Animal Nutrition 5 (2019) 264–269

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Expression of antioxidant genes in broiler chickens fed nettle (*Urtica dioica*) and its link with pulmonary hypertension

Behnam Ahmadipour*, Fariborz Khajali

Department of Animal Science, Shahrekord University, Shahrekord, 88186-34141, Iran

ARTICLE INFO

Article history:
Received 4 November 2018
Received in revised form
19 April 2019
Accepted 24 April 2019
Available online 9 May 2019

Keywords:
Antioxidant enzymes
Chicken
Urtica dioica
Right ventricular failure

ABSTRACT

Nettle (*Urtica dioica*) contains a wide range of chemical constituents that confer a strong antioxidant capacity to the plant. The present study was to investigate the antioxidant gene expression and pulmonary hypertensive responses of broiler chickens to *U. dioica*. A total of 240 one-d-old broilers (Ross 308) were randomly assigned to 4 dietary levels of *U. dioica* (0, 0.5%, 1% and 1.5%). Birds were reared for 6 wk in a high altitude region (2,100 m). The results showed a significant relative overexpression (target gene/β-actin as the arbitrary unit) of catalase (*CAT*) and superoxide dismutase 1 (*SOD1*) in the liver and lung of the chickens fed *U. dioica*. Lipid peroxidation was significantly suppressed, as reflected in reduced circulatory concentrations of malondialdehyde (MDA) in the birds fed *U. dioica*. These birds also had significantly ($P < 0.05$) higher serum nitric oxide (NO) concentrations than those in the control group. Feeding *U. dioica* at 1% and 1.5% also attenuated the right ventricular hypertrophy (reflected in the lower right to total ventricular weight ratio), which was associated with a significant lower rate of mortality from pulmonary hypertension syndrome. Feeding *U. dioica* led to an upregulation of hepatic and pulmonary antioxidant genes.

1. Introduction

In the past several decades, we have witnessed phenomenal growth in the body weight of broiler chickens, mainly due to intensive genetic selection. As a result, chickens tend to mature in a shorter length of time and begin to deposit fat at earlier ages (Hocking, 2014). The excessive accumulation of fat in the body of broiler chickens, which is mainly in the form of poly-unsaturated fatty acids, makes broilers vulnerable to lipid peroxidation and the generation of reactive oxygen species (ROS) (Bottje and Wideman, 1995; Khajali and Wideman, 2016). The generation of ROS is shown to be linked to the development of pulmonary hypertension syndrome (PHS) in broiler chickens (Khajali and Wideman, 2016). The vulnerability of broiler chickens to PHS is exacerbated at high altitudes, where the generation of ROS is accelerated under hypobaric hypoxia (Wu et al., 2007). Pulmonary hypertension causes vascular remodeling in pulmonary arteries and leads to terminal ascites and mortality from PHS, which imposes a significant economic loss to the chicken's meat production industry (Wideman et al., 2011).

Research has shown that PHS can be attenuated by adding exogenous antioxidant supplements to broiler diets (Sharifi et al., 2016). *Urtica dioica* (belonging to the family of Urticaceae) exists in annual and perennial forms and grows in many parts of the world including Asia, Africa, Europe and America. *U. dioica* has strong antioxidant capacity (Gulcin et al., 2004; Alp and Aksu, 2010). The plant has been reported to has various pharmacological activities, such as antioxidant, anti-inflammatory, anti-colitis, antiulcer, anticancer, antiviral, antibacterial, antimicrobial, antifungal, antiandrogenic, insecticide, immunomodulatory, hypocholesterolemic, hypoglycemic, cardiovascular effects, analgesic, natriuretic, hypotensive, hepatoprotective and rheumatoid arthritis (Joshi et al., 2014). Toldy et al. (2005) found *U. dioica* to be an effective antioxidant in reducing the ROS concentration in rats'

* Corresponding author.
E-mail address: Behnam.Ahmadipour@gmail.com (B. Ahmadipour).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2019.04.004

brains. Several studies have reported the use of *U. dioica* to alleviate hypertension in mammals. Legssyer et al. (2002) reported a strong induction of bradycardia through non-cholinergic and non-adrenergic pathways by administering the aqueous extract of *U. dioica*. Thakur et al. (2012) revealed that an ethanolic extract of *U. dioica* reduced the blood pressure of hypertensive rats. The nitric oxide (NO)-mediated vasorelaxation and the calcium channel blocking effects of the methanolic extract of *U. dioica* provide a potential pharmacological base for its medicinal use in the management of hypertension in mammals (Qayyum et al., 2016). To the best of our knowledge, no research has studied the effect of *U. dioica* on pulmonary hypertension in an avian model.

U. dioica has been used in poultry diets as a natural antioxidant. Loetscher et al. (2013) used the herb in broiler diets at 2.5% and observed a significant increase in the tocopherols content of breast meat although the malondialdehyde (MDA) concentration (an index of lipid peroxidation) did not significantly change compared to the control group. *Kelussia odoratissima* Mozzaf was found to be a promising herb to avoid pulmonary hypertension in broiler chickens due to strong antioxidant compounds in the plant (Ahmadipour et al., 2015a, 2015b). In view of the antioxidant properties of *U. dioica*, the present study investigated the antioxidant gene expression of broiler chickens fed different levels of *U. dioica* and its link to pulmonary hypertension.

2. Materials and methods

2.1. Birds and experimental facility

The experiment was carried out at the Poultry Research Center of Shahrekord University, Shahrekord, Iran, a tropical region with an altitude of 2,100 m above sea level. The study was carried out in line with the guidelines of the Care and Use Committee of Shahrekord University.

A total of 240 one-d-old broilers (Ross 308 strain) were randomized across 16 floor pens of 1.8 square meter (15 birds per pen) so that all pens had similar weight at the beginning of the experiment (657 ± 2 g). Each pen was supplied with a bell drinker and a feed trough. The temperature of the experimental house was maintained at 32 °C during wk 1, 25 °C for wk 2, 20 °C for wk 3, and 15 °C thereafter as previously reported (Khajali et al., 2007). All chicks had free access to feed and water and were provided with 23 h of light and 1 h of dark throughout the trial.

2.2. Treatments

A control diet was prepared for the starting (1 to 21 d of age) and growing (22 to 42 d of age) stages based on the National Research Council (1994) recommendations (Table 1).

The control diet consisted of 1.5% wheat bran. Three additional diets were prepared by using 0.5%, 1% and 1.5% *U. dioica* to substitute wheat bran in the control diet. *U. dioica* plants were collected in May 2018 from Pastures Chahrmahal-Va-Bakhtiari province in Iran. The area is at an altitude of 2,100 m above sea level and the average annual precipitation in this area is about 800 mm and the temperature variation is about −20 to 35 °C in the year. The plant was identified by a senior plant taxonomist of University of Shahrekord. Stems and leaves were dried at 25 °C for 4 d without applying any heat treatment to minimize the loss of active components. *U. dioica* is a perennial herb, growing in nitrogen-rich soils. The stem is erect and green, the leaves are opposite, cordate at the base, oblong or ovate, finely toothed, dark green above and paler beneath. Analyzed composition of dried powder of *U. dioica* showed 7.8% crude protein, 8.3% crude fiber, 1.2% Ca, 0.7% P, 0.06% Na, 0.04% Cl, 0.23% S, and 0.7% K. Gas chromatography analysis (using the Agilent 6890 N GC system with Agilent 5975 Mass Selective Detector) (GC-MS) elucidated several active compounds representing 96.7% of the oil (Table 2).

2.3. Measurements

The birds' body weights were recorded at 21 and 42 d of age. Body weight gain (i.e. growth) and feed intake were calculated form 1 to 21 d, 22 to 42 d and 1 to 42 d periods. Feed conversion ratio (amount of feed consumed to produce 1 kg weight gain) was calculated for the same periods taking into account the mortality body weights. At 42 d of age, 10 birds per treatment were selected for blood collection and processing. The selected birds had body weights within approximately 5% of the average pen body weight. Blood samples (3 mL) were taken from the brachial vein and centrifuged at 2,500× *g* for 10 min to collect sera. Nitric oxide and MDA concentrations were measured in serum samples. The concentration of NO in serum was measured according to the method described by Behrooj et al. (2012). The serum MDA concentration was assayed by using the thiobarbituric acid colorimetric method (Nair and Turner, 1984). Moreover, samples of blood were collected in microhematocrit tubes for determining hematocrit. An aliquot of blood was placed on glass slides to prepare the blood smear for the determination of differential leukocyte count. Following the May-Grunwald and Giemsa staining, a total of 100 leukocytes including granular (heterophils) and nongranular (lymphocytes) were enumerated, and the heterophil to lymphocyte (H:L) ratio was subsequently calculated. All chemical reagents were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA).

Table 1
Composition of the basal diet for broilers during starter and grower stages (%, unless noted).

Item	Starter (1 to 21 d of age)	Grower (22 to 42 d of age)
Ingredients		
Corn	51.8	58.4
Soybean meal (44% CP)	38.6	32.5
Soy oil	4	3.9
Dicalcium phosphate	1.7	1.3
Oyster shell	1.5	1.5
Salt	0.3	0.3
DL-Met	0.1	0.1
L-Lys	–	–
Mineral supplement[1]	0.25	0.25
Vitamin supplement[2]	0.25	0.25
Wheat bran	1.5	1.5
Calculated composition		
AME, kcal/kg	3,000	3,100
CP	21.5	19.5
Met	0.48	0.4
Met + Cys	0.9	0.72
Lys	1.26	1.03
Thr	0.92	0.9
Arg	1.38	1.15
Ca	0.95	0.88
Available P	0.43	0.35
Na	0.18	0.15
Cl	0.27	0.29
K	0.9	0.92
Na + K−Cl, mEq/kg	232	233

[1] Provided the following per kilogram of diets: vitamin A (trans retinyl acetate), 3,600 IU; vitamin D_3 (cholecalciferol), 800 IU; vitamin E (DL-α-tocopheryl acetate), 7.2 mg; vitamin K_3, 1.6 mg; thiamine, 0.72 mg; riboflavin, 3.3 mg; niacin, 0.4 mg; pyridoxin, 1.2 mg; cobalamine, 0.6 mg; folic acid, 0.5 mg; choline chloride, 200 mg.

[2] Provided the following per kilogram of diets: Mn (from $MnSO_4 \cdot H_2O$), 40 mg; Zn (from ZnO), 40 mg; Fe (from $FeSO_4 \cdot 7H_2O$), 20 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 4 mg; I [from Ca $(IO_3)_2 \cdot H_2O$], 0.64 mg; Se (from sodium selenite),0.08 mg.

Table 2
Composition of the essential oil of *Urtica dioica* (%).

Compound	Content
Cadina	0.2
Copaene	0.3
2-pentyl furan	0.4
Linalyl acetate	0.4
α-terpineol	0.5
Calamenene	0.7
Nonanal	0.8
β-selinene	0.8
Cumin aldehyde	2.0
Eugenol	1
Kessane	1.1
Limonene	1.3
Cadinene	1.4
Methyl chavicol	1.5
Pentyl benzene	1.5
Bisabolene	1.6
β-caryophyllene	1.9
Linalool	2.1
Furanone	2.1
Caryophyllene oxide	2.8
(E)-Geranyl acetone	2.8
Hexahydrofarnesyl acetone	3
Phytol	3.7
Anethol	4.3
Butyldiene phthalide	5.3
Naphthalene	8.4
Carvone	9.1
Carvacrol	35.7

After the blood collection stage, the birds were euthanized by CO_2. Data recorded at processing included weights of live body, hot carcass, breast, thigh, heart, liver, spleen, the bursa of Fabricious, and abdominal fat. The heart's ventricles were cut and weighed to calculate the right-to-total ventricular weight (RV:TV) ratio. The RV:TV ratio is the main index of pulmonary hypertension (Ahmadipour et al., 2015b, 2018b). In addition, mortality from PHS was checked daily throughout the trial and whenever the RV:TV ratio was greater than 0.29, it was considered as ascites syndrome.

2.4. Quantitative real time PCR analysis

At the end of experiment (42 d of age), 10 chickens from each treatment were randomly selected and slaughtered. The livers and lungs were collected and immediately stored in liquid nitrogen at −70 °C for subsequent RNA analysis. The total RNA from the tissues was extracted using RNX-Plus reagent (Sinaclon Bioscience, Tehran, Iran). The homogenate was mixed with chloroform and centrifuged. The total RNA was separated in the upper aqueous phase of the mixture. The RNA pellet was rinsed with ethanol (75%) and re-suspended in diethyl pyrocarbonate (DEPC) treated water. To remove residual DNA, the RNA was further treated by DNase (Sinaclon Bioscience, Tehran, Iran). The RNA was then measured and qualified spectrophotometrically. Only RNA with an absorbance ratio (A260/A280) greater than 1.9 was used for synthesis of cDNA. Total RNA was reverse transcribed into cDNA using PrimeScript RT Reagent Kit (TaKaRa Bio Inc., Japan). The reverse transcription mix was heated to 85 °C for 5 s to inactivate reverse transcriptase and denature the RNA and then stored at −20 °C.

The levels of superoxide dismutase 1 (*SOD1*), catalase (*CAT*), glutathione peroxidase (*GPX*) and β-actin transcripts were determined by quantitative real time (RT)-PCR using SYBR Premix Ex Taq II (Tli Rnase H Plus) (TaKaRa Bio Inc., Japan). To normalize the input load of cDNA among samples, β-actin was used as an endogenous standard. Specific primers of *SOD1, CAT, GPX1* and β-actin were designed with Primer-Blast (www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC = BlastHome). Details of the primers are listed in Table 3.

PCR were carried out in a RT PCR cycler (Rotor Gene Q 6000, Qiagen, USA) in 3 replicates for each sample of ventricles. One microliter cDNA was added to the 10 μL of SYBR Premix Ex Taq II Mix and 0.5 μmol/L of each specific primer in a total volume of 20 μL. The thermal profile was 95 °C for 30 s, 40 cycles of 94 °C for 40 s, 64 °C for 35 s and 72 °C for 30 s. At the end of each phase, the measurement of fluorescence was carried out and used for quantitative objectives. The gene expression data were normalized to β-actin. The data were analyzed using LinRegPCR software version 2012.0 (Amsterdam, Netherland), to give the threshold cycle number and reaction efficiency (Ruijter et al., 2009). The relative transcript levels and the fold changes in transcript abundance were calculated using efficiency adjusted Paffl methodology (Dorak, 2006).

2.5. Statistical analysis

The results were statistically analyzed by the GLM procedure of SAS (2007) software in a completely randomized design. The means were separated by Duncan's multiple range test.

3. Results

Table 4 represents the growth performance of broilers fed different levels of *U. dioica*. Body weight gain and feed conversion ratio were significantly ($P < 0.05$) improved when *U. dioica* was included in broiler diets at 1% and 1.5%. However, feed intake did not significantly change across the treatments.

Table 5 depicts blood and serum variables of broilers receiving different dietary levels of *U. dioica*. Broilers receiving 0.5% *U. dioica* had a higher ($P < 0.05$) concentration of NO and lower ($P < 0.05$) concentrations of MDA and hematocrit compared to the birds fed the control diet. Feeding *U. dioica* at 1% and 1.5% resulted in a significant ($P < 0.05$) reduction in heterophil to lymphocyte ratio compared to the control.

The hepatic expression of *SOD1* and *CAT* genes in broiler chickens fed *U. dioica* at 1% and 1.5% was significantly ($P < 0.05$) increased compared to their control counterparts (Table 6). However, the hepatic expression of *GPX1* was not influenced by feeding *U. dioica*. Catalase and *SOD1* showed significant over-expression in the lung of birds fed *U. dioica* at 1% and 1.5%.

Table 7 shows carcass characteristics of broilers fed *U. dioica* at 42 d of age. While carcass and thigh yields were not affected by dietary treatments, breast yield was significantly increased by feeding *U. dioica.* Dietary inclusion of *U. dioica* significantly ($P < 0.05$) reduced the proportions of liver, heart, bursa of Fabricious, and abdominal fat in comparison to the control.

The right ventricular hypertrophy (RVH) index and cumulative ascetic mortality up to 42 d of age in broilers fed *U. dioica* at 1% and 1.5% were significantly ($P < 0.05$) lower than those in the control (Table 7).

4. Discussion

An improvement in weight gain and feed conversion ratio of birds fed *U. dioica* is associated with the antioxidative effects of naturally-occurring terpenoid phenols in the plant. Carvacrol and carvone are the main terpenoids found in *U. dioica*, which account for 46.8% of the oil. These compounds exhibit a broad range of biological properties such as growth-promoting, antioxidant, antibacterial and antiviral actions (Upton, 2013). Dietary antioxidants protect gut epithelial cells from pro-apoptotic oxidant stress, which results in increased epithelial cell growth (Miller et al.,

Table 3
Details of the primers used for quantitative real time PCR analysis of chicken mRNA.

Target	Primers	PCR product, bp	Accession No.
β-actin	F:5′-AGCGAACGCCCCCAAAGTTCT-3′ R:5′-AGCTGGGCTGTTGCCTTCACA-3′	139	NM_205518.1
SOD1	F:5′-CACTGCATCATTGGCCGTACCA-3′ R:5′-GCTTGCACACGGAAGAGCAAGT-3′	224	NM_205064.1
CAT	F:5′-TGGCGGTAGGAGTCTGGTCT-3′ R:5′-GTCCCGTCCGTCAGCCATTT-3′	112	NM_001031215.1
GPX1	F:5′-GCTGTTCGCCTTCCTGAGAG-3′ R:5′-GTTCCAGGAGACGTCGTTGC-3′	118	NM_001277853.1

SOD1 = superoxide dismutase 1; *CAT* = catalase; *GPX1* = glutathione peroxidase 1; bp = base pair.

Table 4
Effects of dietary levels of *Urtica diocia* on broiler growth performance.

Item	Dietary levels of *Urtica diocia*, %				SEM
	0 (control)	0.5	1	1.5	
Feed intake, g/bird					
1 to 21 d of age	1,057	1,073	1,037	1,077	10.11
22 to 42 d of age	2,862	2,751	2,806	2,749	30.52
1 to 42 d of age	3,921	3,824	3,843	3,827	70.2
Weight gain, g/bird					
1 to 21 d of age	670^{b}	705^{a}	695^{ab}	724^{a}	7.09
22 to 42 d of age	1,316^{c}	1,443^{b}	1,515^{a}	1,451^{b}	9.73
1 to 42 d of age	1,986^{c}	2,147^{b}	2,210^{a}	2,175^{ab}	10.42
Feed conversion ratio					
1 to 21 d of age	1.58^{a}	1.53^{ab}	1.49^{b}	1.48^{b}	0.012
22 to 42 d of age	2.17^{a}	1.91^{b}	1.85^{b}	1.89^{b}	0.022
1 to 42 d of age	1.97^{a}	1.78^{b}	1.74^{b}	1.76^{b}	0.014

$^{a, b, c}$ Means in the same raw with different superscripts are significantly different ($P < 0.05$).

Table 5
Effect of *Urtica diocia* on serum and blood variables in broiler chickens measured at 42 d of age.[1]

Item	Dietary levels of *Urtica diocia*, %				SEM
	0 (control)	0.5	1	1.5	
Serum nitric oxide, μmol/L	9.58^{b}	14.86^{a}	16.65^{a}	17.5^{a}	1.6
Serum malondialdehyde, μmol/L	4.88^{a}	2.96^{b}	2.74^{b}	2.48^{b}	0.375
Heterophils to lymphocyte, %	0.71^{a}	0.62^{a}	0.45^{b}	0.48^{b}	0.033
Hematocri, %	41.8^{a}	34.6^{b}	31.2^{b}	32.2^{b}	0.78

$^{a, b}$ Means in the same raw with different superscripts are significantly different ($P < 0.05$).
[1] Each mean represents values from 8 replicates.

2001). *U. dioica* possess phytochemicals like phenolic compounds, which have been shown to be effective in scavenging free radicals (Akbay et al., 2003). Environmental conditions and differential geographical distribution, which can change the constitution of plant on phenolic compounds and their derivatives (phenolic acids, flavonoids, etc.), also induce differences in their antioxidant power (Husain and Shah, 2011). Phenolic compounds prevent oxidative stress by the following mechanisms: direct scavenging of ROS, activation of antioxidant enzymes, metal chelating activity, reduction of α-tocopheryl radicals, inhibition of oxidases and increase in uric acid level (Behrooj et al., 2012; Surai, 2014). The improved oxidative stress status in birds fed *U. dioica* can be reflected in a significantly lower H:L ratio in birds fed *U. dioica*. The H:L ratio is the index of stress in the chicken, so a reduction in H:L ratio can be translated into less oxidative stress. In this regard, serum MDA concentration, an index of lipid peroxidation, was significantly reduced in broilers fed *U. dioica*. Gulcin et al. (2004) indicated that the water extract of *U. dioica* at 50 μg/mL inhibited the peroxidation of linoleic acid emulsion by 39% while 60 μg/mL alpha tocopherol exhibited only a 30% inhibition.

The increased serum NO concentration in *U. dioica* groups presumably counterbalanced RVH, as appeared in lower RV:TV ratios. Nitric oxide is a potent vasodilator that slackens the pulmonary vascular resistance by causing vascular smooth muscle to relax, and inhibits the production and release of vasoconstrictors such as serotonin and endothelin-1 (Wideman et al., 2007). It has been suggested that NO insufficiency is associated with the pathophysiology of RVH in broilers with pulmonary hypertension (Ahmadipour et al., 2018b). The reduced hematocrit in *U. dioica* groups might be another factor that counteracted RVH, and together with an increased NO concentration, linked to a lower RV:TV ratio and a reduced proportion of heart observed in the present study. It is not clear, however, whether the decrease in hematocrit results from alteration in erythropoiesis or fluid exudation out of the blood system to the abdominal cavity.

Table 6
Effect of *Urtica diocia* on gene expression in the liver and lung of broiler chickens measured at 42 d of age (%).

Item	Gene	Dietary levels of *Urtica diocia*, %				SEM
		0 (control)	0.5	1	1.5	
Liver	*SOD1*	0.003^{b}	0.214^{ab}	0.745^{a}	0.759^{a}	0.188
	CAT	0.001^{c}	0.017^{bc}	0.062^{a}	0.047^{ab}	0.010
	GPX1	0.003	0.046	0.053	0.089	0.020
Lung	*SOD1*	0.001^{c}	0.007^{bc}	0.028^{ab}	0.033^{a}	0.0065
	CAT	0.003^{b}	0.058^{a}	0.103^{a}	0.620^{a}	0.148

SOD1 = superoxide dismutase1; *CAT* = catalase; *GPX1* = glutathione peroxidase 1.
$^{a, b, c}$ Means in the same raw with different superscripts are significantly different ($P < 0.05$).

Table 7
Effect *Urtica diocia* on carcass characteristics and ascites mortality of broilers raised up to 42 d of age.[1]

Item	Dietary levels of *Urtica diocia*, %				SEM
	0 (control)	0.5	1	1.5	
Carcass yield, %	67.5	69.3	68.9	70.5	0.66
Breast yield, %	36.6^{b}	37.0^{b}	38.0^{a}	38.1^{a}	0.193
Thigh yield, %	32.9	32.6	33.4	32.9	0.21
Abdominal fat, %	2.29^{a}	1.81^{b}	1.67^{b}	1.67^{b}	0.063
Liver, %	2.41^{a}	2.19^{b}	2.14^{b}	2.04^{b}	0.050
Heart, %	0.74^{a}	0.66^{b}	0.65^{b}	0.66^{b}	0.016
Spleen, %	0.095^{c}	0.102^{bc}	0.113^{bc}	0.122^{a}	0.003
Bursa, %	0.209^{a}	0.162^{b}	0.153^{b}	0.156^{b}	0.011
RV:TV ratio	0.30^{a}	0.27^{ab}	0.24^{bc}	0.23^{c}	0.011
PHS mortality, %	37.5^{a}	27.5^{ab}	17.5^{b}	22.5^{b}	3.81

RV:TV ratio = right ventricle to total ventricles weight ratio; PHS mortality = pulmonary hypertension syndrome mortality.
$^{a, b, c}$ Means in the same raw with different superscripts are significantly different ($P < 0.05$).
[1] Each mean represents values from 10 replicates.

A significant relative over-expression (target gene/β-actin as arbitrary unit) of *SOD1* and *CAT* in the liver and lung of broilers fed *U. dioica* is in line with the alleviated RVH (lower RV:TV ratios) and reduced PHS mortality. Chu et al. (2003) reported that an over-expression of *SOD* reduces hypertension, increases the availability of NO and endothelium-dependent relaxation in different models of hypertension. This report explains the link between *SOD* over-expression and the significant reduction in the incidence of PHS mortality in birds fed *U. dioica*. In addition, some research has shown a link between the overexpression of *CAT* and hypertension (Shi et al., 2013). Shi et al. (2013) indicated that the renal over-expression of *CAT*, a key antioxidant enzyme in renal proximal tubular cells, attenuated renal oxidative stress and prevented hypertension in mice. Sundaram et al. (2013) also reported that the upregulation of *CAT* and downregulation of GPX activity in the kidney precede the development of hypertension in pre-hypertensive rats. Adesina et al. (2015) reported that the increased *CAT* activity and reduced hydrogen peroxide generation in mitochondria was effective to prevent hypoxia-induced pulmonary hypertension in mice. These studies explained the link between pulmonary *CAT* overexpression and reduced RV:TV ratio observed in the present study as a result of feeding birds *U. dioica.* Studies have shown that the use of medicinal plants of *K. odoratissima* and *Securigera securidaca* in chickens reared under cold and high altitude conditions increases the expression of *CAT* and *SOD* genes in the heart and lung about 150 fold (Ahmadipour, 2018a; Ahmadipour et al., 2015a), which is agree with the results of this experiment. It must be noted that ascites could considerably change expression of genes and then this plant could probably decrease or even improve these changes (Ahmadipour, 2018a; Ahmadipour et al., 2015a; Hassanpour et al., 2015).

The reduced abdominal fat deposition in chickens fed *U. dioica* clearly indicates the antihyperlipidemic potency of the herb. The abdominal fat is a benchmark of the bird's lipogenesis because it grows more rapidly compared to other adipose tissues and it is highly correlated to the total body fat content in the chicken (Fouad and El-Senousey, 2014). The reduced proportional weight of liver in chickens fed *U. dioica* was in line with a decreased lipogenesis. In fact, the liver is the principal site of lipogenesis in the chicken and a decline in the relative weight of liver can account for less lipogenesis associated with the dietary inclusion of *U. dioica*. The lipolytic effect of *U. dioica* is thought to be attributed to polyphenols (Chen and Li, 2007). The aqueous (150 mg/kg) and ethanolic (100 mg/kg) extracts of *U. dioica* significantly reduced the levels of total cholesterol and low-density lipoprotein (LDL) in hypercholesterolemic rats (Dahar et al., 2006).

5. Conclusion

In conclusion, feeding broilers *U. dioica* significantly improved antioxidant status by overexpression of *SOD1* and *CAT* and remarkably prevented PHS.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This study was financially supported by Shahrekord University, Shahrekord, Iran.

References

Adesina SE, Kang BY, Bijli KM, Ma J, Cheng J, Murphy T, et al. Targeting mitochondrial reactive oxygen species to modulate hypoxia-induced pulmonary hypertension. Free Rad Biol Med 2015;87:36–47.
Ahmadipour B. *Securigera securidaca* seed medicinal herb supplementation o diets improves pulmonary hypertensive response in broiler chickens reared at high altitude. J Anim Physiol Anim Nutr 2018a;102:1601–7.
Ahmadipour B, Hassanpour H, Asadi E, Khajali F, Rafiei F, Khajali F. *Kelussia odoratissima* Mozzaf- A promising medicinal herb to prevent pulmonary hypertension in broiler chickens reared at high altitude. J Ethnopharm 2015a;159:49–54.
Ahmadipour B, Hassanpour H, Rafiei F, Khajali F. Antioxidative, antihyperlipidemic, and growth-promoting effects of *Kelussia odoratissima* in meat-type chickens. Poult Sci J 2015b;3:37–46.
Ahmadipour B, Sharifi M, khajali F. Pulmonary hypertensive response of broiler chickens to arginine and guanidinoacetic acid under high-altitude hypoxia. Acta Vet Hung 2018b;66:116–24.
Akbay P, Basaran A, Undeger U, Basaran N. In vitro immunomodulatory activity of flavonoid glycosides from Urtica dioica L. Phytoth Res 2003;17:34–7.
Alp E, Aksu MI. Effects of water extract of *Urtica dioica* L. and modified atmosphere packaging on the shelf life of ground beef. Meat Sci 2010;86:468–73.
Behrooj N, Khajali F, Hassanpour H. Feeding reduced-protein diets to broilers subjected to hypobaric hypoxia is associated with the development of pulmonary hypertension syndrome. Br Poult Sci 2012;53:658–64.
Bottje WG, Wideman RF. Potential role of free radicals in the pathogenesis of pulmonary hypertension syndrome. Poult Avian Biol Rev 1995;6:211–31.
Chen J, Li X. Hypolipidemic effect of flavonoids from mulberry leaves in triton WR-1339 induced hyperlipidemic mice. Asia Pac J Clin Nutr 2007;16:290–4.
Chu Y, Iida S, Lund DD, Weiss RM, Dibona GF, Watanabe Y, et al. Gene transfer of extracellular superoxide dismutase reduces arterial pressure in spontaneously hypertensive rats: role of heparin-binding domain. Circ Res 2003;92:461–8.
Dahar CF, Baroody KG, Baroody GM. Effect of *Urtica dioica* extract intake upon blood lipid profile in the rats. Fitoterapia 2006;77:183–8.
Dorak M. Real time PCR. 1st ed. Oxford, UK: Taylor & Francis; 2006.
Fouad AM, El-Senousey HK. Nutritional factors affecting abdominal fat deposition in poultry- a review. Asia Aust J Anim Sci 2014;27:1057–68.
Gulcin I, Kufrevioglu OI, Oktay M, Buyukokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J Ethnopharm 2004;90:205–15.
Hassanpour H, Khalaji-Pirbalouty V, Nasiri L, Mohebbi A, Bahadoran S. Oxidant and enzymatic antioxidant status (gene expression and activity) in the brain of chickens with cold-induced pulmonary hypertension. Int J Biometeorol 2015;59:1615–21.
Hocking PM. Unexpected consequences of genetic selection in broilers and turkeys: problems and solutions. Br Poult Sci 2014;55:1–12.
Husain M, Shah M. Study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. Arab J Chem 2011;8:66–71.
Joshi B, Mukhija M, Kalia A. Pharmacognostical review of *Urtica dioica* L. Int J Gre Pharm 2014;8:201–14.
Khajali F, Wideman RF. Nutritional approaches to ameliorate pulmonary hypertension in broiler chickens. J Anim Physiol Anim Nutr 2016;100:3–14.
Khajali F, Zamani-Moghaddam A, Asadi-Khoshoei E. Application of an early skip-a-day feed restriction on physiological parameters, carcass traits and development of ascites in male broilers reared under regular or cold temperatures at high altitude. Anim Sci J 2007;78:159–63.
Legssyer A, Ziyyat A, Mekhfi H, Bnouham M, Tahri A, Serhouchni M, et al. Cardiovascular effects of *Urtica dioica* L. in isolated rat heart and aorta. Phytoth Res 2002;16:503–7.
Loetscher Y, Kreuzer M, Messikommer RE. Oxidative stability of the meat of broilers supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle, and effects on performance and meat quality. Poultry Sci 2013;92:2938–48.
Miller MJ, Angeles FM, Reuter BK, Bobrowski P, Sandoval M. Dietary antioxidants protect gut epithelial cells from oxidant-induced apoptosis. BMC Complement Altern Med 2001;1:11.
Nair V, Turner GA. The thiobarbituric acid test for lipid peroxidation: structure of the adduct with malondialdehyde. Lipids 1984;19:804–5.
National Research Council. Nutrient requirements for poultry. Washington, DC: Ninth Revised Edition; 1994.
Qayyum R, Qamar HM, Khan S, Salma U, Khan T, Shah AJ. Mechanisms underlying the antihypertensive properties of *Urtica dioica*. J Transl Med 2016;14:254.
Ruijter J, Ramakers C, Hoogaars W, Karlen Y, Bakker O, Van den Hoff M, et al. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucl Acid Res 2009;37:1–12.
Sharifi MR, Khajali F, Hassanpour H. Antioxidant supplementation of low-protein diets reduced susceptibility to pulmonary hypertension in broiler chickens raised at high altitude. J Anim Physiol Anim Nutr 2016;100:69–76.
Shi Y, Lo CS, Chenier I, Maachi H, Filep JG, Ingelfinger JR, et al. Overexpression of CAT prevents hypertension and tubulointerstitial fibrosis and normalization of renal angiotensin-converting enzyme-2 expression in Akita mice. Am J Physiol Ren Physiol 2013;304:F1335–46.

Sundaram A, Keah LS, Sirajudeen KN, Singh HJ. Upregulation of CAT and down-regulation of glutathione peroxidase activity in the kidney precede the development of hypertension in pre-hypertensive SHR. Hypertens Res 2013;36:213–8.
Surai PF. Polyphenol compounds in the chicken/animal diet: from the past to the future. J Anim Physiol Anim Nutr 2014;98:19–31.
Thakur RK, Goutman N, Sharma S, Thakur S, Sharma D, Thakur P. Antihypertensive effect of ethanolic extract of *Urtica dioica* L.leaves (Urticaceae) in renal artery occluded hypertensive rats. J Pharm Res 2012;5:3585–7.
Toldy A, Stadler K, Sasvari M, Jakus J, Jung JK, Chung HY, et al. The effect of exercise and nettle supplementation on oxidative stress markers in the rat brain. Brain Res Bull 2005;65:487–93.
Upton R. Stinging nettles leaf (*Urtica dioica* L.): extraordinary vegetable medicine. J Herb Med 2013;3:9–38.
Wideman RF, Chapman MF, Hamal K, Bowen OT, Lorenzoni AG, Erf GF, et al. An inadequate pulmonary vascular capacity and susceptibility to pulmonary arterial hypertension in broilers. Poultry Sci 2007;86:984–98.
Wideman RF, Hamal KR, Bayona MT, Lorenzoni AG, Cross D, Khajali F, et al. Plexiform lesions in the lungs of domestic fowl selected for susceptibility to pulmonary arterial hypertension: incidence and histology. Anat Rec 2011;294:739–55.
Wu W, Platoshyn O, Firth AL, Yuan JX. Hypoxia divergently regulates production of reactive oxygen species in human pulmonary and coronary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2007;293:L952–9.