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Kelussia odoratissima Mozzaf – A promising medicinal herb to prevent pulmonary hypertension in broiler chickens reared at high altitude

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ABSTRACT

Ethnopharmacological relevance: *Kelussia odoratissima* Mozzaf, formerly *Apium odoratissima*, is a plant locally called “Karafs”, found in central Zagros region of Iran. Leaves and stems of the plant are traditionally used in the treatment of hypertension and inflammation. Lowering blood pressure effects of *Kelussia odoratissima* Mozzaf (wild celery) was evaluated in preventing pulmonary hypertension syndrome (PHS) in broiler chickens reared at high altitude (2100 m).

Materials and methods: A total number of 208 day-old male broilers (Ross 308) were randomly assigned to four treatments including different levels of *Kelussia odoratissima* Mozzaf (0%, 0.25%, 0.5%, and 0.75%) in a 42-day trial.

Results: Body weight gain and feed:gain responses significantly ($P < 0.05$) improved when *Kelussia odoratissima* Mozzaf was included in broiler diets at 0.75% in the growing stage and throughout the trial. Over-expression of inducible nitric oxide (NO) synthase in the heart was observed in chickens fed *Kelussia odoratissima* Mozzaf. Birds received *Kelussia odoratissima* Mozzaf at 0.5% and 0.75% had significantly ($P < 0.05$) higher circulatory concentrations of NO though significantly ($P < 0.05$) lower serum malondialdehyde concentration, hematocrit and heterophil to lymphocyte ratio when compared to the birds fed the control diet. Feeding *Kelussia odoratissima* Mozzaf at 0.5% and 0.75% prevented from right ventricular hypertrophy and led to a significant decline in mortality from PHS.

Conclusion: It can be concluded that *Kelussia odoratissima* Mozzaf is a promising medicinal herb to prevent PHS in broiler chickens by improving blood pressure and antioxidant responses.

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1. Introduction

Extraordinary growth rate of today's broiler chickens has disposed these birds to pulmonary hypertension syndrome (PHS) due to the mismatch between oxygen-demanding muscles and oxygen-supplying organs (i.e. heart and lungs). Over the past several decades, intensive genetic selection for rapid growth has remarkably reduced allometric growth of the heart and lungs in

modern broiler chickens when compared to their chronological counterparts (Havenstein et al., 2003a, 2003b). The susceptibility of modern broilers to PHS is augmented whenever they are raised at high altitudes (more than 2000 m above sea level) where the availability of atmospheric oxygen is limited. Pulmonary arterioles in broilers respond to hypoxia by vasoconstriction and if the situation sustains, broiler develop pulmonary hypertension with subsequent right ventricular failure that finally leads to ascites (Wideman et al., 2013). Pulmonary vascular remodeling incurred by pulmonary hypertension intensifies the problem (Wideman et al., 2011). Research has addressed the impact of nutritional factors including energy (Khajali and Fahimi, 2010), protein (Izadinia et al., 2010; Khajali et al., 2011b, 2014; Behrooz et al., 2012; Saki et al., 2013), electrolytes (Saedi and Khajali, 2010; Khajali and Saedi, 2011), feed texture (Baghbanzadeh and Decuyper, 2008), and feed restriction (Khajali et al., 2007; Ozkan et al., 2010) on the development of PHS. However, the effects of herbal medicine in the prevention and control of PHS in

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broiler chickens have not been adequately studied. It is of particular importance to know that the cost to the worldwide broiler chicken industry due to PHS has been estimated to be in excess of hundred billion \$US per year.

Kelussia odoratissima Mozzaf (wild celery; mountain celery) is a medicinal herb that belongs to the family of Umbelliferae and grows at high altitudes (more than 2000 m). This self-growing plant, which grows to height of 150–200 cm, is exclusively found in Iran. In spring seasons, the plant is harvested from its habitat and presented in the market. It has long been recognized as a medicinal plant by indigenous people and local tribes to treat hypertension and inflammation (Le and Elliot, 1991, 1992). The essential oil of *Kelussia odoratissima* Mozzaf consists of bioactive components including Phthalides, mainly z-ligustilide (3-butylidene-4,5-dihydrophthalide) (37.3% of total bioactive components) and sesquiterpenes (14% of total bioactive components) (Shojaei et al., 2011). Alcoholic extract of *Kelussia odoratissima* Mozzaf also contains considerable amount of flavonoids (12.2 mg/g) and polyphenols (102 mg/g), which confer antioxidant properties to the plant (Rabbani et al., 2011; Sajjadi et al., 2012; Pirbalouti et al., 2013). Research has shown that z-ligustilide plays a significant role in arterial smooth muscle relaxation. It also has antiproliferative effects on smooth muscle cells (Kuang et al., 2008) and can attenuate hypertension by inhibiting vascular remodeling (Lu et al., 2006). This is worthy to note that hypoxic broilers develop vascular remodeling in the lungs, which reduces their pulmonary vascular capacity (Wideman et al., 2011). Research has also shown that the antioxidant potential of *Kelussia odoratissima* Mozzaf is comparable to α -tocopherol and butylated hydroxytoluene (BHT) when assayed by various methods (Ahmadi et al., 2007).

Potential role of free radicals in the pathogenesis of PHS in broilers has been addressed (Bottje and Wideman, 1995). Considering the facts that some compounds in *Kelussia odoratissima* Mozzaf have strong antioxidant potential and some of its compounds have lowering blood pressure effects, the objectives of the present study were to examine the effects of different levels of this medicinal plant in preventing pulmonary PHS in broiler chickens. To the best of our knowledge, there has been no report on the effect of *Kelussia odoratissima* Mozzaf on pulmonary hypertension in birds.

2. Materials and methods

2.1. Birds and experimental facility

The experiment was conducted in the experimental facility of Shahrekord University, Shahrekord, Iran, which had an altitude of 2100 m above sea level. The study was carried out in strict accordance with the recommendations in the Guide for the Care and Use Committee of Shahrekord University.

A total of 208 day-old male broilers (Ross 308) were randomized across 16 floor pens measuring 1.5 m² (13 birds per pen). Each pen was equipped with a bell drinker and a feed trough. All chicks were raised up to 5 days of age on a commercial broiler diet. Following an eight-hour fast and removing runts, five-day-old chicks were allocated to pens so that all pens had equal average body weights (89 ± 1.5 g). The temperature of the experimental house was set at about 32 °C during week 1, 25 °C for week 2, 20 °C for week 3, and 15 °C thereafter as previously described (Khajali et al., 2007). All chicks had free access to feed and water and provided with 23 h light and 1 h dark throughout the trial.

2.2. Treatments

A control diet based on corn and soybean meal were formulated for the starting (1–3 weeks of age) and growing (3–6 weeks of age) stages according to NRC (1994) recommendations (Table 1). Three additional diets were prepared by substituting 0.25%, 0.50%, and 0.75% *Kelussia odoratissima* Mozzaf for wheat bran in the control diet. *Kelussia odoratissima* Mozzaf was collected from Koohrang region located in Chahrmahal-Va-Bakhtiari province, Iran. The leaves and shoots of *Kelussia odoratissima* Mozzaf were cut, air-dried, and ground for use in the experimental diets. Proximate analysis of these samples revealed 9% CP, 7.5% CF, 1.8% Ca, 0.6% P, 0.04% Na, 0.04% Cl, 0.28% S, and 2% K.

2.3. Measurements

Body weight of birds was obtained at 21 and 42 days of age. Body weight gain and feed intake were calculated for the 1- to 21-day, 21- to 42-day, and 1- to 42-day periods. Feed:gain data, corrected for mortality body weights, was also calculated for the aforementioned periods. At 42 days 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 collected from the brachial vein and centrifuged at 2500g for 10 min to obtain sera. Serum samples were used for the determination of nitric oxide (NO) and malondialdehyde (MDA). Serum NO was measured according to the method described by Behrooj et al. (2012). Serum MDA concentration as biomarker of oxidative stress was assayed by the method of Nair and Turner (1984).

Table 1

Composition of the basal diet to which *Kelussia odoratissima* Mozzaf added for broilers during starter and grower stages.

| Item (% unless noted) | Starter (1–21 days) | Grower (21–42 days) |
|---------------------------------|---------------------|---------------------|
| Corn | 47 | 55.3 |
| Soybean meal (44% CP) | 37.2 | 32.8 |
| Fish meal (60% CP) | 3.6 | 1 |
| Soy oil | 7.5 | 4.6 |
| Dicalcium phosphate | 1.3 | 1.2 |
| Oyster shell | 1.45 | 1.5 |
| Salt | 0.35 | 0.3 |
| dl-Methionine | 0.1 | 0.1 |
| L-Lysine | – | – |
| Mineral supplement ^a | 0.25 | 0.25 |
| Vitamin supplement ^b | 0.25 | 0.25 |
| Wheat bran ^c | 1 | 1 |
| <i>Kelussia odoratissima</i> | – | – |
| Calculated composition | | |
| AME (kcal/kg) | 3200 | 3200 |
| CP | 23 | 20 |
| Met | 0.5 | 0.4 |
| Met+Cys | 0.85 | 0.73 |
| Lys | 1.3 | 1.08 |
| Thr | 1 | 0.92 |
| Arg | 1.45 | 1.28 |
| Ca | 1 | 0.9 |
| Available P | 0.45 | 0.35 |
| Na | 0.18 | 0.15 |
| Cl | 0.27 | 0.29 |
| K | 0.91 | 0.92 |
| Na+K-Cl (mEq/kg) | 235 | 236 |

^a Provided the following per kilogram of diet: vitamin A (*trans* retinyl acetate), 3600 IU; vitamin D3 (cholecalciferol), 800 IU; vitamin E (dl- α -tocopheryl acetate), 7.2 mg; vitamin K3, 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.

^b Provided the following per kilogram of diet: Mn (from MnSO₄·H₂O), 40 mg; Zn (from ZnO), 40 mg; Fe (from FeSO₄·7H₂O), 20 mg; Cu (from CuSO₄·5H₂O), 4 mg; I (from Ca(IO₃)₂·H₂O), 0.64 mg; Se (from sodium selenite), 0.08 mg.

^c *Kelussia odoratissima* powder was replaced for wheat bran to provide the levels of 2.5, 5 and 7.5 g/kg.

Table 2
Details of the primers used for quantitative real time PCR analysis of chicken mRNAs.

| Target | Primers | PCR product (bp) | Accession no. |
|----------------|--|------------------|---------------|
| β -Actin | 5'-AGCGAACGCCCCAAAGTCT-3' 5'-AGCTGGGCTGTGCCTTACA-3' | 139 | NM_205518.1 |
| SOD1 | 5'-CACTGCATCATTGGCCGTACCA-3' 5'-GCTTGACACGGAAGAGCAAGT-3' | 223 | NM_205064.1 |
| iNOS | 5'-AGGCCAAACATCCTGGAGGTC-3' 5'-TCATAGAGACGCTGCTGCCAG-3' | 371 | U46504 |
| ET-1 | 5'-GGACGAGGAGTGCGTGATT-3' 5'-GCT CCA GCA AGC ATC TCT G-3' | 141 | XM418943 |

SOD1: superoxide dismutase 1; iNOS: inducible nitric oxide synthase; ET-1: endothelin 1; bp: base pair.

Moreover, samples of blood were collected in microhematocrit tubes for measuring hematocrit. An aliquot of blood was also obtained on glass slides to prepare the blood smear for the determination of differential leukocyte count. Following the May–Grunwald and Giemsa staining, 100 leukocytes, including granular (heterophils) and nongranular (lymphocytes) were enumerated and the heterophil to lymphocyte ratio (H:L) was calculated. All chemical reagents were obtained from Sigma-Aldrich Co. (Sigma-Aldrich Co., St. Louis, MO, USA).

After the blood collection, the birds were killed. Data obtained at processing included live body weight, hot carcass weight, breast weight, and thigh weight. The hearts were also removed and the ventricles were dissected and weighed to calculate the right-to-total ventricular weight ratio (RV:TV ratio). The RV:TV is indicative of pulmonary hypertension (Khajali et al., 2011a). In addition, mortality from PHS was checked daily throughout the trial and whenever the RV:TV was greater than 0.25 are considered as pulmonary hypertension (Saedi and Khajali, 2010).

2.4. Quantitative real time PCR analysis

At 42 days of age, 8 chickens from the control group and the group received the highest level of *Kelussia odoratissima* Mozzaf (0.75%) were randomly selected, weighed and killed by decapitation. The hearts were harvested and the right ventricles dissected and immediately frozen in liquid nitrogen and stored at -70°C for subsequent RNA analysis. Total RNA from the right ventricles was extracted using RNX-Plus reagent (Sinaclon Bioscience, Tehran, Iran). Homogenized tissue (100 mg) was prepared in digestion buffer. The homogenate was mixed with chloroform. After centrifuging the mixture, total RNA settled in the upper aqueous phase. Following precipitation with isopropanol, the RNA pellet was rinsed with 75% ethanol. The samples of RNA were resuspended in DEPC-treated water. To remove eventual residual DNA, the RNA was treated by DNase (Sinaclon Bioscience, Tehran, Iran); the RNA was then measured and qualified by spectrophotometry. Only RNA with an absorbance ratio (A260/A280) of > 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), inducible nitric oxide synthase (iNOS), endothelin 1 (ET-1) and β -actin transcripts were determined by real-time reverse transcriptase (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, iNOS 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 2. PCRs were carried out in a real-time PCR cycler (Rotor Gene Q 6000, Qiagen, USA) in three replicates for each sample of ventricles. One microliter cDNA was added to the 10 μl of SYBR[®]

Table 3

Effects of dietary levels of *Kelussia odoratissima* Mozzaf on broiler growth performance

| | Dietary level of <i>Kelussia odoratissima</i> (%) | | | | SEM |
|----------------------|---|-------------------|--------------------|-------------------|-------|
| | Control (0) | 0.25 | 0.5 | 0.75 | |
| Feed intake (g/bird) | | | | | |
| 5–21 days of age | 1040 | 1089 | 1081 | 1082 | 23.5 |
| 21–42 days of age | 3362 | 3238 | 3232 | 3338 | 60.4 |
| 5–42 days of age | 4402 | 4327 | 4314 | 4420 | 70.2 |
| Weight gain (g/bird) | | | | | |
| 5–21 days of age | 663 | 702 | 712 | 705 | 17.9 |
| 21–42 days of age | 1691 ^b | 1679 ^b | 1711 ^{ab} | 1796 ^a | 28.1 |
| 5–42 days of age | 2354 ^b | 2381 ^b | 2422 ^{ab} | 2501 ^a | 33.5 |
| Feed:gain (g:g) | | | | | |
| 5–21 days of age | 1.56 | 1.56 | 1.52 | 1.54 | 0.034 |
| 21–42 days of age | 1.99 ^a | 1.93 ^a | 1.89 ^{bc} | 1.86 ^c | 0.017 |
| 5–42 days of age | 1.87 ^a | 1.82 ^a | 1.78 ^b | 1.77 ^b | 0.017 |

Superscripts in the same row with different letters are significantly different ($P < 0.05$).

Premix Ex Taq II Mix and 0.5 μM 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 done and used for quantitative objectives. Gene expression data were normalized to β -actin. Data were analyzed using LinRegPCR software version 2012.0 (Amsterdam, Netherland), to give the threshold cycle number and reaction efficiency (Ruijter et al., 2009). Relative transcript levels and fold changes in transcript abundance were calculated using efficiency adjusted Paffl methodology (Dorak, 2006).

2.5. Statistical analysis

Results were compared by GLM using SAS (2007) software in a completely randomized design. When there was sampling within pens, data were subjected to a nested design. The statistical model used for growth performance data was $Y_{ij} = \mu + T_i + e_{ij}$. For other variables, the model was $Y_{ijk} = \mu + T_i + e_{ij} + \varepsilon_{ijk}$. In these models, Y_{ij} and Y_{ijk} are observations; μ is the general location parameter (i.e., the mean); T_i is the effect for being in treatment i ; e_{ij} is random error; and ε_{ijk} is subsampling error. Means were separated by Duncan's multiple range test.

3. Results

Effects of dietary levels of *Kelussia odoratissima* Mozzaf on broiler growth performance is shown in Table 3. Body weight gain and feed: gain responses significantly ($P < 0.05$) improved when *Kelussia odoratissima* Mozzaf was included in broiler diets at 0.75% in the growing stage and throughout the trial. However, no significant effect was

observed among the treatments in terms of feed intake in all feeding stages.

Table 4 indicates blood and serum variables of broilers received different levels of *Kelussia odoratissima* Mozzaf in the feed. Broilers received *Kelussia odoratissima* Mozzaf at dietary levels of 0.5% and 0.75% had significantly ($P < 0.05$) higher concentrations of NO though significantly ($P < 0.05$) lower concentrations of MDA than that of the birds fed the control diet. Feeding *Kelussia odoratissima* Mozzaf at 0.5% and 0.75% caused a significant ($P < 0.05$) reduction in heterophil to lymphocyte ratio and hematocrit when compared to the control.

The expression of SOD1, iNOS and ET-1 genes in the heart of broiler chickens has been affected by feeding *Kelussia odoratissima* Mozzaf to broiler chickens (Table 5). Superoxide dismutase 1 has been highly over-expressed in broilers fed *Kelussia odoratissima* Mozzaf at 0.75%. Inducible nitric oxide synthase was also highly over-expressed in the right ventricle of birds fed *Kelussia odoratissima* Mozzaf. On the other hand, *Kelussia odoratissima* Mozzaf significantly suppressed the expression of ET-1.

Table 6 depicts the carcass characteristics of broilers fed with different levels of *Kelussia odoratissima* Mozzaf and slaughtered at 42 days of age. Carcass, breast and thigh yields were not affected by dietary treatments. However, inclusion of *Kelussia odoratissima* Mozzaf in broiler diets significantly ($P < 0.05$) reduced the proportions of liver, heart and abdominal fat when compared to the control. In addition, inclusion of *Kelussia odoratissima* Mozzaf in broiler diets at 0.5% and 0.75% significantly ($P < 0.05$) declined the RV:TV ratio.

Table 7 shows the cumulative mortality of PHS in broilers fed with different levels of *Kelussia odoratissima* Mozzaf and reared up to 42 days of age. Feeding *Kelussia odoratissima* Mozzaf at 0.5% and 0.75% caused a significant ($P < 0.05$) reduction in PHS mortality.

4. Discussion

Significant improvements in body weight gain and feed:gain due to the inclusion of *Kelussia odoratissima* Mozzaf in broiler diets

Table 4
Effect of dietary *Kelussia odoratissima* Mozzaf on serum and blood variables in broiler chickens measured at 42 days of age.

| | Dietary level of <i>Kelussia odoratissima</i> (%) | | | | |
|---|---|---------------------|--------------------|--------------------|-------|
| | Control (0) | 0.25 | 0.5 | 0.75 | SEM |
| Plasma nitric oxide ($\mu\text{mol/L}$) | 15.65 ^b | 21.17 ^{ab} | 22.74 ^a | 23.44 ^a | 2.023 |
| Malonedialdehyde ($\mu\text{mol/L}$) | 3.72 ^a | 2.78 ^{ab} | 2.17 ^b | 2.14 ^b | 0.344 |
| Heterophils to lymphocyte (%) | 0.84 ^a | 0.74 ^{ab} | 0.62 ^{bc} | 0.59 ^c | 0.044 |
| Hematocrit (%) | 43.8 ^a | 40.4 ^b | 39.1 ^b | 38.5 ^b | 0.99 |

Superscripts in the same row with different letters are significantly different ($P < 0.05$).

Each mean represents values from 8 replicates.

Table 5
Effect of *Kelussia odoratissima* Mozzaf on expression of SOD1, iNOS and ET-1 genes in the right ventricle of broiler chickens measured at 42 days of age.

| | SOD1 | iNOS | ET-1 |
|---|----------------------|---------------------|---------------------|
| Control (T1) | 0.9540 ^b | 0.0173 ^b | 2060.6 ^a |
| 0.75% <i>Kelussia odoratissima</i> (T2) | 13.9920 ^a | 0.4193 ^a | 561.3 ^b |
| T2/T1 ratio | 14.67 | 24.24 | 0.272 |
| SEM | 0.913 | 0.039 | 11.67 |
| P-value | 0.0001 | 0.0001 | 0.0001 |

SOD1: superoxide dismutase 1; iNOS: inducible nitric oxide ET-1: endothelin 1
Number of observation = 15

Table 6
Effect of *Kelussia odoratissima* Mozzaf on carcass characteristics of broilers at 42 days of age.

| Item (% unless noted) | Dietary level of <i>Kelussia odoratissima</i> (%) | | | | SEM |
|-----------------------|---|--------------------|--------------------|--------------------|-------|
| | Control (0) | 0.25 | 0.5 | 0.75 | |
| Carcass yield | 67.6 | 67.8 | 70.3 | 70.1 | 1.15 |
| Breast yield | 36.1 | 36.1 | 36.7 | 36.5 | 0.99 |
| Thigh yield | 32.0 | 31.2 | 32.1 | 31.8 | 0.49 |
| Abdominal fat | 1.64 ^a | 1.07 ^b | 1.09 ^b | 1.10 ^b | 0.108 |
| Liver | 2.39 ^a | 2.16 ^b | 2.11 ^b | 2.02 ^b | 0.073 |
| Heart | 0.68 ^a | 0.59 ^b | 0.56 ^b | 0.58 ^b | 0.018 |
| RV:TV | 0.275 ^a | 0.264 ^a | 0.205 ^b | 0.215 ^b | 0.015 |

Superscripts in the same row with different letters are significantly different ($P < 0.05$).

Each mean represents values from 8 replicates.

RV:TV – right ventricle to total ventricle weight ratio.

Table 7
Effect of dietary *Kelussia odoratissima* Mozzaf on cumulative mortality from pulmonary hypertension syndrome (PHS) in broiler chickens reared up to 42 days of age.

| PHS mortality (%) | Dietary level of <i>Kelussia odoratissima</i> (%) | | | | SEM |
|-------------------|---|--------------------|-------------------|-------------------|------|
| | Control (0) | 0.25 | 0.5 | 0.75 | |
| | 32.7 ^a | 21.2 ^{ab} | 13.4 ^b | 13.4 ^b | 5.33 |

Superscripts in the same row with different letters are significantly different ($P < 0.05$).

can be attributed to the higher percentage of healthy birds in this groups. The RV:TV ratio is an index of pulmonary hypertension in chickens so that the RV:TV values greater than 0.25 regarded as pulmonary hypertension (Izadinia et al., 2010; Saedi and Khajali, 2010). The mean value of RV:TV was greater than 0.25 in the control group indicating that numbers of birds in this group had been suffering from pulmonary hypertension. Improvements in growth performance of birds in the groups received *Kelussia odoratissima* Mozzaf can also be attributed to the naturally-occurring polyphenols in *Kelussia odoratissima* Mozzaf. The plant polyphenols including flavonoids and non-flavonoids exhibit a broad spectrum of beneficial biological properties such as growth-promoting, antioxidative, sedative, antibacterial and antiviral actions (Kosmider and Osiecak, 2004; Surai, 2014).

Increased serum concentration of NO as a result of feeding *Kelussia odoratissima* Mozzaf to broilers is due to the over-expression of iNOS gene in broiler's heart. It has been demonstrated that iNOS gene is normally expressed in the heart of broiler chickens and contributed in normal NO production in myocytes. NO is an important regulator of cardiac function by involvement in the control of myocardial energetics, myocardial regeneration, hypertrophic remodeling and improvement of ventricular diastolic distensibility (Belge et al., 2005). It has been suggested that impaired NO synthesis and local reduction of iNOS gene expression in the heart ventricles are involved in the pathophysiology of cardiac failure in broilers with pulmonary hypertension (Hassanpour et al., 2009).

On the other hand, feeding *Kelussia odoratissima* Mozzaf at levels greater than 0.25% caused significant reductions in circulatory level of MDA. MDA is a biomarker of lipid oxidation in the body and it is an index of oxidative stress. It is worth noting that chickens are very susceptible to oxidative stress because firstly they have a metabolic rate that is approximately 2–2.5 times higher than mammals of comparable body size (Lindstedt and Calder, 1976). The higher the metabolic rate, the greater the production of ROS. Second, birds have a body temperature that is about 3 °C higher than in mammals (Holmes and Austad, 1995), which promotes the production of ROS.

Finally, chickens have a high blood sugar concentration that is at least twice as high as that of mammals (Braun and Sweazea, 2008). It is evident that antioxidant defense is crucial to broiler chickens. A number of compounds in *Kelussia odoratissima* Mozzaf contribute to the productive roles against oxidative stress and reduced concentration of MDA. Z-Ligustilide has been reported to increase the activities of the antioxidant enzymes glutathione peroxidase and superoxide dismutase, which counteract the oxidative stress (Peng et al., 2007). Additionally, ferulic acid has ferric reducing ability and by involvement in $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ systems exerts antioxidant activity (Sajjadi et al., 2012). Flavonoids can also prevent oxidative stress by the following mechanisms: direct scavenging of reactive oxygen species (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). Significant reductions observed in the H:L ratio and hematocrit in birds fed *Kelussia odoratissima* Mozzaf are in accordance with decreased oxidative stress. The H:L ratio is an index of stress in the chicken (Khajali et al., 2008). Therefore, feeding *Kelussia odoratissima* Mozzaf suppresses ROS production and alleviates the oxidative stress of birds, which led to improvements in growth performance and reductions in MDA level and H:L ratio.

Abdominal fat deposition was significantly reduced in chickens fed *Kelussia odoratissima* Mozzaf. It is clear that *Kelussia odoratissima* Mozzaf has lipolytic effect. Lipolytic effect of *Kelussia odoratissima* Mozzaf is attributed to flavonoids. Hypolipidemic effects of flavonoids have been well documented (Anila and Vijayalakshmi, 2002; Chen and Li, 2007). Reduction in the proportion of liver weight to live body weight in chickens fed *Kelussia odoratissima* Mozzaf was in line with decreased lipogenesis as appeared in reduced abdominal fat. Liver is the principal site of lipogenesis in the chicken (Behrooj et al., 2012) and decline in the relative weight of liver reflects lower lipogenesis due to the inclusion of *Kelussia odoratissima* Mozzaf in broiler diets. The proportion of heart weight relative to live body weight and RV:TV have been reduced by feeding *Kelussia odoratissima* Mozzaf to birds. These observations confirm that *Kelussia odoratissima* Mozzaf prevents from heart hypertrophy and particularly right ventricular hypertrophy. This is evident from the right ventricular weight ratio (RV:TV). It is evident that birds fed in the control group (RV:TV more than 0.25) are in pre-ascitic condition and this situation has been improved when birds fed *Kelussia odoratissima* Mozzaf at 0.5% and 0.75%. In this regard, a significant decline in mortality from PHS was observed in the groups received *Kelussia odoratissima* Mozzaf at 0.5% and 0.75% when compared to the control group.

Kelussia odoratissima Mozzaf significantly promoted the overexpression of SOD in the heart of chickens. Research has shown that overexpression of SOD reduces hypertension, increases availability of NO and endothelium-dependent relaxation in different models of hypertension (Chu et al., 2003). This finding explains significant reduction in the incidence of PHS in birds fed *Kelussia odoratissima* Mozzaf. Likewise, previous reports show that the vascular remodeling in lung vessel beds contributes to mortality of broilers with pulmonary hypertension syndrome (Wideman et al., 2011; Wideman and Hamal, 2011). Z-ligustilide prevents the proliferation of vascular smooth muscle cells and inhibits thickening of the intima and narrowing of the vessels (Lu et al., 2006). Thus, z-ligustilide contributes to be the effective agent in preventing cardiovascular diseases (Lu et al., 2006). Z-ligustilide has also vasorelaxant potential and considering the fact that z-ligustilide is the main constitute of *Kelussia odoratissima* Mozzaf, this medicinal plant could effectively prevent PHS in broiler chickens. Furthermore, flavonoids as another constitute of *Kelussia odoratissima* Mozzaf have endothelium-independent vasodilating effects and by possessing lowering blood pressure potential (Mladěnka et al., 2010; Jorge et al., 2013) further improved cardiovascular function and helped to prevent PHS. It is worth noting that

the vasodilatory effect of some flavonoids may be intensified by overproduction of NO synthesis (Mladěnka et al., 2010).

Significant decrease in the expression of ET-1 by feeding *Kelussia odoratissima* Mozzaf further suggests the potential of this medicinal herb in preventing pulmonary hypertension. The link between ET-1 and pulmonary hypertension is well established (Stewart et al., 1991).

5. Conclusion

In conclusion, *Kelussia odoratissima* Mozzaf could significantly prevent PHS in broiler chickens reared at high altitudes. Beneficial effects of this medicinal plant are attributed to vasorelaxant and antioxidant actions that mediated through ligustilides and flavonoids. Therefore, *Kelussia odoratissima* Mozzaf is a promising medicinal herb to prevent pulmonary hypertension in broiler chickens reared at high altitude.

Contribution

Behnam Ahmadipour – implementation of the experiments.
Hossein Hassanpour – co-advisor; helped with gene expression techniques.
Ebrahim Asadi – statistical analysis.
Fereidoon Khajal – lab assays and interpretation of data.
Fariba Rafiei – design of the experiments.
Fariborz Khajali – principal advisor.

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