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Full Length Research Paper

Efficiency of different levels of *Satureja hortensis L*. (Savory) in comparison with an antibiotic growth promoter

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This study was conducted to examine the effects of different levels of *Satureja hortensis L*. (Savory) in comparison with an antibiotic growth promoter (flavophospholipol) on performance, carcass characteristics, immune responses and serum biochemical parameters of broiler chicks. In this study, 240 one-day-old mixed sex broiler chicks (Ross 308) were weighed and randomly allocated to the 4 treatment groups, each with 4 replicates and with 15 broilers in each replicate. The dietary treatments consisted of the basal diet (control), antibiotic group receiving 4.5 mg/kg flavophospholipol, and 5 and 10 g/kg savory powder added to the basal diet. Performance parameters were measured in the growth periods. At day 42, two birds per replicate were slaughtered for the determination of carcass traits. Antibody titers against newcastle, influenza viruses and sheep red blood cell (SRBC) were determined. At day 42, biochemical parameters such as albumin, protein, triglyceride, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) of cholesterol were determined. The results show that supplementing 5 g/kg savory improved body weight of broilers at days 14, 28 and 42 but differences did not show statistical significance. Feed intake and feed conversion ratio (FCR) index were not markedly affected by dietary treatments. Internal organ weights and carcass characteristics were not significantly influenced by the dietary treatments at day 42. The use of 5 g/kg savory powder led to the highest antibody titers against SRBC as compared to other groups (P<0.05). The serum biochemical parameters were not affected by dietary treatments. The results suggest that dietary inclusion of 5 g/kg savory can be applied as alternatives to in-feed antibiotics for broiler diets.

Key words: Broiler, growth performance, immunity, *Satureja hortensis* L., biochemical parameters.

INTRODUCTION

In this century, antibiotics growth promoters as feed additives have been banned in many countries.

Antibiotics have been used in animal feed for improving increasing some useful microbial populations such as lactobacillus in intestinal microflora but European Union has banned the use of most antibiotic growth promoters as feed additives because of the secondary effects like resistance and antibiotic residual in animal tissues, therefore alternatives were needed (Nasir and Grashorn, 2006). Phytogenic feed additives have received increa-

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sing attention as possible antibiotics alternatives. The plants have been used traditionally in the therapy of some diseases for a long time in the world, and they have a significant role in maintaining human health. Also, they have been used in research of broiler nutrition to see their effects on their performance and other indices such as immunity and serum biochemical profiles (Toghyani et al., 2011).

Satureja hortensis L. is an annual, herbaceous aromatic and medicinal plant belonging to the Lamiaceae family. It is known as summer savory, native to southern Europe and naturalized in parts of North America (Sefidkon et al., 2006). Also, it is widely distributed in different of the twelve classified *Satureja* species. Its essential oil contains considerable amounts of two phenolic ketones (carvacrol and thymol) (Ghannadi, 2002). With regards to having anti-inflammatory (Hajhashemi et al., 2002), antioxidant (Güllüce et al., 2003), antibacterial (Güllüce et al., 2003; ßahin et al., 2003) and antifungal activities (Güllüce et al., 2003; Boyraz and Ozcan, 2006), it has received major consideration. It is traditionally used in foods for herbal tea and flavor component and in folk and traditional medicine, to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases (Zargari, 1990; Güllüce et al., 2003). Furthermore, Zamani Moghaddam et al. (2007) reported the beneficial effects of savory in poultry production. This study was conducted to evaluate the potential of applying different levels of savory in comparison with an antibiotic growth promoter on performance, some immune responses and biochemical parameters in broiler chicks when used as supplements in the diet.

MATERIALS AND METHODS

Animals and dietary treatments

A total of 240 day-old broiler chicks (Ross 308), were purchased from a local hatchery. On arrival, birds were weighed and randomly allocated to one of the four treatments with four replicates of 15 birds based on a completely randomized design. The 4 treatments were as follows: the basal diet as control, the antibiotic group received 4.5 mg/kg flavophospholipol, *S. hortensis L.* powder at levels of 5 and 10 g/kg respectively as natural growth promoter. Fresh aerial parts of *S. hortensis* were commercially purchased and identified from Center for Advance Studies in Botany, Isfahan University (Herbarium voucher number-17167). Fresh aerial parts of *S. hortensis L*. were sun-shade dried and then ground to obtain powder. Table 1 lists the basal diet formulated to meet or exceed the nutrient requirements of broilers provided by Ross Broiler Manual (2002). Chicks were raised on floor pens (120 × 120 × 80 cm) for 6 weeks and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness. The ambient temperature was gradually decreased from 33 to 25°C on day 21 and was then kept constant.

Performance parameters

The body weight was determined at 14, 28 and 42 days of age. Feed consumption and weight gain were recorded at different periods and feed conversion ratio (FCR) was calculated. Mortality was recorded as it occurred.

Carcass components

At 42 days of age, two birds from each replicate was randomly chosen based on the average weight of the group and sacrificed. Carcass yield was calculated by dividing eviscerated weight by live weight. Abdominal fat, liver, pancreas, gizzard, heart, small intestine and cecum were separated, weighed and calculated as a percentage of live body weight.

Immunity parameters

The birds were vaccinated against newcastle disease (lasota strain) at 8 and 22 days of age and avian influenza (H_9N_2) at 8 days of age, respectively. At 30 days of age, two birds per replicate were randomly chosen and blood samples were collected from brachial vein and centrifuged to obtain serum. Antibody titers against newcastle and influenza viruses were measured using hemagglutination inhibition test (Cunningham, 1971). At day 25 of age, two birds were randomly selected from each replicate, and were inoculated via the brachial vein with 1 ml of 1% sheep red blood cell (SRBC) suspension. At day 6 after inoculation, blood samples were obtained from the brachial vein and SRBC antibody titers were measured by the microtiter procedure of Wegmann and Smithies (1966). Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination.

Serum biochemical parameters

After 12 h fasting, blood samples were collected in non-heparinised tube at day 42 of age from 8 birds in each treatment by puncturing the brachial vein and the blood was centrifuged to obtain serum. Individual serum samples were analyzed for albumin, total protein, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) of cholesterol, and triglyceride using the kit package (Pars Azmoon Company; Tehran, Iran).

Statistical analysis

All data were subjected to ANOVA using the General Linear Models procedure of SAS software (SAS Inst. Inc., Cary, NC). The mean differences among different treatments were separated by Duncan's multiple range tests. A level of $P<0.05$ was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

Immune responses

The results for serum antibody titers against Newcastle and influenza viruses at day 30 and SRBC at day 31 in broilers are presented in Table 2. Treatments failed to induce any marked effect on antibody titers against Newcastle and influenza viruses though it tended to increase in broilers fed on 5 g/kg savory diets ($P>0.05$). The highest antibody titers against SRBC were obtained in broilers fed diet containing 5 g/kg savory. The SRBC antibody titers obtained in birds fed diet containing 5 g/kg

Table 1. The ingredient and chemical composition of basal starter, grower and finisher diets.

Ingredient (g/kg)	Starter (1 - 14 days)	Grower (14 - 28 days)	Finisher (28 - 42 days)
Corn	537.3	533	561.5
Soybean meal	400	396	370
Soybean oil	20	35	35
DCP	19.3	17.1	15.6
$Caco_3$	10.5	8.7	8.5
NaCl	3.5	3	3
Mineral-premix[1]	2.5	2.5	2.5
Vitamin-premix[2]	2.5	2.5	2.5
DL-methionine	3.1	2	1.4
L-lysine	1.3	-	-
Calculated composition			
M.energy (kcal/kg)	2870	2980	3000
Crude protein (g/kg)	221	220	210
Calcium (g/kg)	8.6	7.5	7
Av.phosphorus (g/kg)	4.9	4.4	4.1
Met.+cysteine (g/kg)	10.1	8.9	8
Lysine (g/kg)	13.3	11.9	11.3
Tryptophan (g/kg)	3.2	3.2	3
Sodium (g/kg)	1.5	1.3	1.3

[1]Provided the following per kg of diet: Vit A 10,000 IU; vitamin D3 2000 IU; vitamin E 5 IU; vitamin K 2 mg; riboflavin 4.20 mg; vitamin B12 0.01mg; pantothenic acid 5 mg; nicotinic acid 20 mg; folic acid, 0.5 mg. [2]Provided the following per kg of diet: Choline 3 mg; Mg 56 mg; Fe 20 mg; Cu 10 mg; Zn 50 mg; Co 125 mg; Iodine 0.8 mg.

Table 2. Effect of experimental diets on antibody titers against Newcastle and influenza viruses at day 30 and SRBS at day 31.

Variable	Dietary treatment				
	Control	Antibiotic	5 g/kg Savory	10 g/kg Savory	SEM
New castle (log_2)	2.50	1.87	2.62	2.00	0.26
Influenza (log_2)	3.37	2.87	3.75	3.50	0.35
SRBC (log_2)	6.25^{abc}	4.87^{c}	8.87^{a}	6.87^{ab}	0.56

Values in the same row not sharing a common superscript differ significantly (P<0.05). SEM = Standard error of mean. SRBC, Sheep red blood cell.

diet savory was higher followed by birds fed antibiotic diet (P<0.05).

Use of antibiotic had no statistical effect on antibody titer against Newcastle and influenza viruses, but it tended to decrease in broilers fed diets containing antibiotic. The lowest antibody titers against SRBC were obtained in broilers fed diets containing 4.5 mg/kg flavophospholipol. The SRBC antibody titers obtained in birds fed diet containing 4.5 mg/kg flavophospholipol was lower than those fed diets containing 5 or 10 g savory powder/kg (P<0.05). Dafwang et al. (1985) reported that broilers fed diet supplemented with oxytetracycline, lincomycin, penicillin, bambermycins or tylan had little response to SRBC antigen. Also, Landy at al. (2011b) reported no effect of dietary flavophospholipol on the antibody titers against Newcastle, influenza viruses and SRBC in broiler chicks. Therefore, the influence of enteric conditioners and their effects on gut microflora could be mainly limited to the mucosal immune complement and not the systemic portion of the immune system. Although, the contribution of gut microflora to the development and physiological status of the humoral and cellular mucosal immune system is well understood (Cebra, 1999), the effects on the systemic immune complement may be less dominant.

The savory has been reported to have antibacterial and antioxidant activities (Güllüce et al., 2003; ßahin et al., 2003). Its essential oil contains two phenolic ketones (carvacrol and thymol) (Ghannadi, 2002). The major components of savory essential oil, thymol and carvacrol

Table 3. Effect of experimental diets on performance of broilers at different ages.

Performance	Dietary treatment				
parameter	Control	Antibiotic	5 g/kg Savory	10 g/kg Savory	SEM
Body weight (g)					
14 days	243.75	241.00	251.50	235.75	7.61
28 days	860.00	876.75	891.25	831.00	23.60
42 days	2008.00	1987.75	2041.25	1962.50	56.85
Daily feed intake (g/day)					
0-14 days	26.25	26.50	27.10	25.12	0.78
14-28 days	44.55	47.52	50.27	47.57	1.83
28-42 days	157.30	159.45	165.53	160.18	7.24
0-42 days	66.42	65.45	68.55	66.62	2.93
Feed : gain (g:g)					
0-14 days	1.67	1.71	1.65	1.67	0.05
14-28 days	1.01	1.04	1.10	1.11	0.03
28-42 days	1.91	1.98	1.89	1.98	0.12
0-42 days	1.36	1.36	1.38	1.39	0.075

SEM = Standard error of mean.

have been indicated to possess potent antioxidant properties, and an increase in immune responses of chicks was anticipated. Also, high levels of vitamins especially vitamin A and E in the savory plays a positive role in the antibody production, improve serum antibody titers and phagocytic activity of immune cells of medicated broilers (Elwinger et al., 1998; Tampieri et al., 2005). Zamani Moghaddam et al. (2007) observed immune stimulating effects in broilers by application of savory; this is in agreement with our findings.

Performance and carcass traits

Data on performance parameters are summarized in Table 3. Broilers receiving 5 g/kg savory had higher body weights as compared to other treatments at days 14, 28 and 42 but not significantly. Treatments failed to induce any marked effect on daily feed intake though, it tended to increase in birds fed on 5 g/kg savory diets (P>0.05). The FCR index of broilers was not affected by dietary treatments.

Performance of animals is influenced mainly by the health and immune status, a stressed or weak immune system with a load of infectious causes low weight gain, on the other hand, an enhanced immune system allows maximum performance. Therefore, the application of immune stimulating substances to increase the immune status can result in improved growth performance (Iren, 2000). The improvement in body weight of broilers in group fed 5 g/kg diet savory may be due to optimum immune status of *S. hortensis L*. which led to better weight gain. Other trial with immune stimulating substances application of inactivated poxvirus to calves resulted in better performance of the treated animals (Hanschkf, 1997); this is contrary to our results.

Other factors which could have contributed to the beneficial effects of the herbal products on the growth performance of broilers are their probable antioxidant and antibacterial effects in the intestine (Nascimento et al., 2000). The savory can prevent the diet from oxidation and adverse effects of mycotoxins by antioxidant activity of effective ingredients such as carvacrol and thymol. Also, Skandamis and Nychas (2001) suggested that herbal products alter the permeability of the cell membranes and cause a destruction of the pathogenic bacteria. In this trial, use of 5 g/kg savory led to the highest body weight, but body weight of broilers decreased by use of higher dosage of the savory in feed. Zamani Moghaddam et al. (2007) reported that use of 3 g/kg savory had a beneficial effect on growth performance in broiler chicks, and higher dosage had an adverse effect on performance. Similarly, Landy et al. (2011a) reported that a higher dosage of *Echinacea purpurea L*. in the diet may have had an adverse effect on some beneficial microbial populations such as lactobacillus, preventing the herb from exhibiting its positive influence on performance of broilers.

Table 4 shows relative weight means (as a percentage of live weight at slaughter) of digestive and non-digestive organs as a function of treatments. The carcass traits evaluated including abdominal fat, liver, pancreas, gizzard, heart, proventriculus, small intestine and cecum weights were not markedly affected by dietary treatments. These results are consistent with those observed by Hernandez and Madrid (2004) who did not find any

Table 4. Effect of experimental diets on carcass characteristics and organ weight of broilers at 42 days.

Carcass trait	Dietary treatment				
	Control	Antibiotic	5 g/kg Savory	10 g/kg Savory	SEM
Carcass*	70.62	69.92	71.35	69.40	0.90
Abdominal fat*	1.53	1.62	1.35	1.53	0.16
Liver*	2.18	2.40	2.32	2.16	0.10
Gizzard*	2.22	2.32	2.34	2.21	0.14
Heart*	0.56	0.57	0.63	0.57	0.03
Proventriculus*	0.45	0.53	0.46	0.47	0.03
Small intestine*	3.6	3.8	3.8	3.8	0.23
Pancreas*	0.25	0.24	0.26	0.23	0.018
Cecum*	0.63	0.58	0.64	0.65	0.042

*Percentage of live body weight; SEM = standard error of mean.

Table 5. Effect of experimental diets on serum biochemical parameters of broilers at day 42.

Biochemical parameter	Dietary treatment				
	Control	Antibiotic	5 g/kg Savory	10 g/kg Savory	SEM
Protein*	3.03	3.2	3.2	3.1	0.74
Albumin*	0.87	0.91	0.91	1.07	0.104
Total cholesterol*	158	149	157	152	8.72
LDL-cholesterol*	51.5	61.75	54.25	51.37	4.97
HDL-cholesterol*	56.62	57.37	54.87	61.62	4.62
Triglyceride*	75.88	81.71	68.86	81.57	6.90

* (mg/100 ml). SEM = Standard error of mean.

difference among the control treatment and those containing antibiotic or mixtures of plant extracts for organ weight of 42-day-old broilers. Also, in other trials, use of savory in feed failed to have any statistical effect on relative weight of carcass, breast and thigh muscles (Zamani Moghaddam et al., 2007).

Serum biochemical parameters

Table 5 summarizes data obtained on the effect of experimental treatments on serum biochemical parameters. No significant influence of experimental diets on protein, albumin, triglyceride, total, LDL and HDL of cholesterol was observed (P>0.05). Serum biochemistry is a labile biochemical system which can reflect the condition of the organism and the changes occurring in it under the influence of internal and external factors (Toghyani et al., 2010). Mchedlishvili et al. (2005) reported that flavonoids isolated from *S. hortensis L*. had cholesterol-lowering effect in a situation of rising serum cholesterol. Unfortunately, no more reports are available on the effects of savory on broiler serum biochemical parameters, and further studies are necessary.

In conclusion, results of this study show that dietary inclusion of 5 g/kg savory can be applied as alternatives to in-feed antibiotics for broiler diets and that it is suitable to replace an antibiotic feed additive.

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Abbreviations

SRBC, Sheep red blood cell; **FCR,** feed conversion ratio.

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