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Evaluation of Miswak (*Salvadora persica*) as a Herbal Additive in Broiler Chickens

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Abstract

We determined the effects of dietary supplementation of different doses of Miswak (*Salvadora persica*) steam and leaf powder on the performance, blood parameters, cecal flora, and carcass traits of broilers. Four hundred and eight one-day old Ross 308 broiler chicks were provided one of the following experimental diets over 42 days: a basal diet without any additives, or a basal diet supplemented with 0.1%, 0.2%, 0.4%, 0.6%, or 0.8% Miswak powder. Four replicates of 17 birds were allocated to each treatment. Neither broiler performance (body weight gain, feed intake, and feed conversion ratio) during starter (d 1-21), finisher (d 22-42), and the overall period (d 1-42) of the study, nor blood parameters (glucose, triglyceride, cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, and very low density lipoprotein-cholesterol) were influenced by experimental treatments ($P > 0.05$). Chicks fed diets containing 0.4% Miswak had higher ($P < 0.05$) cecal *Lactobacillus* than the control group at day 42. Furthermore, 0.6% and 0.8% Miswak reduced the number of cecal *E. coli* compared to the control diet ($P < 0.05$). There were no significant differences in carcass yield and the relative weights of thigh, breast, and abdominal fat at day 42 ($P > 0.05$). In conclusion, we found that supplementation with Miswak powder had no beneficial effects on performance and blood parameters of broilers, but could improve cecal bacteria counts at levels greater than 0.4%.

Introduction

The prolonged use of antibiotic growth promoters in livestock production industry has resulted in two main problems: the risk of antibiotic residues in animal products (meat, milk, and egg) and the possibility of developing antibiotic resistance in humans and animals. Hence, there is concern regarding dietary supplementation of these feed additives in livestock. In recent years, application of in-feed antibiotics has been limited and instead, there is growing interest in the use of organic, natural and effective alternatives such as herbal plants or their derivatives (Hippenstiel *et al.*, 2011). Plant

products or phytobiotics are considered natural and residue-free compounds that have less toxic effects than the chemical additives such as antibiotics (Upadhaya and Kim, 2017). Thus, their usage can benefit health (Diaz-Sanchez *et al.*, 2015).

Miswak (*Salvadora persica*) is one of the most important medicinal plants. Different parts of this tree (root, twig, and stem) are extensively used for oral hygiene (Ahmad and Rajagopal, 2013), giving meaning to its name "tooth-cleaning stick" in Arabic. Miswak tree is found in Asian countries such as Iran, Pakistan, Iraq,

and Saudi Arabia (Halawany, 2012). Various biologically active compounds have been identified in different parts of Miswak. Its aqueous extract is rich in saponins, cyanogenic glycosides, alkaloids, vitamin C, salvadorine, salvadoreia, trimethylamine, tannins, and salts (mostly as chlorides; Alali and Al-Lafi, 2003; Ahmad and Rajagopal, 2013). Stem of Miswak contains high levels of carvacrol, benzaldehyde, benzyl isothiocyanate, benzyl nitrile, aniline, and naphthalene (Noumi *et al.*, 2011). The main active components of Miswak leaf include benzyl nitrile, isotymol, thymol, eugenol, β -caryophyllene, eucalyptol, and iso-terpinolene (Alali and Al-Lafi, 2003).

Miswak is reported to have a wide range of biological and pharmacological effects including antidepressant, antiviral, antimicrobial, anti-fever, anti-caries, anti-ulcerogenic, antioxidant, anti-platelet-agression, anti-gingival irritation, wound-healing, and hypoglycemia (Ahmad and Rajagopal, 2013). Miswak fruit has unique health benefits too such as strong antioxidant activity (Kumari *et al.*, 2017). However, little is known about the effects of Miswak on broiler chickens, though some work has been done on layer hens. Yassein *et al.* (2015) showed that supplementation of 0.5% Miswak increased feed intake, egg production, and egg quality (yolk index and Haugh Unit) of Hi-Sex Brown layers. Alm EL-Dein *et al.* (2014) found that Dokki 4 laying hens (an Egyptian strain) had greater body weights than a control group during supplementation of 0.5% Miswak from 18-36 weeks of age. The antibiotic Neomycin (25 mg/kg feed) yielded similar results in the same study. Alm EL-Dein *et al.* (2014) also found that supplementation of a range of Miswak doses (0.5-1%) resulted in earlier sexual maturity. Birds fed 0.75% and 1% Miswak had the highest egg production and these eggs had greater shell thickness, shell and yolk weight percentage, albumin height, and Haugh unit. Similarly, Battaa *et al.* (2013) reported that feeding Dokki4 laying hens 0.5%, 0.75%, and 1% Miswak improved their performance (body weight gain, feed conversion ratio, egg weight, egg mass, and egg production) and the higher concentrations increased digestibility of dietary protein and fat, and improved immune function. In another study, Miswak improved the performance and immunity and decreased plasma total lipid and cholesterol of Dokki 4 layer hens (Battaa *et al.*, 2009). In addition, beneficial effects of Miswak

on other animal species have been reported. El-Kholy *et al.* (2008) showed that Miswak roots (0.2-0.25%) resulted in better performance and higher reproductive capabilities (libido, mating activity, and physical semen characteristics) in male rabbits.

It is foreseeable that Miswak can have similar benefits in broilers. Thus, the objective of this study was to determine the effects of different levels of Miswak powder on the performance, blood parameters, cecal bacteria population, carcass traits, and organ weights of broilers.

Materials and Methods

Birds, diets and experimental design

All experimental procedures were approved by the Yasouj University Institution Animal Care Committee. A total of 408 1-d-old Ross 308 broilers of mixed sex were purchased from a local hatchery and transferred to the experimental site. In a completely randomized design, chicks were allocated to one of six experimental treatments with four replicates, each with 17 birds. The treatments were a control group (basal diet without Miswak), and 0.1%, 0.2%, 0.4%, 0.6%, and 0.8% Miswak powder supplemented into the basal diet. The basal diet for the starter (days 1-21) and finisher (days 22-42) periods were formulated to meet or exceed nutrients requirements (NRC, 1994) using UFFDA Software (Table 1). Feed and water were provided *ad libitum* throughout the experiment. The broiler chickens were reared under similar management condition in floor pens (150 cm length \times 150 cm width) with rice straw as litter.

Preparation of Miswak

Miswak stem and leaf were collected from the nursery of Natural Resources Administration of Larestan, Fars Province, Iran. The Miswak was dried in shade, and then grounded. The powder and dietary micronutrients were thoroughly mixed (as a premix), added to other dietary ingredients, and then mixed again using a mixer.

Measured Parameters

Broiler chickens were weighed by pen at 1, 21, and 42 d of age. Feed intake, body weight gain, and feed conversion ratio were determined for the starter, finisher, and overall periods of the experiment. Mortality was recorded daily and considered in the calculation of feed conversion ratio. It was calculated by dividing feed intake to

body weight gain of live plus dead chicks (Bozkurt *et al.*, 2014). At 21 and 42 days of the study, two birds from each pen (8 birds from each treatment) were randomly selected, weighed, and sacrificed by cervical dislocation. The digestive system was carefully removed from the carcass and the weights of different

organs (proventriculus, gizzard, liver, pancreas, small intestine and spleen) and abdominal fat pad were weighted. At 42 d of age, carcass, legs, and breast were separated and their weights were recorded. Weights of all organs were expressed as a percentage of live body weight.

Table 1. Feed ingredients and nutrient composition of the basal diet

Ingredients (%)	Starter	Finisher
Corn	59.78	65.56
Soybean meal	35.30	28.73
Vegetable oil	1.00	2.38
Limestone	1.28	1.27
Dicalcium phosphate	1.57	1.18
Common salt	0.42	0.32
Vitamins premix ¹	0.25	0.25
Minerals premix ²	0.25	0.25
DL-Methionine	0.15	0.06
<i>Nutrient composition</i>		
ME (Kcal/kg)	2880	2980
Crude protein (%)	20.17	18.25
Calcium (%)	0.90	0.83
Available Phosphorus (%)	0.41	0.33
Lysine (%)	0.99	0.92
Methionine (%)	0.45	0.35
Methionine + Cystine (%)	0.81	0.67
Threonine (%)	0.72	0.69

¹The vitamin premix supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D₃, 1,000 IU; vitamin E (dl- α -tocopherol), 30 IU; vitamin K₃, 2.5 mg; vitamin B₁, 2 mg; vitamin B₂, 5 mg; vitamin B₆, 2 mg; vitamin B₁₂, 0.01 mg; niacin, 30 mg; d-biotin, 0.045 mg; vitamin C, 50 mg; d-pantothenate, 8 mg; folic acid, 0.5 mg.

²The mineral premix supplied the following per kilogram of diet: Mn, 70 mg; Fe, 35 mg; Zn, 70 mg; Cu, 8 mg; I, 1 mg; Se, 0.25 mg; Co, 0.2 mg.

At 21 and 42 days of age, ~4 mL of blood was collected from the jugular vein in a test tube. Blood serum was separated by centrifugation at 3000 \times g for 10 min and kept at -40°C for later assays of blood parameters. Serum glucose, cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-c), very low-density lipoprotein-cholesterol (VLDL-c), and high density lipoprotein-cholesterol (HDL-c) levels were detected using commercially available kits (Pars Azmoon Kits, Iran). At the end of the experiment (d 42), samples of cecal content were collected in the sterile tubes for measuring the populations of *E.coli* and Lactic acid bacteria. 9 mL of phosphate buffered saline was added for each gram of cecal sample, and then homogenized. After serial dilution of samples, 100 μ L of diluted samples were

cultured on specific media. Eosin Methylene Blue (EMB) and De Man, Rogosa and Sharpe (MRS) agar media were used for *E.coli* and Lactic acid bacteria, respectively. All media were incubated at 37°C. EMB and MRS media were incubated for 24 and 48 h under aerobic and anaerobic conditions, respectively (Yang *et al.*, 2012). The results are expressed as log₁₀ colony forming units (CFU) per gram of cecal content.

Statistical analysis

Statistical analyses were done with ANOVA using the General Linear Models (GLM) procedures of SAS software (SAS, 2005). Duncan's multiple range test was used to compare the differences between treatments means (Duncan, 1955). Differences were considered significant at $P < 0.05$.

Results and Discussion

The effects of Miswak on growth performance parameters of broilers are shown in Table 2. Body weight gain, feed intake, and feed conversion ratio were not influenced by supplemental Miswak ($P < 0.05$). In contrast to our findings, Yassein *et al.* (2015) reported that dietary inclusion of Miswak had beneficial

effects on performance and egg quality of Hi-Sex Brown laying hens. Alm EL-Dein *et al.* (2014) showed that 1% Miswak improved the performance as well as egg quality of laying hens. Therefore, there is potential to use Miswak as an alternative to a chemical antibiotic (e.g., Neomycin) or even as a growth promoter in laying hen diets (Battaa *et al.*, 2009; 2013).

Table 2. Effects of experimental diets on the performance of broilers

Parameter	Experimental diets ¹						SEM	P-value
	Ctrl	0.1	0.2	0.4	0.6	0.8		
Body weight gain (g)								
d 1-21	546	575	583	546	577	571	19	0.61
d 22-42	1454	1505	1359	1439	1375	1406	47	0.29
d 1-42	2000	2079	1942	1985	1952	1977	51	0.47
Feed intake (g)								
d 1-21	717	751	776	701	758	745	26	0.37
d 22-42	2948	2959	2929	2964	2870	2912	75	0.94
d 1-42	3666	3711	3706	3665	3630	3658	76	0.97
Feed conversion ratio								
d 1-21	1.315	1.310	1.333	1.283	1.320	1.309	0.047	0.98
d 22-42	2.028	1.969	2.161	2.062	2.095	2.081	0.061	0.37
d 1-42	1.833	1.786	1.910	1.847	1.861	1.857	0.041	0.46

¹ Ctrl: control diet (without Miswak), 0.1, 0.2, 0.4, 0.6, and 0.8: diets supplemented with 0.1%, 0.2%, 0.4%, 0.6%, and 0.8 % Miswak powder, respectively.

There are inconsistent results regarding the application of phyto-biotics in poultry production in the literature. In line with our findings, Varmaghany *et al.* (2015) reported that garlic bulb (0.5%, 1%, or 1.5%) did not affect broiler performance in standard and cold temperature conditions. Similarly, Pourmahmoud *et al.* (2013) found no effects of thyme extract (0.2%, 0.4%, and 0.6%) on feed intake, body weight gain, and feed conversion ratio of broilers. Akbarian *et al.* (2013) also reported no effects of lemon peel extract, orange peel extract, and *Curcuma xanthorrhiza* essential oil on the performance of heat-stressed broiler chickens, though this may be due to inappropriate doses of plants or too short of duration of heat stress exposure. Amouzmehr *et al.* (2012) reported that thyme and garlic extract at 0.3% and 0.6% supplementation had no significant effects on broiler performance, because of clean and hygienic experimental rearing conditions. Other studies have shown lack of effects on broiler chicken performance with supplementation of drinking water with herbal plants such as thyme and satureja extract (alone or in combination) (Souri *et al.*, 2015), cinnamon, thyme, and turmeric (each at 5 g/L; Sadeghi *et al.*, 2012). In contrast, Li *et al.* (2015)

and Jeong and Kim (2015) reported improvement of broilers performance with herbs. Herbs can improve performance in chickens through various mechanisms such as improvement in gastrointestinal morphology and health, resulting in better nutrient digestion, altered digestive secretions (bile salts) and enzymes (trypsin, amylase, lipase), stimulation of beneficial bacteria (lactic acid bacteria and *Bifidobacterium*), prevention of harmful bacteria, and improving the function of vital organs such as liver (Diaz-sanchez *et al.*, 2015).

Feed intake is influenced by different factors such as rearing temperature, nutritive value and visual appearance of the feed, toxicity of feed components, viscosity, saliva release, particle size, and social interactions between the chickens. Feed intake of broilers is differentially affected by different herbs and essential oils (Hippenstiel *et al.*, 2011). The type of active components in plants, their dietary doses, the synergistic effects between the active compounds, the form of administration (e.g. powdered, capsules, *etc.*) and the environmental conditions all have considerable impacts on broilers' feed efficiency (Hashemipour *et al.*, 2013; Lee *et al.*, 2013). For example, Hafeez *et al.* (2016) reported that supplementation of

powdered menthol and anethole at 0.015% did not influence broiler performance, but 0.01% encapsulated form resulted in higher nutrient digestibility and better performance. The importance of herb form is further supported by Yesilbag *et al.* (2011). In the study of Baurhoo *et al.* (2009), broiler performance was not influenced by supplemental antibiotics and prebiotics. It was suggested that broilers reared under hygienic and clean conditions do not require feed additives for maximum growth. Another study that found no significant effects of antibiotics and prebiotics on performance attributed their results to a lack of real microbial challenge in the rearing place (Morales-Lopez *et al.*, 2009). In our study, it is possible that the concentrations of powdered Miswak did not contain enough amounts of active components to improve the performance of the broilers. Perhaps using Miswak as an extract can improve the growth performance of broilers.

Blood parameters

Miswak did not influence blood concentrations of glucose, cholesterol, triglyceride, LDL-c, HDL-c, and VLDL-c at 21 and 42 days of age (Table 3). Our results contrast findings from

previous work that found 0.75% and 1% Miswak significantly reduced levels of cholesterol, total lipids and triglyceride in blood plasma of laying hens (Alm EL-Dein *et al.*, 2014). Moreover, a significant reduction in blood triglyceride was observed in Hi-Sex Brown laying hens fed Miswak-supplemented diets (Yassein *et al.*, 2015). Similar findings have been observed by Khan *et al.* (2014) in hypercholesterolemia rats. They reported hypoglycemic and hypolipidemic effects of Miswak, as diabetic rats fed 0.5% aqueous extracts of Miswak had lower levels of plasma triglycerides, total cholesterol, LDL-c, VLDL-c, and glucose, but higher level of plasma HDL-c after four weeks. Miswak contains fibre and saponins, which form complexes with bile salts, thereby increasing their excretion through faeces. This condition will promote conversion of hepatic cholesterol to bile salts, which reduces blood cholesterol. In addition, the presence of sulphur components in Miswak can increase fat metabolism and bile secretion (Alm EL-Dein *et al.*, 2014). Indeed, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a hepatic key enzyme involved in cholesterol synthesis, is inhibited by some active components of herbs such as thymol and carvacrol (Lee *et al.*, 2004).

Table 3. Effects of experimental diets on blood metabolites (mg/dL)

Parameter	Experimental diets ¹						SEM	P-value
	Ctrl	0.1	0.2	0.4	0.6	0.8		
Glucose								
d 21	244.5	249.9	274.4	284.4	274.9	287.8	21.5	0.08
d 42	246.9	246.4	256.8	250.0	239.1	236.5	6.7	0.32
Triglycerides								
d 21	141.4	103.1	154.9	105.8	90.8	100.1	21.0	0.21
d 42	105.5	92.9	92.9	104.3	103.5	86.5	9.4	0.62
Cholesterol								
d 21	105.3	108.1	120.4	102.1	107.6	110.1	8.6	0.75
d 42	126.3	130.0	129.3	137.4	127.8	129.4	6.3	0.86
LDL-c								
d 21	15.1	15.8	13.6	9.9	12.8	14.6	2.8	0.72
d 42	21.1 ^{ab}	24.0 ^{ab}	18.6 ^{ab}	26.3 ^a	20.8 ^{ab}	17.9 ^b	2.5	0.17
HDL-c								
d 21	72.0	73.6	87.5	76.1	87.1	77.8	6.0	0.55
d 42	80.9	85.8	88.4	90.0	83.4	81.9	3.2	0.28
VLDL-c								
d 21	28.3	20.6	30.1	21.2	18.2	20.0	4.2	0.20
d 42	21.1	18.6	18.6	20.9	20.7	17.3	1.9	0.62

^{ab}Means within a row with different superscripts are significantly different at $P < 0.05$.

¹ Ctrl: control diet (without Miswak), 0.1, 0.2, 0.4, 0.6 and 0.8: diets supplemented with 0.1%, 0.2%, 0.4%, 0.6%, and 0.8% Miswak powder, respectively.

Antidiabetic activity of Miswak extract may be related to the presence of amides in this herb (Khan *et al.*, 2014). Miswak can stimulate glucose

uptake or increase the secretion of insulin. Because of these effects, the peripheral utilization of glucose will be facilitated, giving

Miswak hypoglycemic potential (Trovato *et al.*, 1998). Similar to our results, Galati *et al.* (1999) reported that Miswak had no effect on plasma HDL-c and triglycerides in diet-induced hypercholesterolemia rats.

Population of cecal bacteria

The effects of experimental diets on the population of cecal bacteria at 42 d of age are presented in Table 4. 0.6% and 0.8% Miswak significantly reduced the number of *E. coli* in the ceca compared to the control group. 0.4% Miswak resulted in a higher population of

Lactobacillus than the control group. The antimicrobial activity of Miswak is well documented (Halawany, 2012; Ahmad and Rajagopal, 2013), and herbs are generally known to change the composition and population of gut microflora (Hippenstiel *et al.*, 2011). Due to lipophilic effects of herbal components, they can penetrate cell membranes and mitochondria of the microorganisms, which can lead to the breakdown of the microbial cell membrane, resulting in ion leakage and cell death (Burt, 2004).

Table 4. Effects of experimental diets on population of cecal bacteria at 42 d of age (\log_{10} CFU/g)

Parameter	Experimental diets ¹						SEM	P-value
	Ctrl	0.1	0.2	0.4	0.6	0.8		
<i>E. Coli</i>	11.42 ^a	11.41 ^a	11.39 ^a	11.38 ^a	11.26 ^b	11.27 ^b	0.01	0.008
<i>Lactobacillus</i>	11.14 ^{bc}	11.19 ^{ab}	11.19 ^{ab}	11.42 ^a	11.10 ^c	11.09 ^c	0.02	0.01

^{a-c} Means within a row with different superscripts are significantly different at $P < 0.05$.

¹ Ctrl: control diet (without Miswak), 0.1, 0.2, 0.4, 0.6, and 0.8: diets supplemented with 0.1%, 0.2%, 0.4%, 0.6%, and 0.8 % Miswak powder, respectively.

Herbs are able to influence pathogenic microorganisms and modulate beneficial intestinal bacteria. Various bioactive components have antibacterial activity (including eugenol, thymol, carvacrol, capsaicin, Phenols, alcohols, ketones, aldehydes and cineole), and are present in phytobiotic feed additives (Upadhaya and Kim, 2017). For example, due to the high levels of eugenol in clove, clove oil is used as an antibacterial agent in human and veterinary medicine (Rhayour *et al.*, 2003). Eugenol has the potential to prevent harmful bacterial growth such as intestinal *Salmonella* in broiler chickens (Kollanoor-Johny

et al., 2012). As Miswak is rich in eugenol, it has the potential to beneficially influence intestinal bacteria, as we observed in this study.

Miswak aqueous extract has been shown to inhibit the growth of *Candida Albicans*, possibly due to high levels of sulfate in this herb (Al-Bagieh *et al.*, 1994). Fluoride (one of the active components present in Miswak) can interact with bacterial glycolytic enzymes and their acids which reduce the growth of bacteria. As a compound commonly used in oral hygiene, Miswak extract can have antimicrobial effects by preventing bacteria from attaching to the tooth surface (Halawany, 2012).

Table 5. Effects of experimental diets on relative weight (% live body weight) of carcass traits at 42 d of age and liver at 21 and 42 d of age

Parameter	Experimental diets ¹						SEM	P-value
	Ctrl	0.1	0.2	0.4	0.6	0.8		
Carcass yield	65.3	61.9	64.4	61.7	63.5	64.0	1.79	0.63
Thigh	25.7	25.2	26.9	25.3	27.1	27.3	1.13	0.14
Breast	27.8	25.8	27.4	24.5	27.1	25.9	1.01	0.66
Abdominal fat	1.52	1.34	1.74	1.30	1.54	1.47	0.23	0.75
Liver d 21	3.36 ^c	3.63 ^{bc}	3.58 ^{bc}	3.93 ^{ab}	3.97 ^{ab}	4.27 ^a	0.17	0.01
Liver d 42	2.55	2.49	2.85	2.54	2.67	2.52	0.19	0.92

^{a-c} Means within a row with different superscripts are significantly different at $P < 0.05$.

¹ Ctrl: control diet (without Miswak), 0.1, 0.2, 0.4, 0.6, and 0.8: diets supplemented with 0.1%, 0.2%, 0.4%, 0.6%, and 0.8 % Miswak powder, respectively.

Carcass characteristics

There were no significant differences across experimental treatments in yield of carcass, thigh, breast and abdominal fat at 42 d of age

(Table 5). Similarly, on d 21, 0.1% and 0.2% Miswak had no significant effect on liver weight, though higher levels (more than 0.4%)

significantly increased the weight of the liver compared to the control diet. This increase in liver weight may be an adverse effect in response to certain active components present in Miswak. It may be possible that higher doses of these active ingredients can have toxic effects on the liver- an organ critical for detoxification - causing the liver to enlarge to increase its detoxification potential. However, by day 42, liver weight was similar across treatment groups, which may mean that the toxic effects of Miswak reduced with the bird's age.

The relative weights of proventriculus, gizzard, small intestine, spleen, and pancreas were similar between the control and Miswak-supplemented diets (data not shown). It is expected that the application of herbs and their bioactive components can be variable (Upadhaya and Kim, 2017), leading to inconsistent results across studies. Some factors

that can contribute to the variability include the stability of active compounds in the herbs during feed processing, the origin and dose of the supplemental herb, physiological differences in the gut system, and environmental conditions of the experiment. Climatic conditions, location, harvest and storage conditions can also influence the chemical composition of phytochemical feed additives, thereby affecting the efficacy of herbs (Huyghebaert *et al.*, 2011).

Conclusion

Miswak did not have a significant effect on the performance traits, concentration of blood parameters, carcass traits, and organ weights in broilers. However, Miswak at levels greater than 0.4% had positive effects on population of cecal *Lactobacillus* and *E. coli*. Future studies using higher levels of Miswak or its extract are recommended.

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