Iranian Journal of Applied Animal Science

Reducing Mortality and Improving Performance Due to Ascites by Supplementing with Broccoli Ethanol Extract through Improving Antioxidant Status and Inflammatory in Broiler Chickens

Research Article

M. Fathi[1*], S. Saidian[2], P. Mardani[2] and S. Nikjo[2]

[1] Department of Animal Science, Faculty of Agriculture, Payame Noor University, Tehran, Iran
[2] Department of Biology, Faculty of Agriculture, Payame Noor University, Tehran, Iran

Received on: 24 Feb 2025
Revised on: 13 Jul 2025
Accepted on: 11 Aug 2025
Online Published on: Sep 2025

*Correspondence E-mail: mokhtarfathi@pnu.ac.ir

Online version is available on: www.ijas.ir
https://doi.org/10.71798/ijas.2025.1224404

ABSTRACT

Ascites syndrome is one of the most important metabolic disorders in growing broiler chickens worldwide. This study investigated the effects of dietary supplementation with of broccoli ethanolic extract (BEE) on broiler chickens subjected to pulmonary hypertension syndrome (PHS) induced by cold stress. A total of 500 broiler chickens were used in this study. One hundred birds were reared under thermoneutral conditions and assigned to the negative control group (NT). The remaining 400 birds were exposed to cold stress to induce pulmonary hypertension syndrome (PHS) and were randomly divided into four groups of 100 birds each: (1) the CS group, which received a basal diet and served as the positive control; and three treatment groups-(2) BEE-1, (3) BEE-2, and (4) BEE-3-receiving 1000, 1500, and 3000 mg/kg of broccoli ethanol extract (BEE), respectively. Exposure to cold resulted in a 30% incidence of PHS in the control group, characterized by significant increases in relative heart and right ventricle weights, right ventricle/total ventricle (RV/TV) ratios, and mortality (P<0.01). Supplementation with BEE at all tested levels significantly improved cardiac parameters and reduced mortality (P<0.01). PHS negatively affected performance traits by reducing body weight gain and feed intake while increasing feed conversion ratio (P<0.01); these effects were mitigated by BEE supplementation, which enhanced growth performance and feed efficiency. BEE supplementation significantly enhanced antioxidant enzyme activities and reduced lipid peroxidation (P<0.01). Inflammatory markers TNF-α and IL-1β increased and IL-10 decreased in PHS-affected birds, while BEE treatment reversed these changes, demonstrating anti-inflammatory effects (P<0.01). Hematological disturbances caused by PHS, such as increased WBC, RBC, hemoglobin, hematocrit, and heterophil counts, were also significantly alleviated by BEE (P<0.01). Overall, dietary supplementation with ethanolic extract of broccoli effectively mitigates cardiac dysfunction, oxidative stress, inflammation, and performance losses associated with PHS in broiler chickens.

KEY WORDS anti-inflammatory, antioxidant, broccoli ethanolic extract, broiler, cold stress, PHS.

INTRODUCTION

Fast-growing broiler chickens (*Gallus gallus*), particularly males, possess high metabolic rates due to genetic selection for rapid weight gain (Druyan *et al*. 2008; Hassanzadeh, 2010). This elevated metabolic activity increases their vulnerability to metabolic disorders such as pulmonary hypertension syndrome (PHS), a condition marked by fluid accumulation around the heart and abdominal cavity (Hassanzadeh, 2010). PHS arises from an imbalance between oxygen demand and supply, especially in birds with immature cardiopulmonary systems. Environmental stressors—such as cold temperatures, high altitudes, and dietary salt toxicity—further intensify oxygen demands, with cold stress alone increasing requirements by up to 185% (Xiang *et al.* 2004; Baghbanzadeh and Decuypere, 2008; Fathi *et al.* 2022). This imbalance leads to hypoxemia, elevated pulmonary arterial pressure, right ventricular hypertrophy, and, ultimately, heart failure and death (Wideman *et al.* 2010).

Hypoxemia not only elevates pulmonary arterial pressure and leads to right ventricular hypertrophy and cardiac enlargement, but also contributes to the generation of reactive oxygen species (ROS). Cold stress-induced systemic hypoxia may cause cellular hypoxia, promoting excessive ROS production (Fathi *et al.* 2016; Fathi *et al.* 2022). These oxygen-derived free radicals are key mediators of tissue damage during inflammatory responses (Halliwell and Gutteridge, 1990). Moreover, ROS reduce the half-life of nitric oxide—a crucial vasodilator—thereby impairing vascular relaxation and further predisposing birds to pulmonary hypertension syndrome (PHS) (Lorenzoni and Ruiz-Feria, 2006).

Oxidative stress occurs when the production of free radicals in cells exceeds their antioxidant capacity (Arab *et al.* 2006). It is proposed that PHS might be associated with oxidative stress and lipid peroxidation induced by reactive oxygen species (ROS) (Bottje and Wideman, 1995). The incidence of PHS may be resulting in part from free radical generation in birds, with subsequent depletion of tissue antioxidants (Bottje *et al.* 1995). Thus, adding antioxidant compounds to diet has a major role in protecting cells from the actions of reactive oxygen species (ROS) by reducing chemical radicals and disrupting the process of lipid peroxidation. Using synthetic antioxidants, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), have been restricted long ago, because of their possible carcinogenicity causing liver swelling and changing liver enzyme activities (Rahmani *et al.* 2018). Currently, there is an increasing interest in non-synthetic sources of antioxidants. Medicinal plants and probiotics, due to their strong antioxidant and health-promoting properties, are being widely recognized as natural and effective alternatives (Saeed *et al.* 2017; Babaahmadi Milani *et al.* 2020; Saeed *et al.* 2020; Riaz *et al.* 2022).

Broccoli (*Brassica oleracea* var. italica) is a member of the Brassicaceae family and is rich in bioactive compounds such as vitamin C, carotenoids, glucosinolates, selenium, phenolics, and flavonoids (Dos Reis *et al.* 2015). Among these, flavonoids and phenolic compounds exhibit potent antioxidant properties by scavenging free radicals, reducing oxidative stress, and lowering triglyceride and cholesterol levels—thereby mitigating risks of cardiovascular diseases (Ustundag and Ozdogan, 2015). Broccoli's polyphenolic content contributes to its protective effects, including anticancer and anti-inflammatory actions through modulation of apoptosis and antioxidant enzyme regulation (Bhandari and Kwak, 2014). In poultry, broccoli has been shown to reduce lipid peroxidation and enhance antioxidant defense mechanisms (Cho *et al.* 2006; Babaahmadi Milani *et al.* 2020). However, no study has specifically examined the impact of broccoli extract on the incidence of pulmonary hypertension syndrome (PHS) in broilers. Therefore, the current study aims to evaluate the effects of dietary ethanolic broccoli extract on antioxidant status, inflammatory responses, and growth performance in broilers exposed to cold-induced PHS conditions.

MATERIALS AND METHODS

Animal ethics statement

All animal experiments were performed in accordance with the protocol of the Animal Use Committee of the Iranian Ministry of Science, Research and Technology were approved by the Animal Care Committee of the Department of Animal Science of Payam Noor University. All efforts were made to minimize animal suffering (No: 1404.801).

Birds, diets and experimental design

This study was performed on a total of 500 unsexed one-day-old Ross male 308 broiler chicks with an average body weight of 44 ± 3 g, 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. A total of 100 birds were reared in a thermoneutral environment control (NT group), and the rest of the birds (400 birds in four groups) were subjected to cool environmental temperatures (CS) to induce pulmonary hypertension syndrome (PHS), and were fed a basal diet as CS group and three levels of BEE at rates of 1000, 1500, and 3000 mg/kg (BEE-1, BEE-2, and BEE-3, respectively). The temperature of both houses was set at 32 °C for the first week and then reduced to 29 °C in the second week. A continuous lighting program was set at 23L: 1D throughout the experimental period. Each cage had spherical pen feeders and drinkers and was sized 150 × 200 cm. The broilers were administered vaccines against New Castle disease in addition to other infectious diseases regularly. A mash-based corn-soybean meal diet for starter (1-10), grower (11-24), and finisher (25-42) periods was formulated according to Ross 308 nutrient recommendations (Aviagen, 2014; Table 1). Diets and freshwater were provided *ad libitum*.

Preparing broccoli ethanolic extract

Fresh broccoli (*Brassica oleracea* var. italica) was washed thoroughly with distilled water, chopped, and shade-dried at room temperature for 5-7 days. The dried material was then ground into a fine powder using a mechanical grinder. For extraction, the powdered broccoli (100 g) was subjected to cold maceration with 70% ethanol (v/v) at a 1:10 (w/v) ratio for 72 hours at room temperature (25±2 °C) with intermittent shaking (Dos Reis *et al.* 2015). The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40 °C under reduced pressure to remove the ethanol. The concentrated extract was stored at 4 °C in an amber bottle until further use. The pH of the extract was measured (6.3±0.1), and quality control parameters such as total phenolic content (TPC) and antioxidant capacity (DPPH assay) were determined to ensure consistency across batches (Dasgupta *et al.* 2004; Babaahmadi Milani *et al.* 2020).

Determination of essential oil compounds by GC analysis

Identification of essential oil constituents was done based on the mass spectra of the GC (6890 HP) and comparing them with the standard mass spectra. The compounds in the essential oil were analyzed using gas chromatography. According to the area under the curve of each of the peaks of the GC spectrum and comparing it with the total area under the curve, the relative percentage of each compound of the extracts was determined.

MS-GC analysis showed the presence of 19 compounds in the plant essential oil, of which 13 compounds were identified with a total compound percentage of 92.7%. The three main compositions of essential oils identified carvone (51%), ethyl ether (9.5%) and, Trans-Pinocarveol (8%) (Table 2).

Cold stress induction protocol

On d 16, birds were exposed to cool environmental temperatures to induce PHS as we have previously described (Fathi *et al.* 2022). Briefly, when starting on d 16, the brooding temperature was gradually decreased by 1 °C per day until a final temperature of 15 °C was reached. Bird mortality was recorded daily and necropsies were performed to identify PHS-related deaths from d 16 onward. Diagnosis of ascites generally depends on observation of the following symptoms: cardiac muscle laxation; swollen and stiff liver; clear, yellowish, colloidal fluid in the abdominal activity (Geng *et al.* 2004).

Bird's performance traits

Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded from days 16-42 of age. The FCR was calculated as FI/BWG.

Blood sampling for hematological and biochemistry indices

On day 42, two birds per cage (a total of ten birds per treatment) were randomly selected, and whole blood samples (approximately 2.5 mL) were collected by venipuncture. Whole blood samples were collected by venipuncture into anticoagulation tubes for measuring hematological indices and were analyzed using an automatic blood analyzer (Sysmex KX-21N Automatic blood analyzer, Japan). Leukocyte differential counts were according to the methodology described by Rasha *et al.* (2017). Serum was prepared by centrifugation at 2500 × g for 10 min at room temperature and stored at –20 ˚C for later biochemical analysis (Fathi *et al.* 2022). Serum levels of total protein (TP), and triglyceride (TG) were determined using an autoanalyzer (Abbott alcyon 300, USA) by laboratory kits (Pars Azmoon, Tehran, Iran).

Heart weight and mortality due to PHS

Mortalities were recorded daily, and necropsies were performed to identify related deaths from d 14 onward. Birds that died with an right ventricle/total ventricle (RV/TV) ratio above 0.25 or observation of fluid in the ventricular or pericardium of the heart were included in the PHS mortality (Table 2) (Shao *et al.* 2018; Shao *et al.* 2022). The RV/TV ratio was calculated by dividing the weight of the right ventricle (RV) by the total ventricular weight (TV), which includes the right ventricle, left ventricle, and interventricular septum. On the last day of the experiment (day 42), two birds (a total of ten birds per treatment) were dissected, hearts were weighed, and the atria, pericardium, major vessels, and fat were trimmed off. The left and right ventricles were separated, 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

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) concentration were measured using appropriate laboratory kits (Pars Azmoon, Tehran, Iran). Glutathione peroxidase (GPx) activity was determined using a commercially available enzyme kit (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK), catalase (CAT) and superoxide dismutase (SOD) activity was determined using the commercially available enzyme kit (Ransol, RANDOX/SD-125 supplied by Randox Laboratories) and autoanalyzer (Alcyon 300, USA) according to the manufacturers' protocols.

Table 1 The ingredients and composition of the basal diet

Ingredients (%)	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[1]	0.25	0.25	0.25
Mineral premix[2]	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
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

[1] 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 and Nicotinamide: 5 mg;

[2] 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 and Se (from sodium selenite): 0.3 mg.

Table 2 Results of broccoli ethanolic extract (BEE) analysis using GC/MS

Row	Composition	R.T (min)	Percent
1	Ethyl ether	1.577	9.5
2	Ethyl Acetate	1.88	3.0
3	1-Deoxy-d-mannitol	3.33	1.0
4	Benzaldehyde,3-benzyloxy-2-fluoro-4-methoxy	5.90	1.0
5	Trans-Pinocarveol	6.34	8.0
6	(+)-Dihydrocarvone	7.26	3.0
7	(+)-Grandisol	7.39	6.0
8	(-)-Carvone	7.75	51.0
9	Trans-Pinocarvyl acetate	8.30	1.0
10	Dill- Apiol	12.06	1.0
11	Oleic Acid	14.53	1.0
12	Ethyl iso-allocholate	17.25	1.0
13	β-Glyceryl linolenate	18.13	2.0

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). This method evaluates oxidative stress by measuring MDA, the final product of lipid breakdown caused by oxidative stress. Pro-Inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and anti-inflammatory cytokines such as interleukin-10 (IL-10) concentrations in serum and liver tissue were determined by using ELISA kits (Pars Azmoon, Tehran, Iran) according to the manufacturer's instructions.

Statistical analysis

Data from all response variables were subjected to one-way analysis of variance by applying the SAS program (SAS, 2005) based on a completely randomized design (CRD) with five treatments and five replicates per treatment using a general liner model (GLM). Significant differences among treatment means were separated using Tukey's test at 5% probability.

RESULTS AND DISCUSSION

In this study, we exposed the birds to cold temperatures to induce physiological stress. As shown in Table 3, under this conditions, 30.00% of birds in the CS control group (with PHS-induced) developed PHS. The heart and RV weights as percentages of BW, the RV: TV ratios, and mortality due to PHS were significantly higher in the PHS-induced group broilers compared to the healthy group (P<0.01).

Supplementation with broccoli ethanolic extract (BEE) significantly reduced mortality due to pulmonary hypertension syndrome (PHS) in broilers exposed to cold stress by 49.3%, 60.0%, and 56.7% in BEE-1, BEE-2, and BEE-3 groups, respectively (P<0.01). Additionally, BEE decreased the right ventricle to total ventricle ratio (RV/TV) by 25.0%, 21.9%, and 21.9%, and the right ventricle to body weight ratio (RV/BW) by 12.2%, 14.4%, and 13.3% in these groups compared to cold stress controls (P<0.01). Relative heart weight was also reduced by 6.7%, 9.3%, and 10.7%, respectively (P<0.01). These results demonstrate that BEE supplementation mitigates mortality and cardiac hypertrophy in broilers under cold stress conditions.

Bird's performance traits

As shown in Table 4, PHS induction significantly (P<0.01) reduced BWG and FI and increased FCR (P<0.01). Supplementation with broccoli ethanolic extract (BEE) significantly improved broiler performance under cold stress-induced PHS. Feed conversion ratio (FCR) decreased by 8.5–8.8% in BEE groups compared to the cold stress control (P<0.01). Body weight gain (BWG) increased by 12.0–13.1%, while feed intake (FI) rose by 2.5–4.2% in BEE-treated birds versus the control (P<0.01). These results demonstrate that BEE supplementation effectively mitigates the negative effects of cold stress on growth performance.

Antioxidant status in serum and liver

Based on Tables 5 and 6, broilers exposed to cold stress showed a significant increase in malondialdehyde (MDA) levels by 74.5% in serum and 66.7% in liver compared to thermoneutral controls (P<0.01). At the same time, antioxidant enzyme activities including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) significantly decreased in both serum and liver tissues under cold stress (P<0.01). Supplementation with broccoli ethanolic extract (BEE) at different doses significantly reduced MDA levels by approximately 20–32% and improved activities of CAT, SOD, and GPx compared to the cold stress control group (P<0.01). These results suggest that BEE effectively alleviates oxidative damage by enhancing antioxidant defenses in broilers subjected to pulmonary hypertension syndrome induced by cold stress.

Inflammation response

According to Tables 7 and 8, cold stress significantly increased the levels of pro-inflammatory cytokines TNF-α and IL-1β in both serum and liver tissues of broilers, while significantly decreasing the anti-inflammatory cytokine IL-10 (P<0.01). In the serum, TNF-α and IL-1β levels in the CS group rose by approximately 192% and 619% respectively, compared to the thermoneutral group, while IL-10 dropped by 78.7%. Similar trends were observed in the liver, where TNF-α and IL-1β increased by 278% and 217%, and IL-10 decreased by 75.4% in the CS group compared to the NT group (P<0.01). Supplementation with broccoli ethanolic extract (BEE) significantly attenuated these inflammatory responses. In all BEE-treated groups, TNF-α and IL-1β levels were markedly lower than in the CS group (P<0.01), while IL-10 levels were significantly elevated. For example, in the liver, BEE-3 supplementation reduced TNF-α by about 46% and increased IL-10 by 145% compared to the CS group. These findings suggest that BEE has potent anti-inflammatory effects in broilers under pulmonary hypertension-inducing cold stress conditions.

Biochemical parameters

According to Table 9, cold stress significantly increased serum cholesterol, triglycerides (TG), and liver enzymes (ALP, AST, and ALT) in broilers (P<0.01). However, dietary supplementation with broccoli ethanolic extract (BEE) at 1000–3000 mg/kg markedly reduced cholesterol (by up to 31%) and TG levels (by up to 48%) compared to the cold-stressed control group. Although liver enzyme levels in BEE-treated groups remained higher than in the thermoneutral group, they were significantly lower than in the cold stress group. These findings suggest that BEE mitigates cold stress-induced metabolic and hepatic disturbances in broilers.

Hematological parameters

According to Table 10, cold stress significantly increased the heterophil-to-lymphocyte (H:L) ratio, heterophil count, hematocrit (Hct), hemoglobin (Hb), red blood cells (RBC), and decreased white blood cell (WBC) count compared to the thermoneutral group (P<0.01). Broccoli ethanolic extract (BEE) supplementation (1000–3000 mg/kg) significantly reduced H:L ratio and heterophil count while increasing RBC and stabilizing Hb and Hct levels. WBC counts were also restored to levels similar to or higher than the thermoneutral group. These results indicate that BEE alleviates hematological stress responses induced by cold stress and improves immune status in broilers.

Table 3 Effects of broccoli ethanolic extract (BEE) supplementation on organ relative weight and mortality due to ascites of broilers in thermoneutral and PHS-induced with cold stress

Groups	Relative heart weight (g/100 g BW)	RV/BW (g/100 g BW)	RV/TV Ratio	Mortality due to PHS (%)
NT control	0.61[d]	0.061[d]	0.20[c]	2.00[c]
CS control	0.75[a]	0.090[a]	0.32[a]	30.00[a]
BEE-1	0.70[b]	0.079[b]	0.24[b]	15.20[b]
BEE-2	0.68[b]	0.077[b]	0.25[b]	12.00[b]
BEE-3	0.67[b]	0.078[b]	0.25[b]	13.00[b]
SEM	0.02	0.003	0.02	2.50
P-value	<0.01	<0.01	<0.01	<0.01

RV/BW: right ventricular to body weight ratio; RV/TV: right ventricle/total ventricle; PHS: pulmonary hypertension syndrome; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of broccoli ethanolic extract (BEE) supplementation on performance of broilers in thermoneutral and PHS-induced with cold stress conditions at day 42

Groups	FI (g)	BWG (g)	FCR
NT control	3878[a]	2486[a]	1.56[b]
CS control	3465[c]	2100[c]	1.65[a]
BEE-1	3610[b]	2375[b]	1.52[b]
BEE-2	3562[b]	2359[b]	1.51[b]
BEE-3	3553[b]	2353[b]	1.51[b]
SEM	50	65	0.05
P-value	<0.01	<0.01	<0.01

BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of broccoli ethanolic extract (BEE) supplementation on serum antioxidant capacity of broilers in thermoneutral and PHS-induced with cold stress conditions

Groups	GPx (U/g Hb)	SOD (U/g Hb)	CAT (Nmol/min/mL)	MDA (Nmol/L)
NT control	1409.0[a]	301.57[a]	71.80[a]	10.44[b]
CS control	646.29[d]	222.05[c]	29.08[b]	18.22[a]
BEE-1	1190[a]	287.10[a]	30.52[b]	14.20[b]
BEE-2	1205[a]	289.00[a]	35.12[b]	14.50[b]
BEE-3	1200[a]	285.20[a]	30.12[b]	13.70[b]
SEM	20.1	5.50	6.12	1.70
P-value	<0.01	<0.01	<0.01	<0.01

GPx: glutathione peroxidase; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of broccoli ethanolic extract (BEE) supplementation on liver antioxidant capacity of broilers in thermoneutral and PHS-induced with cold stress conditions

Groups	GPx (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (n mol/mg protein)
NT control	35.2[a]	985[a]	65.2[a]	0.30[c]
CS control	14.0[c]	700[e]	29.2[b]	0.50[a]
BEE-1	18.1[b]	738[c]	30.1[b]	0.35[b]
BEE-2	18.2[b]	821[b]	31.2[b]	0.36[b]
BEE-3	19.1[b]	865[a]	32.2[b]	0.34[b]
SEM	1.10	15.10	2.5	0.02
P-value	<0.01	<0.01	<0.01	<0.01

GPx: glutathione peroxidase; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effects of broccoli ethanolic extract (BEE) supplementation on serum cytokines levels of broilers in thermoneutral and PHS-induced with cold stress conditions

Groups	IL-10 (ug/mL)	IL-1β (ug/mL)	TNF-α (ug/mL)
NT control	57.25[a]	2.10[c]	5.20[c]
CS control	12.20[c]	15.10[a]	15.20[a]
BEE-1	29.10[b]	5.50[b]	10.50[b]
BEE-2	32.20[b]	5.10[b]	10.30[b]
BEE-3	31.20[b]	5.90[b]	9.20[b]
SEM	3.50	2.10	2.90
P-value	<0.01	<0.01	<0.01

IL-10: interleukin-10; IL-1β: interleukin-1β; TNF-α: tumor necrosis factor-alpha.; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effects of broccoli ethanolic extract (BEE) supplementation on liver cytokines levels of broilers in thermoneutral and PHS-induced with cold stress water conditions

Groups	IL-10 (ug/mL)	IL-1β (ug/mL)	TNF-α (ug/mL)
NT control	25.21[a]	0.41[c]	8.51[c]
CS control	6.20[c]	1.30[a]	32.20[a]
BEE-1	13.10[b]	0.75[b]	19.20[b]
BEE-2	14.20[b]	0.71[b]	18.50[b]
BEE-3	15.20[b]	0.72[b]	17.30[b]
SEM	2.5	0.07	4.20
P-value	<0.01	<0.01	<0.01

IL-10: interleukin-10; IL-1β: interleukin-1β; TNF-α: tumor necrosis factor-alpha.; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 9 Effects of broccoli ethanolic extract (BEE) supplementation on serum biochemical parameters of broilers in thermoneutral and PHS-induced with cold stress conditions

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	TG (mg/dL)	Cholesterol (mg/dL)
NT control	5.36[b]	138.8[c]	359.4[c]	31.25[c]	79.2[c]
CS control	7.80[a]	195.98[a]	708.6 [a]	62.78[a]	123.5[a]
BEE-1	7.01[a]	179.23[b]	603.7[b]	35.54[b]	96.21[b]
BEE-2	7.43[a]	183.35[b]	642.1[b]	33.90[b]	89.52[b]
BEE-3	7.45[a]	180.20[b]	640.2[b]	32.50[b]	85.20[b]
SEM	1.10	8.5	45.5	5.50	8.50
P-value	0.19	0.23	0.21	< 0.01	< 0.01

ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; TG: triglyceride; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 10 Effects of broccoli ethanolic extract (BEE) supplementation on hematological parameters of broilers in thermoneutral and PHS-induced with cold stress conditions

Groups	WBCc ($\times 10^3/\mu L$)	RBCc ($\times 10^6/\mu L$)	Hb (g/dL)	Hct (%)	Lymph ($\times 10^3/\mu L$)	Hetro ($\times 10^3/\mu L$)	H:L
NT control	26.8[a]	2.51[c]	8.50[c]	37.50[b]	116.5	45.9[c]	0.39[c]
CS control	19.7[b]	2.98[a]	12.89[a]	41.70[a]	126.5	95.2[a]	0.75[a]
BEE-1	27.2[a]	2.63[b]	10.40[b]	37.47[b]	113.1	55.4[b]	0.49[d]
BEE-2	26.3[a]	2.70[b]	10.42[b]	38.63[b]	109.5	49.2[b]	0.45[b]
BEE-3	29.3[a]	2.76[b]	10.60[b]	36.20[b]	114.5	52.6[b]	0.46[b]
SEM	1.20	0.15	0.62	2.75	14.25	6.25	0.03
P-value	< 0.01	< 0.01	< 0.01	< 0.01	0.19	< 0.01	< 0.01

WBCc: white blood cell count; RBCc: red blood cell count; Hb: hemoglobin; Hct: hematocrit; Lymph: lymphocytes; Hetro: heterophil; H:L: heterophi:lymphocytes; NT: thermoneutral; CS: PHS-induced with cold stress and BEE-1, BEE-2, and BEE-3 indicate the supplementation of broccoli ethanolic extract (BEE) at the rate of 1000, 15000, and 3000 mg/kg respectively.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Induction of cold stress in the present study led to a marked increase in pulmonary hypertension syndrome (PHS) indicators, including RV/TV ratio (0.32), RV/BW (0.090), relative heart weight (0.75%), and ascites-related mortality (30%). These findings are consistent with earlier reports indicating that hypoxia-induced oxidative stress under cold conditions contributes significantly to PHS development (Wideman *et al*. 2010; Hemnes *et al*. 2014; Shao *et al*. 2022). The elevated RV/TV ratio is a key marker of right ventricular hypertrophy and correlates strongly with PHS severity. Cold stress triggers hypoxemia, which promotes reactive oxygen species (ROS) generation in pulmonary tissues, leading to vascular remodeling and right heart overload (Kelley *et al*. 2014; Tanna *et al*. 2022). Consequently, antioxidant intervention has been proposed as a strategy to mitigate PHS progression.

In our study, dietary supplementation with broccoli ethanolic extract (BEE) at all tested levels (1000, 1500, and 3000 mg/kg) significantly reduced mortality and improved heart indices compared to the CS control group. The BEE-2 group (1500 mg/kg) showed the most consistent reduction in RV/TV and RV/BW ratios, suggesting a dose-responsive trend, though the difference among BEE levels was not always statistically significant. This aligns with the concept that optimal antioxidant balance—rather than excessive dosing—is critical for cardiovascular protection. Broccoli is rich in bioactive compounds such as sulforaphane and Carvone, known for their potent antioxidant and anti-inflammatory effects (Fahey *et al*. 2015; Tang *et al*. 2021).

Sulforaphane, for example, activates the Nrf2 pathway, enhancing endogenous antioxidant defense systems, which can attenuate oxidative stress-induced vascular damage (Clarke *et al*. 2008; Hu *et al*. 2020a). Carvone has also been shown to inhibit lipid peroxidation and protect against cardiac hypertrophy in oxidative models (Gaur *et al*. 2019). Thus, the reduction in RV hypertrophy and mortality in BEE-treated birds is likely mediated through suppression of oxidative injury, modulation of inflammatory signaling, and vascular protection by phytochemicals present in broccoli extract.

Despite growing interest in phytogenic feed additives, few studies have addressed their role in modulating the cardiovascular-pulmonary axis, particularly in the context of pulmonary hypertension syndrome (PHS). While broccoli has been widely studied for its antioxidant and anti-inflammatory properties, to our knowledge, this is the first report exploring the effects of broccoli ethanolic extract (BEE) on ascites-related parameters in broilers.

In the current study, BEE supplementation—especially at 1500 and 3000 mg/kg—effectively decreased malondialdehyde (MDA) concentrations and improved antioxidant enzyme activities (SOD and GPx) in both serum and liver.

These changes suggest that BEE helps restore redox balance disrupted by PHS-induced oxidative stress. The dose-dependent response was evident, with BEE-2 (1500 mg/kg) consistently showing optimal antioxidant protection, though BEE-3 (3000 mg/kg) also offered similar benefits, indicating a possible saturation threshold.

This protective effect is likely mediated by sulforaphane, a potent isothiocyanate compound in broccoli that activates Nrf2, a key transcription factor regulating endogenous antioxidant enzymes (Myzak *et al*. 2006; Hu *et al*. 2020b). Carvone, another bioactive component found in broccoli, may further contribute to the reduction in lipid peroxidation by scavenging free radicals and enhancing mitochondrial integrity (Gaur *et al*. 2019). Oxidative stress plays a central role in the pathogenesis of PHS by promoting endothelial dysfunction, lipid peroxidation, and myocardial damage (Yang *et al*. 2011; Tanna *et al*. 2022). BEE's ability to counteract these effects suggests its application as a functional feed additive, particularly in environments where broilers are exposed to cold-induced stress.

The findings also echo the results of Ahmadipour *et al*. (2015), who demonstrated that *Kelussia odoratissima* reduced PHS risk by lowering heart hypertrophy and mortality under hypobaric cold stress. However, BEE may offer broader benefits due to the diverse phytochemical composition of broccoli, including flavonoids, carotenoids, glucosinolates, and essential oils with pleiotropic effects on oxidative pathways. Interestingly, Adesina and Toyo (2014) and Dos Reis *et al*. (2015) previously showed that dietary broccoli can improve growth performance, which may be attributed not only to enhanced antioxidant status but also to better nutrient utilization and immune modulation. This aligns with our observation of reduced oxidative damage and improved systemic antioxidant capacity following BEE supplementation in cold-stressed broilers.

Previous studies have shown that broccoli extract and essential oils improve antioxidant defenses by increasing enzymes like SOD, GPx, and CAT, while reducing lipid peroxidation (Hu *et al*. 2012; Mueller *et al*. 2012a; Mueller *et al*. 2012b). Our results confirm these effects in cold-stressed broilers, likely due to broccoli's bioactive compounds such as flavonoids and glucosinolates, which scavenge reactive oxygen species and activate antioxidant pathways like Nrf2 (Cho *et al*. 2006; Traka and Mithen, 2009; Hu *et al*. 2020b). Different doses of BEE showed dose-dependent improvements in antioxidant enzymes, with moderate doses (1000 mg/kg) already effective and higher doses providing stronger responses, consistent with phytochemical dose-response patterns (Wu *et al*. 2019). These findings suggest broccoli extract is a promising natural antioxidant to mitigate oxidative stress in poultry under cold stress.

Dietary supplementation with BEE significantly reduced pro-inflammatory cytokines (TNF-α and IL-1β) and increased the anti-inflammatory cytokine IL-10 in both serum and liver under cold-induced PHS (Tables 7 and 8). These results suggest that BEE modulates the inflammatory response, likely by attenuating oxidative stress, which is a known trigger of cytokine overexpression and cellular damage (Sun and Karin, 2013; Jiang *et al.* 2018). Previous studies in broilers and rodents have shown that oxidative stress enhances inflammatory cytokine production via ROS overproduction (Tan *et al.* 2005; Ruixia *et al.* 2020a; Ruixia *et al.* 2020b). The upregulation of IL-10 with BEE is particularly notable, as this cytokine plays a central role in dampening inflammation and protecting tissues from immune-mediated injury.

Our findings align with Yuan *et al.* (2021), who demonstrated anti-inflammatory properties of broccoli through *in vitro* models. Additionally, the observed reduction in serum liver enzymes (AST, ALT, ALP) may reflect hepatoprotection conferred by BEE, as oxidative stress is known to cause hepatic injury and enzyme leakage into the bloodstream (Arab *et al.* 2006; Fathi *et al.* 2016; Fathi *et al.* 2022). The antioxidant compounds in broccoli, including sulforaphane and polyphenols, likely contribute to both anti-inflammatory and hepatoprotective effects via modulation of the NF-κB and Nrf2 signaling pathways (Traka and Mithen, 2009; Hu *et al.* 2020a). These combined effects suggest that BEE can reduce both oxidative and inflammatory components of cold stress-induced PHS in broilers.

The lipid-lowering effects of BEE may be attributed to its antioxidant compounds, particularly phenolics and flavonoids, which can inhibit HMG-CoA reductase and reduce cholesterol synthesis. Additionally, these compounds improve lipid profiles by reducing triglycerides and LDL-C while increasing HDL-C levels (Weinbrenner *et al.* 2004; Ustundag and Ozdogan, 2015). In this study, BEE supplementation significantly reduced serum cholesterol and TG in stressed birds, with the 1000–1500 mg/kg doses showing the most effective and balanced response. These findings suggest BEE could mitigate PHS-related cardiovascular risks via antioxidant-mediated lipid regulation.

CONCLUSION

In conclusion, the results of our study indicated supplementation of broccoli ethanolic extract alleviates oxidative stress and inflammation response in broilers exposed to CS. These promising effects may exert through decreased MDA content, increasing SOD and GPx activities, and IL-10 level in vital organs. Therefore, broccoli ethanolic extract supplementation may be a potential agent to relieve oxidative stress and inflammation response in broiler chickens.

ACKNOWLEDGEMENT

The authors would like to express their sincere appreciation to Engineer Vahid Rezaei and the esteemed colleagues of Behshad Afarin Gorgan Chain Company for their valuable cooperation and support in the implementation of this project.

REFERENCES

Adesina O.O. and Toye A.A. (2014). Nutritive effect of cabbage (Brassica oleracea) on growth, obesity, lipidaemia and haematology in broiler and pullet chickens. *Int. J. Agri. Sci.* **13,** 11-25.

Ahmadipour B., Hassanpour H., Asadi E., Khajali F., Rafiei F. and Khajali F. (2015). *Kelussia odoratissima* Mozzaf – A promising medicinal herb to prevent pulmonary hypertension in broiler chickens reared at high altitude. *J. Ethnopharmacol.* **159,** 49-54.

Arab H.A., Jamshidi R., Rassouli A., Shams G. and Hassanzadeh M.H. (2006). Generation of hydroxyl radicals during ascites experimentally. *Br. Poult. Sci.* **47(2),** 216-222.

Aviagen. (2014). Ross Broiler Management Manual. Aviagen Incorporated, Huntsville, Alabama, USA.

Babaahmadi Milani M., Zamani M.A., Zahra K. and Mohebbi A. (2020). Use of broccoli (*Brassica oleracea* L. var. Italica) in comparison to ascorbic acid to decrease pulmonary hypertension syndrome in broiler chickens. *Iranian J. Vet. Med.* **14(4),** 372-385.

Baghbanzadeh A. and Decuypere E. (2008). Ascites syndrome in broilers: physiological and nutritional perspectives. *Avian Pathol.* **37(2),** 117-126.

Bhandari S.R. and Kwak J.H. (2014). Seasonal variation in phytochemicals and antioxidant activities in different tissues of various broccoli cultivars. *African J. Biotechnol.* **13,** 604-615.

Bottje W.B. and Wideman R.F. (1995). Potential role of free radicals in the pathogenesis of pulmonary hypertension syndrome. *Avian Poult. Biol. Rev.* **6,** 221-231.

Bottje W.B., Enkvetchakul B., Moore R. and McNew R. (1995). Effect of alpha-tocopherol on antioxidants, lipid peroxidation, and the incidence of pulmonary hypertension syndrome (ascites) in broilers. *Poult. Sci.* **74,** 1356-1369.

Cho E.J., Lee Y.A., Yoo H.H. and Yokozawa T. (2006). Protective effects of broccoli (*Brassica oleracea*) against oxidative damage *in vitro* and *in vivo*. *J. Nutr. Sci. Vitaminol.* **52,** 437-444.

Clarke J.D., Dashwood R.H. and Ho E. (2008). Multi-targeted prevention of cancer by sulforaphane. *Cancer Lett.* **269(2),** 291-304.

Dasgupta T.A.R., Rao Y. and Yadava P.K. (2004). Chemomodulatory efficacy of basil leaf (*Ocimum basilicum*) on drug-metabolizing and antioxidant enzymes, and on carcinogen-induced skin and forestomach papillomagenesis. *Phytomedicine*. **11,** 139-151.

Dos Reis L.C.R., De Oliveira V.R., Hagen M.E.K., Jablonski A., Flôres S.H. and Oliveira Rios A. (2015). Carotenoids, flavonoids, chlorophylls, phenolic compounds and antioxidant activity in fresh and cooked broccoli (*Brassica oleracea* var. Avenger) and cauliflower (*Brassica oleracea* var. Alphina F1). *Food Sci. Technol.* **63,** 177-183.

Druyan S., Hadad Y. and Cahaner A. (2008). Growth rate of ascites-resistant versus ascites-susceptible broilers in commercial and experimental lines. *Poult. Sci.* **87,** 904-911.

Fahey J.W., Wade K.L., Stephenson K.K. and Chou F.E. (2015). Separation and purification of glucoraphanin from broccoli seed meal. *J. Med. Food.* **18(2),** 198-204.

Fathi M., Haydari M. and Taimour 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. and Saeedyan S. (2022). Influence of dietary lycopene on growth performance, antioxidant status, blood parameters and mortality in broiler chickens with cold-induced ascites. *Arch. Anim. Nutr.* **8,** 1-11.

Gaur P.N., Gupta R.S. and Singh A. (2019). Carvone suppresses oxidative stress and inflammation in isoproterenol-induced cardiac hypertrophy in rats. *Biomed. Pharmacoth.* **109,** 1698-1707.

Geng A.L., Guo Y.M. and Yang Y. (2004). Reduction of ascites mortality in broilers by CoQ10. *Poult. Sci.* **83,** 1587-1593.

Halliwell B. and Gutteridge J.M.C. (1990). Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol.* **186,** 1-85.

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.

Hemnes A.R., Brittain E.L., Trammell A.W., Fessel J.P., Austin E.D. and Penner N. (2014). Evidence for right ventricular lipotoxicity in heritable pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* **189,** 325-334.

Hu C.H., Wang D.G., Pan H.Y., Zheng W.B., Zuo A.Y. and Liu J.X. (2012). Effects of broccoli stem and leaf meal on broiler performance, skin pigmentation, antioxidant function, and meat quality. *Poult. Sci.* **91,** 2229-2234.

Hu C., Eggler A.L., Mesecar A.D. and van Breemen R.B. (2020a). Oxidative stress, inflammation, and Nrf2 signaling in cardiovascular diseases. *Free Radical Biol. Med.* **150,** 197-212.

Hu C., Zawawa M. and Jin H. (2020b). Broccoli-derived sulforaphane activates Nrf2 pathway and suppresses inflammation. *J. Func. Foods.* **68,** 103897-103906.

Jiang F., Gao Y., Dong C. and Xiong S. (2018). ODC1 inhibits the inflammatory response and ROS-induced apoptosis in macrophages. *Biochem. Biophys. Res. Commun.* **504(4),** 734-741.

Kelley N.S., Yoshida Y. and Erickson K.L. (2014). Do n-3 polyunsaturated fatty acids increase or decrease lipid peroxidation in humans? *Metab. Syndr. Relat. Disord.* **12,** 403-415.

Lorenzoni A.G. and Ruiz-Feria C.A. (2006). Effects of vitamin E and L-arginine on cardiopulmonary function and ascites parameters in broiler chickens reared under sub-normal temperatures. *Poult. Sci.* **85,** 2241-2251.

Mueller K., Blum N.M., Kluge H. and Mueller A.S. (2012a). Influence of broccoli extract and various essential oils on performance and expression of xenobiotic- and antioxidant enzymes in broiler chickens. *Br. J. Nutr.* **108,** 588-602.

Mueller K., Blum N.M., Kluge H., Bauerfeind R., Froehlich J., Mader A., Wendler K.R. and Mueller A.S. (2012b). Effects of broccoli extract and various essential oils on intestinal and fecal microflora and on xenobiotic enzymes and the antioxidant system of piglets. *Open J. Anim. Sci.* **2(2),** 78-98.

Myzak M.C., Tong P., Dashwood W.M., Dashwood R.H. and Ho E. (2006). Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp. Biol. Med.* **232(2),** 227-234.

Rahmani M., Golian A., Kermanshahi H. and 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.

Rasha A., Al Wakeel M.S., Abdel Azeez A.A., Mahmoud S.M. and Saad M.F. (2017). Alleviation by gamma aminobutyric acid supplementation of chronic heat stress-induced degenerative changes in jejunum in commercial broiler chickens. *Stress.* **20(6),** 562-572.

Riaz A., Khan M.S., Saeed M., Kamboh A.A., Khan R.U., Farooq Z., Imran S. and Farid M.U. (2022). Importance of *Azolla* plant in poultry production. *World's Poult. Sci. J.* **78(3),** 789-802.

Ruixia L., Linlin W., Qingqing C., Shengnan W. and Zhao Z. (2020a). Effects of dietary chitosan oligosaccharides on oxidative stress and inflammation response in liver and spleen of yellow-feather broilers exposed to high ambient temperature. *Italian J. Anim. Sci.* **19(1),** 1508-1517.

Ruixia L., Qingqing C., Lilong A. and Zhao Z. (2020b). Dietary supplementation with chitosan oligosaccharides alleviates oxidative stress in rats challenged with hydrogen peroxide. *Animals*. **10,** 55-65.

Saeed M., Naveed M., Leskovec J., Kamboh A.A., Kakar I., Ullah K., Ahmad F., Sharif M., Javaid A., Rauf M., Abd El-Hack M.E., Abdel-Latif M.A. and Chao S. (2020). Using Guduchi (*Tinospora cordifolia*) as an eco-friendly feed supplement in human and poultry nutrition. *Poult. Sci.* **99(2),** 801-811.

Saeed M., Arain M.A., Arif M., Lagawany M., Abd El-Hack M.E., Kakar M.U., Manzoor R., Erdenee S. and Chao S. (2017). Jatropha (*Jatropha curcas*) meal is an alternative protein source in poultry nutrition. *World's Poult. Sci. J.* **73,** 1-8.

SAS Institute. (2005). SAS®/STAT Software, Release 7.0. SAS Institute, Inc., Cary, NC. USA.

Shao F.J., Ying Y.T., Tan X., Zhang Q.Y. and Liao W.T. (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. and 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.

Sun B. and Karin M. (2013). Inflammation and liver tumorigenesis. *Front. Med.* 7**(2),** 242-254.

Tan X., Pan J.Q. and Li J.C. (2005). L-arginine inhibiting pulmonary vascular remodeling is associated with promotion of apoptosis in pulmonary arterioles smooth muscle cells in broilers. *Res. Vet. Sci.* **79(3),** 203-209.

Tang L., Zhang Y. and Jobson. H.E. (2021). Bioavailability and bioactivity of sulforaphane: Implications for human health. *Ann. Rev. Food Sci. Technol.* **12,** 497-522.

Tanna A., Sinha D. and Mishra S. (2022). Mechanisms of pulmonary hypertension: emerging role of oxidative stress. *Front. Physiol.* **13,** 867300.

Traka M.H. and Mithen R.F. (2009). Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.* **8(1),** 269-282.

Ustundag A.O. and Ozdogan M. (2015). Usage possibilities of mulberry leaves in poultry nutrition. *Sci. Pap. J. Anim. Sci.* **58,** 170-178.

Wideman R.F., Eanes M.L., Hamal K.R. and Anthony N.B. (2010). Pulmonary vascular pressure profiles in broilers selected for 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 oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J. Nutr.* **134,** 2314-2321.

Wu Q., Liu C. and Feng J. (2019). Dose-dependent effects of phytochemicals on antioxidant capacity in poultry: A review. *Poult. Sci.* **98(7),** 2891-2900.

Xiang R.P., Sun W.D. and Zhang K.C. (2004). Sodium chloride-induced acute and chronic pulmonary hypertension syndrome in broiler chickens. *Poult. Sci.* **83(5),** 732-736.

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

Yuan Y.K., Yuan-Tay K., Shyu S.J. and Wu S.J. (2021). Evaluating the anti-inflammatory and antioxidant effects of broccoli treated with high hydrostatic pressure in cell models. *Foods.* **10,** 167-177.