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Effect of *Scrophularia striata* Extract on Performance, Intestinal Microbial and Histo-morphometry, and Blood Parameters in Broilers under Normal or Challenged Condition with *E. Coli*

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Abstract

The effect of different levels of *Scrophularia striata* extract in normal and challenged conditions with *E. coli* on performance, carcass characteristics, cellular immune response, blood antioxidant status, intestinal histo-morphometry, and microbial population (*E.coli* and Lactobacillus) of Cobb 500 broilers were evaluated. The experiment was performed in a complete block design with a 2 × 4 factorial arrangement with five replications of 10 birds each for 35 days in two separated halls [include challenge (C) and non-challenge (N)] with similar experimental conditions. Experimental treatments in two breeding halls were: 1) basal diet or control (CONT), 2) basal diet + 0.1 g/kg of herbal extract (EXTR 1), 3) basal diet +0.2 g/kg of herbal extract (EXTR 2), 4) Basal diet +0.1 g/kg an antibiotic (Oxytetracycline) (ANTB). On the 16th and 24th days of the experiment, one dose of 1×10^{7} CFU K99 *E. Coli* was gavaged to chickens in a challenged conditioned hall. The results showed that average body weight and daily weight gain in the whole period (days 35 and 1-35) in EXTR 2 was better than the control treatment ($P < 0.05$). The percentage of breast weight in the ANTB was significantly different from the control treatment ($P < 0.05$). Glutathione peroxidase (GPX) in EXTR 2 was better than the control treatment ($P < 0.05$). Malondialdehyde (MDA) in EXTR 1 was significantly lower than the control group ($P < 0.05$). The values of *E. coli* in the treatment ANTB were less than control treatment ($P < 0.05$). Lactobacillus value in treatment EXTR 2 was higher than control treatment ($P < 0.05$). The value of total immunoglobulin in 28 days in ANTB, EXTR 1, and EXTR 2 were significantly higher than that of control treatment ($P < 0.05$). The length of villi was affected by treatments ($P < 0.05$). In conclusion, dietary inclusion of 0.2 g/kg of *Scrophularia striata* extract may improve the health status of the birds during *E. coli* challenge.

Introduction

In recent years, medical plants have become favorite feed additives in the diet of broilers due to the variety of useful mechanisms. In other words, these additives can modulate some intestinal functions, such as the passage rate, digestive fluid secretion, activity of digestive enzymes, thereby changing nutrient digestibility. For example, different types of spices, including turmeric, capsaicin, ginger, and pepper have been shown to stimulate pancreatic digestive enzymes in adult mice (Platel and Srinivason, 2000). In broilers, it has been shown that medicinal plants increase the intestinal activity of trypsin, Lipase, and amylase (Jamroz *et al*., 2005; Lee *et al*., 2003). Adding herbal extracts to feed for 41 days in broilers increased lipase activity by 38 to 46 percent (Jamroz *et al*., 2005). Rosemary essential oil (containing Rosmarinic acid, flavonoids, and monoterpenes) increased liver metabolism and the relative liver weight in mice

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(Debersac *et al*., 2001). Medical plants increase mucus production in the stomach and jejunum, which play a protective role against intestinal pathogens (Jamroz *et al*., 2006). Another mechanism of action is that spices along with organic acids stimulate the proteins breakdown by lowering the pH of stomach and bringing it to the optimal level for pepsin activity (Kamel, 2001). Regarding the antimicrobial properties of medicinal plants, various medicinal plants have been showed to work against a wide range of pathogens such as *Salmonella typhimurium*, *Salmonella enteritis*, *Escherichia coli* H7:0157, *Shigella dysentery*, *Bacillus cypress*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Lambert *et al*., 2001; Burt, 2004; Chorianopoulos *et al*., 2004; Penalver *et al*., 2005; Si *et al*., 2006). The antibacterial properties of herbal essential oils are mainly attributed to their phenolic compounds (Burt 2004; Penalver *et al*., 2005; Si *et al*., 2006) that are generally believed these compounds disrupt cell cytoplasmic membranes and proton displacement, electron flow, active transport and coagulation of cell content (Burt, 2004; Lambert *et al*., 2001). Interestingly, other studies have shown that herbal essential oils selectively inhibit intestinal pathogens, but have no detrimental effect on beneficial intestinal bacteria such as bifidobacterial and lactobacillus (Si *et al*., 2006). 12 plant extracts have been shown that improve performance and reduce ascites and necrotic diarrhea in broilers (Vidanarachchi, 2005). Research has shown that medicinal plants stimulate the growth of immune organs (Takahashi *et al*., 2000; Hevener *et al*., 1999).

The plant that studied in this research is *Scrophularia striata* from the Scrophulariaceae genus, which is mostly herbaceous or shrubby and rarely a tree with intermittent opposite or provide leaves, simple and without earlobes, five-feathered flowers, zygomorph, flower cup with lobes and fruit, usually in the form of capsules with multiple seeds (Amirghofran *et al*., 2000). The results of studies showed that *Scrophularia striata* plant extract inhibits nitric oxide production, that the plant may be used to treat inflammatory disease (Babri *et al*., 2012). *Scrophularia striata* with a local name of Tashnedari is a self-growing perennial plant from snapdragon genus that grows mainly in Ilam province and in some area of Khuzestan and Kermanshah provinces (Mozafarian, 1999). An in vitro study reported a strong inhibitory effect of Scrophularia aqueous extract against E. coli growth following coincubation for a 24 h period. incunated during a 24 h period. (Zamanian Azodi *et al*., 2012). In this study, we intend to investigate the effects of plant extract (*Scrophularia striata*) on the productive performance and some physiological parameters in broilers.

Materials and methods

The current research was conducted in June, when *Scrophularia striata* is in the adult stage. Plant samples (stems, leaves, and fruits) were collected in the required amount from the mountains around the city of Ilam, dried in the shade. Alcoholic extract was prepared by incubating dried powder with ethanol 98% (400: 1000 w/v) (Babri *et al*., 2012). The extract was then passed through a filter and almost green color liquid was obtained, which was kept in a cool place away from sunlight for further analysis. To determine the type and percentage of active ingredients of storage sample, 20 mL was sent to the central laboratory of the Jundishapour University of Ahwaz to perform the GC-MASS test (Table 1). In order to a get a dry pure plant extract without alcohole, wet extracts freeze dried in the laboratory of the faculty of agriculture, and a certain amount of extract was added to the experimental diet.

Birds, management, and treatments

In this experiment 400 male Cobb 500 broilers were used for 35 days in two separate halls but under the same breeding conditions. These experiments include four treatments in each block, five replications of 10 chickens. Chickens were randomly distributed across all 40 pens (pen dimensions) based on the same average weight. The environmental conditions were uniform for all birds in both halls. During the experiment, feed and water were provided *ad libitum*. The experiment was performed in a completely randomized design with eight treatments and five replications. The experimental diets were prepared based on a corn-soybean meal for three periods of 1-10, 11-22, and 23-35 days of starter, grower, and finisher periods according to Cobb 500 breeding guide (Table 2). The experimental diets were prepared by UFFDA software. To create a challenging condition in one of the breeding halls, E. coli K99 strains with a concentration of 1×10^7 CFU/mL were used via oral gavage, 1 mL on 16 and 24 days (Asani *et al*., 2015). Experimental treatments in two breeding halls were: 1) basal diet; CONT, 2) basal diet + 0.1 g/kg of herbal extract; EXTR 1, 3) basal diet + 0.2 g/kg of herbal extract; EXTR 2,and 4) basal diet + 0.1 g/kg of antibiotic (Oxytetracycline 50%, Iran Darou); ANTB. Challenged hall treatments were similar to healthy hall (Challenged hall= C and Non-challenged hall= N).

Recording, slaughtering, and sampling

The performance of chickens (feed intake, weight gain, and feed to gain ratio) was measured weekly at the end of each period. At 35 days of age, one chicken from each replication close to the average replication weight was slaughtered, and the relative weight of carcass components was calculated. To assess intestinal bacteria (*E. coli* and Lactobacillus) and morphologic appearances of jejunum, 2 cm proximal to Meckel's diverticulum was selected. On

the same day, blood samples were collected for malondialdehyde (MDA, TBARS method), glutathione peroxidase (GPX, Ransel kit, Randox company of united kingdom), and corticosteroid hormone levels (Elisa kit, ZellBio Germany). On 21 and 28 days of age, two chickens from each replicate of treatments were selected, and 5 mL of serum blood samples were taken for primary and secondary humoral immune responses, heterophile (H), lymphocyte (L) and their ratio (H/L), and hematocrit (HCT) status of the experimental treatments. All the mentioned indices were analyzed based on GC-MASS (Agilent 7890 Gas Chromatograph Coupled to a 5975 mass selective detector,MSD, USA) methods (Cheema *et al*., 2003; Gross and Siegel, 1983; Paglia and Valentine, 1967; Wawrzyniak *et al*., 2017).

Table 1. The main active ingredients of *Scrophularia striata* extract (based on GC-MASS test)

Active ingredients	Percentage of effective substance
Linalool	43.02
β-phellandrene	8.47
Nerol	4.29
Ethanone	1.40
Pentanal	2.71
Butanal	1.62
Propane	1.99
Butanoic acid	1.21
Pentanone	4.23
Hexanal	2.34
Hexanol	1.77
Xylene	1.31
Benzaldehyde	1.82
Octenol	3.19
Benzene acetaldehyde	2.76
Nanonal	1.45
Δ-Careen	1.50
Octanol	0.49
Limonene	0.37
Cineole	0.13
Corsinitol	0.29
Other compounds	13.64

Table 2. Composition and nutrient content of the experimental diets of broilers from 1-35 days of age

Diet ingredients (%)	Starter (1-10 days)	Grower (11-22 days)	Finisher (23-35 days)
Corn	59.83	63.80	66.40
Soybean meal (CP 44%)	33.80	29.54	26.56
Di-Calcium phosphate	1.78	1.66	1.48
Limestone	0.80	0.74	0.66
Salt	0.37	0.37	0.37
Vegetable oil	2.23	2.78	3.57
DL- Methionine	0.33	0.28	0.24
L- lysine Hydrochloride	0.28	0.26	0.18
L-Threonine	0.08	0.07	0.04
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
Calculated composition			
Metabolizable Energy (kcal/kg)	3008	3086	3167
Crude protein %	21.06	19.31	18.00
Calcium %	0.90	0.84	0.76
Available phosphorous %	0.45	0.42	0.38
Sodium %	0.16	0.16	0.16
Lysine %	1.32	1.19	1.05
Methionine %	0.63	0.57	0.51
Methionine + Cystine %	0.98	0.89	0.82
Threonine %	0.86	0.78	0.71

[1]Each kilogram of vitamin supplement contains 3600000 IU vitamin A, 800000 IU vitamin D3, 14400 IU vitamin E, 800 mg vitamin K, 700 mg vitamin B1, 2640 mg vitamin B2, 3920 mg vitamin B3, 11860 mg vitamin B5, 1176 mg vitamin B6, 400 mg vitamin B9, 40 mg of vitamin H2, 6 mg of vitamin B12, 100,000 mg of choline chloride 60%, 400 mg of antioxidants.
[2]Each kilogram of the mineral supplement contains 39680 mg manganese, 20000 mg iron, 33880 mg zinc, 4000 mg copper, 396 mg iodine, 100000 mg choline chloride, 80 mg selenium, and 1000 g carrier (wheat bran and calcium carbonate).

Statistical analysis

In a randomized complete block design with a 2 × 4 factorial arrangement, the obtained data were analyzed by SAS statistical software (2001) and GLM producer. The Tukey's test was used to compare treatment means when $P < 0.05$.

Results

The effect of treatments on average body weight gain (ABW) during the whole breeding period is shown in Table 3. There was a significant difference between ANTB and CONT treatments in ABW on day 10 of the study ($P < 0.05$). On day 22, the body weight gain in ANTB was more than those of CONT and EXTR 1 ($P<0.05$). On day 35, the EXTR 2 was more than that of CONT ($P < 0.05$). Body weight gain between N and C groups was also significant ($P < 0.05$). A significantly higher daily body weight gain in EXTR 1, EXTR 2 and ANTB was observed during the whole period (1-35) and 23-35 days of the study when they comparted with that of CONT group ($P < 0.05$). Birds in normal conditions (N group) also had significantly higher ABW when compared to those of challenged birds (C group) during the whole period (1-35 days) of the experiment ($P < 0.05$).

Table 3. The effect of *Scrophularia striata* extract on the average body weight and body weight gain of broilers at different ages

	Average body weight (gram/bird)			Daily weight gain (gram/bird/day)			
Treatments *	Day 10	Day 22	Day 35	1-10 days	11-22 days	23-35 days	1-35 days
CONT	194.4 ^{b}	729.5 ^{c}	1447.9 ^{b}	15.6 ^{b}	44.6 ^{c}	55.3 ^{b}	40.3 ^{b}
EXTR1	201.0 ^{ab}	772.3 ^{b}	1595.8 ^{a}	16.3 ^{ab}	47.4 ^{b}	63.4 ^{a}	44.3 ^{a}
EXTR2	202.6 ^{ab}	779.2 ^{ab}	1643.1 ^{a}	16.5 ^{ab}	48.1 ^{ab}	66.5 ^{a}	45.8 ^{a}
ANTB	208.6 ^{a}	814.1 ^{a}	1633.7 ^{a}	17.1 ^{a}	50.5 ^{a}	63.0 ^{a}	45.5 ^{a}
SEM	2.52	10.10	25.85	0.25	0.67	1.90	0.70
P-value	0.004	0.0001	0.0001	0.0042	0.0001	0.0018	0.0001
Challenge with *E. coli*							
N	202.0	779.0	1625.4 ^{a}	16.4	48.0	56.1 ^{a}	45.2 ^{a}
C	201.3	768.6	1534.9 ^{b}	16.3	47.3	59.0 ^{b}	42.7 ^{b}
SEM	1.78	7.14	18.28	0.18	0.48	1.35	0.51
P-value	0.7692	0.3115	0.0014	0.8028	0.3120	0.0028	0.0013
Treatments × Challenge with *E. coli* **							
CONT × N	195.4	737.9	1470.9	15.7	45.2	56.4	40.9
CONT × C	193.4	721.1	1424.9	15.6	44.0	54.1	39.6
EXTR1 × N	202.0	784.1	1648.5	16.4	48.1	66.5	45.7
EXTR1 × C	200.0	760.5	1543.2	16.3	46.7	60.2	43.0
EXTR2 × N	204.4	790.4	1686.8	16.6	48.8	69.0	47.0
EXTR2 × C	200.8	768.1	1599.4	16.3	47.3	63.9	44.6
ANTB × N	206.3	803.5	1695.2	16.9	49.8	68.6	47.4
ANTB × C	210.9	824.6	1572.1	17.3	51.1	57.5	43.6
SEM	3.57	24.29	56.56	0.36	1.95	2.99	1.31
P- value	0.6699	0.3625	0.7485	0.7239	0.3802	0.4316	0.6910

* N: Non-challenging house with *E. coli*; C: Challenging house with *E. coli*; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; *SEM*: Mean standard error.

** Interactions between treatments and E. coli challenge.

$^{a-c}$ Means within each column with different superscripts are significantly different ($P < 0.05$).

The effect of treatments on feed intake and feed to gain ratio of broilers are shown in Table 4. The results showed that in the starter period (1-10 days), daily feed intake in ANTB treatment was significantly different from CONT treatment. In the growing period (11-22 days), feed intake was not affected by treatments. In the finisher period (23-35 days), the control treatments were significantly different from EXTR 1, EXTR 2, and ANTB ($P < 0.05$), and during this period, the treatments were affected by the challenge and the difference between the blocks was significant ($P < 0.05$). Considering daily feed intake during the whole period (1-35 days), there were significant differences among ANTB, EXRT 1 and EXTR 2 with CONT ($P < 0.05$). Although there were no significant differences in the treatments on days 0-10, 23-35, and 1-35 days, but the treatments on 11-22 days and blocks on 23-35 days were significantly different ($P < 0.05$).

Table 4. The effect of *Scrophularia striata* extract on daily feed intake and feed to gain ratio of broilers at different ages

Treatments*	Daily Feed Intake (gram/bird)				Feed to gain ratio			
	1-10 days	11-22 days	23-35 days	1-35 days	1-10 days	11-22 days	23-35 days	1-35 days
CONT	21.8 [b]	60.5	105.2 [b]	66.0 [b]	1.4	1.4 [a]	1.9	1.6
EXTR1	22.4 [a]	61.0	116.1 [a]	70.2 [a]	1.4	1.3 [ab]	1.9	1.6
EXTR2	22.4 [a]	62.7	117.0 [a]	71.3 [a]	1.4	1.3 [ab]	1.8	1.6
ANTB	22.9 [a]	63.2	114.4 [a]	70.5 [a]	1.3	1.3 [b]	1.9	1.6
SEM	0.15	1.11	1.32	0.70	0.02	0.02	0.07	0.03
P-value	0.0002	0.271	0.0001	0.0001	0.1623	0.004	0.6266	0.2098
Challenge with *E. coli*								
N	22.3	62.5	114.5 [a]	70.2	1.4	1.3	1.8 [b]	1.6
C	22.4	61.2	111.8 [b]	68.8	1.4	1.3	1.9 [a]	1.6
SEM	0.10	0.79	0.93	0.49	0.01	0.01	0.03	0.02
P-value	0.342	0.265	0.0468	0.0632	0.4083	0.6598	0.0423	0.0538
Treatments × Challenge with *E. coli* **								
CONT × N	21.9	61.2	105.2	66.3	1.4	1.4	1.9	1.6
CONT × C	21.7	59.7	105.2	65.8	1.4	1.4	2.0	1.7
EXTR1 × N	22.4	62.6	118.0	71.3	1.4	1.3	1.8	1.6
EXTR1 × C	22.5	59.4	114.1	69.2	1.4	1.3	1.9	1.6
EXTR2 × N	22.5	63.7	118.7	72.2	1.4	1.3	1.8	1.5
EXTR2 × C	22.4	61.7	115.2	70.3	1.4	1.3	1.8	1.6
ANTB × N	22.5	62.2	116.1	70.9	1.3	1.3	1.7	1.5
ANTB × C	23.2	64.1	112.7	70.1	1.4	1.3	2.01	1.6
SEM	0.41	1.47	2.86	1.39	0.02	0.03	0.08	0.04
P- value	0.1098	0.4275	0.692	0.8266	0.9715	0.917	0.5686	0.7903

* N: Non-challenging house with *E. coli*; C: Challenging house with *E. coli*; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; *SEM*: Mean standard error.

** Interactions between treatments and E. coli challenge.

[a-b] Means within each column with different superscripts are significantly different ($P < 0.05$).

The effect of experimental treatments on carcass and some internal organs' characteristics are shown in Table 5. The relative weight of breast muscle in birds between ANTB and herbal extract were not significantly different, but they both were significantly higher than the control treatment ($P < 0.05$). Carcass yield in EXTR 2 was significantly different from CONT ($P < 0.05$), but the treatments EXTR 1, EXTR 2, and ANTB had no significant differences. However, dietary treatments had no more effects on other carcass traits and the relative weight of internal organs ($P > 0.05$).

The effect of treatments on blood antioxidants and corticosteroids are shown in Table 6. GPX in EXTR 2 was significantly different compared to CONT ($P< 0.05$), but other treatments were not significantly different. Conversely, MDA in EXTR 1 and EXTR 2 groups were significantly lower than CONT ($P<0.05$). In the case of corticosterone, no significant effect was observed ($P > 0.05$).

The effects of treatments on ileal contents of *E. coli* and *Lactobacillus* are shown in Table 7. The CONT treatment had the highest *E. coli*, count and the ANTB had the lowest *E. coli* counts ($P < 0.05$). In the case of Lactobacillus status among treatments, the group fed 0.2 g/kg of herbal extract had the highest Lactobacillus count and significantly differed with CONT ($P < 0.05$). Also, the effects of block on *E. coli* and *Lactobacillus* factors were significant ($P<0.05$).

Treatments had no effect on blood hematocrit, heterophils, lymphocytes, and the ratio of heterophils to lymphocytes at 21 and 28 days of age (Table 8). The villus height was affected by *E. coli* and herbal extract challenge (Table 9). The height of villi in extract groups were significantly higher than CONT ($P < 0.05$). Villus width was not significantly different among treatments, but crypt depth were significantly higher in unchanged birds compared to challaged ones ($P < 0.05$).

The humoral immune response of chickens (Table 10) was affected by dietary treatments at 28 days of age. On day 28, immunoglobulin G (IgG) in ANTB was significantly different compared to CONT group ($P < 0.05$). Considering total Ig 28 all treatments were significantly higher than control treatment ($P < 0.05$). In other parameters (IgG 21, total Ig 21) between treatments were not significantly different. There was no significant interaction between treatments in all factors. There were interactions between treatments and blocks ($P < 0.05$).

Table 5. Effect of different levels of *Scrophularia striata* extract on relative carcass and some internal organs a of broilers at 35 days of age

	Carcass and some internal organs characteristics (percentage of live body weight)							
Treatments*	Thigh	Breast	Neck and back	Carcass yield	Liver	spleen	Bursa of Fabricious	Pancreas
CONT	18.2	21.6 [b]	20.7	60.5 [b]	2.3	0.1	0.2	0.2
EXTR1	17.6	23.6 [a]	21.2	62.4 [ab]	2.2	0.1	0.2	0.2
EXTR2	18.0	23.8 [a]	21.2	63.0 [a]	2.3	0.1	0.2	0.2
ANTB	17.6	24.0 [a]	21.3	62.9 [a]	2.2	0.1	0.2	0.2
SEM	0.25	0.31	0.29	0.50	0.07	0.01	0.02	0.01
P-value	0.2493	0.001	0.4491	0.0081	0.6933	0.6403	0.9547	0.5054
Challenge with *E. coli*								
N	18.1	23.5	21.4	63.0 [a]	2.2	0.1	0.2	0.2
C	17.7	22.9	20.9	61.4 [b]	2.3	0.1	0.2	0.2
SEM	0.18	0.22	0.21	0.31	0.05	0.01	0.01	0.01
P-value	0.1052	0.0538	0.0877	0.0074	0.254	0.9716	0.1042	0.3672
Treatments × Challenge with *E. coli* **								
CONT × N	18.3	21.9	20.8	60.9	2.2	0.1	0.2	0.2
CONT × C	18.2	21.2	20.6	60.1	2.3	0.1	0.2	0.3
EXTR1 × N	18.2	24.2	21.3	63.7	2.2	0.1	0.2	0.2
EXTR1 × C	17.0	23.0	21.1	61.0	2.2	0.1	0.2	0.2
EXTR2 × N	18.3	23.9	21.8	63.9	2.3	0.12	0.2	0.2
EXTR2 × C	17.9	23.6	20.7	62.2	2.3	0.1	0.2	0.2
ANTB × N	17.7	24.1	21.6	63.4	2.1	0.1	0.2	0.2
ANTB × C	17.5	23.8	21.1	62.4	2.3	0.1	0.2	0.2
SEM	0.35	0.64	0.40	0.78	0.10	0.01	0.02	0.01
P -value	0.3331	0.6829	0.6694	0.6300	0.8304	0.6414	0.8055	0.1912

* N: Non-challenged house; C: Challenged house with *E. coli* ; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg of plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; *SEM*: Mean standard error.

** Interactions between treatments and E. coli challenge.

[a-b] Means within each column with different superscripts are significantly different (P< 0.05).

Table 6. The effect of different levels of *Scrophularia striata* extract on antioxidant status, MDA and corticosterone in broilers at 35 days of age

Treatments*	GPX (U/L)	MDA (nmol/L)	CORTICOSTRON (ng/mL)
CONT	149.0 [b]	9.5 [a]	2.5
EXTR1	167.1 [ab]	8.2 [b]	1.9
EXTR2	172.4 [a]	8.3 [b]	1.8
ANTB	163.7 [ab]	9.1 [ab]	2.2
SEM	4.81	0.26	0.19
P-value	0.0139	0.0043	0.0638
Challenge with *E. coli*			
N	167.1	8.5	1.9
C	159.0	9.0	2.2
SEM	3.40	0.19	0.14
P-value	0.1064	0.0859	0.1162
Treatments × Challenge with *E. coli* **			
CONT × N	153.0	9.3	2.2
CONT × C	145.0	9.6	2.7
EXTR1 × N	171.5	8.0	1.7
EXTR1 × C	162.8	8.5	2.1
EXTR2 × N	180.0	7.8	1.5
EXTR2 × C	164.8	8.7	2.0
ANTB × N	163.8	9.1	2.2
ANTB × C	163.5	9.2	2.1
SEM	7.80	0.37	0.27
P-value	0.7484	0.7506	0.5889

* N: Non-challenged; C: Challenged with *E. coli*; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg of plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; GPX: glutathione peroxidase; MDA: Malondialdehyde; *SEM*: Mean standard error.

** Interactions between treatments and *E. coli* challenge.

[a-b] Means within each column with different superscripts are significantly different ($P < 0.05$).

Table 7. Effect of *Scrophularia striata* extract on *E. coli* and Lactobacillus counts (Log10 CFU/g-) of the ileal contents in broiler chickens at 35 days of age

Treatments*	*E. coli*	Lactobacillus
CONT	141.3 [a]	36.5 [c]
EXTR1	111.2 [b]	54.0 [b]
EXTR2	101.7 [b]	71.0 [a]
ANTB	54.2 [c]	13.2 [d]
SEM	6.07	3.59
P-value	0.0001	0.0001
Challenge with *E. coli*		
N	72.0 [b]	60.2 [a]
C	132.2 [a]	27.2 [b]
SEM	4.30	2.50
P-value	0.0001	0.0001
Treatments × Challenge with *E. coli* **		
CONT × N	94.3 [cd]	56.7 [bc]
CONT × C	188.3 [a]	16.3 [d]
EXTR1 × N	80.3 [cd]	75.7 [ab]
EXTR1 × C	142.0 [b]	32.3 [cd]
EXTR2 × N	81.7 [cd]	92.7 [a]
EXTR2 × C	121.7 [bc]	49.3 [c]
ANTB × N	31.7 [e]	15.7 [d]
ANTB × C	76.7 [d]	10.7 [d]
SEM	8.58	5.07
P-value	0.0258	0.0036

* N: Non-challenging house with *E. coli*; C: Challenging house with *E. coli*; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; *SEM*: Mean standard error.

** Interactions between treatments and E. coli challenge.

[a-e] Means within each column with different superscripts are significantly different ($P < 0.05$).

Table 8. Effect of *Scrophularia striata* extract on blood parameters of broilers at different ages

Treatments*	21 day				28 day			
	H %	L %	H/L	HCT %	H %	L %	H/L	HCT %
CONT	32.8	65.3	0.5	32.1	30.1	67.9	0.5	32.4
EXTR1	31.7	66.4	0.5	34.3	28.7	69.1	0.4	33.9
EXTR2	31.6	66.6	0.5	34.2	28.7	69.0	0.4	33.5
ANTB	30.0	67.9	0.5	33.4	29.1	68.5	0.4	33.4
SEM	1.04	1.02	0.02	0.62	0.97	0.93	0.02	0.56
P-value	0.3171	0.3689	0.4001	0.0633	0.706	0.7892	0.6941	0.2711
Challenge with *E. coli*								
N	31.0	67.2	0.5	33.8	28.8	69.2	0.4	33.4
C	32.1	66.0	0.5	33.3	29.6	68.1	0.4	33.2
SEM	0.74	0.72	0.02	0.43	0.68	0.66	0.01	0.4
P-value	0.3213	0.2494	0.3096	0.4306	0.4132	0.2249	0.3282	0.7311
Treatments × Challenge with *E. coli* **								
CONT × N	32.8	65.0	0.5	32.1	31.6	66.6	0.5	32.2
CONT × C	32.8	65.6	0.5	32.1	28.6	69.2	0.4	32.6
EXTR1 × N	31.2	67.0	0.5	34.6	28.2	70.0	0.4	34.2
EXTR1 × C	32.2	65.8	0.5	34.0	29.2	68.2	0.4	33.5
EXTR2 × N	30.2	68.0	0.5	35.4	27.8	70.0	0.4	33.3
EXTR2 × C	33.0	65.2	0.5	33.0	29.6	68.0	0.4	33.7
ANTB × N	29.8	68.6	0.4	32.9	27.4	70.2	0.4	33.8
ANTB × C	30.2	67.2	0.5	34.0	30.8	66.8	0.5	32.9
SEM	1.47	1.45	0.03	0.88	1.37	1.31	0.03	0.80
P-value	0.7879	0.7086	0.7637	0.2665	0.1352	0.1405	0.1286	0.7700

* N: Non-challenging house with *E. coli*; C: Challenging house with *E. coli*; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg of plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; L: Lymphocytes; H: Heterophile; H/L: Heterophil to Lymphocyte ratio; HCT: Hematocrit; *SEM*: Mean standard error.

** Interactions between treatments and E. coli challenge.

[a-b] Means within each column with different superscripts are significantly different ($P < 0.05$).

Table 9. Effect of *Scrophularia striata* extract on ileal histo-morphometry of broiler chickens at 35 days of age

Treatments*	VH (µm)	VW (µm)	CD (µm)
CONT	1137.4 [c]	224.1	209.4
EXTR1	1609.0 [a]	189.2	194.2
EXTR2	1496.8 [ab]	189.5	193.0
ANTB	1181.8 [bc]	199.8	188.4
SEM	89.59	12.98	11.55
P-value	0.0012	0.2103	0.6054
Challenge with *E. coli*			
N	1447.0	194.2	173.7 [b]
C	1265.5	207.1	218.8 [a]
SEM	63.34	9.20	8.17
P-value	0.0512	0.328	0.0005
Treatments × Challenge with *E. coli* **			
CONT × N	1163.2	221.2	186.8
CONT × C	1111.6	227.0	232.0
EXTR1 × N	1682.8	184.0	163.6
EXTR1 × C	1535.2	194.4	224.8
EXTR2 × N	1712.4	174.8	167.2
EXTR2 × C	1281.2	204.2	218.8
ANTB × N	1229.6	196.8	177.2
ANTB × C	1134.0	202.8	199.6
SEM	136.70	18.37	18.30
P-value	0.4466	0.9051	0.6781

* N: Non-challenging house with *E. coli*; C: Challenging house with *E. coli*; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; VH: Villus Height; VW: Villus Width; CD: Crypt Depth; *SEM*: Mean standard error.
** Interactions between treatments and E. coli challenge.
[a-c] Means within each column with different superscripts are significantly different (P< 0.05).

Table 10. The effect of *Scrophularia striata* extract on the humoral immune response of broilers at different ages

Treatments*	Immunoglobulins on days 21 and 28 (log_2)			
	IgG 21	Total Ig 21	IgG 28	Total Ig 28
CONT	1.2	2.6	2.5 [b]	4.9 [b]
EXTR1	1.7	3.2	3.1 [ab]	5.7 [a]
EXTR2	1.9	3.3	3.3 [ab]	5.8 [a]
ANTB	1.4	2.9	3.4 [a]	5.8 [a]
SEM	0.20	0.19	0.22	0.15
P-value	0.0776	0.0583	0.0262	0.0488
Challenge with *E. coli*				
N	1.7	3.1	3.4 [a]	5.8
C	1.5	2.9	2.8 [b]	5.3
SEM	0.14	0.12	0.10	0.18
P-value	0.3172	0.3014	0.0155	0.0586
Treatments × Challenge with *E. coli* **				
CONT × N	1.4	2.6	2.6	5.0
CONT × C	1.0	2.6	2.4	4.8
EXTR1 × N	1.8	3.4	3.6	6.2
EXTR1 × C	1.6	3.0	2.6	5.2
EXTR2 × N	2.0	3.6	3.8	6.0
EXTR2 × C	1.8	3.0	2.8	5.6
ANTB × N	1.4	2.8	3.4	6.0
ANTB × C	1.4	3.0	3.4	5.6
SEM	0.28	2.70	0.30	0.36
P-value	0.9145	0.4425	0.2345	0.7111

* N: Non-challenging house with *E. coli*; C: Challenging house with *E. coli*; CONT: control treatment; EXTR1: Treatment with 0.1 g/kg of plant extract; EXTR2: Treatment with 0.2 g/kg plant extract; ANTB: Treatment with 0.1 g/kg of antibiotic; IgG: Immunoglobulin G; Total Ig: Total Immunoglobulin; *SEM*: Mean standard error.
** Interactions between treatments and E. coli challenge.
[a-b] Means within each column with different superscripts are significantly different (P< 0.05).

Discussion

The three main components of this extract are Linalool, Betaphellandrene, and Nerol. The composition and percentage of the components of a plant extract may vary depending on factors such as the culture medium, climatic conditions, and the organ of the plant from which the extract is extracted (Khamisabadi and Pourhesabi, 2018). The effects of herbal extract (0.2 g/kg) and antibiotic on average body weight and daily weight gain were consistent with Rostami *et al.* (2015) results. The herbal extract in the birds fed 0.2 g/kg of extract had positive effect on daily feed intake; these results were contrary to the results of Rostami *et al.* (2015) and Beier *et al.* (2014), this may be due to the type and method of using plant materials in the diet (use of plant powder and use of pure linalool in the diet compared to alcoholic extract). The use of plant extract did not affect feed to gain ratio; these results were similar to Beier *et al.* (2014). Consumption of the plant extract in the diet had a positive effect on the carcass yield, the positive effects of herbal extracts on birds performance and carcass yield are already reported by others (Calislar *et al*., 2009; Al-Kassie *et al*., 2010; Roofchaee *et al*., 2011; Erhan *et al*., 2012; Azadegan Mehr *et al*., 2014; Saleh *et al*., 2014; Zeng *et al*., 2015), However in some cases, some reports are showing no effects or adverse effects on chickens (Demir *et al*., 2008; Ocak *et al*., 2008; Brenes and Roura, 2010; Kirkpinar *et al*., 2011; Saleh *et al*., 2014; Zeng *et al*., 2015) that might be related to the type and dosage and variable active ingredients of the herbal extracts used in the studies. Wallace *et al*. (2010) reported that the positive effects of herbal extracts on broiler performance might be related to the better availability of energy offered to the birds.

Herbal extract reduced *E. coli* and increased lactobacillus population of ileal contents of the chickens. Several types of herbal extract components affect the microbial activity of the gastrointestinal tract. The antimicrobial components of these herbal extracts are phenols, alcohols, ketones and aldehydes. There are some reasons describing the effectiveness of these extracts. The mechanism of antimicrobial activities of herbal extracts may depend on the mechanical properties of their active ingredients, and only a single mechanism for their antimicrobial activities cannot be cited, but instead contains a chain of reactions involving the entire bacterial cell activity (Michalina and Danuta, 2017). The main component of the used herbal extract is linalool. Linalool inhibits the pathogenic microorganisms of the gastrointestinal tract (Cabuk *et al*., 2003). Other evidences suggest that herbal extracts reduce the *E. coli* population of the gastrointestinal tract (Lee *et al*., 2004; Ghazanfari *et al*., 2015). The mechanism might be due to destruction of outer cell membrane of microorganism (Helander *et al*., 1998). The antibacterial properties of herbal extracts might also be related to the penetration of k^+ and H^+ ions into the bacteria (Ultee *et al*., 1999). It is suggested that the hydrophobicity property of extracts facilitae their penetration into the lipid structure of microorganisms and kill the bacteria (Smith-Palmer *et al*., 2004). Cross *et al*. (2007) and Kirkpinar *et al*. (2011) showed that feed supplementation with some herbal extracts (Marjoram oregani, Rosemary or Yarrow thyme) did not affect the lactobacilli population. Rostami *et al*. (2015) also showed that diet supplementation with *Scrophularia striata* had no affected on the lactobacilli population.

Consumption of plant extract (0.2 g/kg) was effective on GPX and MDA in chickens' blood. Some studies clearly showed that the inclusion of herbal extract in birds' diet could improve the antioxidant potential of their meats. (Marcinčák *et al*., 2008; Papageorgiou *et al*., 2003). The amount of deposited fat/lipid in the meat of birds is variable. Therefore, the lipid oxidation indices might be different. It was shown that lipids oxidation status of pigs fed diets containing oregano extract was not improved that is possibly due to the differences in the structure of fatty acids fed or deposited by poultry (Janz *et al*., 2007). The relative concentration of unsaturated fatty acids in the poultry meat is high. Therefore, poultry meat is particularly prone to oxidative spoilage and diet supplementation with herbal extract is a good strategy to the oxidation state in poultry meat (Zeng *et al*., 2015). It was shown that diets containing ginger extract improve the total antioxidant capacity of broiler chickens and reduce the amount of MDA (Habibi *et al*., 2014). Some phenolic compounds of pharmaceutical plants can strengthen the antioxidant defense system and prevent the production of free radicals in the body (Sahin *et al*., 2003). Corticosterone acts as the main stress hormone, and it protects the birds against stresses in different ways. By lowering the production of this hormone, more nutrients might be used for egg, growth, and production in the birds (Bains, 1996). Some plants when fed by chickens, reduce the synthesis of corticosteroid hormone Corticosteroid increases the synthesis of fatty acids in the liver during heat stress, and therefore more energy is stored as fat (Lin *et al*., 2006). The ratio of heterophile to lymphocyte is a reliable indicator to measure stress. In the current study, despite the non-significant effect of the herbal extract on this ratio at days 21 and 28, the ratio numerically decreased. It has been shown that heterophile itself is sensitive to stressors and increases during stress conditions (Gray *et al*., 1989). Rostami *et al*. (2015) showed that the use of *Scrophularia striata* powder in the diet of broilers is effective on the status of lymphocytes and the ratio of heterophils to lymphocytes. Perhaps the disagreement between their study and ours is due to the difference

in the amount and method of plant application (powder of the aerial parts of the plant against the alcoholic extract of the plant) in the diet.

The amount of immunoglobulin G and total immunoglobulins for the primary and secondary immunity responses in the chickens fed 0.2 g/kg of herbal extract and chickens fed antibiotic were higher than that of control treatment that is in agreement with those of Rostami *et al*. (2015). They showed that dietery supplementation of *Scrophularia striata* at the level of 8 g/kg improved immune status of broilers. Saleh *et al*. (2014) added 0.1 and 0.2 g/kg of thyme and ginger into the feed of broilers and observed an improvement in the immunological profile of chickens through increased antibody production. It has been reported that herbal extracts can be used to alleviate the stress of vaccination and strengthen the immune system (Barbour *et al*., 2011; Faramarzi *et al*., 2013; Gopi *et al*., 2014).

The length of villi in healthy chickens fed 0.1, and 0.2 g/kg of the herbal extract was higher than that of control birds. Villus height is one of the main indicators of intestinal histo-morphometry. It is shown that nutritional stresses affect intestinal morphology and the absorption ability of intestinal tissue (Burkholder *et al*., 2008). Toxins alter intestinal morphology by reducing the length of villi and increasing the depth of crypt (Xu *et al*., 2003). Kermanshahi *et al*. (2017) showed that the acidic products of yeast reduce the *E. coli* population in the gastrointestinal tract, which leads to the protection and growth of the villi and thus improves the morphology of the gastrointestinal tract. Also, antimicrobial agents (such as herbal extracts) reduce the presence of toxins in the intestine. Cabuk *et al*. (2003) showed that coriander extract containing linalool increases the height of intestinal villi and hence improves the activity of the gastrointestinal tract. Garcia *et al*. (2007) showed that the hydroalcoholic extract of sage, thyme, and rosemary under normal conditions had no effect on the length of villi and the depth of crypt. These contradictory effects might be due to the type or amount of active herbal ingredients.

The effects of treatments, blocks, and interaction showed that *E. coli* effectively reduced the performance, carcass yield, ileal microbial lactobacillus, villus height, secondary IgG responses, and the 0.2 g/kg herbal extract improved these effects. The results are in agreement with those of Michalina and Danuta (2017) who showed that the use of plant extracts under normal and bacterial challenged conditions improve these traits in broiler chickens.

Conclusion

Under the conditions of this study, the use of alcoholic *Scrophularia Striata* extract improved the performance, carcass yield, ileal microbial lactobacillus, villus height, antioxidant status of GPX, and MDA, intestinal morphology, and secondary IGG responses of the chickens. The responses in most cases were better or similar to that of antibiotic effects. Therefore, alcoholic *Scrophularia Striata* extract can be used as an alternative to antibiotics in poultry feeds.

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