Effects of supplementation of *Satureja khuzistanica* Jamzad essential oils in the drinking water on growth performance, serum metabolites and differential counts of white blood cells in broiler chickens

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Abstract This study was carried out to evaluate the effects of *Satureja khuzistanica* essential oils (SKEO) on performance, serum metabolites and differential counts of white blood cells in broiler chickens. A total of 280 one-day-old straight-run Cobb 500 broiler chickens were allocated into seven treatment groups with four replicates of 10 birds each from day 1 to 42 of age. The birds were continuously given drinking water supplemented with 0 (Control⁻), 100, 200, 300, 400 and 500 mg/L SKEO or 1,000 mg/L Tween-80 (control⁺) as seven experimental treatments. During overall period (1 to 42 days) of the study, broilers received water supplemented with 300, 400 and 500 mg/L SKEO consumed less (P < 0.05) feed compared with those given water supplemented with 100 mg/L SKEO. However, feed intake for the control broilers was intermediate and was not statistically different (P > 0.05) from either of the groups. Broilers received water supplemented with 300 mg/kg SKEO had lower (P < 0.05) weight gain compared with the control broilers, while feed conversion ratio was higher (P < 0.05) in broilers received water supplemented with 100 mg/kg SKEO compared with the control broilers. The serum concentrations of glucose, triglycerides, and total cholesterol were not affected (P > 0.05) by treatments at days 21 and 42 of age. Similarly, water supplementation with SKEO had no effect on differential counts of white blood cells and heterophil to lymphocyte (H/L) ratio at days 21 and 42 of age. Overall, results of the present study showed that drinking water supplementation with SKEO had no beneficial effect on growth performance, serum metabolite concentrations and differential counts of white blood cells in broiler chickens and water supplementation with 100 and 300 mg/L SKEO decreased broiler growth performance.

Keywords: broilers, growth performance, *Satureja khuzistanica*, serum metabolites, white blood cells

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Introduction

The use of in-feed antibiotics was banned to prevent the risk of developing pathogens resistant to antibiotics and to satisfy consumer demand for a food chain free of drugs in the European Union since January 2006 (da Costa et al., 2010). The same policy is being considered in other parts of the world. As a result, poultry industry seeks alternatives for antibiotics as growth promoters. In recent years, the use of phytogenic compounds has gained momentum for their potential role as natural alternatives to antibiotic growth promoters in poultry nutrition (Wallace et al., 2010; Hashemi and Davoodi, 2012).

The genus *Satureja* (Lamiaceae, tribe Mentheae) consists of more than 200 species of aromatic herbs and shrubs, widely diffused in Mediterranean region (Ghannadi et al., 2000). This genus is represented in the flora of Iran by 12 species, eight of them being endemic and exclusive of Iran (Ghannadi, 2002). *Satureja khuzistanica* Jamzad is widely distributed in the southern and western parts of Iran (Jamzad, 1994). It is famous for its therapeutic value as an analgesic and antiseptic in Iranian traditional medicine (Zargari, 1990).

The *Satureja khuzistanica* essential oils (SKEO) have been reported to have a wide range of effects; they

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are anti-inflammatory and anti-nociceptive (Amanlou et al., 2005), antioxidant and antihyperlipidemic (Abdollahi et al., 2003). It was also reported that SKEO exerts stimulatory effect on male rat fertility (Haeri et al., 2006), and lowers blood glucose and triglycerides levels in diabetic rats (Abdollahi et al., 2003). Previous works reported carvacrol the main component of SKEO (Farsam et al., 2004; Hadian et al., 2011; Khosravinia et al., 2013), ranging from 92.2 to 93.9%, along with p-cymene (0.6 to 1.3%) and thymol (0.2 to 0.6%).

A previous study examining five concentrations (500, 1,000, 1,500, 2,000 or 2,500 mg/L) of SKEO in drinking water of broilers reported that water supplementation with SKEO suppressed the productive performance of the chickens (Khosravinia et al., 2013). Parvar et al. (2013a) tested four concentrations (200, 300, 400 or 500 mg/L) of SKEO through drinking water of broiler chickens and found that water supplementation with 400 mg/L SKEO improved breast weight of broilers under a tropical climate. Details on production performance were, however, not reported. Moreover, the effects of SKEO supplementation on the physiological responses of broilers have not been well documented. Khosravinia et al. (2015) reported that water supplementation with SKEO has a potential to affect the serum lipid profile in broiler chickens because serum concentration of estradiol and the weight of abdominal fat were decreased by SKEO supplementation (500 or 1,000 mg/L). In addition, there is some evidence that *Satureja khuzistanica* ethanolic extract (Souri et al., 2015) or selected SKEO components, carvacrol and thymol (Major et al., 2011; Machado Junior et al., 2014), have immunomodulatory effects in broiler chickens. Therefore, this study was carried out to evaluate the effects of water supplementation with SKEO on performance, serum biochemical parameters and differential counts of white blood cells in broiler chickens.

Materials and methods

Birds, management and experimental design

A total of 280 one-day-old straight-run Cobb 500 broiler chickens were obtained from a commercial hatchery. The chickens were weighed and randomly allocated to seven treatments in a completely randomized design. Each treatment consisted of four replicates (battery cage) of 10 birds each. Seven experimental treatments were based on supplementing drinking water with 0 (control⁻), 100, 200, 300, 400 or 500 mg/L SKEO and 1,000 mg/L Tween-80 (control⁺). Tween-80 (Sigma, Munich, Germany) is an emulsifier which was used to dissolve SKEO in water at a 2: 1 ratio (v: v). The SKEO

Table 1. Ingredient and calculated nutrients composition of basal diets (g/kg, unless stated otherwise)

Period	Starter	Grower	Finisher
Age	1 to 11 days	12 to 28 days	29 to 42 days
Ingredients			
Corn	558.8	605.6	616.4
Soybean meal (440 g CP/kg)	374.9	329.7	312.5
Soybean oil	20.4	19.7	25.0
Oyster shell	15.2	14.7	13.9
Dicalcium phosphate	18.7	18.0	16.6
Salt	3.1	2.9	2.6
Sodium bicarbonate	0.8	1.2	5.0
Mineral-vitamin premix ¹	5.0	5.0	5.0
DL-methionine	2.4	2.3	2.3
Lysine-HCl	0.7	0.9	0.7
Calculated nutrients composition			
Metabolizable energy (MJ/kg)	12.13	12.34	12.55
Crude protein	210.0	190.0	180.0
Calcium	10.0	9.8	9.0
Available phosphorous	5.0	4.6	4.5
Lysine	12.0	11.0	10.5
Methionine	4.6	4.4	4.3
Methionine + Cysteine	8.9	8.4	8.2

¹ Mineral-vitamin premix provided the following per kg of diet: vitamin A, 9,000 IU; vitamin D₃, 2,100 IU; vitamin E, 30 mg; nicotinic acid, 30 mg; vitamin B₁₂, 0.12 mg; calcium pantothenate, 10 mg; vitamin K₃, 5 mg; thiamine, 1.1 mg; riboflavin, 4.5 mg; vitamin B₆, 2.0 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 50 mg; Cu, 10 mg; Mn, 70 mg; Zn, 50 mg; Co, 0.2 mg; I, 1.0 mg; Se, 0.3 mg.

was prepared according to the methods previously described (Hadian et al., 2011). Broilers had *ad libitum* access to water and feed (in mash form) according to a three-phase feeding program on a starter, grower and finisher diet during the periods of 1 to 11, 12 to 28 and 29 to 42 days of age, respectively. All the diets (Table 1) were formulated to meet or exceed Cobb 500 strain nutrient requirements (Cobb, 2008). The ambient temperature was set at 32°C for the first week and 30°C for the second week, which was further decreased to 21°C until the end of the study. A 23: 1 lighting: darkness program was followed throughout the experimental period.

Growth performance

Feed intake and body weights were recorded by replicate at days 21 and 42 of age, whereas mortality was recorded daily. From these data, average feed intake and body weight gain and feed conversion ratio were calculated per pen for each period.

Serum biochemical parameters and leukocyte profile

Blood samples were collected from the wing vein of four randomly selected chickens per treatment (one chickens per replicate) at days 21 and 42 of age. Blood samples for serum metabolites were collected into tubes containing no anti-coagulant and then centrifuged (15 min, 3,000 rpm). The sera were removed and stored at – 20°C until further analysis. Serum concentrations of glucose, triglycerides and total cholesterol were measured using the diagnostic kit (Pars Azmun, Tehran, Iran), and enzymatic methods. Blood samples for differential counts of white blood cells (leukocyte profile) were collected into tubes pretreated with heparin, as anti-coagulant. Briefly, two drops of blood were placed on a slide, spin prepared, and stained with May-Grünwald-Giemsa stain. One hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes), were counted on one slide using a light microscope (Olympus CX31, Tokyo, Japan), and the heterophil to lymphocyte (H/L) ratio was calculated (Gross and Siegel, 1983).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the SAS software (SAS Institute, 2003). Tukey's test was used to compare and separate treatment means. All statements of significance were based on a probability of less than 0.05.

Results

The effects of treatments on broiler growth performance are presented in Table 2. Broilers received water supplemented with 400 or 500 mg/L SKEO consumed less (P < 0.05) feed compared with the control broilers during 1 to 21 days of age. However, body weight gain and feed conversion ratio were not affected (P > 0.05) by treatments during this period of age. Broilers received water supplemented with 400 or 500 mg/L SKEO consumed less (P < 0.05) feed compared with those received water supplemented with 100 mg/L SKEO during 22 to 42 days of age. However, feed intake for the control broilers was not statistically different (P > 0.05)from either of groups. Nevertheless, broilers received water supplemented with 300 or 500 mg/kg SKEO had lower (P < 0.05) weight gain compared with the control broilers during this period of age. Meanwhile, feed conversion ratio was higher (P < 0.05) in broilers given water supplemented with 100 or 300 mg/kg SKEO compared with the control broilers. During the overall period (1 to 42 days), broilers received water supplemented with 300, 400 or 500 mg/L SKEO consumed less (P < 0.05) feed compared with those given water supplemented with 100 mg/L SKEO. However, feed intake for the control broilers was not statistically different (P > 0.05) from either of groups. Broilers received water supplemented with 300 mg/kg SKEO had lower (P < 0.05) weight gain compared with the control broilers during this period of age. At the same time, feed conversion ratio was higher (P < 0.05) in broilers given water supplemented with 100 mg/kg SKEO compared with the control. No effect (P > 0.05) of Tween-80 supplemented water (Control⁺) was found on performance criteria thought day 42 of experiment.

Serum concentrations of glucose, triglycerides, and total cholesterol were not affected (P > 0.05) by treatments at days 21 and 42 of age (Table 3). Similarly, water supplementation with SKEO had no effect (P > 0.05) on differential counts of white blood cells and H/L ratio at days 21 and 42 of age (Table 4).

Discussion

The results of the present study indicated that drinking water supplementation with SKEO at 300 or 500 mg/L adversely affected body weight gain in broiler chickens from 22 to 42 days of age. Broilers received water supplemented with 300 mg/L SKEO also exhibited lower body weight gain during the overall period (1 to 42 days) of the study. These findings are similar to those of Khosravinia et al. (2013), who showed that body weight

Table 2. Effects of *Satureja khuzistanica* (Jamzad) essential oils (SKEO) supplementation into the drinking water on body weight gain (g), feed intake (g) and feed conversion ratio (g/g) in broiler chickens

					SKEC	SKEO (mg/L)			
Item	Control	Control+	100	200	300	400	500	SEM	P values
Feed intake									
day 1 to 21	743.6 ± 36.7^{ab}	763.1 ± 24.6^{a}	$766.1\pm13.5^{\mathrm{a}}$	$700.1 \pm 67.5^{abc} 668.5 \pm 34.4^{bc}$	668.5 ± 34.4^{bc}	$649.6 \pm 12.8^{\circ}$	$656.3 \pm 20.1^{\circ}$ 10.7	10.7	<0.0001
day 22 to 42	3025.4 ± 110.9^{ab}	3007.1 ± 43.3^{ab}	3268.5 ± 317.4^{a}	$3025.4 \pm 110.9^{ab} \ 3007.1 \pm 43.3^{ab} \ 3268.5 \pm 317.4^{a} \ 2937.0 \pm 278.0^{ab} \ 2724.5 \pm 23.5^{b} \ 2809.60 \pm 120.8^{b} \ 2830.3 \pm 81.6^{b} \ 43.4$	2724.5 ± 23.5^{b} ?	2809.60 ± 120.8^{b}	2830.3 ± 81.6^{b}	43.4	900.0
day 1 to 42	3769.0 ± 143.4^{ab}	3770.2 ± 66.8^{ab}	4034.6 ± 315.0^{a}	$3769.0 \pm 143.4^{ab} \ 3770.2 \pm 66.8^{ab} \ 4034.6 \pm 315.0^{a} \ 3637.1 \pm 343.5^{ab} \ 3399.0 \pm 12.2^{b} \ 3459.2 \pm 121.8^{b} \ 3486.5 \pm 74.7^{b}$	3393.0 ± 12.2^{b}	3459.2 ± 121.8^{b}	3486.5 ± 74.7^{b}	51.6	0.002
Body weight gain									
day 1 to 21	447.5 ± 51.2	463.2 ± 24.3	449.3 ± 87.6	427.7 ± 55.8	407.7 ± 35.7	395.2 ± 15.5	424.4 ± 25.9	9.10	0.44
day 22 to 42	1502.1 ± 88.9^{a}	1439.3 ± 63.3^{ab}	1426.0 ± 90.4^{ab}	$1439.3 \pm 63.3^{ab} 1426.0 \pm 90.4^{ab} 1351.9 \pm 128.4^{abc} 1215.1 \pm 43.9^{c} 1402.4 \pm 98.9^{abc} 1292.1 \pm 81.7^{bc}$	$1215.1 \pm 43.9^{\circ}$	1402.4 ± 98.9^{abc}	1292.1 ± 81.7^{bc}	22.7	0.003
day 1 to 42	1949.6 ± 114.8^{a}	$1902.4\pm77.6^{\mathrm{a}}$	1875.3 ± 44.3^{a}	$1949.6 \pm 114.8^{a} 1902.4 \pm 77.6^{a} 1875.3 \pm 44.3^{a} 1779.6 \pm 181.7^{ab} 1622.8 \pm 73.4^{b} 1797.6 \pm 109.9^{ab} 1716.4 \pm 94.2^{ab} 26.97 + 109.9^{ab} 1716.8 \pm 109.9^{ab} 1716.8 $	1622.8 ± 73.4^{b}	1797.6 ± 109.9^{ab}	1716.4 ± 94.2^{ab}	26.97	0.005
Feed conversion ratio									
day 1 to 21	1.67 ± 0.120	1.65 ± 0.061	1.75 ± 0.291	1.64 ± 0.056	1.64 ± 0.067	1.65 ± 0.058	1.55 ± 0.049	0.024	0.57
day 21 to 42	2.02 ± 0.094^b	2.09 ± 0.067^{ab}	$2.29 \pm 0.143^{\mathrm{a}}$	2.17 ± 0.026^{ab}	$2.25\pm0.091^{\rm a}$	2.01 ± 0.085^b	$2.20 \pm 0.099^{ab} \ 0.025$	0.025	0.001
day 1 to 42	1.94 ± 0.071^{b}	1.99 ± 0.053^{ab}	$2.15\pm0.164^{\rm a}$	2.05 ± 0.025^{ab}	2.10 ± 0.086^{ab}	1.93 ± 0.066^b	$2.04 \pm 0.082^{ab} \ 0.021$	0.021	0.01

 $^{a-c}$ means (\pm SD) within a column showing different superscripts are significantly different (P < 0.05), Tukey test was applied to compare means. SEM, Standard error of the mean.

Table 3. Effect of *Satureja khuzistanica* (Jamzad) essential oils (SKEO) supplementation into the drinking water on serum metabolite concentrations (mmol/L) in broiler chickens

			SKEO (mg/L)						
Item	Control	Control+	100	200	300	400	500	SEM	SEM P values
Day 21									
Glucose	14.51 ± 1.18	12.42 ± 1.41	12.66 ± 1.71	12.16 ± 2.25	12.76 ± 1.64	12.76 ± 1.64 13.79 ± 1.27	13.56 ± 1.99	0.318	0.43
Triglycerides	1.93 ± 0.23	1.51 ± 0.63	1.62 ± 0.56	1.28 ± 0.39	1.22 ± 0.43	1.70 ± 0.40	1.66 ± 0.49	0.089	0.37
Total cholesterol	4.25 ± 0.38	4.45 ± 0.62	3.96 ± 0.72	4.03 ± 0.59	3.86 ± 0.75	3.61 ± 0.91	3.48 ± 0.62	0.127	0.44
Day 42									
Glucose	12.28 ± 1.04	12.21 ± 0.92	11.82 ± 1.89	12.46 ± 0.87	11.85 ± 0.98 12.32 ± 1.20	12.32 ± 1.20	13.32 ± 0.76	0.211	09.0
Triglycerides	3.81 ± 0.54	3.92 ± 0.53	3.55 ± 0.73	3.73 ± 0.61	3.72 ± 0.32	3.15 ± 0.47	3.25 ± 0.55	0.105	0.39
Total cholesterol	4.87 ± 0.53	5.20 ± 0.78	5.16 ± 0.75	4.80 ± 0.20	4.34 ± 0.49	4.37 ± 0.51	4.40 ± 0.56	0.116	0.18

SEM, Standard error of the mean.

Table 4. Effect of *Satureja khuzistanica* (Jamzad) essential oils (SKEO) supplementation into the drinking water on differential counts of white blood cells in broiler chickens

COLOR IN CLOSED CHICKORY	CINCINS								
140.00	100	Contract			SKE	SKEO (mg/L)			
Ilem	Control	Control+	100	200	300	400	500	SEM	P values
Day 21									
Monocytes	5.50 ± 1.29	3.75 ± 1.71	4.50 ± 2.65	5.25 ± 2.75	5.00 ± 2.16	4.75 ± 1.50	6.25 + 1.70	0.367	0.72
Eosinophils	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.050	0.70
Basophils	0.75 ± 0.96	0.75 ± 1.50	0.00 ± 0.00	0.00 ± 0.00	0.75 ± 1.50	0.00 ± 0.00	0.50 ± 1.00	0.173	0.55
Heterophils	37.75 ± 2.22	34.00 ± 3.37	34.25 ± 3.86	37.50 ± 4.65	35.00 ± 6.38	37.50 ± 6.25	34.00 ± 6.98	0.905	0.80
Lymphocytes	56.00 ± 1.83	61.25 ± 1.71	61.25 ± 4.78	57.25 ± 3.77	59.00 ± 6.58	57.75 ± 4.44	59.25 ± 6.34	0.864	0.65
H/L ratio	0.68 ± 0.06	0.56 ± 0.07	0.56 ± 0.10	0.66 ± 0.12	0.61 ± 0.17	0.66 ± 0.16	0.59 ± 0.17	0.024	0.73
Day 42									
Monocytes	5.75 ± 0.96	5.25 ± 0.50	7.25 ± 3.50	6.00 ± 2.58	6.75 ± 2.63	5.75 ± 2.22	6.50 ± 2.65	0.412	06.0
Eosinophils	0.00 ± 0.00	0.000	0.70						
Basophils	0.50 ± 1.00	0.00 ± 0.00	0.25 ± 0.50	1.00 ± 2.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 1.00	0.171	06.0
Heterophils	34.25 ± 5.06	36.00 ± 6.38	36.00 ± 6.98	36.50 ± 5.92	35.50 ± 1.91	35.00 ± 2.45	34.5 ± 4.04	0.848	0.99
Lymphocytes	59.50 ± 5.45	58.75 ± 6.29	56.50 ± 9.75	56.5 ± 7.23	57.75 ± 1.71	59.25 ± 2.99	58.00 ± 4.73	1.021	0.98
H/L ratio	0.59 ± 0.13	0.63 ± 0.19	0.67 ± 0.23	0.66 ± 0.18	0.62 ± 0.04	0.59 ± 0.07	0.60 ± 0.11	0.026	0.97
	,								

SEM, Standard error of the mean.

gain in broilers was reduced by continual supplementation with SKEO into the drinking water (500 to 2,500 mg/L) and attributed this effect to lower water intake by the broilers received treated water. The SKEO consists of a wide range of volatile lipophilic compounds, including carvacrol, which comprises more than 92% of the entire extract (Farsam et al., 2004; Hadian et al., 2011; Khosravinia et al., 2013). This monoterpenoid phenol is bitter-tasting and pungent, which obviously changed the flavor of water and caused significant reduction in water intake in broilers (Khosravinia et al., 2013). It is well known that water intake has strong correlation with feed intake, so that when water intake drop, the feed intake will decrease. In the present study, water intake was not measured, however, except for the group given 100 mg/L SKEO, the overall feed intake in broilers kept on SKEO-supplemented water were lower (about 3.5 to 10%) compared with the control broilers, although these were not statistically different. In an overview on feeding phytogenic compounds, Windisch et al. (2008) reported that the majority of experimental results indicate reduced feed intake at largely unchanged body weight gain or final body weight, leading to an improved feed conversion ratio. Therefore, the results of the present study are in agreement with the conclusion drawn by Windisch et al. (2008), and support the statement that it is generally not justified to assume that phytogenic extracts improve the palatability of feed. Moreover, in the present study water supplementation with SKEO significantly affected the feed conversion ratio. It was higher in broilers received water supplemented with 100 mg/L SKEO than in the control broilers. The SKEO effect on feed conversion ratio could relate to altered gut function or altered carcass composition. However, these were not examined in the present study, and, thus, no plausible interpretation can be given. This topic clearly merits further study.

No significant effect of treatments was found on serum concentrations of glucose, triglycerides and total cholesterol. These results are in accordance with those of Khosravinia (2015), who demonstrated no significant difference in serum concentrations of triglycerides and total cholesterol between broilers given SKEO–supplemented water (500 to 2,500 mg/L) and broilers from the control group. Similarly, Goodarzi et al. (2014) observed no significant differences in serum concentrations of glucose, triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol in broiler chickens due to dietary supplementation with 500 mg/kg SKEO. Souri et al. (2015) also found that the serum concentrations of glucose, triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol in broiler chickens

were not changed by dietary supplementation with 10 or 20 g/kg Satureja khuzistanica ethanolic extract. However, studies in other animal models and humans, suggest that SKEO may have a benefit in reducing serum levels of glucose, triglycerides and cholesterol. Abdollahi et al. (2003) who evaluated the effects of SKEO (1,000 mg/L of drinking water) on rats, reported no significant effects of treatment on serum total cholesterol levels; however, average concentration of serum glucose and triglycerides were reduced by 20.6 and 17.4% in rats given SKEO-treated water, respectively. Similar results were reported by Nazari et al. (2005) who added SKEO to diets (250 mg/kg) of male Wistar rats and Shahsavari et al. (2009) who administered SKEO orally at different doses (50 and 100 mg/kg/day) to normal and diabetic rats. Conversely, Vosough-Ghanbari et al. (2010), working with 21 hyperlipidemic patients with type 2 diabetes mellitus, observed no differences in serum concentrations of glucose and triglycerides, while serum concentrations of total cholesterol showed significant decrease in patients received Satureja khuzistanica (tablets contain 250 mg dried leaves). The lack of agreement among these studies may be, in part, explained by both the route and dosage of administration. Nevertheless, these results also suggest that there may be inherent differences among species with regard to their response to SKEO, or other similar additives.

Leucocytes are known to increase sharply when infection occurs because they are one of the first lines of defense in the body (Sahu et al., 2007). In the present study, differential counts of white blood cells and H/L ratio were not influenced by water supplementation with SKEO. These results are in general agreement with those of Parvar et al. (2013b), who reported that the count of heterophils and lymphocytes and H/L ratio were not affected by water supplementation with SKEO (200 to 500 mg/L). Similarly, Souri et al. (2015) observed no significant differences in differential counts of white blood cells and H/L ratio in broiler chickens due to dietary supplementation with 10 or 20 mg/kg Satureja khuzistanica ethanolic extract. Also, Hashemipour et al. (2013) were not able to demonstrate a beneficial effect from carvacrol on white blood cells counts. However, Boskabady and Jalali (2013) showed that supplementation of 80 mg/L SKEO was able to reduce total and differential counts of white blood cells in guinea pigs. The reasons for these differences are not clear; however, several factors may affect the efficacy of an herbal essential oil application, as indicated previously.

Conclusions

The results of the present study showed that water supp-

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lementation with SKEO had an adverse effect growth performance of broilers, as their body weight gain was decreased by supplementation with SKEO at 300 or 500 mg/L and their feed conversion ratio was increased by supplementation with SKEO at 100 mg/L when compared with the control broilers throughout the study. The mechanisms associated with the negative effects of SKEO on broiler growth performance need to be elucidated.

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References

- Abdollahi, M., Salehnia, A., Mortazavi, S.H., Ebrahimi, M., Shafiee, A., Fouladian, F., Keshavarz, K., Sorouri, S., Khorasani, R., Kazemi, A., 2003. Antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of *Satureja khuzestanica* in rat in vivo: a oxicopharmacological study. *Medical Science Monitor* 9, BR331-BR335.
- Amanlou, M., Dadkhah, F., Salehnia, A., Farsam, H., Dehpour, A.R., 2005. An anti-inflammatory and anti-nociceptive effects of hydroalcoholic extract of *Satureja khuzistanica* Jamzad extract. *Journal of Pharmacy & Pharmaceutical Sciences* 8, 102-106.
- Boskabady, M.H., Jalali, S., 2013. Effect of carvacrol on tracheal responsiveness, inflammatory mediators, total and differential WBC count in blood of sensitized guinea pigs. *Experimental Biology and Medicine* 238, 200-208.
- Cobb, 2008. Broiler performance and nutrition supplement: Cobb 500. Available at: http://www.cobbvantress.com/contactus/brochures/Cobb500_BPN_Supp_2008%2 8lbs% 29pdf. [Verified 30 May 2011].
- Da Costa, P.M., Bica, A., Vaz-Pires, P., Bernardo, F., 2010. Changes in antimicrobial resistance among faecal enterococci isolated from growing broilers prophylactically medicated with three commercial antimicrobials. *Preventive Veterinary Medicine* 93, 71-76.
- Farsam, H., Amanlou, M., Radpour, M.R., Salehinia, A.N., Shafiee, A., 2004. Composition of the essential oils of wild and cultivated *Satureja khuzistanica* Jamzad from Iran. *Flavour and Fragrance Journal* 19, 308-310.
- Ghannadi, A., 2002. Composition of the essential oil of *Satureja hortensis* L. seeds from Iran. *Journal of Essential Oil Research* 14, 35-36.
 - Ghannadi, A., Hajhashemi, V., Sadraei, H., Mohseni, M., 2000. Composition of the essential oil of *Satureja hortensis* L. grown in Kashan. *Iranian Journal of Basic Medical Sciences* 23, 166-169.

- Goodarzi, M., Mohtashami Pour, N., Modiri, D., 2014. The effect of savory (*Satureja khuzistanica*) essential oils on performance and some blood biochemical parameters of Ross and Cobb broilers. *Annual Research & Review in Biology* 4, 4336-4343.
- Gross, W.B., Siegel, H.S., 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases* 27, 972-978.
- Hadian, J., Mirjalili, M.H., Kanani, M.R., Salehnia, A., Ganjipoor, P., 2011. Phytochemical and morphological characterization of *Satureja khuzistanica* Jamzad populations from Iran. *Chemistry & Biodiversity* 8, 902-915.
- Haeri, S., Minaie, B., Amin, G., Nikfar, S., Khorasani, R., Esmaily, H., Salehnia, A., Abdollahi, M., 2006. Effect of *Satureja khuzestanica* essential oil on male rat fertility. *Fitoterapia* 77, 495-499.
- Hashemi, S.R., Davoodi, H., 2012. Herbal plants as new immuno-stimulator in poultry industry: a review. *Asian Journal of Animal and Veterinary Advances* 7, 105-116.
- Hashemipour, H., Kermanshahi, H., Golian, A., Veldkamp, T., 2013. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry Science* 92, 2059-2069.
- Jamzad, Z., 1994. A new species of the genus *Satureja* (Labiatae) from Iran. *Iranian Journal of Botany* 6, 215-218.
- Khosravinia, H., 2015. Hypolipidemic effects of *Satureja khuzistanica* essential oil in broiler chicken are realized through alteration in steroid hormones. *Kafkas Universitesi Veteriner Faku Itesi Dergisi* 21, 203-209.
- Khosravinia, H., Ghasemi, S., Rafiei Alavi, E., 2013. The effect of savory (*Satureja khuzistanica*) essential oils on performance, liver and kidney functions in broiler chickens. *Journal of Animal and Feed Sciences* 22, 50-55.
- Machado Junior, P.C., Beirão, B.C.B., Fernandes Filho, T., Lourenço, M.C., Joineau, M.L., Santin, E., Caron L.F., 2014. Use of blends of organic acids and oregano extracts in feed and water of broiler chickens to control *Salmonella Enteritidis* persistence in the crop and ceca of experimentally infected birds. *Journal of Applied Poultry Research* 23, 671-682.
- Major, P., Revajová, V., Levkut, M., Ševčíková, Z., Spišáková, V., Faixová, Z., Levkutová, M., Kožárová, I., Goldová, M., Levkut, M., 2011. Intestinal mucin dynamic and leukocytic responses of chickens infected with *Eimeria acervulina* and fed oregano supplemented diet. *Acta Veterinaria Brno* 80, 147-156.
- Nazari, A., Delfan, B., Shirkhani, Y., Kiyanei, A.A., Mandegary, A., 2005. Effect of decoction of *Satureja khuz-*

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- estanica Jamzad on blood coagulation time, triglyceride and glucose levels in rats. Pakistan Journal of Biological Sciences 8, 790-792.
- Parvar, R., Khosravinia, H., Azarfar, A., 2013a. Effect of *Satureja khuzestanica* essential oils on postmortem pH and antioxidative potential of breast muscle from heat stressed broiler. *Asian Journal of Poultry Science* 7, 83-89.
- Parvar, R., Khosravinia, H., Azarfar, A., 2013b. Impact of different level of *Satureja* essential oils on immune response of broiler chickens reared in different age periods. *International Journal of Life Sciences Biotechnology and Pharma Research* 2, 214-220.
- Sahu, S., Das, B.K., Mishra, B.K., Pradhan, J., Sarangi, N., 2007. Effect of *Allium sativum* on the immunity of *Labeo rohita* infected with *Aeromonas hydrophila*. *Journal of Applied Ichthyology* 23, 80-86.
- SAS Institute., 2003. SAS User's Guide: Statistics. Version 9.1. SAS Institute Inc. Cary, North Carolina, NC.
- Shahsavari, R., Ehsani-Zonouz, A., Houshmand, M., Salehnia, A., Ahangari, G., Firoozrai, M., 2009. Plasma glucose lowering effect of the wild *Satureja khuzestanica* Jamzad essential oil in diabetic rats: role of decreased gluconeogenesis. *Pakistan Journal of Biological Sciences* 12, 140-145.

- Souri, H., Khatibjoo, A., Taherpoor, K., Hassan Abadi, A., Fattahnia, F., Askari, M., 2015. Effect of *Thymus vulgaris* and *Satureja khuzestanica* ethanolic extracts on broiler chickens' performance and immune response. *Iranian Journal of Applied Animal Science* 5, 437-446.
- Vosough-Ghanbari, S., Rahimi, R., Kharabaf, S., Zeinali, S., Mohammadirad, A., Amini, S., Yasa, N., Salehnia, A., Toliat, T., Nikfar, S., Larijani, B., Abdollahi, M., 2010. Effects of *Satureja khuzestanica* on serum glucose, lipids and markers of oxidative stress in patients with type 2 diabetes mellitus: a double-blind randomized controlled trial. *Evidence-Based Complementary and Alternative Medicine* 7, 465-470.
- Wallace, R.J., Oleszek, W., Franz, C., Hahn, I., Baser, K.H.C., Mathe, A., Teichmann, K., 2010. Dietary plant bioactives for poultry health and productivity. *British Poultry Science* 51, 461-487.
- Windisch, W., Schedle, K., Plitzner, C., Kroismayr, A., 2008.
 Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science* 86, 140-148.
- Zargari, A., 1990. Medicinal Plants. Tehran University Publications. Tehran, Iran.

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تاثیر مکمل کردن روغنهای اسانسی مرزه خوزستانی در آب آشامیدنی بر عملکرد رشد، متابولیتهای سرم و شمارش تفریخی گلبولهای سفید در جوجههای گوشتی شد. قاضی، ر. مهدوی، ع. ناصری و م. حبیبان*

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چکیده این مطالعه به منظور ارزیابی اثرات روغنهای اسانسی مرزه خوزستانی بر عملکرد، متابولیتهای سرم و شمارش تفریخی گلبولهای سفید در جوجههای گوشتی انجام شد. تعداد ۲۸۰ قطعه جوجه گوشتی کاب ۵۰۰ (مخلوط هر دو جنس) به هفت گروه تیماری با چهار تکرار و هر تکرار شامل ۱۰ یرنده تخصیص یافتند و از سن ۱ تا ۴۲ روزگی نگهداری شدند. یرندهها به طور پیوسته آب آشامیدنی مکمل شده با سطوح ۰ (شاهد منفی)، ۱۰۰، ۲۰۰، ۳۰۰، ۴۰۰ و ۵۰۰ میلی گرم بر لیتر روغنهای اسانسی مرزه خوزستانی یا ۱۰۰۰ میلی گرم بر لیتر تویین-۸۰ (شاهد مثبت) را به عنوان هفت تیمار آزمایشی دریافت کردند. در کل دوره یرورش (۱ تا ۴۲ روزگی)، جوجههای گوشتی دریافت کننده آب مکمل شده با ۳۰۰، ۳۰۰ و ۵۰۰ میلیگرم بر لیتر روغنهای اسانسی مرزه خوزستانی کمتر (۲۰۰۵) از جوجههای دریافت کننده آب مکمل شده با ۱۰۰ میلیگرم بر لیتر روغنهای اسانسی مرزه خوزستانی خوراک مصرف کردند. اما مصرف خوراک جوجههای شــاهد در حد میانگین این گروهها بود و از نظر آماری تفاوت معنیداری با هیچ یک از آنها نداشــت (۲۰/۰۵). جوجههای گوشتی دریافت کننده آب مکمل شده با ۳۰۰ میلی گرم بر لیتر روغنهای اسانسی مرزه خوزستانی در مقایسه با جوجههای شاهد افزایش وزن کمتری داشتند ($P < \cdot \cdot \cdot \circ \circ$)، در حالی که ضریب تبدیل خوراک در جوجههای گوشتی دریافت کننده آب مکمل شده با ۳۰۰ میلی گرم بر لیتر روغنهای اسانسی مرزه خوزستانی بالاتر از جوجههای شاهد بود (۲۰۵۵). غلظتهای سرمی گلوکز، تریگلیسریدها و کلسترول کل در سنین ۲۱ و ۴۲ روزگی تحت تاثیر تیمارها قرار نگرفت (۹>٠/٠٥). به طور مشابه، مکمل کردن آب آشامیدنی با روغنهای اسانسی مرزه خوزستانی تاثیری بر شمارش تفریخی گلبولهای سفید و نسبت هتروفیل به لنفوسیت در سنین ۲۱ و ۴۲ روزگی نداشت (۲۰/۰۵). در مجموع، نتایج مطالعه حاضر نشان داد که مکمل کردن آب آشامیدنی با روغنهای اسانسی مرزه خوزستانی اثر سودمندی بر عملکرد رشد، غلظت متابولیتهای سرم و شمارش تفریخی گلبول های سفید در جوجههای گوشتی نداشت، حتی آب آشامیدنی مکمل شده با ۱۰۰ و ۳۰۰ میلیگرم بر لیتر روغنهای اسانسی مرزه خوزستانی عملكرد رشد جوجهها را كاهش داد.