

Effect of sumac (*Rhus coriaria* L.) seed powder on growth, carcass traits, blood parameters, immune system and selected ileal microorganisms of broilers

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Summary

Sumac (*Rhus coriaria* L.) is a plant species belong to Anacardiaceae family that is worldwide diffused. The sumac seed powder (SSP), produced by grinding dried fruits, is recognized to have defensive and beneficial effects on numerous health-related problems. In this study, SSP was included in broilers basal-diet to investigate the comparative effects of different levels of SSP on performance, carcass characteristics, blood parameters, immune system and ileal microorganisms. A total of 225, one day-old male broilers (Ross 308) were randomly assigned to the five dietary treatments with three replicates per treatment. The experimental diets were: basal-diet (BD); and BD including 0.05, 0.10, 0.15 and 0.20% SSP, respectively. During the whole feeding period (42 days), birds fed corn-based grower (1-21 days) and finisher (22-42 days) diets, respectively. Results indicated that supplementing SSP had no effect on broiler body weight gain, feed intake and feed conversion as well as carcass characteristics ($P > 0.05$). Similarly, blood total protein, albumin, glucose and triglyceride were not influenced by dietary SSP. Conversely, serum total cholesterol and LDL-cholesterol levels were decreased, while HDL-cholesterol increased in all SSP fed groups compared to control ($P < 0.05$). In this study the addition of SSP in broilers diets did not show any effect on blood heterophils and lymphocyte. Moreover, the lactobacillus count remained unaffected by dietary treatments, while *E. coli* count in broiler ileal content was lower when fed 0.10% SSP than the other groups ($P < 0.05$). Thus, the present findings indicated a positive effect of feeding SSP (especially at 0.10% diet) on blood cholesterol levels and *E. coli* count in broiler chickens.

Introduction

In recent years, phytogetic and herbal products have been accepted by consumers as natural additives and have received increased attention (Landy *et al.* 2011, Dhama *et al.* 2015). A variety of herbal supplements have been widely used to sustain and improve health of humans (Freeman *et al.* 1995) and animals (Gardzielewska *et al.* 2003). Beneficial effects of bioactive medicinal or herbal

plants and their products, including plant extracts, fruits or seeds, in animal nutrition and health may include the stimulation of appetite and intake, the improvement of endogenous digestive enzyme secretion, activation of immune response, as well the antibacterial, antiviral and antioxidant action (Nouzarian *et al.* 2011).

Over the past few decades, a number of studies have focused on the biological activity of sumac extract. Sumac (*Rhus coriaria* L.) is a plant belonging

to Anacardiaceae family, growing worldwide, especially in temperate and sub-tropical regions. Traditionally, sumac has been used as medicine (Zargari 1997) by native North Americans for the treatment of bacterial diseases, such as syphilis, gonorrhoea, dysentery, and gangrene (Erichsen-Brown 1989). Research indicated that sumac extracts have various health-promoting benefits, due to a multitude of compounds with antifibrogenic, antifungal, anti-inflammatory, antimicrobial, antimutagenic, antioxidant, antiviral, cytotoxic, hypoglycaemic and leukopenic properties (Rayne and Mazza 2007, Marech *et al.* 2018). Sumac fruits contain flavonols, phenolic acids, hydrolysable tannins, anthocyanins, and organic acids such as malic, citric and tartaric acids (Jung 1998, Greathead 2003, Ozcan and Haciseferogullari 2004). More recently, there has been a resurgence in interest in sumac seed due to the aforementioned potential bioactive properties (Rayne and Mazza 2007). Some researchers have proved an increase in body weight and decrease in feed efficiency, when supplementing sumac in broilers diet (Gulmez *et al.* 2006, Ghasemi *et al.* 2014).

Therefore, keeping in view the positive effects of sumac or its potential bioactive components, the present research was designed to evaluate the impact of different levels of sumac seed powder (SSP) on growth performance, carcass traits, hematology, immunity, and ileal microflora of broiler chickens.

Materials and methods

Animals, diets and management

All experimental procedures conducted were approved by the Islamic Azad University (Rasht Branch, Rasht, Iran) in accordance with the institutional animal welfare procedure for experimental rearing and handling of poultry.

A total of 225 one day-old male chicks of Ross 308 strain (Aviagen) were allotted to five dietary treatment groups with three replicates per treatment. Groups were formed by animals with similar mean body weight (BW).

The sumac was locally purchased and it was ground to a fine powder and then mixed with the basal diet. The dietary treatments were as follows: a basal diet (BD) without SSP as control, and the BD including 0.05, 0.10, 0.15 and 0.20% SSP, respectively. During the whole experimental period (42 days), birds were fed corn-based grower (1-21 days) and finisher (22-42 days) diets, and covering the nutrient requirements as suggested by the Ross breeder manual (Aviagen). Diets provided similar metabolizable energy (ME kcal/kg) and

crude protein (CP). Energy levels were adjusted with vegetable oil and digestible amino acid levels with soybean meal, fish meal and synthetic amino acids. The composition and nutrient specifications of diets are reported in Table I.

Chicks had *ad libitum* access to water and feed. Day old chicks were brooded using heaters, while humidity was kept at 55 to 65% by adding water to floor. The temperature and humidity program were set according to the instructions for Ross 308 broilers (Aviagen). The lighting program was based as suggested by the Ross management manual (Aviagen) and comprised 23:1 h light:darkness until slaughter at day 42. Birds were vaccinated against bronchitis disease (at 1 day of age), Newcastle disease (at 11 and 21 days of age), influenza disease (9 days of age) and Gumboro disease (14 and 23 days of age).

Growth performance and carcass traits

Average individual BW and average daily gain (ADG), and cage average daily feed intake (ADFI) as well feed conversion ratio (FCR) were recorded on a weekly basis. The FCR values were corrected for

Table I. Ingredients and nutrient analysis of diets fed to broiler chickens.

Ingredients (%)	Starter	Finisher
Corn	56.9	58.7
Soybean meal (43% CP)	33.1	30
Fish meal	3.4	3.5
Vegetable oil	2.0	3.5
Dicalcium phosphate	1.55	1.55
Oyster shell	1.03	1.18
DL-methionine	0.01	0.01
Vitamin premix*	0.5	0.5
Mineral premix**	0.5	0.5
Salt	0.26	0.26
Sand	0.75	0.75
Nutritional content		
ME (kcal/kg)	2,910	3,030
Crude protein (%)	20.1	19
Crude fat (%)	4.60	6.14
Ca (%)	0.95	0.9
Total P (%)	1.23	1.06
Available P	0.45	0.36
Meth	0.50	0.38
Lys	1.01	1.01
Met + Cys	0.83	0.71

* Vitamin A, 7.2 mg; D3, 1.6 mg; vitamin E, 14.4 mg; vitamin K3, 1.6 mg; vitamin B1, 0.72 mg; vitamin B2, 3.13 mg; vitamin B3, 4 mg; vitamin B6, 1.2 mg; vitamin B9, 0.5 mg; vitamin B12, 6 mg; vitamin B5, 12 mg; H2, 2 mg; choline chloride, 3 mg and Antioxidant, 10 mg.

** Mn, 13227 mg; Fe, 100 mg; Zn, 4235 mg; Cu, 16 mg; I, 0.64 mg and Se, 0.2 mg.

the BW of any bird that died during the experiment. At the age of 42 days after 4 h of fasting for complete evacuation of the gut, one bird from each replicate was selected. Care was taken to choose the most representative male birds with respect to BW compared to the group mean BW. These animals were used for measuring carcass yield and gastrointestinal tract characteristics. Birds were fully plucked using a dry plucking method. Feet were separated from the carcass at the tibio-tarsal joint. Neck, wing tips, gut and liver were removed, and the empty or edible carcass was weighed, and intestinal segments were recorded. The carcass parts were dissected and separately weighed.

Blood biochemical parameters

Before blood collection, feed was removed from all birds for a period of 4 h in an attempt to allow stabilization of plasma constituents, and blood sampling was done in the morning to further reduce the variability of plasma traits. At 42 days of age, a 5 ml venous blood sample was collected from the basilic vein in the wing of three birds taken from each replicate. Care was taken to choose the most representative male birds with respect to BW compared to the group mean BW. The whole blood sample was transferred into a tube coated with 10 mg of the anticoagulant ethylene diamine-tetra acetic acid (EDTA). Blood samples were centrifuged at 3,000 rpm \times 20 min and plasma was collected and stored at - 20 °C until analyses. Plasma cholesterol and triglyceride levels were determined using enzymatic methods (TeifAzmoon Pars, Co., Tehran, Iran), and HDL- and LDL-cholesterol were measured using diagnostic kits (TeifAzmoon Pars Co, Tehran, Iran). The colorimetric determination of cholesterol in plasma samples involved the use of cholesterol oxidase procedure of Barham and Trinder (Barham and Trinder 1972), which is based on the formation of a colored red-purple quinoneimine dye, produced by oxidative condensation of a phenolic compound with 4-aminoantipyrine in the presence of hydrogen peroxide. The absorbance of the quinoneimine dye, measured spectrophotometrically, has a direct relationship with the amount of cholesterol in the sample. Plasma triglycerides were measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. The glycerol is converted to pyruvate and then to lactate. Decreased absorbance, measured spectrophotometrically, is proportional to the triglyceride concentration in the sample (Schmid and Forstner 1986). A glucose oxidase kit (TeifAzmoon Pars, Co., Tehran, Iran), based on oxidase-peroxidase procedure was used to measure plasma glucose. In this assay, glucose is oxidized in the presence of the glucose oxidase catalyst into H₂O₂ and gluconic acid. The

reaction among gluconic acid, hydrogen peroxide, a phenolic compound, and 4-aminoantipyrine forms a red-violet colored quinoneimine, and the absorbance of quinoneimine chromagen, measured by spectrophotometry, is directly associated with the amount of glucose in the sample.

Immunity response

Humoral immune response of broiler chickens to the Newcastle vaccine at days 28 and 42 was assessed by hemagglutination inhibition (HI) test. Sheep red blood cells (SRBC) were used as a test antigen to quantify specific antibody responses. On days 21 and 35, broiler chickens were injected with SRBC and sampled at days 28 and 42 to assess the humoral immune response. For the SRBC injection, initially 1 ml of PBS along with 10 ml of SRBC was mixed, and 0.5 mL of the obtained solution was drawn into the syringe and injected under the skin of the broiler chicken's breast. Three birds per treatment group were randomly selected for blood sampling from the brachial vein for measuring antibody titres against Newcastle disease virus (Seidavi et al. 2014).

Ileal microflora

At the end of the trial, three birds from each repetition were euthanized and ileums removed for further cultures. The ileal contents were placed on agar plates for determination of bacterial growth and colony counts. The culture media were prepared 24 h before collection as follows: Man Rogosa Sharpe (MRS, 1.10660.500) agar was used to culture lactobacilli, Eosin Methylene Blue (EMB, 1.01347.0500) agar to culture *E. coli*, respectively. The cultures of *Lactobacillus* and *E. coli* bacteria were made an aerobically form. The plates were incubated at 37.5 °C for 48 h. A colony counter was used to count bacterial colonies on plates. After counting the number of colonies in each plate, the number so obtained was multiplied by inverse of the dilution and the result was stated as the number of colony forming unit (CFU) in 1 g of sample (Downes and Ito 2001).

Statistical analysis

Data were analyzed using a completely randomized experimental design involving five treatments, and subjected to statistical analysis using the GLM and linear and quadratic response procedures of the Statistical Analysis System v8 (SPSS). Differences among main effect means were assessed via Duncan's multiple range test. Statements of significance were set at $P \leq 0.05$.

Results and discussion

The effects of feeding SSP on growth performance of broilers are presented in Table II. Sumac seed powder supplemented diets had no effect on body weight, FCR, and feed intake of chickens from 1-21 (grower phase) and 22-42 (finisher phase) days of age ($P > 0.05$). Similarly, the growth performance of birds considering the overall rearing period (1-42 days of age) was not significantly influenced by SSP-supplemented diet. Our findings are confirmed by a recent study of Cakmak and colleagues (Cakmak *et al.* 2017), reporting no effect ($P > 0.05$) of sumac powder on body weight gain, feed intake and efficiency; conversely Ghasemi and colleagues (Ghasemi *et al.* 2014) reported significant ($P < 0.05$) influence of sumac extract on growth traits of broiler chickens. These results might be explained by differences in form and levels of sumac supplemented in poultry diet.

The effects of dietary SSP on broiler chicks carcass characteristics are shown in Table III. Carcass traits, such as hot carcass, cooked carcass and meat cut (breast, thigh and wing) weight, were not influenced by dietary SSP ($P > 0.05$). Similarly, internal organ

weight such as liver, gizzard and abdominal fat pad of broilers were not affected by dietary SSP ($P > 0.05$). In agreement with our results, Sharbati and colleagues (Sharbati *et al.* 2013) reported no significant differences in carcass characteristics in broilers fed diets including sumac extract.

As shown in Figure 1, at the end of the experimental period, blood total protein, albumin, triglycerides and glucose were not influenced ($P > 0.05$) by treatments, while lower blood cholesterol ($P < 0.05$) was found in SSP supplemented groups. The lower blood cholesterol level in broilers fed SSP supplemented diets might be due to its polyphenolic components. Some studies have demonstrated that polyphenols could have beneficial effects on cardiovascular disease (Hertog *et al.* 1995, Laudadio *et al.* 2015, Tufarelli *et al.* 2017), and could be regarded as bioactive compounds with a high potential health-promoting capacity. In previous studies, Tebib and colleagues (Tebib *et al.* 1994), Bravo (Bravo 1998) and Mansoob (Mansoob 2012) also reported that polyphenols have been shown to depress the reverse cholesterol transport, reduce the intestinal cholesterol absorption and even increase bile acid excretion. Similarly, Valiollahi and colleagues

Table II. Effects of different levels of sumac seed powder (SSP) on growth performance of broiler chickens.

Item	Control	0.05% SSP	0.10% SSP	0.15% SSP	0.20% SSP	SEM	P-value
Day 1-21							
Body weight (g/bird) at day 21	715.8	697.8	682.1	712.5	683.1	15.84	0.450
Average feed intake (g)	1,002.3	974.0	958.6	996.0	962.3	20.46	0.481
Feed conversion ratio (FCR)	1.40	1.39	1.40	1.39	1.40	0.008	0.890
Day 21-42							
Body weight (g/bird) at day 42	1,241.3	1,061.0	1,208.3	1,072.0	1,178.6	97.30	0.602
Average feed intake (g)	2,512.6	2,162.3	2,418.0	2,254.3	2,379.6	170.00	0.634
Feed conversion ratio (FCR)	2.02	2.04	2.01	2.10	2.02	0.037	0.461
Day 1-42							
Body weight (g/bird)	1,957.1	1,758.8	1,890.5	1,784.50	1,861.8	90.6	0.561
Average feed intake (g)	3,515.0	3,136.3	3,376.6	3,250.3	3,342.6	160.6	0.563
Feed conversion ratio (FCR)	1.79	1.78	1.78	1.82	1.79	0.016	0.522

Table III. Effects of different levels of sumac seed powder (SSP) on carcass characteristics (g) of broiler chickens.

Item	Control	0.05% SSP	0.10% SSP	0.15% SSP	0.20% SSP	SEM	P-value	Linear	Quadratic
Hot carcass	1,713	1,440	1,516	1,534	1,509	104	0.470	0.216	0.276
Cooked carcass	1,129	1,078	1,165	1,139	1,148	66	0.902	0.943	0.980
Breast	391	345	408	378	374	39	0.837	0.957	0.957
Thighs	173	185	184	188	191	9.5	0.740	0.555	0.736
Wings	50	49	56	49	57	4.7	0.615	0.975	0.823
Liver	54	54	54	55	57	5.4	0.988	0.830	0.752
Gizzard	40	44	45	47	45	3.74	0.738	0.291	0.385
Abdominal fat	67	33	34	33	66	5.74	0.255	0.783	0.939

(Valiollahi *et al.* 2014) and Kheiri and colleagues (Kheiri *et al.* 2015) found a lower blood cholesterol level in broiler chickens fed sumac powder. Furthermore, research indicated that D-limonene, a monocyclic monoterpene component of sumac, has hypocholesterolemic effects in the body (Kurucu *et al.* 1993, Marshall 1995, Santiago *et al.* 2011).

Results of plasma LDL and HDL cholesterol in the current study are presented Figure 1. Data

demonstrated that dietary SSP had important influences on plasma LDL and HDL of broilers ($P < 0.05$). After consumption of SSP, the level of LDL decreased, whereas HDL increased in SSP supplemented group compared to control ($P < 0.05$). These findings are in accordance with Kheiri and colleagues (Kheiri *et al.* 2015) who demonstrated that blood HDL concentrations were significantly increased and LDL decreased in SSP-treated groups.

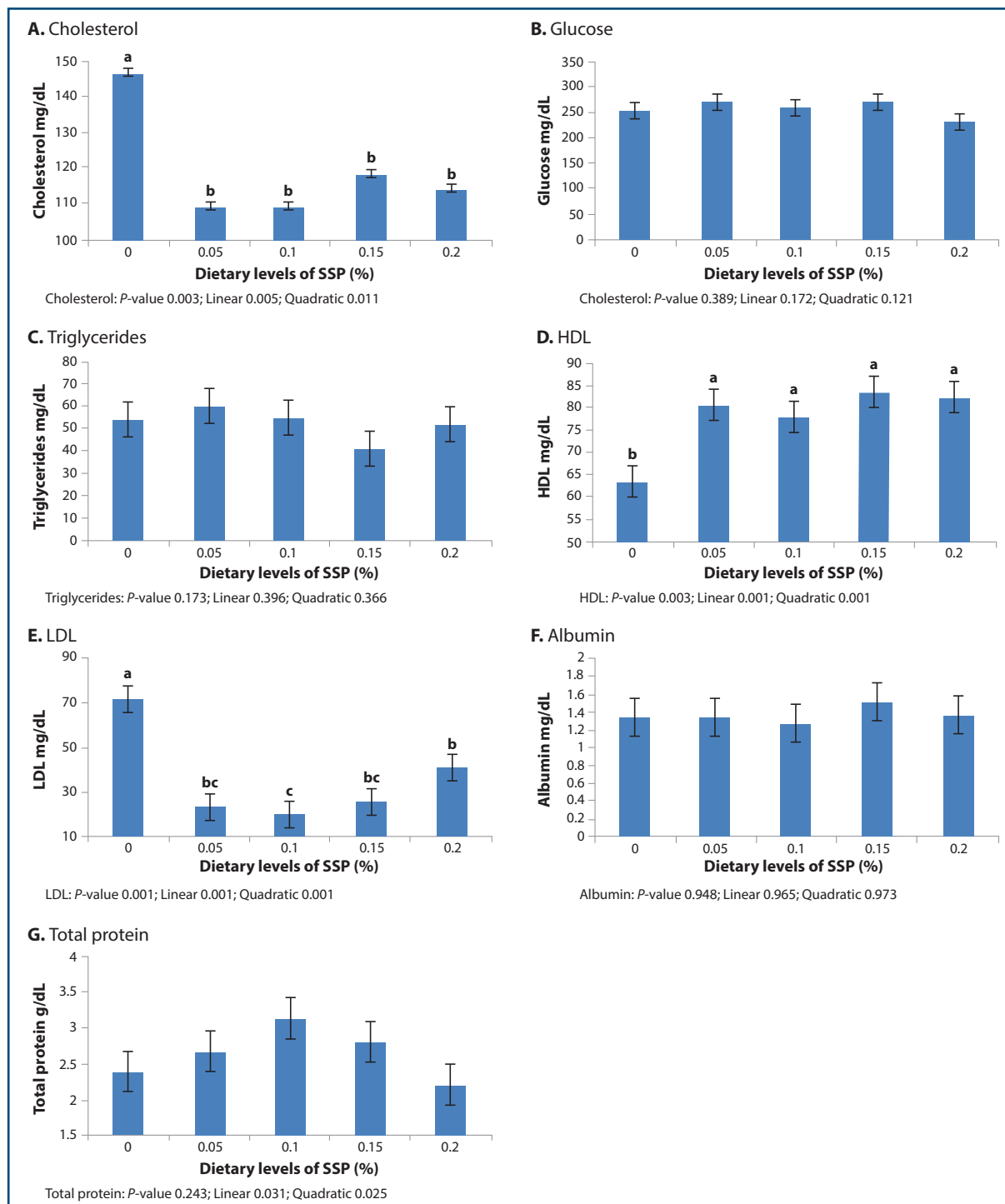


Figure 1. Effect of dietary sumac seed powder (SSP) on blood biochemistry in broiler chickens.

Table VI. Effects of different levels of SSP on blood parameters (mg/dl) of broiler chickens.

Item	Control	0.05% SSP	0.10% SSP	0.15% SSP	0.20% SSP	SEM	P-value	Linear	Quadratic
Heterophil	28.3	32.7	29.3	34.0	33.7	3.14	0.620	0.728	0.909
Monocyte	2.33	3.33	3.67	3.67	2.00	0.745	0.408	0.055	0.048
Lymphocyte	69.3	64.0	67.0	62.3	64.3	3.33	0.616	0.443	0.572
H/L	42.2	51.5	44.3	55.2	53.1	7.41	0.674	0.704	0.866

H/L = heterophil to lymphocyte ratio.

Table V. Effects of different levels of SSP on ileum microflora (log CFU/g) of broiler chickens.

Item	Control	0.05% SSP	0.10% SSP	0.15% SSP	0.20% SSP	SEM	P-value	Linear	Quadratic
<i>E. coli</i>	6.63	6.43	5.16	6.67	5.54	0.294	0.013	0.432	0.590
<i>Lactobacilli</i> spp.	7.02	7.63	7.30	7.42	7.13	0.269	0.553	0.200	0.192
L:E	1.05	1.19	1.41	1.11	1.29	0.168	0.082	0.365	0.289

L:E = *Lactobacilli* to *E. coli* ratio.

Moreover, it was assessed a negative correlation between the dietary sumac powder consumption and plasma total cholesterol and LDL concentrations in broiler chickens (Golzadeh et al. 2012). It was also demonstrated that high level of sumac consumption might have a protective effect on atherosclerosis and oxidative stress (Setorki et al. 2012). Studies indicated a strong relationship between total fat intake and cellular cholesterol concentration and several diseases, including atherosclerosis, cancer, diabetes, depression in human (Leaf and Kang 1998, Katan 2000, Ayerza et al. 2002). Low cholesterol containing diet has become an important concern for people with atherosclerosis, and poultry meat is one of the main products consumed, given its low in fat and cholesterol content compared to red meat.

The effects of dietary SSP on immune related parameters are presented in Table IV. Addition of sumac had no effect on some selected haematological parameters (lymphocyte and monocyte) of broilers ($P > 0.05$). We have not found other evidences in literature related to the effect of sumac on haematological traits in chickens. In the present study, the heterophil to lymphocyte (H/L) ratio was unaffected by dietary SSP ($P > 0.05$). As well demonstrated, heterophils increased and lymphocytes decrease when birds are stressed, so that the H/L ratio is a valuable index of response to a stressor (Maxwell and Robertson 1998). Results of our study indicated that dietary supplementation of SSP had no harmful effects on birds health status.

Natural inhibitors for pathogenic microorganisms have been explored in many plants (Al-Zoreky 2009). Among plant constituents, polyphenols have received a great deal of attention in recent years, due to their biological functions. Tannins are high molecular weight phenolic compounds, which are

present in many plant species (Tufarelli et al. 2017), including sumac, whose tannins have remarkable antimicrobial activity. A direct correlation between total phenols and antimicrobial activity is well documented, and research indicated that sumac is effective against both gram positive and negative bacteria (Ahmadian-attari et al. 2007). The effect of dietary SSP on intestinal microbial population is presented in Table V. Findings of the present study indicated a lowest *E. coli* counts in 0.10% SSP supplemented group ($P < 0.05$), while *Lactobacillus* population was unchanged in all dietary groups ($P > 0.05$). In agreement with Mansoob (Mansoob 2012), sumac has significant antimicrobial activity that can reduce the pathogenic bacteria in gut tract. Therefore, the differences in *E. coli* populations under SSP treatments may possibly be attributed to the bioactive components of plant product; however, it is necessary to conduct further research regarding the effect of SSP on poultry, specially on the effect on broiler gut morphology.

In conclusion, this study demonstrated that dietary supplementation of sumac seed powder influenced positively blood cholesterol in broiler chickens, supporting their growth and carcass traits. Moreover, a significant reduction of *E. coli* in small intestine by supplementing the plant extract was found, and this could be interesting as it may have the potential to be an alternative natural feed additive for broiler chickens.

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