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Serum Biochemical Profile of Broiler Chickens Fed Diets Containing Rosemary and Rosemary Volatile Oil

Umit Polat[1], Derya Yesilbag[21] and Mustafa Eren[2]
[1]Department of Biochemistry, Faculty of Veterinary Medicine, University of Uludag, 16059 Bursa,TURKEY
[2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, University of Uludag, 16059 Bursa,TURKEY

ABSTRACT

The study was conducted to determine the effects of dietary supplementation rosemary aromatic plant, rosemary volatile oil and α-tocopherol acetate (Vitamin E) on serum variables of broilers fed on maize-soybean meal based diets. Eight hundred 1-d-old Ross-308 male chickens were weighed and randomly divided into 1 control and 7 experimental groups each with 10 replicates of 10 birds. There were 8 dietary treatments: (VitE1) control without rosemary and rosemary volatile oil only with 50 mg/kg vitamin E; (R1) 5.7 g/kg ground rosemary leaves; (R2) 8.6 g/kg ground rosemary leaves; (R3) 11.5 g/kg ground rosemary leaves; (RO1) 100 mg/kg rosemary volatile oil; (RO2) 150 mg/kg rosemary volatile oil; (RO3) 200 mg/kg rosemary volatile oil and (VitE2) 200 mg/kg vitamin E. Broilers consumed the diets and water *ad libitum*. After 42 days, 80 animals were randomly selected for serum biochemical profile analysis involving ceruloplasmin, superoxide dismutase activity (SOD), transferring, albumin globulins ratio (A/G), total cholesterol, creatin, urea, alanine aminotransferase (ALT) and aspartate amino transferase (AST). While serum transferrin, urea level and ALT-AST activity were not statistically different among groups serum ceruloplasmin ($p< 0.000$), SOD activity ($p<0.05$), albumin/globulin ratio ($p< 0.000$), total cholesterol ($p<0.001$), creatinin ($p<0.05$) and AST ($p< 0.000$) level were found to be significantly different. In conclusion, the *Rosmarinus officinalis* plant and its volatile oil have increasing effect on serum SOD activity and effect positively oxidation mechanism. On the other hand, it can be assumed that rosemary plant created hypocholesterolemic effect in this study.

Key Words: Biochemical, broiler, rosemary, serum, volatile oil

INTRODUCTION

The use of antibiotics as growth promoters in animal nutrition is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria; antibiotic use has been banned in the European Union since January 2006. Bans on the use of antibiotics as feed additives have accelerated and led to investigations of alternative feed additives in animal production. As one alternative, essential oils (EO), which are already used as feed supplements, can improve growth performance under intensive management systems (William and Losa 2001). Natural feed additives of plant origin are generally believed to be safer, healthier and less subject to hazards for humans and animals. Many herbs and plant extracts have antimicrobial activities and antioxidant properties which make them useful as natural animal feed additives (Faixova and Faix 2008). Today, there is increasing interest in the use of natural antioxidants such as rosemary (*Rosmarinus officinalis* L.), flavonoid and tocopherol extracts for food preservation (Hras et al. 2000, Williams et al. 2004) because these natural antioxidants avoid undesirable health problems that may arise from the use of synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, which may have toxic effects (Aruoma et al. 1992). Antioxidant effect of aromatic plants is due to the presence of hydroxyl groups in their phenolic compounds (Shahidi and Wanasundara 1992). Rosemary, belonging to the Lamiaceae family, is well known for its antioxidative properties, is used for flavouring foods and beverages, and is also used in several pharmaceutical applications. These polyphenols also have important biological activities *in vitro* such as anti-tumour, chemo-preventive and anti-inflammatory activities (Shuang-sheng and Rong-liang 2006, Cheung and Tai 2007). It has been proposed that polyphenols from rosemary may greatly increase the functionality of food in terms of health and wellness. It has been extensively reported that rosemary essential oils have antimicrobial properties against a wide range of microorganisms, although there is little information regarding the specificity and efficacy of non-volatile phenolic compounds such as microbicides (Shahidi and Naczk 2004, Santoyo et al. 2005). Only limited studies have been conducted to investigate the effects of EO on biochemical serum parameters in broiler chickens. In consequence present study was arranged to evaluate the effects of dietary rosemary, rosemary volatile oil and α-tocopherol acetate supplementation on biochemical serum parameters of broiler chickens

[1] Corresponding author: dyesilbag@uludag.edu.tr

MATERIALS AND METHODS

Animals, Diets and Experimental Design

Eight hundred one-day-old Ross-308 male broilers fed on a starter diet for 3 weeks grower diet for 2 weeks and finisher diet for 1 week. The ingredients and the nutrient composition of the basal diet are presented in Table 1. The broiler chicks were assigned into one control and seven treatment groups that were replicated ten times with ten animals per replicate. A basal diet including basic level of vitamin E (50 mg/kg) was given to broiler chicks in the group VitE1 (control group). The remaining seven groups were given the same basal diet further supplemented with rosemary plant, rosemary essential oil or vitamin E (α-tocopherol acetate). Rosemary plant was added to the diets at 5.7 g/kg (R1), 8.6 g/kg (R2) or 11.5 g/kg (R3). These amounts were calculated according to essential oil ingredients of the plant as for 100, 150 and 200 mg/kg rosemary essential oil in the final diets, respectively. The diets of groups RO1, RO2 and RO3 were supplemented with 100, 150 and 200 mg/kg rosemary essential oil extracted from the rosemary aromatic plant used in the study. The diet of the group VitE2 was supplemented with 200 mg/kg of vitamin E. Rosemary consisted of leaves of *Rosmarinus officinalis* that had been dried and ground to pass through a 2-mm screen. In this study, rosemary and rosemary oils supplementing the experimental diets were provided from a producer of essential oil in Mersin. The rosemary was harvested in Mersin, Turkey between late May and the end of June. Experimental diets were prepared weekly by a special feed factory. Vitamin and mineral premixes did not include anticoccidials or antioxidants. During the feeding period of 42 days, diets and water were provided *ad libitum.* Diets were formulated to meet or exceed the requirements of the National Research Council (NRC 1994) for broilers at this age. Ten broilers were kept in pens (1 m x 1 m) in a ventilated broiler house containing wood shavings as litter material.

Table 1 . Ingredient and nutrient (g/kg) composition of the starter, grower and finisher diets

Ingredients (g/kg)	**Starter diet (0-21 d)**	**Grower diet (22-35 d)**	**Finisher diet (35-42 d)**
Maize	411.6	442.2	492.5
Wheat	99.5	94.2	93.8
Soybean meal	317.2	185.4	158.1
Full-fat soybean	39.7	148.6	132.4
Meat and Bone meal	29.8	24.7	14.8
Chick meal	29.8	24.7	22.7
Vegetable oil	39.7	44.6	44.4
NaCl	3.0	3.0	3.0
Limestone	6.1	6.1	6.1
Monocalcium phosphate	6.2	6.2	9.0
DL-methionine	3.9	3.9	3.9
L Lysine	3.5	3.5	3.5
L Treonine	0.8	0.8	0.8
Vitamin – mineral premix*	3.5	3.5	3.5
Rosemary	5.7	8.6	11.5
Calculated analysis (g/kg)			
Metabolisable Energy, Mj/kg	12.5	13.3	13.3
Crude protein	230.9	210.0	191.8
Lysine	12.8	11.3	10.5
Methionine + Cystine	7.6	6.9	6.4
Calcium	10.8	9.7	9.5
Available phosphorus	5.5	5.0	5.0

[1]: Provides per kg of diet trans-retinol: 3125 µg , cholecalciferol: 75 µg , α-tocopherol acetate : 50 mg, Vit K_3: 5 mg, Vit B_1: 3 mg, Vit B_2: 6 mg, Vit B_6: 5 mg, Vit B_{12}: 0.003 mg, Pantothenic acid: 10 mg, Niacin: 50 mg, Folic acid: 1 mg, Biotin: 0.1 mg, Cu: 5 mg, İ: 2 mg, Co: 0.5 mg, Se: 0.15 mg, Mn: 90 mg, Fe: 50 mg, Zn: 70 mg

Determination of chemical evaluation and total Phenolics in rosemary

Rosemary leaves samples were finely ground and subjected to proximate analysis according to the methods of AOAC (1980). The amount of total phenolic compounds in rosemary was determined with the Folin-Ciocalteau reagent according to a slightly modified method (Paneri et al. 2006). Ground rosemary (0.05 g) was extracted with 25 ml 80% aqueous methanol using a mortar. The homogenate was first centrifuged at 2000 g for 5 min and then filtered, and 0.05 ml from the filtrate was mixed with 6.45 ml distilled water and

0.5 ml of the Folin-Ciocalteau reagent. The mixture was allowed to stand for 3 min, after which 3 ml of a 7.5% aqueous Na_2CO_3 solution was added. The absorbance of the mixture was measured at 760 nm after 60 min of incubation at room temperature. The amount of total phenolics is expressed as gallic acid equivalents/g herb, using a gallic acid calibration curve.

Identification of Volatile Oils in Rosemary

Plant material was hydrodistilled in a glass apparatus according to the method recommended in the TS ISO 356. GC analysis was carried out on a MS-Thermo PolarisQ GC- Thermo Trace GC ultra-fitted with a fused HP5-MS capillary column (30 x 0.25 x 0.5 mic.m). The temperature was programmed to increase from 95^{o}C to 240^{o}C at 4^{o}C/min. Injection was performed at 250^{o}C in the split mode. Helium gas was used as a carrier at 20 psi. Detection was performed by FID at 250^{o}C. The injection volume for all samples was 0.1 µl. Chromatograms were determined using MS (mass spectrometer) or MS/MS. Data were calculated using internal standards (Pala-Paul 2004).

Serum Samples

At 42 days of age individual blood samples were collected from the jugular vein (blood samples were collected one animal from each subgroup) and serum was separated for determination of ceruloplasmin, superoxide dismutase, transferrin, albumin globulin ratio, total cholesterol, creatinin, urea, ALT and AST using commercial kits. SOD activity was determined using the Bio Vision-Superoxide Dismutase Activity Assay Kit. This sensitive SOD assay kit utilises WST-1 to produce a water-soluble Formosan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to xanthine oxidase (XO) activity and is inhibited by SOD. Therefore, the inhibitory activity of SOD can be determined via a colorimetric method. The results are expressed as the inhibition rate (%). Other biochemical parameters were measured using a Roche Cobas Integra 400 Plus autoanalyser (Roche Diagnostics, GmbH, Mannheim Germany).

Statistical Analysis

The data were considered by a 3-factor incomplete factorial design were the main effects were either 3 levels of rosemary (5.7, 8.6 or 11.5 g/kg) or rosemary oil (100,150 or 200 mg/kg) and 2 level of Vitamin E (50 mg/kg (control) and 200 mg/kg). All data were carried out by the GLM procedure of the SPSS package program for Windows (SPSS Inc., 1997, Chicago, IL), assuming the following model:

$$Yijk = \mu + t_i + l_j + t_i \times l_j + eijk$$

where *Yijk* are observations; μ is the population average; *ti* is the effect of the *i*th treatment group; *lj* is the effect of the *j*th level; $t_i \times l_j$ is the interaction of treatment x level and *eijk* is the random error. Moreover, linear contrasts were determined for treatment, level and treatment x level interactions. When the effect was significant, the differences between dietary treatments means were separated by Tukey's (Honestly Significant Differences) multiple range tests. Mean values and SEM are reported. All statements of significance were based on $p < 0.05$.

RESULTS

The ingredients and chemical compositions (%) of the starter, grower and finisher diets are shown in Table 1. The 1,8-cineole (43.96%), α-pinene (25.33%) and camphene (11.09%) were determined as main active components for rosemary (Table 2). In this study the total phenolic content and chemical composition of rosemary plant were determined as 6.25 g GAE/100g (Table 2). Some biochemical serum parameters of broiler chickens fed dietary rosemary; rosemary volatile oil and vitamin E were shown in Table 3. The result of serum biochemistry revealed a significant variation ($p< 0.05$) except for transferrin, urea, ALT which did not differ significantly. The ceruloplasmin concentration was higher in animals that received rosemary, rosemary volatile oil and high level vitamin E in groups R1,R2, RO1, RO2 and VitE2 ($p< 0.001$) than in the control animals. The values of SOD activity in R2, RO1 and VitE2 were significantly ($p< 0.05$) higher than control and other groups. Moreover, the values of A/G ratio and total cholesterol were in control, R2, R3 and RO3 than in the R1, RO1, RO2 and VitE1 ($p< 0.001$) groups. The serum creatinin values were lower in control, R1, RO1 and RO2 ($p< 0.05$) groups than the other experimental groups. The lowest AST concentration ($p< 0.001$) was determined in animals treated with rosemary (R1, R2 and R3).

Table 2. Chemical analysis of rosemary leaves and volatile oil active components and total phenolic content of rosemary plant

Chemical analysis, %		*Active components of volatile oil, %*		*Total phenolic content*
Moisture	6.0	α-Pinene	25.33	6.25 g GAE/100 g
Crude protein	5.53	Camphene	11.09	
Ether extract	16.08	β-Pinene	1.40	
Crude fiber	25.24	Limonene	1.77	
Ash	7.95	D-limonene	2.50	
Nitrogen free extract	39.4	1,8-Cineole (Eucalyptol)	43.96	
Metabolisable energy, (Mj/kg)	12.44	3-Carene	10.70	
		Camphor	0.73	
Essential oil	1.75	Ocimen	1.68	
		Borneol	0.03	
		Isoborneol	0.02	
		Caryophyllene	0.02	
		Bornyl acetate	0.04	

GAE: gallic acid equivalent

Table 3. The effects of diets including rosemary, rosemary oil and Vitamin E on some serum parameters

Groups		N	Ceruloplasmin (mg/l)	SOD (%)	Transferrin (mg/l)	A/G ratio	T.Cholesterol (mg/dl)	Creatinin (mg/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)
Rosemary	RI	10	10.70^{a}	442.0^{ab}	142.5	1.05^{b}	96.40^{b}	0.23^{b}	4.00	5.91	203.5
	R2	10	11.10^{a}	468.0^{a}	125.0	1.12^{a}	115.0^{a}	0.24^{a}	4.10	5.80	227.4
	R3	10	9.80^{b}	437.5^{b}	138.3	1.12^{a}	116.8^{a}	0.25^{a}	4.00	6.10	213.0
	Total	30	10.53A	448.4	135.2	1.09B	109.4B	0.24A	4.03	5.93	214.6B
Rosemary Oil	RO1	10	11.50^{a}	527.5^{a}	144.7	1.20^{b}	106.8^{b}	0.23^{b}	4.15	6.00	229.5
	RO2	10	10.50^{a}	450.0^{ab}	141.8	1.23^{b}	108.1^{b}	0.23^{b}	4.08	6.20	252.6
	RO3	10	9.70^{b}	370.0^{b}	128.5	1.26^{a}	129.0^{a}	0.24^{a}	4.00	5.90	247.2
	Total	30	10.56A	449.8	138.3	1.23A	114.6A	0.23AB	4.07	6.03	243.1A
Vitamin E	VitE1(control)	10	8.10^{b}	420.0^{b}	140.4	1.29^{a}	136.3^{a}	0.21^{b}	4.1	5.82	245.8
	VitE2	10	11.20^{a}	465.0^{a}	144.5	0.99^{b}	86.50^{b}	0.24^{a}	4.1	6.00	236.2
	Total	20	9.65B	442.5	142.4	1.14B	111.4 AB	0.22B	4.1	5.91	241.0A
Pooled SEM			0.14	11.66	1.78	0.014	1.80	0.0003	0.03	0.04	3.35
P-value											
Source											
Treatment			0.000	0.511	0.468	0.000	0.000	0.049	0.838	0.502	0.000
Level			0.000	0.040	0.184	0.000	0.000	0.005	0.605	0.643	0.189
Interaction			0.000	0.049	0.017	0.000	0.000	0.812	0.812	0.094	0.144

R1: 5.7 g/kg rosemary R2: 8.6 g/kg rosemary R3: 11.5 g/kg rosemary RO1: 100 mg/kg rosemary oil RO2: 150 mg/kg rosemary oil RO3: 200 mg/kg rosemary oil VitE1: 50 mg/kg VitE2: 200 mg/kg
A/G: Albumin Globulin ratio
ALT: Alanine aminotransferase AST: Aspartate aminotransferase
A,B: Different letters within the same column indicate significant differences among treatment groups according to Tukey's test ($p \leq 0.05$)
a,b: Different letters within the same column indicate significant differences among levels according to Tukey's test ($p \leq 0.05$)

DISCUSSION

The effects of aromatic plants and plant volatile oils in the clinical chemistry of broiler are still unclear. Therefore, the aim of this study was to evaluate the effects of different doses of rosemary, rosemary volatile oil and α-tocopherol acetate as dietary supplements on renal and hepatic functions of broilers by analyzing their serum biochemical profile.

Results from chemical analysis shown in Table 2 indicate that rosemary leaves contain 6% moisture, 5.5% crude protein, 16.08 ether extract, 7.95% ash, 25.24% crude fiber and 39.4 nitrogen free extra. The metabolisable energy 12.44 Mj/kg. The volatile oil yield of rosemary plant leaves were 1.75% and main active components were defined 1,8-cineole (43.96%), α-pinene (25.33%) and camphene (11.09%). In this study the total phenolic content of rosemary plant were determined as 6.25 g GAE/100g. Ghazalah and Ali (2008) stated that rosemary leaves meal ranged 1.4-1.6% essential oil and main active components were camphor (11-16%), pinene (15-20%) and cineole (30-35%). These findings agree with those obtained by Wolski et al. (2000) and Porte et al. (2000). Farag et al. (1989) stated that these active compounds have high antioxidant activity due to the presence of phenolic groups in their structure. Yesil-Celiktas (2007) found that total phenol values of rosemary harvested in June and September was 9.51- 9.53 g/100 g. It is extremely important to point out that there is a positive correlation between antioxidant activity and the total phenolic content of the plants. Plants belonging to the *Lamiaceae* family are very rich in polyphenolic compounds (Lu and Fo 2001). Differences in the volatile oil composition and total phenol content of rosemary could be attributed to climatic effects on the plants that are growing in different habitats. Additionally harvest time and type of distillation influence the qualitative composition of the volatile oil produced.

The ceruloplasmin is an acute phase proteins. The acute phase proteins (APPs) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress (Eckersall 2004). The APPs assay may have potential for monitoring adverse environmental and /or management stressors, thus enabling better control of animal welfare (Murata 2007). They are mainly synthesized in the liver, mediated by pro inflammatory cytokines and their concentration can increase (positive APPs) or decrease (negative APPs) as a consequence of inflammatory stimuli. It has been suggested that APPs may be useful in the assessment of animal welfare (Murata 2007). In this study, the ceruloplasmin concentration was significantly higher in rosemary, rosemary volatile oil and VitE2 experimental groups in comparison with VitE1 (control).

Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), are synthesised and regulated endogenously, but require dietary supplies of the relevant nutrients. SOD plays an important role in protecting cells from damage caused by reactive oxygen species. In this study, serum SOD activities were statistically different between the control and RO1, R2 and VitE2 groups. The highest SOD activity value was found in the 100 mg/kg rosemary oil experimental group (RO1). However, when we increased rosemary oil up to 150 mg/kg (RO2 group) or 200 mg/kg (RO3 group), SOD activity was reduced to the lowest levels seen in the treatment groups.

Serum proteins divided into two groups, albumin and globulin. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition proteins help balance the osmotic pressure of the blood tissue. Most of the circulating cholesterol is carried in birds by high-density lipoprotein cholesterol (α-2globulin fraction) and LDL (β-globulin fraction) (Zantop 1997). These lipoproteins became the principal cholesterol transport and carried about 40 to 44% of the total serum proteins. In this study, data showed that there was an decrease in A/G ratio in all broiler groups fed on the rosemary, rosemary volatile oil and 200 mg/kg Vit E (VitE2) when compared with in VitE1 groups(control). This reduction in A/G ratio observed by inclusion of rosemary diets in our study was also accompanied by a significant lower total cholesterol level. A decrease in A/G ratio related to a decrease in total cholesterol levels was reported by (Ghazalah and Ali 2008).

Serum cholesterol levels were determined to be 136.30 mg/dl in the VitE1 (control) and between 86.50 and 129.0 mg/dl in the other groups. Total cholesterol levels were significantly different between the rosemary and rosemary volatile oil. Rosemary leaves addition to the diet decreased total cholesterol levels. The reduced content of total cholesterol may reflect the hypocholesterolemic properties attributed to the defatted part of the leaves which are rich in fibrous (25.24 %) content and may block intestinal cholesterol absorption (Lansky et al. 1993). This finding is consistent with a study of total cholesterol performed by Ghazalah and Ali (2008), who fed chickens diets supplemented with 0.5, 1.0 and 2.0 % dried *Rosmarinus officinalis L.* Their results showed addition of rosemary leaves in the broiler diet lowered plasma content of

total cholesterol levels. In another study dietary anise seeds supplementation in broiler diet not significantly reduced serum cholesterol level (Soltan et al. 2008).

Serum creatinin levels were significantly different between the experimental groups. All serum creatinin levels increased with diets containing dietary rosemary rosemary oil and high dosage vitamin E (VitE2) compared to the control group. Creatinine is a chemical waste molecule that is generated from muscle metabolism. The kidneys maintain the blood creatinine in a normal range. The lower values derived that no muscular wastage which might have been possibly cause by inadequacy of protein in animals. The creatinin levels of Arbor Acres broiler chickens fed different levels (0.5, 1.0 and 2.0%) of rosemary and rosemary oil were determined to be 1.12-1.22 mg/dl by Ghazalah and Ali (2008). Creatinine levels were all reduced by dietary rosemary leaves compared to controls (Ghazalah and Ali 2008). Another study anise supplementation at 0.75 and 1.0 g/kg diet significantly increased creatinine concentration values compared with the control.

In conclusion, our findings suggest that rosemary, rosemary volatile oil and vitamin E at level of 200 mg/kg may cause amendatory effect renal and hepatic functions. In addition dietary rosemary aromatic plant lowered total cholesterol level in broiler. Therefore, it can be concluded that due to the contributions of rosemary and rosemary oil to antioxidant and other metabolites activities, the addition of these natural products to chicken rations could be important to chicken and human health. More studies are necessary using plant and plant extracts to optimize that will provide benefits to the animal without being harmful and

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