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Effect of *Ferulago angulata* sub. *carduchorum* on total serum antioxidant activity and some of the humoral immune responses in broiler chicks

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ABSTRACT

Background & Aim: *Ferulago angulata* sub. *carduchorum* (locally name: Chavir or Chavil) is an important medicinal plant in Iran. The powder of aerial parts of this plant contains variety of components with different therapeutical effects such as antioxidant activities (AOA). This study was conducted to investigate the effect of dietary supplementation powder of aerial parts of this plant on total activities of serum antioxidant and some of the humoral immune responses in broiler chicks.

Experimental: Three hundred of 16 days-old commercial chicks (Ross 308) were randomly allocated to six treatments with five replicates. The dietary treatments consisted of the basal diet as control (A), 100 ppm (150 IU) of vitamin E (B), 70 g / 100 kg Flavofosfolipol (C), 0.3% (D), 0.6% (E), and 1% (F) of the powder of the aerial parts of this plant added to the basal diet. At 25 days, from each treatment of experiment, two chicks were randomly selected and sheep red blood cell (SRBC) was injected through their wings veins. At 30 days, blood samples (ten sample of per treatment) for the test of antibody measurement produced against SRBC, the influenza virus and Newcastle were prepared. Moreover, with the blood extension and numbering of Hetrophil and Lymphocyte calculated and hetrophil to lymphocyte ratio were estimated. At 42 days, blood samples (ten samples per treatment) were taken for measuring total activities of serum antioxidant.

Results & Discussion: Results indicated that supplementing of 0.6% of *F. angulata* significantly increased total activities of serum antioxidant compared to control birds ($p \leq 0.05$). There is no significant difference among the treatments of experiment in the number of Hetrophil, Lymphocyte their ratio and the antibody against the virus of Newcastle and SRBC. However, this antibody against the influenza virus at the treatment of 3 g/kg *F. angulata* in comparison to control treatment was more.

Recommended applications/industries: Use of *F. angulata* in broiler diet could be increase total serum antioxidant activity and improve humoral immunity.

1. Introduction

The use of antibiotic growth promoters (AGP) to improve animal performance has been a usual practice for more than half century. After 2005, in the Europe Union the use of all such antibiotics was banned by regulatory measures (Isabel *et al.*, 2009). This is because of the potential risks associated with their use and development of resistant strains of bacteria, mainly in humans. There are various products for replacing AGP, such as medicine herbs. There is sufficient evidence to show that potential herbs are effective for enhancement of the immune system and increasing antioxidant activity for poultry (Ghalamkari *et al.*, 2011). *Ferulago angulata* subsp. *carduchorum*, belonging to the family Apiaceae, is an important medicinal plant of Iran. *Ferulago* species are used in folk medicine for their sedative, tonic, digestive and anti-parasitic effects (Yesilbag *et al.*, 2011). In addition, the antibacterial, antifungal, and anti-oxidant effects of this plant have been reported (Ghasempour *et al.*, 2007; Yesilbag *et al*., 2011). The main constituents of this plant are monoterpens and among, α-pinene, bornyl acetate and *cis*-ocimene (Dorman *et al*., 2000) and β- pinene (Homann *et al*., 1999; Lopez *et al*., 1994; Billany *et al*., 1995) have the most antioxidant activity. The mechanism of these compounds is to damage the membrane of lipoprotein cell bacteria that is lead to leaking and decreasing of cytoplasm compound (Hosseini Mansob *et al*., 2011). On the other hand, the antioxidant compounds, with adding of some parts to free radicals to deactivate these poisonous materials. Moreover, the herb has the compounds with antibacterial activity, such as α- terpineol, terpenen-4-ol (Cosentino *et al*., 1999), α-pinene, β-pinene, and *ρ*-cymene have anti-fungous (Adam *et al*., 1998; Lis-Balchin *et al*., 1998). Antibacterial and antifungal activities of the plant are also presented on both positive -Gram like *Staphylococcus* and negative-Gram like *E. coli*, and fungi like *C. albicans* (Ghasempour *et al*., 2007). The chemical composition of *F. angulata* such as β-hydroxy-13-epi-manoyloxide, α-pinene, 2,5-dimethoxy-*p*-cymene, *p*-cymene, methyl carvacrol, transchrysanthenyl, transchrysanthenyl acetate, γ-terpinene, (*Z*)-β ocimene, α-pinene, myrcene, terpinolene, 2,4,5-trimethylbenzaldehyde, and α-phellandrene was reported by Taran et al. (2010) and, Ghasempour et al. (2007). Since there have not been any study on the effects of antioxidant activity and humoral immunize of *F. angulata* in broiler chicks ROOS (308), this study was conducted to investigate the effect of powder of aerial parts of this plant on total activities of serum antioxidant and immune system function of broiler chicks.

2. Materials and Methods

2.1. Bird, diets and management

The aerial parts of *Ferulago angulata* were collected from Shahoo Mountain western Iran, dried in shed and added to broiler diet as a powder. In present research, 360 one-day old broiler chicks (Ross-308) randomly allocated into six treatments, each with free replicates with 12 broiler chickens. Vitamin E, Flavofosfolipol, and the aerial parts of *F. angulata* were supplemented to no additive added basal diet. Six treatments were as follows:

a) Basal diet (control)
b) Basal diet + 100 mg / kg (150 IU) Vitamin E;
c) Basal diet + 70 g Flavofosfolipol / 100 kg diet;
d) Basal diet + 0.3 kg *F. angulata* / 100 kg diet;
e) Basal diet + 0.6 kg *F. angulata* / 100 kg diet;
f) Basal diet +1 kg *F. angulata* / 100 kg diet;

The trial comprised three 2-weeks periods. The birds were fed a starter diet from days 1-14, a grower diet from days 14-28 and finisher from days 28-42 (Table 1). The diets were formulated to meet the requirements of broilers as recommended by the Catalog Ross (Ross Co., 2007). The dried aerial parts of *F. angulata* were added at the expense of powder. The birds were allowed to free access to feed and water during the 42-d grow out period. The lighting cycle was 23 h/d maintained. The ambient temperature in experimental house was maintained at 32 ˚C during the first week and gradually decreased by 3 ˚C in the second and third week, and then fixed at 22 ˚C.

2.2. Total activities of serum antioxidant

At 42 days from each repetition of treatments, two chicks were randomly selected, and the blood samples were taken by cylinder 2.5 ml from wings vein. The blood samples of serum were prepared by centrifuge for Fenton type reaction. AOA was measured by the spectrophotometry method. In summary, standard solution of Fe–EDTA complex reacts with hydrogen peroxide by a Fenton type reaction, leading to the

formation of hydroxyl radicals. These reactive oxygen species degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample of broiler serum cause suppression of the production of TBARS. This reaction can be measured by spectrophotometer and the inhibition of color development defined as the AOA (Ghalamkari *et al*., 2011).

2.3. Calculation of Hetrophil to lymphocyte ratio

At 30 days from each repetition of experiment, two chicks were randomly selected and the blood samples were taken from wings vein. Blood extensions samples were prepared and colored based on Gimsa method (Thrall *et al*., 2004). One hundred white blood cells for each sample with the separation of Heterophil and lymphocyte were counted, and the Heterophil to lymphocyte ratio was calculated and then registered. Then data gained were analyzed statistically.

2.4. The measurement of the anti-body produced against SRBC

At 25 days from each treatment of experiment, two chicks were randomly selected and then 1 ml SRBC 1% was injected through the wings veins. At 30 days, taking blood process was performed from the wings veins of the same chicks for determining of antibody against SRBC by the method of Hemaglutination (HA) method. Then the blood samples were placed in centrifuge 1500 T/m for 15 min. Their serums were separated, and kept in the temperature of - 20 ^{o}C for experiment. Then from the average of each repetition, was under the analysis and survey statistics.

2.5. The measurement of the anti-body produced against Newcastle and Influenza virus

At 30 days from each treatment of experiment, two chicks were randomly selected and taking blood through the wings vein was performed. After separation of serum from blood samples, the measurement of special antibody produced against Newcastle and Influenza vaccine were measured separately by the Hemaglutination inhibition (HI) method.

2.6. The measurement of Lymphatic organs weight

At the end of the experiment (at 42 days), two chicks that their average weight was about the average of their group, were randomly selected (10 chicks from each treatment), and their carcasses were examined. For each bird, the weight of effective glands in immune system of the chicks (Bursa of fabricius and spleen) was measured. In final, the data was calculated in percent of body weight and analyzed statistically.

2.7. Statistical analysis

Data subjected to one–way analysis of variance using SAS statistical package (version 6.08, 1989). Significant effect of dietary treatments was compared with Least Significant Difference Test (LSD) with $p < 0.05$.

3. Results and Discussion

3.1. Total activities of serum antioxidant

The AOA of serum in broiler chicks under six g *F. angulata* / kg treatment significantly ($p \leq 0.05$) increased than those control and antibiotic treatments (Table 2). But, there was no significant difference between *F. angulata* treatment and vitamin E treatment.

Table 2. The effect of dietary inclusion of feed additives on AOA (mmol/lit) of serum in broiler chicks.

Treatments	AOA
Control	0.45^{b¶}
Vitamin E	0.54^{ab}
Flavofosfolipol	0.35^{b}
0.3% *F. angulata*	0.40^{b}
0.6% *F. angulata*	0.67^{a}
1% *F. angulata*	0.56^{ab}
SEM	±0.03

¶ Mean values followed by the same letters in the column do not differ according to LSD test.

3.2. Heterophil and Lymphocyte ratio

There was no significant difference among the treatments ($p > 0.05$) (Table 3). However, the least numbers of Heterophil among treatments were belonged to the control treatment, and the most numbers of Lymphocyte were belonged to 3 and 6 g/kg of *F. angulata*. In addition, the least Heterophil to Lymphocyte ratio was belonged to 3 g/kg *F. angulata* treatment, and the most were belonged to antibiotic treatment ($p > 0.05$).

3.3. The measurement of antibody produced against Newcastle, Influenza (HI) and SRBC (HA)

The measurement of antibody produced against the Influenza virus in the chicks of treatment 3 g/kg *F. angulata* in comparison to other groups of treatments was significant ($p \leq 0.05$) (Table 4). No significant effect on the antibody against Newcastle virus and, SRBC also were seen ($p > 0.05$). However, the most measurement of the antibody produced against the Newcastle virus was observed in the chicks diet with 3 g/kg *F. angulata* and the vitamin E treatment ($p > 0.05$). The measurement of the antibody produced against SRBC was not affected by treatment experiment significantly ($p > 0.05$). It is clear that the most measurement of the antibody produced against SRBC, in the treatment 6 g/kg *F. angulate* (Table 5).The least measurement of antibody produced was belonged to the treatment of 3 g/kg *F. angulata* ($p > 0.05$).

*3.4. Weight comparison of Lymph*atic organs

Treatments had no significant effect on weight average of Lymphatic organs at 42 days (Table 5). At 42 days, the maximum weight of fabricius bursa was in control group and in treatment that received 6 g/kg *F. angulate*. The minimum weight of fabricius bursa belonged to treatment of vitamin E. In addition, among the treatments, the maximum weight average of Bursa of fabricius was for 6 g/kg *F. angulata*, and the minimum weight of that was for 3 g/kg *F. angulata*. Moreover, there was no significant difference between the weights of spleen at 42 days in different treatments experiments. The maximum of spleen weight is for antibiotic treatment, and the minimum of that, is for the treatment of 10 g/kg *F. angulata*. The maximum of spleen weight in chicks of 3 g/kg *F. angulata* and the minimum of that is for 10 g/kg *F. angulata*.

Since there was no experiment on the effect of this plant on the total serum antioxidant activity and immune responses in broiler chickens, the effects of other plants that are similar to *F. angulata* were chosen and discussed. The maximum total serum antioxidant activity belonged to the treatment of 6 g/kg dietary and had significant effect to decreasing oxidation of tissues specially lipid tissues (Table 2).

The active ingredients of a medicinal plant are mainly its phenolic compound that is also an important antioxidant (Huda-Faujan *et al*., 2009; Khanavi *et al*., 2009). The phenolic compounds are generic term for multiple aromatic groups including mainly flavonoids, phenols acid, isoflavonoids, and anthocyanins. These ingredients are naturally produced during a plant's growth metabolic process, the active substances with antioxidant function such as scavenging reactive oxygen species, free radicals or non-free radical reactive oxygen species production from body metabolism (Rath *et al*., 2006).

Vossen et al. (2009) clarified that the chicks, under the test of oxidative plasma, have more antioxidant properties in serum when they applied *Rosmarinces officinalis* in their diet. They reported that the chicks, which got 500 g/kg oil of the herb, had less meat cholesterol oxidation in comparison to control treatment ($p \leq 0.05$). In contrast, Roofchaee et al. (2011) in their experiment showed that adding oregano essential oil level 300, 600, and 1200 mg/kg dietary, had no significant effect on the serum antioxidant activity.

Botsoglou et al. (2002) claim that high concentration of unsaturated fatty acids in poultry meat increases susceptibility of chicken meat lipids to oxidative deterioration during storage. In this study, it seems that the antioxidant activity of *F. angulata* due to the presence of phenolic components having off group these components act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation thus retarding the hydroxy peroxide formation (Farag *et al*., 1989).

F. angulata treatments had no significant effect on the titer of produced antibody against Newcastle virus and sheep red blood cell (SBRC) (Table 5). However, the treatments of *F. angulata* had significant effect on the titer of produced antibody against Newcastle virus. In this way, AL-ankari et al. (2004) reported the titer of produced antibody against Newcastle antigene in the chicks fed by mint was higher. They also suggested the herb could stimulate immune system and improve the responses of immune system against Newcastle virus.

Results of a study by Mathivanan and Kalaiarasi (2007) showed that the titer of produced antibody against SRBC, in the dietary completed by herbs in comparison to the dietary with antibiotics of Virginiamycin was increased. Kabir et al. (2004) and Ramarao et al. (2004) concluded that increasing antibody in the herbal treatments in comparison to control and dietaries is due to hypertrophy and hyperplasia of lymphatic organs in herbal treatments.

Table 1. The ingredient and chemical composition of basal starter, grower and finisher diets

Ingredients	Starter	Grower	Finisher
Corn	55.18	57.94	62.4
Soybean meal	39.2	36.4	32.4
Oil	1.32	2.1	2
DCP	1.66	1.4	1.3
$Caco_3$	1.29	1.12	1
Vitamin-Premix[1]	0.25	0.25	0.25
Mineral -Premix[2]	0.25	0.25	0.25
NaCl	0.35	0.3	0.25
DL-Methionine	0.31	0.2	0.15
L-Lysine	0.19	0.04	-
Calculated composition (mg/kg)			
M. energy (kcal/kg)	2830	2900	3000
Crude protein(%)	21.8	20.7	19
Calcium(%)	0.98	0.84	0.81
Av. phosphorus(%)	0.46	0.42	0.39
Meth.+ cysteine(%)	0.96	0.87	0.76
Lysine (%)	1.33	1.77	1.0

[1]To provide the following per kg of diet: Vit A 10,000 IU, vitamin D3 2000 IU, vitamin E 5 IU, vitamin K 2 mg, riboflavin 4. 20 mg; vitamin B12 0.01 mg; pantothenic acid 5 mg; nicotinic acid 20 mg; folic acid 0.5 mg.

[2]To provide the following per kg of diet: choline 3 mg; Mg 56 mg; Fe 20 mg; Cu, 10 mg; Zn 50 mg; Co 125 mg; Iodine 0.8 mg.

Table 3. The effect of dietary inclusion of feed additives on Heterophil, Lymphocyte and their ratio at 42 days

Treatment	H/L	Lymphocyte%	Heterophil%
Control	0.47	72	32
Vitamin E	0.52	70	35
Flavofosfolipol	0.66	65	38
F. angulata 3g/Kg	0.45	76	34
F. angulata 6g/Kg	0.46	76	35
F. angulata 10g/Kg	0.51	75	37
SEM	0.03	1.54	1.4

Table 4. The effect of dietary inclusion of feed additives on the antibody produced against Newcastel, Influenzae Virus and SRBC of chicks at 30 days

Treatment	The measurement of the antibody produced against SRBC	The measurement of the antibody produced against Influenza Virus	The measurement of the antibody produced against Newcastle Virus
Control	6.6	4.8 [b]	5.0
Vitamin E	6.2	6.7 [a]	5.2
flavofosfolipol	5.6	6.2 [ab]	4.6
F. angulata 3 gr/kg	5.4	7.2 [a]	5.0
F. angulata 6 gr/kg	5.8	3.3 [c]	4.2
F. angulata 10 gr/kg	5.7	4.8 [b]	4.3
SEM	0.17	0.27	0.13

Followed by the same letters in the column do not differ according to LSD test ($p \leq 0.05$).

Table 5. The effect of dietary inclusion of feed additives on weight average of lymphatic organs at 42 days

Treatment	Spleen	Bursa of fabricius
Control	0.115	0.18
Vitamine E	0.115	0.15
Flavofosfolipol	0.126	0.16
F. angulata 3g/kg	0.116	0.16
F. angulata 6g/kg	0.113	0.18
F. angulata 10g/kg	0.112	0.17
SEM	0.004	0.005

AbdEl – Moteal et al. (2008) in their experiments preformed on brown laying hens observed that adding eucalyptus leaves powder to the dietary, decreased hetrophil to Lymphocyte ratio significantly. In birds, the heterophil are phagocytic cells whose main is protection against invading microorganisms, whereas primary functions of Lymphocyte is cell mediated and humoral immunity. The H/L ratio is a recognized measure of stress in birds (Davison *et al*., 1983; Gross *et al*., 1983; Maxwell *et al*., 1993) that has become a valuable tool in stress research especially when combined with the convenience and repeatability of automated blood cell count. As it is clear from the results of the comparison of the weight averages related to Lymphotic organs (Bursa of Fabricius and spleen) experiment treatments (Table 5), had no significant effect on the weight of these organs, and the maximum weight of Bursa Fabricius in the chicks fed by control treatment and treatment of 6 g/kg *F. angulata* was observed. The maximum spleen weight was for the chicks of 3 g/kg treatment.

4. Conclusion

According to this result, the treatment of 6 g/kg *F. angulata* has increased the total serum antioxidant activity in broilers and has proper effects on the function of their immune system. In addition, it could be improving meat oxidative stability but a further research is necessary.

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