Journal of Medicinal Plants and By-products (2023) 3: 217-223
https://doi.org/10.22092/jmpb.2022.355089.1372

Original Article

Can Broiler Chicken Growth, Immunity, and Meat Quality be Enhanced by *Salvia mirzayanii*?

Running title: *Salvia mirzayanii* as Feed Additive

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Article History

Received: 02 July 2021
Accepted: 10 January 2022

Keywords
Broilers
immune response
meat peroxidation
performance
Salvia mirzayanii

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ABSTRACT

Many natural phytobiotics of medicinal herbs possess antibiotic and radical scavenger properties for poultry species as nutritional additives. A bioassay study was conducted to evaluate the potential effects of *Salvia mirzayanii* Rech.f. & Esfand. (SM) on some physiological responses in broiler chickens including adaptive immune responses, blood parameters, and meat quality. A total of 200, one-day-old broiler chicks received five experimental diets containing 0.0, 0.25, 0.50, 0.75, and 1.0% SM in diet for a 42 d assay. Consumption of 0.50% and 0.75% of SM, were able to produce higher antibodies against sheep red blood cells and the Newcastle disease virus ($P \leq 0.05$). A substantial increase was observed in antibody titers against bronchitis virus in birds fed SM compared to those birds not receiving SM ($P \leq 0.05$). Using 0.50% SM in the diet increased skin thickness after dinitrochlorobenzene challenge ($P \leq 0.05$). The use of 0.50% SM in the diet increased the relative weight of the bursa of Fabricius ($P \leq 0.05$). The blood concentration of triglyceride, cholesterol, total protein, and albumin was maximized using SM in diet, while blood glucose decreased by increasing dietary levels of SM ($P \leq 0.05$). Meat malondialdehyde concentration has been minimized at 0.25%, 0.50% and 0.75% SM in diet ($P \leq 0.05$). In conclusion, SM consumption did not have a positive effect on the growth performance of broilers, but the use of its proper level in the diet improved the humoral and cellular immune responses, as well as meat quality.

INTRODUCTION

Usually, growth-promoting antibiotics are used to increase meat production in meat-type chickens and decrease the risk of diseases at the grower and finisher periods [1,2]. Residues of growth-promoting antibiotics in poultry products are harmful compounds for human health [3,4], which encouraged nutritionists to find safe alternatives to antibiotics. Medicinal plants have shown positive responses in preserving the meat quality and microbial populations in the gut of poultry species [5-10]. However, medicinal herbs may have little influence on bird performance [10,11] and phytochemical compounds in those herbs possess protective effects, which are synthesized to protect them against insects, fungi, disease, and herbivorous mammals. The essential oils in medicinal plants impact the physiological pathways, especially redox reactions at the cellular level, profile of blood metabolites, and immune system [8,10-13].

Lamiaceae is known for its aromatic compounds such as essential oils. *Salvia mirzayanii* (SM) is one of the members of the Lamiaceae family that mainly grown in warm and semiarid climates [14]. The most important components of essential oil in SM are comprised of cineole, linalool, alpha-pinene, carvacrol, thymol, linalyl acetate, spathulenol, delta-cadinene, alpha-terpinyl acetate, alpha-cadinol, alpha-terpineol, beta-eudesmol, cubenol, and eucalyptol [15]. Essential oils play a varied role in the body that may impact immunity responses and oxidant stability of the cells, improving the immune system and meat quality [16-19].

However, there is scanty data on SM properties in poultry diet, the present study was carried out to

evaluate the effect of dietary leaf powder of SM on some physiological responses of broiler chicken.

MATERIALS AND METHODS

S. mirzayanii leaves were manually harvested before the flowering stage in the Fasa region of Fars province, Iran. The fresh plant leaves were dried to 10% moisture content in a dark room with good ventilation, relative humidity of 40%, and temperature of 28 °C for five days.

Two hundred day-old Ross 308 broiler chicks were randomly distributed into 20-floor pens of five treatments and four replicates, and 10 chicks each. As shown in Table 1, the basal diets were adjusted for the starter (1-10 d), grower (11-24 d), and finisher (25-42 d) periods according to the commercial recommendation of Ross 308 [20]; (Table 1). Five dietary treatments including 0.0, 0.25, 0.50, 0.75, and 1.00% SM (w:w) in diet were prepared and dried SM was added to the basal diet at the expense of corn starch. During the experimental period, feed intake and bodyweight gain (BWG) were measured at 10, 24, 29, and 42 d of age, and then feed conversion ratio (FCR) was calculated from the feed intake and BWG records.

At the age of 17 and 27 days, three birds from each replicate were injected 0.2 mL of 1% sheep red blood cells suspension (SRBC) washed in a sterile phosphate buffer through a wing vein. After 15 days of the second injection, 2 mL of blood was taken from the birds and blood serum samples were separated by centrifuging at 1500 rpm for 10 minutes and then kept frozen at -20 °C until further analyses. Antibody titer against SRBC antigen was determined by agglutination test using 96 U-shaped well microplates. Newcastle disease vaccine (NDV; B_1strain) was given at 7 d of age while LaSota strain of NDV was given at 21 day of age. Infectious bronchitis vaccine H-120 for poultry was given at 14 d of age by eye drop. At 42 d of age, blood was taken from the wing vein of three birds from each experimental group. After the separation of serum from the blood clot, the generated antibody titer against NDV and infectious bronchitis vaccines (IBV) was measured by the Hemagglutination Inhibition (HI) test. In brief, 25 µl of phosphate-buffered saline (PBS) was poured into all cavities of the horizontal rows of 96-well microplates, using a sampler. Then 25 µl of serum was added to the first well of each row, and after mixing, 25 µl of the contents of the first well was removed and added to the second well, and this operation was repeated for all wells. In the next step, 25 µl of virus concentration was added to all cavities. The plate was then placed at room temperature for 30 minutes without moving, after which the results were read [21].

On day 42, three birds in each pen were randomly chosen, weighed, and euthanized using CO_2 asphyxiation. After defeathering, the bursa of Fabricius was separated and its weight was measured by a digital scale with a precision of 0.001 g and reported as a percentage of live weight. At d 40, three birds from each experimental unit were labeled with different colors to measure skin thickness following 2,4-dinitrochlorobenzene (DNCB) challenge. The solution of DNCB 0.1% was prepared by a mixture of 1 mg of DNCB in 1 mL of acetone and olive at the ratio of 4:1.

In this method, a region with relatively no feather with an approximate area of 10 cm^2 was treated by DNCB on the right side of the body.

Table 1 Nutrient composition of the basal diet

	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
ME (MJ/kg)	12.68	13.18	13.39
CP (g/kg)	244.70	220.00	200.00
Lys (g/kg)	14.50	12.80	11.00
Met (g/kg)	6.40	5.90	5.20
TSAA (g/kg)	10.80	9.80	8.80
Thr (g/kg)	9.90	8.50	7.40
Trp (g/kg)	2.60	2.50	2.30
Ca (g/kg)	10.50	9.00	8.50
Available P (g/kg)	5.00	4.50	4.20
DEB (mEq/kg)*	250.00	250.00	250.00

*DEB: Dietary electrolyte balance (Na + K – Cl).

Skin thickness was measured with an electronic micrometer with a precision of 0.01 mm before and 12, 24, and 48 hours after DNCB challenge. The average increase in skin thickness of 3 birds was obtained from the difference in thickness before and after DNCB challenge [22].

At day 42, three birds were randomly selected from each experimental unit, and 2 mL of blood was taken from the wing vein. The concentration of triglyceride, cholesterol, total protein, albumin, and glucose in sera was determined using spectrophotometric method (UNIKON 933, Kontron Co. Ltd., Milan, Italy) and Pars Azmun (Tehran, Iran) biochemical kits.

One gram of breast meat was homogenized in the presence of 5 mL of an aqueous solution containing 5% of trichloroacetic acid (Merck, Darmstadt, Germany) and 5 mL of butylated hydroxytoluene (BHT) solution in hexane (Merck Co., Germany) at the concentration of 0.8 g per 100 mL. The resulting mixture was then centrifuged at 3000 rpm for 10 minutes, and after dispensing the upper layer, 2.5 mL of the lower layer was mixed with 1.5 mL of aqueous solution of 2-thiobarbituric acid (Merck Co., Germany) at the concentration of 0.8 g per 100 mL. Thereafter, the mixture was incubated in a warm-water bath of 70 °C for 30 minutes. After cooling, the optical absorption of the solution was measured at the wavelength of 532 nm, and the amount of malondialdehyde (MDA) was calculated using the standard material 1, 1, 3, and 3-Tetraethoxypropane (Merck, Hohenbrunn, Germany) as μg/g of meat [23].

Data analysis was performed using GLM procedure of SAS software [24] and averages differences were compared using Tukey test at $P < 0.05$. Skin thickness data were analyzed by the repeated measurement method.

Experimental diets influenced the either humoral (i.e., SRBC: P=0.003; NDV: $P < 0.001$; IBV: $P < 0.005$) or cell-mediated (i.e., skin thickness: P = 0.002) immunity systems of broiler chickens (Tables 3 and 4); and also changed the relative weight of bursa of Fabricius ($P < 0.001$).

RESULTS

As shown in Table 2, the use of SM up to 0.50% of the diet increased BWG at 29-42 d of age while BWG sharply dropped at 1.0% of the diet ($P < 0.0001$). Dietary SM up to 0.50% showed a stimulating effect on BWG during the finisher period (i.e., 29-42 d of age) but the inhibitory effect on BWG during the starter (i.e., 1-14 d of age) and grower (i.e., 15-28 d of age) periods resulted in the best performance of 0.50% SM at 1-42 d of age. By applying 0.50% and 0.75% of SM in the diet, birds were able to produce higher antibodies against sheep red blood cells.

Table 2 Effect of *S. mirzayanii* Rech.f. & Esfand. on the performance of broilers

Item	*S. mirzayanii* (%)					SEM *	P-value†
	0	0.25	0.50	0.75	1.00		
1-14 days							
Body weight gain (g/day)	20.44 a	18.52 a	19.06 a	18.67 a	14.69 b	0.845	0.0032
Feed intake (g/day)	33.52 a	33.08 a	33.46 a	32.13 a	28.98 b	1.075	0.0469
Feed conversion ratio	1.64 b	1.78 ab	1.76 ab	1.74 ab	1.97 a	0.066	0.0378
15-28 days							
Body weight gain (g/day)	62.35 a	60.58 a	61.02 a	57.69 a	50.67 b	1.524	0.0005
Feed intake (g/day)	98.95 a	92.74 ab	94.98 ab	92.79 ab	86.96 b	2.294	0.0304
Feed conversion ratio	1.59 b	1.53b	1.55 b	1.61b	1.72 a	0.024	0.0007
29-42 days							
Body weight gain (g/day)	70.91 bc	72.39 b	77.17 a	70.19 bc	67.73 c	0.946	<0.0001
Feed intake (g/day)	125.77	124.41	125.56	125.07	122.97	1.876	0.8331
Feed conversion ratio	1.77 a	1.72 ab	1.62 b	1.78 a	1.81 a	0.028	0.0030
1-42 days							
Body weight gain (g/day)	51.23 a	50.49 a	52.41 a	48.85 a	44.36 b	0.921	0.0002
Feed intake (g/day)	86.08	83.41	84.67	83.33	79.64	1.508	0.0846
Feed conversion ratio	1.68 cb	1.65 cb	1.61 c	1.71 ab	1.79 a	0.019	0.0002

*SEM: Standard error of the means.

† Means in the same column with different superscripts differ ($P < 0.05$).

Table 3 Effect of *S. mirzayanii* Rech.f. & Esfand. on immune response to sheep red blood cells (SRBC), Newcastle disease virus (NDV) and infectious bronchitis disease virus of growing broilers

S. mirzayanii (%)	Anti-SRBC titer	Anti-NDV titer	Anti-IBV titer
0.00	3.87 c	5.50 c	4.00 b
0.25	5.12 bc	7.50 b	6.00 a
0.50	6.65 a	8.50 a	6.75 a
0.75	6.50 ab	8.50 a	6.25 a
1.00	5.25 abc	5.50 c	6.00 a
SEM *	0.29	0.33	0.28
P-value†	0.003	<0.001	0.005

*SEM: Standard error of the means.

† Means in the same column with different superscripts differ ($P < 0.05$).

Table 4 Effect of dietary treatments on the relative weight of lymphatic organs (%) and increase in mean skin thickness (mm) after 2,4-Dinitro 1-chlorobenzene (DNCB) challenge

S. mirzayanii (%)	Skin thickness	Spleen	Bursa of Fabricius
0.00	0.207 c	0.159	0.216 b
0.25	0.249 bc	0.132	0.217 b
0.50	0.333 a	0.142	0.260 a
0.75	0.310 a	0.160	0.216 b
1.00	0.286 ab	0.149	0.199 bc
SEM *	0.012	0.009	0.006
P-value†	0.002	0.856	<0.001

*SEM: Standard error of the means.

† Means in the same column with different superscripts differ ($P < 0.05$).

Table 5 Effect of dietary treatments on some blood factors in broilers

S. mirzayanii (%)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Glucose (mg/dl)
0.00	59.0 b	104 c	4.70 bc	2.10 b	222 a
0.25	81.5 a	123 b	5.20 ab	2.52 a	215 ab
0.50	77.0 a	124 b	5.62 a	2.55 a	213 b
0.75	78.2 a	138 a	5.12 ab	2.57 a	209 b
1.00	78.2 a	133 ab	4.52 c	2.10 b	202 c
SEM *	1.92	3.08	0.11	0.06	1.77
P-value†	<0.001	<0.001	0.002	<0.001	<0.001

*SEM: Standard error of the means.

† Means in the same column with different superscripts differ ($P < 0.05$).

Table 6 Effect of dietary treatments on malondialdehyde (MDA) concentration in meat of broilers (µg/g)

S. mirzayanii (%)	MDA
0.00	0.327 a
0.25	0.277 b
0.50	0.262 b
0.75	0.276 b
1.00	0.354 a
SEM *	0.009
P-value†	<0.001

*SEM: Standard error of the means.

† Means in the same column with different superscripts differ ($P < 0.05$).

Moreover, the use of 0.50% and 0.75% SM in the diet resulted in a significant increase in antibody titer against the Newcastle virus. As well, a substantial elevation was observed in antibody titers against bronchitis virus in birds fed SM compared to those birds not receiving SM. Using 0.50% SM in the diet increased skin thickness after DNCB challenge. In addition, the use of 0.50% SM in the diet increased relative weight of the bursa of Fabricius.

Five blood parameters including triglyceride ($P < 0.001$), cholesterol ($P < 0.001$), total protein ($P = 0.002$), albumin ($P < 0.001$), and glucose ($P <$

0.001) have been affected by dietary treatments (Table 5). The blood concentration of triglyceride, cholesterol, total protein, and albumin was maximized using SM in diet, while blood glucose decreased by increasing dietary levels of SM.

The use of SM in diet changed the production of MDA ($P < 0.001$) in meat samples (Table 6), in which the measured MDA has been minimized at 0.25%, 0.50%, and 0.75% SM in diet.

DISCUSSION

The results of this research showed that the use of SM powder in diet impacts the performance and qualitative responses of broiler chickens including immunity and meat peroxidation as a quality index. Based on previous studies on medicinal plants containing a variety of bioactive compounds especially alkaloids, glycosides, polyphenols, and terpenes as the major phytochemicals, the medicinal herbs had more profound effects on qualitative traits than the performance of poultry [8,10,11]. Correspondingly, the results of the present study implying that SM as medicinal plants had more effects on immunity and product quality than performance response.

The modulatory effects of medicinal herbs are attributed to the biochemical natural compounds and one of the most important sesquiterpenes of SM samples in tropical regions is spathulenol, an aromatic essential oil [25] with antioxidant activity in aqueous media like physiological condition in the cell [26]. However, in some cases, SM may show weak antioxidant properties, which may be related to the high concentration of flavonoids in the herb samples [25]. Yu *et al*. reported that the high concentrations of essential oils such as sesquiterpenes may result in cytotoxicity [27], inhibitory effects on lymphocyte proliferation, and subsequently apoptosis [28, 29]. Another biochemical compound in SM is teuclatriol that in high concentrations may have a synergistic effect with spathulenol to suppress the immune functions [30]. This dual role of SM supplementation in the broiler diet that has been revealed in the present study clearly showed the utmost importance of using optimal values for the addition of such medicinal plant into the broiler diet.

The boosted immune system caused by the use of medicinal plants in diet may be achieved by the various mechanism at a cellular level. Reduction of reactive oxygen species (ROS) in the cell by the antioxidant action of biochemical molecules in medicinal herbs could prevent oxidative damage of cellular membrane [31]. It should be noted that *Lamiaceae* family could change ileal microbial population in favor of beneficial bacteria such as lactobacillus [11] contributing in a healthy environment of the digestive system and intestinal immune system [32].

The results of the present experiment show that the use of SM in broiler diet increases the serum triglyceride and cholesterol but decreases serum glucose. Nikavar *et al*. suggested that SM could decrease alpha-amylase activity resulting in reduce carbohydrate digestion and reduction of glucose absorption in digestive system [33]. Low concentration of glucose in circulation system may suppress the expression of SREBP-1c gene in skeletal muscles, decreasing muscular lipogenesis [34]. This process may result in accumulating the lipid compounds in blood. An important blood marker of the immunity status of the birds is the concentration of serum albumin, which may correlate with both humoral and cell-mediated responses in this study. Taking together, the highest positive relationships with optimal use of dietary SM were observed for serum albumin, and immune responses whereas the highest negative relationships with the SM supplementation were obtained for serum glucose and MDA production, proving antioxidant and immune stimulator properties for SM as an additive in the broiler chicken diet. Serum albumin and globulin are vital elements for maintaining a healthy immune system [35]. The increment of serum levels of various peptides such as lysozymes, antibodies, and complement factors is the first line of defense to prevent adherence and colonization of microorganisms resulting in the prevention of infection and diseases [36].

CONCLUSION

The growth performance of broilers was not affected by SM. However, the use of 0.5 to 0.75 levels of SM in the diet improved the immune responses and meat quality, providing evidence that this medicinal plant was more needed for physiological responses rather than growth performance and negligible amount of active substances in this herb may greatly impact the the humoral and cellular immunity, as well as meat quality.

CONFLIC OF INTEREST

There are no known conflicts of interest.

Animal Welfare Statement

Animal handling and experimental procedures were performed according to the Iranian Council of Animal Care (1995).

ACKNOWLEDGEMENTS

The authors would like to acknowledge the University of Zabol for providing experimental facilities and financial support (grant numbers UOZ-GR-9718-56).

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