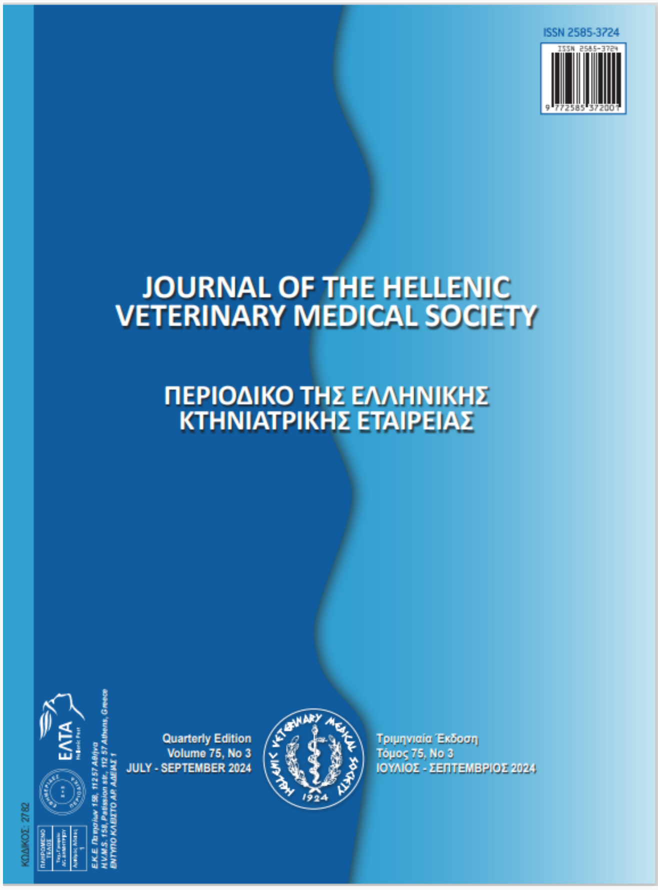
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 3 (2024)



Improving antioxidant function, inflammatory responses, growth performance, and mortality by supplementing black cumin (Nigella Sativa) seed in broiler chickens exposed to low ambient temperature

M Fathi, K Zarrinkavyani, Z Biranvand, Y Mohmmadi, M Jameel Hilal

doi: [10.12681/jhvms.35662](https://doi.org/10.12681/jhvms.35662)

J HELLENIC VET MED SOC 2024, 75 (3) : 7845-7856
ΠΕΚΕ 2024, 75 (3) : 7845-7856

Research article
Ερευνητικό άρθρο

Improving antioxidant function, inflammatory responses, growth performance, and mortality by supplementing black cumin (*Nigella Sativa*) seed in broiler chickens exposed to low ambient temperature

M. Fathi[1*], K. Zarrinkavyani[2], Z. Biranvand[3], Y. Mohmmadi[2], M. Jameel Hilal[2]

[1]*Department of Animal Science, Payam Noor University, Tehran, Iran*

[2]*Department of Animal Science, Faculty of Agriculture, Ilam University, Ilam, Iran*

[3]*Graduated PhD in Animal Breeding and Genetics, Guilan University, Rasht, Iran*

ABSTRACT: This study was done to investigate the effects of black cumin (*Nigella sativa*) seed powder (BCP) on oxidative stress, inflammation cytokines, hematological and biochemical change and growth performance of broilers under low ambient temperature. *Total of 375 one -day-old male Ross 308 broilers were randomly allocated into five treatments with five replicationpens (with 15 broilers per pen).* A total of 75 birds were reared in a thermoneutral (TN group) environment, and the rest of the birds (300 birds in four groups) were exposed to cold stress (CS).On d 14, CS birds' groups were fed a control diet and three levels of BCP *at rates of 5, 10, and 15 g/kg (*BCP-*5,*BCP-*10, and* BCP-*15,* respectively*). The* blood and heart samples were collected*. On* day 42, two birds per cage (n = 10/treatment) were slaughtered, and were collected samples for blood and tissues (heart and liver).Dietary BCP modulated cold-induced effects on growth performance parameters, relative weight of heart weight, right ventricle / total ventricle ratio, right ventricle /body weight and mortality. The activities of superoxide dismutase, catalase, and glutathione peroxidase in serum and liver were enhanced, and malondialdehyde *was* reduced by BCP supplementation compared to the CS group. The cold stress-induced effect on serum and liver levels of TNF-α and interleukin-6 were reduced, and interleukin-10 was enhanced by dietary BCP supplementation. Moreover, supplementing of diets with BCP alleviated the adverse effect of cold stress as reflected by a reduction in alanine transaminase, aspartate transaminase, alkaline phosphatase, triglyceride, and cholesterolcompared totheCS group. In conclusion, BCP supplementation during cold stress may be used to alleviate cold stress- related changes in broiler chickens.

***Keywords*:** Antioxidant capacity; *broiler;* black cumin; *cold stress; inflammation response*

Corresponding Author:
Mokhtar Fathi, Department of Animal Science, Payam Noor University, Tehran, Iran
E-mail address: Mokhtarfathi@pnu.ac.ir

Date of initial submission: 20-10-2023
Date of acceptance: 29-01-2024

INTRODUCTION

Genetic selection to maximize the rate of growth today's broiler chickens has led to a considerable increase in the growth rate of these birds. The organs in the cardiovascular system in birds, have not yet grown to the same extent as this increase in growth rate. In addition, these birds are susceptible to various types of environmental stress, including cold stress. (Rahmani et al., 2018).It has been accepted that birds increase thyroid hormone secretion in order to deal with cold stress by increasing the body metabolism and heat production. Increasing of basal metabolic rates caused to increasing of tissue necessity for oxygen in a situation that cannot be prepared by immature cardiopulmonary systems of broilers (Nemati et al., 2017). The lack of balance between oxygen requirement and oxygen supply, it will be result in hypoxemia (Hassanzadeh, 2010). During hypoxemia, the heart rate to increased compensate for hypoxemia, and subsequently, these cascading actions will be hypertrophy of the heart, increased pulmonary hypertension syndrome (PHS) , and right ventricle inefficiency. Finally, death occurs due to heart failure and increased pulmonary blood pressure) Wideman et al., 2010).

Moreover, it is argued that hypoxemia in all types of tissues, especially the involved tissues such as lungs, heart, and liver, can lead to the generation of free radicals - especially reactive oxygen species (Shao et al., 2022; Fathi et al., 2022). Overwhelming accumulation of reactive oxygen species (ROS) leading to diverse disorders represents distress (Wang et al., 2020) , while levels of ROS necessary for physiological redox signaling important for proper cellular/tissue/organism functioning (Acaroz et al., 2019; Jaganjac et al., 2022). So that one of the leading causes of PHS is the occurrence of lung cell damage (Fathi et al., 2022). In addition, free radicals from oxygen derivatives decrease the half-life of nitric oxide (a vasodilator agent) , causing a decrease in the ability of blood vessels to expand and provide the basis for the occurrence of PHS (Lorenzoni and Ruiz- Feria. 2006).

There are also some reports show that following the occurrence of oxidative stress, severe inflammatory reactions occur in the involved cells, which lead to more severe tissue damage and can activate tissue apoptosis (Jiang et al. 2018; Sun and Karin 2013). Oxidative stress plays an essential role in the emergence of a number of chronic disorders such as diabetes and cancer by inducing inflammation (Acaroz et al., 2019; Jaganjac et al., 2022).

To protect against free radicals, living organisms have a combined antioxidant defense system including enzyme system (including, glutathione peroxidase, superoxide dismutase and, catalase enzymes in the cytosol and, cell membrane structure) and a non-enzymatic system (including glutathione, polyphenol, carotenoids, special dipeptides, proteins containing thiol group, polyamines, ubiquinol, flavonoids, bilirubin, uric acid, vitamin E with selenium and, vitamin C in serum and tissues (Nemati et al., 2017; Ramazani et al., 2023).

Black cumin (Nigella sativa) seed have been used as an alternative medicine due to its multi-systemic beneficial actions for more than 2000 years. It is commonly known as black cumin seed and is the seed of an annual herb that belongs to the botanical family Ranunculaceae (Adam et al., 2016). Many active components of black cumin have been identified, including dithymoquinone, thymoquinone, nigellone, thymohydroquinone, nigilline, melanthin, nigelamine, damascenone, pinene and p-cymene. Black cumin contains minerals such as, calcium, magnesium, potassium, iron, phosphorus, cobalt, zinc and manganese and vitamins A, B, C, D and E (Guler et al., 2006; Cheikh-Rouhou et al., 2007; Khan et al., 2012). Moreover, black cumin is rich in both stable and essential oils, proteins, alkaloids, saponins, flavonoids, and polyphenols. The seeds have anti-inflammatory, analgesic, anthelmintic, hypocholesteremia, appetite and digestive stimulant, antidiarrheal, diuretic, antiulcer, spasmolytic and bronchodilatory, antimicrobial, antihypertensive, antidiabetic, anticancer, hepatoprotective and renal protective activities and possess antioxidant properties, including free radical scavenging (Guler et al., 2006; Adam et al., 2016).

As a result, the purpose of this study was to determine effects of black cumin powder (BCP) supplementation on growth performance, antioxidant status, inflammation response, and mortality of broilers under cold stress-induced.

MATERIALS AND METHODS

All experimental protocols used in this research were approved by the Animal Ethics Committee of Payam Noor University, Tehran, Iran (P.L.N:1401.30.11).

Birds, treatments, and experimental design

The experiment was carried out between January 1, and February 12, at Gorgan-Iran. A total of 375 day-old broiler chicken (Ross, 308) with approximately 424.0±2g body weight was assigned randomly into 5 treatments, included 5 replicates of 15 birds per pen. A group of 75 birds were reared in a thermoneutral environment (TN group) , and rest of the birds (300 birds in four groups) were treated in condition of cold stress (CS). The birds were raised at 32ºC, for the first 3 days, and the temperature was gradually reduced by 2ºC at 3 days' intervals until 24ºC. Birds in the control group (TN) were housed in an environmentally controlled room at 22±1 °C and fed the basal diet. On d 14, CS birds' groups were fed with a control diet and three levels of BCPatrates of 5, 10 and, 15 g/kg (BCP-5BCP-10 and, BCP-15, respectively) until to 42 days of age. Black cumin seeds were obtained from a local market and used in the diets after grinding. A sample of the BCP was then analysed to determine the chemical composition and phytochemical composition (Table 2).

The temperature of both environment was set at 32°C during the first week and in the following reduced to 29°C during the second week. The thermoneutral temperature (TN) was gradually decreased by approximately 0.5°C every other day. Decreasing of temperature occurs from day of 14 until it reached 22°C on 28 days of age and after that remained constant. The temperature of cold stress (CS) condition, gradually dropped to 15°C at day 14 and does not change between 13 and 15°C (Rahmani et al., 2018).

Table 1. The ingredients and composition of the basal diet

	Starter (0-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Ingredients (%)			
Mize, 8% CP	47.53	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	0.00
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
Methionine‡cysteine, %	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 seeds

Chemical composition	Contents (%)
Dry matter	93.40
Ethereal 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
DPPH (mg TE/g)	1.367

A continuous exposure of lighting program was set at 23L:1D throughout the duration of experiment. For each cage of under experiment was used spherical pen feeders and drinkers, So the size of each one was 150 × 100 cm. Vaccinated against New Castle disease and other infectious diseases regularly. A diet base on corn and soybean puree was formulated according to Ross 308 nutrient recommendations, so these diet was used for starter (1-10) , grower (11-24) , and finisher (25-42) periods (Aviagen, 2014; Table 1).Diets and freshwater were provided adlibitum.

Performance traits

During the research, parameters as body weight gain (BWG) , feed intake (FI) , and feed conversion ratio (FCR) were evaluated for the experiment period (42 days). The FCR was calculated as FI/BWG.

Sampling for biochemistry indices

On day 42, two birds that were treated in each cage were randomly selected, So, whole blood samples (approximately 2.5 mL) were collected by venipuncture. Blood was centrifuged and the plasma samples stored at -20°C for later analysis (Fathi et al., 2022).

Heart weight and mortality due to PHS[1]

Mortalities in the level of chickens was recorded daily, and autopsy were performed in order to specify related death of mortalities from d 14 onward. Birds that died with an RV/TV[2] ratio above 0.25 or observation of fluid in the ventricular or pericardium of the heart were included in the PHS mortality (Table 3) (Shao et al., 2018, 2022). On the last day of the assessment, it means day 42, Two of ten birds that were under treatment were dissected. By doing these dissection, hearts of birds were weighed, and the atria, pericardium, major vessels, and fat were trimmed off. Separations of left and right ventricles was done, So, their weights were measured on an analytical balance (Scaltec SBA41, Goettingen, Germany; precision 10^{-3} g) , and the RV/TV and RV/BW ratio were calculated (Fathi et al., 2022).

Biochemical indices

Enzymes activities alanine aminotransferase (ALT) , aspartate aminotransferase (AST) , and alkaline phosphatase (ALP) , cholesterol and triglyceride (TG) were considered by Vitros 350 autoanalyser (Alcyon 300, USA) and commercialkits (ParsAzmun Co,Tehran, Iran).Determination of glutathione peroxidase (GPx) , catalase (CAT) , and superoxide dismutase (SOD) activity were measured by commercial kits (Randox, Randox, Co. Antrim, United Kingdom). The level of malondialdehyde (MDA) in serum and liver tissue, as an indicator of lipid peroxidation, was performed according to the methodology described by Fathi et al. (2022). Pro-Inflammatory cytokines such astumor necrosis factor-α (TNF-α) , interleukin-6 (IL-6) , andanti-inflammatory cytokinessuch asinterleukin-10 (IL-10) concentrations in serum and liver tissue were determined by using ELISA kits which was prepared from Pars Azmoon, Tehran, Iran, according to the manufacturer's instructions.

Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) using the General Linear Model (GLM) command

1

2 Right ventricle / total ventricle

in the SAS statistical package (SAS Inst. Inc., Cary, NC, US (SAS 2001).The pen was used as the experimental unit for the growth performance and blood parameters data. The significant difference between treatment means was determined by the Duncan test (P<.01). Mortality analyses were performed based on Chi-Square.

RESULTS

Heart weight and mortality due to PHS

As shown in Table3, The heart and RV weights was shown as percentages of BW, the RV:TV ratios, and mortality due to PHS were worthy of attention higher in CS group broilers compared to the TN group (P< 0.01). Supplementing of diet with BCP improved cardiac parameters and reduced mortality due to PHS for all levels of BCP than the CS group (P< 0.05). There was no significant difference between different levels of black seed powder (P> 0.05).

Bird's performance traits

BWG and FI significantly reduced and FCR increased by cold stress (P<0.05) (Table 4). The highest value of feed consumption and body weight belongs to chickens of BCP-10, and BCP-15 compared to CS broilers (P < 0.01). The birds of the TN and alllevel of BCP in compared to the CS group birds showed the best feed conversion ratio with lowest number (P < 0.01).

Antioxidant status in serum and liver

Cold stress significantly reduced activities of GPx, SOD and, CAT in serum (Table 5) and in liver (Table 6) (P <0.01). Values for MDA in serum and liver for CS regimen also increased in compared with the TN regimen (P < 0.01). The supplementation ofBCP, especially in the level of BCP-15, significantly improved the antioxidant status in serum, liver and spleen (P< 0.01).

Inflammation response

The results of treatments on pro-Inflammatory cytokines such astumor necrosis factor-α (TNF-α) , interleukin-6 (IL-6) , andanti-inflammatory cytokinessuch asinterleukin-10 (IL-10) concentrations in serum and liver tissue of birdsare summarized in Table 7, 8.Birds under cold stress (CS group) had significantly high concentra-

Table 3: Effects of black cumin (*Nigella sativa*) seed powder supplementation on organ relative weight and mortality rate due to ascites of broilers in thermoneutral and cold stress conditions

Groups	Heart	RV/BW	RV/TV	Mortality
TN control	0.61^{d}	0.061^{d}	0.20^{c}	2.50^{b}
CS control	0.76^{a}	0.091^{a}	0.31^{a}	10.00^{a}
BCP-5	0.72^{b}	0.081^{b}	0.24^{b}	2.50^{b}
BCP-10	0.66^{c}	0.078^{c}	0.24^{b}	2.50^{b}
BCP-15	0.65^{c}	0.076^{c}	0.25^{b}	2.50^{b}
SEM	0.02	0.001	0.02	0.007
P Value	< 0.01	< 0.01	0.01>	0.01>

a, b, c Mean values in the same columnwith different superscript letters were significantly different (n=10).
RV/BW, Right ventricular to body weight ratio; RV/TV, right ventricle/total ventricle; PHS, pulmonary hypertension syndrome; TN, thermoneutral; CS, cold stress, BCP-5, BCP-10, and BCP-15; chicken in these groups were kept in the cold environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black cumin (*Nigella sativa*) seed powder respectively.

Table 4: Effects of black cumin (*Nigella sativa*) seed powder supplementation on performance of broilers in thermoneutral and cold stress conditions at day 42

Groups	FI (g)	BWG (g)	FCR
TN control	4970^{b}	3186^{a}	1.56^{c}
CS control	5760^{a}	2642^{c}	2.18^{a}
BCP-5	4737^{c}	2850^{b}	1.66^{b}
BCP-10	5152^{b}	3052^{a}	1.68^{b}
BCP-15	5155^{b}	3005^{a}	1.71^{b}
SEM	92.79	34.04	0.05
P Value	< 0.01	< 0.01	< 0.01

a, b, c Mean values in the same columnwith different superscript letters were significantly different (n=10).
BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; TN; thermoneutral, CS; cold stress, BCP-5, BCP-10, and BCP-15; chicken in these groups were kept in the cold environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black cumin (*Nigella sativa*) seed powder respectively.

tions of cytokines such asTNF-α and IL-6and reduced IL-10 in serum (Table 7) and liver (Table 8) compared to those under thermoneutral condition (TN group). Birds in BCP-10 and 15 had lower serum TNF-α, IL-6,and higherIL-10than CS group birds (P<0.01). Meanwhile, Supplementation of all levels of BCP had no significant effect on TNF-α, IL-6 levels of liver, BCP-15 significantly increased the IL-10 level (P<0.01).

Biochemical parameters

Cold stress significantly increased the serum's enzyme contents of ALT, AST, ALP, TG, and cholesterol in CS group birds than the TN group birds (Table 8) (P<0.01). Supplementation of BCP in all levels caused a significant decrease in these biochemical parameters in serum (P<0.01).

Table 5: Effects of black cumin (*Nigella sativa*) seed powdersupplementation on serum antioxidant capacity of broilers in thermoneutral and cold stress conditions

groups	GPx (U/g Hb)	SOD (U/g Hb)	CAT (Nmol/min/mL)	MDA (Nmol/L)
TN control	1409.0^{a}	301.57^{a}	71.80^{a}	10.44^{c}
CS control	505^{d}	248^{c}	61.91^{b}	17.18^{a}
BCP-5	838^{b}	260^{bc}	67.81^{a}	17.14^{a}
BCP-10	730^{c}	267^{bc}	67.54^{a}	17.16^{a}
BCP-15	893^{b}	276^{b}	68.63^{a}	16.84^{b}
SEM	52	19	3.9	0.34
P Value	0.01>	0.01>	0.73	0.01>

a, b, c Mean values in the same columnwith different superscript letters were significantly different (n=10).
GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; MDA,malondialdehyde; TN; thermoneutral, CS; cold stress, BCP-5, BCP-10, and BCP-15; chicken in these groups were kept in the cold environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black cumin (*Nigella sativa*) seed powder respectively.

Table 6: Effects of black cumin seed (*Nigella sativa*) powder supplementation on liver antioxidant capacity of broilers in thermoneutral and cold stress conditions

groups	GPx (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/mg protein)
TN control	1850^{a}	320.1^{a}	124.1^{a}	14.50^{c}
CS control	1285^{c}	214^{b}	81.70^{c}	17.01^{a}
BCP-5	1231^{c}	196^{b}	80.07^{c}	16.81^{a}
BCP-10	1538^{b}	200^{b}	88.00^{b}	16.97^{a}
BCP-15	1778^{a}	189^{b}	89.24^{b}	16.45^{b}
SEM	85	21	4.15	0.21
P Value	0.01>	0.31	0.38	0.01>

a, b, c Mean values in the same columnwith different superscript letters were significantly different (n=10).
GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; MDA,malondialdehyde; TN; thermoneutral, CS; cold stress, BCP-5, BCP-10, and BCP-15; Chicken in these groups were kept in the cold environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black cumin (*Nigella sativa*) seed powder respectively.

Table 7: Effects of black cumin (*Nigella sativa*) seed powder supplementation on serum cytokines levels of broilers in thermoneutral and cold stress conditions

groups	IL-10 (ug/mL)	IL-6 (ug/mL)	TNF-α (ug/mL)
TN control	57.25^{a}	9.10^{d}	5.20^{d}
CS control	14.18^{d}	15.27^{a}	17.43^{a}
BCP-5	15.92^{d}	15.29^{a}	16.32^{a}
BCP-10	16.09^{c}	13.8^{b}	14.26^{b}
BCP-15	18.95^{b}	11.20^{c}	10.21^{c}
SEM	1.02	0.98	1.5
P Value	0.01>	0.01>	0.01>

a, b, c Mean values in the same columnwith different superscript letters were significantly different (n=10).
IL-10, interleukin-10; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.; TN; thermoneutral, CS; cold stress, BCP-5, BCP-10, and BCP-15; chicken in these groups were kept in the cold environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black cumin (*Nigella sativa*) seed powder respectively.

Table 8: Effects of black cumin (*Nigella sativa*) seedsupplementaion on liver cytokines levels of broilers in thermoneutral and cold stress conditions

groups	IL-10 (ug/mL)	IL-6 (ug/mL)	TNF-α (ug/mL)
TN control	20.22^{a}	1.31^{b}	11.21^{b}
CS control	10.20^{c}	2.80^{a}	29.50^{a}
BCP-5	10.90^{c}	2.75^{a}	28.30^{a}
BCP-10	14.10^{bc}	2.71^{a}	29.37^{a}
BCP-15	15.40^{b}	2.72^{a}	28.20^{a}
SEM	1.95	0.28	2.80
P Value	0.01>	0.01>	0.01>

$^{a, b, c}$ Mean values in the same column with different superscript letters were significantly different (n=10).
IL-10, interleukin-10; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.; TN; thermoneutral, CS; cold stress, BCP-5, BCP-10, and BCP-15; chicken in these groups were kept in the cold environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black cumin (*Nigellasativa*) seedpowder respectively.

Table 9: Effects of black cumin (*Nigella sativa*) seed supplementaion on serum biochemical parameters of broilers in thermoneutral and cold stress conditions

groups	ALT (U/L)	AST (U/L)	ALP (U/L)	TG (mg/dl)	Cholesterol (mg/dl)
TN control	5.36^{c}	58.8^{c}	159.4^{c}	31.25^{c}	79.2^{c}
CS control	18.50^{a}	154.8^{a}	223.0^{a}	194.2^{a}	706.5^{a}
BCP-5	14.02^{b}	127.5^{b}	215.2^{b}	123.7^{b}	398.2^{b}
BCP-10	15.25^{b}	128.0^{b}	215.3^{b}	121.5^{b}	387.2^{b}
BCP-15	15.12^{b}	131.3^{b}	216.1^{b}	125.2^{b}	392.2^{b}
SEM	1.95	8.25	3.5	4.1	25.5
P Value	0.01>	0.01>	0.01>	0.01>	0.01>

$^{a, b, c}$ Mean values in the same column with different superscript letters were significantly different (n=10).
ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; TG: Triglyceride; TN; thermoneutral, CS; cold stress, BCP-5, BCP-10, and BCP-15; Chicken in these groups were kept in the cold environment and fed a basal diet supplemented with 5, 10, and 15 g/kg black cumin (*Nigella sativa*) seed powder respectively.

DISCUSSION

Maintain the broilers' health status, improve production performance and immune response was the main goal of broiler production (Abdel-Wareth et al., 2019). In this research, by experimentally inducing cold stress, we investigated the hematological, biochemical, and anatomical changes of the heart due to cold stress, and, simultaneously, the effects of supplementing black cumin at different levels on these changes. In this regard, 10% of the birds in the cold induction group showed signs and symptoms of PHS syndrome and died. In dead chickens after necropsy, it was observed that the right ventricle to the total ventricles ratio and the relative weight of the heart were much higher compared to the TN group birds. Previously, many researchers reported that cold stress induces PHS syndrome, so they suggested that an increase in the relative weight of the right ventricle, heart hypertrophy, and mortality (Shao et al., 2022; Fathi et al., 2016, 2022; Arab et al., 2006; Nemati et al., 2017; Wang et al., 2020; Hassanzadeh, 2010; Wideman et al., 2010).These researchers suggested that one of the most important causes of PHS mortality is related to the occurrence of oxidative stress during cold stress caused by hypoxemia. Therefore, they suggested that antioxidant therapy in birds involved with oxidative stress caused by induced PHS can effectively reduce mortality caused by PHS. In our experiment, PHS mortality in supplemented with BCP groups was reduced to comparable CS group, Probably due to the antioxidant effects of BCP (Burits and Bucar, 2000; Mansour et al., 2001; Mabrouk et al., 2002; Badary et al., 2003; Ozogurlu et al., 2005;Ilhan et al., 2005; Guler et al., 2007).

The addition of BCPin this research significantly improved the growth performance of chickens subjected to cold stress, which indicates the improving effect of BCP on the undesirable effects of cold stress. Similar to the present results, there are reports of the positive effects of adding black cuminon the growth quality of broiler chickens. BCP supplementation in 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 (Khan et al. 2012; Salam et al., 2013; Rahman and Kim, 2016; El-Hack et al., 2018). Improvement in broiler performance due to black cumin may related to high essential oils levels in black seed as they increased the digesta retention time in the gizzard (Gonzalez-Alvarado et al., 2007). The main active constituent thymoquinone (approximately 60%) and other components (anethole, carvone, 4-terpineol, and carvacrol) of black cumin essential oil have such biological functions that they may serve not only as antioxidants and antibacterials effects, but also as stimulants of digestive enzymes in the pancreas and intestinal mucosa. This improves the nutrients digestibility and feed efficiency, thus increasing the growth performance (Guler et al., 2007; Abu-Dieyeh and Abu-Darwish, 2008; Abdou and Rasher, 2015).

Moreover, the inclusion of active components (anethole) and essential oil, which promote the production of digestive enzymes and bile acid, may explain the enhanced feed intake in the BCP supplemented groups (Amin and Hosseinzadeh, 2016). 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. (Mahmud et al., 2014; Asghar et al., 2022).

In the current study, all BCP groups significantly reduced mortality compared to CS group broiler (Table 3). Antibacterial effects of black cumin reduced the pathogenic bacteria and improve immunity by increasing the production of immunoglobulins (Guler et al., 2007; Abu-Dieyeh and Abu-Darwish, 2008; Khan et al., 2012).

Exposure to cold stress is a result of overproduction of free radicals and equilibrium distribution between ROS production and antioxidant defense system, eventually contributing to oxidative stress and damage (Yang et al., 2011; Rahmani et al., 2018; Fathi et al., 2016, 2022).In this study, cold stress induced a significant increased MDA levels in serum, liver and spleen of cold-stressed broilers and oxidative stress developed which is consistent with the previous study (Yang et al., 2011; Rahmani et al., 2018; Fathi et al., 2016, 2022) who reported exposure to heat stress resulted in reactive ROS radicals overproduction and disturbing the balance between antioxidant defense system and ROS production via increasing lipid peroxidation and depletion of intracellular antioxidant system reserves, eventually contributed to oxidative stress and multiple tissue damage such as heart, liver and lung tissue. The MDA, the end product of lipid peroxidation, served as the marker of oxidative stress (Cheng et al., 2019a). Previous studies indicated that antioxidant enzymes were decreased and MDA levels was increased in cold-stressed broilers (Fathi et al., 2016, 2022).

The results of the present study clearly revealed that supplementing the diet with 1% and 2% BCP, along with increasing the activity of antioxidant enzymes (SOD, GPx, and CAT) , significantly reduced MDA levels in the serum, liver, and spleen of broilers under heat stress. Antioxidant effects exerted by black seed in this research, could be due to the main active constituent thymoquinone and other components: anethole, carvacrol, and 4-terpinol. Black cumin seeds contain about 0.5-1.6% essential oil and approximately 60-80% of it is thymoquinone. Thymoquinone and other components; anethole,4-terpineol, and carvacrol have strong antioxidant potentials through the scavenging ability of different free radicals (Burits and Bucar, 2000; Mansour et al., 2001; Badary et al., 2003; Guler et al., 2007). Similarly, Ilhan et al. (2005) revealed that black seed oil increased superoxide dismutase and glutathione peroxidase activities and reduced MDA levels in mice. Also, they revealed that black seed oil may by decreasing xanthine oxidase and adenosine deaminase activities, which are major potential source of oxygen free radicals, prevent ATP degradation in cell. Our results are consistent with previous reports who indicated that black cumin seeds and its extracts were shown to effective antioxidant and reduce MDA levels in rat (Mabrouk et al., 2002) , in brain and medulla spinals tissues (Ozogurlu et al., 2005) and in A549 cells (Farah et al., 2005).

Cold stress also induced inflammatory response (Fathi et al., 2023b). Former studies indicated that the pro-inflammatory cytokines production was associated with the overproduction of ROS under heat stress (Jang et al., 2014; Ohtsu et al., 2015; Wang et al. 2018)

, physiological stress (Fathi et al., 2023a) and cold stress (Fathi et al., 2023b). Moreover, it has been suggested that mRNA expression for pro-inflammatory cytokines is increased during heat stress, and leads to inflammatory response which may lead to serum and liver inflammation (Kawai and Akira, 2010; Bharati et al., 2017). Also, evidence demonstrated that during heat stress period, the increased cytokines such as tumor necrosis factor alpha (TNF-a) and interleukins (ILs) regulate perturbation of intercellular tight junctions, leading to enhanced cellular permeability and lead to cell damage (Yi et al., 2016).

In the present study, BCP played a beneficial role in alleviating the inflammatory response of cold-stressed broilers, so that, in serum and liver, the increased Il-6 and TNF-α and decreased IL-10 of cold-stressed broilers were attenuated by BCP inclusion, which may indicate that proteins and gene expression levels of pro-inflammatory cytokines are changing in the same way. Agree with our 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 seeds have been proven in many reports of researchers and the present research, it is possible that the anti-inflammatory effects of black seeds are also due to its antioxidant effects. There are already reports that indicate that the supplementation of antioxidant compounds such as chitosan oligosaccharide can significantly increase the level of IL-10 cytokine and decrease IL-1βand TNF-ain broilers under cold stress (Fathi et al., 2023b) and in rats that subjected to heat stress (Lan et al., 2020).

Cold stress was known to induce liver damage and increased serum AST and ALT levels and simultaneous increase in serum cholesterol and triglycerides in broiler chickens (Arab et al., 2006; Fathi et al. 2016, 2022, 2023a,2023b). In present study, dietary BCP supplementation alleviated liver damage by decreasing serum ALT and AST levels due to its antioxidant effects in birds under cold stress. Other studies also reported that dietary antioxidant supplementation decreased ALT and AST levels in in serum of broiler under various stress (Huang et al., 2018; Cheng et al. 2019a; Lan et al., 2020; Fathi et al., 2022). So there is a possibility that the BCP, through its antioxidant effects, has caused a decrease in the serum level of liver enzymes in birds under cold stress. The reduction in serum triglyceride and cholesterol due to BCP supplementaion, may be attributed to the lowering effect of monosaturated fatty acids and thymoquinone on the synthesis of cholesterol by hepatocytes or the fractional reabsorption from the small intestine. Moreover, BCP also contain an appreciable amount of sterols, especially β-sitosterol, that has the ability to inhibit the absorption of dietary cholesterol (Brunton, 1999). Reducing in cholesterol by antioxidant compounds is by inhibiting biosynthesis of cholesterol, because cholesterol synthesis is applied at the beginning of its biosynthesispathway, namely hydroxyl methyl glutathionecoenzyme a reductase (HMG-COA). The reaction of HMG-COA conversion to mevalonate is done under nicotinamide adenine dinucleotide phosphate (NADPH) and HMG-COA reductase. It has been shown that antioxidantcom pounds by reducing the activity of HMG-COA, decrease production of mevalonate. Phenolic compounds of medical plant can increase serum HDL-C levels and decrease LDL-C oxidation (Weinbrenner et al., 2004). In addition, it has revealed that antioxidant compounds can reduce serum triglyceride levels by increasing the flow of triglycerides from the blood into the cells, and by decreasing the production of pancreatic lipase (Ahmadipour et al., 2015).Studies have shown that anthocyanins and flavonoids can prevent atherosclerosis and chronic cardiovascular disease by trapping free radicals, inhibiting LDL-C oxidation, and lowering cholesterol and triglyceride levels in the blood (Ustundag and Ozdogan, 2015).

CONCLUSIONS

In conclusion, the results of this experiment showed that cold stress causes an increase in basal metabolism, consequence, hypoxemia, and the occurrence of PHS. The PHS significantly induces oxidative stress, followed by inflammation in broilers. Decreased growth performance and increased hypertrophy of the right ventricle and heart, as well as decreased growth performance, are the consequences of oxidative stress and inflammation caused by PHS. Meanwhile, black cumin (Nigella Sativa) seeds powder (BCP) supplementation significantly moderated the related adverse effects. In general, the best positive effect was related to the level 10 g/kg BCP of diet.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Abdel-Wareth AA, Hammad S, Khalaphallah R, Salem WM, Lohakare J. 2019. Synbiotic as eco-friendly feed additive in diets of chickens under hot climatic conditions. Poult Sci. 98 (10) :4575-4583.

Abdou, A., Rashed, G.G. 2015. Effect of black seed (Nigella sativa) and garlic (Allium sativum) feed supplements on productive performance and some physiological and immunological responses of japanese quail. Egypt. J. Nutr. Feed, 18, 129-141.

Abu-Dieyeh, Z.H.M., Abu-Darwish, M.S., 2008. Effect of feeding powdered black cumin seeds (Nigella sativa L.) on growth performance of 4-8 week-old broilers. J. Anim.Vet. Adv. 7:286-290.

Acaroz, U., Ince, S., Arslan-Acaroz, D., Gurler, Z., Demirel, H. H., Kucukkurt, I., Zhu, K. 2019. Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: protective role of boron. Toxicology research, 8 (2) , 262-269.

Adam GO, Rahman MM, Kim GB, Kang HS, Kim JS, Kim SJ. 2016. Hepatoprotective effects of Nigella sativa seed extract against acetaminophen-induced oxidative stress. Asian Pac J Trop Med. [In press]. doi: 10.1016/ j. apjtm.2016.01.039.

Ahmadipour B, Hassanpour H, Asadi E,Khajali F, Rafiei, F, Khajali F. 2015.Kelussia odoratissima Mozzaf-A promisingmedicinal herb to prevent pulmonary hypertension in broiler chickens reared at high altitude. J Ethnophar. 159: 49-54.

Amin, B and Hosseinzadeh H. 2016. Black Cumin (Nigella sativa) and Its Active Constituent, Thymoquinone: An Overview on the Analgesic and Anti-inflammatory Effects. Planta Med, 82: 8-16.

Arab, H. A., R. Jamshidi, A. Rassouli, G. Shams., Hassanzadeh, M.H., 2006. Generation of hydroxyl radicals during ascites experimentally. Br. Poul. Sci, 47, 2: 216-222.

Aviagen. 2014. Ross 308 broiler performance objectives. AL, USA: Aviagen Group.

Azeem T, Ur-Rehman Z, Umar Z, Asif S, Arif MM, A. Rahman A. 2014. Effect of Nigella Sativa on poultry health and production: a review. Science Letters, 2 (2) : 76-82.

Badary OA, Taha RA, Gamal-el-Din AM, Abdel-Wahab MH. 2003. Thymoquinone is a potent superoxide anion scavenger. Drug Chemstry and Toxicolgy. 26:87-98.

Bharati, J., Dangi, S.S., Mishra, S.R., Chouhan, V.S., Verma, V., Shankar, O., Bharti, M.K., Paul, A., Mahato, D.K., Rajesh, G., Singh, G., Maurya, V.P., Bag, S., Kumar, P., Sarkar, M., 2017. Expression analysis of Toll like receptors and interleukins in Tharparkar cattle during acclimation to heat stress exposure. J. Therm. Biol. 65, 48-56.

Brunton LL. 1999. Agents affecting gastrointestinal water flux and motility, digestants and bile acids. In: A.G. Goodman, T.W. Rall, A.S. Nies, P. Taylor (eds.) The pharmacological basis of therapeutics. 8th ed. Pregman Press, Oxford, UK, pp 914-932.

Burits M, Bucar F.2000. Antioxidantactivity of Nigella sativa essential oil. Phytotherapy Research. 14: 323-328.

Cheikh-Rouhou S, Besbes S, Hentati B, Blecker C, Deroanne C, Attia H. 2007. Nigella sativa L.: chemical composition and physicochemical characteristics of lipid fraction. Food Chem. 101:673-681.

Cheng, K, Yan E, Song Z, Li S, Zhang H, Zhang L, Wang C, Wang T.

2019a. Protective effect of resveratrol against hepatic damage induced by heat stress in a rat model is associated with the regulation of oxidative stress and inflammation. J Therm Biol. 82:70-75.

Cheng Y, Chen Y, Chen R, Su Y, Zhang R, He Q, Wang K, Wen C, Zhou Y. 2019b. Dietary mannan oligosaccharide ameliorates cyclic heat stress-induced damages on intestinal oxidative status and barrier integrity of broilers. Poult Sci. 98 (10) :4767-4776.

El-Hack, A., Mohamed, E.; Mahgoub, S.A.; Hussein, M.; Saadeldin, I.M. 2018. Improving growth performance and health status of meat-type quail by supplementing the diet with black cumin cold-pressed oil as a natural alternative for antibiotics. Environ. Sci. Pollut. Res. 25, 1157-1167.

Farah N., Benghuzzi H., Tucci M., Cason Z. 2005. The effects of isolated antioxidants from black seed on the cellular metabolism of A549 cells. Biomed. Sci. Instrum, 41, 211-216.

Fathi, M., Haydari, M., Tanha, T., 2016. Influence of Dietary Aspirin on Growth Performance, Antioxidant Status, and Mortality due to Ascites in Broiler Chickens. Poult. Sci. J. 4 (2) : 139-146.

Fathi, M., Tanha, T., Saeedyan, S., 2022. Influence of dietary lycopene on growth performance, antioxidant status, blood parameters and mortality in broiler chicken with cold-induced ascites. Arch. Anim. Nutr. 8:1-11. doi: 10.1080/1745039X.2022.2046451.

Fathi, M., Saeedyan, S., Kaoosim M. 2023a. Gamma-amino butyric acid (GABA) supplementation alleviates dexamethasone treatment-induced oxidative stress and inflammation response in broiler chickens. Stress, 26 (1) : 2185861 https://doi.org/10.1080/10253890.2023.2185861

Fathi, M., Saeedyan, S., Baghaeifar, Z., Varzandeh, S., 2023b. Chitosan Oligosaccharides in the Diet of Broiler Chickens Under Cold Stress Had Anti-Oxidant and Anti-Inflammatory Effects and Improved Hematological and Biochemical Indices, Cardiac Index, and Growth Performance. SSRN Electronic Journal. DOI: 10.2139/ssrn.4383540.

Gonzalez-Alvarado, J.M., Jiménez-Moreno, E., Lázaro, R., Mateos, G.G., 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poultry Sci. 86:1705-1715.

Guler, T., Ertas, O.N., Kızıl, M., Dalkılıc, B., Ciftci, M., 2007. Effect of dietary supple- mental black cumin seeds on antioxidant activity in broilers. Med. Weter. 63:1060-1063.

Guler T, Dalkilic B, Ertas ON, Ciftci M. 2006. The effect of dietary black cumin seeds (Nigella sativa L.) on the performance of broilers. Asian Austral J Anim. 19:425-430.

Hassanzadeh, M. 2010. Endogenous and environmental factors interactions that contribute to the development of ascites in broiler chickens: a review. Int. J. Vet. Res. 4:117-126.

Huang, S.C., He-ping Yang., Mujeeb Ur Rehman. and Zong-xi Tong. 2018. Acute heat stress in broiler chickens and its impact on serum biochemical and electrolyte parameters. B-690 [1-4]. DOI: 10.18805/ijar. v0iOF.8490

Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. 2005. Antiepileptogenic and antioxidant effects of Nigella sativa oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology. 49: 456-464.

Jang IS, Ko YH, Moon YS, Sohn SH. 2014. Effects of vitamin C or E on the pro-inflammatory cytokines, heat shock protein 70 and antioxidant status in broiler chicks under summer conditions. Asian Australas J Anim Sci. 27 (5) :749-760.

Jaganjac, M., Milkovic, L., Zarkovic, N., & Zarkovic, K. 2022. Oxidative stress and regeneration. Free Radical Biology and Medicine, 181, 154-165.

Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity:update on toll-like receptors. Nat. Immunol. 11, 373-384.

Khan, S.H., Ansari, J., Haq, A.U., Abbas G. 2012. Black cumin seedsas phytogenic product in broiler diets and its effects onperformance, blood constituents, immunity and caecalmicrobial population. Ital J Anim Sci. 11:77.

Lan, R., Linlin Wei, Qingqing Chang, Shengnan Wu., Zhao Zhihui. 2020. Effects of dietary chitosan oligosaccharides onoxidative stress and inflammation response inliver and spleen of yellow-feather broilers exposedto high ambient temperature. Italian Journal of Animal Science. 19 (1) : 1508-1517. doi.org/10.1080/1828051X.2020.1850215.

Lorenzoni, A. G., & Ruiz-Feria, C. A. 2006. Effects of vitamin E and l-arginine on Cardiopulmonary function and ascites parameters in broilers chickens reared under sub-normal temperatures. Poultry Science, 85, 2241-2250. DOI: 10.1093/ps/85.12.2241.

Mabrouk GM, Moselhy SS, Zohny SF, Ali EM, Helal TE, Amin AA, Khalifa AA. 2002. Inhibition of methylnitrosourea (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and Nigella grains in Sprague Dawely rats. J Exp. Clin Cancer Res. 21: 341-346.

Mansour M. A., Ginawi O. T., El-Hadiyah T., El-Khatib A. S., Al-Shabanah O. A., Al-Sawaf H. A. 2001. Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Res. Commun. Mol. Pathol. Pharmacol, 110, 239-251.

Mahmud, H.A., 2014. Response of growing Japanese quail to different level of fennel seed meal. Egypt. Poult. Sci. J. 15 (6) , 795-807. https://doi.org/10.21608/ epsj.2014.5362.

Nemati, M.H., Shahir, M.H.I., Harakinezhad, M.T.I., Lotfalhian, H.I., 2017. Cold-Induced Ascites in Broilers: Effects of Vitamin C and Coenzyme Q10. Brazi. J.Poul. Scie. 9 (3) : 537-544.

Ozogurlu F, Sahin S, Idýz N, Akyol O, Ilhan A, Yigitoglu R, Isýk B.2005. The effect of Nigella sativa oil against experimental allergic encephalomyelitis via nitric oxide and other oxidative stress parameters. Cell Mol Biol. 51: 337-342

Ojueromi, O. O., Ganiyu Oboh., Ayokunle Olubode Ademosun. 2022. Effect of black seeds (Nigella sativa) on inflammatory and immunomodulatory markers in Plasmodium berghei-infected mice. J Food Biochem. 2022;00: e14300. https://doi.org/10.1111/jfbc.14300.

Ohtsu, H, Yamazaki M, Abe H, Murakami H, Toyomizu M. 2015. Heat stress modulates cytokine gene expression in the spleen of broiler chickens. Jpn Poult Sci. 52 (4) : 282-287.

Ustundag AO, Ozdogan M. 2015. Usage possibilities of mulberry leaves in poultry nutrition.Scientific Papers. J Anim Scie. 58:170-178.

Rahmani, M., Golian, A., Kermanshahi, H., Bassami, M.R., 2018. Effects of curcumin or nanocurcumin on blood biochemical parameters, intestinal morphology and microbial population of broiler chickens reared under normal and cold stress conditions. J. Appl. Poult. Sci. 46 (1) , 200-209. https://doi.org/10.1080/09712119.2017.1284077.

Rahman, M. M. and Shang-Jin Kim. 2016. Effects of dietary Nigella sativa seed supplementation on broiler productive performance, oxidative status and qualitative characteristics of thighs meat. ITALIAN JOURNAL OF ANIMAL SCIENCE ,15, NO. 2, 241-247

Ramazani, N., Mahd Gharebagh, F., Soleimanzadeh, A., Arslan, H. O., Keles, E., Gradinarska-Yanakieva, D. G., Dinç, D. A. 2023. The influence of L-proline and fulvic acid on oxidative stress and semen quality of buffalo bull semen following cryopreservation. Veterinary Medicine and Science.9 (4) : 1791-1802.doi: 10.1002/vms3.1158.

Shao FJ, Ying YT, Tan X, Zhang QY, Liao WT. 2018. Metabonomics profiling reveals biochemical pathways associated with pulmonary arterial hypertension in broiler chickens. J Proteome Res. 17 (10) :3445-3453.

Shao, F., Liu, R., Tan, X., Zhang, Q., Ye, L., Yan, B., Zhuang, Y., Xu, J., 2022.MSC Transplantation Attenuates Inflammation, Prevents Endothelial Damage and Enhances the Angiogenic Potency of Endogenous MSCs in a Model of Pulmonary Arterial Hypertension. J. Inflamm. Res, 15:2087-2101.

Salam, S., Sunarti, D. 2013. Isroli, I. Physiological responses of blood and immune organs of broiler chicken fed dietary black cumin powder (Nigella sativa) during dry seasons. J. Indones. Trop. Anim. Agric, 38, 185-191.

Wang W, Yan F, Hu J, Amen O, Cheng H. 2018. Supplementation of Bacillus subtilis-based probiotic reduces heat stress-related behaviors and inflammatory response in broiler chickens. J Anim Sci. 96 (5) :1654-1666.

Wang, C., Liu, H., Yang, M., 2020. RNA-seq based transcriptome analysis of endothelial differentiation of bone marrow mesenchymal stem cells. Eur. J. Vasc. Endovasc. Surg. 59 (5) :834-842.

Wideman, R.F., Eanes, M.L., Hamal. K.R., Anthony NB., 2010.Pulmonary vascular pressure profiles in broilers selectedfor susceptibility to pulmonary hypertension syndrome:age and sex comparisons. Poult Sci. 89:1815-1824.

Weinbrenner, T., Fito, M., Torre, R.D., Saez, G.T.,Rijken, P. and Tormos, C., 2004. Olive oilshigh in phenolic compounds modulate oxidative/antioxidative status in men. J. Nutr. 134,2314-2321.

Yang, X. J., W. L. Li, Y. Feng., Yao, J.H., 2011. Effects ofimmune stress on growth performance, immunity, and cecal microflora in chickens. Poult. Sci. 90:2740-2746.

Yi, D., Hou, Y.Q., Tan, L.L., Liao, M., Xie, J.Q., Wang, L., Ding, B.Y., Yang, Y., Gong, J., 2016. N-acetylcysteine improves the growth performance and intestinal function in the heat-stressed broilers. Anim. Feed Sci. Technol. 220, 83-92.