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Original Research Article

Influence of Various Volatile Oils as a Dietary Supplement on Biochemical and Performance Parameters in Broilers

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ABSTRACT

Volatile oils like rosemary, thyme and fennel oils have positive effects on poultry health and yields. The aim of this study were to investigate the effects of volatile oils on some serum biochemical parameters, growth hormone activities and growth performance in broiler chickens. A total of 180 broilers were randomly divided into 6 groups and 3 replicates of 10 animals according to supplementation of thyme oil, rosemary oil, fennel oil, oil mixture 100 ppm dosage and oil mixture 200 ppm dosage. Serum growth hormone activity and glucose concentration were not affected by dietary addition of volatile oils according to groups. However, addition of rosemary oil, thyme oil and fennel oil showed an effect on decreased serum cholesterol and partially lipid concentrations in the present study. In conclusion, supplementation of volatile oils had impressive effects on serum total cholesterol and total lipids levels in broiler diets. Whereas, growth performance results were not affected by dietary addition of volatile oils in our study.

Keywords: Biochemical parameters, Broiler, Volatile oil, Performance

Çeşitli Uçucu Yağ Asitlerinin Biyokimyasal ve Büyüme Performansı Parametreleri Üzerine Etkisi

ÖZET

Biberiye kekik, ve rezene gibi uçucu yağ asitleri, kanatlı sağlığı ve verimi açısından pozitif etkilere sahiptir. Bu çalışmanın amacı, uçucu yağ asitlerinin broiler tavuklarda, büyüme hormonu aktivitesi, bazı serum biyokimyasal ve büyüme performansı parametreleri üzerine etkilerinin araştırılmasıdır. Toplam 180 adet broiler 3 tekrarlı olacak şekilde 6 gruba ayrıldı. Her grupta 10 adet hayvan olacak şekilde kontrol biberiye, kekik, rezene ve karışım uçucu yağ asitleri (100 ppm, 200 ppm) gruplarına bölündü. Bizim çalışmamızda uçucu yağ asitlerinin yeme katılmasının büyüme hormonu aktivitesi ve glukoz konsantrasyonları açısından herhangi bir etkisi yoktur. Fakat biberiye, kekik ve rezenenin yeme katılması, kolesterol ve kısmen lipid konsantrasyonları üzerinde düşme sağlamıştır. Sonuç olarak broiler yemlerine uçucu yağ asitlerinin katılması, total kolesterol ve lipid konsantrasyonlarında etkili bir şekilde düşme göstermiştir. Bununla birlikte uçucu yağ asitlerinin yeme eklenmesinin büyüme performans parametreleri üzerine herhangi bir etki görülmemiştir.

Anahtar Kelimeler: Biyokimyasal parametreler, Broiler, Uçucu yağ, Performans

INTRODUCTION

The use of herbal feed supplements has become very popular as alternative feeding strategy for poultry diets in recent years (Cabuk *et al.*, 2014). After banning the use of antibiotics on animal feed in the European Union (EU) at 2006, alternative promoters have been thoroughly prominence to investigate. Phytogenic feed additives consist groups of plants including herbs, spices, and the volatile oils (Mueller *et al*., 2012). In these days, natural antioxidants are preferred to prevent lipid oxidation instead of synthetic antioxidants (Botsoglou *et al.*, 2002). Phenolic compounds like rosemary, thyme and fennel play role to scavenge free radicals and this feature provides protection from oxidative stress (Labban *et al.*, 2014). Especially aromatic plant and plant extracts are frequently used because of their antioxidant activity, antimicrobial, growth and immune stimulation, anti-inflammatory hypocholestrolemic effects (Lopez Bote *et al.*, 1998; Craig 1999; Botsoglou *et al*., 2002; Labban *et al*., 2014). The effects of essential oil mixture on poultry health and yields related to administered dose are also indicated (Williams and Rosa 2001). This positive effect of volatile oils defines more advantageous compare to used conventional growth factors.

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Rosemary (*Rosemarinus officinals*) is nowadays widely used for food aromatic and medicinal beneficial that has known large antioxidant activity (Huisman et al., 1994; Lopez-Bote *et al.*, 1998; Basmacıoglu *et al.*, 2004; Carvalho *et al.*, 2005; Yesilbag *et al.*, 2011). Demir *et al.* (2005) determined that herbal natural additives such as thyme, rosemary, garlic and cinnamon significantly increased broiler performance and consumers can benefit of these poultry product's antioxidant effects. Thyme (*Thymus vulgaris*) has antibacterial and antioxidant properties due to containing thymol and carvacrol components (Wareth *et al.*, 2012). Fennel oil (*Foeniculum vulgare Mill*) is an essential oil crop using as spices and medicinal herb. It has also antioxidant and anti-inflammatory specialties (Zheljazkov *et al.*, 2013).

There haven't been sufficient studies effects of essential oils on evaluating blood lipids, blood glucose and blood protein in broiler chickens. In this study rosemary, thyme, fennel and mixture oils with dosages (100 ppm and 200 ppm) were utilized to find out the effects of these volatile oils.

The present study was designed to investigate the effects of volatile oils (thyme, rosemary, and fennel oils) on growth hormone, some serum biochemical and growth performance parameters in broiler chickens.

MATERIALS AND METHODS

Animals and Feeding

A total of 180 Ross 308 broiler 1-day-old chicks were used in this experiment. Chicks were randomly allocated into 6 treatment groups and 3 replicates. Each group included 10 animals. All of the animals were fed until 42 day of slaughter time. The room temperature was maintained at 24-33°C. The light timing was set up at 23 hours light /1 hour dark. Animals were obtained from the Uludag University Animal Health and Production, Research and Application Centre (Bursa, Turkey). The experiment was approved by the Ethics Committee of Uludag University. All animals were fed ad libitum with same basal diet and supported free access of water. Volatile oils were added 5 experimental groups. The determined dosages of supplemented groups and control group were shown in Table 1.

Table 1. Doses of the volatile oils according to the groups.

	Groups	***Doses of Volatile Oils***
1	*Control*	-
2	*Thyme Oil*	100 ppm
3	*Rosemary Oil*	100 ppm
4	*Fennel Oil*	100 ppm
5	*Oil Mixture (thyme,rosemary, fennel)*	100 ppm
6	*Oil Mixture (thyme,rosemary,fennel)*	200 ppm

Growth Performance and Blood Parameters

Animals were weighed individually at the beginning and every week of the experiment. Live body weights and feed consumptions were recorded weekly and calculated live body weight gain and feed conversion ratio.

Blood samples were taken from animal's jugular veins during slaughter time (42th day). Serum was separated by centrifugation at 3,000 × g for 10 min. and stored at -20°C until time of analyses. Serum samples were analyzed for glucose (Cayman Chemical Company Colorimetric Assay Kit, Cat No.10009582, USA), total lipid (Far Diagnostics Company, Cat No. 22501, Italy), total protein (Ben Biochemicals Enterprise Cat No. PT 371, Italy) albumin (Ben Biochemicals Enterprise Cat. No. Al BG6045, Italy), total cholesterol (Ben Biochemicals Enterprise Cat. No. C20T5, Italy) and triglyceride (Ben Biochemicals Enterprise Cat No. TG381, Italy) levels by using spectrophotometer (Schimadzu UV 1601, Kyoto, Japan). Serum Growth hormone activity was measured by Cusabio Biotech Chicken Growth Hormone ELISA Kit (Cat. No. CSB-E09866Ch, China). Microplate reader (Biotek ELx 808, USA) was used for growth hormone analysis.

Volatile Oils

Rosemary and Thyme volatile oils were obtained from local commercial company of Sesim Health Products Ankara, Turkey. Fennel volatile oil was obtained from local company of Düzey Laboratory Products İstanbul, Turkey.

The ingredients and chemical composition of the basal diet are presented in Table 2.

Table 2. Ingredients and Chemical Composition of the basal diet.

Ingredients (g)	***Starter diet (0-21d)***	***Grower diet (22-35 d)***	***Finisher diet (36-42 d)***
Maize	484.4	530.29	559.21
Wheat	75	75	100
Full Fat Soy	50	66.37	77.77
Soybean Meal	281.49	171.01	107.18
Vegetable Oil	20	20	20
Salt	2.93	2.96	2.99
Sodium Bicarbonate	1	1	1
Limestone	14.7	12.47	12.08
DL-Methionine	2.39	1.2	0.7
Maise Gluten	47.06	100	100
DCP % 22	9.82	8.17	7.31
Fitase	1	1	1
L-Lysine	4.21	4.53	4.06
Choline Chloride % 70	1.5	1.5	2.2
Vitamin Premix	3	3	3
Mineral Premix	1	1	1
Total	1000	1000	1000
Calculated nutrient concentration (%)			
Dry Matter	88.11	88.22	88.17
Crude Protein	22	21	19
Crude Lipid	4.73	5.20	5.47
Crude Cellulose	2.96	2.96	3
Meth+Cys	1.07	0.95	0.83
Lysine	1.43	1.24	1.06
Calcium, Ca	1.05	0.90	0.85
Total Phosphorus, P	0.79	0.73	0.66
Metabolic Energy, ME, kcal/kg	3025	3150	3200

[1]Contained 70% liquid choline chloride; [2]Contained 22% DCP: Dicalcium phosphate; [3]Vitamin Premix providing per 3 kg of diet: 11 000 IU Vitamin A, 1 5000 IU Vitamin D3, 50 mg Vitamin E, 3 mg Vitamin K, 3 mg Vitamin B1, 8 mg Vitamin B2, 4 mg Vitamin B6, 0.016 mg Vitamin B12, Folic acid 2 mg, 15 mg Pantothenic acid, 0.015 mg Biotin, 60 mg Nicotinamide; [4]Mineral Premix providing per 1 kg of diet: 120 mg Manganese, 40 mg Iron, 100 mg Zinc, 16 mg Copper, 1.25 mg Iodide, 0.3 mg Selenium.

Statistical Analyses

The Statistical Package for the Social Sciences version 22.0 (SPSS, Chicago, IL, USA) was used for data analysis. Values were expressed as arithmetic means ± standard error of mean (SEM). A one-way analysis of variance (ANOVA) was used to evaluate the growth performance and blood parameters. The Tukey test was used as a post hoc test and the level of significance used in all of the tests was $p< 0.05$.

RESULTS

The serum growth hormone activity, total lipid, total protein albumin, triglyceride, glucose and total cholesterol concentrations were investigated in the experimental and control groups. They were presented in the Table 3.

GH concentrations were observed generally similar in all groups. However, serum GH concentrations were the highest in the control group (1189.74 $\pm$ 40.22) although no significant differences were observed among groups. Also, low values of serum GH concentration were observed in the rosemary oil supplemented group and oil mixture supplemented group 200 ppm (1092.82 $\pm$ 37.46 and 1111.13 $\pm$ 22.00).

The serum glucose levels did not significantly differ among the experimental groups. The oil mixture 100 ppm dosage supplemented group and rosemary oil supplemented group showed lower levels of serum glucose and there wasn’t any significantly difference between treatment groups.

Table 3. Serum biochemical parameters during feeding in broilers subjected to the adding of volatile oils to diet.

Groups	*Growth Hormone (pg/ml)*	*Blood Parameters*					
		Total Lipid (mg/dl)	*Total Protein (g/dl)*	*Albumin (g/dl)*	*Triglyceride (mg/dl)*	*Glucose (mg/dl)*	*Total Cholesterol (mg/dl)*
			Mean ± SE				
Control	1189.74 ± 40.22	333.16 ± 31.59^{ac}	4.38 ± 0.30^{ade}	2.23 ± 0.08^{ad}	166.23 ± 8.35	232.43 ± 15.69	85.42 ± 2.70^{a}
Thyme Oil	1118.05 ± 29.23	264.64 ± 25.36^{a}	3.10 ± 0.11^{bc}	3.09 ± 0.11^{bc}	173.34 ± 13.67	248.53 ± 19.20	68.15 ± 4.43^{b}
Rosemary Oil	1092.82 ± 37.46	546.38 ± 27.73^{b}	6.15 ± 0.26^{a}	2.87 ± 0.19^{ac}	209.99 ± 13.50	197.56 ± 18.21	47.83 ± 4.96^{c}
Fennel Oil	1167.07 ± 22.79	389.55 ± 23.27^{c}	4.94 ± 0.29^{d}	2.95 ± 0.14^{bc}	203.14 ± 13.82	248.04 ± 22.18	61.53 ± 5.53^{bc}
Oil Mixture (100 ppm)	1135.32 ± 20.94	336.91 ± 31.80^{ac}	3.86 ± 0.29^{ce}	2.59 ± 0.21^{acd}	196.66 ± 17.29	195.60 ± 9.70	124.53 ± 2.11^{d}
Oil Mixture (200 ppm)	1111.13 ± 22.00	513.33 ± 21.84^{b}	4.56 ± 0.14^{de}	2.06 ± 0.14^{d}	199.99 ± 17.29	217.19 ± 11.47	73.51 ± 2.40^{ab}

^{a,b,c,d,e} :Different superscripts indicate statistical significance at in the same column

Total protein values were higher in rosemary oil supplemented group than other treatment groups ($p<0.001$). However serum total protein levels had the lowest values in thyme oil supplemented group ($p<0.001$). Nevertheless, serum albumin levels had the highest values in the thyme oil supplemented group and albumin levels significantly decreased in oil mixture 200 ppm dosage supplemented group compare to other experimental groups ($p<0.001$).

We observed statistical differences between total lipid level in the supplemented groups ($p<0.001$) compare to control group. The highest level of total lipid concentration were determined in rosemary oil supplemented group ($p<0.001$) while the lowest levels were observed in thyme oil supplemented group to compare other experimental groups.

In the oil mixture 100 ppm supplemented group showed the highest peak values of serum total cholesterol according to the other groups ($p<0.001$) and this values significantly decreased in rosemary oil supplemented group ($p<0.001$).

The effects of volatile oils on growth performance are shown in Table 4. Live weight gain, feed intake and feed conversion ratio results were not affected by supplementations of volatile oil to broiler diet and there were no significant differences among groups.

Table 4. Comparison of growth performance parameters in broilers.

Groups	***Time Period (d)***	***Live Weight Gain (g)***	***Feed Intake (FI) (g/d)***	***Feed Conversion Ratio (FCR) (g/g)***
		Mean±SE		
Control	*0-21 d*	779.55 ± 14.28	1178.33 ± 31.80	1.64 ± 0.03
	21-42 d	1820.30 ± 103.91	3394.52 ± 96.22	1.87 ± 0.03
	0-42 d	2599.85 ± 111.22	4572.85 ± 96.48	1.80 ± 0.02
Thyme Oil	*0-21 d*	773.80 ± 19.66	1226.12 ± 22.25	1.60 ± 0.04
	21-42 d	1717.60 ± 113.74	3429.86 ±105.36	1.89 ± 0.04
	0-42 d	2491.40 ± 106.81	4655.98 ±106.47	1.78 ± 0.01
Rosemary Oil	*0-21 d*	865.70 ± 18.07	1206.52 ± 41.85	1.57 ± 0.05
	21-42 d	1746.90 ± 94.84	3305.56 ± 90.65	1.83 ± 0.01
	0-42 d	2612.60 ± 93.83	4512.08 ± 90.65	1.75 ± 0.02
Fennel Oil	*0-21 d*	839.85 ± 14.33	1280.01 ± 36.82	1.63 ± 0.04
	21-42 d	1779.60 ± 119.41	3311.84 ± 60.05	1.85 ± 0.01
	0-42 d	2619.45 ± 120.88	4591.85 ± 93.56	1.77 ± 0.01
Oil Mixture (100ppm)	*0-21 d*	654.85 ± 17.13	1164.74 ± 35.26	1.63 ± 0.03
	21-42 d	1678.20 ± 136.53	3312.81 ± 64.91	1.82 ± 0.03
	0-42 d	2333.05 ± 140.61	4477.55 ± 63.63	1.76 ± 0.02
Oil Mixture (200 ppm)	*0-21 d*	895.40 ± 21.50	1228.78 ± 51.32	1.61 ± 0.07
	21-42 d	1762.80 ± 120.85	3266.75 ±117.92	1.81 ± 0.01
	0-42 d	2658.20 ± 123.34	4495.53 ±111.73	1.75 ± 0.03

DISCUSSION

In our study, serum glucose levels were lower in rosemary oil supplemented group than other groups although there were no significant changes for these levels, and total cholesterol levels were also determined very low in rosemary supplemented group similarly other studies (Lanksy *et al.*, 1993; Ghazalah *et al.*, 2008; Polat *et al.*, 2011). Furthermore serum triglyceride and total lipid levels were determined statistically higher in rosemary oil supplemented group than other groups. Thus, addition of rosemary oil to diet decreased blood cholesterol levels, also blood lipid levels were increased at the same time. The reason is that the body might be supplied energy needed from lipolysis due to decreased glucose levels. Ghazalah *et al.* (2008) suggested that plasma total cholesterol levels after addition of rosemary leaves might show hypocholesterolemic properties in broilers. Some

researchers indicated that rosemary has hypolipidemic and hypoglycemic activities (Dearlove *et al.*, 2008). However, hypolipidemic effects of rosemary were not observed in our study while this group showed hypocholesterolemic and hypoglycemic effects. Manafi *et al.* (2014) reported that, when presence of rosemary oil in the broiler diets exhibited good effects on reducing cholesterol, HDL, LDL. Akiba and Matsumoto (1982) and Ashan (2012) showed that, rosemary has effect on decreased cholesterol and triglyceride in broilers blood. Additionally, in rosemary oil supplemented group, serum total protein values were the highest levels compare to other groups ($p<0.001$) although serum albumin levels were within normal levels. This result showed that increasing of the serum proteins are probably due to the high serum globulin levels, which also explains partially the anti-inflammatory effect of rosemary. Serum growth hormone levels were no statistical difference in this group because growth hormone shows pulsatile secretion, therefore blood samples should be taken several times during the day for determination of growth hormone's effect exactly. Further work will be necessary to investigate with regards to the rosemary supplemented to broiler diets and its effect on growth hormone secretion need to better investigate.

In thyme oil supplemented group, there were no significant changes for serum growth hormone and glucose levels. However, serum total lipid levels were the lowest in this group ($p<0.001$). Additionally, serum triglyceride and total cholesterol levels were found to be one of low levels ($p<0.01$) in thyme oil supplemented group. This means that, addition of thyme oil to broiler diet leads to prominently lower blood lipids, hence this finding showed that supplementation of thyme oil cause hypolipidemic effect in the body. The results of present study are similar with some studies (Ali *et al.*, 2007; Radwan *et al.*, 2008; Khosravinia 2014). Ali *et al.* (2007) and Radwan *et al.* (2008) reported that addition of thyme oil to broilers diet decreased plasma triglycerides, total cholesterol and total lipid levels. Thyme oil supplemented group also showed low serum total protein levels compare to other treatment groups ($p<0.001$) while serum albumin levels were higher than control and oil mixture 200 ppm supplemented groups ($p<0.001$), even so serum albumin levels remained within normal values in this group. Briefly, addition of thyme oil to diet showed noticeable hypolipidemic agent according to rosemary oil supplementation to diet because serum total lipid levels of thyme oil supplemented group were lower than rosemary oil supplemented group. That mean, thyme oil were found out the strongest hypolipidemic effect in current study.

The statistical differences were not determined on growth hormone and glucose concentrations in fennel oil supplemented group. However, total lipid levels were also higher than thyme oil supplemented group ($p<0.05$) and lower than oil mixture 200 ppm dosage group ($p<0.001$) in this group. Fennel oil supplementation to diet reduced serum total cholesterol levels. Oulmouden *et al.* (2014) reported that addition extract of fennel to mice diet causes lower levels lipids, cholesterol and triglycerides and they suggested that the extracts can restore the catabolism of β-lipoproteins in liver. Furthermore, dietary addition of fennel oil was not observed any effect on serum total protein and albumin concentrations in current study. Fennel oil additives emerged more cholesterol-lowering effect in present study.

Serum growth hormone, total lipids, total cholesterol, triglyceride, total protein and albumin levels were normal in oil mixture 100 ppm dosage supplemented group. Exclusively, serum glucose concentrations had low levels although there was no significant changes. In this way, oil mixture 100 ppm dosage supplementation just showed effect on serum glucose values.

In oil mixture 200 ppm dosage supplemented group had the lowest serum albumin levels although total protein levels were within normal levels. It might be considered that oil mixture 200 ppm dosage additive enhanced the concentrations of serum globulin due to low albumin levels in this group. This oil mixture supplementation might be positive effect on immune system depending on the present study. Besides serum total cholesterol levels of oil mixture 200 ppm dosage supplemented group were higher than rosemary oil supplemented group ($p<0.001$) and lower than oil mixture 100 ppm dosage supplemented group. Serum total lipid and triglyceride levels of this group were also normal values. In conclusion, this dosage of oil mixture of adding to broiler rations were more effective for serum total cholesterol and total protein levels than oil mixture 100 ppm dosage.

There were no significantly changes with regards to the effects of volatile oil additives to broiler rations on growth performance. Live weight gain, feed intake and feed conversion ratio were not affected by adding of

volatile oils to diet. Whereas it was determined that addition of volatile oils to broiler rations were increased live weight gain compare to control group similar studies using different and similar volatile oils (Jamroz *et al.*, 2003; Jamroz *et al.*, 2005; Ciftci *et al.*, 2005; Bozkurt *et al.*, 2009). Some researchers indicated that adding of individual or mixture of volatile oils did not provide significant improvement on growth performance (Basmacıoglu *et al.*, 2004; Botsoglu *et al.*, 2004; Hernandez *et al.*, 2004).

Base on the results of the current study, supplementation of volatile oils most impressive effects on total cholesterol and total lipids levels in broiler diets and the results of present study suggest that especially rosemary and thyme oil additives to broiler ration provide to prevent of some diseases due to hypolipidemic, hypocholesterolemic and hypoglycemic effects. According to present study, dietary addition of volatile oils has not effects on growth performance, serum growth hormone and glucose levels. We consider that volatile oils might be used efficiently in feeding programs to benefit their hypolipidemic, hypoglisemic and antioxidant properties.

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