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Effects of purslane (*Portulaca oleracea* L.) powder on growth performance, blood indices, and antioxidant status in broiler chickens with triiodothyronine-induced ascites

Mahmood Habibian, Ghorbanali Sadeghi, and Ahmad Karimi

Department of Animal Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, 661715175, Iran

Correspondence to: Ghorbanali Sadeghi (ghorbanalis@yahoo.com, gsadeghi@uok.ac.ir)

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Abstract. This study was carried out to evaluate the effects of dietary supplementation of purslane powder (PP) on performance, blood indices, and antioxidant status in broilers with triiodothyronine (T3)-induced ascites. In total, 240 one-day-old male broiler chicks (Ross 308) were randomly assigned to four treatments, with four replicates per treatment and 15 birds per replicate. The experimental diets included (i) a control diet, (ii) a control diet plus 1.5 mg kg^{-1} of T3 (T3 diet), (iii) a T3 diet with the addition of 1.5 g kg^{-1} of PP, and (iv) a T3 diet with the addition of 3 g kg^{-1} of PP. Feed intake and body weight were measured at 10, 24, 39, and 49 days of experiment. Blood and liver samples were collected from two birds in each replicate at 24 and 49 days of experiment. The T3-treated birds had higher ($P < 0.05$) right ventricle to total ventricle (RV / TV) ratio and mortality due to ascites compared with the control. In addition, during the entire experimental period (0 to 49 days of experiment) the T3-treated birds had lower ($P < 0.05$) feed intake, body weight gain, and production efficiency index and higher ($P < 0.05$) feed conversion ratio compared with the control. Dietary supplementation of PP reduced ($P < 0.05$) mortality due to ascites and RV / TV ratio, while the production efficiency index was increased ($P < 0.05$) by the addition of PP to the diet. The T3-treated birds had higher ($P < 0.05$) red blood cell counts, hematocrit percentage, and hemoglobin concentration compared with the control at 24 and 49 days of experiment. Dietary supplementation of PP substantially alleviated ($P < 0.05$) the negative effects of T3 on hematocrit and hemoglobin values at both 24 and 49 days of experiment and on red blood cells counts at 49 days of experiment. The T3 birds showed an increase ($P < 0.05$) in activities of lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase at 49 days of experiment. However, the detrimental effect of T3 on alanine aminotransferase activity was attenuated ($P < 0.05$) by dietary supplementation of PP. The plasma and liver activities of superoxide dismutase, catalase, and glutathione peroxidase were lower ($P < 0.05$) in T3-treated birds compared with the control at 24 and 49 days of experiment, whereas malondialdehyde concentrations were elevated ($P < 0.05$) by dietary T3 administration. Dietary supplementation of PP, especially at 3 g kg^{-1}, increased ($P < 0.05$) the plasma and liver activities of antioxidant enzymes, and reduced ($P < 0.05$) the plasma and liver concentrations of malondialdehyde near to the control levels. It is concluded that the supplementation of 3 g kg^{-1} of PP in diet improves oxidative status and reduces ascites incidence in broiler chickens without impairing their growth performance.

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1 Introduction

Intensive genetic selection for fast growth rate over the past several decades markedly increased body weight, whereas it hardly reduced allometric growth of the heart and lungs in modern broilers compared with older breeds (Havenstein et al., 2003). As a result, an imbalance between oxygen requirement by tissues and oxygen supply has emerged and resulted in increased blood pressure within the pulmonary arteries, which can subsequently lead to the progressive development of pulmonary hypertension (ascites) syndrome. Ascites is a metabolic disorder, characterized by hypoxemia, increased workload of the cardiopulmonary system, central venous congestion, right ventricular hypertrophy and a flaccid heart, an excessive accumulation of plasma-like fluid in the abdominal cavity, and finally death (Baghbanzadeh and Decuypere, 2008). It accounts for over one-quarter of overall broiler mortality across the world (Zheng et al., 2007).

Before ascites becomes evident clinically, common anatomical and hematological changes can be detected in a bird (Maxwell et al., 1986, 1987). A right ventricle to total ventricle (RV / TV) ratio of more than 0.299 is considered an accurate measure of the onset of ascites (Walton et al., 2001). Alterations in blood gas volumes, hemoglobin, hematocrit, and systemic red blood cell counts have been demonstrated between healthy and ascetic broiler chickens (Yersin et al., 1992; Wideman et al., 1998; Daneshyar et al., 2007, 2009). In addition, some other variables may be affected by hypoxemia and ascites. Arab et al. (2006) observed that broilers with triiodothyronine (T3)-induced ascites had higher plasma activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) than in healthy ones, whereas Fathi et al. (2011) reported higher serum activities of ALT, AST, and lactate dehydrogenase (LDH) in cold-induced ascitic broilers compared with control.

Moreover, there are reports indicating that free-radical-mediated mechanisms are involved in the etiology of ascites (Bottje and Wideman, 1995; Grobe et al., 2006; Nain et al., 2008). The rate of generation of free radicals has been demonstrated to increase by systemic hypoxia and inflammation (Bottje and Wideman, 1995). Therefore, it has been hypothesized that the development of ascites may be due, in part, to the oxygen free radical produced by the mitochondrial cardiomyocytes of hypoxic birds, with subsequent depletion of tissue antioxidants (Xiang et al., 2002). In addition, previous studies have shown that during an episode of inflammation, increased numbers of activated white blood cells secrete a variety of cytokines that promote generation of reactive oxidants into surrounding tissues, which may, in turn, alter tissue antioxidant status (Maxwell et al., 1986; Bottje and Wideman, 1995).

Common purslane (*Portulaca oleracea* L.), belonging to the family Portulacaceae, is an important traditional drug that has been used in many parts of the world, showing therapeutic activity against diseases related to the intestine, liver, and stomach; coughing; shortness of breath; and asthma (Uddin et al., 2012). The therapeutic value of purslane is mainly attributed to the presence of many biologically active compounds, including phenolic acids, flavonoids, alkaloids, saponins, vitamins, minerals, and high content of n-3 fatty acids (Okafor et al., 2014). Purslane is also a good source of β-carotene, glutathione, coenzyme Q_{10}, and melatonin (Naeem and Khan, 2013). All these compounds together contribute to the antioxidant properties and free-radical scavenging activities of purslane (Yang et al., 2009). A purslane extract has been shown to be superior to vitamin C and β-carotene in scavenging reactive oxygen species (Zhang et al., 2008). A wide range of other pharmacological effects of purslane, such as antibacterial, analgesic, anti-inflammatory, and bronchodilatory effects, have also been reported (Chan et al., 2000; Malek et al., 2004; Peng et al., 2014). It also has anti-proliferative effects on smooth muscle cells (Parry et al., 1988) and can attenuate hypertension by inhibiting vascular remodeling (Lee et al., 2012). It is noteworthy that hypoxic broilers develop vascular remodeling in the lungs, which reduces their pulmonary vascular capacity (Wideman et al., 2011). Furthermore, when searching for promising antihypoxic drugs, it was noticed that aqueous and alcoholic extracts of purslane exhibited potent antihypoxic properties and that they improve survivability and antioxidant status of the lung and the brain in hypoxic mice (Jin et al., 2010; Yue et al., 2015).

Up to now, no study has reported the effect of purslane herb on performance and ascites incidence in broiler chickens. Thus, in the present study, we evaluated the effects dietary supplementation of purslane powder on ascites mortality, growth performance, blood indices, and antioxidative status in T3-induced ascitic broilers.

2 Materials and methods

All procedures used in the present study were approved by the Animal Care and Use Committee of the University of Kurdistan (Sanandaj, Iran).

2.1 Purslane herb

Fresh, mature, wild purslane plants were collected at the seedling stage from a local field in Sanandaj (Kurdistan Province, Iran). The plants (including seeds, leaves, stems, and roots) were cleaned of soil particles and other pollutants, dried, and finely ground to a size of 2 mm using a typical mill (model IKH.S1, Iran Khodsaz Company, Tehran, Iran). Dried purslane powder (PP) was stored in air-tight containers at room temperature ($25 \pm 2\,^{\circ}$C) prior to use. Antioxidant compounds of PP, including total phenolics content (TPC; Zhou and Yu, 2006), total flavonoids content (TFC; Abu Bakar et al., 2009), vitamin E (Kayden et al., 1973), and vitamin C (Awe et al., 2013) were determined in six replicates using a spectrophotometer (Hitachi U-2001, Tokyo,

Japan) as indicated. The PP contained 14.9 ± 0.40 mg gallic acid equivalents g^{-1} TPC, 8.5 ± 0.19 quercetin equivalents g^{-1} TFC, 0.27 ± 0.006 mg α-tocopherol equivalents g^{-1} vitamin E, and 0.24 ± 0.007 mg ascorbic acid equivalents g^{-1} vitamin C.

2.2 Birds, management, and experimental diets

In total, 240 one-day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery and placed in 16 floor pens (1.25 m × 1.25 m). All chickens were vaccinated against infectious bronchitis, Gumboro, and Newcastle diseases. Each pen was covered with wood shavings to a height of approximately 5 cm as litter base and equipped with two nipple drinkers and one hanging tube feeder. During the first 7 days, the light regimen was continuous, which was reduced to 23 h of light afterward. Temperature was set initially at 30 °C and gradually reduced by 3 °C/7 days until 22 °C was reached.

Birds were randomly assigned to four treatments (four replicate pens; 15 birds per pen) and kept for 49 days. The four experimental diets included the following: (i) control diet, without any supplement; (ii) control diet plus 1.5 mg kg^{-1} of T3 (T3 diet); (iii) T3 diet with the addition of 1.5 g kg^{-1} of PP; and (iv) T3 diet with the addition of 3 g kg^{-1} of PP. Liothyronine sodium (Iran Hormone Company, Tehran, Iran) was used as the source of T3 and included in the basal diets to induce ascites from 7 to 49 days of experiment (Hassanzadeh et al., 2000). The PP supplemental levels were chosen according to a previous study conducted in our laboratory (Sadeghi et al., 2016). The birds were provided ad libitum access to mash feed and water according to a four-phase feeding program during 0 to 10, 10 to 24, 24 to 39, and 39 to 49 days of experiment. Diets (Table 1) were formulated to meet nutrient requirements according to breeder nutrient requirements (Aviagen®, 2014). The nutrient contents for all ingredients were taken from AminoDat 5.0, gold version (Evonik-Degussa GmbH, Hanau, Germany).

2.3 Sampling procedure

Feed intake and body weight were recorded by pen at 10, 24, 39, and 49 days of experiment. Mortality was recorded daily, and feed conversion ratio was corrected for mortality, and represent weight of feed consumed by all birds in a pen divided by body weight gain per pen plus the body weight of the dead birds. European production efficiency index was determined as cited by Attia et al. (2012). All dead birds were necropsied to identify the cause of death. The diagnosis of ascites was based on the following symptoms: (i) right ventricle hypertrophy, and cardiac muscle laxation; (ii) swollen and stiff liver; and (iii) clear, yellowish, colloidal fluid in the abdominal cavity (Geng et al., 2004).

At 24 and 49 days of experiment, eight birds from each treatment (two birds per replicate) were randomly selected and bled by wing vein puncture. The blood samples were transferred into EDTA-coated tubes. A portion of blood was used for determination of hematocrit, hemoglobin, and red blood cell counts, whereas the other portion of the blood was centrifuged at $2000 \times g$ for 15 min at room temperature and plasma was collected in labeled tubes and stored at −40 °C until further analysis.

After blood sampling, the birds were slaughtered by severing the jugular veins and carotid arteries, and the thorax and abdomen were opened and inspected for signs of heart failure and ascites. The heart was dissected and removed from the body to determine the RV / TV ratio. Birds having RV / TV ratios more than 0.299 were classified as having ascites (Walton et al., 2001). Moreover, a portion of liver was obtained, frozen in liquid nitrogen, and stored at −80 °C until use.

2.4 Laboratory analysis

Hemoglobin, hematocrit, and red blood cells counts were measured by the routine methods (Baker and Silverton, 1985; Jain, 1986). The activities of LDH, ALT, and AST in plasma were determined using spectrophotometric kits (Pars Azmun, Tehran, Iran) as recommended by the supplier.

For antioxidant assays, liver tissues (10 g) were homogenized using a mortar on ice in freezing isotonic physiological saline to form homogenates at the concentration of 0.1 g mL^{-1}. The samples were centrifuged at $700 \times g$, after which the supernatants and plasma were measured for malondialdehyde (MDA) concentrations and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) using spectrophotometric methods. The activity of SOD was measured by the xanthine oxidase method, which monitors the inhibition of reduction of nitroblue tetrazolium and the change of absorbance at 560 nm (Sun et al., 1988). The CAT activity was measured following the decrease in absorbance at 240 nm due to hydrogen peroxide decomposition (Aebi, 1984). The GPx activity was determined by measuring the rate of oxidation of reduced glutathione to oxidized glutathione at 412 nm (Hafeman et al., 1974). The thiobarbital method (Placer et al., 1966) was used to determine the MDA concentration with a wavelength of 532 nm to determine absorbance. Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

2.5 Statistical analysis

Data were analyzed as a completely randomized design and subjected to ANOVA using the general linear model procedure of SAS software (SAS Institute, 2003). Differences among means were tested using Tukey's test ($P < 0.05$).

Table 1. Ingredients and nutrients level of the basal diets at different periods of experiment.

Item (%, unless otherwise noted)	0–10 days	10–24 days	24–39 days	39–49 days
Ingredients				
Corn	52.35	56.94	63.46	65.99
Soybean meal (44 % CP)	39.74	35.71	29.98	27.28
Soybean oil	3.08	3.00	2.58	2.89
DL-methionine	0.40	0.35	0.31	0.28
L-lysine-HCl	0.27	0.20	0.21	0.20
L-threonine	0.11	0.08	0.06	0.05
Dicalcium phosphate	2.21	1.95	1.71	1.63
Calcium carbonate	1.04	0.97	0.90	0.88
Common salt	0.29	0.29	0.30	0.30
Vitamin–mineral premix[1]	0.50	0.50	0.50	0.50
Calculated composition				
Metabolizable energy (kcal kg^{-1})	2950	3000	3050	3100
Crude protein	22.50	21.00	19.00	18.00
Methionine + cysteine	1.08	0.99	0.90	0.85
Lysine	1.44	1.29	1.15	1.08
Threonine	0.97	0.88	0.78	0.73
Tryptophan	0.28	0.25	0.22	0.21
Arginine	1.51	1.39	1.23	1.15
Isoleucine	0.96	0.89	0.79	0.74
Valine	1.05	0.98	0.89	0.84
Calcium	0.96	0.87	0.78	0.75
Available phosphorus	0.48	0.44	0.39	0.38
Sodium	0.14	0.14	0.14	0.14

[1] The vitamin–mineral premix provided the following quantities per kg of diet: vitamin A, 10 000 IU (all-trans-retinal); cholecalciferol, 2000 IU; vitamin E, 15 mg; vitamin K_3, 3.0 mg, riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B_{12}, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; iron, 60 mg; zinc, 90 mg; copper, 10 mg; iodine, 0.46 mg; selenium, 0.2 mg.

3 Results

3.1 Ascites mortality and RV / TV ratio

The effects of dietary treatments on mortality rates and the RV / TV ratio in surviving birds that were selected randomly at 24 and 49 days of experiment are presented in Table 2. Both total mortality and mortality due to ascites were greater ($P < 0.05$) in the T3 birds compared with the control throughout the experiment. The T3-treated birds also exhibited a greater ($P < 0.05$) RV / TV ratio than did the control. However, the negative effects of T3 on mortality related to ascites, total mortality, and RV / TV ratio were substantially alleviated ($P < 0.05$) by dietary PP supplementation, although RV / TV ratios were still higher ($P < 0.05$) than the control. Mortality due to causes other than ascites was not affected ($P > 0.05$) by dietary treatments.

3.2 Growth performance

The effects of dietary treatments on growth performance are summarized in Table 3. During 0 to 10 days of experiment, feed intake, body weight gain, and feed conversion ratio did not differ among dietary treatments ($P > 0.05$). During 10 to 24, 24 to 39, 39 to 49, and 0 to 49 days of experiment, the T3-treated birds consumed markedly lower ($P < 0.05$) feed and exhibited lower ($P < 0.05$) body weight gain compared with the control. In addition, during 24 to 39 and 0 to 49 days of experiment, the T3-treated birds had higher ($P < 0.05$) feed conversion ratio compared with the control. Also, the T3-treated birds exhibited lower ($P < 0.05$) production efficiency index compared with the control. Feed intake, body weight gain, and feed conversion ratio were not affected ($P > 0.05$) by dietary PP supplementation in any period of experiment. However, the production efficiency index was somewhat ($P < 0.05$) improved by the addition of PP to the diet.

3.3 Hematological indices

The effects of dietary treatments on hematological parameters are presented in Table 3. The T3-treated birds had higher ($P < 0.05$) red blood cell counts, hematocrit percentage, and hemoglobin concentration compared with the control at 24 and 49 days of experiment ($P < 0.05$). Dietary supplemen-

Table 2. Effects of dietary treatments on mortality rate (0–49 days of experiment) and right ventricle to total ventricle (RV / TV) ratio in broiler chickens slaughtered at 24 and 49 days of experiment.

Items	Diets[1]					
	C	T3	T3 + PP1.5	T3 + PP3.0	SEM[3]	*P* value
Mortality (%)[2]						
Ascites	0.00[b]	11.67[a]	3.34[b]	3.34[b]	1.322	0.004
Other cases	0.00	5.00	3.33	1.67	1.031	0.290
Total	0.00[b]	16.67[a]	6.67[a, b]	5.00[b]	1.872	0.003
RV / TV ratio						
Day 24	0.14[c]	0.31[a]	0.24[b]	0.19[b]	0.013	< 0.001
Day 49	0.15[c]	0.32[a]	0.27[b]	0.26[b]	0.012	< 0.001

[a–c] Means within row with different superscripts are significantly different ($P < 0.05$); Tukey's test was applied to compare means. [1] C, control (basal diet); T3, basal diet + 1.5 mg kg^{-1} T3; T3 + PP1.5, T3 + 1.5 g kg^{-1} purslane powder; T3 + PP3.0, T3 + 3 g kg^{-1} purslane powder. [2] Number of dead birds/number of total birds ×100. [3] SEM, standard error of means.

tation of PP substantially ($P < 0.05$) alleviated the negative effects of T3 on hematocrit and hemoglobin values at both recorded days and on red blood cells counts at 49 days of experiment. Dietary supplementation of PP also ameliorated the negative effect of T3 on the counts red blood cells at 24 days of experiment, but the values obtained in PP-treated birds were intermediate and did not reach statistical significance from either the control or the T3-treated birds ($P > 0.05$).

3.4 ALT, AST, and LDH activities

The effects of dietary treatments on the plasma activities of LDH, ALT, and AST are also shown in Table 4. At 24 days of experiment, the T3 birds showed an increase in ALT activity ($P < 0.05$), whereas no change ($P > 0.05$) in activities of LDH and AST were detected due to T3 treatment compared with the control ($P > 0.05$). At 49 days of experiment, the activities of all three enzymes were higher in the T3 birds compared with the control ($P < 0.05$). Dietary supplementation of PP had no significant ($P > 0.05$) effects on AST and LDH activities. However, the detrimental effect of T3 on ALT activity was attenuated ($P < 0.05$) by dietary PP supplementation at 49 days of experiment, although it could not restore the ALT activity to levels observed in the control ($P < 0.05$).

3.5 Antioxidant enzyme activities and lipid peroxidation

The effects of treatments on plasma and liver antioxidant indices are presented in Tables 5 and 6, respectively. The plasma and liver activities of SOD, CAT, and GPx were lower ($P < 0.05$) in the T3-treated birds compared with the control at 24 and 49 days of experiment, whereas MDA concentrations were higher in the T3-treated birds than in the control. At 24 days of experiment, dietary supplementation of PP, especially at 3 g kg^{-1}, increased ($P < 0.05$) the plasma and liver activities of SOD and GPx, and reduced ($P < 0.05$) the concentrations of MDA near to the control levels. In addition, administration of PP-supplemented diet restored the liver activity of CAT near to the control levels ($P < 0.05$). At 49 days of experiment, the patterns of liver activities of SOD, CAT, and GPx and plasma and liver concentrations of MDA in response to PP supplementation were similar to those observed at 24 days of experiment. Also, the negative effects of T3 treatment on plasma activities of SOD and GPx were ameliorated ($P < 0.05$) by dietary supplementation of PP, but PP supplementation could not restore the reduced activities of SOD and GPx towards close the control levels ($P > 0.05$).

4 Discussion

Hypoxia is believed to be the primary cause of the development of ascites (Wideman, 2000); therefore, situations which impose greater metabolic demand or reduced oxygen availability increase incidence of ascites in broilers. Dietary supplementation of T3 induces an increased basic metabolic rate and high cardiac output, which may cause the conditions of hypoxemia (Decuypere et al., 1994; Hassanzadeh et al., 2000; Arab et al., 2006). Hypoxemia results in a series of reactions that leads to ascites (Wideman, 2000). Before death, a significant decline in the performance of the birds occurs (Hassanzadeh et al., 2000; Luger et al., 2001). Lower feed intake, body weight gain, and production efficiency index and higher feed conversion ratio in the T3-treated birds also confirmed these detrimental effects in the present study. Increases in mortality rate and RV / TV ratio are also accompanied by ascites (Hassanzadeh et al., 2000; Fathi et al., 2016). Birds with RV / TV ratios greater than 0.299 are considered

Table 3. Effects of dietary treatments on feed intake, body weight gain, and feed conversion ratio in broiler chickens at different periods of experiment.

Items	Diets[1]					
	C	T3	T3 + PP1.5	T3 + PP3.0	SEM[2]	*P* value
Feed intake (g)						
0–10 days	248	247	246	246	0.9	0.844
10–24 days	998[a]	898[b]	895[b]	885[b]	13.0	< 0.001
24–39 days	2618[a]	2453[b]	2452[b]	2457[b]	21.6	0.001
39–49 days	1924[a]	1764[b]	1764[b]	1771[b]	18.3	< 0.001
0–49 days	5789[a]	5362[b]	5357[b]	5359[b]	50.6	< 0.001
Body weight gain (g)						
0–10 days	186	189	187	191	1.4	0.724
10–24 days	580[a]	545[b]	550[b]	547[b]	4.7	0.007
24–39 days	1418[a]	1168[b]	1165[b]	1164[b]	31.1	< 0.001
39–49 days	993[a]	766[b]	765[b]	763[b]	32.9	0.009
0–49 days	3176[a]	2667[b]	2666[b]	2664[b]	59.8	< 0.001
Feed conversion ratio ($g\,g^{-1}$)						
0–10 days	1.33	1.31	1.32	1.29	0.010	0.601
10–24 days	1.72	1.65	1.63	1.62	0.016	0.103
24–39 days	1.85[b]	2.10[a]	2.12[a]	2.11[a]	0.037	0.005
39–49 days	1.94	2.30	2.37	2.36	0.079	0.177
0–49 days	1.82[b]	2.01[a]	2.01[a]	2.02[a]	0.025	< 0.001
Production efficiency index						
1–49 days	360[a]	229[c]	257[b]	261[b]	8.7	< 0.001

[a–c] Means within row with different superscripts are significantly different ($P < 0.05$); Tukey's test was applied to compare means. [1] C, control (basal diet); T3, basal diet + 1.5 mg kg^{-1} T3; T3 + PP1.5, T3 + 1.5 g kg^{-1} purslane powder; T3 + PP3.0, T3 + 3 g kg^{-1} purslane powder. [2] SEM, standard error of means.

to have ascites (Walton et al., 2001). In this study, the mean value of RV / TV ratio was greater than 0.299 in the T3-treated group, indicating that numbers of birds in this group had been suffering from ascites. Body weight gain, feed intake, and feed conversion ratio were not influenced by dietary PP supplementation during the experiment. However, the bird groups that received dietary PP supplementation had greater production efficiency index compared with the T3-treated birds. In addition, total mortality, mortality due to ascites, and RV / TV ratio were substantially decreased by dietary PP supplementation, especially at 3 g kg^{-1}, though the RV / TV ratios were still much higher than the control levels.

Our results showed higher values hematocrit, hemoglobin, and red blood cell counts in the T3-treated birds compared with the control. These results concur with those of Hassanzadeh et al. (2000) and Arab et al. (2006), who found higher hematocrit levels and red blood cell counts in broilers with T3-induced ascites. This may represent higher blood viscosity associated with a noticeable increase in hematocrit, which was postulated to be one of the main causes of ascites (Baghbanzadeh and Decuypere, 2008). Broilers are particularly sensitive to an increase in blood viscosity because their lung volumes are limited by the size of the thoracic cavity and their blood capillaries are small and relatively non-compliant (Zhou et al., 2008; Fathi et al., 2016). Dietary supplementation of PP markedly alleviated the negative effects of T3 on values of hematocrit and hemoglobin and red blood cells counts. These effects may be probably because of antioxidant constituents of PP such as phenolic acids, flavonoids, vitamin E, and vitamin C. A number of antioxidant compounds have been shown to suppress erythropoiesis occurs in response to hypoxia (Xiang et al., 2002; Geng et al., 2004; Ahmadipour et al., 2015; Varmaghany et al., 2015). Another major reason for this effect of PP on hematological indices must be its high content of n-3 fatty acids (Okafor et al., 2014). The increased content of n-3 fatty acids probably increases the fluidity of the erythrocyte membrane and alters membrane function to increase the deformability and transportation ability of erythrocytes, hence reducing red blood cell counts and subsequently hematocrit and hemoglobin (Baghbanzadeh and Decuypere, 2008).

Table 4. Effects of dietary treatments on red blood cell counts (RBC), hemoglobin (HB), and hematocrit (HCT), and plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in broiler chickens at 24 and 49 days of experiment.

Items	Diets[1]					
	C	T3	T3+PP1.5	T3+PP3.0	SEM[2]	*P* value
Day 24						
RBC (10^6 µL^{-1})	1.78^{b}	2.27^{a}	2.10$^{a, b}$	1.97$^{a, b}$	0.054	0.005
HB (g dL^{-1})	8.74^{b}	11.50^{a}	8.67^{b}	8.29^{b}	0.351	0.001
HCT (%)	23.9^{b}	33.3^{a}	28.8$^{a, b}$	24.7^{b}	1.017	0.001
ALT (L^{-1})	4.80^{b}	5.89^{a}	5.57$^{a, b}$	5.56$^{a, b}$	0.125	0.008
AST (L^{-1})	304	302	309	306	1.5	0.453
LDH (L^{-1})	2884	2844	2835	2892	22.3	0.767
Day 49						
RBC (10^6 µL^{-1})	2.41^{b}	2.89^{a}	2.50^{b}	2.50^{b}	0.049	< 0.001
HB (g dL^{-1})	11.2^{b}	13.7^{a}	11.4^{b}	11.2^{b}	0.276	< 0.001
HCT (%)	30.3^{b}	38.7^{a}	30.8^{b}	31.1^{b}	0.872	< 0.001
ALT (L^{-1})	10.2^{c}	16.5^{a}	13.4^{b}	13.7^{b}	0.42	< 0.001
AST (L^{-1})	317^{b}	458^{a}	457^{a}	452^{a}	11.1	< 0.001
LDH (L^{-1})	3128^{b}	4282^{a}	4254^{a}	4261^{a}	90.2	< 0.001

$^{a–c}$ Means within row with different superscripts are significantly different ($P < 0.05$); Tukey's test was applied to compare means. [1] C, control (basal diet); T3, basal diet + 1.5 mg kg^{-1} T3; T3 + PP1.5, T3 + 1.5 g kg^{-1} purslane powder; T3 + PP3.0, T3 + 3 g kg^{-1} purslane powder. [2] SEM, standard error of means.

Table 5. Effects of dietary treatments on superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, and malondialdehyde (MDA) concentrations of plasma in broiler chickens at 24 and 49 days of experiment.

Items	Diets[1]					
	C	T3	T3 + PP1.5	T3 + PP3.0	SEM[2]	*P* value
Day 24						
SOD (mL^{-1})	155^{a}	131^{c}	145^{b}	151$^{a, b}$	1.8	< 0.001
CAT (mL^{-1})	4.37^{a}	3.61^{b}	3.64^{b}	3.62^{b}	0.106	0.019
GPx (mL^{-1})	178^{a}	161^{c}	173^{b}	174$^{a, b}$	1.3	< 0.001
MDA (nmol mL^{-1})	3.53^{b}	4.76^{a}	3.80^{b}	3.75^{b}	0.134	0.002
Day 49						
SOD (mL^{-1})	177^{a}	149^{d}	160^{c}	165^{b}	1.9	< 0.001
CAT (mL^{-1})	7.32^{a}	6.24^{b}	6.25^{b}	6.24^{b}	0.104	< 0.001
GPx (mL^{-1})	216^{a}	181^{d}	189^{c}	196^{b}	2.5	< 0.001
MDA (nmol mL^{-1})	4.12^{b}	5.54^{a}	4.50^{b}	4.42^{b}	0.113	< 0.001

$^{a–d}$ Means within row with different superscripts are significantly different ($P < 0.05$); Tukey's test was applied to compare means. [1] C, control (basal diet); T3, basal diet + 1.5 mg kg^{-1} T3; T3 + PP1.5, T3 + 1.5 g kg^{-1} purslane powder; T3 + PP3.0, T3 + 3 g kg^{-1} purslane powder. [2] SEM, standard error of means.

Moreover, the present study showed a significant increase in the activities of LDH, ALT, and AST in plasma of the T3-treated birds compared with the control. The LDH activity result of our study is consistent with those of Hassanzadeh et al. (1997), indicating a higher rate of anaerobic glycolysis in the T3-treated birds. Similar findings were observed by Arab et al. (2006), who reported higher ALT and AST activities in T3-induced ascitic broilers and by Fathi et al. (2011), who detected elevated LDH, ALT, and AST activities in cold-induced ascitic broilers. It has also been recognized that AST activity increases in damaged heart (Wirz et al., 1990) and damaged liver (Pratt and Kaplan, 2000), and that ALT activity increases in damaged liver (Zantop, 1997). It has been found that the liver, heart, and pulmonary system are affected

Table 6. Effects of dietary treatments on superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities as well as malondialdehyde (MDA) concentrations of liver in broiler chickens at 24 and 49 days of experiment.

Items	Diets[1]					
	C	T3	T3 + PP1.5	T3 + PP3.0	SEM[2]	*P* value
Day 24						
SOD (mg protein^{-1})	137^{a}	92^{b}	123^{a}	135^{a}	4.1	< 0.001
CAT (mg protein^{-1})	17.4^{a}	11.5^{b}	15.2^{a}	16.2^{a}	0.56	< 0.001
GPx (mg protein^{-1})	19.6^{a}	13.2^{c}	17.1^{b}	18.4$^{a, b}$	0.49	< 0.001
MDA (nmol mg protein^{-1})	0.98^{b}	1.19^{a}	1.01^{b}	1.00^{b}	0.030	0.047
Day 49						
SOD (mg protein^{-1})	207^{a}	180^{b}	191$^{a, b}$	190$^{a, b}$	3.2	0.014
CAT (mg protein^{-1})	20.8^{a}	17.5^{c}	17.6^{bc}	18.5^{b}	0.27	< 0.001
GPx (mg protein^{-1})	21.2^{a}	15.2^{c}	18.8^{b}	19.9$^{a, b}$	0.48	< 0.001
MDA (nmol mg protein^{-1})	1.51^{b}	2.13^{a}	1.81$^{a, b}$	1.73^{b}	0.059	< 0.001

$^{a–c}$ Means within row with different superscripts are significantly different ($P < 0.05$); Tukey's test was applied to compare means. [1] C, control (basal diet); T3, basal diet + 1.5 mg kg^{-1} T3; T3 + PP1.5, T3 + 1.5 g kg^{-1} purslane powder; T3 + PP3.0, T3 + 3 g kg^{-1} purslane powder. [2] SEM, standard error of means.

in ascites (Maxwell et al., 1986, 1987); therefore, it would be expected that these plasma enzyme activities change with ascites. Dietary supplementation of PP was found to reduce the release of ALT into the systemic circulation at 49 days of experiment, although it could not restore the ALT activity to levels found in the control. These results are in agreement with those of Hanan et al. (2014), who reported that oral administration of purslane aqueous extract significantly attenuated carbon tetrachloride-induced increase in serum ALT activity and liver injury without any alteration in serum AST activity in mice. The release of these diagnostic enzymes reflects a non-specific aberration in the plasma membrane integrity (Kumar and Anandan, 2007). Therefore, our results indicate that dietary supplementation of PP cannot fully protect the cells against harmful effects of T3.

In addition, a significant increase in the concentrations of MDA with a parallel decrease in activities of SOD, CAT, and GPx was observed in the plasma and liver tissue of the T3-treated birds, which is indicative of increased oxidative stress (Geng et al., 2004; Pan et al., 2005). These results support the previous findings, which showed higher generation of reactive oxygen intermediates (Iqbal et al., 2001; Arab et al., 2006), lower activity of enzymatic (Peng et al., 2013) and non-enzymatic (Nain et al., 2008) antioxidative systems, and elevated levels of oxidized lipids (Peng et al., 2013; Fathi et al., 2016) in broilers with naturally and experimentally induced ascites. Dietary supplementation of PP, especially at 3 g kg^{-1}, increased the liver activities of SOD, CAT, and GPx, and reduced the plasma and liver concentrations of MDA near to the control levels at 24 and 49 days of experiment. The negative effects of T3 treatment on plasma activities of SOD and GPx were also ameliorated by dietary PP supplementation, but it could not restore the reduced activities of SOD and GPx towards close the control levels at 49 days of experiment. These results are in accordance with previous studies (Jin et al., 2010; Yue et al., 2015), in which aqueous and alcoholic extracts of purslane improved survivability and antioxidant status of the lung and the brain in hypoxic mice. Similarly, in previous studies, supplementation of dried aerial parts of purslane powder to diet positively affected the antioxidant enzymes activities, decreased oxidative damage to lipids, and improved antioxidant status in healthy broilers (Ghorbani et al., 2013; Sadeghi et al., 2016). Some studies have found that phenolic constituents, including flavonoids, phenolic acids, and alkaloids, have a strong correlation with the antioxidant capacity of purslane (Lim and Quah, 2007; Uddin et al., 2012), whereas others have suggested that other compounds such as saponins, proteins, amino acids, melatonin, vitamin C, vitamin E, and trace mineral contents may also contribute to antioxidant capacity of this plant (Yang et al., 2009; Uddin et al., 2012). Purslane is also known as a rich source of glutathione and coenzyme Q_{10} (Okafor et al., 2014). The glutathione obtained from the feed is absorbed intact from the gut and acts as a substrate for GPx in animal cells (Simopoulos et al., 1992). Coenzyme Q_{10}, as a necessary component of the respiratory chain in the inner mitochondrial membrane, not only functions as an electron and proton carrier and drives ATP synthesis but also, in its reduced form (ubiquinol), can be an important antioxidant to reduce the accumulation of free radicals, in particular reactive oxygen intermediates, and lessen the peroxidative damage in the body (Choudhury et al., 1991; Geng and Guo, 2005). Studies have shown that the antioxidative status in ascitic broilers can be attenuated by dietary supplementation of antioxidants, such as vitamin C and vitamin E (Villar-Patiño et al., 2002; Xiang et al., 2002), coenzyme Q_{10} (Geng

and Guo, 2005), and natural plant products (Daneshyar et al., 2012; Ahmadipour et al., 2015; Varmaghany et al., 2015).

5 Conclusions

In this study, supplementation of PP to diets could not prevent the negative effect of T3 on broiler growth performance. However, supplementation of 1.5 or 3 g kg^{-1} of PP to broiler diets significantly attenuated the increasing effect of T3 administration on the RV / TV ratio, total mortality, and ascites-related mortality as well as the values of hematocrit, hemoglobin, and red blood cell counts. Moreover, the detrimental effects of T3 treatment on plasma and liver antioxidative status were substantially alleviated by dietary supplementation of PP, especially at 3 g kg^{-1}, although dietary PP supplementation had no effect on plasma activity of CAT and could not restore the reduced plasma activities of SOD and GPx towards close the control levels. It is concluded that supplementation of PP to diet improves oxidative status and reduces ascites incidence in broilers without impairing their growth performance.

Data availability. The original data of the paper are available upon request from the corresponding author.

Competing interests. The authors declare that they have no conflict of interest.

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