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The effects of adding *Salvia mirzayanii* (Lamiaceae) in feed on growth performance, carcass traits, and humoral immune responses of broiler chickens

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ABSTRACT

Background & Aim: The present study was carried out to evaluate the effects of *Salvia mirzayanii* (SM) as an antibiotic growth promoter's alternative on growth performance, carcass characteristics, and some of the immune responses in broiler chickens.

Experimental: A total of 192 one-d-old female chicks (Ross 308) were raised in 16 randomly allotted cages. Treatments were as follows: basal diet, basal diet mixed with 4.5 mg flavophospholipol/kg of diet, and basal diet mixed with 5 or 10 g SM leaves powder/kg of diet. Body weights (BW) of broilers were measured at 1, 14, 28 and 42 d of ages to calculate daily weight gain (DWG), daily feed intake (DFI) was also determined and feed conversion ratio (FCR) calculated accordingly. Antibody titers against avian influenza (AI), Newcastle disease (NDV) viruses and sheep red blood cell (SRBC) and albumin to globulin ratio were assessed as immune responses.

Results: At 14 d of age, the broilers fed diets containing antibiotic had significantly higher (P<0.05) BW compared to those fed basal diet or basal diet mixed with 10 g SM/kg of diet. BW of broilers at 28 and 42 d of ages were not affected by the dietary treatments although it tended to increase in broilers fed diets containing antibiotic (P>0.05). DFI and FCR were not affected by the dietary treatments (P>0.05). Carcass traits were not affected by the dietary treatments except for relative weight of cecum which increased in broilers fed diets containing 10 g SM/kg (P<0.05). Antibody titers against NDV, AI, and SRBC were not affected by the dietary treatments.

Recommended applications/industries: In conclusion, the results indicated that supplementing broiler diet with 5 or 10 g SM leaves/kg of diet couldn't induce any positive effects on assessed parameters and it's not a suitable alternative for in-feed antibiotics.

1. Introduction

In-feed antibiotics (IFA) have been largely consumed at less than therapeutic doses in poultry breeding to improve growth performance and preserve healthiness of the birds. (Mosavi *et al.*, 2023; Afiouni *et al.*, 2023; Moradpour *et al.*, 2024). IFA were presumed to enhance rate of growth as a consequence of promoted multiple positive aspects of the gastrointestinal, causing in greater nutrient usage and feed conversion ratio (Landy and Kheiri, 2023). Although, there is a concern that the constant sub-therapeutic application of IFA could conduct to the development of strains of bacteria which have developed the ability to

withstand the effects of antibiotics (Kavyani *et al.*, 2014). Hence, attempts have been carried out in various corners of the world to ban the application of all kinds of IFA in the diet. As a consequence of the ban on the application of IFA in poultry production, and the considerable pressure on poultry producers in various regions of the world natural nutritional products have been investigated. Among the available options to replace IFA, special attention has been paid to probiotics (Landy and Kavyani, 2014; Toghyani *et al.*, 2015), medicinal herbs and their products (Landy and Kheiri, 2019), organic acids (Pham *et al.*, 2022) prebiotics (Zhen *et al.*, 2023) and bioactive peptides (Landy and Kheiri, 2021). The use of medicinal herbs and their products have received enhanced consideration in the last few years since they have been approved by users as organic ingredient (Landy *et al.*, 2020).

Salvia mirzayanii (Lamiaceae) is an endemic herbaceous plant belonging to the Lamiaceae family native to the center and south of Iran (Persia). Lamiaceae possess α-cadinol, 5-neo-cedranol, α-terpinyl acetate, linalyl acetate, linalool, caryophyllene oxide, bicyclogermacrene, epi-α-cadinol, and δ-cadinene (Taghizadeh *et al.*, 2024). Most of the plant parts such as its leaves containing compounds with proven many pharmacological effects including antioxidant, anti-cholinesterase, antimicrobial, anticancer, anti-inflammatory and enhancing cognition and memory (Jafari and Ghanbarian, 2015).

Furthermore, Omidikia *et al.* (2023) reported that the application of Lamiaceae in broiler diets couldn't induce any positive effects on broiler performance whereas it improved the humoral and cellular immune responses. In contrast, Bayati *et al.* (2017) reported that administration of *Salvia mirzayanii* (SM) essential oil in broilers' diet could improve feed consumption and feed conversion ratio. This experiment was conducted to evaluate the possibility of using dried powder of SM leaves as an alternative for IFA on growth performance, carcass traits, and immune responses of broiler chickens.

2. Materials and Methods

2.1. Animals and dietary treatments

A total of 192 one-d-old female broiler chicks (Ross 308) were raised for 42 d in 16 randomly allotted cages. The treatments were as follows: basal diet as a control, basal diet mixed with 4.5 mg flavophospholipol/kg of diet, and basal diet mixed with 5 or 10 g SM leaves powder/kg of diet. Table 1 lists the formulation of basal diet which provides the nutrients requirements of broilers as recommended by the Aviagen (2022).

Table 1. The constituent and computed composition of basal starter, grower, and finisher diets.

Item	Starter	Grower	Finisher
Ingredient, g/kg			
Corn	543.4	592.3	627.9
Soybean meal	395.2	355.5	314.8
Soybean oil	11.3	10.7	19.6
Dicalcium phosphate	21.9	17.4	14.1
$CaCO_3$	9.6	7.2	6.7
NaCl	1.9	2.2	2.1
NaHCO3	2.9	2.6	2.7
Trace mineral premix[1]	2.5	2.5	2.5
Vitamin premix[2]	2.5	2.5	2.5
DL-Methionine	3.7	3.3	3.2
L-Lysine-HCl	2.4	1.7	2.0
L-Threonine	1.4	0.9	0.9
Choline chloride	1.3	1.2	1.0
Calculated composition			
Metabolizable energy (kcal/kg)	2,845	2,908	3,020
Crude protein (g/kg)	220	205	190
Available phosphorus (g/kg)	5.0	4.2	3.6
Calcium (g/kg)	9.5	7.5	6.5
Sodium (g/kg)	1.8	1.8	1.8
Chloride (g/kg)	2.1	2.1	2.1
Digestible amino acids			
Methionine + cysteine (g/kg)	9.5	8.8	8.4
Lysine (g/kg)	12.6	11.2	10.5
Threonine (g/kg)	8.4	7.5	7.0

[1] Provided the following per kg of diet: Mg, 120 mg; Fe, 20 mg; Cu, 16 mg; Zn, 120 mg; Se, 0.3 mg; I, 1.25 mg.
[2] Provided the following per kg of diet: vitamin A, 13,000 IU; vitamin D_3, 5000 IU; vitamin E, 80 IU; vitamin K, 4.0 mg; riboflavin, 9.0 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 25 mg; nicotinic acid, 70 mg; folic acid, 2.5 mg

The broilers received starter diet from the beginning until 14 d of age, a grower diet from 14 to 28 d of age, and finisher diet from 28 to 42 d of age. Antibiotic or SM leaves powder were supplemented to the basal diets in replacement of maize. The chickens were raised in cages (120 × 120 × 80 cm) for 6 wks and had

free access to water and feed throughout the experiment. The lighting schedule consisted of 23 hours of light and one hour of darkness. The temperature of the boiler house was 32ºC at the beginning of the experiment and decreased by 3ºC per wk until it reached 22ºC and this temperature was maintained during the experiment.

2.2. Performance and carcass components

The body weight (BW) of the chicks was measured at 14, 28, and 42 d of ages to measure the average daily weight gain (DWG) during different raising periods. The daily feed intake (DFI) was measured in different raising periods and feed conversion ratio (DFI/DWG) was computed accordingly. The mortality was daily recorded and the DFI was adjusted accordingly.

At 42 d of the experiment, 2 chickens from each cage were selected and killed with a manual neck cutter. Carcass yield was computed by dividing the eviscerated weight by the live weight. Empty gizzard, empty proventriculus, empty small intestine, empty cecum, heart, liver, abdominal fat, and pancreas were weighed and expressed as a percentage of live BW. Small intestine and cecum lengths were also measured.

2.3. Immunity

At 7 d of age, 0.2 mL of the oil-adjuvant injectable emulsion against Newcastle (NDV) and influenza (AIV) viruses was subcutaneously injected. Also, at 21 d of age, the broilers were orally vaccinated against NDV (B1 type, LaSota strain). Antigen–antibody reactions against NDV, AIV, and sheep red blood cells (SRBC), and albumin–globulin (A/G) ratio which is a typical biomarker for monitoring inflammation and nutritional status were also measured as immune responses. At 25 d of age, 1 mL of 1% SRBC suspension was injected into the brachial vein and 6 d post-SRBC, the blood samples were obtained and serum samples were removed and frozen. Hemagglutination, a measure of SRBC specific immunoglobulin production was used to measure humoral immune function as described by Wegmann and Smithies (1966). Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination. At 28 d of age, blood samples were collected (2 samples/cage) to obtain sera by puncture of the brachial vein. Antigen–antibody reactions against NDV, and AIV were measured by hemagglutination inhibition test (HI), and HI antibodies were then converted to $\log_2$.

To measure the A/G ratio, at 42 d of age blood samples were taken from the wing vein and after serum separation, albumin and protein concentrations were measured via spectrophotometer and the kit (Pars Azmoon Company; Tehran, Iran). The difference between albumin and protein concentrations was measured and expressed as globulin.

2.4. Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Means were compared using Tukey test. Statements of statistical significance are based on $P<0.05$.

3. Results and discussion

3.1. Performance and carcass traits

As shown in Table 2 supplementation of antibiotic or different levels of SM leaves had not any marked effects on DFI, DWG and FCR of broilers at different growth periods. At 14 d of age the broilers fed diets containing antibiotic had higher BW compared to those fed basal diet or basal diet supplemented with 10 g SM/kg ($P<0.05$). At 28 d of age, the broilers fed diets containing antibiotic or 5 g SM/kg had higher BW compared to those fed basal diet whereases the results were not statistically significant ($P>0.05$). At 42 d of age the broilers fed diets containing antibiotic had none significantly higher BW compared to those fed basal diet ($P>0.05$). Antibiotics may limit the growth and colonization of invasive species of microorganisms and harmless microorganism in the digestive tract of poultry (Ferket, 2004). A more balanced flora in the digestive tract of poultry can lead to increased digestive efficiency and increased digestion and absorption of nutrients, which will ultimately improve growth (Bedford, 2000). Zhang *et al.* (2021) reported that administration of 20 mg colistin sulfate and 20 mg virginiamycin/kg of feed could increase DWG of broiler chickens. Similarly, Miles *et al.* (2006) reported that supplementation of bacitracin methylene disalicylate or virginiamycin to a corn-soybean meal diet increased BW and decreased intestinal length and weight of broiler chickens.

In the current experiment addition of 5 or 10 g SM/kg of diet did not induce any marked effects on broiler performance. Similarly, Omidikia *et al.* (2023) reported that supplementation of SM in broiler diets did not induce any positive effects on broiler growth performance. Bayati *et al.* (2018) stated that inclusion of 300 ppm SM essential oil could indue favorable influences on DFI and FCR of broiler chickens raised under heat stress condition. Bagherzadeh *et al.* (2015) reported that inclusion of 5 g SM leaf powder/kg of diet could improve growth performance and cecal microbial population of broiler chickens. The differences between these investigations are subject to a number of complicated factors, particularly breed, use dosage, conditions of the experiment, and the formula of basal diet (easy-to-digest or hard-to-digest). Considering the marginal (unremarkable) positive effects of IFA in the present study in broiler performance, we don't recommend to use IFA in easy-to-digest diets.

Table 2. Effect of antibiotic or different levels of *Salvia mirzayanii* on performance criteria of broilers at different ages.

Variables	Dietary treatments				SEM[1]
	Control	Antibiotic	5 g SM* /kg	10 g SM /kg	
Body weight (g)					
14 d	285[b]	307[a]	296[ab]	287[b]	6.5
28 d	881	941	939	916	32.4
42 d	1808	1868	1808	1820	42.2
Daily feed intake (g/d)					
0-14 d	29	29	30	29	0.7
14-28 d	94	70	94	70	10
28-42 d	126	128	126	127	2.3
0-42 d	75	75	75	76	1.1
Daily weight gain (g/d)					
0-14 d	17.2[b]	18.8[a]	17.9[ab]	17.3[ab]	0.4
14-28 d	42	45	47	44	2.2
28-42 d	66	66	62	62	2.02
0-42 d	41	43	42	42	1.1
Feed:gain (g:g)					
0-14 d	1.72	1.56	1.67	1.71	0.1
14-28 d	1.63	1.55	1.46	1.56	0.4
28-42 d	1.92	1.94	2.04	2.06	0.1
0-42 d	1.79	1.74	1.76	1.84	0.03

[1] Standard error of mean.
[*] *Salvia mirzayanii*

Table 3 illustrated the effects of antibiotic and two levels of SM on carcass traits, and the length of small intestine and cecum. The carcass yield and relative weight of internal organs were not affected by the dietary treatments except for the percentage of cecum which has been increased in broilers fed diets containing 5 g SM/kg of diet ($P<0.05$). Furthermore, the length of small intestine and cecum tended to enhance in broilers fed diets containing 5 g SM/kg of diet ($P<0.05$).

Table 3. Effect of antibiotic or different levels of *Salvia mirzayanii* on carcass traits of broilers at 42 d.

Relative organ weight	Dietary treatments				SEM[1]
	Control	Antibiotic	5 g SM* /kg	10 g SM /kg	
Carcass (%)	79.3	80.2	79.3	79.9	0.6
Heart (%)	0.47	0.48	0.47	0.46	0.2
Liver (%)	2.37	2.45	2.40	2.27	0.2
Abdominal fat (%)	0.97	0.96	0.87	0.97	0.6
Gizzard (%)	1.94	1.73	1.74	1.89	0.03
Pancreas (%)	0.28	0.27	0.25	0.26	0.04
Proventriculus (%)	0.43	0.43	0.51	0.49	0.07
Small intestine (%)	6.44	5.87	6.71	6.47	2.1
Cecum (%)	0.81[b]	0.87[ab]	1.09[a]	0.89[ab]	0.1
Small intestine (cm)	247.0	231.0	243.0	233.0	11.1
Cecum (cm)	41	42	45	44	1.7

[1] Standard error of mean.
[*] *Salvia mirzayanii*

Mosaddegh *et al.* (2013) reported that addition of different levels of SM essence had not any significant effects on carcass traits of broiler chickens. Hernandez *et al.* (2004) reported that supplementation of broilers diet with 200 ppm essential oil extract from oregano, cinnamon, and pepper; and 5,000 ppm extract from sage, thyme, and rosemary had not any marked effects on internal organ weights. As reported by Rybicka *et al.* (2024) cecal development and consequently capacity of its fermentation depends on physicochemical properties of diet specially the percentage and type of fiber; thus, the increments in length and relative weight of cecum in the current experiment may be due to the dietary fiber content which increased by the use of SM.

3.2. Immune responses

The obtained data related to immune responses are summarized in Table 4. As indicated in the Table 4 antibody titers against NDV, AIV and SRBC and A/G ratio were not significantly affected by the dietary treatments. The antibody titers against SRBC tended to increase in broiler chickens supplemented with 10 g SM/kg of diet (P>0.05). In contrast with our results Omidiki *et al.* (2023) reported that supplementation of SM in broilers diet increased antibody titers against NDV and SRBC. Zarshenas and Krenn (2015) stated that low levels intake of SM essential oil may improve the immunological function. Bayati *et al.* (2018) investigated the effects of different inclusion rate of SM essential oils in broilers diet; the results indicated that by increasing levels of essential oil the percentage of heterophile, heterophil/lymphocyte ratio reduced, and percentages of lymphocyte and monocyte enhanced. The SM contains some effective ingredients which indicated antioxidant, anti-cholinesterase, antimicrobial, anticancer, anti-inflammatory, immunomodulatory and suppressing effects (Amirghofran *et al.*, 2010; Jafari and Ghanbarian, 2015; Ziaei *et al.*, 2011). Yu *et al.* (2011) stated that some effective ingredients such as sesquiterpenes may result in cytotoxicity, inhibitory effects on lymphocyte proliferation, and subsequently apoptosis. Ziaei *et al.* (2011) stated that teuclatriol another biochemical compound in SM indicated immune suppressing effect. According to the mentioned reports, it seems that the difference in the obtained results may be due to different extraction methods or, in cases which the aerial parts of the plant were used, the region and cultivation conditions.

Table 4. Effect of experimental diets on immune responses of broiler chickens.

Variables	Dietary treatments				SEM[1]
	Control	Antibiotic	5 g Lamiaceae /kg	10 g Lamiaceae /kg	
New castle (log_2)	2.75	2.25	2.60	2.12	0.2
Influenza (log_2)	2.37	2.00	2.00	2.50	2.2
SRBC (log_2)	7.75	7.62	7.50	8.75	0.5
Albumin to globulin ratio	0.89	0.92	0.86	0.91	0.1

[1] Standard error of mean.

In contrast with our results Omidiki *et al.* (2023) reported that supplementation of SM in broilers diet increased antibody titers against NDV and SRBC. Zarshenas and Krenn (2015) stated that low levels intake of SM essential oil may improve the immunological function. Bayati *et al.* (2018) investigated the effects of different inclusion rate of SM essential oils in broilers diet; the results indicated that by increasing levels of essential oil the percentage of heterophile, heterophil/lymphocyte ratio reduced, and percentages of lymphocyte and monocyte enhanced. The SM contains some effective ingredients which indicated antioxidant, anti-cholinesterase, antimicrobial, anticancer, anti-inflammatory, immunomodulatory and suppressing effects (Amirghofran *et al.*, 2010; Jafari and Ghanbarian, 2015; Ziaei *et al.*, 2011). Yu *et al.* (2011) stated that some effective ingredients such as sesquiterpenes may result in cytotoxicity, inhibitory effects on lymphocyte proliferation, and subsequently apoptosis. Ziaei *et al.* (2011) stated that teuclatriol another biochemical compound in SM indicated immune suppressing effect. According to the mentioned reports, it seems that the difference in the obtained results may be due to different extraction methods or, in cases which the aerial parts of the plant were used, the region and cultivation conditions.

4. Conclusion

In conclusion, the results indicated that supplementing broiler diet with 5 or 10 g SM leaves/kg couldn’t induce any positive effects on assessed

parameters and it's not a suitable alternative for in-feed antibiotics.

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