

Full Length Research Paper

Effect of supplementation of *Satureja* essential oils in drinking water on immune performance of broiler chickens reared under heat stress

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Phytogenic feed additives, also called as phytobiotics or botanicals, are plant-derived compounds added into diets to enhance the productivity of poultry. This study was conducted to investigate the effect of administration of *Satureja khuzestanica* essential oils (SKEO) through drinking water on immune performance of broiler chicken using 720 one-day-old Arian chicks. The birds were raised under standard production practices up to 42 days of age. The birds continuously received drinking water treated with Tween 80 (Cont+; 500 ppm) or SKEO at 0 (Cont-), 200, 300, 400 and 500 ppm. The mean hemagglutinin inhibition (HI) antibody titer was not significantly different among the treatments at 15, 21, 28 and 35 days of age ($P>0.05$). No significant differences were pointed out for relative weight of spleen, bursa and thymus at 21 days. Thymus weight reduced for the birds that received treated water but a difference was only significant for 400 ppm SKEO. The mean heterophyle lymphocyte ratio was not significantly differing among the treatments. It is concluded that supplementation of broiler chicken drinking water with *S. khuzestanica* essential oils has no favorite impact on immune response of heat stressed broiler chicken.

Key words: *Satureja khuzestanica*, immune system, broiler chicken.

INTRODUCTION

Feed additives including antibiotics have been widely used in poultry industry for several decades. Manipulations of gut function and microbial habitants of domestic animal with feed additives have been recognized as an important tool to improve growth performance and feed efficiency (Collington et al., 1990). The ban of antibiotics use as feed additives has led to investigations for alternatives to antibiotics. Herbal extracts have already been used as feed supplements to improve growth performance under intensive management systems (William and Losa, 2001). In recent years, epidemic infectious diseases have been important challenges throughout the world and were the cause of substantial financial failure for many poultry producers. Such a situation becomes

more crucial for poultry producers when we realize that factors such as vaccination failure, infections by immune suppressive diseases and the abuse of antibiotics have led to immunodeficiency in poultry. Utilization of immune stimulants is one solution which improves the immune system of animals and decreases their susceptibility to infectious disease (Liu, 1999). In some studies, medical plants efficiency on broiler immune system has been reported (Windisch et al., 2008). Dietary inclusion of polysavone (alfalfa extract) in the diets of broiler chickens improved the relative weights of thymus, bursa and spleen and increased the proliferation of T and B lymphocytes when compared with the control group (Dong et al., 2007). Moreover, inclusion of polysavone increased

serum antibody titer of Newcastle disease virus (Dong et al., 2007). Khaligh et al. (2011) indicated that addition of 10 g/kg blend of alfalfa, liquorice root, great burdock, cinnamon to broiler diets resulted in an improvement in antibody titer against Newcastle disease virus when compared with the control.

Satureja khuzistanica Jamzad is a plant which belongs to the family of *Lamiaceae* and is widely distributed in the southern part of Iran. In Iran, it has been used in ethno-medicine as an analgesic and antiseptic agent (Abdollahi et al., 2003). The essential oils of *S. khuzistanica* Jamzad is mainly consisted of carvacrol (80.6%), p-cymene (4.8%), myrcene (1.5%), γ -terpinene (2.1%) and terpinene-4-ol (2.1%) (Farsam et al., 2003). Extract and essential oil of this plant as well as carvacrol, the main constituent of its essential oil, have shown propitious antimicrobial, analgesic and antioxidant activity (Abdollahi et al., 2003; Radonic et al., 2003). The aim of this study was to evaluate the effect of *S. khuzestanica* essential oils on immune system of broiler chickens reared under heat stress.

MATERIALS AND METHODS

A total of 720 day-old mixed sex broiler chicks (Arian) were weighed and based on completely randomized design assigned to 6 treatment groups with 6 replicate and 20 bird (5 male and 5 female) per each. Chopped barley stalks top dressed with 2 cm wood shavings were used as bedding material. Corn and soybean meal based super starter (24.28% CP and 2962 Kcal ME/kg, 1 to 7 day), starter (21.15% CP and 2880 Kcal ME/kg, 7 to 21 d), grower (18.82% CP and 2952 Kcal ME/kg, 22 to 35 d) and finisher (17.63% CP and 2993 Kcal ME/kg, 36 to 42 d) diets and water were provided for *ad libitum* consumption throughout the experimental period. The shed was equipped with wet pad-and-fan cooling system to decline the ambient temperature. Nonetheless, average temperature during day and night hours ranged from 32 to 35 and 28 to 30°C during 21 to 42 days. Therefore, from 21 days of age the birds were exposed to seasonal extreme ambient temperatures. The effect of six experimental treatments consisted of supplementation of drinking water with 0 (control-), 200, 300, 400 and 500 ppm SkEO or 500 ppm Polysorbate- 80 (control+) were examined in 6 replicates of 20 birds each. Polysorbate-80 is an emulsifier which was used to disperse SkEO in water at 1:1 ratio (v/v). All treatments (drinking water) were prepared daily. Newcastle vaccination against Newcastle virus was done on the 15th and 28th days (as eye drop), day of the experimental period. At 21 and 42 days of age, two birds per pen (a male and a female) were selected, weighed and killed by decapitation to obtain the immune organs relative weights such as spleen, thymus and bursa Fabricius (percentage of live body weight). At 15, 21, 28 and 42 days of age, blood samples were collected in anticoagulant tubes (citrate sodium 3.6% solution) during a forty minute period. After centrifugation (5000 rpm) for 7 min, blood serum was separated and then Newcastle virus antibody titers were measured by using the hemagglutinin inhibition (HI) method.

Statistical analysis

The statistical model used to analyze the collected data was: $Y_{ijk} = \mu + SkEO_i + S_j + B_k + \epsilon_{ijk}$,

Where Y_{ijk} is the dependent variable, μ is the general mean, SkEO_i is the fixed effect of SkEO (i = 6; control + and 0, 200, 300, 400, 500 ppm SkEO), S_j is the fixed effect of sex (j = 2), B_k is the random effect of block (j = 6; 1, 2, 3, 4, 5 and 6) and ϵ_{ijk} is the residual

Table 1. The effect of different level of essential oils of *Satureja khuzistanica* on antibody titers against Newcastle.

Treatment	15 day	21 day	28 day	35 day
CON ⁻¹	5.833 ^a	2.500 ^a	1.000 ^a	6.333 ^a
CON ⁺¹	5.667 ^a	2.667 ^a	0.667 ^a	5.833 ^{ab}
200	5.500 ^a	2.167 ^a	0.833 ^a	4.500 ^{ab}
300	6.167 ^a	2.833 ^a	1.000 ^a	5.667 ^{ab}
400	6.167 ^a	2.833 ^a	1.000 ^a	5.833 ^{ab}
500	5.667 ^a	3.000 ^a	1.333 ^a	5.333 ^b
SEM ²	0.152	0.169	0.171	0.237
P value	0.7391	0.7863	0.9309	0.3446

¹Control⁻¹: The birds received drinking water with no additive and Control⁺¹: The birds received drinking water supplemented with 500 ppm polysorbate-80 throughout the trial. ²Standard error for overall mean. ^{a-b}Means within a column without a common superscript differ significantly (p<0.05).

error. For the variables evaluated at 21 days of age, sex was omitted from the model. The data were analyzed using PROC MIXED of SAS 9.1 (2002). The LSD test was used for multiple treatment comparisons using the LSMEANS statement of SAS 9.1 with letter grouping obtained using the SAS pdmix800 macro (Saxton, 1998). For the different statistical tests, significance was declared at P ≤ 0.05. The REG procedure of SAS 9.1 was used to provide regression models for assessment of relation between SkEO and water consumption.

RESULTS AND DISCUSSION

The effect of different treatments on antibody Newcastle titer

No significant differences between treatments were found for the measured factors at 15, 21 and 28 days of age (Table 1). At 35 day of ages, the treatments of 0- and 200 ppm contained max and min titer when compared with other group. The results of this study revealed that *S. khuzistanica* consumption in drinking water could not stimulate the immune system response in broiler chickens. In agreement with our results, a herbal extract with a blend of cinnamon, thyme and oregano did not have a significant effect on the serum concentration of immunoglobulin G in pigs (Namkung et al., 2004). In the same way, serum antibody titer level against NDV in broilers supplemented with 0.2, 0.4, 0.6, 0.8 and 1% garlic powder did not differ with control birds at 14, 28 and 42 days of age (Pourali et al., 2010). Khaligh et al (2011) indicated that addition of 10 g/kg a blend of alfalfa, liquorice root, great burdock, cinnamon to the broiler diet resulted in the most consistent improvement in antibody titer against Newcastle disease virus (p < 0.05) when compared with the control.

The effect of different treatments on relative weight of immune organ

No significant differences were pointed out for relative

Table 2. The effect of different level of essential oils of *S. khuzistanica* on relative weight (% body weight) of lymphoid organs.

Treatment	Burse		Thymus		Spleen	
	21 day	42 day	21 day	42 day	21 day	42 day
CON - ¹	0.259	0.130	0.148	0.107	0.145	0.125
CON+ ¹	0.270	0.122	0.138	0.093	0.120	0.108
200	0.328	0.132	0.155	0.107	0.139	0.128
300	0.298	0.138	0.189	0.109	0.149	0.118
400	0.312	0.151	0.180	0.103	0.146	0.105
500	0.248	0.156	0.159	0.090	0.144	0.110
SEM ¹	0.013	0.007	0.009	0.002	0.005	0.005
P value	0.481	0.791	0.576	0.134	0.589	0.186

Control¹:- The birds received drinking water with no additive and ¹Control+: The birds received drinking water supplemented with 500 ppm polysorbate-80 throughout the trial. ²Standard error for overall mean.

weight of spleen, bursa and thymus at 21 days of age ($P>0.05$; Table 2). Adding of level 200-400 P.P.M of SKEO showed a significant decrease on relative weight of thymus in 42 days of age as compared to that in con (-) group ($P<0.05$; Table 2). Thymus weight reduced for the birds that received treated water but a difference was only significant for 400 ppm SKEO. Overall use of SKEO to 21 days had a positive effect but on lymphoid organs, although this difference was not significant. During 21 to 42 days, with heat stress adding of SKEO on contract of spleen reduced weight of thymus and burse of fabricius. Rahimi et al. (2011) reported that dietary thyme extract (0.1%) soluble in water did not affect immune system, but relative weight of bursa fabricius was significantly affected by garlic group and antibody response to sheep red blood cells (SRBC) was higher in coneflower group when compared with control group ($P<0.05$).

In some studies carried out in animal fields, the influence of medical plants on improvement of immune system had been reported (Rivera et al., 2003; Schuberth et al., 2002). Furthermore, Rahimi et al (2011) reported that dietary thyme extract (0.1%) soluble in water did not affect immune system, but relative weight of bursa fabricius was significantly affected by garlic group and antibody response to SRBC was higher in coneflower group when compared with control group ($P<0.05$). Die-tary birds with polysavone (alfalfa extract) improved the relative thymus, bursa and spleen weights and led to increase in proliferation of T and B lymphocytes when compared with the control group ($P<0.05$). Moreover, polysavone consumption resulted in a significant increase ($P<0.05$) in serum antibody titer of Newcastle disease virus (Dong et al., 2007). Regarding this fact that a few reports are available on the impact of *S. khuzestanica* on bird immune response, more studies will be needed to investigate SKEO immunomodulatory properties and principal components on broiler health.

Effect of SKEO on white blood cell

Heterophile phagocytic cells act to destroy infectious agents. Heterophil: lymphocyte ratio in the safety assessment is an important indicator of low index number indicates better performance than the lymphocytes in the blood and the immune system to produce antibody. In comparison of counts of white blood cells in two different time periods (1 - 21 and 22 - 42 days), the result shows that in the second period (22 to 42 days) when poultry were exposed to heat stress, number of white blood cells were decreased, the decrease being due to unbearable temperatures and low air resistance in poultry. Greater number of lymphocytes in the blood indicated better immune system performance in terms of antibody production. Treatments of 200 to 500 ppm by using treated SKEO drinking water for broiler chickens had no significant effect on the percentage of heterophile, lymphocytes and heterophil : lymphocyte ratio ($P>0.05$) (Table 3).

Hens under heat stress need to consume more water. Each element of water and feed additives that stimulate water consumption is greater for hens exposed to heat stress in birds helps. It seems that adding SKEO to water to create savory taste causes sharp decrease in water consumption (data not reported). Reduced water consumption exacerbates the negative consequences of heat stress, including immune system activity reduction.

This adverse effect on the age of 42 days when compared with 21 days (until the appropriate environmental conditions for growth in hens at this time) is evident. Al-Kassie (2010) showed that feeding diets were supplemented with oil extract derived from thyme and cinnamon to broilers, which significantly increased RBC, HCT, Hb and WBC values when compared with the control group.

In conclusion, results of the present study showed that supplementation of *S. khuzestanica* in drinking water did not improve the immune status in broiler chickens in the

Table 3. The effect of different level of essential oils of *S. khuzistanica* on WBC count (%) and heterophile to lymphocyte ratio.

Treatment	Heterophyle		Lymphocyte		Heterophile : Lymphocyte	
	21 day	42 day	21 day	42 day	21 day	42 day
CON ⁻¹	43.000	36.667	50.000	52.667	0.905	0.796
CON ⁺¹	46.333	28.833	48.667	55.500	1.019	0.591
200	43.000	30.000	51.667	56.667	0.843	0.582
300	42.333	32.000	53.667	58.667	0.827	0.622
400	42.000	27.333	51.333	95.000	0.924	0.531
500	41.333	34.000	52.000	55.5000	0.866 ^a	0.694
SEM ¹	1.628	1.756	1.566	2.193	0.057	0.060
P value	0.9648	0.6377	0.9614	0.9732	0.9418	0.8620

Control¹:- The birds received drinking water with no additive and ¹Control+: The birds received drinking water supplemented with 500 ppm polysorbate-80 throughout the trial. ²Standard error for overall mean.

whole experimental period. Despite some indications observed, further studies are necessary to confirm the immune performance effect of SkEO in broiler chicken.

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