Effect of *Scrophularia striata* and *Ferulago angulata*, as alternatives to virginiamycin, on growth performance, intestinal microbial population, immune response, and blood constituents of broiler chickens

Farhad Rostami,[*] Hossein A. Ghasemi,[†] and Kamran Taherpour[*,1]

[*]*Department of Animal Science, Faculty of Agriculture, Ilam University, Ilam, Iran; and* [†]*Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349 Arak, Iran*

ABSTRACT An experiment was conducted to investigate the comparative effect of *Scrophularia striata*, *Ferulago angulata*, and virginiamycin (VM) on performance, intestinal microbial population, immune response, and blood constituents of broilers. A total of 300 Ross 308 male broiler chickens were randomly assigned to 5 treatments, with 5 replicates/treatment (10 chickens/pen). Birds were fed either a corn–soybean meal basal diet (control) or the basal diet supplemented with 200 mg/kg VM; 4 g/kg *S. striata* (SS_1); 8 g/kg *S. striata* (SS_2); 4 g/kg *F. angulata* (FA_1); or 8 g/kg *F. angulata* (FA_2). After 6 wk, the BW, ADG, and feed-to-gain ratio (F:G) of the VM, SS_1, and FA_1 groups were better ($P < 0.01$) compared with the control group. At 42 d, cecal lactobacillus counts were higher ($P = 0.032$) in SS_2 and FA_2 groups compared with the control and VM groups. In addition, broilers fed any of the diets exhibited lower coliform counts ($P < 0.05$) in the ileum and ceca than those fed the control diet. Total and IgG antibody titers against SRBC for secondary responses, relative spleen weight, and lymphocyte counts were higher ($P < 0.05$) in birds fed the SS_2 or FA_2 diet compared with the control group. Moreover, feeding the SS_2 or FA_2 diet decreased ($P < 0.05$) the blood heterophil/lymphocyte ratio and plasma triglyceride level, whereas only the SS_2 diet increased ($P = 0.037$) the white blood cell counts compared with the control diet. All diets, except for the VM diet, decreased ($P = 0.009$) the plasma cholesterol level compared to the control treatment. The plasma high-density lipoprotein cholesterol level was also increased ($P = 0.042$) in the SS_2 and FA_2 groups. In conclusion, dietary *S. striata* or *F. angulata* at a level of 4 g/kg diet enhanced growth performance, which was comparable to that of VM used as an antibiotic growth promoter. Furthermore, a high dose of both herbs (8 g/kg diet) could beneficially affect the intestinal health and immune status of broilers.

Key words: broiler performance, *Ferulago angulata*, immune response, intestinal microflora, *Scrophularia striata*

2015 Poultry Science 94:2202–2209
http://dx.doi.org/10.3382/ps/pev198

INTRODUCTION

Among the possible alternatives to antibiotic growth promoters (AGPs), phytogenic and herbal products are considered interesting because they have acquired more reliability and acceptability among consumers as safe and natural additives (Hashemi and Davoodi, 2011).

The Scrophulariaceae is a large angiosperm family, which is widely distributed in deciduous forests of central Asia, central Europe, and North America and is represented by about 3,000 species and 220 genera (Richman et al., 1997). *Scrophularia striata*, an herbaceous flowering plant from the Scrophulariaceae, grows in the northeastern part of Iran and is used as a traditional herb in the treatment of rheumatism, allergies, and chronic inflammatory diseases (Mahboubi et al., 2013). The immunomodulatory activities of some species of *Scrophularia* have also been reported by other investigators (Azadmehr et al., 2011, 2012). Bahrami and Valadi (2010) reported the antibacterial activities of *S. striata* against both gram-positive bacteria (like *Staphylococcus aureus*) and gram-negative bacteria (like *Escherichia coli*). Furthermore, flavonoids, quercetin, isorhamnetin 3-O-rutinoside, and phenolic compounds, which play important roles as antioxidant active substances, were isolated from *S. striata* (Monsef-Esfahani et al., 2010).

Ferulago angulata (locally called chavir) is widespread in the high altitudes of several Asian countries, such as Iran, Turkey, and Iraq. The most abundant constituents of this herb are α-terpineol, terpenen-4-ol, α-pinene, β-pinene, and ρ-cymene, which have antibacterial and anti-inflammatory activities (Govahi et al., 2013). The antimicrobial activities of the aerial parts of *F. angulata* against

Received December 15, 2014.
Accepted June 1, 2015.
[1]Corresponding author: k.taherpour@ilam.ac.ir; kamran_taherpour@yahoo.com

E. coli, *Pseudomonas aeruginosa*, and *Salmonella typhi* have been confirmed (Taran et al., 2010). This herb is also a rich source of polyphenolic compounds, such as α-pinene, bornyl acetate, cis-ocimene, and β-pinene, which possess strong antioxidant properties (Khanahmadi and janfeshan, 2006). In a study on rats, Rafieian-kopaei et al. (2014) found that the use of a hydroalcoholic extract of *F. angulata* inhibited lipid peroxidation and resulted in lower blood levels of cholesterol, triglycerides, and LDL.

Gut microflora can affect both health and growth performance of chickens in many ways (Choct, 2009). Changes in the composition of gut microbiota could also result in modulations of the immune responses in broiler chickens (Brisbin et al., 2008). Thus, since the aforementioned herbs are reported to have antimicrobial factors that could reduce pathogenic bacteria counts (Bahrami and Valadi, 2010; Taran et al., 2010), they may beneficially affect the immune function in broilers. In this connection, Govahi et al. (2013) reported that the addition of 3 and 6 g/kg *F. angulata* to the diet increased serum antibody titers against the influenza virus without affecting antibody response to the Newcastle disease virus.

As described, owing to (1) a lack of research about the influence of *S. striata* and *F. angulata* on broiler performance and (2) the importance of improving the gut health and immune status of broilers in order to prevent infectious diseases, this study was conducted to evaluate the effect of *S. striata* and *F. angulata* on male broiler growth performance, intestinal microbial population, immunity, and blood constituents, and to compare them with a well-documented AGP (virginiamycin, or VM).

MATERIALS AND METHODS

Animals and Diets

A total of 300-d-old male broiler chicks (Ross 308) were purchased from a local hatchery. On arrival, all birds were weighed (44±0.5 g) and randomly assigned to 1 of 6 treatments, with 5 replicate pens/treatment and 12 chickens/pen. Chickens were fed either a corn–soybean meal basal diet (control) or the basal diet supplemented with 200 mg/kg virginiamycin (VM) (Tolide Darouhai Dami Iran Co., Tehran, Iran; the manufacturer provided the following/kilogram of product: 100 g VM powder and 900 g wheat shorts as carrier), 4 g/kg *S. striata* (SS_1), 8 g/kg *S. striata* (SS_2), 4 g/kg *F. angulata* (FA_1), or 8 g/kg *F. angulata* (FA_2). The aerial parts of *S. striata* and *F. angulata* were collected from the Chardavol region (northeastern part of Iran) and Sirvan Mountains (Kermanshah province, Iran), respectively. Both herbs were air-dried at room temperature and then ground into a fine powder. A sample of each herb was then analyzed to determine the proximate composition (AOAC International, 2000). *Scrophularia striata* contained 96.6% DM, 5.4% CP, 16.9% crude fiber, 36.4% NDF, 26.4% ADF, 3.5% ether extract, 3.9% ash, and 3,514 kcal/kg gross energy. Chemical analysis of *F. angulata* was as follows: 94.1% DM, 12.3% CP, 15.1% crude fiber, 34.7% NDF, 23.2% ADF, 5.3% ether extract, 10.6% ash, and 4,037 kcal/kg of gross energy. No antimicrobial or anticoccidial supplements were included in the formulation. Diets were fed in mash form. A 3-phase feeding program was used, with a starter diet from day 0 to day 10, a grower diet from day 11 to day 24, and a finisher diet from day 25 to day 42 (Table 1). Chickens were raised on floor pens (100 × 120 cm) and had free access to feed and water for the entire experimental period (days 0–42). The room temperature was gradually decreased from 33 to 22°C on day 28 and then remained constant thereafter. The lighting program was a period of 23 h of light and 1 h of darkness.

Performance Data

Birds and feed were weighed at 10, 24, and 42 d of age. The values of AFDI and ADG were recorded in different periods, and the feed-to-gain ratio (F:G) was calculated. Mortality was also recorded as it occurred. Since there were only 5 deaths during the entire growth period, the mortality rate was not analyzed. However, AFDI and F:G were corrected for the mortality of related groups.

Bacteriological Analysis

Ten birds/replicate on day 42 were selected and killed by cervical dislocation. The carcasses were subsequently opened and the entire gastrointestinal tract was removed aseptically. The jejunum (from the distal-most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), the ileum (from the junction with Meckel's diverticulum to the ileocecal junction), and the ceca (from the ostium to the tip of each ceca) were then excised. Samples (1 g) from the contents of the jejunum, ileum, and both ceca were immediately collected into glass containers. Digesta samples from each segment were diluted in a 0.85% sterile saline solution for the enumeration of lactobacilli and coliforms by conventional microbiological techniques using selective agar media as described by Jin et al. (1998). All microbiological analyses were conducted in duplicate, and the average values were used in the statistical analysis. *Lactobacillus* bacteria were grown on Rogosa SL agar (Merck, Darmstadt, Germany), and coliforms were grown on McConkey agar (Merck, Darmstadt, Germany). Selective agar used to enumerate *Lactobacillus* spp. was incubated anaerobically for 48 h at 37°C, whereas selective agar used to enumerate coliforms was incubated aerobically for

Table 1. Diet composition and calculated analysis of basal diet.

Ingredient g/kg of diet	Starter (days 1–10)	Grower (days 11–24)	Finisher (days 29–42)
Corn	57.00	59.30	63.73
Soybean meal (44% CP)	31.46	30.69	27.39
Fish meal (60% CP)	5.66	4.31	2.62
Soybean oil	1.94	2.38	2.90
Limestone	1.16	0.96	0.94
Dicalcium phosphate[1]	1.25	1.09	1.18
Sodium chloride	0.21	0.24	0.27
Bicarbonate sodium	0.16	0.14	0.14
Vitamin premix[2]	0.25	0.25	0.25
Mineral premix[3]	0.25	0.25	0.25
DL-Methionin	0.28	0.19	0.14
L-Lysin, HCL	0.20	0.05	0.04
Choline chloride-50%	0.18	0.15	0.15
Calculated analysis			
ME, Mj/kg	12.62	13.08	13.27
Crude protein, g/kg	22.84	21.65	19.46
Ca, g/kg	1.02	0.86	0.80
Available phosphorous, g/kg	0.49	0.43	0.40
Digestible Lysine, g/kg	1.23	1.06	0.92
Digestible Methionine + cystine, g/kg	0.92	0.81	0.70
DEB[4], mEq/kg	245	237	220

[1]Dicalcium phosphate contained 16% phosphorus and 23% calcium.

[2]Vitamin premix/kg diet: vitamin A, 9000 IU; vitamin D3, 2000 IU; vitamin E, 18 IU; vitamin K_3, 2 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; niacin, 30 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; biotin, 0.1 mg; folic acid, 1 mg; cyanocobalamin, 0.015 mg.

[3]Mineral premix/kg diet: Zn, 100 mg; Mn, 100 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg.

[4]DEB = (Na^+, mEq/kg + K^+, mEq/kg) – CL^-, mEq/kg.

24 h at 37°C. Bacterial numbers were expressed as log 10 cfu/g of jejunal, ileal, or cecal digesta.

Immunological Tests

On day 42, 2 birds/replicate were randomly selected and slaughtered, and the immune organs (thymus, spleen, and bursa of Fabricius) were collected. The thymus, spleen, and bursa indices were calculated as the immune organ weight (g) divided by the BW (kg) of the same bird and used as indicators of immune function.

To assay the primary and secondary antibody responses against SRBC, at 14 and 35 d of age, 2 birds/replicate were immunized intramuscularly with 0.25 mL 10% SRBC in PBS (Leshchinsky and Klasing, 2001). Blood samples (1.5 mL/bird) were obtained from the brachial vein at 7 d following each injection (days 21 and 42). Serum was separated by centrifugation for 10 min at 3000×g and stored at −20°C for further analysis. Total antibody titers against SRBC were determined by a hemagglutination method using 96-well, U-bottomed microtiter plates according to Wegmann and Smithies (1966). The 2-mercaptoethanol-resistant (MER) antibodies in the serum were measured according to the method described by Bartlett and Smith (2003). The 2-mercaptoethanol-sensitive (MES) antibodies were also estimated as the difference between the total and MER antibody titers.

MER antibodies are presumed to be a measure of IgG titers, whereas MES antibodies consist primarily of IgM titers. Anti-SRBC titers were expressed as the reciprocal of the highest serum dilution resulting in complete agglutination.

Blood Analyses

At 42 d of age, blood samples were collected from 2 birds/replicate into vials containing EDTA to avoid blood clot formation. The red blood cell (RBC) and white blood cell (WBC) counts were measured by a hemocytometer method using Natt-Herrick solution; hemoglobin (Hb) and hematocrit (Hct) values were determined by cyanmethemoglobin and microhematocrit methods, respectively (Kececi et al., 1998). RBC indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were also computed according to Campbell (1988). To determine blood leucocyte profiles, 100 leucocytes/sample were counted using an optical microscope for heterophil and lymphocyte separation according to the protocol described by Lucas and Jamroz (1961), and then the heterophil/lymphocyte (H/L) ratio was calculated.

On day 42, 3 mL of blood was harvested from the brachial vein of 2 birds/replicate following approximately 8 h fasting to avoid diurnal influences. Blood samples were collected into plasma-separating tubes containing lithium heparin (100 IU/5 mL) and centrifuged for 10 min at 2000×g; then plasma was collected and stored at −20°C until the analysis. The concentrations of total protein, glucose, triglycerides (TG), total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein

Table 2. Effects of dietary treatment on growth performance in broilers up to the age of 42 d.

Item	Dietary treatment[1]						SEM	*P*-value
	Control	VM	SS_1	SS_2	FA_1	FA_2		
BW, g								
10 d	200.9	199.2	200.7	194.7	195.1	195.8	3.967	0.773
24 d	925.4^{b}	992.7^{a}	977.76^{a}	904.5^{b}	971.7^{a}	894.8^{b}	15.740	<0.001
42 d	2169.4^{b}	2332.7^{a}	2305.4^{a}	2159.5^{b}	2306.3^{a}	2156.8^{b}	41.959	0.008
ADG, g								
Starter (0–10 d)	15.74	15.56	15.72	15.10	15.14	15.20	0.396	0.729
Grower (11–24 d)	51.76^{b}	56.68^{a}	55.50^{a}	50.70^{b}	55.47^{a}	49.93^{b}	1.102	<0.001
Finisher (25–42 d)	69.11	74.44	73.77	69.72	74.14	70.11	2.061	0.244
Overall (0–42 d)	50.62^{b}	54.50^{a}	53.85^{a}	50.37^{b}	53.87^{a}	50.31^{b}	0.996	0.008
ADFI, g								
Starter (0–10 d)	22.87	21.52	21.18	20.80	20.72	21.00	0.604	0.160
Grower (11–24 d)	91.46^{a,b}	95.16^{a}	87.59^{b,c}	84.83^{c}	86.15^{b,c}	83.25^{c}	2.194	0.008
Finisher (25–42 d)	138.14	141.48	136.64	136.60	136.99	135.20	3.041	0.770
Overall (0–42 d)	95.14^{a,b}	97.48^{a}	92.80^{b,c}	91.77^{b,c}	92.36^{b,c}	90.69^{c}	1.328	0.016
F:G[2], g:g								
Starter (0–10 d)	1.46	1.38	1.35	1.38	1.37	1.38	0.025	0.081
Grower (11–24 d)	1.77^{a}	1.68^{b}	1.58^{c}	1.68^{b}	1.56^{c}	1.67^{b}	0.028	<0.001
Finisher (25–42 d)	2.01	1.90	1.85	1.96	1.85	1.93	0.051	0.226
Overall (0–42 d)	1.88^{a}	1.79^{b,c}	1.72^{c}	1.83^{a}	1.72^{c}	1.81^{a,b}	0.030	0.004

$^{a–c}$Means in the same row without a common superscript differ significantly ($P < 0.05$).

[1]Control, none of the additives; VM, 200 mg/kg VM; SS_1, 4 g/kg *S. striata*; SS_2, 8 g/kg *S. striata*; FA_1, 4 g/kg *F. angulata*; FA_2, 8 g/kg *F. angulata*.

[2]Feed:gain = feed intake/pen/total pen BW gain, corrected for weights of mortalities that occurred during each time period.

cholesterol (LDL-C) in plasma samples were analyzed using an automatic biochemical analyzer (Clima; Ral. Co, Barcelona, Spain), following the instructions of the corresponding reagent kit (Pars Azmoon Co., Tehran, Iran).

Statistical Analysis

Data were analyzed in a completely randomized design using the General Linear Models procedures of SAS (SAS Institute Inc., 2001). Data on growth performance parameters (BW, ADG, ADFI, and F:G) were analyzed on a pen basis, whereas data on microbial population, immune response, and blood constituents were based on individual broilers. Log_2 transformations were performed on antibody titers prior to statistical analysis. When the differences were significant ($P < 0.05$), mean values between treatments were compared using Tukey's test.

RESULTS

Growth Performance

The effects of different dietary treatments on broiler BW, ADG, AFDI, and F:G are shown in Table 2. At 24 and 42 d of age, the BW of the VM, SS_1, and FA_1 groups was higher ($P < 0.01$) compared with other experimental groups. Furthermore, broilers fed the VM, SS_1, or FA_1 diet had higher ($P < 0.01$) ADG in the grower (days 11–24) and the overall (days 0–42) experimental periods compared with all other treatments. In the same periods, VM-fed broilers had the highest ADFI ($P < 0.05$) compared with other groups, except for the control group. Moreover, overall, the ADFI of the FA_2 group was lower ($P = 0.016$) compared with the control group. In the grower period, all diets decreased ($P < 0.001$) the F:G compared with the control diet, where the birds fed the SS_1 or FA_1 diet had the best F:G. Moreover, the F:G of VM, SS_1, and FA_1 groups were better ($P = 0.004$) compared with the control and SS_2 groups during the overall experimental period. In contrast, the addition of VM and herbal additives did not significantly affect ADG, ADFI, and F:G during the starter (days 0–10) and finisher (days 25–42) periods.

Population of Intestinal Lactobacillus and Coliform Bacteria

Bacteriological analysis results at 42 d of age are detailed in Table 3. No differences were found in the viable counts of total *Lactobacillus* in the jejunum and ileum. However, the total count of *Lactobacillus* in the jejunum tended to decrease ($P = 0.083$) when the broilers were fed the VM diet. The cecal populations of *Lactobacillus* were also higher ($P = 0.032$) in broilers fed the SS_2 or FA_2 diet compared with birds fed the control diet at 42 d of age. In contrast, VM-fed broilers exhibited lower ($P = 0.032$) cecal *Lactobacillus* counts than other experimental groups. The numbers of coliforms in the ileum and ceca of birds fed any of the experimental diets were significantly lower ($P < 0.05$) than in birds fed the control diet, where the birds fed the VM diet had the lowest coliform counts. However, there was no

Table 3. Effects of dietary treatment on populations of lactobacilli and coliforms in the jejunum, ileum, and ceca of broilers at 42 d of age (log10 cfu/g fresh digesta).

	Dietary treatment[1]							
Item	Control	VM	SS_1	SS_2	FA_1	FA_2	SEM	*P*-value
Lactobacillus								
Jejunum	8.14	7.64	7.93	8.25	8.04	8.23	0.195	0.083
Ileum	8.47	8.02	8.45	8.65	8.44	8.70	0.321	0.182
Ceca	$8.49^{b,c}$	8.15^{c}	$8.70^{a,b}$	9.12^{a}	$8.74^{a,b}$	9.08^{a}	0.166	0.032
Coliform								
Jejunum	6.29	5.84	6.24	6.37	5.96	6.34	0.313	0.349
Ileum	7.93^{a}	6.50^{c}	7.22^{b}	$6.95^{b,c}$	7.14^{b}	$6.90^{b,c}$	0.268	0.019
Ceca	8.76^{a}	7.58^{c}	8.15^{b}	$7.90^{b,c}$	8.13^{b}	$7.98^{b,c}$	0.192	0.007

[a–c]Means in the same row without a common superscript differ significantly ($P < 0.05$).

[1]Control, none of the additives; VM, 200 mg/kg VM; SS_1, 4 g/kg *S. striata*; SS_2, 8 g/kg *S. striata*; FA_1, 4 g/kg *F. angulata*; FA_2, 8 g/kg *F. angulata*.

significant difference in the coliform counts in the jejunum among any of the treatments.

Immune Response

The effects of dietary treatment on lymphoid organ weights and secondary antibody responses against SRBC are shown in Table 4. Spleen index was higher ($P = 0.017$) in birds fed the SS_2 or FA_2 diet compared with those fed the control diet. However, bursa and thymus indices were unaffected by dietary treatment.

The additives used in this study failed to have any significant impact on the anti-SRBC titers of total, IgM, and IgG antibodies at 35 d of age (data not shown). In the case of secondary response, there was also an increase ($P < 0.001$) in total anti-SRBC titers in broilers fed the SS_2 or FA_2 diet compared with other experimental groups (Table 4). Moreover, serum IgG levels were similar in birds fed the SS_2 (3.80) or FA_2 (3.60) diet but were higher ($P = 0.003$) than IgG levels of the control (2.80), VM (2.50), and FA_1 (2.80) groups with respect to secondary response. In contrast, during the secondary challenge, the SRBC-specific IgM titer in the broiler serum showed no differences across treatments.

Blood Characteristics

There were no significant differences in hematological parameters, including, RBC, Hb, Hct, and RBC indices (MCV, MCH, and MCHC), among any of the treatments (data not shown). In contrast, WBC counts were significantly higher ($P = 0.037$) in birds fed the SS_2 diet compared with birds fed the control diet (Table 5). Feeding the SS_2 or FA_2 diet also increased ($P = 0.022$) the lymphocyte count and decreased ($P = 0.043$) the H/L ratio compared with the VM and control diets. However, heterophil count was not affected by any of the treatments.

There were no differences in plasma total protein and glucose levels among experimental groups (Table 5). All diets, except for the VM diet, decreased ($P = 0.009$) plasma CHOL level compared to the control diet. Feeding the SS_2 or FA_2 diet reduced ($P = 0.026$) plasma TG levels compared with the control and VM diets, but only

Table 4. Effect of dietary treatment on lymphoid organ weights and secondary antibody response against sheep red blood cell in broilers at 42 d of age.

	Dietary treatment[1]							
Item	CD	VM	SS_1	SS_2	FA_1	FA_2	SEM	*P*-value
Lymphoid organ weight[2]								
Spleen index	0.91^{b}	$1.15^{a,b}$	$1.24^{a,b}$	1.43^{a}	$1.30^{a,b}$	1.39^{a}	0.145	0.017
Bursa index	1.92	2.15	2.16	2.13	2.20	2.36	0.267	0.326
Thymus index[3]	3.65	3.81	3.83	4.10	3.93	4.07	0.321	0.152
Secondary anti-SRBC titer[4]								
Total antibody	6.40^{b}	6.10^{b}	7.00^{b}	8.10^{a}	6.90^{b}	8.00^{a}	0.322	<0.001
IgM	3.60	3.60	3.70	4.30	4.10	4.40	0.286	0.177
IgG	$2.80^{b,c}$	2.50^{c}	$3.30^{a,b}$	3.80^{a}	$2.80^{b,c}$	3.60^{a}	0.250	0.003

[a–c]Means in the same row without a common superscript differ significantly ($P < 0.05$).

[1]Control, none of the additives; VM, 200 mg/kg VM; SS_1, 4 g/kg *S. striata*; SS_2, 8 g/kg *S. striata*; FA_1, 4 g/kg *F. angulata*; FA_2, 8 g/kg *F. angulata*.

[2]The organ index is expressed as immune organ weight (g) per BW (kg).

[3]All thymic lobes from both sides of neck were weighed for each chick.

[4]Values are means of $\log_2$ of reciprocal of last dilution exhibiting agglutination.

Table 5. Effect of dietary treatment on blood characteristics of broilers at 42 d of age.

	Dietary treatment[1]							
Item[2]	Control	VM	SS_1	SS_2	FA_1	FA_2	SEM	*P*-value
WBC, $\times 10^3/\mu L$	15.11[b]	15.43[a,b]	15.76[a,b]	16.27[a]	15.66[a,b]	15.24[a,b]	0.385	0.037
Lymphocyte[3], %	58.91[b]	59.17[b]	66.03[a,b]	68.72[a]	63.80[a,b]	68.02[a]	2.74	0.022
Heterophil[3], %	31.24	30.72	25.33	23.50	27.82	24.00	2.66	0.128
H/L	0.53[a]	0.52[a]	0.39[a,b]	0.34[b]	0.44[a,b]	0.35[b]	0.055	0.043
Total protein, g/dl	3.77	3.95	4.33	4.19	4.55	4.03	0.42	0.432
Glucose, mg/dl	197.94	213.33	187.32	205.21	203.10	182.11	15.44	0.320
Triglyceride, mg/dl	97.98[a]	93.92[a]	80.77[a,b]	75.95[b]	84.26[a,b]	68.57[b]	6.45	0.026
CHOL, mg/dl	138.53[a]	128.67[a,b]	105.89[c]	103.36[c]	119.34[b,c]	112.68[b,c]	5.98	0.009
HDL-C, mg/dl	56.74[b]	69.92[a,b]	68.39[a,b]	71.51[a]	63.24[a,b]	76.07[a]	5.70	0.042
LDL-C, mg/dl	66.5[a]	63.7[a]	53.4[a,b]	42.5[b]	54.3[a,b]	50.1[a,b]	7.93	0.006

[a–c]Means in the same row without a common superscript differ significantly ($P < 0.05$).

[1]Control, none of the additives; VM, 200 mg/kg VM; SS_1, 4 g/kg *S. striata*; SS_2, 8 g/kg *S. striata*; FA_1, 4 g/kg *F. angulata*; FA_2, 8 g/kg *F. angulata*.

[2]WBC, white blood cell; H/L, heterophil/lymphocyte; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

[3]Values presented as percentage of total WBC count.

the SS_2 diet reduced ($P = 0.006$) plasma LDL-C levels. The plasma HDL-C level was also higher ($P = 0.042$) in birds fed the SS_2 or FA_2 diet than in birds fed the control diet.

DISCUSSION

The higher BW and ADG in broilers fed 4 g/kg *S. striata* or *F. angulata* reveal that the impact of phytogenic products on performance could be related to the presence of growth-promoting substances in these herbs. The improvements were of similar magnitude to those determined for treatment containing VM, a well-documented AGP in broiler diets (Belay and Teeter, 1994). The present findings also indicate that the SS_1 and FA_1 treatments resulted in an improved F:G by 8.5% in the overall period compared to the unsupplemented control treatment. In general, an improvement in F:G in broilers when feeding phytogenic additives is evidenced in the majority of the literature recently reviewed by Brenes and Roura (2010), who stated that in most studies the improvement in feed efficiency comes as result of a decreased feed intake at a largely unaffected BW gain. In this study, the improved F:G in the grower period and the overall experiment was associated more with an increase in ADG and less with a decrease in ADFI (Table 2).

However, in the current study, broilers receiving the SS_2 or FA_2 diet reached a lower BW and grew more slowly than those receiving the SS_1 or FA_1 diet, mainly because of a lower feed intake. This might be due to the adverse effects of toxic components, such as glycoside terpenoids, present in the genus *Scrophularia* (Ardeshiry Lajimi et al., 2010) or benzenoid derivatives contained in the genus *Ferulago* (Sajjadi et al., 2012). Golfakhrabadi et al. (2015) also reported that some plants from the genus *Ferulago* exhibited promising cytotoxic activity against the human carcinoma cell line. However, the point that should be discussed here is that the toxicity dose of selected herbs in this study was not clear because the dietary herbal supplements causes no performance losses or mortality, even with their high levels in the diet (8 g/kg).

In terms of the gut, the antimicrobial properties of *S. striata* and *F. angulata* in this study are supposed to favor a healthy gut, which in turn could be related to the F:G improvement. However, whether such an antimicrobial activity could have a positive effect on the beneficial gut bacteria remains an open question. It is suggested that essential oil compounds might exhibit strong antimicrobial activity against gut pathogens while not harming beneficial bacteria such as lactobacilli and bifidobacteria (Si et al., 2006). In this study, it was shown that herbal additives modulated the ileal and cecal microflora composition via the reduction of coliforms at the age of 42 d. Those birds fed the diets supplemented with *S. striata* or *F. angulata* at the level of 8 g/kg showed a similar magnitude of reduction in the coliform counts when compared to those fed the antibiotic-supplemented diets. The antibacterial properties of the aerial parts of both herbs could be attributed mainly to their essential oil components (Mohebat et al., 2010; Monsef-Esfahani et al., 2010). The increase in the levels of cecal lactobacilli in the SS_2 or FA_2 group, compared to the control group, is an interesting result that is hard to interpret. Under in vivo studies, the effect of herbal additives on the chicken gastrointestinal microbiota appears to be inconsistent (Cross et al., 2007; Giannenas et al., 2010; Khalaji et al., 2011; Abdel-Wareth et al., 2012), even though active components from the herbs have been generally recognized as an antimicrobial agent. Therefore, it is hypothesized that the in vivo antimicrobial property of herbal active components in broilers can be influenced by environmental conditions and basal diet.

Another notable result from this study was the highest secondary antibody response observed in birds fed the SS_2 or FA_2 diet. This could be another factor

explaining the lack of effect of these treatments on BW and ADG. Whenan immune response is stimulated, nutrients are used for the production of immunoglobulin antibodies, and thus the growth rate is retarded. Some reports indicate that the use of 3g/kg *F. angulata* in the broiler diet may promote humoral immunity by increasing antibody titers against the influenza virus (Govahi et al., 2013). Immunomodulatory activities of some species of *Scrophularia* have also been demonstrated by other investigators (Azadmehr et al., 2011, 2012). As suggested, enhancement of the growth of intestinal lactic acid bacteria will boost the immune system's response (Perdigón et al., 2001; Herich and Levkut, 2002). The increase in the relative weight of spleen recorded in broilers fed diets supplemented with 8 g/kg *S. striata* or *F. angulata* is likely attributable to higher lymphocyte proliferation. Analogous results were also obtained in terms of H/L ratios. Monsef-Esfahani et al. (2010) discovered that the essential oils from aerial parts of *S. striata* contain quercetin and isorhamnetin 3-O-rutinoside, which play important roles as antioxidant active substances. Therefore, enhanced lymphocyte proliferation by the SS_2 treatment, along with the possible protection of the cells from oxidative stress, seemed to contribute to the increased WBC count reported in this study.

In relation to the results of plasma lipid profile, it can be deduced that *S. striata* and *F. angulata* have favorable effects on lipid and cholesterol metabolism. Like the results of this study, administration of a hydroalcoholic extract of *F. angulata* reduced blood levels of total cholesterol and triglycerides in male Wistar rats (Rafieian-kopaei et al., 2014). The mechanism by which dietary herbal supplements affect the concentrations of plasma lipids is not fully understood. However, herbs and herbal products are known to induce hypocholesterolemic effects by reducing the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol synthesis (Hong et al., 2012). The hypolipidemic effects of herbs used in this study might not originate from their direct effect on lipid metabolism; however, it could be mediated by their effect on lowering oxidized lipid products. This effect may be associated with an increase in the plasma HDL-C concentration of the respective treatment groups. HDL carries important antioxidant enzymes, especially glutathione peroxidase and phospholipid hydroperoxide, and appears to exchange undamaged phospholipids for oxidized phospholipids in LDL (Moradi et al., 2009).

In conclusion, the inclusion of 4 g/kg *S. striata* or *F. angulata* in broiler diet resulted in an improved overall ADG and F:G comparable to that of VM used as an AGP. In particular, the beneficial effects of these herbs on growth performance became evident in the grower period. The gut microbial study revealed that, irrespective of inclusion level, adding the mentioned herbs to the diet decreased coliform bacteria counts in the ileum and ceca, with the high inclusion level of 8 g/kg resulting in increased cecal *Lactobacillus* counts compared with the control and VM groups. Interestingly, the dietary inclusion of 8 g/kg of herbal supplements resulted in a beneficial modulation of the immune response and lipid metabolism. These findings justify further investigation on the aforementioned herbs in varying dosages and diverse conditions to achieve more comprehensive results.

ACKNOWLEDGMENTS

The authors are grateful to Ilam University for the financial support that allowed them to carry out this work.

REFERENCES

Abdel-Wareth, A. A. A., S. Kehraus, F. Hippenstiel, and K. H. Südekum. 2012. Effects of thyme and oregano on growth performance of broilers from 4 to 42 d of age and on microbial counts in crop, small intestine and caecum of 42-d-old broilers. Anim. Feed Sci. Technol. 178:198–202.

AOAC International. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Ardeshiry Lajimi, A., M. Rezaie-Tavirani, S. A. Mortazavi, M. Barzegar, S. H. Moghadamnia, and M. B. Rezaee. 2010. Study of anti cancer property of *Scrophularia striata* extract on the human astrocytoma cell line (1321). Iran. J. Pharm. Res. 9: 403–410.

Azadmehr, A., R. Hajiaghaee, A. Afshari, Z. Amirghofran, M. Refieian-Kopaei, H. yousofi Darani, and H. Shirzad. 2011. Evaluation of *in vivo* immune response activity and *in vitro* anticancer effect by *Scrophularia megalantha*. J. Med. Pl. Res. 5: 2365–2368.

Azadmehr, A., G. H. Maliji, R. Hajiaghaee, M. Shahnazi, and A. Afaghi. 2012. Inhibition of pro-inflammatory cytokines by ethyl acetate extract of *Scrophularia striata*. Trop. J. Pharm. Res. 11:893–897.

Bahrami, A., and A. Valadi. 2010. Effect of *Scrophularia ethanolic* leaves extracts on *Staphylococcus aureus*. Int. J. Pharmacol. 6:393–396.

Bartlett, J. R., and M. O. Smith. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult. Sci. 82:1580–1588.

Belay, T., and R. G. Teeter. 1994. Virginiamycin effects on performance and salable carcass of broilers. J. Appl. Poult. Res. 3:111–116.

Brenes, A., and E. Roura. 2010. Essential oils in poultry nutrition: Main effects and modes of action. Anim. Feed Sci. Technol. 158:1–14.

Brisbin, J. T., J. Gong, C. A. Lusty, P. Sabour, B. Sanei, Y. Han, P. E. Shewen, and S. Sharif. 2008. Influence of in-feed virginiamycin on the systemic and mucosal antibody response of chickens. Poult. Sci. 87:1995–1999.

Campbell, T.W. 1988. Avian Hematology and Cytology. Iowa State University Press, Iowa City, IA, USA.

Choct, M. 2009. Managing gut health through nutrition. Br. Poult. Sci. 50:9–15.

Cross, D. E., R. M. McDevitt, K. Hillman, and T. Acamovic. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br. Poult. Sci. 48:496–506.

Giannenas, I., D. Tontis, E. Tsalie, E. F. Chronis, D. Doukas, and I. Kyriazakis. 2010. Influence of dietary mushroom *Agaricus bisporus* on intestinal morphology and microflora composition in broiler chickens. Res. Vet. Sci. 89:78–84.

Golfakhrabadi, F., M. Khanavi, S. N. Ostad, S. Saeidnia, H. Vatandoost, M. R. Abai, M. Hafizi, F. Yousefbeyk, Y. Razzaghi Rad, A. Baghenegadian, and M. R. Shams Ardekani. 2015. Biological

activities and composition of *Ferulago carduchorum* essential oil. J. Arthropod-Borne Dis. 9:104–115.

Govahi, R., G. Ghalamkari, M. Toghyani, S. Eghbal Saied, M. Mohammadrezaei, M. Shahryari, and A. Dehghani Abari. 2013. Effect of *Ferulago angulata* sub. *carduchorum* on total serum antioxidant activity and some of the humoral immune responses in broiler chicks. J. Herb. Drugs 4:119–126.

Hashemi, S. R., and H. Davoodi. 2011. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet. Res. Commun. 35:169–180.

Herich, R., and M. Levkut. 2002. Lactic acid bacteria, probiotics and immune system. Vet. Med.–Czech 47:169–180.

Hong, J. C., T. Steiner, A. Aufy, and T. F. Lien. 2012. Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. Livest. Sci. 144:253–262.

Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1998. Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. Poult. Sci. 77:1259–1265.

Kececi, O., H. Oguz, V. Kurtoglu, and Ö. Demet. 1998. Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br. Poult. Sci. 39:452–458.

Khalaji, S., M. Zaghari, K. Hatami, S. Hedari-Dastjerdi, L. Lotfi, and H. Nazarian. 2011. Black cumin seeds, *Artemisia* leaves (*Artemisia sieberi*), and *Camellia* L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. Poult. Sci. 90, 2500–2510.

Khanahmadi, M., and K. Janfeshan. 2006. Study of antioxidant property of *Ferulago angulata* plant. Asian J. Plant Sci. 5:521–526.

Leshchinsky, T. V., and K. C. Klasing. 2001. Relationship between the level of dietary vitamin E and the immune response of broiler chickens. Poult. Sci. 80:1590–1599.

Lucas, A. M., and C. Jamroz. 1961. Atlas of Avian Hematology. Agriculture Monograph 25. USDA, Washington, DC.

Mahboubi, M., N. Kazempour, and A. R. Boland Nazar. 2013. Total phenolic, total flavonoids, antioxidant and antimicrobial activities of *Scrophularia Striata* Boiss Extracts. Jundishapur J. Nat. Pharm. Prod. 8:15–19.

Mohebat, R., M. H. Mosslemin, S. Masoudi, V. Dad, and A. H. Rustaiyan. 2010. Composition and antibacterial activity of the essential oils from aerial parts, stems, flowers and leaves of *Ferulago contracta* from Iran. J Essent. Oil Bear. Pl. 13:607–614.

Monsef-Esfahani, H., R. Hajiaghaee, A. R. Shahverdi, M. R. Khorramizadeh, and M. Amini. 2010. Flavonoids, cinnamic acid and phenyl propanoid from aerial parts of *Scrophularia striata*. Pharm. Biol. 48:333–336.

Moradi, H., M. V. Pahl, R. Elahimehr, and N. D. Vaziri. 2009. Impaired antioxidant activity of high-density lipoprotein in chronic kidney disease. Trans. Res. 153:77–85.

Perdigón, G., R. Fuller, and R. Raya. 2001. Lactic acid bacteria and their effect on the immune system. Curr. Issues Intest. Microbiol. 2:27–42.

Rafieian-kopaei, M., N. Shahinfard, H. Rouhi-Boroujeni, M. Gharipour, and P. Darvishzadeh-Boroujeni. 2014. Effects of *Ferulago angulata* extract on serum lipids and lipid peroxidation. Evid. Based Complement. Alternat. Med. Article ID 680856. http://dx.doi.org/10.1155/2014/680856.

Richman, A. D., W. Broothaerts, and J. R. Kohn. 1997. Self-incompatibility mases from three plant families homology or convergence? Am. J. Bot. 84:912–917.

Sajjadi, S. E., Y. Shokoohinia, and M. Jamali. 2012. Chemical composition of essential oil of *Ferulago macrocarpa* (Fenzl) Boiss. fruits. Res. Pharm. Sci. 7:197–200.

SAS Institute Inc. 2001. SAS User's Guide: Statistics. Version 8. Cary, NC.

Si, W., J. Gong, R. Tsao, T. Zhou, H. Yu, C. Poppe, R. Johnson, and Z. Du. 2006. Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. J. Appl. Microbiol. 100:296–305.

Taran, M., H. R. Ghasempour, and E. Shirinpour. 2010. Antimicrobial activity of essential oils of *Ferulago angulata* subsp. carduchorum. Jundishapur J. Microbiol. 3:10–14.

Wegmann, T., and O. Smithies. 1966. A simple hemagglutination system requiring small amounts of red cells and antibodies. Transfusion 6:67–75.