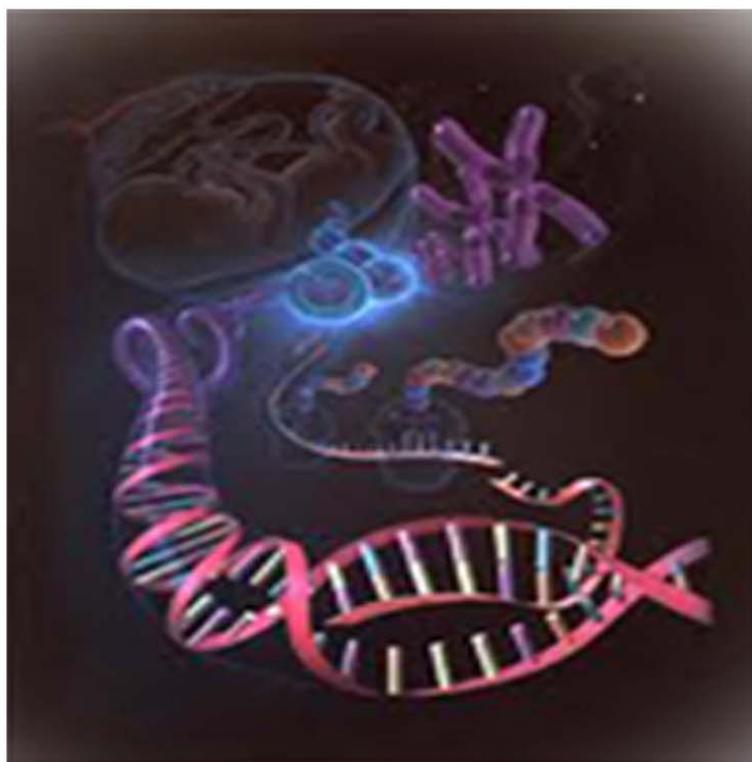




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Research Paper

IMPACT OF DIFFERENT LEVEL OF SATUREJA ESSENTIAL OILS ON IMMUNE RESPONSE OF BROILER CHICKENS REARED IN DIFFERENT AGE PERIODS

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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 raised under standard production practices up to 42 days of age. The birds were 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 differ 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 21d. Thymus weight reduced for the birds received treated water but a difference was only significant for 400 ppm SKEO. The mean Hetrophyle / lymphocyte ratio was not significantly differing among the treatments. It was concluded that supplementation of broiler chicken drinking water with *Satureja khuzestanica* essential oils has no favorite impact on immune response of heat stressed broiler chicken.

Keywords: *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

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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 compared with 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 a blend of alfalfa, liquorice root, great burdock, cinnamon to broiler diets resulted in an improvement in antibody titer against Newcastle disease virus compared with the control.

Satureja khuzestanica 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 ethnomedicine as an analgesic and antiseptic agent (Abdollahi *et al.*, 2003). The essential oils of *Satureja 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 *Satureja 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 were 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 it 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 40 min period. After centrifugation (5000 rpm) for 7 min, Blood serum was separated and then Newcastle virus antibody titers were measured by using the Hem agglutinin Inhibition (HI) method.

Statistical Analysis

The statistical model used to analyze the collected data was

$$Y_{ijk} = \mu + SkEO_i + Sj + B_k + \varepsilon_{ijk}$$

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 ($k=6$; 1, 2, 3, 4, 5 and 6) and ε_{ijk} is the residual 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 pdmix 800 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 was 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 were contain maximum and minimum titer the compare with other group. The results of this study revealed that *Satureja 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 didn't has a significant effect on the serum concentration of

Table 1: The Effect 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

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

immunoglobulin G in pigs (Namkung *et al.*, 2004). In same way, serum antibody titer level against NDV in broilers that supplemented with 0.2, 0.4, 0.6, 0.8 and 1% garlic powder didn't 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 ($p < 0.05$) compared with the control.

The Effect of Different Treatments on Relative Weight of Immune Organ

No significant differences were pointed out for relative weight of spleen, Bursa and thymus at 21 day of age ($P > 0.05$; Table 2). Adding of level 200-400 ppm of SKEO showed a significant decrease on Relative weight of thymus in 42 day of age as compared with that in con (-) group ($P < 0.05$; Table 2). Thymus weight reduced for the birds received treated water but a difference was only significant for 400 ppm SKEO. Overall

use of SKEO to 21 d had a positive effect but on lymphoid organs, although this difference was not significant. During 21 to 42 d, 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 significantly affected by garlic group and antibody response to Sheep Red Blood Cells (SRBC) was higher in coneflower group compared with control group ($P < 0.05$).

In the some studies that 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 significantly affected by garlic group and antibody response to SRBC was higher in coneflower group compared with control

Table 2: The of Effect Different Level of Essential Oils of Satureja Khuzistanica on Relative Weight (% Body Weight) of Lymphoid Organs

Treatment	Spleen		Thymus		Burse	
	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 ²	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

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

group ($P < 0.05$). Dietary birds with polysavone (alfalfa extract) improved the relative thymus, bursa and spleen weights and led to increase in proliferation of T and B lymphocytes 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 *Satureja 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 that 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 antibodies. Comparison of counts of white blood cells in two different time periods (1-21 and 22- 42 days) The result is that in the second period (22 to 42 days) when poultry were exposed to heat stress in number of white blood cells they were decreased, the decrease being due to unbearable temperatures and low air resistance was 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 was no significant effect on the percentage of heterophile, lymphocytes, and heterophil: lymphocyte ratio ($P > 0.05$; Table 3).

Hens under heat stress is the 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 is sharp decrease in water consumption (data not reported). Reduce water

Table 3: The Effect of Different Level of Essential Oils of *Satureja Khuzistanica* on WBCCount (%) 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
SEM1	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

Note: CON⁻¹: The birds received drinking water with no additive; and CON⁺¹: The birds received drinking water supplemented with 500 ppm polysorbate-80 throughout the trial. ^a Standard error for overall mean.

consumption exacerbate the negative consequences of heat stress, including immune system activity is reduced. This adverse effect on the age of 42 days compared with 21 days (until the appropriate environmental conditions for growth have been in hens at this time) is evident. Al-Kassie (2009) 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 compared with the control group.

In conclusion, results of the present study showed that supplementation of *Satureja khuzestanica* in drinking water did not improve the immune status in broiler chickens in the whole experimental period. Despite of some indications observed, the further studies are necessary to confirm the immune performance effect of SkEO in broiler chicken.

REFERENCES

1. Abdollahi M, Salehnia A, Mortazavi S H, Ebrahimi M, Shafiee A, Fouladian F, Keshavarz K, Sorouri S, Khorasani R and Kazemi A (2003), "Antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of *Satureja khuzestanica* in rat in vivo: a toxicopharmacological study", *Med Sci Monit.*, Vol. 9, pp. 331-335.
2. Al-Kassie G A M (2009), "The role of peppermint (*Mentha piperita*) on performance in broiler diets", *Agric. Biol. J. N. Am.*, Vol. 15, pp. 1009-1013.
3. Burt S (2004), "Essential oils: Their antibacterial properties and potential applications in food-A review", *Int J Food Microbiol.*, Vol. 94, pp. 223-253.
4. Collington G K, Park D S and Armstrong D G (1990), "The influence of inclusion of both an antibiotic and a probiotic in the diet on the development of digestive enzyme activity in the pig", *Br. J. Nutr.*, Vol. 64: pp. 59-70.
5. Cuppett S L and Hall C A (1998), "Antioxidant activity of Labiatae", *Adv Food Nutr Res.*, Vol. 42, pp. 245-271.
6. Dong X F, Gao W W, Tong J M, Jia H Q, Sa R N and Zhang Q (2007), "Effect of polysavone (Alfalfa extract) on abdominal fat deposition and immunity in broiler chickens", *Journal of Poultry Science*, Vol. 86, pp. 1955-1959.
7. Farsam H, Amanlou M, Radpour M R, Salehnia A and Shafiee A (2004), "Essential oil composition of wild and cultivated *Satureja khuzistanica* Jamzad from Iran", *Flavour Fragr J.*, Vol. 19, pp. 308-10.
8. Khaligh F, Ghorbanali S, Karimi A and Vaziry A (2011), "Evaluation of different medicinal plants blends in diets for broiler chickens", *Journal of Medicinal Plants Research*, Vol. 5, No. 10, pp. 1971-1977.
9. Liu X Y (1999), "Stress and Immunity", In *Poultry Immunology*, (Ed.): Yin T B, China Agriculture Press, Beijing, China, pp. 230-252.
10. Namkung H and Li M (2004), "Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs", *Canadian Journal of Animal Science*, Vol. 84(4), pp. 697-704.
11. Pourali M, Mirghelenj S A and Kermanshahi H (2010), "The effect of productive

- performance and immune response of broiler chickens challenged with Newcastle disease virus”, *Global veterinaria*, Vol. 4(6): pp. 616-621.
12. Radonic A and Milos M (2003), “Chemical composition and in vitro evaluation of antioxidant effect of free volatile compounds from *Satureja montana* L.”, *Free Radic Res.*, Vol. 37, pp. 673–9.
 13. Rahimi S, Teymouri Zadeh Z, Karimi Torshizi M A, Omidbaigi R, Rokni H (2011), “Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens”, *Journal of Agricultural Science and Technology*, Vol. 13, pp. 527-539.
 14. Rivera E and Hu S (2003), “Ginseng and aluminium hydroxide act synergistically as vaccine adjuvants”, *Vaccine*, Vol. 21, pp. 1149-1157.
 15. SAS Institute (2002), *SAS/STAT® Guide for personal computers*. Version 9.1 Edition. SAS Institute, Inc., Cary, NC.
 16. Saxton A M (1998), “A macro for converting mean separation output to letter grouping in Proc Mixed”, pp. 1243-a264 in Proc. 23rd SAS User Group Intl. SAS Institute, Cary, NC.
 17. Schubert H J and Riedel Caspari G (2002), “Flow cytometric testing of immunological effects of a phytochemical combination (EquiMun) and its compounds on bovine leucocytes”, *Journal of Veterinary Medicine Series*, Vol. 49(6), pp. 291-298.
 18. Windisch W, Schedle K, Plitzner C and Kroismayr (2008), “Use of phytochemical products as feed additives for swine and poultry”, *Journal of Animal Science*, Vol. 86, pp. 140-148.
 19. Williams P and Losa R (2001), “The use of essential oils and their compounds in poultry nutrition”, *World Poultry*, Vol. 17, pp. 14-15.



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