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Hawthorn (Crataegus Oxyacantha) Extract in the Drinking Water of Broilers on Growth and Incidence of Pulmonary Hypertension Syndrome (PHS)

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ABSTRACT

Hawthorn extract has been used for ameliorating cardiac disorders and pulmonary hypertension. Flavonoids and oligomeric proanthocyanidins are considered to be responsible for the positive health effects of hawthorn extract. The effect of Hawthorn extract in the water supply on feed intake, growth, carcass traits, internal organ weight, cardiac indices, the concentration of serum proteins and the incidence of pulmonary hypertension syndrome was evaluated in broiler chickens. At one day-of-age, 225 chickens were assigned to one of three experimental groups where 0, 0.1 and 0.2 ML of hawthorn extract was added per one liter of drinking water. Feed intake, live weight gain and carcass weight increased when hawthorn extract was included in the drinking water at a level of 0.1 and 0.2 ML/L (p<0.05). Compared to no extract, the addition of Hawthorn extract in the drinking water (p<0.05) reduced the proportion of the body attributed to abdominal fat, liver and heart (p<0.05), and decreased the percentage of birds that died or showed clinical symptoms of pulmonary hypertension syndrome (p<0.05). Serum protein concentration was (p<0.05) higher in chickens that received the hawthorn extract in the drinking water compared to no addition of extract in the drinking water. Hawthorn extract has shown potential for use as a herbal medicine to aid in the prevention of physiological cardiac disorders and pulmonary hypertension in chickens.

INTRODUCTION

Ascites as a result of pulmonary hypertension syndrome (PHS) and its subsequent physiological disorders lead to mortality and slow growth of broiler chickens especially at high altitude areas with low oxygen pressure (Ahmadipour *et al.*, 2015). Intensive genetic selection for growth rate in broiler chickens has created a situation where the development of the heart and lung is unsynchronized, increasing the sensitivity of broilers to PHS (Sharifi *et al.*, 2015). Advanced pulmonary hypertension and right ventricular failure (RVF) are the final impacts of hypoxia and vaso-disorders which leads to ascites and PHS (Wideman, *et al.*, 2013).

Hawthorn (*Crataegus oxyacantha*) is a member of the *Rosaceae* family which grows in Asia and other parts of the world (Chang *et al.*, 2002). Excluding the fruit, parts of this medical plant are traditionally used to treat cardiac disorders and cardiovascular problems and used as antihypertensive or antiatherosclerotic agent (Chang *et al.*, 2007). Other positive implications of hawthorn extract have included improving blood circulation, elimination of blood stasis, improved recovery after heart failure and myocardial injuries, and amelioration of hypertension, arrhythmia and inflammation (Chen *et al.*, 1998; Kao *et al.*, 2005; Brixius *et al.*, 2006). The most important chemical constituents in Hawthorn extract are flavonoids and oligomeric proanthocyanidins (OPCs) which are present at a concentration of 1-3% of the dry matter (Chang *et al.*,

2002; Kao *et al.*, 2005; Barros *et al.*, 2011; Kirakosyan *et al.*, 2003). Other bioactive components which have been found at varying concentrations in hawthorn extracts include: triterpene acids (Kao *et al.*, 2007), organic acids (Liu *et al.*, 2011), sterols, and cardio-active amines (Long *et al.*, 2006). Mostly, hawthorn extract is recognized for its flavonoid and OPC content (Long *et al.*, 2006; Surai *et al.*, 2014) and these compounds reported to have several pharmacological effects (Kirakosyan *et al.*, 2003; Chang *et al.*, 2007).

Because the bioactive compounds in *Crataegus oxyacantha* have known antioxidant activity with positive pharmacological effects and in particular the capacity to moderate serological and immunological parameters and mediate the negative effects of PHS, the objectives of the present study were to examine the effects of different levels of Hawthorn extract in drinking water (HE) of broiler chickens raised in high altitude conditions in respect to the incidence of PHS and associated physiological disorders.

MATERIALS AND METHODS

Birds and experimental facility

This experiment was performed in the experimental facility of Shahrekord University, Iran in accordance with the recommendations in the Guide for the Care and Use Committee of Shahrekord University. The effect of Hawthorn extract in the water supply on feed intake, growth, carcass traits, internal organ weight, cardiac indices, incidence of pulmonary hypertension syndrome and the concentration of serum enzymes and proteins was evaluated in the reared broiler chickens. A total of 225 unsexed broiler chicks (Ross 308) were raised under commercial conditions according to Ross 308 broiler management manual (2009) for a 42-day experimental period. At one-day of age the birds were assigned to one of 15 floor pens. The birds had an average body weight of 702 ± 16g. The birds were reared at high altitude (2,100 m above sea level) and the temperature of the experimental shed was set at about 32ºC for the first week then reduced to 29ºC for week 2 to 4, and finally fixed at 22ºC until the end of the trial. All birds had free access to feed and water and provided with 23h light and 1h dark throughout the trial.

Treatments

For all experimental groups the diet was based on corn and soybean meal formulated and balanced for nutrients for starting (1–3 weeks of age) and growing (3–6 weeks of age) periods according to the recommendations for broiler chickens (National Research Council 1994). Treatments were prepared by adding 0, 0.1 and 0.2 ML of Hawthorn (*Crataegus oxyacantha*) extract per liter of drinking water. The 0 ML/L treatment was the control group in this experiment. According to the manufacturer, the Hawthorn extract used contained biologically active flavonoid compounds (polyphenols) such as anthocyanidins and proanthocyanidins. Each ML of Hawthorn extract (Iran-Darouk Pharmacy Co, Iran, Tehran, Production Code: 3067-88-02) contained 2.5 mg of total flavonoids compounds in form of hyperoside.

Measurements

Feed intake (FI), average daily gain (ADG) and feed conversion ratio (FCR) were calculated as a daily mean for the 42-day period. At the end of the trial, 10 birds per treatment were selected for blood sampling 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 serum enzyme concentration including aspartate amino transferase, alanine amino transferase and alkaline phosphatase. Serum protein concentrations were also determined including total protein, albumin and globulin using an auto-analyser (Model Biotecnica BT-3000, Biotecnica Instruments SpA, Germany).

After the blood collection, the selected birds were euthanized and body weight, hot carcass weight, breast, thigh, liver, heart and abdominal fat weights were obtained. For calculating the right-to-total ventricular weight ratio (RV: TV ratio), the heart was also dissected and the ventricles weighed. The RV:TV is an important index for evaluating pulmonary hypertension. Pulmonary hypertension is said to be evident when the RV:TV is greater than 0.25 (Ahmadipour *et al.*, 2015).

Statistical analysis

Results were analyzed using GLM of the SAS (2007) software. Data were subjected to a nested design due to potential sampling effects within pens. The statistical model used for growth traits without sampling effects was $Y_{ij}=\mu+T_i+e_{ij}$. For carcass traits, organ weights and blood parameters which had sampling effects, 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 mean; T_i is the effect for being in treatment i; e_{ij} is random error; and ε_{ijk} is sub sampling error. Significance was considered to occur when $p<0.05$ and means were separated by Duncan's multiple range test.

RESULTS

Effects of different levels of Hawthorn extract on chicken daily growth performance are shown in Table 1. Feed intake, ADG and FCR (p<0.05) improved when Hawthorn extract was included in drinking water at a level of 0.1 and 0.2 ML throughout the trial.

Table 1 – Effects of Hawthorn extract in drinking water on mean growth performance of Ross 308 broiler chickens

	Concentration of hawthorn extract in drinking water (ML/L)			
Item	0	0.1	0.2	SEM
FI (g/bird/day)	94.82^{b}	97.60^{a}	96.61^{ab}	1.35
ADG (g/bird/day)	48.74^{b}	53.33^{a}	54.59^{a}	0.98
FCR (g/g)	1.95^{a}	1.83^{b}	1.77^{c}	0.03

Superscripts in the same row with different letters are significantly different (p<0.05).
FI=Feed Intake; ADG= Average Daily Gain; FCR=Feed Conversion Ratio.

Live weight and hot carcass yield was higher and the proportion of abdominal fat in the body weight was lower in broilers that had 0.1 and 0.2ML/L Hawthorn extract in the drinking water compared to the control group (p<0.05; Table 2).

Table 2 – Effect of Hawthorn extract in the drinking water on carcass traits of Ross 308 broiler chickens[1]

	Concentration of hawthorn extract in drinking water (ML/L)			
Item (% of body weight unless noted)	0	0.1	0.2	SEM
Live body weight (gram)	2101.3^{b}	2152.5^{a}	2260.1^{a}	44.48
Hot carcass yield	77.83^{b}	80.73^{a}	80.77^{a}	1.16
Breast yield	35.27	35.05	36.35	0.87
Thigh yield	30.21	30.54	30.22	0.45
Abdominal fat	1.39^{a}	1.14^{b}	1.05^{b}	0.10

Superscripts in the same row with different letters are signficantly different (p<0.05).
[1]Each mean represents values from 10 replicates.

The percentage that the heart and liver comprised of the body weight was lower and the percentage as spleen and bursa of fabricus was greater in broilers that had 0.1 and 0.2 ML/L of Hawthorn extract in the drinking water compared to the control group (p<0.05; Table 3).

Table 3 – Effect of Hawthorn extract in the drinking water on internal organ weights of Ross 308 broiler chickens[1]

	Concentration of hawthorn extract in drinking water (ML/L)			
Item (% of body weight unless noted)	Control (0)	0.1	0.2	SEM
Heart	0.83^{a}	0.71^{ab}	0.63^{b}	0.06
Liver	2.88^{a}	2.52^{b}	2.26^{c}	0.07
Spleen (gram)	2.44^{b}	2.66^{b}	3.15^{a}	0.17
Bursa of fabricius (gram)	1.99^{c}	2.25^{b}	2.80^{aa}	0.13

Superscripts in the same row with different letters are significantly different (p<0.05).
[1]Each mean represents values from 10 replicates.

The broilers that had drinking water with 0.1 and 0.2 ML/L of Hawthorn extract had a lower RV:TV ratio compared to the control group (p<0.05; Table 4). The birds observed to have pulmonary hypertension and the proportion of birds dying from PHS was lower with the inclusion of Hawthorn extract in the drinking water at levels of 0.1 and 0.2 ML/L (p<0.05; Table 4).

Table 4 – Effect of Hawthorn extract in the drinking water on RV: TV ratio and PHS indices of Ross 308 broiler chickens

	Concentration of Hawthorn extract in drinking water (ML/L)			
	0	0.1	0.2	SEM
RV:TV (ratio)	0.32^{a}	0.25^{b}	0.22^{b}	0.02
Birds with Pulmonary hypertension (%)	57.3^{a}	44.7^{b}	36.8^{c}	3.85
PHS mortality (%)	28.7^{a}	22.3^{b}	18.4^{b}	2.88

Superscripts in the same row with different letters are significantly different (p<0.05).
Each mean represents values from 10 replicates.
RV:TV=right ventricle to total ventricle weight ratio.

The concentration of AST, ALT and ALP in chickens that had Hawthorn extract in the drinking water at a level of 0.1 and 0.2 ML/L was lower than the control group (p<0.05; Table 5). The AST/ALT ratio between the experimental groups was not different (Table 5).

Table 5 – Effects of Hawthorn extract in the drinking water on serum enzyme concentration of Ross 308 broiler chickens[1]

Concentration of HE in drinking water (ML/L)	Aspartate Amino Transferase	Alanine Amino Transferase	Alkaline Phosphatase	Aspartate Amino Transferase: Alanine Amino Transferase
0	232.63^{a}	4.26^{a}	2502.51^{a}	54.61
0.1	214.00^{b}	3.56^{b}	2434.14^{a}	60.11
0.2	174.50^{c}	3.02^{c}	2034.26^{b}	57.78
SEM	6.42	0.15	101.51	3.57

Superscripts in the same column with different letters are significantly different (p<0.05).
[1]Each mean represents values from 10 replicates.

Chickens that received Hawthorn extract in the drinking water at a level of 0.1 and 0.2 ML/L had a higher concentration of total protein, albumin and globulin than the control group (p<0.05; Table 6).

Table 6 – Effects of Hawthorn extract in the drinking water on serum protein concentration of Ross 308 broiler chickens[1]

Concentration of HE in drinking water (ML/L)	TPr	Albu	Glob	Albu/Glob
0	3.84b	2.00c	1.84b	1.09b
0.1	4.55a	2.27b	2.28a	0.99b
0.2	4.85a	2.75a	2.10a	1.31a
SEM	0.23	0.11	0.13	0.11

Superscripts in the same column with different letters are significantly different (p<0.05). [1]Each mean represents values from 10 replicates. TPr=: Total Protein; Albu=Albumin; Glob=Globulin; Albu/Glob= The ratio of Albumin to Globulin.

DISCUSSION

Improvements were observed in growth (FI, ADG and FCR) and carcass (live body weight and hot carcass yield) characteristics when Hawthorn extract was included in the drinking water at 0.1 and 0.2 ML/L for broilers raised under high altitude conditions. The improved growth performance are likely to be a consequence of the naturally occurring polyphenols (flavonoids) and oligomeric proanthocyanidins (OPCs) in the Hawthorn (*Crateagus oxyacantha)* extract. Polyphenols and flavonoids have been noted to be promotors of growth due to their anti-oxidative and antibacterial properties (Barros *et al.*, 2001; Surai *et al.*, 2014).

Abdominal fat deposition and liver growth was reduced when chickens consumed Hawthorn extract in the drinking water. Hawthorn extract has a lipolytic effect which is attributed to flavonoids and OPCs as well as phenolic compounds (Rajendran *et al.*, 1996). Lipid-lowering effects of bioactive compounds such as flavonoids have been well demonstrated (Rajendran *et al.*, 1996; Anila *et al.*, 2002). Because the liver in the chicken is the major organ of lipogenesis (Sharifi *et al.*, 2015) the reduced liver weight reflects lower lipogenic activity.

An increased spleen weight is considered an indicator of the body combating and trying to ward against diseases or stresses (Corrier *et al.*, 1990; Rezaei *et al.*, 2014). Medicinal plant extracts especially flavonoid extracts inhibit the growth and proliferation of pathogenic micro-organisms and protect the gut epithelium directly (Nofrarias *et al.*, 2006). The reduced incursion of pathogens and microbes into the body which plant extracts such as Hawthorn provides, modify the weight and activity of immune organs such as the bursa of fabricius and thymus indirectly (Rezaei *et al.*, 2014; Fathi*et al.*, 2015).

Hawthorn extract seems to control heart hypertrophy and particularly right ventricular hypertrophy which results in a lower heart weight. The birds of the control group with a RV:TV ratio of 0.32 can be considered to be in a preascitic condition and this was prevented when birds consumed Hawthorn extract in the drinking water because the RV:TV was at 0.25 or below. The effect of the Hawthorn extract on preventing mortality via PHS and Ascites was evident.

Positive pharmacological effects of hyperoside, which is the bioactive constituent of Hawthorn include potent antioxidant and free radical scavenging activities to prevent oxidative damage caused by pulmonary hypertension or cardiovascular injuries (Chang *et al.*, 2007; Barros *et al.*, 2011).

One of the most important ways to diagnose pathological conditions in birds is the serum enzyme activity (Senanayake *et al.*, 2015). Alkaline phosphatase is one of the serum enzymes involved in dephosphorylation and active in the organs of liver, intestine and bones, but the highest activity of this enzyme is in the alkaline environment of the liver. Often, increased levels of alkaline phosphatase indicates liver damage (Sorbi *et al.*, 1999; Senanayake *et al.*, 2015). Higher serum levels in ascitic broilers indicate a higher tissue peroxidation rate which results in liver, lung and heart damage. The Hawthorn extract reduced the serum alkaline phosphatase and it has been reported that the flavonoids and oligometric proanthocyanidins such as those found in Hawthorn have protective effects on hepatocytes by scavenging the free radicals in the body.

Aspartate aminotransferase and alanine aminotransferase are liver enzymes which are released into the blood stream when there is liver damage. These two serum enzymes are the main indicators to diagnose liver damage. The production of free radicals increases in hypoxic conditions which decreased the potency of body antioxidant systems, which eventually lead to oxidative stress and extensive destruction of cell membranes and damage of internal organs (Janeway *et al.*, 2001). In the hypoxic state prior to ascites in chickens the serum levels of aspartate aminotransferase and alanine aminotransferase increase due to a higher demand for oxygen and this increases the incidence of PHS (Fathi *et al.*, 2015).

Higher total serum protein of the birds that received the Hawthorn extract suggests an improved ability of the hepatocytes to synthesize protein and consequently

improves the physiological and immunological responses to disease or biological stressors (Corrier *et al.*, 1990; Rezaei *et al.*, 2014). Serum globulin concentrations along with immunoglobulin (IgG and IgM) are components of total serum immunoglobulin which guard the body against foreign antigens (Jahanian *et al.*, 2009). Higher serum globulin level in the birds that received Hawthorn extract had a potentially better immune status (Janeway *et al.*, 2001; Jahanian *et al.*, 2009) which is further substantiated by the fact that liver percentage and immune organs (spleen and bursa of fabricius) were heavier for weight in with the birds that had access to the Hawthorn extract. The increased albumin level in this study could have positive effects on the colloidal state of blood as well as capillary permeability, which assists in preventing pulmonary hypertensive birds.

CONCLUSION

The findings of this study suggests that antioxidants such as those found in Hawthorn help maintain the health and vitality of broiler chicken raised in high altitude zones. The bioactive flavonoids and OPCs in Hawthorn extracts protect against oxidative stress and lipid peroxidation. For commercial broiler chickens consuming Hawthorn extract in drinking water at a concentration between of 0.1 and 0.2 ML/L, free radical production is suppressed, growth and carcass performance is promoted and the serological and immunological characteristics are improved providing an amelioration of pulmonary hypertensive and ascitic chickens.

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AUTHOR CONTRIBUTIONS

Behnam Ahmadipour and Majid Kalantar conceived and designed the experiments. Behnam Ahmadipour performed the experiments and wrote the manuscript. Seyed Mahdi Hosseini and Li Guo Yang assisted in analyzing the data. Mohammadhassan Kalantar helped to collect the samples and data. Sayed Haidar Abbas Raza and Nicola M. Schreurs provided constructive suggestions for the discussion and reviewed the manuscript for language usage and grammar.

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