

The Effects of Savory (*Satureja khuzistanica*) Extract on Performance, Organ Weight, Blood Parameters and Immune Function in Heat Stressed Broilers

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Abstract

An experiment was conducted to study the effect of savory (*Satureja khuzistanica*) extract on the performance, organ weight, immune response and hepatic enzymes in broiler chickens. 320 day-old Ross chickens were assigned to four distinct treatments in a completely randomized design. Each treatment was administered to four replicates of twenty birds. The variables were heat stress (34 ± 2 °C for 8 hours) and savory extract (0.4 ml/L) in drinking water. Feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were measured in successive weeks of the trial. The relative weights of different organs (dressing, breast, thigh, liver, heart, spleen and bursa of Fabricius) determined at 42 days. The serum glucose and blood plasma content of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured by blood sampling at 42 days. Plasma IgG were quantified on days 21, 28, 35 and 42. The savory extract did not affect FCR, or the relative weights of different organs ($P>0.05$). BW and FI increased with savory oil inclusion ($P<0.05$). Further, the savory extract reduced plasma glucose, AST and ALT in heat stressed broilers significantly ($P<0.05$). ALP content also declined, but not significantly ($P>0.05$). Blood IgG in heat stressed broilers, increased in every case of treatment with savory extract ($P<0.05$). In conclusion, in conditions of heat stress, 0.4 ml/L of savory extract improves economic proficiency in broiler flocks due to the accumulation of minute advantages in increased WG, FI, improved IG and reduced hepatic enzymes.

Keywords: heat stressed broilers, hepatic enzymes, immune function, performance, savory extract

Introduction

Heat stress (HS) is one of the most important negative factors in poultry growth and immune function in tropical countries, as well as in the warm seasons in temperate countries that can be very costly. The main problem caused by HS in young birds is depressed weight gain, occurring mainly due to reduced feed intake (FI) and elevated energy use in reducing body temperature (May and Lott 1992; Belay and Teeter 1993). Nutritional manipulation may be the cheapest way to negate the harmful effects of heat stress.

The general health and performance of broilers exposed to heat stress responded positively to diets supplemented by antibiotics (Männer and Wang, 1991; Çabuk et al., 2006). In poultry feed, the extracts and essential oils of some herbs have also attracted considerable interest as unique feed additive alternatives to AGP (Suderman and Solikhah 2011; Zeinali et al. 2011).

Savory (*Satureja khuzistanica* Jamzad) is a plant identified for its therapeutic effects in traditional medicine (Abdollahi et al., 2003). The upper (aerial) parts of the savory plant collectively include up to 3% of an essential oil spectacularly rich in carvacrol (Khosravinia, 2016). Carvacrol is a phenolic, bitter-tasting and caustic component with good stability demonstrating antioxidant and antimicrobial properties (Khosravinia, 2016). Correspondingly, it has been reported, mainly in experiments conducted under standard directorial practice and normal environmental situations, that Savory essential oils have antioxidant and antibacterial effects (Abdollahi *et al.*, 2003; Radonic & Milos 2003; Azaz *et al.*, 2002). Furthermore, some reports have indicated that savory extract is beneficial in heat stress situations and also helpful in overcoming the harmful effects of this stressor (Khosravinia, 2016). Therefore, the purpose of this experiment was to evaluate the effects of savory extract in drinking water on the performance, organ weight, immune response and hepatic enzymes in broilers under heat stress.

Materials and Methods

Experimental design

The experiment was conducted in the Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Iran. A total of 320, one-day-old, male, Ross 308 broiler chickens were used. During the experiment, feed and water was accessible *ad libitum*. The savory extract was obtained from a local company. Chicks from 21 to 42 days old (800 ± 100 gr) were used in completely randomized fashion. The 4

treatments included a control treatment with no heat stress (Control+); a control treatment with heat stress (Control-), in which the temperature was about 34 ± 2 °C for 8 hours per day; a treatment containing 0.4 ml savory extract mixed with drinking water with no heat stress (Control+ + 0.4 ml savory extract); and a treatment containing 0.4 ml savory extract mixed with drinking water with heat stress (Control- + 0.4 ml savory extract). Each treatment was administered to four replicates of twenty birds. The broilers' diet (Table 1) was formulated in consideration of Ross 308 catalogue guidelines (Ross 308, 2007). During the experimental period, there were periods of 23 hours of light and 1 hour of darkness.

Table 1. The nutrient composition and ingredients of the experimental diets

Ingredients (%)	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Corn	56.12	58.89	62.61
Soybean meal (44%)	37.9	33.80	30.04
Soy oil	1.23	2.83	3.25
L-lysine	0.30	0.33	0.19
DL-methionine	0.18	0.23	0.17
Dical phosphate	2.13	1.88	1.74
Oyster shell	1.28	1.17	1.13
Salt	0.36	0.37	0.37
Vit premix ¹	0.25	0.25	0.25
Min premix ²	0.25	0.25	0.25
Composition analysis			
AMEn ³ (kcal/kg)	2845	2990	3060
CP (%)	22.00	20.50	19.00
Lysine (%)	1.38	1.30	1.10
Methionine (%)	0.55	0.58	0.50
Methionine + cysteine (%)	0.92	0.92	0.82
Calcium (%)	1.00	0.9	0.85
Available phosphorus (%)	0.50	0.45	0.42
Sodium (%)	0.16	0.16	0.16
DCAD ⁴	222	201	192

¹ Provided per kg of diet: vitamin A: 9000 IU; vitamin D: 2000 IU; vitamin E: 18 IU; vitamin K : 3 mg; vitamin B : 1.78 mg; vitamin B : 6.6 mg; vitamin B 3 1 2 6 : 3 mg; vitamin B12 : 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. ³ AMEn : apparent metabolizable energy corrected for nitrogen. ⁴DCAD: dietary cation anion difference.

Performance parameters

Feed intake (FI) and Body weight (BW), were determined and body weight gain (BWG), and feed conversion ratio (FCR) were computed based on hen day per period.

Organ weights

At 42 days old, two birds from each pen were caught, weighed and killed by decapitation to obtain the Dressing and relative weights of organs. The organs consisted of breast, thigh, heart, liver, spleen and bursa Fabricius (percentage of live body weight).

Biochemical analysis

Blood samples were collected on day 42, in sterile vacutainers (heparinised) for analysis of blood glucose, ALP, ALT, and AST. Blood samples were centrifuged at $1,500\times g$ for 10 minutes, within 30 minutes of blood collection. Plasma was harvested and stored at $-20^{\circ}C$ until analysis. All biochemical analyses were assayed on an automated biochemical analyzer.

IgG assay

At days 28, 35 and 42, two chicks from each pen were chosen at random, and blood samples were taken from a wing vein. Before harvesting the serum, blood samples were allowed to coagulate at $4^{\circ}C$, and then centrifuged at $3,000 \times g$ for 10 minutes at $4^{\circ}C$. All serum samples were stored at $-20^{\circ}C$ until they were analyzed. Serum concentrations of IgG were determined by a sandwich ELISA set, using chicken-specific IgG ELISA quantitation kits and microtiter plates (Jiancheng Biological Engineering Research Institute, Nanjing, China, Cat. No. H106). The ELISA procedure was carried out in consideration of the producer's protocol, and absorbance was determined at 450 nm.

Statistical analysis

The results obtained were subjected to variance analysis procedures suitable for a completely randomized design via the general linear model procedures of SAS (2004). Duncan's multiple-range test was applied to determine the statistical significance of differences between treatments.

Results

Growth performance

The results of growth performance by broiler chickens during the experiment are displayed in Tables 2 and 3. The results available indicate no significant difference ($P > 0.05$) for BWG and FCR between treatments. However, there were significant differences between control+ vs. control- diet ($P=0.009$) and 0.4 ml savory extract vs. control+ diet ($P=0.101$) for BWG.

Also, there was significant difference in feed intake values between different treatments ($P < 0.05$). As shown in table 3, the feed intakes by broilers for 3 weeks and the whole period of experiment (21 to 42 days) differ significantly ($P < 0.05$) and the control+ group (basal diet with no heat stress) had the highest feed intake.

Table 2. The effect of different treatments on weight gain (WG) by experimental broilers at different weeks during the experiment (gr).

Treatments	21-28 d	29-35	36-42	21-42 d
Control+ ¹	396.25	439.5	458.00	1293.75 ^a
Control- ¹	359.00	404.5	375.50	1139.25 ^b
Control+ + 0.4 ml savory extract	396.00	459.0	420.25	1275.25 ^a
Control- + 0.4 ml savory extract	389.00	433.5	410.75	1233.25 ^{ab}
SEM	3.36	4.9	12.32	16.52
Probability	0.34	0.27	0.95	0.22
Independent comparisons	Probability			
Control+ vs control-	Ns	Ns	Ns	0.009
Control+ vs savory	Ns	Ns	Ns	0.101
Savory vs control-	Ns	Ns	Ns	Ns

¹Control+ = birds grown in normal temperatures throughout the trial (21-42 days), and Control- = birds grown under heat stress throughout the trial (21-42 days); the means within the same column with different letters, have significant differences ($P < 0.05$). SEM: standard error of the mean.

Table 3. Table 2. Effect of different treatments on feed intake (FI) and feed conversion ratio (FCR) by experimental broilers at different weeks during the experiment.

Treatments	Feed intake (gr)				Feed conversion ratio			
	21-28 d	29-35 d	36-42 d	21-42 d	21-28 d	29-35 d	36-42 d	21-42 d
Control+ ¹	723.00 ^a	847.25 ^a	1101.5 ^a	2671.75 ^a	1.82	1.93	2.42	2.05
Control- ¹	698.00 ^b	815.50 ^b	1016.5 ^b	2530.00 ^b	1.95	2.01	2.71	2.22
Control+ + 0.4 ml savory extract	712.25 ^{ab}	847.00 ^a	1098.75 ^a	2658.00 ^a	1.81	1.87	2.63	2.10
Control+ + 0.4 ml savory extract	714.00 ^{ab}	829.75 ^{ab}	1072.0 ^{ab}	2615.75 ^a	1.84	1.93	2.66	2.14
SEM	4.15	6.53	15.39	21.05	0.03	0.04	0.08	0.03
Probability	0.63	0.4	0.19	0.07	0.39	0.36	0.60	0.78
Independent comparisons	Probability							
Control+ vs control-	Ns	0.008	0.01	0.003	Ns	Ns	Ns	Ns
Control+ vs savory	Ns	0.37	0.15	0.02	Ns	Ns	Ns	Ns
Savory vs control-	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

¹Control+ = birds grown in normal temperatures throughout the trial (21-42 days), and Control- = birds grown under heat stress throughout the trial (21-42 days); the means within the same column with different letters, have significant differences (P<0.05). SEM: standard error of the mean.

Organ weights

No significant differences in relative weights were indicated for dressing, breast, thigh, liver, heart, bursa of Fabricius and spleen in experimental broilers at 42 days old (P>0.05; Table 4). Adding 0.4 ml savory extract gave an increase in relative weight of thigh and breast at 42 days old, as compared with other treatments, but not significantly so (P>0.05). Further, heart and liver weights increased in birds that received treated water, but the differences were not significant (P>0.05). Overall, the use of savory extract for 21 days had a positive effect on lymphoid organs (bursa of Fabricius and spleen), although the differences were not significant.

Table 4. Effect of different treatments on relative weights of dressing, breast, thigh, liver, heart, spleen and bursa of Fabricius at 42 days old. (% body weight).

Treatments	Dressing	Breast	Thigh	Liver	Heart	Spleen	Bursa
Control+ ¹	65.46	25.16	20.92	2.33	0.46	0.11	0.17
Control- ¹	64.59	24.76	19.33	2.37	0.44	0.13	0.18
Control+ + 0.4 ml savory extract	64.59	26.07	19.42	2.41	0.43	0.13	0.20
Control- + 0.4 ml savory extract	63.57	31.62	24.79	2.95	0.58	0.14	0.16
SEM	0.08	1.68	1.5	0.15	0.03	0.01	0.01
Probability	0.61	0.27	0.58	0.26	0.47	0.46	0.95
Independent comparisons	Probability						
Control+ vs control-	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Control+ vs savory	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Savory vs control-	Ns	Ns	Ns	Ns	Ns	Ns	Ns

¹Control+ = birds grown in normal temperatures throughout the trial (21-42 d), and Control- = birds grown under heat stress throughout the trial (21-42 d); the means within the same column with different letters, have significant differences ($P < 0.05$). SEM: standard error of the mean.

Biochemical results

The effects of different treatments on blood plasma glucose content and liver function, which were monitored by determining the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are shown in table 5. The results obtained indicate that the glucose, AST and ALT contents of blood plasma in broilers in group 4 (Control- + 0.4 ml savory extract) reduced significantly compared with the control- group ($P < 0.05$). Additionally, there was no significant difference between the different treatments in the ALP content of blood plasma in the experimental broilers ($P > 0.05$).

Table 5. The effects of different treatments on blood plasma activity in the experimental broilers for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and blood plasma glucose content at 42 days old.

Treatments	Glucose	AST (U/L)	ALT (U/L)	ALP (U/L)
Control+ ¹	188.5 ^b	234.25 ^b	13.00 ^a	1400.50
Control- ¹	264.75 ^a	273.75 ^a	20.00 ^c	1871.75
Control+ + 0.4 ml savory extract	189.00 ^b	227.25 ^b	13.75 ^{bc}	1672.75
Control- + 0.4 ml savory extract	232.5 ^{ab}	228.75 ^b	16.25 ^b	1580.50
SEM	10.64	6.12	0.83	78.11
Probability	0.3	0.007	0.14	0.94
Independent comparisons	Probability			
Control+ vs control-	0.001	0.02	0.0004	Ns
Control+ vs savory	0.29	0.03	0.03	Ns
Savory vs control-	Ns	0.017	Ns	Ns

¹Control+ = birds grown in normal temperatures throughout the trial (21-42 days), and Control- = birds grown under heat stress throughout the trial (21-42 days); the means within the same column with different letters, have significant differences (P<0.05). SEM: standard error of the mean.

IgG

IgG measurements are shown in Table 6. As is clear, the IgG contents of chickens' blood were significantly affected by different treatments in different weeks (P<0.05). IgG rose with the inclusion of savory extract in broilers' drinking water, and in the 42-day treatment 3, (Control+ + 0.4 ml savory extract), had the highest IgG content (P<0.05). Also, at 42 day the IgG extent of treatment 4 (Control- + 0.4 ml savory extract) improved significantly in compare with control- group (P<0.05).

Table 6. Table 4. Effect of different treatments on IgG of experimental broilers at 42 day of age. (mg/ml).

Treatments	21d	28d	35d	42 d
Control+ ¹	2.35	1.93 ^{ab}	1.81 ^b	2.03 ^a
Control- ¹	1.79	1.38 ^c	1.55 ^c	1.57 ^c
Control+ + 0.4 ml savory extract	2.46	2.17 ^a	1.98 ^a	2.20 ^a

Control- + 0.4 ml savory extract	1.91	1.65 ^{bc}	1.90 ^{ab}	1.82 ^b
SEM	0.11	0.09	0.05	0.07
Probability	0.15	0.04	0.0003	0.005
Independent comparisons	Probability			
Control+ vs control-	2.40 ^a	2.05 ^a	Ns	2.12 ^a
Control+ vs savory	1.85 ^b	1.52 ^b	Ns	1.70 ^b
Savory vs control-	Ns	1.91 ^a	1.94 ^a	2.01 ^a

¹Control+ = birds grown in normal temperature throughout the trial (21-42 days), and Control- = the birds grown under heat stress throughout the trial (21-42 days); the means within the same column with different letters, have significant differences ($P < 0.05$). SEM: standard error of the mean.

Discussion

In the current study, savory extract exhibited hopeful effects on WG in broiler chickens from 21–42 days old when the birds suffered extreme heat stress. The savory was identified as a natural product rich in essential oils and carvacrol; practically all its attributes are characterised by the carvacrol (Khosravinia, 2016). It has been shown that supplementing drinking water with 200, 300, 400 or 500 mg/L of savory essential oils over 1–28 days, may effectively influence and increase weight gain from 29–42 days old (Khosravinia *et al.*, 2013). Our findings are also consistent with the results in Lee *et al.* (2003) which found a 2% improvement in average daily weight gain in broiler chickens with the inclusion of 0.2 g/kg carvacrol in the diet. Although, some reports recorded no positive effects from savory on weight gain in broiler chickens (Khosravinia, 2016). Nevertheless, the differences in observations may be due to the physiological status of broilers; thus, in the current study chicks were raised in conditions of heat stress. The improved FI in treated chickens in this study, corresponded with results observed by Basmacioglu *et al.* (2004), who found that an addition of 0.2 g/kg carvacrol and 0.15 g/kg oregano extract produced +2% and –6% difference in the FI of treated birds, compared with control groups. The results for FI in this study were not in line with the findings of Lee *et al.* (2003) or (Khosravinia, 2016). Furthermore, the improvement in FI in this study may be due to the phytogetic properties of savory; there are suggestions that a dietary inclusion of phytogetic foodstuffs may improve digestion processes in avian species (Mellor, 2000). The data on FCR in this study differed from the results of almost all other reports. In this study, Savory-treated water caused no change in FCR. These findings disagree to some extent with

reports in Basmacioglu *et al.* (2004) and Lee *et al.* (2003), who observed a decline in FCR in chickens treated with carvacrol.

Furthermore, the use of savory oil up to 21 days had a positive effect on organs, although this influence was not significant. Mohammad *et al.* (2013) reported that the inclusion of savory essential oil for 21 days (days 21-42) in the diet of heat stressed broilers had no significant effect on the lymphoid organs (spleen, bursa of Fabricius and thymus,) and weight, and our observations confirm that. Besides, in some studies conducted in fields, an influence by medicinal plants on increases in the relative weights of different organs has been reported (Schuberth *et al.*, 2002; Rivera *et al.*, 2003). Rahimi *et al.* (2011) reported that thyme extract (0.1%), dissolved in drinking water, affected the relative weight of the bursa of fabricius in broilers significantly. Moreover, broilers treated with dietary polysavone (alfalfa extract) saw increases in the relative weights of the thymus, bursa and spleen (Dong *et al.*, 2007).

In the current study, blood glucose in experimental birds was influenced by savory essence and decreased significantly; while these observations are dissimilar to results obtained by Ghazi *et al.* (2015) and Saadat *et al.* (2004), who reported no effect by savory on blood glucose in broilers. A disturbance of glucose metabolism in the liver was suggested to be a result of an anti-diabetic effect of savory oil, which might be related to savory's antioxidative property (Souri, 2015). Thus, any medication to alter hepatic gluconeogenesis or glycogenolysis might affect glucose homeostasis (Souri, 2015). Further, reductions in fasting blood glucose and triglyceride were reported when savory essential oil was given to diabetic and hyperlipidemic rats (Abdollahi *et al.*, 2003).

The present results showed statistically significant reductions in activity by aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following the addition of savory. Alkaline phosphatase (ALP) also declined in activity with the addition of savory to drinking water, albeit not significantly ($P > 0.05$). It is clear that the highly active AST, ALP and ALT in the blood are bioindicators of liver damage (Rosa *et al.*, 2001; Safameher *et al.*, 2008; Mohamed & Mohamed, 2009; Valchev *et al.*, 2014) so a reduction of these hepatic enzymes by adding savory, indicated a positive effect by savory essence on reparation of the liver.

The IgG measurements from experimental broilers revealed a significant improvement in IgG content in the blood of those treated with savory. Observations in Souri *et al.* (2015) confirm our findings. It is vital for the poultry industry to improve immunity and avoid infectious diseases. A range of factors including vaccination failure, infection by immunosuppressive diseases and abuse

of antibiotics can induce immunodeficiency. The use of immunity stimulants is one way of increasing immunity in animals and reducing their vulnerability to infectious disease (Chen *et al.*, 2003). Plants with a high content of flavonoids, such as *T. vulgaris*, expand the activity of vitamin C, act as antioxidants and may therefore enhance functions of immunity (Cook & Samman, 1996; Manach *et al.*, 1996). The addition of 0.3% savory to the broilers' diet elevated chickens' Newcastle disease titers because the high volumes of vitamin A and vitamin E in the herb assist antibody production, improving serum antibody levels and the phagocytic activity of immune cells (Tampieri *et al.*, 2005). Flavonoids and polyphenolic complexes exhibit several pharmacological properties, including antioxidant activity, the inhibition of histamine release from mast cells and of arachidonic acid metabolism (Amresh *et al.*, 2007). Essential oil extracted from savory reversed oxidative damage to rat lymphocytes induced by hydrogen peroxide (Hajhashemi *et al.*, 2011).

Conclusion

In conclusion, the present study has shown that the inclusion of savory extract at 4 ml/L in drinking water to broilers under heat stress may improve economic efficiency in broiler flocks. Advantageous effects: greater WG and FI, improved IG over 21–42 days of age, as well as reduced hepatic enzymes, are due to the accumulation of minute quantities of savory extract.

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