

HYPOLIPIDEMIC EFFECTS OF CARVACROL IN RELATION WITH SEX HORMONES IN BROILER CHICKEN

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Original scientific paper

Abstract: Two experiments were conducted to evaluate the effects of high and low doses of carvacrol on blood lipid constituents and sex hormones in broiler chicks. Inclusion of carvacrol into the drinking water 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 the corresponding control groups ($P > 0.05$). Carvacrol at 0.5 g/ L significantly decrease (16%) abdominal fat percentage of the birds at 28 d (Trial 1; $P < 0.05$). In trail 2, concentration of estradiol in serum significantly reduced in all carvacrol-receiving birds compared with the control- birds ($P > 0.05$). Serum testosterone, however, increased in the birds received carvacrol at doses greater than 0.2 g/ L in comparison with the control birds ($P < 0.05$). The results propose the possibility of testosterone-coupled hypolipidemic properties for carvacrol in broiler chicken.

Key words: broiler chicken, carvacrol, estradiol, hypolipidemic effect, testosterone

Introduction

Carvacrol is described as a phenolic, caustic and bitter tasting compound (ARS, 2000) which demonstrates significant antioxidant (Guppert and Hall, 1998) and antimicrobial (Burt, 2004) properties. Accordingly, it has been reported that the carvacrol-bearing essential oils from Lamiaceae family plants such as savory has antioxidant (Abdollahi, et al., 2003, Radonic and Milos, 2003), antiviral (Yamasaki et al., 1998), antibacterial (Azaz et al., 2002) and antifungal (Skocibusic and Bezic, 2004) effects.

Recently particular attention has been focused on hypolipidemic effects of phytogetic remedies in poultry meat and egg. Among many herbal spices or

extracts examined, essential oils of onion and garlic (*Sklan et al., 1992, Konjufca et al., 1997*), thyme (*Case et al., 1995, Lee et al., 2004a, Lee et al., 2004b*), turmeric (*Honda et al., 2006, Sugiharto et al., 2011*) and oregano (*Brenes and Roura, 2010*) 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 (*Qureshi et al., 1983, Elson and Qureshi 1995, Crowell, 1999*). On the other hand, many clinical investigations showed that certain herbal extracts are able to alter the reproductive functions in animals through affecting sex hormones secretion and their physiological balance (*Dehghani et al., 2008, Grigorova et al., 2008*). 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 (*Abdollahi, et al., 2003*), 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 carvacrol, on blood fat constituents and sex hormones, while it was administrated through drinking water into broiler chicks.

Materials and methods

Preparation of carvacrol

Carvacrol, with 94 percent purity, was freshly provided from a particular species of savory herbs known as *Satureja khuzistanica* Jamzad, an endemic plant distributed in southern part of Iran (*Hadian et al., 2011, Zargari, 1990*). 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) (*Khosravinia et al., 2013*). 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. (2011). 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 γ -Terpenene (0.51 %) (Table 1).

Table 1. Composition of *Satureja khuzistanica* essential oil.

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

¹ RI; Retention indices determined relative to n-alkanes (C₆-C₂₄) on a DB-5GC column.

² RI; Retention indices, MS; mass spectra, CoI; co-injection.

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 m²/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 carvacrol or 3.0 g/ L Polysorbate-80 as emulsifier agent (control+) were administrated *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 (1994) recommendations (Table 2). The Diets and water were provided for *ad libitum* consumption throughout the 28-d experimental period.

Table 2. The ingredients and the nutrient composition of the experimental diets.

Item	Experiment 1		Experiment 2			
	Starter (1-14d)	Grower (15-28d)	Pre-starter (1-7d)	Starter (8-21d)	Grower (22-35d)	Finisher (36-42d)
Ingredient						
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 ¹	0.25	0.30	0.28	0.25	0.26	0.27
Mineral permix ²	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
Calculated value						
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

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

²Supplied per kg of diet: vitamin A, 18000 IU; vitamin D₃, 4000 IU; vitamin E, 36 mg; vitamin K₃, 4 mg; vitamin B₁₂, 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.

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 super 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 carvacrol 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.

Data collection

At the end of Experiment 1 (28 d) two male and two female birds per pen, ± 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 replicate / 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 Tassugny – 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 (SAS, 2003). The LSD test was used for multiple treatment comparisons using the LSMEANS statement of SAS 9.3 (2003) with letter grouping obtained using the SAS pdmix800 macro (1998). 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 $P \leq 0.05$.

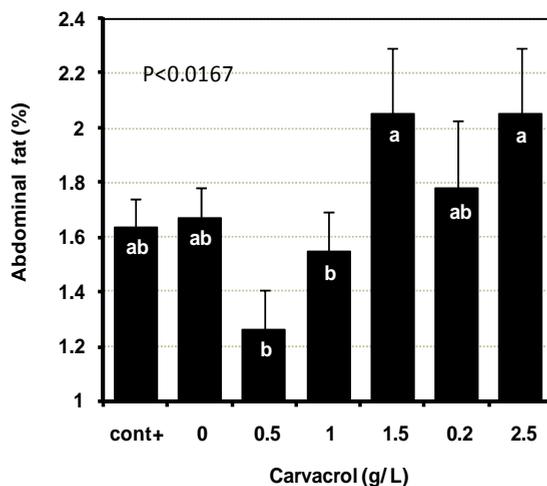


Figure 1. Effect of high doses of carvacrol in drinking water on abdominal fat (%) in broiler chicks at 28 days of age (Experiment 1). Means without a common superscript (^{a-b}) differ significantly ($P < 0.0167$).

Results and discussion

Experiment 1

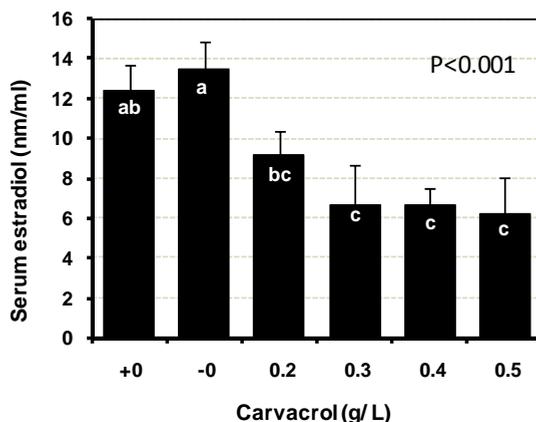


Figure 2. Effect of low doses of carvacrol in drinking water on serum estradiol level in male broiler chicken at day 42 of age (Experiment 2). Means without a common superscript (^{a-c}) differ significantly ($P<0.001$).

Data for Experiment 1 are presented in Tables 3 and 4, and Figures 1 and 2. Administration of carvacrol 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 carvacrol 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 carvacrol-treated water in dissimilar ways (Table 4). As shown in figure 1, accumulation of fat in abdominal cavity of the birds was reduced by carvacrol-added water ($P=0.0262$). Supplementation of 0.5 g/ L carvacrol caused approximately 16% decrease in abdominal fat-to-body weight ratio (AFP) at 28 d. Addition of carvacrol in drinking water at doses >0.5 g/ L exhibited adverse effect of AFP (Figure 1). The serum TC and HDL level was significantly influenced by the sex of the birds. The Male broilers were found to have 8.55 and 9.01 percent lower TC and HDL, respectively, in serum. No parameter of consideration was affected by sex \times carvacrol interaction in the Experiment 1 (Table 3).

Table 3. Effect of high doses of carvacrol 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).

Factor\level	TG	TC	LDL	HDL
	mg/ 100 ml			
Carvacrol (g/ L)				
Control ⁺ ¹	35.50	150.16 ^a	62.25	75.58 ^a
Control ⁻ ¹	36.83	157.26 ^a	66.83	74.91 ^a
0.5	40.17	122.75 ^b	52.08	60.92 ^b
1.0	37.08	125.51 ^b	56.50	65.25 ^{ab}
1.5	38.16	132.59 ^{ab}	55.83	71.50 ^{ab}
2.0	40.50	128.83 ^{ab}	56.67	66.25 ^{ab}
2.5	37.33	129.41 ^{ab}	57.58	65.25 ^{ab}
Sex				
Male	38.77	135.46	57.39	68.97
Female	37.11	136.35	52.32	68.10
SEM²				
	1.087	2.546	1.300	1.321
P > F				
Carvacrol	0.8859	0.0094	0.1463	0.0270
Sex	0.4765	0.8464	0.1135	0.7257
Carvacrol × Sex	0.2480	0.0511	0.0115	0.0344

¹Control⁺; 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.

² Standard error for overall mean.

^{a-b} Means within a column for each factor without a common superscript differ significantly (P<0.05).

Experiment 2

Incorporation of low doses of carvacrol (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 carvacrol-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.

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

Table 4. Effect of high doses of Carvacrol (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 28 days of age separated by sex (Experiment 1).

Factor\level	TG	TC	LDL	HDL
	mg/ 100 ml			
Males				
Control ⁺ ¹	37.37	141.25	55.62	71.62
Control ⁻ ¹	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 ²	2.606	3.944	1.940	1.881
P > F	0.8893	0.3009	0.1597	0.2223
Females				
Control ⁺ ¹	31.75	125.50	53.00	61.00
Control ⁻ ¹	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 ²	1.492	2.637	1.823	1.422
P > F	0.5365	0.1874	0.1598	0.3944

¹Control⁺; 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.

² Standard error for overall mean.

^{a-b} Means within a column for each factor without a common superscript differ significantly (P<0.05).

The results of analysis of variance in the Experiments 1 and 2 (Tables 3, 4 and 5) indicated that the administrated doses of carvacrol 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 carvacrol (Table 3). These observations were coincided with the significantly reduced abdominal fat at 0.5 g/ L carvacrol in Figure 1, indicating the potential of carvacrol as a hypolipidemic water additive. Thus, from the results in table 3 and figure 3, it appears that the “optimum inclusion level” for carvacrol in water for broilers is between 0.3 to 0.5 g/ L water.

Table 5. Effect of low doses of Carvacrol 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 42 days of age (Experiment 2).

Factor\level	TG	TC	LDL	HDL
	µg/ 100 ml			
Carvacrol (g/ L)				
Control ⁺	80.66	147.33 ^{ab}	70.17 ^{ab}	74.00 ^{ab}
Control ⁻	84.50	155.01 ^a	74.50 ^a	78.33 ^a
0.2	83.50	134.33 ^{bc}	63.83 ^{abc}	71.17 ^{abc}
0.3	76.83	132.33 ^{bc}	56.50 ^c	66.83 ^{bc}
0.4	81.83	152.83 ^c	58.67 ^{bc}	66.17 ^{bc}
0.5	80.50	122.67 ^c	53.83 ^c	61.17 ^c
SEM ²	0.412	2.833	1.692	1.974
	<i>P > F</i>			
SkEO	0.9886	0.0004	0.0064	0.0445

¹Control⁺; The birds received drinking water supplemented with 0.5 g/ L polysorbate-80 throughout the experiment, and Control⁻; The birds received drinking water with no additive.

² Standard error for overall mean.

^{a-b} Means within a column for each factor without a common superscript differ significantly ($P < 0.05$).

Although there was no alteration in plasma lipids, the pronounced decrease in abdominal fat of the birds received 0.5 g/ L carvacrol in Experiment 1 (Figure 1), indicates that carvacrol 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 (*Saadoun and Leclercq, 1987*). 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 (*Griffin et al., 1992, Hermier, 1997*). We suggest the significant decrease (15%) in the abdominal fat of the 0.5 g/ L carvacrol- treated birds was a response to decreased plasma LDL and HDL. Carvacrol seems to affect LDL and/or HDL metabolism in extra hepatic metabolic routes (*Hotta et al., 2010*). These results are compliant with other reports which shown that dietary carvacrol significantly affect fat metabolism in chicken (*Case et al., 1995, Baser, 2008*). Brenes and Roura, (2010) reported that oregano extract, which it is also rich in carvacrol, exhibit significant hypocholesterolemic effects in chicken. 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 carvacrol were concur with opposite alteration in plasma estradiol and testosterone levels (Figures 2 and 3). These results are in consistent with the finding of Haeri et al. (2006) 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 (*Chen et al., 2005*). 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 (*Middleton and Hui, 1982*).

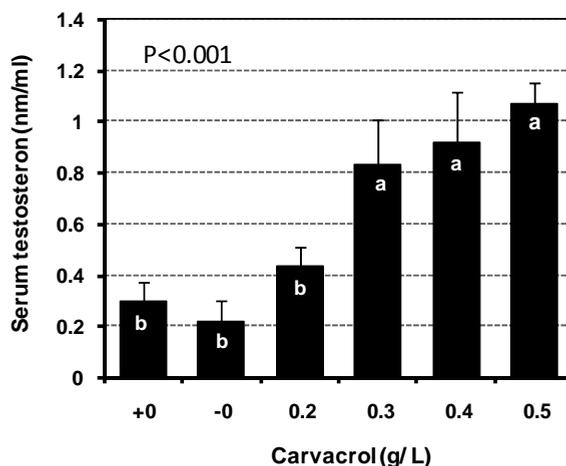


Figure 3. Effect of low doses of carvacrol in drinking water on serum testosterone level in male broiler chicken at 42 days of age (Experiment 2). Means without a common superscript (^{a-b}) differ significantly ($P < 0.001$).

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 carvacrol-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. (2005)* in conformity with the idea confirmed by *Whitehead et al. (1984)* 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 carvacrol in broiler chicken under the circumstances which the current experiments were conducted. 1) The decreased abdominal fat in 0.5 g/ L carvacrol-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 carvacrol-treated birds could be associated with elevated serum testosterone, as an anabolic hormone, in trial 2.

Conclusion

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.

Acknowledgment

This study was supported by a grant from Lorestan Medical Plant Lab., Khorraman, Khorramabad-68135-579, Iran.

Hipolipidemijski efekti karvakrola na polne hormone kod brojlerskih pilića

H. Khosravinia

Rezime

Dva eksperimenta su sprovedena kako bi se procenio uticaj visokih i niskih doza karvakrola na sastojke lipida u krvi i polne hormone u brojlerskih pilića. Uključivanje karvakrola u pijaću vodu u količini od 0,5 i 0,3 g/L u oglecima 1 i 2, respektivno, uticalo je na serumski holesterol i nivo lipoproteina velike gustine (HDL), mada razlike nisu bile značajne u odnosu na odgovarajuće kontrolne grupe ($P > 0.05$). Karvakrol u količini od 0,5 g/L značajno smanjuje (16%) sadržaj abdominalne masnoće živine, u uzrastu od 28 dana (ogled 1, $P < 0.05$). U ogledu 2, koncentracija estradiola u serumu značajno je smanjena u svim grlima koja su dobijala karvakrol u poređenju sa kontrolnim-grlima ($P > 0.05$). Serum testosteron, međutim, se povećao kod pilića koji su dobili karvakrol u dozama većim od 0,2 g/L u poređenju sa kontrolnim pilićima ($P < 0.05$). Rezultati ukazuju na mogućnost hipolipidemijskih svojstva testosterona vezanog za karvakrol u brojlerskih pilića.

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