

# Hypolipidemic Effects of *Satureja khuzistanica* Essential Oil in Broiler Chicken are Realized Through Alteration in Steroid Hormones

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## Abstract

Two experiments were conducted to evaluate the effects of high and low doses of *Satureja Khuzistanica* essential oil (SEO) on blood lipid constituents and sex hormones in broiler chicks. In trail 1, 420 one-day-old Cobb 500 chicks of both sexes were randomly placed in 21 floor pens to examine the effect of supplementation of drinking water with 0, 0.5, 1.0, 1.5, 2.0, and 2.5 g/L SEO or 3.0 g/L Polysorbate-80 (control+) in 3 replicates of 20 birds each. In Experiment 2, 720 newly hatched male Arian chicks were randomly assigned to 36 floor pens to examine the effect of 0, 0.2, 0.3, 0.4 and 0.5, g/L SEO or 0.5 g/L Polysorbate-80 (control+) in 6 replicates of 20 birds each. As a result of this study, SEO at 0.5 and 0.3 g/L in Experiments 1 and 2, respectively, modulated serum cholesterol and high density lipoproteins (HDL) levels, albeit the differences were not significant when compared to appropriate control groups ( $P>0.05$ ). The SEO at 0.5 g/L caused significant decrease (16%) in abdominal fat percentage of the birds at 28 d (Trial 1;  $P<0.05$ ). In trail 2, inclusion of SEO into drinking water at 0.2, 0.3, 0.4 and 0.5 g/L significantly reduced the serum estradiol to 50, 50 and 45 percent, respectively, and increased serum testosterone level by about 2, 4, 4 and 5 folds, respectively, in comparison with the control- birds ( $P>0.05$ ). The results propose the possibility of testosterone-coupled hypolipidemic properties for SEO in broiler chicken.

**Keywords:** Broiler chicken, Carvacrol, Estradiol, Hypolipidemic effect, Testosterone

## Broyler Tavuklarda *Satureja khuzistanica* Esansiyel Yağın Hipolipidemik Etkileri Steroid Hormonlardaki Değişim Yoluyla Oluşturulur

### Özet

Yüksek ve düşük doz *Satureja Khuzistanica* esansiyel yağ (SEO) uygulamasının broyler civcivlerde kan lipid bileşenleri ve seks hormonları üzerine etkilerini değerlendirmek üzere iki çalışma yürütüldü. Birinci çalışmada, 420 adet her iki cinsiyetten bir günlük Cobb 500 civciv rastgele olarak her birinde 20 adet civciv bulunan 21 adet yer kümeslerine yerleştirildi ve 3 tekrar olmak üzere içme suyu içerisinde 0, 0.5, 1.0, 1.5, 2.0 ve 2.5 g/L SEO veya 3.0 g/L Polysorbate-80 (kontrol+) uygulamasının etkileri incelendi. İkinci çalışmada, 720 adet yumurtadan yeni çıkmış erkek Arian civciv rastgele olarak her birinde 20 adet civciv bulunan 36 adet yer kümeslerine yerleştirildi ve 6 tekrar olmak üzere 0, 0.2, 0.3, 0.4 ve 0.5, g/L SEO veya 0.5 g/L Polysorbate-80 (kontrol+) uygulamasının etkileri incelendi. Çalışma sonucunda 1 çalışmada 0.5 ve 2. çalışmada 0.3 g/L SEO uygulamasının serum kolesterol ve yüksek yoğunluklu lipoprotein (HDL) seviyelerini azalttığı ancak farkların kontrol grupları ile karşılaştırıldığında anlamlı olmadığı ( $P>0.05$ ) tespit edilmiştir. 0.5 g/L SEO 28. günde civcivlerin abdominal yağ yüzdesinde (%16) anlamlı bir düşmeye neden oldu (Deney 1;  $P<0.05$ ). İkinci deneyde, kontrol grubuyla karşılaştırıldığında 0.2, 0.3, 0.4 ve 0.5 g/L SEO azalmaları serum östradiyol seviyelerini yüzde 50, 50 ve 45 oranlarında anlamlı derecelerde azaltırken serum testosteron seviyelerini sırasıyla 2, 4, 4 ve 5 katlarında arttırdı ( $P>0.05$ ). Elde edilen sonuçlar broyler tavuklarda SEO için testosteron ilişkili hipolipidemik etkilerin olabileceğini göstermektedir.

**Anahtar sözcükler:** Broyler tavuk, Carvacrol, Östradiyol, Hipolipidemik etki, Testosteron

## INTRODUCTION

The essential oil derived from *Satureja Khuzistanica* contains above 90 percent carvacrol. Carvacrol is described

as a phenolic, caustic and bitter tasting compound <sup>[1]</sup> which demonstrates significant antioxidant <sup>[2]</sup> and antimicrobial <sup>[3]</sup> properties. Accordingly, it has been reported that the carvacrol-reached essential oils from Lamiaceae



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family plants such as savory has antioxidant [4,5], antiviral [6], antibacterial [7] and antifungal [8] effects.

Recently, particular attention has been focused on hypolipidemic effects of phytochemical remedies in poultry meat and egg. Among many herbal spices or extracts examined, essential oils of onion and garlic [9,10], thyme [11-13], turmeric [14,15] and oregano [16] exhibited superior hypocholesterolemic effects in chicken. It has been suggested that such effects are mainly induced through the inhibition of the key enzymes in cholesterol and lipid synthesis [17-19]. On the other hand, many clinical investigations showed that herbal extracts are able to alter the reproductive functions in mice [20] and white plymouth rock-mini cocks [21] through affecting sex hormones secretion and their physiological balance. Considering the anabolic effects of androgens, the hypolipidemic effects of herbal extracts may be the consequence of abovementioned alterations in the sex hormones.

In spite of a significant decrease in serum triglyceride levels observed with a carvacrol reached plant extract from savory in diabetic and hyperlipidemic rats and no change in cholesterol level in hyperlipidemic rats [4], the hypolipidemic properties of carvacrol and carvacrol-reached plant extracts remain largely uninvestigated. In view of the scarce experimental results on hypolipidemic properties of carvacrol in connection with sex hormones in avian species, two studies were undertaken to examine the effect of high and low doses of *Satureja khuzistanica* essential oil containing 94.16 percent carvacrol, on blood fat constituents and sex hormones, while it was administered through drinking water into broiler chicks.

## MATERIAL and METHODS

### Preparation of Essential Oil

The essential oils used for this experiment freshly provided from a particular species of savory herbs known as *Satureja khuzistanica* Jamzad, an endemic plant distributed in southern part of Iran [22,23]. The aerial parts of the plant collectively contain up to 3 percent of essential oils which is spectacularly rich in carvacrol (up to 95 percent) [24]. The aerial parts of *Satureja khuzistanica* were manually harvested during the flowering stage of plant. The collected materials 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 random sample of the stored oil was analyzed for the composition of essential oils using the methods described by Hadian et al. [22]. The resulting composition verified that it is highly-reached in carvacrol by >94 percent. The major constituents in the remaining impurity were determined as *p*-Cymene (0.96%) and  $\gamma$ -Terpinene (0.51%) (Table 1).

### Experimental Flocks

In Experiment 1, 420 day-old Cobb 500, broiler chicks (43.65±1.2 g) were provided from a local commercial hatchery. The birds were housed in a concrete floor, cross-ventilated windowless shed where they were randomly placed in 21 pens (90×180 cm; at density of 0.08 m<sup>2</sup>/bird). Each pen was equipped with an infra-red brooder. The treatments were arranged into 3 blocks to account for variations in the ventilation system. Seven experimental treatments including 0 (control-), 0.5, 1.0, 1.5, 2.0, and 2.5 g/L SEO or 3.0 g/L Polysorbate-80 as emulsifier agent (control+) were administered *ad libitum* via drinking water to 3 replicate pens of 20 birds each, up to the day 28 of age. The solution was prepared for each treatment in a daily basis and the remaining was discarded. The chicks were maintained on a 24-h light schedule. Feed and water supplied to the birds through a tube feeder and a manual waterer in each pen, respectively. Corn and soybean meal based starter and grower diets were formulated using UFFDA software according to the NRC [25] recommendations (Table 2). The Diets and water were provided for *ad libitum* consumption throughout the 28-d experimental period.

In the second experiment, 720 one-day-old Arian broiler chicks were obtained from a commercial hatchery and housed in the same shed with similar flocking density as Experiment 1 up to 42 days of age. The chicks were randomly assigned to 36 pens arranged in 6 rows (blocks/replicates). Corn and soybean meal based pre starter, starter, grower and finisher diets (Table 2) and water was provided for *ad libitum* consumption throughout the 42-d experimental period. Diets were pelleted and the pellet sizes adjusted to the age of the birds. The six experimental treatments consisting 0 (control-), 0.2, 0.3, 0.4 and 0.5 g/L SEO or 0.5 g/L Polysorbate-80 (at 1:1 ratio v/v; control+) were continuously provided (through drinking water) for 6 replicate pens of 20 birds each, up to 42 days of age.

Table 1. Composition of *Satureja khuzistanica* essential oil

Tablo 1. *Satureja khuzistanica* esansiyel yağın bileşeni

Compound	RI <sup>1</sup>	Composition (%)	Identification <sup>2</sup>
Carvacrol	1282	94.16±0.46	RI, MS, Col
<i>p</i> -Cymene	1017	0.96±0.86	RI, MS, Col
$\gamma$ -Terpinene	1053	0.51±0.23	RI, MS, Col
(Z)- $\beta$ -Oeimene	1036	0.42±0.08	RI, MS
$\alpha$ -terpinole	1175	0.32±0.45	RI, MS
Myrene	981	0.21±0.19	RI, MS
$\alpha$ -Terpinene	1013	0.18±0.12	RI, MS, Col
$\alpha$ -Thujene	925	0.14±0.14	RI, MS
$\alpha$ -Pinene	933	0.12±0.05	RI, MS, Col

<sup>1</sup> RI; Retention indices determined relative to *n*-alkanes (C<sub>6</sub>-C<sub>24</sub>) on a DB-5GC column

<sup>2</sup> RI; Retention indices, MS; mass spectra, Col; co-injection

**Table 2.** The ingredients and the nutrient composition of the experimental diets**Tablo 2.** Deneysel diyetin içerik ve besin bileşenleri

Item	Experiment 1		Experiment 2			
	Starter (1-14d)	Grower (15-28d)	Prestarter (1-7d)	Starter (8-21d)	Grower (22-35d)	Finisher (36-42d)
<b>Ingredient (%)</b>						
Corn	55.00	63.00	45.3	47.9	46.7	47.8
Soybean meal	36.00	28.10	34.8	33.9	26.9	23.6
Fish meal	3.17	3.20	-	-	-	-
Wheat	-	-	7	12	20	22
Soybean oil	3.40	3.20	1.3	1.2	1.3	1.4
Corn gluten	-	-	6	-	-	-
Calcium carbonate	-	-	1.20	1.10	1.11	1.15
Dicalcium phosphate	1.20	1.00	2.16	1.94	2.01	2.07
DL-methionine	0.10	0.15	0.34	0.31	0.32	0.33
L-lysine	0.15	0.15	0.15	0.13	0.14	0.14
Vitamin permix <sup>1</sup>	0.25	0.30	0.28	0.25	0.26	0.27
Mineral permix <sup>2</sup>	0.30	0.30	0.28	0.25	0.26	0.27
Salt	0.25	0.25	0.39	0.35	0.36	0.38
Coline chloride	-	-	0.14	0.13	0.13	0.14
<b>Calculated value</b>						
ME (kcal/kg)	3100	3220	2962	2880	2952	2993
Crude protein (%)	23.00	19.12	24.28	21.15	18.82	17.63
Crude fat (%)	3.90	3.70	4.32	3.13	3.50	3.74
Crude fiber (%)	3.01	2.87	3.74	3.75	3.48	3.33
Calcium (%)	0.85	1.00	1.10	1.00	1.00	1.00
Available P (%)	0.42	0.50	0.55	0.50	0.50	0.50
Methionine (%)	0.51	0.40	0.59	0.51	0.45	0.43
Lysine (%)	1.44	1.03	1.29	1.09	0.95	0.88

<sup>1</sup> Supplied per kg of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg

<sup>2</sup> Supplied per kg of diet: vitamin A, 18000 IU; vitamin D<sub>3</sub>, 4000 IU; vitamin E, 36 mg; vitamin K<sub>3</sub>, 4 mg; vitamin B<sub>12</sub>, 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

### Data Collection

At the end of Experiment 1 (28 d) two male and two female birds per pen,  $\pm 50$  g of the mean pen weight for each sex, and at close of Experiment 2 (42 d), one male bird with the closest mean to the mean pen weight for males were killed for blood and abdominal fat collection. Abdominal fat (in Experiment 1) was manually collected and recorded as the summation of fat deposited around proventriculus and gizzard plus fat pad for each bird. Serum low-density lipoprotein (LDL), High-density lipoprotein (HDL), total cholesterol (TC), and triglyceride (TG) concentrations were estimated in both experiments using SEPPIM Diagnostic Kits (SEPPIM S.A.S., Zone Industrielle, 61500, SEES, France) in two replicates/sex per pen, at 25°C. The concentration of estradiol and testosterone in serum were measured by a solid-phase RIA in Experiment 2 using reagents provided by IMMUNOTECH kits (IMMUNOTECH SAS, 130 av. De Lattre de Tassigny – B.P. 177 – 13276 Marseille Cedex 9 France) in 6 male birds per treatment.

### Statistical Analysis

The collected data were analyzed using PROC MIXED of SAS 9.3 [26]. The LSD test was used for multiple treatment comparisons using the LSMEANS statement of SAS 9.1 [26] with letter grouping obtained using the SAS pdmix800 macro [27]. For all variables in Experiment 2, the effect of birds' live weight before slaughter, as a continuous random variable, was also included in the statistical model. For the different statistical tests, significance was declared at  $P \leq 0.05$ . Linear and quadratic contrasts were used for the effects of SEO levels on the studied variables.

## RESULTS

### Experiment 1

Data for Experiment 1 are presented in [Tables 3 and 4](#), and [Fig. 1](#) and [Fig. 2](#). Administration of SEO through

drinking water had no significant effect on serum TG, LDL, HDL and total cholesterol (TC) levels of the birds at day 28 of age ( $P>0.05$ ). However, the concentrations of TC and HDL (pooled data over sexes) were reduced by 1.41 and 8.50%, respectively, in the birds received 0.5 g/L SEO compared to control- birds (Table 3). In sexwise analysis of data, male and female serum LDL, HDL and TC levels, but not triglycerides, were affected by SEO-treated water in dissimilar ways (Table 4). As shown in Fig. 1, accumulation of fat in abdominal cavity of the birds was reduced by SEO-added water ( $P=0.0262$ ). Supplementation of 0.5 g/L SEO caused approximately 16% decrease in abdominal fat-to-body weight ratio (AFP) at 28 d. Addition of SEO in drinking water at doses  $>0.5$  g/L exhibited adverse effect of AFP (Fig. 1). The serum TC and HDL level was significantly influenced by the sex of the birds. The male broilers were

**Table 3.** Effect of high doses of Satureja khuzistanica essential oil (SEO) in drinking water on serum concentration of triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL) and high density lipoproteins (HDL) in broiler chicks at day 28 of age (Experiment 1)

**Tablo 3.** İçme suyu içerisinde yüksek doz Satureja khuzistanica esansiyel yağ (SEO) uygulamasının 28. günde Broyler tavukların trigliserid (TG), total kolesterol (TC), düşük yoğunluklu lipoproteinler (LDL) ve yüksek yoğunluklu lipoproteinler (HDL) serum konsantrasyonlarına olan etkileri (Deney 1)

Factor\Level	TG	TC	LDL	HDL
	mg/ 100 ml			
<b>SEO (g/ L)</b>				
Control+ <sup>1</sup>	35.50	150.16 <sup>a</sup>	62.25	75.58 <sup>a</sup>
0.0	36.83	157.26 <sup>a</sup>	66.83	74.91 <sup>a</sup>
0.5	40.17	122.75 <sup>b</sup>	52.08	60.92 <sup>b</sup>
1.0	37.08	125.51 <sup>b</sup>	56.50	65.25 <sup>ab</sup>
1.5	38.16	132.59 <sup>ab</sup>	55.83	71.50 <sup>ab</sup>
2.0	40.50	128.83 <sup>ab</sup>	56.67	66.25 <sup>ab</sup>
2.5	37.33	129.41 <sup>ab</sup>	57.58	65.25 <sup>ab</sup>
<b>Sex</b>				
Male	38.77	135.46	57.39	68.97
Female	37.11	136.35	52.32	68.10
SEM <sup>2</sup>	1.087	2.546	1.300	1.321
$P > F$				
<b>ANOVA results</b>				
SEO	0.8859	0.0094	0.1463	0.0270
Sex	0.4765	0.8464	0.1135	0.7257
SEO × Sex	0.2480	0.0511	0.0115	0.0344
<b>Trends<sup>3</sup></b>				
Linear	0.8721	0.0923	0.1291	0.1007
Quadratic	0.5710	0.0946	0.0874	0.0426

<sup>1</sup> Control+; The birds received drinking water supplemented with 3.0 g/L polysorbate-80 throughout the experiment, and Control-; The birds received drinking water with no additive  
<sup>2</sup> Standard error for overall mean  
<sup>3</sup> Trend analysis was done for SEO levels  
<sup>a-b</sup> Means within a column for each factor without a common superscript differ significantly ( $P<0.05$ )

**Table 4.** Effect of high doses of Satureja khuzistanica essential oil (SEO; g/L) in drinking water on serum concentration of triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL) and high density lipoproteins (HDL) in male and female broiler chicks at day 28 of age (Experiment 1)

**Tablo 4.** İçme suyu içerisinde yüksek doz Satureja khuzistanica esansiyel yağ (SEO; g/L) uygulamasının 28. günde dişi ve erkek Broyler tavukların trigliserid (TG), total kolesterol (TC), düşük yoğunluklu lipoproteinler (LDL) ve yüksek yoğunluklu lipoproteinler (HDL) serum konsantrasyonlarına olan etkileri (Deney 1)

Factor\Level	TG	TC	LDL	HDL
	mg/ 100 ml			
<b>Males</b>				
Control+ <sup>1</sup>	37.37	141.25	55.62	71.62
0.0	41.00	131.33	56.00	68.67
0.5	42.50	130.33	53.17	62.83
1.0	38.67	140.50	60.17	72.67
1.5	40.33	156.83	70.50	78.50
2.0	41.25	126.62	55.62	65.25
2.5	50.33	123.33	51.17	62.83
SEM <sup>2</sup>	2.606	3.944	1.940	1.881
$P>F$	0.8893	0.3009	0.1597	0.2223
<b>Trends</b>				
Linear	0.0985	0.3491	0.3251	0.2008
Quadratic	0.1529	0.1528	0.1280	0.1134
<b>Females</b>				
Control+ <sup>1</sup>	31.75	125.50	53.00	61.00
0.0	35.44	122.22	45.22	63.67
0.5	37.83	115.17	51.00	59.00
1.0	35.50	118.83	52.83	57.83
1.5	36.00	125.00	51.17	64.50
2.0	39.00	138.25	58.75	68.25
2.5	44.17	137.17	62.33	67.67
SEM <sup>2</sup>	1.492	2.637	1.823	1.422
$P>F$	0.5365	0.1874	0.1598	0.3944
<b>Trends</b>				
Linear	0.5201	0.4612	0.2381	0.3011
Quadratic	0.0970	0.1036	0.0872	0.1905

<sup>1</sup> Control+; The birds received drinking water supplemented with 3.0 g/L polysorbate-80 throughout the experiment  
<sup>2</sup> Standard error for overall mean  
<sup>a-b</sup> Means within a column for each factor without a common superscript differ significantly ( $P<0.05$ )

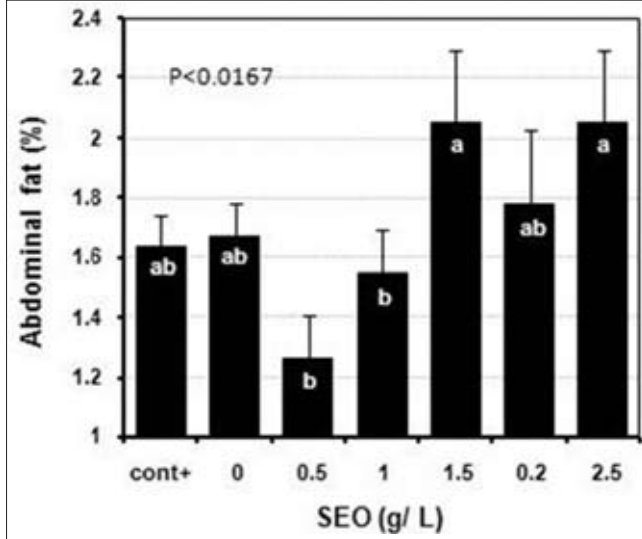
found to have 8.55 and 9.01 percent lower TC and HDL, respectively, in serum. No parameter of consideration was affected by sex × SEO interaction in the Experiment 1 (Table 3).

## Experiment 2

Incorporation of low doses of SEO (ranging from 0.2 to 0.5 g/L) in drinking water did not affect plasma TG, LDL, HDL and TC levels of the birds at the day 42 of age

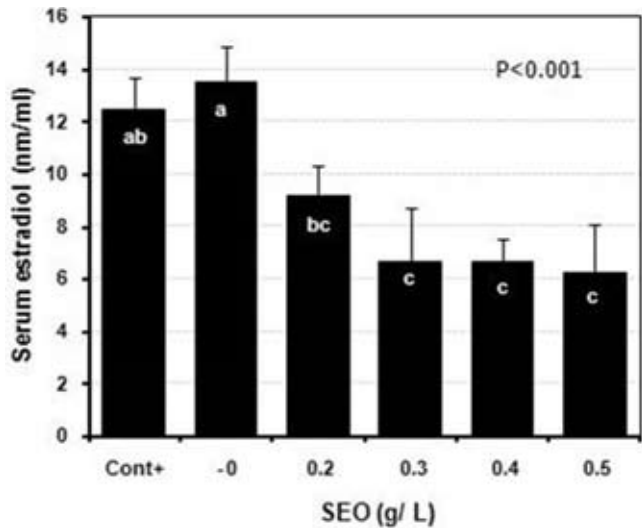


( $P>0.05$ ; Table 5). The concentrations of TC, LDL, and HDL, nevertheless, were modulated by approximately 8, 9 and 5%, respectively, by SEO-treated water at 0.3 g/L compared to the control- birds (Table 5). In contrast to the Experiment 1, live body weight of the birds before slaughter showed significant effects on serum LDL and TC levels.



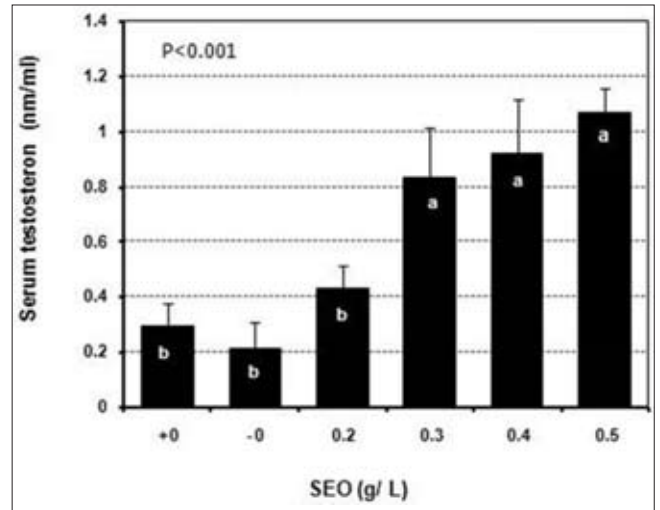
**Fig 1.** Effect of high doses of *Satureja khuzistanica* essential oil (SEO) in drinking water on abdominal fat (%) in broiler chicks at 28 days of age (Experiment 1). Means without a common superscript (<sup>a-b</sup>) differ significantly ( $P<0.017$ )

**Şekil 1.** İçme suyu içerisinde yüksek doz *Satureja khuzistanica* esansiyel yağ (SEO) uygulamasının 28. günde Broiler tavukların abdominal yağı (%) üzerine etkileri (Deney 1). Aynı üstsimgeye (<sup>a-b</sup>) sahip olmayan gruplar anlamlı oranda birbirlerinden farklıdır ( $P<0.017$ )



**Fig 2.** Effect of low doses of *Satureja khuzistanica* essential oil (SEO) in drinking water on serum estradiol level in male broiler chicken at day 42 of age (Experiment 2). Means without a common superscript (<sup>a-c</sup>) differ significantly ( $P<0.001$ )

**Şekil 2.** İçme suyu içerisinde düşük doz *Satureja khuzistanica* esansiyel yağ (SEO) uygulamasının 42. günde erkek Broiler tavukların serum östradiyol seviyeleri üzerine etkileri (Deney 2). Aynı üstsimgeye (<sup>a-c</sup>) sahip olmayan gruplar anlamlı oranda birbirlerinden farklıdır ( $P<0.001$ )



**Fig 3.** Effect of low doses of *Satureja khuzistanica* essential oil (SEO) in drinking water on serum testosterone level in male broiler chicken at 42 days of age (Experiment 2). Means without a common superscript (<sup>a-b</sup>) differ significantly ( $P<0.001$ )

**Şekil 3.** İçme suyu içerisinde düşük doz *Satureja khuzistanica* esansiyel yağ (SEO) uygulamasının 42. günde erkek broiler tavukların serum testosteron seviyeleri üzerine etkileri (Deney 2). Aynı üstsimgeye (<sup>a-c</sup>) sahip olmayan gruplar anlamlı oranda birbirlerinden farklıdır ( $P<0.001$ )

**Table 5.** Effect of low doses of *Satureja khuzistanica* essential oil (SEO) in drinking water on serum concentration of triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL) and high density lipoproteins (HDL) in male broiler chicks at day 42 of age (Experiment 2)

**Tablo 5.** İçme suyu içerisinde düşük doz *Satureja khuzistanica* esansiyel yağ (SEO) uygulamasının 42. günde erkek broiler tavukların trigliserid (TG), total kolesterol (TC), düşük yoğunluklu lipoproteinler (LDL) ve yüksek yoğunluklu lipoproteinler (HDL) serum konsantrasyonlarına olan etkileri (Deney 2)

Factor\Level	TG	TC	LDL	HDL
	µg/ 100 ml			
SEO (g/ L)				
Control+ <sup>1</sup>	80.66	147.33 <sup>ab</sup>	70.17 <sup>ab</sup>	74.00 <sup>ab</sup>
0.0	84.50	155.01 <sup>a</sup>	74.50 <sup>a</sup>	78.33 <sup>a</sup>
0.2	83.50	134.33 <sup>bc</sup>	63.83 <sup>abc</sup>	71.17 <sup>abc</sup>
0.3	76.83	132.33 <sup>bc</sup>	56.50 <sup>c</sup>	66.83 <sup>bc</sup>
0.4	81.83	152.83 <sup>c</sup>	58.67 <sup>bc</sup>	66.17 <sup>bc</sup>
0.5	80.50	122.67 <sup>c</sup>	53.83 <sup>c</sup>	61.17 <sup>c</sup>
SEM <sup>2</sup>	0.412	2.833	1.692	1.974
$P > F$				
ANOVA Result				
SEO	0.9886	0.0004	0.0064	0.0445
Trends				
Linear	0.3461	0.3471	0.0016	0.0876
Quadratic	0.1023	0.0120	0.2468	0.0432

<sup>1</sup> Control+; The birds received drinking water supplemented with 0.5 g/L polysorbate-80 throughout the experiment

<sup>2</sup> Standard error for overall mean

<sup>a-b</sup> Means within a column for each factor without a common superscript differ significantly ( $P<0.05$ )

Significant differences were found among treatments in mean serum estradiol and testosterone levels. Administration of SEO into drinking water at 0.3, 0.4 and 0.5 g/L significantly reduced the serum estradiol to 52, 50 and 48 percent, respectively, in comparison with the control- birds (Fig. 2). The mean serum testosterone significantly elevated in the birds received 0.2, 0.3, 0.4 and 0.5 g/L SEO by about 2, 4, 4 and 5 folds, respectively, compared to the control- birds (Fig. 3).

## DISCUSSION

The results of analysis of variance in the Experiments 1 and 2 (Table 3, 4 and 5) indicated that the administered doses of SEO had no effect on plasma lipid constituents. However, the serum cholesterol and HDL levels were the lowest for the birds receiving 0.5 g/L SEO (Table 3). These observations were coincided with the significantly reduced abdominal fat at 0.5 g/L SEO in Fig. 1, indicating the potential of SEO as a hypolipidemic water additive. Thus, from the results in Table 3 and Fig. 3, it appears that the "optimum inclusion level" for SEO in water for broilers is between 0.3 to 0.5 g/L water.

Although there was no alteration in plasma lipids, the pronounced decrease in abdominal fat of the birds received 0.5 g/L SEO in Experiment 1 (Fig. 1), indicates that SEO may affect lipid metabolism in broiler chicken. In broiler, lipids and especially triglycerides are mainly stored in adipocytes of the abdominal fat. It has been shown that *de novo* lipogenesis, i.e., synthesis of fatty acids, is very limited in abdominal fat [28]. Thus, triglyceride storage in abdominal fat compartments depends on the availability of a plasma lipid substrates originating from either the diet or lipogenesis in the liver [29,30]. We suggest the significant decrease (15%) in the abdominal fat of the 0.5 g/L SEO- treated birds was a response to decreased plasma LDL and HDL. Carvacrol as the main component of SEO, seems to affect LDL and/or HDL metabolism in extra hepatic metabolic routes [31]. These results are compliant with other reports which shown that dietary carvacrol significantly affect fat metabolism in chicken [11,32]. It has been revealed that oregano extract, which it is also rich in carvacrol, exhibit significant hypocholesterolemic effects in chicken [16]. From the results of the second experiment, remarkable decrease (Table 5) in plasma cholesterol, LDL, and HDL by 8, 9 and 5%, respectively, with 0.3 g/L SEO were concur with opposite alteration in plasma estradiol and testosterone levels (Fig. 2 and Fig. 3). These results are in consistent with the finding of Haeri et al. [33] who reported that oral administration of 150 and 225 mg/kg per day *Satureja khuzistanica* essential oils through drinking water significantly increased plasma testosterone concentration in male rats. In the study of caponization and testosterone effects on blood lipid in male chicken it has been demonstrated that testosterone decreases lipid storage capacity and inhibit lipid accumulation in

male chicks [34]. These results are interesting since the current knowledge proposed that the inhibitory action of essential oils on lipid metabolism regulatory enzymes is independent of the diurnal cycle of many hormones such as insulin, glucocorticoids, T3 and glucagons [35].

The modulated serum LDL and cholesterol in the first experiment could be attributed to the elevated serum testosterone level in the birds received 0.3 g/L SEO-added water. The results from sex-disconnect analysis of data in Experiment 1 also supported the above conclusion where an apparent dose-dependent response in serum LDL, HDL and TC levels were exhibited in male chicks, but not in females (Table 4). Chen et al. [34] in conformity with the idea confirmed by Whitehead et al. [36] reported that testosterone implantation in capons decreased the serum LDL and cholesterol level while triglycerides remained unaffected. Considering all variables in Experiments 1 and 2, it is barely credible to attribute the differences between the treated and control- birds as regards blood fat constituents to random variability. Therefore, two reasons could be pointed out to propose the possibility of hypolipidemic properties for SEO in broiler chicken under the circumstances which the current experiments were conducted. 1) The decreased abdominal fat in 0.5 g/L SEO-treated birds could be caused by modulated serum cholesterol, LDL, and HDL in trial 1. 2) The decreased levels of the same blood lipid constituents in 0.3 g/L SEO-treated birds could be associated with elevated serum testosterone, as an anabolic hormone, in trial 2.

Results propose the possibility of testosterone-linked hypolipidemic properties for carvacrol as well as carvacrol-reached plant extracts in broiler chicken under the circumstances which the current experiments were conducted.

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