10.22059/ijvm.2020.293989.1005046

Iranian Journal of Veterinary Medicine
Volume 14- Issue 04

Original Article
Online ISSN: 2252-554

Use of Broccoli (*Brassica oleracea* L. var. Italica) in Comparison to Ascorbic Acid to Decrease Pulmonary Hypertension Syndrome in Broiler Chickens

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Abstract

BACKGROUND: Ascites syndrome is one of the most important metabolic disorders in growing broiler chickens worldwide.

OBJECTIVES: This study investigated the effect of broccoli in comparison to ascorbic acid on controlling pulmonary hypertension syndrome (PHS) in broiler chickens.

METHODS: A total of 144 one-day old chicks were randomly divided into four groups. Each group included 36 chicks with three replicates of 12 birds. Experimental treatments were control, ascorbic acid (500 mg/kg dietary), and two levels of broccoli (0.5% and 1% dietary broccoli powder). Growth performance, hematocrit or packed cell volume (PCV), heterophil to lymphocyte (H:L) ratio, right ventricle to total ventricle weight ratio (RV:TV), serum biochemical factors, lipid peroxidation, total antioxidant capacity, as well as humoral and cellular immune responses were evaluated.

RESULTS: Growth performance parameters improved in experimental treatments compared to control, even though it was not statistically significant. The RV:TV ratio in treatment groups was lower than control, and it was significant in ascorbic acid and broccoli 1% ($P \leq 0.05$). H:L ratio decreased in treatment groups than control ($P \leq 0.05$). Nitric oxide and HDL-C levels increased at both broccoli levels than control ($P \leq 0.05$), but LDL-C and triglyceride levels decreased ($P \leq 0.05$). Serum malondialdehyde (MDA) and total antioxidant capacity were lower and higher in treatment groups than control, respectively ($P \leq 0.05$). There were no significant changes in other parameters.

CONCLUSIONS: This research showed that addition of broccoli to the diet of broiler chickens, through improving antioxidant parameters, increasing serum nitric oxide levels, and decreasing blood pressure, blood lipids, RV:TV ratio, and mortality, could be effective to prevent PHS.

KEYWORDS: Ascites, Ascorbic acid, Broccoli, Broiler chicken, Syndrome

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Received: 2020-03-18
Accepted: 2020-06-10

How to Cite This Article

Babaahmadi Milani, M ., Zamani Moghadam, A., Khosravi, Z., Mohebbi, A., A. (2020). Use of Broccoli (*Brassica oleracea* L. var. Italica) in Comparison to Ascorbic Acid to Decrease Pulmonary Hypertension Syndrome in Broiler Chickens, ***Iranian Journal of Veterinary Medicine***, 14(4), 372-385

Introduction

The growth rate of broiler chickens has increased surprisingly over the past years as a result of intensive genetic selection, so that a newly-hatched chick can reach to 50 times of initial weight in five to six weeks. This broiler growth rate is almost distinctive compared with all animal breeding (Janwari, 2018). In contrast to body weight, the relative weight of the heart and lung in modern broiler chickens is increased; in addition, a significant reduction in relative weight of the heart and lungs is created due to genetic selection, resulting in a high pressure on the cardiovascular system, followed by incidence of pulmonary hypertension syndrome (PHS) or ascites syndrome (Julian, 1989; Hassanzadeh *et al.*, 2005). PHS is one of the serious problems of the poultry industry worldwide. The incidence of this syndrome is high in chicks raised in high altitude (above 1500 m of sea level) and cold, indicating exacerbation of the syndrome in hypoxia conditions (Ozkan *et al.*, 2010; Hassanzadeh *et al.*, 2013; Bahadoran *et al.*, 2010).

Different physiological mechanisms in response to hypoxia are formed in the body of the bird, most notably stimulating alpha adrenergic receptors in the smooth muscle of the pulmonary artery wall, which occurs as a pulmonary vasoconstriction, resulting in pulmonary hypertension (Ladmakhi *et al.*, 1997). Many attempts have been made to determine the chemical factors of vasoconstrictor and vasodilator that cause pathological changes in the pulmonary arteries (Khajali and Wideman, 2016). Any substance increasing the resistance of the pulmonary arteries can cause or exacerbate pulmonary hypertension. Factors such as oxygen deficiency (Khajali and Wideman, 2016), adrenergic neurotransmitter, (Hassanzadeh *et al.*, 2002; Wideman *et al.*, 2013) eicosanoids, endotoxins, lesions and diseases of the lung, and methyl glyoxal cause vascular resistance to blood flow in the lungs of the broiler chickens (Behrooj *et al.*, 2012). Moreover, it seems that such factors as cholinergic neurotransmitters (Odom *et al.*, 2004), I2 and E2 prostaglandin (Stebel and Wideman, 2008), and nitric oxide (Ahmadipour *et al.*, 2015) reduce the pulmonary vascular resistance against the blood flow in the lungs, which has a positive effect in preventing the occurrence of pulmonary hypertension. Ascites is the result of malfunctioning heart and circulatory system and is caused by an imbalance between oxygen supply and the oxygen required to sustain rapid growth rates and high food efficiencies in broiler chickens (Decuypere *et al.*, 2000).

The latest research on the cause of ascites syndrome incidence in chickens has focused on three aspects: high pulmonary blood pressure, various cardiac injuries, and various types of cell damage caused by oxygen disorders and the production of oxygen free radicals (Arab *et al.* 2006; Fang *et al.*, 2002). Oxidative stress is one of the important factors in the development of PHS. The oxidation reactions release free radicals, which cause damage to the vascular cells. Inadequate levels of antioxidants or reduced antioxidant enzymes may cause oxidative stress, cell damage, or even death (Fang *et al.*, 2002). Thus, adding antioxidant compounds to diet, especially in stressed conditions, can help body health. Plants and fruits with strong antioxidant properties can be considered as a rich source of antioxidants and anticancer .agents. Broccoli (*Brassica oleracea* L. var *Italica*) (green cauliflower) is a variety of vegetable in the family of Brassicaceae and the Brassicales order. It has different compounds ,such as vitamin C, carotenoids, glucosinolates selenium, phenolic compounds, and flavonoids (Dos Reis *et al.*, 2015). One of the non-nutrient factors that prevent cancer and cardiovascular disease is flavonoids, which are abundant in broccoli (Ouyang *et al.*, 2016). Flavonoids and phenolic compounds act as strong antioxidants by clearing the free radicals under oxidative stress conditions. These compounds also reduce triglycerides and cholesterol, and as a result, they reduce chronic heart disease and atherosclerosis (Ustundag and Ozdogan, 2015). As broccoli contains a variety of polyphenols, it is a great source of ingredients for the body health. The anticancer effects of broccoli are probably related to direct and indirect antioxidant effects of regulating enzymes, as well as controlling apoptosis and cell cycle (Bhandari and Kwak, 2014). It has been reported that broccoli can reduce lipid peroxidation and increase the activity of antioxidant enzymes in broiler chickens (Cho *et al.*, 2006). The effect of vitamin C, as a strong antioxidant in reducing the incidence of ascites syndrome, has been demonstrated (Nemati *et al.*, 2017). As far as the researchers investigated, there is no study to evaluate the effects of broccoli on reducing the incidence of PHS in poultry industry. Hence, this study aimed to evaluate the antioxidant effects of broccoli in controlling PHS in broiler chickens.

Materials and Methods

This study was carried out with a randomized design in a poultry house of Shahrekord, Iran (altitude: 2,100 meters above sea level). A total of 144 one-day-old broiler chicks (Ross 308 strain) were divided into 4 groups (n=36 in each group) with 3 replicates of 12 birds. The breeding room temperature was set at 32°C until 3 days of age, then decreased to 29°C until the end of the first week. From the beginning of the second week (day 8), the temperature decreased by 2°C daily, until the end of the second week (day 14); thus, the temperature decreased to 15°C and remained constant until the end of the breeding period (day 35). The birds were exposed to 23 hours of light and 1 hour of darkness. Water and feeding were freely available to birds during breeding. Fresh broccoli was prepared and completely dried (under the shade) and powdered. The ration formula was based on maize-soybean, and was divided into three starting, growing, and finishing stages according to Nutrient Requirements of *Poultry* National research council (NRC) recommendations (1994). (i) Control group received the base diet. (ii) 500 mg/kg dietary Vitamin C, (iii) dietary 0.5 % broccoli powder, (iiii) dietary 1% broccoli powder.

Weight gain, feed intake, and feed conversion ratio (FCR) were measured in three stages of breeding (7-21, 22-35, and 7-35 days of age). At 35 days of age, 3 birds from each replicate (9 birds from each treatment) with a body weight close to the average weight of treatment were selected for determination of organs weight and were slaughtered after blood collection. The weights of the heart, liver, spleen, and bursa of Fabricius were measured using a digital scale with accuracy of 0.01 g. To determine the ratio of right ventricle to total ventricular mass (RV:TV), the hearts were picked up and ventricles were dissected and weighed. This ratio is used as an indicator of pulmonary hypertension (Khajali, 2014). Hematocrit or packed cell volume (PCV) was determined by using micro centrifuge (BMC 24) and PCV ruler. Moreover, heterophil to lymphocyte (H:L) ratio was determined by counting them according to the method proposed by Lucas and Jamroz (1961). Serum samples were collected for determination of cholesterol, triglyceride, HDL-C, LDL-C, uric acid, and total protein, and were evaluated by Auto Analyzer (mindray bs380). Nitric oxide (nitrate+nitrite) was determined according to Behrooj *et al.* (2012). Serum malondialdehyde (MDA) levels were determined as an indicator of lipid peroxidation by Buege and Aust (1978). The serum total antioxidant capacity was measured by Naxifer^{TM} commercial kit (Navand Salamat Co., Urmia, Iran), according to the manufacturer's instructions. To evaluate the humoral immune response, 0.5 ml sheep red blood cells (SRBC 10%) suspension was intramuscularly injected to 9 birds of each group at day 28, then hemagglutination test (HA) was done 7 days after injection (35 days of age) (Munns and Lamont, 1991). Cellular immunity was evaluated by skin hypersensitivity reaction 24 hours after subcutaneous injection of phytohemagglutinin P (Sigma-Aldrich Co., St. Louis, MO, USA) (0.1 ml) to foot toe web at 28 days of age according to Yousefi *et al.* (2013). All mortalities were collected and the carcasses were autopsied to determine the cause of death, and examination of carcasses was done in terms of fluid accumulation in the abdominal and pericardia cavity. The RV:TV ratio was measured to confirm the incidence of ascites.

Data were analyzed in a completely randomized design by SAS software (2007). Means were separated by Duncan's multiple range test at the significance level of P-value≥0.05.

Results

Weight gain and feed intake of all treatment groups were higher than control group during the breeding period (7-35 days), though not statistically significant (P≥0.05) (Table 1).

Ascorbic acid treatment had the highest weight gain and feed intake. The FCR of ascorbic acid and broccoli 1% was less than control, but these results were not statistically significant (P≥0.05). (Table 2).

Table 1. Composition of the experimental diets fed to broilers in the starting, growing and finishing stages

Ingredient %	1-10 days	11-24 days	25-35 days
Yellow maize	44.8	49.04	58.94
Soybean meal	43.5	38.81	33.04
Oyster shell	1.1	0.98	0.77
DL-methionine	0.35	0.31	0.17
L-lysine HCl	0.15	0.15	0.15
Mineral premixa	0.3	0.3	0.3
Vitamin premixb	0.3	0.3	0.3
Sodium chloride	0.4	0.39	0.34
Oil	7	8	4.46
Dicalcium phosphate	2.1	1.72	1.49
Energy (kcal/kg)	3000	3100	3200
Protein	23	21.5	19.5

[a] Provided the following per kilogram of diet: vitamin A (trans retinyl acetate), 3600 IU; vitamin D3 (cholecalciferol), 800 IU; vitamin K3, 1.6 mg; vitamin E (dl-α-tocopheryl acetate), 7.2 mg; riboflavin, 3.3 mg; niacin, 0.4 mg; thiamine, 0.72 mg; choline chloride, 200 mg; pyridoxin, 1.2 mg; cobalamine, 0.6 mg; folicacid,0.5 mg;

[b] Provided the following per kilogram of diet: Fe (from FeSO4 _7H2O), 20 mg; Zn (from ZnO), 40 mg; Mn (from MnSO4 _H2O), 40 mg; Cu (from CuSO4 _5H2O), 4 mg; Se (from sodium selenite), 0.08 mg; I [from Ca(IO3)2 _H2O], 0.64 mg;

Table 2. Effects of dietary broccoli and vitamin C on growth performance of broiler chickens (Mean ± SEM)

	Control	Vit C	bro 0.05 %	bro 1 %
7 -21 days				
Weight gain (g/bird)	432 ± 28.74	468.33 ± 37.90	433 ± 5.56	457.33 ± 30.27
Feed intake (g/bird)	639.66 ± 11.5	692 ± 26.10	640 ± 23.90	678.66 ± 29.47
Feed:gain (FCR)	1.49 ± 0.08	1.48 ± 0.06	1.47 ± 0.03	1.49 ± 0.04
22-35 days				
Weight gain (g/bird)	766.33 ± 46.39	915.33 ± 35.59	777.33 ± 51.40	787.66 ± 22.54
Feed intake (g/bird)	1344.33 ± 65.82	1528.66 ± 58.67	1390.66 ± 80.08	1348.66 ± 61.48
Feed:gain (FCR)	1.77 ± 0.183	1.67 ± 0.01	1.79 ± 0.09	1.71 ± 0.05
7-35 days				
Weight gain (g/bird)	1198.33 ± 67.89	1383.66 ± 70.86	1258 ± 16.00	1244 ± 43.14
Feed intake (g/bird)	1984.66 ± 61.07	2220.66 ± 84.47	2031.33 ± 57.20	2027.33 ± 62.86
Feed:gain (FCR)	1.66 ± 0.11	1.60 ± 0.02	1.66 ± 0.02	1.63 ± 0.05

Liver weight was lower in treatment groups than control, though it was not statistically significant. While the weights of the spleen and bursa of Fabricius in the treatment groups were higher than the control and the control group had the lowest mean in both indices, there was no significant difference between the weight of the spleen and bursa of Fabricius (Table 3). The RV:TV ratio, as an indicator of PHS, was significantly higher in the control group (Table 3). The lowest mean ratio of RV:TV was observed in the ascorbic acid group (0.241), followed by broccoli 1% group (0.244), which was significantly different with control (0.331) ($P \leq 0.05$). Although the broccoli 0.5% group had a lower RV:TV ratio (0.272) than control, no significant difference was observed in this regard ($P \geq 0.05$). The PCV level of the groups did not show any significant difference, but the control group showed a higher hematocrit level than treatment groups. The H:L ratio in control was higher than other groups, and this difference was significant ($P \leq 0.05$) (Table 4). Serum nitric oxide increased in both broccoli levels compared to control, and this difference was statistically significant ($P \leq 0.05$) (Table 4). Serum uric acid and total serum cholesterol levels were lower and total protein was higher than control, but there was no significant difference ($P \geq 0.05$). Serum triglyceride in both broccoli levels were significantly lower than control ($P \leq 0.05$), this parameter was lower in the ascorbic acid group than control, but it was not significantly different ($P \geq 0.05$). (Table 4). Serum HDL-C levels of treatments were statistically higher than control ($P \leq 0.05$) and serum LDL-C levels were statistically lower in broccoli 1% and broccoli 0.5% than control ($P \leq 0.05$) (Table 4).

Table 3. Effect of dietary broccoli and vitamin C on different organ weight and RV:TV ratio in broiler chickens measured at 35 days of age (Mean ± SEM)

	Control	Vit C	bro 0.5 %	bro 1 %
Liver (%)	2.73 ± 0.14	2.40 ± 0.10	2.59 ± 0.08	2.50 ± 0.08
Spleen (%)	0.07 ± 0.004	0.088 ± 0.007	0.102 ± 0.005	0.088 ± 0.007
Bursa of fabricius (%)	0.17 ± 0.026	0.196 ± 0.017	0.183 ± 0.023	0.229 ± 0.02
RV/TV (%)	0.313 ± 0.012a	0.241 ± 0.001b	0.272 ± 0.025	0.244 ± 0.006b

[a-b] Values within the same row with no common superscripts differ significantly (P<0.05).

Table 4. Effects of dietary broccoli and vitamin C on serum and blood parameters in broiler chickens measured at 35 days of age (Mean ± SEM)

	Control	Vit C	bro 0.5 %	bro 1 %
Hematocrit (%)	48.44 ± 1.04	48.07 ± 0.73	48.14 ± 1.79	47.44 ± 0.74
Heterophils to lymphocyte (%)	0.65 ± 0.07a	0.34 ± 0.02b	0.43 ± 0.02b	0.39 ± 0.02b
Serum nitric oxide (µmol/L)	47.44 ± 6.50b	49.23 ± 3.63b	97.80 ± 8.66a	91.69 ± 7.32a
Serum uric acid (mg/dl)	6.00 ± 0.48	5.75 ± 0.48	4.50 ± 0.86	5.00 ± 0.28
Total serum protein (g/dl)	4.06 ± 0.39	4.78 ± 0.49	4.46 ± 0.35	5.23 ± 0.34
Triglyceride (mg/dl)	94.00 ± 8.19a	86.42 ± 2.84ac	73.75 ± 3.04bc	65.16 ± 4.43b
Cholesterol (mg/dl)	124.25 ± 6.14	120.61 ± 6.07	114.00 ± 5.26	122.00 ± 7.50
HDL-C (µmol/L)	61.33 ± 2.75b	125.44 ± 3.09a	78.25 ± 3.35c	112.75 ±3.74a
LDL-C (µmol/L)	57.77 ± 1.69a	56.62 ± 1.40a	36.33 ± 1.02b	41.12 ± 1.4b

[a-c] Values within the same row with no common superscripts differ significantly (P<0.05).

The levels of serum MDA and serum total antioxidant capacity of the treatments were lower and higher than control group, respectively, indicating a statistically significant association (Table 5) ($P \leq 0.05$).

Table 5. Effects of dietary broccoli and vitamin C on serum antioxidant parameters measured at 35 days of age (Mean ± SEM)

	Control	Vit C	bro 0.5%	bro 1%
MDA (μmol/L)	58.389 ± 2.607a	34.601 ± 3.545b	36.752 ± 3.989b	34.843 ± 4.311b
TAC (μmol/L)	0.564 ± 0.121b	1.336 ± 0.115a	1.418 ± 0.189a	1.348 ± 0.077a

[a-b] Values within the same row with no common superscripts differ significantly ($P<0.05$).

The antibody response to SRBC (humoral immunity) and cellular immune responses to phytohaemagglutinin-P (PHA-P) injection for treatments are presented in Table 6. Although toe web thickness index in all treatment groups and antibody titers in ascorbic acid and broccoli 1% groups were numerically higher than control group, they were not statistically significant ($P≥0.05$). Birds fed with ascorbic acid and broccoli had a lower rate of PHS mortality, and these differences were statistically significant between ascorbic acid and control groups ($P≤0.05$) (Table 7).

Table 6. Effect of dietary broccoli and vitamin C on antibody titer against SRBC and toe web thickness (Mean ± SEM)

	Control	Vit C	bro 0.5 %	bro 1 %
HA titer	2.375 ± 0.324	3.111 ± 0.455	1.667 ± 0.408	3.111 ± 0.423
Toe web thickness	0.190 ± 0.245	0.247 ± 0.081	0.337 ± 0.103	0.260 ± 0.039

Table 7. Effect of dietary broccoli and vitamin C on cumulative mortality from pulmonary hypertension syndrome (PHS) in broiler chickens reared up to 35 days of age (Mean ± SEM)

	Control	Vit C	bro 0.5 %	bro 1 %
PHS Mortality (%)	27.27 ± 0.27a	6.06 ± 0.57b	12.12 ± 0.40	15.55 ± 0.36

[a-b] Values within the same row with no common superscripts differ significantly ($P<0.05$).

Discussion

In recent years, numerous studies have been conducted to find compounds like antioxidants to reduce the economic costs of PHS, especially in high-altitude areas. One of these sources of antioxidants is herbs. Antioxidant compounds are abundantly found in plants; these compounds include carotenoids, flavonoids, phenolic compounds, glutathione, vitamins, amino acids, and some minerals that protect body against harmful free radicals (Cho *et al.*, 2006). Broccoli has most of these compounds and it is one of the most valuable food sources for the body health preventing many diseases. Several experiments have been conducted to prove the presence of potent antioxidant compounds in broccoli (Dos Reis *et al.*, 2015).

In this research, addition of broccoli and ascorbic acid to diet relatively improved growth performance parameters, but it was not statistically significant ($P≥0.05$). The effects of ascorbic acid and broccoli on growth performance in different studies are different. Growth performance results are numerically consistent with some studies. Nemati *et al.* (2017) showed that using a diet containing 300 mg/kg of vitamin C in broiler chickens under cold stress can improve weight gain and FCR. Adesina and Toyo (2014) reported that using dietary broccoli 3% could increase weight gain, especially at 4 weeks of age, and improve the growth of broiler chickens. On the other hand, no effects of vitamin C and broccoli on growth performance match with studies of Hu *et al.* (2012)

and Nemati *et al.* (2017). Furthermore, Ladmakhi *et al.* (1997) reported that adding of 500 mg/kg vitamin C to the diet had no significant effect on growth performance of broiler chickens.

Liver weight was lower in the groups which received ascorbic acid and broccoli in comparison with the control. It has been proved that 20% of the body oxygen is used for the metabolic process of the liver. Liver is the main organ of lipogenesis, and its weight loss is indicative of a reduction in lipogenesis. Therefore, reduction of lipogenesis and insignificant reduction of liver size was observed in experimental treatments. Oxygen consumption decrease in high altitude regions like Shahrekord, Iran; so the result is the incidence of pulmonary hypertension syndrome in broiler chickens which are susceptible to this disorder (Suzuki *et al.*, 2014). Mueller *et al.* (2012) showed that using broccoli extract at the level of 0.075 g/kg increases the antioxidant capacity of the liver and intestine and reduces the iron induced liver peroxidation in liver.

It has been reported that different stresses cause corticosteroid secretion, resulting in impaired immune and lymphoid function and reduction size of immune organs (Fang *et al.*, 2002). The relative weights of the bursa of Fabricius and spleen, as immune organs, were measured; the results indicated an increase in treatment groups compared to the control group, though this increase was not statistically significant ($P \geq 0.05$).

The RV:TV ratio is an indicator for determining pulmonary arterial hypertension in broiler chickens (Saedi and Khajali, 2010). In this research, ascorbic acid and broccoli 1% significantly reduced the RV:TV ratio in broiler chicks raised in conditions predisposing pulmonary hypertension ($P \leq 0.05$). When the RV:TV ratio was greater than 0.25, it was considered as pulmonary hypertension. It has been proven that antioxidants such as vitamin C reduce congestion and blood flow in the cardiovascular-pulmonary system, subsequently decreasing PHS and ascites (Biswas, 1993; Ladmakhi *et al.*, 1997). Ladmakhi *et al.* (1997) also reported that vitamin C can reduce the activities of thyroid hormones in broiler chickens (as indicator of metabolic rate), and therefore it can decrease PHS and ascites.

Low temperatures increase metabolism and require more oxygen, so it develops ascites. On the other hand, oxidative stress produces free radicals from mitochondria, which is one of the major causes of ascites syndrome in broiler chickens (Babu, 2019). Therefore, the use of dietary sources containing antioxidants can reduce this syndrome by preventing the production of free radicals and maintaining the stability of tissue cells.

Nemati *et al.* (2017) showed that vitamin C can reduce the risk of ascites by decreasing RV:TV ratio. These researchers expressed that oxidative stress due to increased reactive oxygen species (ROS) production from mitochondrial electron leak is one of the main causes of the etiology of ascites in the poultry exposed to cold temperatures. Ascorbic acid is required for the synthesis of collagen, carnitine, corticosterone, and several neurotransmitters. It is also involved in the metabolism of tyrosine, iron, and thyroid hormones. More importantly, it is a powerful antioxidant and its ability to protect lipids against peroxidation in plasma is higher than vitamin E.

Few studies have been conducted on the effect of medicinal herbs on cardiovascular system and PHS. Since there is no study on the effects of broccoli on PHS, it can be noted that little attention has been paid to the role of medicinal herbs on the cardiovascular-pulmonary system. Accordingly, we hypothesized that broccoli supplementation to broiler chickens could alleviate the negative effects of cold stress by powerful antioxidant effects. Ahmadipour *et al.* (2015) demonstrated that use of *Kelussia odoratissima Mozzaf* in broiler chicken's diets at cold-stressed and high-altitude condition could reduce the risk of PHS by decreasing the RV:TV ratio, blood pressure, and mortality. In addition, Varmaghany *et al.* (2015) reported that dietary garlic recued ascites in broiler chickens by reducing systolic blood pressure.

Another issue for reducing the incidence of this syndrome is the increased activity of endothelial nitric oxide synthase enzyme (eNOS) and the formation of nitric oxide (NO). NO is a potent endogenous dilator for vessels, and it tends to relax smooth vascular muscles. Thus, it can decrease pulmonary vascular resistance, pulmonary hypertension, and cardiac output resulting in reduced right ventricular hypertrophy, RV:TV ratio, and death (Odom *et al.*, 2004). NO reduces pulmonary vascular resistance by vascular dilatation and reduces their response to endothelial-dependent vasoconstrictor factors, such as endothelin-1 and thromboxane A2 (Odom *et al.*, 2004). The study of Hassanpour *et al.* (2009) showed that plasma NO concentration decreases in chickens involved with PHS. In this study, serum NO concentration in broccoli groups was higher than control ($P≤0.05$). Possibly, it can be related to the higher level of arginine in broccoli, because arginine is a precursor of NO, which is a potent vasodilator reducing the incidence and severity of PHS. NO levels in the blood increase as a consequence of arginine supplementation. This observation suggests that extra arginine has become available for formation of NO through the action of NO synthetase (eNOS, NOS-3; EC 1.14.13.39) in the vascular bed. Thus, an increased NO could reduce the blood flow and PHS (Khajali *et al.*, 2014). Besides, the activation of eNOS can be initiated by increasing intracellular calcium, which is by calcium-boosting agonists such as bradykinin followed by bonding calcium-calmodulin complex with eNOS and isolating the enzyme from the Caveolin inhibitor (Michel *et al.*, 1997). Therefore, the highest NO concentration in broccoli groups can be related to the high calcium content in broccoli. So, the reduction of mortality rates in the treatments compared to control indicate the positive effects of broccoli in preventing PHS.

The high level of hematocrit can partially be related to the incidence of ascites, even though it was not confirmed in this research and several other studies (Nemati *et al.*, 2017; Imaseun *et al.*, 2017; Adesina and Toyo, 2014).

The H:L ratio is an indicator of the level of stress in birds. Hypoxia and cold temperature as stressors can increase the amount of corticosteroid secretion. In addition, the increase of steroid hormones in the blood reduce the production of lymphocytes, also steroid hormones effect on heterophil receptors release them in the blood, which increases the heterophil percentage in the blood (Ahmadipour *et al.*, 2015). According to Maxwell *et al.* (1990), the H:L ratio increases as a result of PHS and hypoxia. It can be concluded that decreasing the H:L ratio in chickens fed with ascorbic acid, broccoli 1%, and broccoli 0.5% is somehow consistent with the reduction of oxidative stress. These results are consistent with the findings of some other researchers. Lohakare *et al.* (2005) and Ladmakhi *et al.* (1997) reported a decrease in H:L ratio in broiler chickens fed with 500 ppm and 10-200 ppm of vitamin C in diet. Ahmadipour *et al.* (2015) reported a reduction of hematocrit and H:L ratio in broiler chickens involved with PHS by using *Kelussia odoratissima* Mozzaf.

There was no significant difference in uric acid, total protein, and serum cholesterol levels in different treatments. However, the levels of triglyceride in broccoli 1% and broccoli 0.5% decreased significantly ($P≤0.05$). HDL-C levels, as useful cholesterol, increased significantly in ascorbic acid and broccoli treatments ($P≤0.05$). Moreover, serum LDL-C levels, as harmful cholesterol, significantly decreased in broccoli treatments ($P≤0.05$).

The mechanism of antioxidant compounds to reduce cholesterol is by inhibiting biosynthesis of cholesterol, because cholesterol synthesis is applied at the beginning of its biosynthesis pathway, namely hydroxyl methyl glutathione coenzyme A reductase (HMG-COA). The reaction of HMG-COA conversion to mevalonate is done under HMG-COA reductase and nicotinamide adenine dinucleotide phosphate (NADPH). It has been shown that antioxidant compounds decrease production of mevalonate by reducing the activity of HMG-COA. Plant phenolic compounds can decrease LDL-C oxidation and increase serum HDL-C levels (Weinbrenner *et al.*, 2004). Furthermore, antioxidants decrease triglyceride levels by increasing the absorption of triglycerides and decreasing the activity of the hepatic triacylglycerol lipase (Ahmadipour *et al.*, 2015). The results of serum triglyceride levels in birds also confirmed this principle, and the control group had the highest levels of triglyceride ($P \leq 0.05$) and cholesterol, even though the level of cholesterol was not statistically significant.

Studies have shown that flavonoids and anthocyanins can prevent chronic cardiovascular disease and atherosclerosis by trapping free radicals, inhibiting LDL-C oxidation, and lowering triglyceride and cholesterol levels in the blood (Ustundag and Ozdogan, 2015). Therefore, broccoli can potentially prevent the occurrence of cardiovascular disorders associated with ascites because of its high levels of antioxidant compounds like phenols and flavonoids.

Adesina and Toyo (2012) reported a decrease in total serum cholesterol and LDL-C by adding broccoli at the levels of 0.03, 0.06, and 0.12 to the broiler and laying chicken's diet. In addition, Habibian *et al.* (2014) showed that vitamin C can lower total cholesterol and serum LDL-C. Another possible reason for lowering cholesterol and triglyceride is the increase in dietary fiber that has been reported by some researchers. Adding fiber to diet can affect the metabolism and blood cholesterol concentration and reduce cholesterol (Panja, 2013). MDA is a combination of lipids peroxidation in the body and is an indicator of oxidative stress. It should be noted that poultry are more susceptible to oxidative stress and MDA production. Since the metabolism rate in poultry is higher than mammals, the probability of producing free radicals increases. Thus, antioxidants play an important role in poultry. It has been shown that inhibiting ROS production prevents proliferation induced by hypoxia in the pulmonary arteries and subsequent pulmonary hypertension (Kelley *et al.*, 2014). Meanwhile, studies have shown that increased lipid peroxidation, due to decreased antioxidant activity in the tissues, causes coronary degeneration and right ventricular hypertrophy (Hemnes *et al.*, 2014). In this study, serum MDA concentration significantly decreased in treatment groups than control ($P \leq 0.05$). The antioxidant activity is carried out by breaking the chain, decomposing peroxides, chelating metals, and freeing radicals trapping. The antioxidant function of polyphenolic plants is multiple and can act as reduction agents, hydrogen donating, and free oxygen diluents (Ravikumar, 2015). The decrease in serum MDA observed in this research was consistent with the study conducted by Cho *et al.* (2006), that reported a decrease in lipid and protein peroxidation, decreased serum, liver and kidney MDA, and decreased glucose levels in diabetic rats. These researchers indicated that broccoli contains flavonoids, hydroxy-cinnamic acids, and sulfur compounds such as glucosinolates, and sulfur fans preserves the body against oxidative stress and cancer by capturing free radicals (Cho *et al.*, 2006). Mueller *et al.* (2012) reported an increase in iron induced lipid peroxidation and MDA in the liver by using of broccoli extract in broiler chicken's diet.

In this study, serum total antioxidant capacity increased significantly in ascorbic acid, broccoli

1%, and broccoli 0.5% groups compared to control ($P \leq 0.05$), which can be attributed to antioxidant compounds of broccoli.

Mueller *et al.* (2012) examined the effect of broccoli extract on the performance and antioxidant status of broiler chicks and found that the amount of 0.075 g/kg of extract could increase the expression of Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) in the liver and intestine and reduce lipid peroxidation in the liver. Hu *et al.* (2012) reported an increase in the activity of SOD and catalase (CAT), reducing MDA, and improving the qualitative properties of broiler chicken's breast meat by adding 4%, 8%, and 12% broccoli levels to diets. Moreover, Imaseun and Ijeh (2017) showed that the total serum antioxidant capacity increased and serum MDA concentrations decreased at the level of 0.5% ascorbic acid, ginger, and black pepper in broiler chicks.

Toe web thickness index reflects the cellular immunity in the poultry (Corrier and Deloach, 1990). In the present study, although the toe web thickness index and antibody titer against SRBC was higher in treatment groups compared to control, no significant difference was found between them. In addition, the results of ascorbic acid were consistent with those of Lohakare *et al.* (2005). Kumar *et al.* (2018) indicated that the pomegranate peel extract had no effect on the toe web thickness and antibody titer against Newcastle. Furthermore, Hong *et al.* (2012) did not show the effect of oregano, anis, and citrus peel on the antibody titer against SRBC in broiler chickens.

The results of this study showed that addition of broccoli to the diet of broiler chickens, through improving antioxidant parameters, increasing serum nitric oxide levels, and decreasing blood pressure, blood lipids, RV:TV ratio, and mortality, could be effective to prevent PHS.

Acknowledgments

The authors thank the staff of the faculty of Veterinary Medicine and Veterinary Clinic of the University of Shahrekord, Iran who collaborated on this research.

Conflict of Interest

The author declared no conflict of interest.

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10.22059/ijvm.2020.293989.1005046
Iranian Journal of Veterinary Medicine
Abstracts in Persian Language
Online ISSN 2252-0554

مجله طب دامی ایران، ۱۳۹۹، دوره ۱۴، شماره ۴، ۳۸۵-۳۷۲

استفاده از کلم بروکلی (*Brassica oleracea L. var. Italica*) در مقایسه با اسید آسکوربیک بر کاهش سندرم افزایش فشار خون ریوی در جوجه‌های گوشتی

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زمینه مطالعه: سندرم آسیت یکی از مهم‌ترین اختلالات متابولیکی در جوجه‌های گوشتی در حال رشد در سراسر جهان است.

هدف: در این مطالعه، تاثیر کلم بروکلی در مقایسه با اسید آسکوربیک بر کنترل سندرم افزایش فشار خون ریوی در جوجه‌های گوشتی بررسی شد.

روش کار: نمونه‌های سرمی، از ۲۰ فرد مختلف در سه گروه زنبورداران، افراد آلرژیک و افراد سالم جمع‌آوری گردید. زهرهای زنبورعسل معمولی و وحشی اطراف شهر تهران جمع‌آوری شد. همچنین زهرهای تجاری زنبورعسل (مستخرج از *آپیس ملیفرا*) و زنبورهای وحشی (مستخرج از *پولیستس و وسپولا*) از کشور فرانسه خریداری گردید. ایمونوبلات با استفاده از آنتی‌بادی‌های ضدایمونوگلوبولین G انسان و ضدایمونوگلوبولین E انسان کونژوگه شده با HRP انجام شد.

نتایج: عملکرد رشد هر چند در تیمارها بهبود یافت ولی از نظر آماری معنی دار نبود. نسبت RV/TV در گروه‌های اسید آسکوربیک و بروکلی کمتر از کنترل بود که این اختلاف در گروه‌های اسید آسکوربیک و بروکلی ۱٪ معنی‌داری بود ($P≤۰/۰۵$). نسبت H/L در تیمارهای آزمایشی نسبت به کنترل کاهش داشت ($P≤۰/۰۵$). میزان نیتریک اکساید و HDL-C سرم در هر دو سطح بروکلی افزایش یافت. ($P≤۰/۰۵$). اما LDL-C و تری‌گلیسرید در هر دو سطح بروکلی کاهش معنی‌دار داشت ($P≤۰/۰۵$). میزان مالون‌دی‌آلدهید و ظرفیت آنتی‌اکسیدانی تام سرم به ترتیب در تمامی تیمارها نسبت به کنترل پایین‌تر و بالاتر و از نظر آماری معنی‌داری بود ($P≤۰/۰۵$). در سایر شاخص‌های اندازه‌گیری شده تغییر معنی‌داری مشاهده نشد.

نتیجه‌گیری نهایی: مطالعه حاضر نشان داد که افزودن پودر کلم بروکلی به جیره جوجه‌های گوشتی با بهبود شاخص‌های آنتی‌اکسیدانی و افزایش نیتریک‌اکساید، کاهش نسبت RV/TV، فشار خون، چربی خون و تلفات می‌تواند در پیشگیری از سندرم افزایش فشار خون ریوی موثر باشد.

واژه‌های کلیدی: آسیت، اسید آسکوربیک، بروکلی، جوجه گوشتی، سندرم

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