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(RESEARCH ARTICLE)



The effect of Rosemary (*Rosmarinus officinalis* L.) extract supplemented into broiler diets, on performance and blood parameters

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Abstract

This experiment was carried out to determine the effect of dietary supplementation of Rosemary (*Rosmarinus officinalis*) ethanol extract, on performance and blood parameters of broilers. One hundred twenty 1-day-old male Ross-308 broiler chicks were used in this experiment. The chickens were divided into 3 groups, including one control and 2 treatment containing 40 birds each. Each group was divided into four replicates and the study lasted 42 days. Group 1 (Control group) was fed with only basal diets. Group 2 (ROE 100mg/kg) was fed basal diet supplemented with 100 mg/kg *R. officinalis* L. Group 3 (ROE 200mg/kg), was fed basal diet supplemented with 200 mg/kg *R. officinalis* L. At the end of experiment blood samples collected. Body weight gain ($P < 0.01$) and feed consumption ($P < 0.001$) were decreased when compared with control group, while feed conversion ratio is not affected. Besides, it is detected that rosemary ethanol extract increases the enzymes antioxidant (SOD, GPx, CAT), GSH; reduces lipid peroxidation (MDA) and reduces the ALT, AST, blood glucose level ($P > 0.05$) and LDL-cholesterol ($P < 0.01$). The results of this study showed that lipid peroxidation, oxidative stress parameters significantly decreased after ROE application. Rosemary (*R. officinalis* L.) ethanol extract at 200 mg/kg administered group was found to create a more positive effect on some biochemical parameters and can be a viable alternative growth promoter in the feeding of broilers.

Keywords: Blood parameters; Broiler; Performance; Rosemary

1. Introduction

The word rosemary is derived from latin word rosmarinus, it means sea dew. In ancient times, it used to be called by Greeks as “antos” which means perfect flower [1]. Rosemary (*Rosmarinus officinalis*) is also used in recent times widely for its medical benefits and for its aromatic properties together with its antioxidant features [2]. An aspect that renders many plants and plant extracts useful as natural animal feed additives, is the fact that they have antimicrobial activity and antioxidant properties [3]. Its antioxidative property is due to the presence of phenolic terpenes [4] such as rosmarinic acid [3] and rosmarole [5-6]. Rosemarinic acid is very well absorbed from the skin and gastrointestinal tract [7]. It is reported that this plant and its plant extracts show a positive influence on immunity [3], some blood parameters, egg quality properties, egg development and egg production performance of poultry [8].

Rosemary has been used for the treatment of hyperglycemia in traditional Turkish folk medicine [9]. The compounds contained in essential oils also shows biological properties such as antimicrobial and antioxidant properties [2, 4-5, 10-12]. It has been established that the *in vitro* antimicrobial activities of essential oils from Labiatae family such as oregano

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and rosemary show antimicrobial properties only against pathogenic bacteria [13-14]. Bölükbaşı and Erhan [13], ascertained with their studies that the essential oils of oregano and rosemary are active against pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*. Rosemary oil, besides of having a high level of inhibition against 25 species of bacteria and fungus, can also be used as a gas eliminator, as a perfume and as a flavorant [1]. Products, including essential oils and plant extracts for poultry rations, are being used in many countries because they have different functions in relation to poultry performance, easy implementation and low cost [3]. At the same time, it is shown with studies made that the essential oils increase the effects of digestive enzymes by stimulating the digestive system of animals [1, 12, 15].

The reactive oxygen species (ROS) are produced as the products of oxidative metabolism in mitochondria. ROS present a wide category of molecules to give rise to reactive free radicals such as hydroxyl radicals, hydrogen peroxide and superoxide. Free radicals join in oxidative reactions that damage organic substrates, such as proteins, DNA and lipids of organisms, resulting in a corruptive biological condition called oxidative stress [16]. The biological damage of oxidative stress is harmful at the cellular level. It has been included as the cause of several degenerative diseases which influence livestock productivity [17-18]. Dietary antioxidants prevent mortality and *in vivo* it prevents oxidative rancidity and oxidative stress in production animals [19-20]. There are many different antioxidants in plants which are classified as natural antioxidants and counteract the negative effects of oxidative stress [21]. The *in vivo* effect of antioxidants will improve the welfare and health of broilers, the meat quality and performance of broilers especially under intensive production systems. Achieving optimal broiler performance under conditions of oxidative stress is a challenge to prevent economic loss in the poultry industry [22].

This experiment was carried out to determine the effect of dietary supplementation of rosemary (*Rosmarinus officinalis* L.) ethanol extract performance and blood parameters of broiler chicks.

2. Material and methods

2.1. Chemicals

All chemicals used were of analytical grade and were purchased from the Sigma Chemical Co. (St. Louis, MO).

2.2. Animals

The experiment was carried out at Ataturk University in Research and Application Unit of School of Veterinary Faculty in accordance with approval by Ataturk University Local Ethics Committee for Animal Experiments (Number: 25.07.2013 36643897-656-ATA-100). In this research, only one-day 120 Ross 308 commercial hybrid female broiler chicks were used. During the research, one control group and two experimental groups were formed and each group consisted of forty chicks. Each group consisted of 4 replicates (subgroups). Before the trial started, a 7-day adaptation period was allowed. Feed and water for animals were provided *ad libitum*. The effect of *R. officinalis* L. ethanol extract on performance and blood parameters in broiler on ingredients and analyzed nutrient composition of the experimental basal diets are presented in Table 1. Feeder and drinker spaces were identical in each pen, and lighting was continuous (12 h light/dark cycle under a temperature of 25 ± 2 °C). The trial period was 42 days.

Table 1 Ingredients and chemical composition of the experimental diets

Ingredient	Experiment diet composition (%)			
	7-14 day	14-21 day	21-28 day	>28 day
Corn,	35.72	36.67	36.46	32.81
Full fat soybean	27.68	21.17	19.10	21.11
Dry soybean	12.78	14.92	17.48	16.53
Wheat	8.33	11.02	12.65	16.03
Soybean fat	0.75	3.05	5.25	5.50
Poultry meal	1.50	2.00	3.00	3.50
Corn glutenin	8.30	6.60	1.50	-
Meat and bone meal	1.50	2.00	2.44	2.65
DCP (Dicalcium phosphate	1.44	1.00	0.72	0.50
Methionine,	0.24	0.22	0.21	0.25
Vitamin mineral premix	0.50	0.50	0.50	0.50
Salt,	0.21	-	0.18	0.17
Sodium bicarbonate,	0.15	0.15	0.15	0.15
Marble,	0.32	0.16	-	-
Antitoxin,	0.10	0.10	0.10	0.10
Choline chloride,	0.09	0.09	0.09	0.09
Threonine,	0.08	0.08	0.07	0.07
Lysine,	0.31	0.27	0.10	0.04
TOTAL	100.00	100.00	100.00	100.00
Analyzed values				
Dry matter	89.11	89.54	90.41	89.95
Crude protein	25.71	23.96	22.04	22.10
Metabolizable energy (kcal/kg)	3065	3215	3320	3355
Crude fat	7.96	9.18	11.35	11.85
Crude fiber	3.39	3.10	3.26	3.45
Calcium	1.00	0.90	0.90	0.82
Phosphorous	0.50	0.45	0.45	0.41

Provided per kilogram of diet: retinyl acetate, 6 000 000 IU; cholecalciferol, 800 000 IU; DL- α -tocopheryl acetate, 8 000 mg; menadione, 2000 mg; riboflavin, 1000 mg; D-calcium pantothenate, 4000 mg; niacin, 10 000 mg; pyridoxine, 2000 mg; folic acid, 300 mg; D-biotin, 20 mg; thiamine, 3000 mg; cyanocobalamin, 8 mg; ascorbic acid, 20 000 mg; choline chloride, 400 000 mg. Provided per kilogram of diet: Mn, 80 000 mg; Zn, 60 000 mg; Fe, 30 000 mg; Cu, 5 mg; I, 2000 mg; Co, 500 mg; Se, 200 mg.

2.3. Plant material

Rosmarinus officinalis L. aerial parts were collected in July 2013 from Kop Mountain and identified by one of the authors Saban KORDALI (Ataturk University, Faculty of Agriculture, Department of Plant Protection, Erzurum).

2.4. Preparation of the test samples

Rosmarinus officinalis L. aerial parts were dried in the shade for 3–4 days. The dried plant samples were powdered in a blender and then 100 g of plant sample was extracted individually with 500 mL ethanol at room temperature. The extract was filtered, evaporated, and dried in a vacuum at 40 °C with a rotary evaporator after 48 hours. Filtration, the organic solvents were evaporated under reduced pressure and temperature. The dried extracts were stored at +4 °C until used [23].

2.5. Experimental protocol

In total, one hundred and twenty broiler (Ross 308) chicks were randomly assigned into three experimental groups of forty birds each and adapted for 7 days under similar housing and managemental conditions. The experiment consisted of 4 replicates, and birds were allocated in a completely randomized design. The 3 groups were control (C), 100 mg/kg diet *R. officinalis* L. (Group 2) ethanol extract and 200 mg/kg *R. officinalis* L. (Group 3) extract. Chickens were divided into the following groups:

Group I: Control group. Received standard basal diet.

Group II: ROE1 administered group. ROE 100 mg/kg/day days

Group III: ROE2 administered group. ROE 200 mg/kg/day days

To determine the performance parameters the chickens were weighed every 7 days, and daily feed consumption calculated. At the end of trial all broilers were slaughtered and blood samples were taken at slaughter, collected into vacuum tubes anticoagulant centrifuged at 3000 rpm for 10 minutes, at 4 °C and serum stored at -20 °C until laboratory analyses. Serum parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), glucose (GLU), cholesterol (CHOL), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) analysis were measured with autoanalyzer called Mindray Perfect Plus 400.

Levels of malondialdehyde (MDA) were measured according to Yoshioka et al. [24] method, reduced glutathione (GSH) according to Tietze [25], superoxide dismutase (SOD) levels were measured according to Sun et al. [26], glutathione peroxidase (GPx) according to Mates et al. [27] method and catalase (CAT) was measured according to Goth's [28], method with Biotek Elisa Reader (Bio Tek μ Quant MQX200 Elisa reader/USA).

2.6. Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed using SPSS software package, version 20.00. Post-hoc Tukey's test was used to compare the biochemical parameters between the groups. P values < 0.05 were considered as significant. The results are expressed as mean \pm standard deviation (SD) for each group.

3. Results

As shown in Table 2, administration of ROE (100 mg/ kg and 200 mg/ kg diet) significantly ($P < 0.01$) reduced body weight and feed consumption, while feed conversion ratio did not affect.

Table 2 Means of body weight, feed consumption and feed conversion ratio for all groups of broilers

Groups	Body weight (g)	Feed consumption (g)	Feed conversion ratio
Group 1	1670.88 \pm 9.78 ^a	3612.98 \pm 162.94 ^a	2.06 \pm 0.10
Group 2	1491.71 \pm 38.11 ^b	2930.88 \pm 37.93 ^b	1.97 \pm 0.07
Group 3	1430.23 \pm 42.32 ^b	2905.48 \pm 69.19 ^b	2.03 \pm 0.03
P- Value	**	***	NS

Group 1= Control; Group 2=100mg/kg ROE; Group 3= 200mg/kg ROE

a,b,c Values followed by the different letters in the same columns are significantly different (NS: non-significant, ** $P < 0.01$; *** $P < 0.001$).

Table 3 Effects of Rosemary extract supplementation on metabolic profile in broiler serum

Groups	ALT (U/L)	AST (U/L)	TP (g/dL)	ALB (g/dL)	GLU (mg/dL)	CHOL (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Group 1	6.92±0.29 ^a	432.35±19.22 ^a	3.05±0.05 ^b	1.30±0.05 ^b	274.35±6.90 ^a	128.35±2.22	18.35±0.57 ^c	99.67±1.97	25.01±0.71 ^a
Group 2	6.58±0.22 ^a	393.88±16.89 ^a	3.30±0.04 ^a	1.50±0.03 ^a	269.46±8.21 ^a	127.67±1.30	19.00±1.77 ^b	99.10±1.64	24.77±0.87 ^a
Group 3	5.69±0.12 ^b	318.62±10.13 ^b	3.25±0.08 ^a	1.52±0.05 ^a	249.19±1.69 ^b	127.69±2.20	22.38±0.80 ^a	102.18±1.87	21.03±1.05 ^b
P	**	***	**	***	*	NS	***	NS	**

^{a, b, c} Values followed by the different letters in the same columns are significantly different (NS: non-significant, * P<0.05; ** P<0.01; *** P<0.001).
Group 1= Control; Group 2=100mg/kg ROE; Group 3= 200mg/kg ROE

Table 4 The effects of Rosemary extract on serum some biochemical parameters

Groups	MDA (nmol/L)	GSH (mmol/L)	SOD (U/L)	GPX (U/L)	CAT (KU/L)
Group 1	7.70±0.33 ^a	2.27±0.11 ^a	46.66±0.54 ^a	1.94±0.02 ^a	89.23±5.71 ^a
Group 2	7.11±0.33 ^b	2.78±0.19 ^b	51.62±1.38 ^b	2.12±0.01 ^b	122.81±3.69 ^b
Group 3	5.79±0.23 ^b	3.03±0.14 ^b	52.88±1.38 ^b	2.13±0.01 ^b	131.05±1.57 ^b
P	***	**	***	***	***

^{a, b, c} Values followed by the different letters in the same columns are significantly different (NS: non-significant, ** P<0.01; *** P<0.001).
Group 1= Control; Group 2=100mg/kg ROE; Group 3= 200mg/kg ROE

As shown in Table 3-4, administration of ROE (100 mg/ kg and 200 mg/ kg) significantly reduced serum ALT, AST, GLU, LDL and MDA levels, increased serum TP, ALB, TG, GSH, SOD, GPx and CAT levels, and that CHOL and HDL levels weren't affected compared to control group. It was found that administration serum LDL and MDA levels of ROE 200 mg/kg group were more significantly than ROE 100 mg/kg group and prevented the lipid peroxidation more than ROE 100 mg/kg group.

4. Discussion

Accelerating growth, improving feed conversion, increasing carcass weight in poultry production is among targets of top priority. In recent years it has been given importance to efforts for improving meat quality. One of the practices resorted to for improving meat quality is to use different feed additives [29].

In a study where 500 ppm rosemary extract is added to broiler diets by Manafi et al. [10], it has been obtained highest feed consumption and live weight in rosemary group as compared to control group in 42 days of age. Feed conversion ratio was improved with rosemary group as compared to control group. In broiler chickens, Abd El-Latif et al. [3], found that the live weight and body weight gain were lowered with rosemary oil at 200 mg/kg diet as compared to control. The latest authors found that feed consumption and feed conversion ratio were increased in rosemary oil groups at 100 and 200 mg. Al-Kassie et al. [7], found in a study where they used a mixture of anise oil and rosemary oil that in groups, where a mixture of 0, 75 of anise and rosemary oil is used, the live weight in broilers increased significantly as compared to control group and to a mixture of 1.25%. They have found out that a difference is formed between groups as to feed consumption in the study of Al-Kassie et al. [7], where they added rosemary to broiler rations and it is also found out that the highest feed consumption is in the group with 1% of anise, the highest live weight gain is still in the group with 1% anise and that it is followed by 0.5% anise, 1% rosemary and 0.5% rosemary groups. In the same way they have determined the conversion rate of feeds are improved. In Franciosini et al. [4] study with broiler rations they determined that 2 gr/kg rosemary additives do not have any effect on body weight and feed conversion ratio, and that it lowered the average daily feed consumption as compared to control group. They established that the rosemary oil does not have an effect on live weight and feed consumption, that it had a positive effect on feed conversion, and that the hot and cold carcass yield is obtained in the group where 250 ppm rosemary was used in the study made by Ciftci et al. [30], in relation with the effect of rosemary oil on quails under temperature stress. In the study of Mathlouthi et al. [6], where different levels of rosemary was added to broiler rations, it was demonstrated that it had no effect on broilers' performance. Şimşek et al. [12], showed that feed consumption was similar between groups and that it improved egg production and feed conversion rate when they added rosemary oil in the laying hen rations. Algawany and Abd El-Hack [31], in his study where they added 6 and 9 gr/kg of rosemary powder to laying hen rations, it is determined that the additives and the applied dose do not have any influence on feed conversion rate and egg weight. Bugdayci and Ergun [32], observed that additives did not influence live weight, live weight increase, carcass yield, internal organ weight abdominal fat ratios and small intestine pH when they added rosemary oil and probiotic to the broiler rations. In this study we showed that the addition of rosemary extract to broiler rations it does not have a negative effect on animal health, we have seen that the feed consumption and body weight decreased in groups with rosemary addition in comparison to control group and it also seen that it does not have any influence on feed conversion rate.

Ciftci et al. [30], has found that LDL, HDL, cholesterol and triglyceride were not significantly affected by the diet containing 250 ppm rosemary oil in comparison with control group. Algawany and Abd El-Hack [31], determined that total cholesterol, which is one blood parameters, is not influenced by additives. It is determined that the 200 mg/kg dose of rosemary extract decreases the serum cholesterol level numerically and the LDL-cholesterol levels in a statistically significant manner, and increases the triglyceride level in the study performed. However it is not regarded important even though there has been a numeric increase in HDL-cholesterol level. Belenli et al. [2], determined that rosemary caused a decrease in cholesterol and partially on lipid concentration in broiler rations. Bugdayci and Ergun [32], added rosemary oil and probiotics to broiler rations, and observed that these additives did not affect total cholesterol and triglyceride levels in blood serum and humoral immune response. Gazalah and Ali [1], in a study where they added rosemary leaves to broiler rations at a level of 0.5% determined that it increased plasma total protein, albumin and globulin level while it decreased plasma glucose, total lipid and cholesterol content. And also it is determined that the rosemary has a strong antioxidant property in the study where Loetcher et al. [33] and Yasar et al. [34], added rosemary to broiler ration after drying and grinding them. In a study where Polat et al. [35], added rosemary oil and rosemary itself to broiler rations, it was showed that rosemary additives did not have any statistically important effect on serum ALT and AST activity but a significant difference in serum albumin/globulin ratio, total cholesterol and SOD levels. As a result it is determined that rosemary added to broiler feed increased antioxidant enzymes of ethanol extract, prevented lipid peroxidation, lowered blood glucose level and LDL "bad" cholesterol. It is determined that especially rosemary has some more positive effects on some biochemical parameters in groups where 200 mg/kg is applied.

As there are academic publications supporting blood and performance parameters of the study performed, there are also academic publications that are reporting the complete contrary. It is thought that this difference arises from the variety of the different rosemary forms (powder, leaf, extract and oil) that are used during studies and especially from the fact that the active ingredient (phenolic terpenes such as rosmarinic acid) per unit volume is different from each other. The effects of Rosemary ethanol extract on nonenzymatic, enzymatic and lipid peroxidation parameters of serum in broiler had not previously been reported.

5. Conclusion

In conclusion, it is thought that Rosemary extract added to broiler feed has a positive effect on animal health, yet it has a negative effect on performance. This kind of studies must increase in number and must be more detailed in order to fully establish this kind of conclusions.

Compliance with Ethical Standards

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Disclosure of conflict of interest

Betul Apaydın Yıldırım, Muhammed Ali Tunc, Mehmet Gül, Fatih Yildirim and Ahmet Yıldız declare that they have no conflict of interest.

Statement of ethical approval

The experiment was carried out at Ataturk University in Research and Application Unit of School of Veterinary Faculty in accordance with approval by Ataturk University Local Ethics Committee for Animal Experiments (Number: 25.07.2013 36643897-656-ATA-100).

Abbreviations

Reactive oxygen species (ROS), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total protein (TP), Albumin (ALB), Glucose (GLU), Cholesterol (CHOL), Triglyceride (TG), High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Malondialdehyde (MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT).

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