The effect of hydroalcoholic extract of angelica (Heracleum persicum) fruit on performance, immune responses, small intestine histology, haematological parameters and carcass characteristics of broiler chickens

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ABSTRACT
This study was carried out to investigate the effects of dietary supplementation of hydroalcoholic extract of Heracleum Persicum fruit (HPE) on performance, immune responses, small intestine morphology, haematological parameters and carcass characteristics of broiler chickens. A total of 300 day-old Ross 308 broiler chicks were used in a completely randomized design with five treatments and five replicates of 12 chicks each. The treatments were comprised of a corn-soybean meal base diet supplemented with five levels of HPE at 0 (control), 100, 200, 300 and 400 mg/kg diet. Average daily feed intake (ADFI) was increased by 100 mg/kg of HPE comparing to control group during 11–24 d (p < 0.05). HPE at the levels of 200, 300 and 400 mg/kg, decreased (p < 0.05) serum cholesterol comparing to control group and showed a linear response to different levels of HPE (p = 0.0007). Also, LDL was decreased by 300 mg/kg of HPE (p < 0.05). The villus height to crypt depth ratio (VH/CD) was increased by 400 mg/kg of HPE, comparing to control group at 42 d (p < 0.05). Also, this ratio showed a linear response to different levels of HPE (p = 0.007). The results of this study indicated that HPE improves performance and intestine morphometrical and haematological parameters of broiler chickens.

1. Introduction

With restrictions on the use of antibiotics as growth promoters, some feed additives have been included in the diet of broiler chickens to improve the performance. Among those used on a wider basis, are organic acids, probiotics, and plant extracts (Chaveerach et al. 2004). Medicinal plants are one of the possible alternatives that show antimicrobial and growth promoting effect in poultry (Windisch et al. 2008). Medicinal plants, such as herbs or their extracts are being investigated as natural sources of biologically important substances that may positively influence poultry health and productivity.

The genus Heracleum includes more than 70 species all around the world (Evans 1996) and is represented in Iran by 10 species which four of them being endemic (Mozaffarian 1996). Heracleum persicum Desf. ex Fisher (Umbelliferae) that is commonly known as Persian Hogweed is an annual plant and native to Iran with a wide distribution especially in northern mountainous regions with an altitude ranging 1500–2500 m (Parsa 1948). This plant has a long reputation as a natural remedy in the Iranian folk medicine, and its fruits are commonly used as a food spice, pickling and food additive (Asgarpanah et al. 2012). A number of chemical constituents, such as alkaloids, volatile substances, terpenoids, triterpenes and furanocoumarins have been isolated from fruit and different part of the plant (Sefidkon et al. 2002; Mojab and Nickavar 2003; Sefidkon et al. 2004; Hajhashemi et al. 2009). Because of the diversity of phytochemicals that are present in this plant, several evidence have shown the antimicrobial (Kousha and Bayat 2012), immunostimulatory (Sharififar et al. 2009; Naeini et al. 2013), lipid-modifying (Panahi et al. 2011), anti-inflammatory (Hajhashemi et al. 2009), anticonvulsant (Sayyah et al. 2005) and antioxidant activity (Souri et al. 2004) of Heracleum persicum.

Some reports indicate the presence of six furanocoumarins and flavonoids in the fruits of Heracleum persicum (Merijanian et al. 1980; Brunton et al. 2006). These compounds have an antioxidant activity especially in hydroalcoholic extract (Souri et al. 2004). The findings of Sharififar et al. (2009) showed that aqueous extract of Heracleum persicum has stimulatory effect on both humoral and cellular immune functions in mice. Short-term dietary supplementation of Heracleum persicum fruit extract showed that this extract can be used as an adjunctive treatment for patients with hypertriglyceridemia (Dadjo et al. 2015). It has been also shown that the hydroalcoholic extract and essential oil of Heracleum persicum have antinociceptive and anti-inflammatory effects in rats and these results support the traditional use of this plant in relieving pain and inflammation (Hajhashemi et al. 2009).

According to the aforementioned results, it could be hypothesized that the administration of angelica extract might boost the beneficial effects on growth performance, small intestine histology, haematological parameters, carcass characteristic and immune responses in broiler chickens.
2. Materials and methods

2.1. Preparation of plant extract

Fruits of *Heracleum persicum* were collected from Khorasan Razavi province (Iran) and were authenticated at the Herbarium of Botany Directorate in Ferdowsi University of Mashhad, Iran. For the preparation of hydroalcoholic extract, each 300 g air-dried and powdered fruits of the plant were macerated with 1500 mL 50% ethanol (1w:5v) with occasional shaking at room temperature for 48 h. Then the ethanol soluble materials were passed through a Whatman No. 41 filter paper and were concentrated using a rotary evaporator at 50°C (Laborota 4000, Heidolph Germany), and then freeze-dried for 24 h to yield HP extract powder. The dried extract was stored at −20°C until use for experimental work.

2.2. Analysis of angelica extract

The GC/MS analyses (Hewlett-Packard 6890) were performed to identify the composition of hydroalcoholic extract of angelica. Gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; coating thickness 0.25 mm). The oven temperature was programmed from 60°C to 280°C at 4°C/min. Helium was used as carrier gas at a flow rate of 2 mL/min. The gas chromatograph was coupled to a Hewlett-Packard 6890 mass selective detector. Components were identified by using different parameters such as retention time, comparison of the mass spectra with those of standards, Wiley 2001 library data of the GC/MS system and literature data (Adams 2001).

2.3. Birds, housing and diets

All procedures used were approved by the Ferdowsi University of Mashhad Animal Care and Use Committee. The experiment was arranged as a completely randomized design with 5 treatments, 5 replicates and 12 birds in each floor pen (initial body weights: 42 ± 1.2 g). Each pen was equipped with a hanging feeder and drinker and used litter, and the birds were allowed to consume feed and water on ad libitum basis. Mash corn-soybean meal base diets were prepared for starter (1–10 d), grower (11–24 d) and finisher (25–42 d) periods (Table 1) to meet or exceed Ross 308 nutrient recommendations (Aviagen 2014). Experimental treatments consisted of five dietary supplemental levels of HPE powder at 0 (control), 100, 200, 300 and 400 mg/kg of basal diet.

The lighting programme was 23L:1D. The temperature was set at 32°C on day one and was gradually decreased by approximately 3°C per week to reach 22°C on d 28 and was remained constant thereafter. The accuracy of the scale for weighing the birds and feed was 10 g. Feeders were filled as much as one-third of their capacity to decrease feed wasting. Probable wasted feed was carefully collected and separated from the litter materials and added to the unconsumed feed at the end of each feeding phase for each pen.

2.4. Experimental procedures

2.4.1. Growth performance and sample collection

All birds were weighed at the beginning and at the end of each experimental period. Feed intake was calculated as the difference between the amount of feed offered and the feed residue at the end of each period. Feed conversion ratio (FCR) was calculated by dividing feed intake by weight gain and corrected for mortality.

2.4.2. Immune response (humoral immunity)

Sheep red blood cells were administered as a test antigen to quantify specific antibody responses. On day 25, two birds were immunized intravenously with 1 mL of 7% suspension of SRBC in 0.85% saline via the brachial vein. Seven days postinjection, the same birds were bled by brachial venipuncture, 3 mL of blood was collected for primary antibody response, and the antigenic challenge was repeated. On day 32, injection of SRBC suspension was repeated in the same birds via the brachial vein and then blood samples were taken at 39 d to measure secondary antibody response. The blood was left at room temperature for 2 h to clot and then placed in a 4°C refrigerator overnight for maximum sera yield. The serum samples were collected and kept frozen at −20°C until serological analysis. Total antibody response, IgM, and IgG were determined using microhemagglutination assay in 96-well plates as previously described (Lepage et al. 1996). Briefly, 2-mercaptoethanol-resistant antibodies (presumably IgG) were determined by incubating 0.05 mL serum with an equal volume of PBS (pH 7.5) and 0.2M 2-mercaptoethanol at 37°C for 30 min prior to the hemagglutination test. The 2-mercaptoethanol-sensitive antibody (presumably IgM) levels were determined by subtracting the 2-mercaptoethanol-resistant antibody titres from the total titres. The antibody titres were expressed as

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**Table 1. Composition and calculated analysis of experimental diets (as-fed basis)**

| Ingredient (g/100 kg) | Starter (1–10 d) | Grower (11–24 d) | Finisher (25–42 d) |
|-----------------------|------------------|------------------|-------------------|
| Maize                 | 51.04            | 53.20            | 53.00             |
| Soybean meal (44% CP) | 42.15            | 37.00            | 36.90             |
| Soybean oil           | 2.38             | 5.80             | 6.56              |
| Dicalcium phosphate   | 1.52             | 1.55             | 1.40              |
| Limestone             | 1.44             | 1.07             | 1.03              |
| Common salt           | 0.30             | 0.47             | 0.41              |
| DL-Methionine (98%)   | 0.42             | 0.28             | 0.20              |
| L-Lysine HCl (78%)    | 0.19             | 0.13             | –                 |
| L-Threonine (99%)     | 0.06             | –                | –                 |
| Vitamin premix^b      | 0.25             | 0.25             | 0.25              |
| Mineral premix^c       | 0.25             | 0.25             | 0.25              |
| Crude protein         | 23.2             | 21.15            | 20.91             |
| Available phosphorus  | 0.48             | 0.45             | 0.42              |
| Calcium               | 0.96             | 0.90             | 0.85              |
| Sodium                | 0.16             | 0.19             | 0.17              |
| Methionine            | 0.77             | 0.61             | 0.52              |
| Methionine + Cystine  | 1.08             | 0.95             | 0.86              |
| Lysine                | 1.45             | 1.24             | 1.13              |
| Threonine             | 0.97             | 0.85             | 0.83              |

^a Hydroalcoholic extract of angelica was added to the diets by the levels of 100, 200, 300 and 400 mg/kg diet.

^b The vitamin premix supplied the followings per kilograms of diet: vitamin A, 9000 IU; vitamin D3, 1000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6.5 mg; vitamin B6, 2 mg; vitamin B12, 0.01 mg; niacin, 30 mg; choline chloride, 500 mg; vitamin C, 50 mg; calcium D-pantothenate, 8 mg; folic acid, 0.5 mg.

^c The mineral premix supplied the followings per kilograms of diet: Mn, 100 mg; Fe, 50 mg; Zn, 70 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg.
the log 2 of the highest dilution of serum that agglutinated 0.05 mL of 2.5% suspension of SRBC in PBS.

2.4.3. Intestinal morphology
To carry out a histological morphometric analysis of the jejunal mid epithelium, formalin-fixed jejunal tissue samples were dehydrated, embedded in paraffin, sectioned (10 μm), and stained with haematoxylin and eosin. Morphometric indices were determined on these sections by means of a computer-aided light microscope (Olympus microscope, Olympus Corporation, Tokyo, Japan) by 4 magnifications, image analyser (Olysiya Soft Imaging System, Germany). The criterion for villus selection (10 villi per section) was based on the presence of intact lamina propria. At least three sections with 10 observations for each sample were viewed and the values were averaged to constitute a single observation. The analysed morphometric variables included: villus height (from the tip of the villus to the villus–crypt junction), crypt depth (defined as the depth of the invagination between adjacent villi), villus width and the ratio of villus height to crypt depth (VH/CD). The obtained readings in millimetre were finally expressed into micrometre (μm).

2.4.4. Carcass characteristics
At 42 d of age, 10 birds per treatment (2 chicks per replicate), were randomly selected, weighed, and euthanized by cervical dislocation. The carcass and different organs including breast, thighs, liver (without gall bladder), pancreas, gizzard, and immune organs including spleen and bursa of Fabricius were removed and weighed. Organ weights were expressed as a percentage of live body weight (Alipour khesht et al. 2015).

2.4.5. Serum metabolites
Blood samples were collected from the brachial vein at 42 days of age. Serum samples were separated by centrifugation at 2000g for 15 min at 4°C, and frozen at −24°C until use. Concentrations of glucose (GLU), total protein (TP) and serum lipids including cholesterol (CHL), triglycerides (TG), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) were assessed using commercial assay kits according to the manufacturer’s instructions (Pars Azmoon Co. kits, Tehran, Iran; http://parsazmun.ir/Products/Index/2022).

### Table 2

| No. | Components       | RI  | Percentage |
|-----|------------------|-----|------------|
| 1   | 1-Hexanol        | 894 | 1.3        |
| 2   | Pinene           | 914 | 0.1        |
| 3   | Terpinene        | 1020| 1.7        |
| 4   | Limonen          | 1095| 0.2        |
| 5   | Linalool         | 1132| 0.2        |
| 6   | Hexyl isobutyrate| 1137| 4.3        |
| 7   | Hexyl butyrate   | 1183| 48.8       |
| 8   | p-Cymene         | 1192| 0.8        |
| 9   | Octyl acetate    | 1215| 18.2       |
| 10  | Trans-Anethole   | 1256| 0.5        |
| 11  | Hexyl 2-methylbutyrate | 1278 | 2.2 |
| 12  | n-Hexyl hexanoate| 1368| 0.9        |
| 13  | Pulegone         | 1391| 0.5        |
| 14  | n-Octyl butyrate | 1415| 1.6        |
| 15  | Octyl ester      | 1478| 4.1        |

Note: RI: retention indices on HP-5 capillary column.

2.5. Statistical analysis
All data were tested for normality using PROC UNIVARIATE of SAS (SAS 9.2) before analysis. A completely randomized design was used and the data were analysed by the GLM procedure of SAS (SAS Institute Inc. 2009). Tukey’s test was performed for comparison of the means. Differences were considered significant when p < 0.05. The responses of chickens to HPE were investigated through orthogonal polynomial contrasts (linear and quadratic effects of HPE supplementation levels). Statistical significance was declared at p < 0.05.

3. Results

3.1. Identification of chemical composition of angelica extract
Table 2 lists 15 components identified in the angelica extract by GC–MS analysis. Hexyl butyrate (48.8%), Octyl acetate (18.2%), Hexyl isobutyrate (4.3%) and Octyl ester (4.1%) were detected as the dominant compounds.

3.2. Growth performance
The effects of dietary treatments on growth performance of the birds are shown in Table 3. Average daily weight gain (ADG) were not significantly affected by dietary treatments in different phases of the experiment (p = 0.08). Birds received 300 mg/kg HPE grew significantly more than control birds (51.58 vs. 47.83 g/b/d) during 1–42 d. The increasing dietary levels of HPE resulted in a positive linear response (p < 0.05) of the ADG during 1–42 d. ADFI was significantly affected by the treatments during 11 to 24 days of age (p < 0.05). Birds fed diet supplemented with 100 mg/kg HPE consumed more feed than the control group and the ADFI showed both linear (p < 0.05) and quadratic (p < 0.01) responses to HPE levels during this period. HPE supplementation had no significant effect on ADFI during starter, finisher and whole experimental periods. Also, the analysis of orthogonal polynomial contrasts for ADFI revealed a linear response (p < 0.05) to HPE inclusion levels in the whole experimental period. No significant differences were observed in FCR by different dietary supplementation levels of angelica extract (p > 0.05).

3.3. Carcass characteristics
The results of the carcass analysis at 42 days of age are shown in Table 4. Different dietary levels of HPE powder did not significantly affect the percentages of edible carcass, breast yield and thighs. Also, there were no significant differences among dietary treatments regarding to liver, gizzard, pancreas, spleen and bursa weights (p > 0.05). However, relative weight of liver showed a negative linear response (p < 0.05) to the HPE supplementation levels.

3.4. Intestinal morphology
Table 5 summarized the effects of HPE on various histological measurements of the jejunum at 42 days of age. The ratio of villus height to crypt depth (VH/CD) was significantly increased
by 400 mg/kg HPE in compare to control group at 42 days of age (p < 0.05). This ratio showed a linear response to different levels of HPE (p = 0.007); so that it was increased as the level of HPE increased. Villus height, villus width and crypt depth were not significantly affected by experimental treatments (p > 0.05). There was a significant increasing linear response to increasing dietary levels of HPE in the case of crypt depth (p < 0.05). Our results indicated that HPE increased villus height and decreased crypt depth.

### 3.5. Serum profile

The effect of dietary HPE on serum profile at 42 days of age is shown in Table 6. Supplementation of angelica extract at the levels of 200, 300 and 400 mg/kg, significantly decreased cholesterol concentration (p < 0.05) comparing to the control group and showed a linear response to different levels of HPE (p = 0.0007). Also, concentration of LDL was significantly decreased by 300 mg/kg of angelica extract (p < 0.05); while the concentrations of glucose, HDL, triglycerides and total protein were not affected by dietary treatments (p > 0.05). Also, the analysis of orthogonal polynomial contrasts for total protein and LDL concentrations revealed quadratic (p < 0.05) and linear responses (p < 0.01) to HPE inclusion levels, respectively.

### 3.6. Immune response

Titres of anti-SRBC (Humoral immunity) in the experimental groups are presented in Table 7. Supplementation of HPE had no significant effect on primary and secondary antibody responses (p > 0.05) and there were no linear or quadratic trends among the treatments (p > 0.05).

### 4. Discussion

#### 4.1. Analysis of angelica extract

The identity of the components of angelica extract by GC–MS revealed that hexyl butyrate (48.8%) and octyl acetate (18.2%) were its major components. Previously, Scheffer et al. (1984) and Hajhashemi et al. (2009) reported these two components as the major constituents. A number of chemical constituents, such as alkaloids, volatile substances, terpenoids, triterpenes and furanocoumarins have been isolated from fruit and different parts of the anglica (Sefidkon et al. 2002; Mojab and Nickavar 2003; Sefidkon et al. 2004; Hajhashemi et al. 2009). Phytopgenic feed additives usually show considerable variation in their chemical composition, depending on their ingredients and the influences of climatic conditions, location, harvest stage, or storage conditions.

#### 4.2. Growth performance

The results of this study indicated that dietary supplementation of HPE (100, 200, 300, or 400 mg/kg of the diet) had no remarkable effect on FCR at all recorded ages (p > 0.05). However, the growth performance results showed that HPE increased ADG during 1–42 d (p = 0.08) and ADFI during 11–24 d. Increasing

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**Table 3. Effect of different levels of angelica extract on growth performance of broiler chickens during different ages (d).**

| Treatment | ADG (g/b/d) | ADFI (g/b/d) | FCR (g/g) |
|-----------|-------------|--------------|-----------|
| Angelica extract (mg/kg diet) | 1–10 | 11–24 | 25–42 | 1–42 | 1–10 | 11–24 | 25–42 | 1–42 | 1–10 | 11–24 | 25–42 | 1–42 |
| 0, Control | 20.46 | 34.78 | 73.12 | 47.83 | 23.29 | 54.59 | 156.42 | 90.55 | 1.14 | 1.61 | 2.14 | 1.89 |
| 100 | 21.82 | 41.42 | 73.03 | 49.97 | 26.95 | 63.55 | 153.87 | 92.90 | 1.23 | 1.54 | 2.12 | 1.86 |
| 200 | 21.51 | 38.21 | 77.15 | 50.31 | 26.08 | 58.34 | 156.84 | 91.44 | 1.21 | 1.54 | 2.03 | 1.81 |
| 300 | 22.53 | 40.47 | 76.81 | 51.58 | 25.61 | 63.19 | 160.88 | 95.78 | 1.17 | 1.57 | 2.09 | 1.85 |
| 400 | 21.89 | 40.48 | 74.34 | 50.18 | 25.60 | 59.97 | 163.45 | 95.08 | 1.17 | 1.49 | 2.21 | 1.89 |
| SEM | 0.32 | 0.96 | 1.07 | 0.43 | 0.51 | 0.82 | 1.89 | 0.77 | 0.01 | 0.03 | 0.02 | 0.01 |

**Notes:** a–c represents values within a column with different letters differ significantly (p < 0.05) by Tukey’s test. ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; g/b/d: gram/bird/day. SEM: standard error of the means.
dietary levels of HPE resulted in a positive linear response (p < 0.05) in the case of ADG and ADFI during 1–42 d. Although there are no other available data reporting the effects of dietary hydroalcoholic extract of HPE on chickens performance, there are experiments by angelica extract in drinking water of broiler chickens. Jamshidparvar et al. (2017) showed that the increasing levels of angelica extract in drinking water (1, 1.5, 2 and 2.5 mL/L) resulted in a positive linear response (p < 0.01) in the body weight gain and final body weight of broilers. Also, the performance traits of broilers were improved in response to different levels of HPE (100, 150 and 200 mg/L) in drinking water (Kheiri et al. 2014). Although, our findings showed an increase in ADG during 1–42 d (p = 0.08) and ADFI during grower period (p < 0.05), we did not observe significant differences in other growth parameters. It seems that these differences might be attributed to different forms of HPE administration via feed or drinking water (powder and liquid) and the doses of usage. Khodambashi-Emami et al. (2012) indicated that the addition of essential oil of peppermint (200 mg/kg) had significant positive effects on FCR.

It has been reported that extracts and essential oils of medicinal plants reduce feed transit time and enhance the activity of digestive enzymes and secretion of bile acids (Manzanilla et al. 2004). The increase of digestive enzymes secretion in the intestine not only improves feed digestibility but also has a significant effect on reducing digesta viscosity (Lee et al. 2003). Inamori et al. (2007) revealed that the addition of spice additives enhances gastric emptying because of reduction in gastric antral spasms. Shahrani (2006) stated that a significant increase occurs in pepsin and acid secretion in rats that received a methanolic extract of angelica comparing to control group. Phytogenic feed additives are often claimed to improve the flavor and palatability of feed (Windisch et al. 2008). Although angelica is a strong flavor it seems unlikely that these levels of extract can be effective on palatability of the diet.

4.3. Carcass characteristics

Dietary supplementation of HPE had no significant effect on the relative weights of immune organs and carcass characteristics comparing to the control group (p > 0.05). Moreover, the relative weight of liver showed a negative linear response (p < 0.05) to the HPE supplementation levels. In contrast to our results, Jamshidparvar et al. (2017) reported that the relative weights of the liver and gizzard were increased in a quadratic manner in response to angelica extract supplementation in broiler chickens’ diet. A significant increase was reported in the relative weight of the spleen at the doses of 100 and 200 mg/kg of diet in mice (Sharififar et al. 2009). In agreement with the present study, no differences were observed in carcass characteristic of broiler chickens by some other plant extracts (Ocak et al. 2008; Toghyani et al. 2010; Alipour khesht et al. 2015).

4.4. Intestinal morphology

Dietary treatments had a significant effect on VH/CD of the jejunum (p < 0.05). Supplementation of 400 mg/kg diet HPE, significantly increased VH/CD ratio in comparison to control group. Results from the current study are inconsistent with the earlier reports of workers who reported villus height (Garcia et al. 2007) and VH/CD ratio (Chowdhury et al. 2018) are increased by plant extracts supplementation. On the other hand, some researchers found no significant differences in intestinal morphology by plant extracts (Guo et al. 2004; Barreto et al. 2008; Ahmadi et al. 2012; Akbarian et al. 2013). VH/CD ratio (p = 0.007) and CD (p < 0.05) showed linear

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**Table 5.** Effect of different level of angelica extract on gut morphology (μm) of broiler chickens at 42 d of age.

| Treatment | VH | VW | CD | VH/CD |
|-----------|----|----|----|-------|
| Angelica extract (mg/kg diet) | 1054.0 | 122.0 | 209.4 | 5.03<sup>a</sup> |
| 0, Control | 1257.3 | 156.2 | 204.4 | 6.19<sup>a</sup> |
| 200 | 109.6 | 154.0 | 192.8 | 6.29<sup>ab</sup> |
| 300 | 1185.4 | 154.0 | 193.8 | 6.15<sup>ab</sup> |
| 400 | 1233.6 | 182.8 | 182.4 | 6.81<sup>a</sup> |
| SEM | 26.34 | 10.67 | 3.57 | 0.19 |

*p*-value

ANOVA 0.10

Linear 0.13

Quadratic 0.39

**Notes:** a-b represents values within a column with different letters differ significantly (p < 0.05) by Tukey’s test. SEM: standard error of the means.

**Table 6.** Effect of different level of angelica extract on serum metabolites of broiler chickens at 42 d of age.

| Treatment | Glu (mg/dl) | TC (mg/dl) | TG (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | TP (g/dl) |
|-----------|-------------|------------|------------|-------------|-------------|-----------|
| Angelica extract (mg/kg diet) | 54.27 | 91.91 | 22.36<sup>a</sup> | 2.71 |
| 0, Control | 133.23<sup>a</sup> | 54.27 | 91.91 | 22.36<sup>a</sup> | 2.71 |
| 100 | 51.08 | 88.91 | 22.13<sup>a</sup> | 3.27 |
| 200 | 47.06 | 92.82 | 16.98<sup>ab</sup> | 3.00 |
| 300 | 50.51 | 86.72 | 15.86<sup>ab</sup> | 3.10 |
| 400 | 48.87 | 94.65 | 17.76<sup>ab</sup> | 2.88 |
| SEM | 3.95 | 2.33 | 1.09 | 0.32 | 0.78 | 0.07 |

*p*-value

ANOVA 0.55

Linear 0.22

Quadratic 0.12

**Notes:** a-b represents values within a column with different letters differ significantly (p < 0.05) by Tukey’s test. SEM: standard error of the means.

**Table 7.** Effect of different level of angelica extract on primary (32 d) and secondary (39 d) antibody responses.

| Treatment | primary antibody response | Secondary antibody response |
|-----------|---------------------------|-----------------------------|
| Angelica extract (mg/kg diet) | IgY | IgM total | IgY | IgM total |
| 0, Control | 2.0 | 7.0 | 2.4 | 5.6 | 8.0 |
| 100 | 1.4 | 5.4 | 6.8 | 1.8 | 4.8 | 6.6 |
| 200 | 1.8 | 5.4 | 7.2 | 2.0 | 6.0 | 8.0 |
| 300 | 2.0 | 6.2 | 8.2 | 2.2 | 6.2 | 8.4 |
| 400 | 2.2 | 5.8 | 8.0 | 2.2 | 6.4 | 8.6 |
| SEM | 0.16 | 0.23 | 0.31 | 0.14 | 0.27 | 0.34 |

*p*-value

ANOVA 0.65

Linear 0.42

Quadratic 0.34

**Notes:** SEM: standard error of the means.
responses to different dietary levels of HPE. These results indicate that the mentioned parameters may be increased by higher levels of HPE in future studies.

Dietary constituents change not only digestibility of nutrients but also in ontogeny and morphology of the gastrointestinal walls (Noy and Sklan 1995; Uni et al. 1998). Intestinal villi are the main site of nutrient absorption (Ray et al. 2002) and their better development could be the reason for higher nutrient absorption (Mekbungwan et al. 2002). In this study, we did not observe a significant increase in villus height by the treatments. There is a relationship between villus height and nutrient absorption and digestibility (Mekbungwan et al. 2002) because increased villus height can be considered as an indicator of increased surface area available for nutrient absorption (Amat et al. 1996). Perhaps, an increased villus height is paralleled by an increased expression of brush border enzymes and improved nutrient transport systems (Viveros et al. 2011).

In this study, birds receiving HPE supplemented diets showed an increase in VH/CD ratio ($p < 0.05$). Enterocytes show a secretory function when they are in the crypt and absorptive function when they migrate to the villi, which imply that net absorption in the small intestine depends on the VH/CD ratio (Buddle and Bolton 1992).

Jamroz et al. (2006) showed that the increased releasing of large amounts of mucus and the creation of a thick layer of mucus on glandular stomach and wall of jejunum in chickens fed diets containing active substances of plant extract. Antimicrobial agents are known to reduce the intestinal microbial count, which in turn reduces the presence of toxins at this level (Xu et al. 2003). In the several studies, it has been shown that the essential oils and extract of angelica have antibacterial (Zandi and Mehrizi 2012) and antifungal activities. So, this can explain the reduced possibility of microorganism adhesion to gut epithelium and number of Escherichia coli, Clostridium perfringens and fungi in the intestinal content of the birds fed with angelica extract supplemented diets. In addition, it may explain increases in the villus height or crypt depth and better functions of secretion, digestion, and absorption of nutrients by the small intestine. On the other hand, it has been suggested that the essential oils and herbal extracts improve villus height due to their antioxidant activities (Windisch et al. 2008). Oxygen radicals liberated during digestive processes, act on the superficial mucous of the intestine and shorten the intestinal villi. The phytochemical products may protect the villi from oxidative damage by stimulating the activity of antioxidant agents, and the phenolic group may act as hydrogen donor showing antioxidant activity (Windisch et al. 2008).

### 4.5. Serum profile

Diet containing HPE, in the current study, changed the concentration of cholesterol and LDL ($p < 0.05$). In agreement with these findings, the addition of HPE (500 mg/day) to the diet of people with dyslipidemia (high level of cholesterol in blood) for 8 weeks resulted in significant decreases in serum LDL-C and total cholesterol levels (Panahi et al. 2011). Also, Kheiri et al. (2014) reported that serum triglycerides, cholesterol and LDL concentrations in broiler chickens were significantly decreased ($p < 0.05$) in response to different levels of HPE treatment (100, 150 and 200 mg/L in drinking water). The antioxidative properties of plant extracts can positively affect the secretion of endogenous digestive enzymes and influence the blood metabolites (Lee et al. 1995; Grassmann et al. 2000). According to the previous phytochemical investigations, furanocoumarins and flavonoids are among the constituents of *H. persicum* (Ghodsi 1976; Aynehchi et al. 1978; Merijanian et al. 1980). Therefore, the possible hypocholesterolemic activity of this plant may be attributed, at least in part, to the presence of flavonoids, as these phytochemicals have been reported to exert numerous activities, including lipid-lowering effects (Kim et al. 2009; Li et al. 2009; Zhou et al. 2009). Recently, there has been more focus on the effects of angelica on different parameters in humans, such as its hypocholesterolemic effect. The analysis of orthogonal polynomial contrast for total protein content revealed a positive quadratic ($p < 0.05$) response to HPE supplementation levels in our study.

These results are in agreement with findings of AL-Kassie (2009) who reported that, when 200 ppm essential oil derived from *C. Verum* is added to a standard diet of broiler chicks for 42 days, a significant increase in serum total protein concentration is observed. In some cases, increased serum total protein may be associated with acute inflammation, dehydration, or some other types of tissue damages (Murray et al. 2000). However, no adverse clinical signs were observed in the birds of this study. According to findings of this investigation, it can be suggested that elevated serum total protein levels in HPE treated birds may be due to increased body weight gain (Kapelanski et al. 2004).

Physiological studies have shown a correlation between some of the blood parameters with the degree of fatness in broiler chickens. In this regard, Whithead and Griffin (1984) and Whithead et al. (1986) indicated that plasma VLDL and LDL was a useful parameter to infer the degree of fatness in chickens as causes decreasing abdominal fat in broiler chickens. Chicken meat is healthier than other meat sources for human consumption because of its low cholesterol and fat content (Ponte et al. 2004). The quality and quantity of lipids and their fatty acid composition in meat are influenced by internal (age, gender, genotype and castration) and external (temperature, feeding) factors (Mašek et al. 2013). Several studies have been used to decrease the saturated fatty acids and cholesterol content of broiler meat. Dietary inclusion of phytogenic substances in broiler diets has been used to manipulate the fatty acid composition and decrease the detrimental ingredients in meat. Blood lipid profile has an important role in the performance and carcass quality of broilers. On the other hand, the obtained results may be helpful in the evaluation of changes in the metabolic profile, health condition and production patterns in growing broiler chickens reared under farm conditions. Also, these findings may be useful in human nutrition.

### 4.6. Immune responses

There were no significant differences among the birds received dietary treatments in regard to primary and secondary antibody responses in the current study ($p > 0.05$). In contrast to our results, Sharirfaff et al. (2009) reported that aqueous extract of angelica by levels of 50, 100 and 200 mg/kg of diet showed a
stimulatory effect on both humoral and cellular immune functions in mice. This activity could be due to the presence of flavonoids or coumarins, which can augment the humoral response by stimulating the macrophages and B-lymphocytes involved in antibody synthesis (Makare et al. 2001). Another study revealed that Heracleum maximum and Heracleum neapolense have immunomodulatory effects (Fortier 1996). The discrepancies among our findings with these studies are likely due to different levels of angelica extract supplementation or differences in chemical composition of angelica samples, as well as experimental conditions and immune status of the flock.

5. Conclusion
It was concluded that dietary supplementation of 400 mg/kg HPE significantly increased the ratio of villus height to crypt depth. 300 mg/kg of diet HPE improved average daily weight gain of broiler chickens and significantly decreased the serum concentration of cholesterol and LDL. The results revealed that the supplementation of HPE in amounts used in the present study had a beneficial effect on intestinal morphology, haematological parameters and growth performance of broiler chickens. Our results justify further researches in this area to determine the optimal dietary level and the mechanism of action of Heracleum persicum fruit extract.

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