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Productive performance, litter characteristics and carcass defects of the broiler chickens given drinking water supplemented with *Satureja khuzistanica* essential oils

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Two trials were conducted to examine the effect of *Satureja khuzistanica* essential oils (SkEO) on productive performance using 420 Cobb 500 broiler chicks. SkEO was added into drinking water at high doses (0.5 to 2.5 g/L) up to day 28 (Trial 1) and then it was reduced to 0.5 < g/L up to day 42 of age (Trial 2). The SkEO-treated water significantly decreased weight gain, feed intake, water intake and feed-to-gain, and carcass weight in a dose-dependent linear trend up to day 28 of age (Trial 1; P < 0.05). The mean abdominal fat percent significantly decreased in the treated birds and it was lower for the male birds which received 0.5 g/L SkEO compared to the other birds of the same sex. The birds regained somewhat in all zootechnical indices by lowering the SkEO doses (0.2, 0.3 and 0.5 g/L) in Trial 2. In conclusion, SkEO at high doses (0.5 to 2.5 g/L) adversely affected production performance of broiler chicks up to day 28 of age. The adverse effect was found to be fairly reversible by lowering the SkEO doses in advanced ages.

Key words: Broiler chicken, carcass defects, productive performance, *Satureja khuzistanica.*

INTRODUCTION

Satureja khuzistanica Jamzad, known as *'Marzeh khuzestani*' in Persian, is an endemic plant which resources are abundant in the rangelands of southern part of Iran. It is distinguished for its therapeutic virtues as an analgesic and antiseptic in traditional medicine (Zargari, 1990). Aiming at mass production, the plant has been domesticated and cultivated by Lorestan Medical Plant Laboratory, Khoraman, Khoramabad, Iran, since 2006. The aerial parts of *S. khuzistanica* collectively contain up to 3 percent essential oils while it undergoes hydro-distilled process (Hadian et al., 2011). The essential oils obtained were found to be astoundingly rich in carvacrol (84 to 94%) (Farsam et al., 2004; Hadian et al., 2011). Therefore, the plant acts as a bioreactor for carvacrol biosynthesis. However, the *S. khuzistanica* essential oils (SkEO) have shown to contain a wide range of other active compounds including phenols, flavones, triterpenoids, steroids and tannins (Moghaddam et al., 2007; Hadian et al., 2011).

Carvacrol was reported to show significant antioxidant (Cuppett and Hall, 1998), antiviral (Yamasaki et al., 1998), antibacterial (Burt, 2004) and antifungal

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(Skocibusic and Bezic, 2004) effects. Studies on administration of carvacrol to avian species are scarce. Lee et al. (2003) reported that inclusion of 0.2 g per kg carvacrol in broiler diets resulted in 2 percent increase in feed intake and average daily gain and one percent decrease in feed conversion ratio. Addition of thymole, an isomer of carvacrol, at the same dose caused greater reduction in all three parameters by 5, 3 and 3%, respectively. Windisch et al. (2008) reviewed the effect of dietary supplementation of many phytogenic extracts and concluded that despite conflicting results on feed intake and weight gain, almost all herbal extracts showed adverse effect on feed conversion ratio.

Oregano is also a medical plant whose extract is rich in carvacrol. It has been reported that at dietary dose of 0.15 g/kg, essential oils from organo caused 6, 2 and 4% decrease in feed intake, average daily gain and feed conversion, respectively, in broiler chicken compared to the control birds (Basmacioglu et al., 2004). With doubling-up the dietary dose (0.3 g/kg), the same researchers observed -3, +2 and -2 percent difference in the same parameters.

Khosravinia et al. (2013) revealed that continual addition of high doses of SkEO, up to 2.0 g/L, in drinking water caused no elevation in serum marker enzymes indicating no hepatotoxic effects in broiler chicks up to 28 the day of age. However, effects of the same doses on productive performance of broiler chicken remained uninvestigated.

This study, consisting of two trials, formulated to examine the effect of SkEO on productive performance, litter characteristics and carcass defects in broiler chickens when it was administrated through drinking water at high doses (0.5 to 2.5 g/L) up to day 28 and then reduced to 0.5 < g/L up to day 42 of age.

MATERIALS AND METHODS

Preparation of essential oil

The aerial parts of *S. khuzistanica* were collected during the flowering stage of the plant from Khorraman farm, Kashkan, Lorestan, Iran. The plant was identified by the Department of Botany of the Research Institute of Forests and Rangelands (TARI), Tehran. A voucher specimen (No. 58416) has been deposited at the Herbarium of TARI.

The aerial parts were air dried at ambient temperature in the shade and hydrodistilled using a Clevenger type apparatus for 5 h, giving yellow oil in 3 percent yield. The oils were dried over anhydrous sodium sulfate and stored at 4°C.

A sample of the stored oil was analyzed based on the method used by Hadian et al. (2001) and the resulting composition is presented in Table 1.

Table 1. Essential oil composition of *S. khuzistanica*.

Compound	RI[1]	Composition (%)
α-Thujene	925	0.24±0.14
α- Pinene	933	0.15±0.05
Myreene	981	0.26±0.19
α-Terpinene	1013	0.24±0.12
p-Cymene	1017	1.26±0.86
Limonene	1026	0.13±0.04
(Z)- *β*-Oeimene	1036	0.54±0.08
γ-Terpenene	1053	0.74±0.23
trans-Sabinene hydrate	1081	0.17±0.02
Terpin-4-ol	1163	tr
α -terpinole	1175	0.42±0.45
Thymol	1266	tr
Carvacrol	1282	92.16±0.46
Thymyl acetate	1329	tr
β -Caryophyllence	1425	0.16±0.01
α - Humulene	1427	tr
β -Bisabolene	1501	tr
Trans- *β*- Bisabolene	1522	0.10±0.01

[1] RI; Retention indices determined relative to n-alkanes (C_6-C_{24}) on a DB-5GC column.
tr; Trace (<0.05%).

Experimental flock and treatments

In trial 1, 420 one-day-old Cobb 500 unsexed broiler chicks were randomly allocated to 21 pens (90 × 180 cm; at density of 0.08 m^2/bird) belonging to 3 blocks up to day 28 of age. The pens were located in a cross-ventilated negative-pressure house equipped with infrared brooders. Corn and soybean meal based starter and grower diets (Table 2) formulated according to NRC (1994) recommendations were used. Feed and water supplied to the birds through a tube feeder and a manual waterer in each pen, respectively, for *ad libitum* consumption under round the clock lighting regime was administered. Seven experimental treatments were supplementation of drinking water with 0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 g/L SkEO or 3.0 g/L polysorbate 80 (control+). Polysorbate 80 is an emulsifier which is used to dissolve SkEO in water at 2: 1 ratio (*v/v*). The data on average daily weight gain (WG), feed intake (FI), water intake (WI), feed conversion ratio (FCR) and FI-to-WI ratio were gathered and analyzed for the 1 to 7, 8 to 14, 15 to 21, 22 to 28 and 1 to 28 day periods. At day 28 of age, 12 birds per treatment (6 males and 6 females) were killed and carcass weight and abdominal fat recorded individually. Mortality was monitored on a daily basis. In the second trial, the birds belonging to each of the treatments in trial 1(except for control- and control+) were divided into three groups of 20 birds each and received water supplemented with 0.2, 0.3 or 0.4 g/L SkEO up to day 42 of age. So, the number of treatments increased to 17. Body weight was individually recorded at day 42 of age.

Statistical analysis

The statistical model used to analyze the collected data was

$$Y_{ijk} = \mu + SEO_i + B_j + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable, μ is the general mean, SEO_i is

Table 2. Percentage inclusion and calculated composition of basal starter (1 to 21 d) and grower (22 to 42 d) diets.

Ingredient (%)	Starter	Grower
Corn	52.00	56.00
Soybean meal	25.00	22.5
Fish meal	7.00	5.00
Soybean oil	4.00	4.00
Wheat	3.50	3.50
Calcium carbonate	2.00	2.00
Wheat bran	2.50	3.00
Dicalcium phosphate	2.00	2.00
DL-Methoinine	0.20	0.20
L- Lysine	0.10	0.10
Common salt	0.25	0.25
Mineral premix[1]	0.50	0.50
Vitamin permix[2]	0.85	0.85
Coccidiostat	0.10	0.10
Calculated composition		
ME, MJ/kg	12.444	12.991
Crude Protein %	22.24	20.02
Calcium %	1.10	1.00
Available P %	0.45	0.40
Methionine %	0.52	0.40
Lysine %	1.15	1.10

[1]Mineral mix supplied / kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg. [2]Vitamins mix supplied / kg diet: vitamin A, 18000 IU; vitamin D_3, 4000 IU; vitamin E, 36 mg; vitamin K_3, 4 mg; vitamin B_{12}, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.

the fixed effect of essential oils from *S. khuzistanica* (i = 7; cont-, cont+, 0.5, 1.0, 1.5, 2.0, 2.5 g/L), B_j is the random effect of block (j = 3; 1, 2 and 3) and ε_{ijk} is the residual error. The data were analyzed using PROC MIXED of SAS 9.1 (SAS institute, 2002). The least significant difference (LSD) test was used for multiple treatment comparisons using the LSMEANS statement of SAS 9.1 (SAS institute, 2002) with letter grouping obtained using the SAS pdmix800 macro (Saxton, 1998). For the different statistical tests, significance was declared at $P \leq 0.05$. The REG procedure of SAS 9.1 (SAS institute, 2002) was used to provide regression models for assessment of relation between SkEO and water consumption.

RESULTS

In trail 1, average daily weight gain (WG), feed intake(FI), water intake (WI), feed conversion ratio (FCR) and FI-to-WI ratio were gathered and analyzed for the 1 to 7, 8 to 14, 15 to 21, 22 to 28 and 1 to 28 day periods. Addition of SkEO into drinking water significantly decreased WG of the birds ($P < 0.05$). Significant adverse effects associated with WG and WI was consistent in all weekly periods in a dose-dependent linear fashion (Table 3). The mean FI was significantly reduced by increasing levels of SkEO in drinking water of the birds during 1 to 7, 8 to 14 and 15 to 21 days of age ($P < 0.05$) but the same pattern was not observed for 22 to 28 as well as 1 to 28 days. Supplementation of drinking water with SkEO significantly increased FCR ($P < 0.05$) but the difference were not dose-dependent (Table 3). The most obvious adverse effect of SkEO was reduced WI (Table 4) where the significant negative dose-responses were pointed out by regressing WI versus the level of SkEO for the 1 to 7, 8 to 14, 15 to 21, 22 to 28 as well as 1 to 28 days of age (Figure 1). The mean FI: WI ratio were also reduced for the birds received the treated water, however, there was no consistent negative decrease in FI: WI for increasing levels of SkEO in drinking water (Table 4).

Mortality data were gathered daily but they were not analyzed. During the first trial, only 2 and 1 dead chicks were observed in 1.5 and 1.0 g/L SkEO-treatments, respectively. There was also no mortality for 2.0 and 2.5 g/L SkEO-treatments in the second trial. Carcass weight (CW) in male and female birds significantly reduced by addition of SkEO in drinking water at day 28 of age ($P < 0.05$). Patterns of decrease in CW were similar in both sexes, with the differences observed in pre-slaughter live body weight of the birds (Table 5). The mean carcass yield at day 28 was not different among the treated and control birds except for those which received 2.5 g/L SkEO. They showed significant decreased CY in both male and females by 4.1 and 3.57%, respectively, compared to control birds. The mean abdominal fat percent significantly decreased in the treated birds and it was lower for the male birds which received 0.5 g/L SkEO compared to the other birds of the same sex.

In females, abdominal fat was not significantly altered in the birds which received SkEO-treated water but the lowest value was found for the same treatment as males (Table 5). At day 28 of age, it was clear that exposure of the broiler chicks to high doses of SkEO through drinking water significantly suppressed almost all production parameters. To assess the reversibility of such unfavorable effect, the treatments rearranged into the second trial.

At day 42 of age, the mean live body weight was significantly increased for all birds especially for those which received 0.5 g/L SkEO during 1 to 28 days of age (Figure 2). The mean body weight for the birds received 0.5 g/L during 1 to 28, and 0.4 g/L during 28 to 42 days was 0.35% (8 g) greater than the control birds.

DISCUSSION

Supplementation of drinking water with high doses of

Table 3. Effect of essential oils of *Satureja khuzistanica* on weight gain, feed intake and feed conversion ratio in broiler chicks up to 28 days of age.

Days (d)	Cont+*	Cont-*	Essential oils of *S. khuzistanica* (g/L)					SEM[1]	P-value
			0.5	1.0	1.5	2.0	2.5		
Average weight gain (g/day per bird)									
1-7	13.25^{b}	15.03^{a}	12.81^{bc}	13.12^{bc}	12.04^{cd}	12.43^{bcd}	11.62^{d}	0.261	0.0005
8-14	28.35^{ab}	29.99^{a}	27.96^{bc}	26.33^{cd}	24.78^{de}	23.62^{ef}	22.20^{f}	o.608	0.0001
15-21	74.04^{b}	78.66^{a}	70.10b^{c}	67.21^{c}	62.62^{d}	61.65^{d}	59.19^{d}	1.536	0.0001
22-28	66.64^{a}	66.42^{a}	66.00^{a}	58.03^{b}	57.45^{bc}	52.66^{bc}	50.40^{c}	1.610	0.0010
1-28	40.86^{ab}	42.35^{a}	39.64^{b}	36.52^{c}	34.83^{c}	33.06^{d}	31.56^{d}	0.866	0.0001
Feed intake (g/day per bird)									
1-7	17.39^{ab}	18.99^{a}	16.78^{bc}	16.64^{bc}	15.89^{c}	16.34^{c}	15.77^{c}	0.252	0.0002
8-14	46.98^{ab}	48.43^{a}	46.29^{b}	45.50^{b}	43.62^{cd}	43.74^{b}	42.00^{d}	0.492	0.0001
15-21	75.74^{abc}	80.18^{a}	71.47^{cd}	72.02^{cd}	73.68^{bcd}	78.32^{ab}	69.55^{d}	0.966	0.0064
22-28	138.51^{cde}	123.95^{e}	144.67^{abc}	145.67^{ab}	141.52^{bcd}	130.00^{de}	153.67^{a}	0.465	0.0015
1-28	68.42^{bc}	67.89^{c}	69.80^{ab}	69.95^{ab}	68.68^{abc}	67.10^{c}	70.25^{a}	0.308	0.0169
Feed conversion ratio (g feed: g gain)									
1-7	1.31^{ab}	1.26^{b}	1.30^{ab}	1.27^{b}	1.32^{ab}	1.31^{ab}	1.36^{a}	0.010	0.0842
8-14	1.66^{cd}	1.61^{d}	1.66^{cd}	1.73^{c}	1.76^{bc}	1.85^{ab}	1.89^{a}	0.025	0.0007
15-21	1.38^{c}	1.39^{c}	1.38^{c}	1.49^{c}	1.64^{b}	1.80^{b}	1.65^{a}	0.038	0.0001
22-28	2.00^{c}	1.87^{c}	2.19^{bc}	2.51^{b}	2.47^{b}	2.54^{b}	3.05^{a}	0.093	0.0007
1-28	1.67^{cd}	1.60^{d}	1.76^{c}	1.92^{b}	1.97^{b}	2.03^{a}	2.23^{a}	0.047	0.0001

*Control +, The birds received drinking water supplemented with 3000 g/ L polysorbate-80 throughout the trial; Control -, The birds received drinking water with no additive.[1]Standard error for overall mean. [a-g]Means within a raw without a common superscript differ significantly (P<0.05).

Table 4. Effect of essential oils of *Satureja khuzistanica* on water consumption and feed-to-water ratio in broiler chicks up to 28 days of age.

Days (d)	Cont+*	Cont-*	Essential oils of *S. khuzistanica* (g/ L)					SEM[1]	P-value
			0.5	1.0	1.5	2.0	2.5		
Water intake (ml/bird/day)									
1-7	58.67^{a}	47.14^{b}	46.52^{c}	43.67^{d}	39.76^{f}	41.90^{e}	39.09^{g}	1.381	0.0001
8-14	127.88^{a}	126.19^{b}	110.48^{c}	104.81^{d}	99.50^{f}	100.24^{e}	94.50^{g}	2.737	0.0001
15-21	226.19^{a}	216.19^{b}	181.31^{c}	169.86^{d}	161.90^{e}	154.05^{f}	147.69^{g}	6.320	0.0001
22-28	436.67^{a}	450.00^{b}	326.67^{c}	305.00^{d}	293.00^{e}	292.50^{f}	261.67^{g}	15.45	0.0001
1-28	212.35^{a}	209.88^{b}	166.24^{c}	155.83^{d}	148.52^{e}	147.17^{f}	135.74^{g}	6.395	0.0001
Water intake (ml) : feed intake (g) ratio									
1-7	3.38^{a}	2.48^{c}	2.78^{b}	2.63^{bc}	2.50^{c}	2.57^{c}	2.48^{c}	0.070	0.0001
8-14	2.72^{a}	2.61^{b}	2.39^{c}	2.30^{cd}	2.28^{d}	2.29^{d}	2.25^{d}	0.039	0.0001
15-21	2.99^{a}	2.70^{b}	2.54^{b}	2.36^{c}	2.20^{cd}	1.97^{e}	2.13^{de}	0.075	0.0001
22-28	4.68^{b}	5.19^{a}	3.23^{c}	3.00^{c}	2.96^{c}	3.22^{c}	2.44^{d}	0.210	0.0001
1-28	3.44^{a}	3.24^{b}	2.73^{c}	2.57^{d}	2.47^{e}	2.51^{de}	2.32^{f}	0.087	0.0001

*Control+, The birds received drinking water supplemented with 3000 g/ L polysorbate-80 throughout the trial; Control-, The birds received drinking water with no additive.[1]Standard error for overall mean. [a-g]Means within a raw without a common superscript differ significantly (P<0.05).

SkEO (ranging from 0.5 to 2.5 g/L) for broiler chicks unfavorably affected almost all production parameters. The poultry nutrition researchers usually attribute unappreciated production performance to gastro-intestinal related events including interaction of essential oils with digestion process, counteracting with some

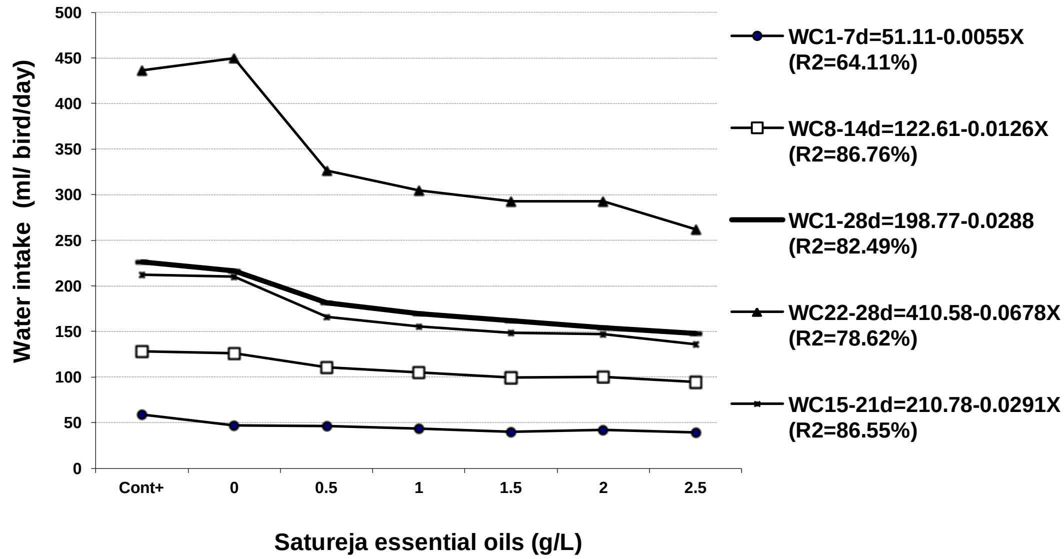

Figure 1. Effect of essential oils of *Satureja khuzistanica* on average weekly water intake in broiler chicks up to 28 days of age.

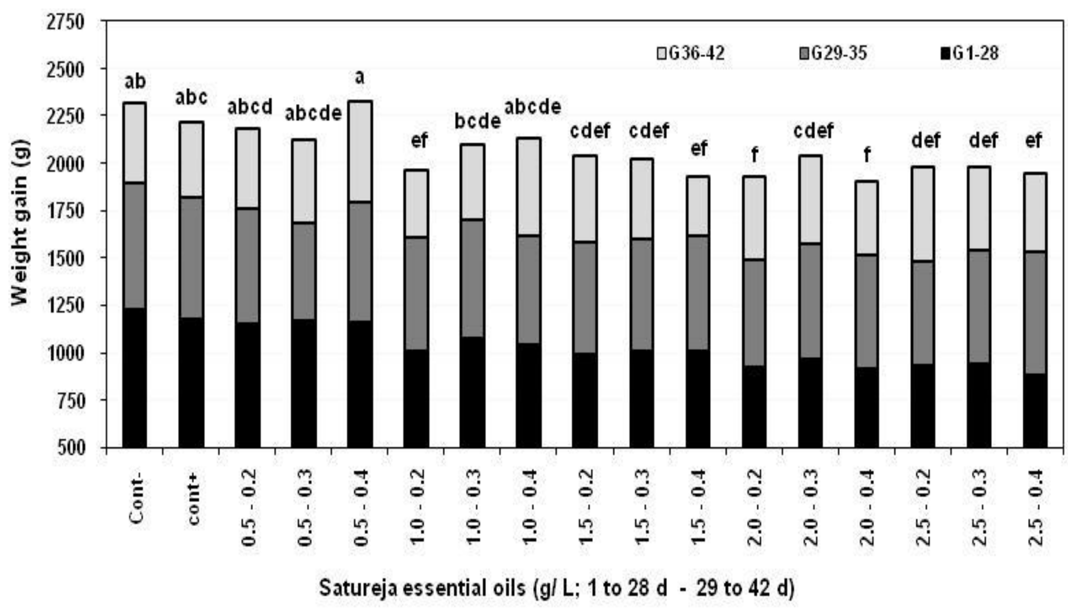

Figure 2. Effect of essential oils of *Satureja khuzistanica* on weight gain of broiler chicken during 1 to 28 (G 1-28), 29 to 35 days (G 29-35) and 36 to 42 days (G 36-42). The letters above the bars represent difference treatments for 1- to 42 days of age. bars (means) not sharing a common letter are significantly different (P<0.05).

aspects of metabolism, disruption in some metabolic pathways. Nevertheless, during the last 2 decades, it has been frequently reported that oral administration of essential oils to chicks usually improves digestion (Lee et al., 2004a). Especially most of those essential oils which contain pungent compound, such as carvacrol, have been shown to stimulate bile secretion (Sambaiah and Srinivasan, 1991) and to stimulate digestive enzyme activities of intestinal mucosa and of pancreas in chicken (Platel and Srinivasan, 2000).

Despite of the above findings, the digestive tonic capability of essential oils may differ by the nature of active constitutes and their whole composition. For instance, it has been reveled that the dietary levels of thymol, carvacrol and cinnamaldehyde have dissimilar effects on enzyme activities in broilers chicks (Lee et al., 2003, 2004a). In the present study, the most prominent effect of treatments was reduced water consumption for all the birds grown on SkEO-added water. Such effect was clearly attributed to water flavor. The bitter and pungent tasting principles in SkEO caused significant drop in WI. The birds showed no adaptation with water flavor as the rate of decreased WI was elevated in advanced ages (Figure 1). Considering the vital role of water in all aspects of metabolism, reduced WI could by itselfcause all adverse effects monitored in productive performance indications. It has to be mentioned that there are evidences suggesting reduced WI imposed by high doses of SkEO which may impair kidney function (Khosravinia et al., 2012).

Specific effects of flavors on chickens performance has not received much attention because poultry may not actually respond to flavor when compared to mammals, and the effect of flavors on performance of birds are regarded as irrelevant (Moran, 1982). Despite early evidences that flavors could affect feed intake (Deyone et al., 1962), recently it has been shown that dietary administration of carvacrol to broiler chicks reduced feed intake by modulating the birds appetite (Lee et al., 2003). These researchers did not measure the bird's WI. However, others verified that the bitter-tasting essential oils adversely affect WI in broiler flocks (Lee et al., 2004a, 2004b).

Windisch et al. (2008) reviewed the effect of dietary supplementation of many phytogenic extracts and concluded that despite conflicting results on feed intake and weight gain, almost all herbal extracts showed adverse effect on feed conversion ratio. Therefore, in the finding of the present study, in accordance with the conclusion made by Lee et al. (2004a) and Windisch et al. (2008), it is confirmed that the assumption that phytogenic extracts improve the palatability of feed but does not seem to be justified in general.

By attributing the adverse effects of SkEO-treated water on broiler performance to reduced WI in a dose-dependent pattern, it was postulated that decreased SkEO doses in water may provide opportunity to the birds to compensate the weight gain. The results from Trial 2 verified the idea as the lowered doses of SkEO significantly enhanced weight gain in the birds which received 0.5 g/L SkEO during 1 to 28 days of age (Figure 2). The birds which received 1.0 and 1.0 < g/L SkEO also exhibited improved weight gain but it seems that they needed more time to entirely compensate the reduced weight gain. These findings reveal that the adverse effects of high doses of SkEO on broiler performance are reversible. Moreover, they give an indication that SkEO has to be administered through drinking water to broiler chicken at doses lower than 0.5 g/L.

It has been reported that dietary phytogenic oils such as anice oil (El-Deek et al., 2003) or their active compounds such as carvacrol affect fat metabolism in chicken (Case et al., 1995). In broiler chicken, lipids and specially triglycerides are stored in adipocytes (mainly abdominal fat) and hepatocytes while de novo lipogenesis is very limited in adipose tissue (Saadoun and Leclercq, 1978). There is an apparently general assumption that almost all the fat accumulates in broiler adipose tissue including abdominal fat is synthesized in the liver or derived from the diet (Griffin et al., 1992; Hermier, 1997). In the current study, the mean relative abdominal fat weight was 19 and 8% lower for the male and female birds which received 0.5 g/L SkEO compared to the control birds of the same sex, respectively (Table 5). The differences were statistically significant in males ($P < 0.0238$), indicative that SkEO-added water exhibit hyperlipidemia properties by affecting lipids metabolism. As far as excessive accumulation of fat in abdominal cavity of broiler chickens is a major concern for poultrymen, processing units as well as consumers (Weltzien, 2002), such effect may exert economic benefits and mask some aspects of depressed productive performance.

Conclusion

The present study revealed that administration of SkEO at 0.5 to 2.5 g/L through drinking water adversely affected production performance of broiler chicken up to day 28 of age. The adverse effect, however, is reversible. By lowering the SkEO dose to below 0.5 g/L, the birds could be able to regain the performance over a course of time depending on the earlier SkEO doses received. Our observation implies that the reduced water intake due to pungent and caustic flavor of SkEO-treated water is the main cause for suppressed performance but the molecular bases remains unknown.

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