Article

Effects of savory aqueous extract on performance, carcass traits, some blood biochemical and immune parameters of broiler chickens under heat stress condition

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Abstract

An experiment was done to study effects of savory aqueous extract on performance, carcass traits, some blood biochemical and immune parameters of broiler chickens under heat stress condition. To do this 320 day-old Ross chickens were assigned to four distinct treatments in a complete randomized design. Each treatment was given to four replicates of twenty birds. Variables were heat stress (34 ± 2˚C for 8 hours) and savory extract (0.4 ml/L) in drinking water. At different weeks of trial, feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) was measured. Some relative weight of different organs (dressing, breast, thigh, liver, heart, spleen and bursa of Fabricus) determined at d 42 of age. The blood serum glucose and plasma content of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured after blood sampling at d 42 of age. 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 ($p<0.05$). Besides, the savory extract decreased the plasma glucose, AST and ALT of heat stressed broilers significantly ($p<0.05$). Also the ALP content decreased but not significantly ($p>0.05$). Totally, blood IgG of heat stressed broilers, increased with savory extract treating ($p<0.05$). In conclusion, under heat stress situations, 0.4 ml/L of savory extract improves economic proficiency in broiler flocks due to accumulation of minute advantages in greater WG, FI, and improved IG and lowered hepatic enzymes.

Keywords heat stress; hepatic enzymes; immune function; performance; savory; aqueous extract; broiler chickens.

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1 Introduction

Heat stress condition is one of the most important negative prepossessing of poultry growth factors and immune function in tropical countries, also at the warm seasons in clement countries that could be so much

costly. The main concern of heat stress in chicks is depressed weight gain occurring mainly due to reduced feed intake (FI) and elevated energy spending to decrease body temperature (May and Loot, 1992; Belay and Teteer, 1993). The nutritional manipulations could be the cheapest way to outcome the harmful effects of heat stress. The general healthiness condition and performance of broilers exposed to heat-stressed environmental situations positively responded to diets supplemented with antibiotics (Cubak et al., 2006; Maner and Wang, 1991). Extracts and essential oils of some herbs received considerable interest in poultry feeding as a unique feed additive alternative to AGP (Suderman and Solikhah, 2001; Zienali et al., 2011). Savory (*Satureja khuzistanica* Jamzad L.) has therapeutic effects in traditional medicine (Abdollahi et al., 2003). The aerial parts of savory plant concertedly include up to 3% of an essential oil spectacularly rich in carvacrol that it is a phenolic, bitter-tasting and caustic component with good stability demonstrating antioxidant (Mellor, 2000) and antimicrobial properties (Schuberth et al., 2002). It has been reported that savory essential oils have antioxidant and antibacterial effects (Mohammad et al.,2013) mainly in experiments directed under standard directorial practices and normal environmental situations (Azzaz et al., 2002; Radonic et al., 2003). Furthermore, there is some reports indicated that savory extract is beneficial in heat stress condition and helpful to overcome the harmful effects of mentioned stressor (Khosravinia et al., 2016). Therefore, the purpose of this experiment was to evaluate study effects of savory aqueous extract on performance, carcass traits, some blood biochemical and immune parameters of broiler chickens under heat stress condition.

2 Materials and Methods

The experiment was administered in the Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Iran. A total of 320 one-day-old male Ross 308 broiler chicken were used. At the period of experiment, accessibility to feed and water was *ad libitum*. Savory aqueous extract was obtained from local company. Experimental chicks from 21 to 42 days of age (800 ± 100 gr) were used in a completely randomized design. There were 4 treatments included a control treatment with no heat stress (control+), control treatment with heat stress (control-) and the temperature was about 34 ± 2˚C for 8 hours in day, treatment containing 0.4 ml savory aqueous extract mixed with drinking water with no heat stress ($Control^{+}$ + 0.4 ml savory aqueous extract), and treatment containing 0.4 ml savory aqueous extract mixed with drinking water with heat stress ($Control^{-}$ + 0.4 ml savory aqueous extract). Each treatment was given to four replicates of twenty chicks. The broilers diet (Table 1) was formulated in consider with Ross 308 catalog guidelines. During the experimental period, the lighting method involved of a period of 23 h light and 1 h of darkness.

Table 1 The nutrient composition and ingredients of 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
Calculated compositions content			
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 phosphorous (%)	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. [4] DCAD: dietary cation anion differences.

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 for each period.

At 42 day of age, two birds from each pen were catch, weighed and killed by decapitation to obtain the dressing and organs relative weights. The organs were consisted of breast, Thigh, heart, liver and spleen and bursa fabricius as a percentage of live body weight.

Blood samples were collected on d 42, in sterile containers and heparinized for analysis of blood glucose, ALP, ALT, and AST. Within 30 min right after blood collection, blood samples were centrifuged at 1,500×g for 10 min. Plasma was harvested and stored at –20 $^{\circ}C$ until analysis. All biochemical analyses were assayed on an automated biochemical analyzer.

At d 28, 35 and 42, two chicks from each pen were chosen by random, and blood samples were catch from the wing vein. Earlier to harvesting the serum, blood samples were be allowed to clog at 4°C, and then centrifuged at 3,000 × g just for 10 min at 4°C. The all serum samples were stored up at −20°C up to they were analyzed. Serum concentrations of IgG were determined by a sandwich ELISA set, using chicken-specific IgG ELISA quantization kits and micro-titer plates (Jiancheng Biological Engineering Research Institute, Nanjing, China, Cat. No. H106). The ELISA procedure was done considering with the protocol of the producer, and absorbance was determined at 450 nm. The obtained results were subjected to variance procedures analysis, suitable for a completely randomized design via the general linear model procedures of SAS (2004). The

Multi-domain Duncan's (1995) test was applied to determine the statistical significance of differences between treatments.

3 Results

3.1 Growth performance

The results of growth performance of broiler chickens during the experiment are displayed in Table 2 and 3.

According to available results there was no significant difference ($p>0.05$) for BWG and FCR among treatments. However, there was significant difference in comparison of control+ *vs*. control- diet ($p=0.009$) and 0.4 ml savory extract vs. control+ diet ($p=0.101$) for BWG.

Table 2 Effect of different treatments on weight gain (WG) of experimental broilers at different weeks of experiment (g).

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

[1]Control+ = the birds grown in normal temperature throughout the trial (21-42 d), and Control- = the birds grown in heat stress situation 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 means.

Also there was a significant difference for feed intake values among different treatments ($p<0.05$). As it shown in table 3 the feed intakes of broilers for 3 weeks and the whole experiment period (21 to 42 days) are different significantly ($p<0.05$) and the control+ group (basal diet with no heat stress) had the uppermost feed intake.

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

	Feed intake (gr)				Feed conversion ratio			
Treatments	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]	1101.50[a]	2671.75[a]	1.82	1.93	2.42	2.05
Control-[1]	698.00[b]	815.50[b]	1016.50[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]	1072.00[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

[1]Control+ = the birds grown in normal temperature throughout the trial (21-42 d), and Control- = the birds grown in heat stress situation 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 means.

3.2 Organs weight

No significant differences were pointed out for relative weight of dressing, breast, thigh, liver, heart, bursa of Fabricus and spleen of experimental broilers at d 42 of age ($p>0.05$; Table 4). Adding of 0.4 ml savory extract showed an increase on relative weight of thigh and breast in d 42 of age as compared to other treatments, but not significant ($p>0.05$). Also, heart and liver weights improved for the birds that received treated water but differences were not significant ($p>0.05$). Overall use of savory extract for 21 days had a positive effect on lymphoid organs (bursa of fabricius and spleen), although the difference was not significant.

Table 4 Effect of different treatments on relative weight of dressing, breast, thigh, liver, heart, spleen and bursa of Fabricus at 42 days of age (% 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

[1]Control+ = the birds grown in normal temperature throughout the trial (21-42 d), and Control- = the birds grown in heat stress situation 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 means.

3.3 Blood biochemical

The Effects of different treatments on blood plasma glucose content and liver function that monitored by determining the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) showed in table 5. The obtained results indicated that the glucose, AST and ALT content of blood plasma of broilers in group 4 (Control^{-} + 0.4 ml savory extract) reduced significantly in compare with control- group ($p<0.05$). Also there was no significant difference between different treatments in ALP content of blood plasma of experimental broilers ($p>0.05$).

Table 5 Effects of different treatments on experimental broilers blood plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and blood plasma glucose content on 42 day of age.

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

[1]Control+ = the birds grown in normal temperature throughout the trial (21-42 d), and Control- = the birds grown in heat stress situation 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 means.

Table 6 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]

[1]Control+ = the birds grown in normal temperature throughout the trial (21-42 d), and Control- = the birds grown in heat stress situation 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 means.

3.4 IgG

IgG measurements are shown in Table 6. As it clear, the IgG content of chickens blood were significantly affected by different treatments on different weeks ($p<0.05$). IgG were rise with including savory aqueous extract to drinking water of broilers and at 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 ($p<0.05$).

4 Discussion

In the current study, savory extract exhibited hopeful effects on WG of the broiler chicken in days 21–42 of age when birds suffered from extreme heat stress. The savory was specified as a natural product rich in essential oils and carvacrol component so that practically all its characteristics could be attributed by carvacrol features (Movahhedkhah et al., 2019). It has been shown that supplementation of drinking water with 200, 300, 400 or 500 mg/L savory essential oils during 1–28 days could effectively influence and compensate their weight gain through 29–42 days of age (Khosravinia et al., 2013; Basmacioglu et al., 2004). Also, our findings are consistent with results of Lee et al., who stated 2% improvement in average daily gain of broiler chicken by inclusion of 0.2 g/kg carvacrol in the diet (Mozafari et al., 2018; Lee et al., 2003). Although, some reports showed no positive effects of savory on weight gain of broiler chickens (Rivera and Hu, 2003; Rahimi et al., 2013). Nevertheless, the differences in observations may be endorsed by physiological status of the broilers where in the current study chicks were raised in heat stress conditions. The improved FI for the treated chickens in this study, was in agreement with the results observed by Basmacioglu et al., who find out that addition of 0.2 g/kg carvacrol and 0.15 g/kg oregano extract produced +2% and −6% difference in FI of treated birds in compare with the control groups (Basmacioglu et al., 2004). The results on FI in this study were not in harmony with the findings of Lee et al. (2003) and Abdollahi et al. (2003). Furthermore, the improvement in FI in this study may be due to the phytogenic properties of savory, witch there are suggestions that dietary inclusion of phytogenic foodstuffs may improve digestion processes in avian species (Mellor, 2000). The data on FCR in this study were disagreed with results of almost all other reports. In this study, Savory-treated water caused no change in FCR. These findings are to some extent in disagreement with the reports of (Lee et al., 2003; Mohammad et al., 2013) who found declined FCR for the chickens treated with carvacrol.

Furthermore, use of savory oil to 21 days had a positive effect on organs, although this influence was not significant. Other researchers reported that, inclusion of savory essential oil for 21 days (21-42 days) in diet of heat stressed broilers had no significant effect on lymphoid organs (spleen, bursa of fabricius and thymus,) weight and our observations confirm it (Mohammad et al., 2013). Besides, in some studies performed in animal fields, the influence of medical plants on improvement of relative weights of different organs had been reported (Rivera and Hu, 2003; Schuberth et al., 2002). It was reported by (Rahimi et al., 2011) that the thyme extracts (0.1%), solved in drinking water, affect relative weight of bursa of fabric us of broilers significantly. Moreover, the broilers treated with dietary polysavone in Alfalfa aqueous extract improved the relative thymus, bursa and spleen weights (Dong et al., 2007).

In the current study, blood glucose of experimental birds influenced by savory essence and decreased significantly, while, these observations dissimilar to (Ghazi et al., 2015 and Saadat et al., 2004) results, who reported no effect of savory on blood glucose of broilers. Disturbance of glucose metabolism in liver was suggested as a mechanism of the anti-diabetic action of savory oil, which might be related to the anti-oxidative influence of savory (Souri et al., 2015; Yeganeparast et al., 2019). Therefore any medicine to change hepatic gluconeogenesis or glycogenolysis might affect glucose homeostasis (Rosa et al., 2011; Mohamed et al., 2007).

Besides, reductions in fasting blood glucose and triglyceride were reported when savory essential oil was given to diabetic and hyperlipidemia rats (Abdollahi et al., 2003).

The present results showed statistically significant demotion in activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with savory inclusion. Also the activity of alkaline phosphatase (ALP) was decline with savory addition to drinking water but not significantly ($p>0.05$). As it cleared, the high activities of AST, ALP and ALT in blood are bio-indicators of liver damage (Safameher, 2008; Mohamed and Mohamed, 2009) so the reduction of these hepatic enzymes with savory addition, indicated the positive effects of savory essence on reparation of liver (Chen et al., 2003).

The IgG measurements of experimental broilers showed a significant improvement in IgG content in blood of savory treated broilers. Some researcher observations confirm our findings (Valchev et al., 2014). A range of factors such as vaccination failure, infection by immunosuppressive diseases, and abuse of antibiotics can induce immunodeficiency. Immune stimulants usage is one of the solutions to increase the immunity of animals and to reduce their weakness to infectious disease (Chen et al., 2003). Plants with much flavonoid such as *T. vulgaris* spread out the activity of vitamin C, perform as antioxidants and may therefore enhance immune function (Cook and Samman, 1996; Manach et al., 1996). The addition of 0.3% savory to broilers 'diet elevated chickens' Newcastle disease titers because high volumes of vitamin A and vitamin E in this herb play a helpful role in antibody production, improving serum antibody levels and the phagocytic activity of immune cells (Tampieri et al.,2005). The flavonoids and polyphenolic complexes exhibit several pharmacological influences, including antioxidant activity, inhibition of histamine release from mast cells and inhibition 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).

5 Conclusions

In conclusion, the result of current study showed that inclusion of savory aqueous extract at 4 ml/L through drinking water to broilers under heat stress maybe improves economic proficiency in broiler flocks. Some advantageous properties are due to accumulation of minute advantages of savory extract in greater WG and FI and also improved IG over days 21–42 of age and lowered hepatic enzymes.

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