maintaining the health status, and immune response are the main goals of broiler production (Abdel-Wareth *et al*., 2019). Severe physiological and behavioral response changes caused by various stresses, including heat stress, can affect metabolism and growth performance (Rhoads *et al*., 2013). Also, in high environmental temperatures, the ability of animals to regulate body temperature is disrupted and depends on their physiological, anatomical and behavioral settings (Babinszky *et al*., 2011). In addition, heat stress can affect the economic indicators of breeding such as growth performance, production efficiency, and economic indicators of production in broiler chickens. (Fatima *et al*., 2022). When broilers are exposed to heat stress (Temperatures above 41◦C), They dissipate heat to their surrounding environment. But when the ambient temperature reaches above 42◦C, their ability to dissipate heat is reduced and in fact, heat stress occurs, on the other hand, causing mortality (Fatima *et al*., 2022). The response of birds to heat stress is to reduce feed consumption so that the metabolic heat produced from feed metabolism will decrease, and as a result, body weight will decrease (Saki *et al*., 2014; Chand *et al*., 2018; AL-Sagan *et al*., 2020; Fatima *et al*., 2022).

In the present study, the supplement of BSP could enhance the growth performance index and reduce the mortality of broilers under heat stress, So, birds in BSP groups had higher feed consumption and weight gain than the HS group, consistent with previous studies (Khan *et al*., 2012; Salam *et al*., 2013; Rahman and Kim, 2016; El-Hack *et al*., 2018) who found that black seed supplementation in the diet increased the digestive enzyme production including lipase and protease, improving the digestibility and finally, led to the improvement of the growth performance in broilers. Improvement in broiler performance due to black seed may related to high essential oils levels in black seed as they increased the digesta retention time in the gizzard (Abu-Dieyeh and Abu-Darwish, 2008).

The main active compound (thymoquinone (approximately 60%) and other components (including carone, entole, carvacrol and 4-terpineol) of black seed essential oil have such biological functions that they may act not only as antibacterial and antioxidant effects but also as stimulant for the secretion of digestive enzymes in the pancreas and intestinal mucosa and this improves the nutrients digestibility, feed efficiency, thus improving the growth performance (Abu-Dieyeh and Abu-Darwish, 2008; Abdou and Rashed, 2015).

Moreover, the inclusion of active components (anethole) and essential oil in BSP, may stimulate the secretion of digestive enzymes and the secretion of bile acids. It has been able to increase the efficiency of digestion and digestion of feed and improve growth performance (Amin and Hosseinzadeh, 2016). The microbial balance of the cecum and reducing pathogenic bacteria caused by black seed supplementation can strengthen the immune system in the intestines and subsequently improve the mechanisms of digestion and absorption in the small intestine. (Asghar *et al*., 2022). In the current study, all BSP groups on reduced mortality compared to the HS group broiler (Table 3). Black seed significantly suppressed the population of pathogenic bacteria in the intestine through antibacterial effects and improved immunity in broilers (Abu-Dieyeh and Abu-Darwish, 2008; Khan *et al*., 2012).

In the present study, heat stress increased malondialdehyde (MDA) concentration in the serum, liver and spleen of heat-stressed broilers. Increased level of MDA production is a reliable marker of lipid peroxidation during oxidative stress that occurs during heat stress in broilers (Fathi *et al*., 2022). Our result is consistent with the previous study (Liu *et al*., 2014; Lan *et al*., 2020; Fatima *et al*., 2022; Uyanga *et al*., 2022) which reported heat stress induces high production of free radicals, including reactive oxygen species (ROS), So that the body's antioxidant system is not able to neutralize this high volume of free radicals, the oxidant-antioxidant balance of the body is disturbed, and the eventually contributed to oxidative stress. Increased levels of MDA production are a reliable marker of lipid peroxidation during oxidative stress that occurs during heat stress in broilers. (Cheng *et al*., 2019 a,b).

The current study clearly revealed that the inclusion of a diet with 10 and 15 g/kg BSP, caused an increase in the activity of antioxidant enzymes (GPx, SOD and CAT) and significantly reduced the MDA concentration (serum, liver and spleen) of broiler chickens exposed to heat stress. The presence of active compounds, including anethole, thymoquinone, carvacrol, and 4-terpinol in black seed can be the main reason for its antioxidant effects. Previously, many researchers have considered the antioxidant properties of black seed due to its active compounds, including thymoquinone. Thymoquinone, anethole, 4-terpineol, and carvacrol (Burits and Bucar, 2000; Mansour *et al*., 2001; Badary *et al*., 2003). Similarly, Ilhan *et al*. (2005) revealed that black seed due to having essential oil (0.5-1.6%) increased antioxidant enzyme activity (superoxide dismutase and glutathione peroxidase) and reduced MDA levels in mice. Also, the essential oils in the black seed can suppress one of the potential sources of oxygen-free radical production, including the activity of xanthine oxidase enzymes, and thus contribute to the cellular oxidant-antioxidant balance, preventing ATP degradation in cells (Ilhan *et al*., 2005). Similar to the results of this research, it has been shown that black seed and its extracts were shown to be effective antioxidants and reduce MDA levels in rats (Mabrouk *et al*., 2002), in brain and medulla spinal tissues (Ozogurlu *et al*., 2005) and A549 cells (Farah *et al*., 2005).

In the present study, heat stress significantly triggered inflammatory responses in different tissues. Previous studies (Kawai and Akira, 2010; Bharati *et al*., 2017) showed that during heat stress and due to the induction of oxygen free radicals, the expression of genes expressing pro-inflammatory cytokines is increased. The result will be an increase in the levels of pro-inflammatory cytokines in the involved tissues and leads to inflammatory response which may lead to serum and liver inflammation and damage. Moreover, other evidence showed that during inflammatory responses and increased production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), regulate perturbation of intercellular tight junctions, leading to enhanced cellular permeability and lead to cell damage (Yi *et al*., 2016).

In the present experiment, the inclusion of black seed increased the level of IL-10 and decreased Il-6 and TNF-α levels of broilers subjected to heat stress. Agree with the present results, Ojueromi *et al*. (2022) reported that supplementing the diet of mice suffering from malaria significantly decreased the inflammatory IL-6 and TNF-α and increased the anti-inflammatory IL-10 in the serum. Since the antioxidant effects of black seed have been proven in many reports of researchers and the present research, the anti-inflammatory effects of black seed may also be due to its antioxidant effects.

Liver damage followed by an increase in serum levels of liver enzymes, including ALT and AST levels (Cheng *et al*. 2019a; Lan *et al*., 2020) and an increase in serum cholesterol and triglycerides has been reported during heat stress in broiler chickens (Kutlu and Forbes, 1993). In the present study, dietary BSP inclusion decreasing serum AST and ALT levels may due to its antioxidant effects in broilers. Previous studies (Cheng *et al*., 2019a; Lan *et al*., 2020) have reported that dietary antioxidant supplementation decreased serum AST and ALT levels under heat stress and cold stress in broilers (Fathi *et al*., 2022). We guessed that it is a possibility that the BSP, through its antioxidant effects, has caused a decrease in the serum level of liver enzymes in birds under heat stress.

The positive effect of antioxidant compounds in reducing blood cholesterol and triglycerides has already been investigated (Weinbrenner *et al***.**, 2004; Al-Beitawi *et al*., 2009; Babaahmadi Milani *et al*., 2020). It has been shown that antioxidant compounds reduce cholesterol synthesis by reducing the activity of the enzyme hydroxyl methyl glutathione coenzyme A reductase (HMG-COA) and finally by reducing the biosynthesis of mevalonate (Babaahmadi Milani *et al*., 2020). Furthermore, the increase in triglyceride absorption and the decrease in liver triglyceride lipase activity and the decrease in triglyceride levels have been attributed to antioxidants. (Ahmadipour *et al.*, 2015; Weinbrenner *et al.*, 2004).

Conclusion

Based on the present results, the inclusion of BSP in the diet improved growth performance and antioxidant status and reduced mortality in chicks subjected to high temperatures. These effects may be exerted through enhanced antioxidant capacity and anti-inflammatory effects.

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