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Influence of Using Sage Powder (*Salvia officinalis*) on Performance, Blood Cells, Immunity Titers, Biochemical Parameters and Small Intestine Morphology in Broiler Chickens

Research Article

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ABSTRACT

An experiment was performed to investigate the effect of using sage powder (*Salvia officinalis*) on performance, blood cells, immunity titers, biochemical parameters and small intestine morphology in broiler chickens. A total of 200 Ross 308 broiler chickens were assigned in a completely randomized design with four treatments and five replicates and ten chicks in each replicate. Experiment has been done from 1 to 42 days old. The experimental groups consisted of a control group receiving basal diet and basal diet supplemented with 0.2, 0.5 and, 1.2% of sage powder. The results of the experiment revealed that supplementation of 0.5% of salvia powder significantly ($P \leq 0.05$) increased body weight (BW) and reduced feed conversion ratio (FCR). An increase in eosinophil, monocyte and, heterophil as well as immunity titers against Newcastle disease and avian influenza viruses were observed where in the control diet was supplement with 0.2% of Salvia powder. The plasma cholesterol, triglyceride, and low-density lipoprotein (LDL) concentration were reduced and high-density lipoprotein (HDL) concentration was increased significantly by inclusion of 0.2% sage powder. Additionally, ileal villus height and intestinal health index was increased and the number of gablet cells and crypt depth was reduced significantly ($P \leq 0.05$) by incorporation of 0.2% sage powder in basal diet. In conclusion, supplementation of 0.2-0.5% sage powder had meaningful impact on broiler performance, ileal morphology and vaccine titer against Newcastle disease and avian influenza.

KEY WORDS blood characteristic, ileal morphology, immunity, performance, sage powder.

INTRODUCTION

The incorporation of medicinal plants or their essential oils into chicken ration have been proven that effectively could improve the health of intestinal tract of broiler chickens by altering the gut microbial flora, consequently could improve the immune system and reduce the blood cholesterol. In addition to antimicrobial effects, medicinal herbs also have other beneficial effects such as: the stimulating of growth, reduction of blood lipids and improving blood factors (Amoozmehr and Dastar, 2009).

Consumption of medicinal plants to enhance the performance, and immunity response of birds has been reported previously (Alcicek *et al.* 2004). Extracts and essences of herbal medicine plants have extensive applications that inhibit pathogens, therapeutic effects and activity in various body systems such as endocrine and immune system (Frankic *et al.* 2009). Investigating the effect of medicinal plants, including powder, essential oil, oil extract as a growth-stimulating substances, antioxidant and immune system booster, is an active field in poultry research (Warshafsky *et al.* 1993).

Sage with the scientific name of *Salvia officinalis*, belong to Labiatae family, is a dark-toothed plant, which grows herbaceous, plant-like and multi-year-old with a cracked and up to 60 cm height (Ahmadi and Abdullahi, 2012).

As reported by Khakpour and Khosravi (2014), the consumption of *Salvia officinalis* delayed the degradation of nerve cells, stunt bloating and gastric gas, reduced inflammatory factors, and boost the immune system in humans. Ryzner *et al*. (2013) reported that the combination of salvia powder and sodium selenite resulted in a significant decrease in the activity of superoxide dismutase in erythrocytes and activity of glutathione peroxidase in the liver and duodenal mucosa compared to the control group. In another study ČapkovIcova *et al*. (2014) examined the effects of salvia (1 and 0.05 percent of diets) on the intestinal mucus thickness and the amount of mucin in broiler chickens and showed that the effect of salvia powder on the thickness of mucus and distribution of mucin in the chicken intestine.

MATERIALS AND METHODS

Housing and experimental diets

The two hundred Ross 308 male broiler chicks with average mean weight of 40 ± 2 were used in the experiment. Chicks were divided into floor pens on a completely randomized design with four experimental treatments (basal diet and diet supplement with 0.2, 0.5 and, 1.2% of sage powder) and five replicates and 10 chicks in each experimental unit with floor space area of 1 m^2. Chicks in all the replicates were reared up to six weeks of age under similar standard conditions throughout the trail. Each pen was fitted with an automatic bell type drinker and a tubular feeder. Chicks had free access to feed and water throughout the trial. Feeding of test diets commenced at first day of age and continued till the termination of experiment at six weeks of age. A three phase (0-10, 11-24 and 25-42) experimental diets were formulated according to the nutrient specification recommended by national research council (NRC, 1994) (Table 1).

Saliva powder preparation

Fresh leaves of salvia were obtained from local company and dried in a dark room with suitable ventilation under relative humidity 40% and temperature of 28 ˚C for five days, and then were ground through a 1mm screen and analyzed in Laboratory of Malayer University (Table2).

Performance parameters

The mortality of each pen and feed intake of each pen were recorded daily. Weight gain and feed conversion ratio (FCR) were calculated at the end of each week. For this purpose, at the end of each week of each test unit, all chickens with a digital scale were measured with a precision of 0.001 grams. Feed conversion ratio per week based on the proportion of feed intake according to gram, the mean weight of chicks-in each test unit was calculated in gram.

Blood analysis

Two chicks from each pen were selected from all groups, and individual blood samples from brachial vein were collected separately into non-heparinized tubes at 6 weeks of age. The sera were separated from centrifuged 3000 xg, 10 min, and isolated serum stored at −20 ˚C for further analysis. Each serum was analyzed for cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride using commercial kits (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan), and antibody titers against Newcastle disease (ND) and avian Influenza (AI) viruses were done by hemagglutination inhibition test (HI) technique in immunology lab. Differential blood cells count in the hematology laboratory, performed with Hemacytometer slide (neuobauer®) (Hedayati and Manafi, 2018).

Ileal morphology

On d 42, 2 birds from each pen were randomly selected, and killed (cervical vertebra displacement) for evaluation of ileal histology. The digestive tract along with contents was removed aseptically and the ileum was separated from the Meckel's diverticulum up to 1 cm proximal to the ileocecal junction and then dried with desiccant paper. A 5 cm section of ileum was taken from the middle of the ileum and gently flushed with phosphate-buffered saline (PBS) (pH 7.2). The samples were stored in containers containing formalin 10%. Tissue sections (2 µm) were cut by microtome (Leitz-1512 Microtome, Leitz, Wetzlar, Germany), floated onto slides, and stained with H&E, haematoxylin (Gill no. 2, Sigma, St. Louis, MO), and eosin (Sigma). To measure villus height and crypt depth, images from samples were taken using a digital camera that had light microscopy. Twelve images from 4 tissue sections of each ileal section were taken and 24 villus heights and crypt depths were measured by imaging software (ToupView 7.1). Measurements for each villus length were taken from the tip of the villus to the valley and measurements for crypt depth were taken from the valley to the basal layer (Hedayati and Manafi, 2018).

Statistical analysis

Collected data were analyzed using the following statistical model using SAS software (SAS, 2009). Comparison of treatments means were performed using Duncan-range Test (1995) and the significance level was considered at $P \leq 0.05$.

Table 1 Ingredients and chemical compositions of the basal diet in starter, grower and finisher periods

Item	Starter diet (0-10 d)	Grower diet (11-24 d)	Finisher diet (25-42 d)
Ingredient (%)			
Corn	56.70	58.78	63.70
Soybean meal	37.34	34.62	29.32
Soybean oil	1.66	2.78	3.39
Dicalcium phosphate	1.83	1.63	1.46
Limestone	0.87	0.78	0.74
Sodium chloride	0.30	0.30	0.30
Sodium bicarbonate	0.10	0.10	0.10
L-lysine-HCl (98%)	0.23	0.15	0.16
DL-methionine (98%)	0.34	0.28	0.26
L-threonine	0.13	0.08	0.07
Vitamin and mineral premix[1]	0.50	0.50	0.50
Calculated composition (%, unless otherwise stated)			
Metabolizable energy (MJ/kg)	12.18	12.60	13.02
Crude protein	22.00	20.80	18.90
Calcium	0.93	0.84	0.78
Available phosphorous	0.46	0.42	0.38
Sodium	0.16	0.16	0.16
Lysine	1.24	1.11	1.00
Methionine	0.63	0.56	0.52
Methionine + cysteine	0.92	0.84	0.78
Threonine	0.83	0.75	0.67
Dietary cation anion balance (mEq/kg)	232	224	200

[1] Supplied per kg diet: vitamin A: 9000 IU; vitamin D_3: 2000 IU; vitamin E: 18 IU; vitamin K_3: 2 mg; vitamin B_{12}: 15 µg; Riboflavin: 6.6 mg; Pantothenic acid: 10 mg; Pyridoxine: 3 mg; Folic acid: 1 mg; Thiamin: 1.8 mg; Biotin: 0.1 mg; Niacin: 30 mg; Choline: 500 mg; Selenium: 0.2 mg; Iodine: 1 mg; Copper: 10 mg; Iron: 50 mg; Zinc: 85 mg and Manganese: 100 mg.

Table 2 Composition of Sage powder (*Salvia officinalis*) (analysed in Malayer University lab)

Item	Ingredients (%)
Crude protein	1.50
Ash	29.34
Crude fiber	6.66
Crude fat	1.04
Phenol	0.239

RESULTS AND DISCUSSION

Performance parameters

The influence of sage powder on broiler performance is shown in Tables 3, 4 and 5. Sage powder inclusion reduced body weight and suppressed feed intake significantly during the trial except for 0.5% of salvia powder, which significantly increased weight ($P \leq 0.05$). Significantly increased the weight gain and FCR has to be were observed when 1.2% sage powder incorporated to diets ($P \leq 0.001$). Nonetheless, the same feed intake was achieved among the treatment at the end of experimental period. Except for diet contained 1.2% sage powder, feed conversion ratio was almost similar among the treatments. The feed intake-suppressing effect of sage powder might be the result of its high fiber. The high fiber in broiler diets increases digesta retention time and therefore stimulates mechanical receptors of the proventriculus, promotes HCl production, and reduces gizzard pH (Hajati *et al*. 2014).

Additionally, the major phytochemicals in flowers, leaves of sage powder are well identified (Ghorbani and Esmaeilizadeh, 2017).

Aphyto chemical compound of this plant mainly consist of alkaloids, carbohydrate, fatty acids, glycosidic derivatives (e.g., cardiac glycosides, flavonoid glycosides and saponins) and phenolic compounds from which mono-di and triterpenoids such as saponins are major constituent which possess feed intake suppressing effects. The extract of sage powder contained high levels of triterpenoid saponins and studies have proven that the inclusion levels of saponins in diets as well as the type of saponin affected the broiler response. Previous studies have suggested that alfalfa saponins have adverse effects on the performance of birds and the adverse effects of saponins have been attributed to suppressing feed consumption because of the bitter taste (Martins *et al*. 2003). On the other hand, it seems that herbal additives for broiler diets because palatability and increase feed intake (Saki *et al*. 2014).

Table 3 Body weight (g) of broilers fed different levels of sage powder

Experimental groups	1-7	1-14	1-21	1-28	1-35	1-42
Control	190.50	384.25^{a}	737	1217.50^{a}	1826.75^{a}	2422^{b}
Sage powder 0.2%	185	379^{a}	688	1158^{a}	1683^{b}	2193^{b}
Sage powder 0.5%	180	373^{a}	717	1169.50^{a}	1643.25^{b}	2478.50^{a}
Sage powder 1.2%	178.75	344.50^{b}	687	1047.50^{b}	1495^{c}	1936.41^{c}
P-value	0.2556	0.0370	0.2474	0.0110	0.0033	0.0025
SEM	4.19	8.97	19.10	29.86	43.39	73.06

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Feed intake (g/bird) of broilers fed different levels of sage powder

Experimental groups	1-7	1-14	1-21	1-28	1-35	1-42
Control	145.25	436.50	1050.50^{a}	1867.50^{a}	2914^{a}	3976.3
Sage powder 0.2%	145.25	420.50	949.25^{b}	1621.25^{c}	2510.75^{c}	3694.8
Sage powder 0.5%	144.25	425.50	989.50^{b}	1653.75^{bc}	2587^{bc}	3879.8
Sage powder 1.2%	147.35	422.25	972.25^{b}	1694^{b}	2658.67^{b}	3714
P-value	0.7452	0.6238	0.0016	0.0006	0.0001	0.3291
SEM	1.95	9.31	13.90	19.55	31.27	126.11

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Feed conversion ratio (FCR) of broilers fed different levels of sage powder

Experimental groups	1-7	1-14	1-21	1-28	1-35	1-42
Control	0.80	1.26^{a}	1.42	1.53^{a}	1.60^{b}	1.63^{b}
Sage powder 0.2%	0.80	1.14^{b}	1.37	1.39^{b}	1.50^{b}	1.68^{b}
Sage powder 0.5%	0.75	1.50^{b}	1.37	1.40^{b}	1.57^{b}	1.56^{b}
Sage powder 1.2%	0.79	1.10^{b}	1.41	1.61^{a}	1.77^{a}	1.93^{a}
P-value	0.2504	0.0019	0.5001	0.0014	0.0013	0.0015
SEM	0.01	0.02	0.02	0.03	0.04	0.10

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Studies have shown that the use of herbs containing flavonoid in the diets of broiler increases feed intake (Starcevic *et al*. 2015). Thymol and carvacrol of thyme and savory oil essence (Lee, 2003) by regulating the control centers of appetite reduced the amount of consumed feed in experimental treatments (Platel and Srinivasan, 2003).

On the other hand, the existing fructoides in medicinal plants have stimulatory effects on digestive enzymes, pancreas and intestinal mucus, which leads to improvement of digestion and uptake of the nutrients and increase nutrient absorption. Herbs and herbal extracts because accelerated digestion and shortening the passage of digestive substances from the length of the gastrointestinal tract (Platel and Srinivasan, 2003). The labiatae family plants are content that these compounds improve oxidative stability in animal production, which has also been proven in poultry meat and eggs and pork and rabbits (Platel and Srinivasan, 2003). Sadeghi *et al*. (2012) in research, sage extract was used with several different doses and observed significant results on functional traits in broiler chickens also, reported that the use of combined diets (garlic, sage, purple, thyme, oregano) had significant effects on weight gain and feed conversion ratio of broiler chickens.

Demir *et al*. (2008) reported that the use of combined diets (peppermint, salvia and thyme) had significant effects on weight gain and feed intake as well as significant effect on the conversion ratio from 21 to 42 days.

Bagherzadeh Kasmani *et al*. (2014) reported that the use of Salvia leaf powder until the age of 21 days decreased feed intake and lower weight gain but the better conversion ratio and age of 21 to 42 days of weight gain and better conversion ratio but on feed intake did not affect. Nobakht and Mehman-Navaz (2010) showed that the use of the oregano powder in diet of laying hens has positive effects on their performance traits.

Blood cell analysis

The effect of different experimental groups on blood cells in broiler chickens was shown in Table 6. The most significant amounts of eosinophil and monocytes were found in experimental groups received 0.2% sage powder and the lowest amount was observed in birds were fed with 1.2% of sage powder. The highest amount of lymphocytes were observed in birds fed diets containing 1.2% sage powder and the lowest value obtained in birds received a 0.5% sage powder.

Table 6 Effect of sage powder on blood cells in broiler at 42 days

Experimental groups	Basophil	Heterophil	Lymphocyte	Monocyte	Eosinophil
Control	2.50^{b}	24.25^{b}	61.50^{b}	4.25^{b}	4.50^{b}
Sage powder 0.2%	1^{c}	36.50^{a}	57.25^{c}	8.25^{a}	5.10^{a}
Sage powder 0.5%	3.75^{a}	34.50^{a}	57^{c}	2^{c}	4.75^{ab}
Sage powder 1.2%	3.25^{a}	21^{b}	72.50^{a}	1.25^{d}	4.25^{b}
P-value	0.004	0.001	0.002	0.038	0.004
SEM	0.03	0.67	1.49	0.06	0.08

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Natural antioxidants such as sage powder protect cells against reactive oxygen over production and therefore can counteract oxidative stress-mediated tissue damage. Evidence from several studies suggests that sage powder has potent antioxidant activities and could alter the heterophils/lymphocyte ratio (Horváthová *et al*. 2016). Enriching the drinking water of rats with *S. officinalis* extract increases resistance of rat hepatocytes against oxidative stress. Fanimakki *et al*. (2013) said, increased the number of lymphocytes and heterophils in poultry receiving compound containing sage and thyme. Capek and Hríbalová (2004) the he effects of oregano on the immune system of broiler chickens showed that there was a significant difference in the ratio of heterophilic to lymphocyte among experimental groups and oregano was effective on the immune system of broiler chickens.

Mirzavand *et al*. (2015) The effects of medicinal plants such: mint, coriander, garlic, basil and parsley on the immune system of broiler chickens, the ratio of heterophilic to lymphocyte in garlic was minimal and in the control of the highest and the antibody was not affected by the experimental groups.

Nobakht and Mehman-Navaz (2010) the use of mixed three medicinal plants of peppermint, thyme and oregano increased the percentage of lymphocytes and reduction of heterophilic percentages and the ratio of heterophilic to lymphocytes, which suggests its beneficial effects three plants are on the immunity system of laying hens.

Humeral antibody titer

The immunity response of blood titers against conventional pathogenic viruses in broiler chickens are shown in Table 7. Results showed a significant effect of sage powder on the immune response of blood titers against the Newcastle virus and avian influenza virus in broiler chickens. The highest (P≤0.05) immunity titer against Newcastle and influenza diseases was seen when birds recived 0.2 percent of sage powder and the lowest immunity response was observed in the control group.

Serum biochemical parameters

Results of serum biochemical analysis are shown in Table 8. Significant (P≤0.05) reduction of serum cholesterol, triglyceride and LDL concentration were observed when sage powder included in diets. Data related to high-density lipoprotein blood parameters also showed significant reduction compared to control diets. The highest amount of HDL was observed when basal diet was included with 0.2% sage powder compared to the control group.

Pharmacological studies also revealed that different extracts of *S. officinalis* reduces serum lipids (Ghorbani and Esmaeilizadeh, 2017). Hernandez-Saavedra *et al*. (2016) mentioned that sage powder reduced serum triglycerides, total cholesterol, and low density lipoproteins (LDL) levels in diet-induced obese rats. It also decreased body weight and abdominal fat mass in these animals. Eidi *et al*. (2006) and Khattab *et al*. (2012) reported some therapeutic effects of sage powder on lipid profile in diabetic animals. They reported decrease the level of triglyceride, cholesterol, urea, uric acid, creatinine, aspartate amino transferase (AST), and alanine amino transferase (ALT) in streptozotocin-induced diabetic rats. In clinical trials, extract of sage powder leaf could lower the blood levels of triglyceride, total cholesterol, LDL, very low density lipoproteins (VLDL) and 2 h postprandial glucose in patients with hyperlipidemia and diabetes (Kianbakht and Dabaghian, 2013). Mirzavand *et al*. (2015) The effects of peppermint, parsley, dill, coriander, garlic and basil on broiler chickens and reported that serum cholesterol of plant-groups showed significant differences compared to the control group, but triglycerides, HDL and LDL were none of the significant differences.

Pish Jang (2011) reported the use of different coriander levels has significant effects on total cholesterol concentrations in broiler chickens. As well as Pish Jang, it was reported that using coriander blood triglycerides significantly reduced the broiler chickens compared to the control group.

In a study Dhanapakiam *et al*. (2008) reported that a decrease in LDL in broilers fed with coriander, while the HDL level was increased.

Table 7 Effect of sage powder on immunity titers in broiler at 42 days

Experimental groups	Avian influenza	Newcastle
Control	1.15[b]	2.75[c]
Sage powder 0.2%	1[b]	3.50[c]
Sage powder 0.5%	1.50[a]	5.50[b]
Sage powder 1.2%	1.25[ab]	6.50[a]
P-value	0.003	0.026
SEM	0.06	0.15

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Biochemical parameters (mg/dL) of broilers fed different levels of sage powder at 42 days

Experimental groups	HDL	LDL	Triglyceride	Cholesterol
Control	83.75[b]	75.75[a]	132.25[a]	153.75[a]
Sage powder 0.2%	89[a]	39.25[c]	91.75[c]	127.50[d]
Sage powder 0.5%	88[a]	47[b]	94.50[b]	135.50[c]
Sage powder 1.2%	87.50[a]	48.75[b]	92.50[c]	141.65[b]
P-value	0.001	0.013	0.028	0.006
SEM	0.002	6.17	9.30	12.78

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 9 Effect of sage powder on morphology of ileum at 42 days

Experimental groups	Villi height (micrometer)	Crypt depth (micrometer)	Villi height to crypt depth	Goblet cells (millimeter)
Control	320[b]	60[a]	5.33[b]	8[a]
Sage powder 0.2%	382[a]	50[b]	7.64[a]	3[c]
Sage powder 0.5%	376[a]	65[a]	5.78[b]	4[bc]
Sage powder 1.2%	373[a]	52[b]	7.17[a]	5[b]
P-value	0.0325	0.0023	0.0231	0.0256
SEM	54.23	7.61	1.17	0.96

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Warshafsky *et al*. (1993) reported that the total concentration of LDL, triglyceride and cholesterol significantly decreased, however there was no significant increase in HDL concentration.

Ileal morphology

In birds fed sage powder, crypt depth was reduced and villus height and villi to crypt ratio were increased (P≤0.001) compared with the basal diet. Also, addition of sage powder reduced the numbers of goblet cell significantly (P≤0.001). As an important finding of this experiment, sage powder, improved the ileum structure as indicated by an increase in villi length and crypt depth.

Baurhoo *et al*. (2007) reported that an increase in villi length has been associated with increased lactobacilli and bifidobacteria colonization of broiler intestines. Several studies have proven the antimicrobial effects of sage powder (Hayouni *et al*. 2008). The essential oil and ethanolic extract of sage powder show strong bactericidal and bacteriostatic effects against both gram-positive and gram-negative bacteria an due to these effect it can promote the health of intestine through increased in villi growth.

Nutrients and edible additives are used to change the structure of small intestine. The length and depth of the crypt are the criteria for detecting the health of the digestive system that is directly related to the adsorption process of mucosa membranes. The greater the height of the villus, the more absorption capacity caused (Pelicano *et al*. 2007). The higher villus prevents the faster transit of food, reduce the moisture of the material and improve the conversion coefficient. The ratio of the length of villus to crypt depth can indicate the capacity of intestinal digestion. So that whenever this ratio increases, digestion and absorption will also increase (Montagne, 2003).

Demir *et al*. (2003) reported that in broiler chickens that had used garlic and thyme, the depth of crypt was reduced in the ileum. In contrast to our results, inclusion of essential oils in the diet of broilers increased goblet cell numbers reported by Garcia *et al*. (2007) and Reisinger *et al*. (2011).

Garcia *et al*. (2007) reported that using medicinal plants in the diet of broiler increases the height of villi, so it seems that the cause of the reduction of harmful bacteria in the intestinal wall, which reduces the production of toxic and less damage to the intestinal epithelial cells.

Mirzavand *et al.* (2015) the effects of using peppermint, dill, coriander, and garlic on the intestinal morphology of broiler chickens were investigated and reported that the height of the intestinal villus in none of the intestinal parts was not significant and the ratio of the height of the intestinal villus and the ratio of villus to its width was significantly.

CONCLUSION

Based on the results of present study was determined that supplementation of 0.2-0.5% sage powder had meaningful impact on broiler performance, ileal morphology and vaccine titer against Newcastle disease and avian influenza virus.

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