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The Effect of Powder and Essential Oil of Savory Medicinal Plant (*Satureja hortensis*) on Performance and Antioxidant Status of Broiler Chicks under Heat Stress

Research Article

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ABSTRACT

This study was conducted to investigate the effect of different levels of savory (*Satureja hortensis*) powder and its essential oil on performance and antioxidant status of broiler chickens under heat stress (34±2 ˚C for 8 hours per day). A total of 336 one-day-old male broiler chicks (Ross-308) were divided into 6 treatments and 4 replications (14 birds per replicate). Experimental diets were a corn-soybean based diet with no supplement (control), diet containing 200-ppm vitamin E as positive control, diets containing 1 and 2% of savory powder and diets containing 100 and 200 ppm of savory essential oil. Antioxidant status was measured by analysis of malondialdehyde (MDA), glutathione peroxidase (GPX) and super oxide dismutase (SOD) activities in liver tissue. Results indicated that body weight, weight gain, feed intake and feed conversion ratio were not significantly affected by the experimental treatments. Experimental diets except diet containing 1% savory powder, decreased (P<0.05) MDA concentrations and increased SOD activities (P<0.05) in liver tissue as compared to the control diet. However, there were no significant differences in liver GPX activities between treatments. The results of this study concluded that, supplementation of diet with savory powder and its essential oil improved antioxidant status in broiler chickens reared under heat stress and it can be considered as a replacement for synthetic antioxidant in poultry diet in heat stress conditions.

KEY WORDS antioxidant status, broiler, heat stress, performance, savory.

INTRODUCTION

The wide use of antibiotics and other chemical compounds have been experienced throughout the last 50 years which research have been directed back to natural antimicrobial products as indispensable resources (Ferrini *et al.* 2008). Consequently, there is considerable research interest in the possible use of natural products, such as essential oils and extracts of edible and medicinal plants, herbs and spices, for the development of new additives in animal feeding. Aromatic plants like Sea-buckthorn contain flavonoids described previously as stimulators of the immune response, these dietary flavones having an effect against microbial infection (Grabley and Thierick, 1999; Kamel, 2001) showed that the essential oil extracted from Satureja hortensis reversed oxidative damage to rat lymphocytes induced by hydrogen peroxide.

Recently, aromatic plants, and their associated essential oils or extracts are used as potentially growth promoters. At present, the scientists are working to improve feed efficiency and growth rate of livestock using useful herbs (Bunyapraphatsara, 2007). Some plants were found to have natural effects, for example, tonics, antiparasitic, antibacterial, stimulant, carminative, antifungal, anti-microbial and antiseptic (El Emary, 1993; Soliman *et al.* 1995). Researches on the use of herbal mixtures in poultry diets have produced positive effect on performance of broiler chicks. In an experiment, broilers fed with 0.5% of peppermint, grow faster performed better than those fed with 1.5% peppermint. In other research, using 0.5% of *Mentha pulegium* aerial parts powder significantly improved the performance and carcass traits and reduced the blood glucose of broilers (Nobakht, 2011).

The stress responses in poultry are excitated mainly by the activation of hypothalamic pituitary adrenal axis and of the orthosympathic nervous system. Moreover, heat stress caused a series of physiological and metabolic changes in broiler chickens such as elevated body temperature, panting and respiratory alkalosis (Deyhim and Teeter, 1991), that declines the performance and immune system of chicks. Some research reported that, supplementation of essential oils from medicinal plants improves the immune-defense in poultry (Lavinia *et al.* 2009).

Savory herb contains a number of compounds that exert varying biological activities, including antioxidant (Suhaj, 2006), and various pharmacological effects (Nobakht, 2011). Although there was some research about of savory and its effects on performance and immune system on broilers (Nobakht, 2011); however, there are no reported studies on the effect of savory powder and its essential oils on growth performance and antioxidant indices in broiler chickens reared under heat stress. Therefore, in the present study, our aim was to investigate the effect of different levels of savory (*Satureja hortensis*) powder and its essential oil on performance and antioxidant status in broiler chicks under heat stress.

MATERIALS AND METHODS

Experimental design and husbandry

The experiment was performed in the department of animal science, Astara branch, Islamic Azad University, Iran. A total of 336 one-day-old male Ross 308 broiler chicken were used.

Feed and water were offered *ad libitum* throughout the experimental period. Dried aerial parts of medicinal plants were obtained from local market. After fine milling, savory mixed with other ingredients.

Chicks from 1 to 42 days of age were used in a completely randomized design. There were 6 treatments included a control diet with no medicinal plant supplementation, diet containing 200 mg/kg vitamin E as positive control and diets containing 1 and 2% savory powder or 100 and 200 mg/kg savory essential oil (Table 1). For preparation diets of essential oil, first essential oil was mixed with vegetable oil and subsequently mixed with the other ingridients of diet. Therefore, the six diets were fed to four groups of fourteen male chicks from 1 to 10, 11 to 24 and 25 to 42 days post hatching.

Table 1 The ingredients and nutrients composition of starter diets of broilers

Ingredients (%)	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Yellow corn	56.12	58.89	62.61
Soybean meal (44% CP)	37.9	33.80	30.04
Vegetable oil	1.23	2.83	3.25
L-lysine	0.30	0.33	0.19
DL-methionine	0.18	0.23	0.17
Dicalcium phosphate	2.13	1.88	1.74
Oyster shell	1.28	1.17	1.13
Common salt	0.36	0.37	0.37
Vitamin premix [1]	0.25	0.25	0.25
Mineral premix [2]	0.25	0.25	0.25
Analyzed composition			
AMEn (kcal/kg)	2845	2990	3060
Crude protein (%)	22.00	20.50	19.00
Lysine (%)	1.38	1.30	1.10
Methionine (%)	0.55	0.58	0.50
Methionine + cystine (%)	0.92	0.92	0.82
Calcium (%)	1.00	0.9	0.85
Available phosphorous (%)	0.50	0.45	0.42
Sodium (%)	0.16	0.16	0.16
DCAD	222	201	192

[1] Provided per kg of diet: vitamin A: 9000 IU; vitamin D: 2000 IU; vitamin E: 18 IU; vitamin K_3: 3 mg; vitamin B_1: 1.78 mg; vitamin B_2: 6.6 mg; vitamin B_6: 3 mg; vitamin B_{12}: 0.015 mg; Niacin: 30 mg; Pantothenic acid: 10 mg; Biotin: 0.15 mg and Choline: 1500 mg.

[2] Provided per kg of diet: Cu: 10 mg; I: 0.99 mg; Fe: 50 mg; Mn: 100 mg; Se: 0.08 mg; and Zn: 100 mg.

DCAD: dietary cation anion differences and AME_n: apparent metabolizable energy corrected for nitrogen.

The diets were formulated to meet the requirements of broilers as established by Ross 308 guideline (Ross 308, 2007). The lighting program during the experimental period consisted of a period of 23 h light and 1 h of darkness. Environmental temperature after 14 d was gradually decreased from 33 to 25 ˚C and from 14 to 42 d chickens kept under heat stress condition at 34 ± 2 ˚C for 8 hours in day.

Performance parameters

Body weight (BW), feed intake (FI) and mortality were measured and body weight gain (BWG), and feed conversion ratio (FCR) were calculated based on hen day for each period.

Sample analysis

The activity of liver glutathione peroxidase (GPX, EC 1.11.1.9) was determined using the method of Paglia and Valentine (1967) with a Ransel kit (Randox, UK). Tissue samples of liver for malondialdehyde (MDA) determination were homogenized with deionizer distilled water and 50 Ml of butylated hydroxytoluene. The MDA concentrations in homogenates were measured by the modified fluorimetric method in accordance with Jo and Ahn (1998). In addition, superoxide dismutase activities (SOD, EC 1.15.1.1) in the liver were analyzed using kits from Randox, UK (Arthur and Boyne 1985). The protein concentrations in the tissues examined were measured by the spectrophotometric method of Bradford (1976).

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design by using the general linear model procedures of SAS (2004). Statistical significance of differences among treatments was assessed using the Duncan's test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Growth performance

The effects of experimental treatments during heat stress on growth performance of broilers are shown in Table 2 and 3. There were no significant differences among treatments for body weight, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR). Growth performance was not significantly affected by savory powder and its essential oil, but there was a tendency of broilers consuming a savory-supplemented diet to grow faster during the overall phase as compared with broilers fed the control diet.

In contrast, there was significant difference in comparison of essential oil *vs*. control diet (P=0.037) and powder *vs*. control diet (P=0.045) for body weight gain and feed conversion ratio.

In other orthogonal comparisons, there was no significant difference. These results agree with the findings of Zhang *et al.* (2009), who studied the influence of ginger powder usage on production performance, carcass traits and reported no significant differences in body weight gain, cumulative feed consumption and cumulative feed conversion ratio, between treatments. Similarly, Botsoglou *et al.* (2002) observed that supplementation of broiler feed with oregano essential oil for thirty-eight days had no growth-promoting effects.

Antioxidant status

Supplementation of savory powder and essential oil significantly (P<0.05) increased activities of SOD in liver, but GPX activity was not affected by experimental treatments.

Table 2 Effect of savory powder and essential oil on performance of broiler chicks under heat stress

Treatments	Average body weight (g)			Average body gain (g)			
	10 d	24 d	42 d	1-10 d	11-24 d	25-42 d	1-42 d
Control	250.34	851.21	2223.97	208.93	602.86	1370.76	2182.55
200 ppm vitamin E	244.50	873.33	2274.31	202.98	628.83	1400.98	2232.79
1% of savory powder	247.67	860.83	2262.98	206.24	613.16	1402.15	2221.55
2% of savory powder	244.47	858.60	2239.01	203.17	614.13	1380.41	2197.71
100 ppm of savory essential oil	254.23	895.38	2280.78	212.77	641.15	1385.40	2239.32
200 ppm of savory essential oil	249.59	873.98	2318.48	208.24	624.38	1444.50	2277.13
SEM	1.488	5.471	16.958	1.487	4.693	16.203	16.941
Probability	0.407	0.267	0.711	0.409	0.223	0.861	0.710
Independent comparisons	Probability						
powder *vs*. control	NS	NS	NS	NS	NS	NS	NS
Essential oil *vs*. control	NS	NS	NS	NS	0.037	NS	NS
powder vs. vitamin E	NS	NS	NS	NS	NS	NS	NS
essential oil *vs*. vitamin E	NS	NS	NS	NS	NS	NS	NS
powder *vs*. essential	NS	NS	NS	NS	NS	NS	NS

NS: non significant and SEM: standard error of mean.

Table 3 Effect of savory powder and essential oil on average feed intake and feed conversion ratio of broiler chicks under heat stress

Treatments	Average feed intake (gr)				Feed conversion ratio			
	1-10 d	11-24 d	25-42 d	1-42 d	1-10 d	11-24 d	25-42 d	1-42 d
Control	275.65	956.26	2571.59	3803.50	1.32	1.59	1.88	1.74
200 ppm vitamin E	273.64	953.46	2647.34	3874.44	1.35	1.51	1.89	1.73
1% of savory powder	277.62	942.75	2572.93	3793.30	1.34	1.54	1.84	1.71
2% of savory powder	266.18	929.42	2571.40	3767.00	1.31	1.51	1.87	1.71
100 ppm of savory essential oil	274.59	963.22	2579.10	3816.92	1.29	1.50	1.87	1.70
200 ppm of savory essential oil	268.23	989.99	2605.73	3863.95	1.29	1.58	1.81	1.70
SEM	2.809	9.443	21.081	24.482	0.012	0.011	0.023	0.014
Probability	0.867	0.604	0.910	0.826	0.680	0.067	0.926	0.938
Independent comparisons	Probability							
powder *vs*. control	NS	NS	NS	NS	NS	0.045	NS	NS
essential oil *vs*. control	NS	NS	NS	NS	NS	NS	NS	NS
powder *vs*. vitamin E	NS	NS	NS	NS	NS	NS	NS	NS
essential oil *vs*. vitamin E	NS	NS	NS	NS	NS	NS	NS	NS
powder *vs*. essential	NS	NS	NS	NS	NS	NS	NS	NS

NS: non significant and SEM: standard error of mean.

Dietary treatments, except diet containing 1% savory powder significantly (P<0.05) increased SOD activity in liver tissue than control diet. Among the savory-supplemented groups, liver of broilers diet containing 200 ppm savory essential oil had the highest SOD enzyme activity. In addition, the MDA concentration of liver significantly decreased (P<0.05) by all of treatments except diet containing 1% savory powder (Table 4).

In contrast, comparisons diets containing savory powder *vs*. control diet and essential oil *vs*. control diet significantly improved SOD activity and decreased MDA concentration. Comparing essential oil *to* vitamin E, the treatments containing essential oil significantly decreased (P=0.009) MDA concentrations compared with diet containing vitamin E. There was not significant difference in residual contrast comparisons.

Alizadeh *et al.* (2010) observed that supplementation of savory significantly improved antioxidant status. In addition, Faix *et al.* (2009) indicated that addition of cinnamon essential oil to broiler diets, significantly increased SOD and GPX activity than untreated group in red blood cells broilers. Although reduced MDA concentration in the liver could partially be attributed to the increased antioxidant enzymatic activity, the reason why savory powder and its essential increased these antioxidant enzymatic activities remains unknown. A body of literature has shown that plant polyphenolic flavonoids were one of the major groups of compounds acting as primary antioxidant free-radical terminators (Huang and Frankel, 1997; Singh *et al.* 2005). The potential active constituents in savory are the thymol, carvacrol and some related phenolic ketone derivatives (Suhaj, 2006).

Table 4 Effect of savory powder and essential oil on antioxidant status of broiler chicks under heat stress

Treatments	Superoxide dismutase (SOD) unit/mg protein	Glutathione peroxidase (GPX) unit/mg protein	Malondialdehyde (MDA) nmol/mg protein
Control	3.56[c]	0.48	5.22[a]
200 ppm vitamin E	4.20[ab]	0.49	4.07[b]
1% of savory powder	3.85[bc]	0.52	4.69[ab]
2% of savory powder	4.21[ab]	0.51	2.87[c]
100 ppm of savory essential oil	4.20[ab]	0.52	3.16[c]
200 ppm of savory essential oil	4.60[a]	0.52	2.93[c]
SEM	0.093	0.009	0.214
Probability	0.011	0.878	< 0.0001
Independent comparisons		Probability	
Powder *vs*. control	0.045	NS	0.0007
Essential oil *vs*. control	0.001	NS	< 0.0001
Powder *vs*. vitamin E	NS	NS	NS
Essential oil *vs*. vitamin E	NS	NS	0.009
Powder *vs*. essential	0.049	NS	0.019

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

The higher SOD activity in chickens fed diets containing powder and essential oil of savory compared with that chicks fed control diet indicated that savory powder and its essential oil enhanced antioxidant enzymatic activity in the liver. On the other hand, the antioxidant defenses include natural and synthetic antioxidants and the antioxidant enzymes are present in the biological system (Sies, 1991). Free radicals are produced during normal metabolism but can in turn induce body damage if they are present in excessive levels. It has been generally recognized that SOD, GPX are main antioxidant enzymes in scavenging the oxygen free radical (McCord, 1979). Increasing activities of SOD would subsequently enhance the capacity of broilers to clear out the oxygen free radicals. Consistent with the increased activity of liver SOD, the MDA concentration in the liver was reduced by inclusion of savory powder and its essential oil in broiler diets. Malondialdehyde is formed as an end product of lipid peroxidation and therefore the extent of lipid peroxidation by reactive oxygen species can be monitored by MDA levels (Sumida *et al.* 1989). Hence, the reduced liver MDA in chickens fed savory diets as compared with control diet indicated that lipid peroxidation was reduced by savory different forms (powder and essential oil) via enhancing antioxidative action. All together, these results demonstrated that savory powder and its essential oil supplemented at the used level improved antioxidant status of broiler chickens.

CONCLUSION

It is concluded that dietary supplemental savory powder (1 and 2%) and its essential oil (100 and 200 ppm) significantly improved antioxidant status of broiler chicks. Therefore, the powder and essential oil of savory (*Satureja hortensis*) could be considered as a replacement for synthetic antioxidant in broiler diet under heat stress conditions.

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