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Effect of dietary extract and dried areal parts of *Rosmarinus officinalis* on performance, immune responses and total serum antioxidant activity in broiler chicks

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ABSTRACT

Objective: To investigate effects of *Rosmarinus officinalis* compared with antibiotic and vitamin E on performance, immune responses and total serum antioxidant activity in broiler chicks.

Methods: A total of 455 day-old broiler chicks (Ross 308) were assigned to one of 7 treatments, comprising 5 replicates in a completely randomized design including a basal diet (control) or either an antibiotic group; 4.5 mg/kg flavophospholipol, vitamin E; 150 IU/kg α-tocopherol acetate and also two different levels of rosemary containing 3 (RP1) and 7 g/kg (RP2) powder added to a basal diet. Furthermore, two different levels of rosemary either encompass of 2.5 (RE1) and 5.0 g/L (RE2) ethanolic extract were added to drinking water. Body weight, daily feed intake and feed conversion ratio were evaluated in different phases of the experiment. Antibody titers against sheep red blood cells, Newcastle and influenza disease viruses were measured on day 32 of age.

Results: Birds receiving RP1 possessed lower body weight than control group during starter and growing periods ($P < 0.05$). Supplementing RE1 decreased body weight on Days 28 and 42 of age compared with control group ($P < 0.05$). In comparison to control birds, carcass yield impaired in broilers given different levels of rosemary powder and rosemary ethanolic extract ($P < 0.05$). Although antibody titers were not affected by dietary treatments, supplementing RP2 and RE1 considerably improved total serum antioxidant activity compared with other dietary treatments ($P < 0.05$).

Conclusions: In conclusion, RP1 and RE1 deteriorated body weight of chickens in different periods. Inclusion of RP2 and RE1 resulted in improved total serum antioxidant activity.

1. Introduction

Application of antibiotics for animal nutrition has been widely banned in European Union due to concerns over emergence of resistant bacteria and possible residues in animal tissues. Furthermore, because suspicions over carcinogenic impact of synthetic antioxidants and also general rejection of synthetic food additives by consumers, new commercial additives of plant origin have been considered to be natural products[1]. Phytogenic feed additives have received attention as possible replacements for antibiotic growth promoters[2]. Moreover, aromatic plants and their components in animal nutrition are potential to beneficially influence appetite and daily feed intake of broilers. Additionally, these medical herbs are able to stimulate immune responses and antioxidant actions.

Rosemary (*Rosmarinus officinalis*) is known to be a dense, aromatic, and evergreen perennial small shrub which grows up to 2 m high with branched, sticky, and narrow leaves. It has small, pale purple or bluish flowers which appear in cymose inflorescence. Fresh or dried leaves (greyish green), whole, chopped, crushed or ground, and essential oil are among the usable forms of rosemary. Rosemary contains phenolic diterpenes such as carnasol, rosmanol, 7-methyl-epirosmanol, isorosmanol and carnosic acid, and the phenolic acids, such as rosmarinic and

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caffeic acids[3]. These substances are associated with antioxidant activity of rosemary. Furthermore, monoterpenes such as α-pinene, myrcene, 1,8-cineole and borneol are components of rosemary volatile oil which are known for their antibacterial, antimicrobial and antifungal activities[4]. Dietary inclusion of rosemary extracts and its triterpenes has been reported to exert anti-inflammatory properties[5]. Additionally, rosemary leaves were shown to improve weight gain and also increase feed utilization as added to broiler diets[6]. Yesilbag *et al.*[7] found that supplementation of 100 mg/kg rosemary oil resulted in higher serum superoxide dismutase activity than other dietary treatments. Furthermore, thiobarbituric acid values decreased in meat of broilers[7] and quails[1], when rosemary was added to the basal diets. Despite noted studies on the antioxidative effect of rosemary, to our knowledge there is no report considering total serum antioxidant activity in broilers supplemented with rosemary. Furthermore, little information is available about the effect of rosemary ethanolic extract.

As such, we expected that rosemary ethanolic extract and rosemary ground plant might beneficially influence the performance, humoral immunity and total serum antioxidant activity in comparison to control group, antibiotic and vitamin E. Thus, the objective of this experiment was to determine the effect of 3 (RP1) and 7 g/kg (RP2) rosemary ground plant and also 2.5 (RE1) and 5 g/L (RE2) rosemary ethanolic extract on performance, humoral immunity and total serum antioxidant activity of broiler chicks.

2. Materials and methods

2.1. Plant extraction

Rosemary areal parts were dried under standard conditions (45 ℃) and then pressed to cobs to provide optimal storage conditions. Also, rosemary areal parts were carefully grinded afterward and added to the experimental diets. The ethanolic extract was extracted using a percolation method at room temperature with 70% ethanol. The extract was concentrated under reduced pressure (bath temperature 50 ℃) and dried in a vacuum desiccator. The residue was dissolved in distilled water and filtered, after which the filtrate was evaporated to dryness[8].

2.2. Birds, diets and management

A total of 455 day-old broiler chicks (Ross 308) were purchased from a commercial hatchery and used in this experiment. At arrival, chicks were weighed, wing banded and assigned to treatment groups so that the initial weight was similar among different treatments. Five replicates comprising 35 pens of 13 chicks each were randomly allotted to 7 dietary treatments in completely randomized design across starter (1–14), growing (14–21) and finishing (21–42) periods. Dietary treatments including: a basal diet (control) or either an antibiotic [4.5 mg/kg flavophospholipol, vitamin E (150 IU/kg α-tocopherol acetate)] and also 2 different levels of rosemary containing RP1 and RP2 powder added to a basal diet. Furthermore, 2 different levels of rosemary either encompass of RE1 and RE2 ethanolic extract were added to drinking water. Experimental diets were formulated to provide nutritional requirements for broiler chicks based on Ross Broiler Manual[9] and fed in mash form (Table 1). Chicks were housed in 1.2 cm × 1.2 cm wire floor pens covered with paper roll and had free access to feed and water throughout the trial. The ambient temperature was gradually decreased from 33 ℃ at first week to 25 ℃ on Day 21 and was then kept constant afterwards. The lighting was programmed as a continuous 24 h light. All the experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee from the Islamic Azad University, Isfahan (Khorasgan) Branch.

Table 1
Ingredients and composition of the basal diets during different periods.

Ingredient/composition		Starter (1–14)	Grower (14–28)	Finisher (28–42)
Diet ingredients (%)	Corn	53.60	56.50	62.30
	Soybean meal	40.40	36.60	32.00
	Soybean oil	2.40	2.99	2.26
	Dicalcium phosphate	1.70	1.84	1.59
	Calcium carbonate	1.17	1.00	0.91
	DL-methionine	0.29	0.22	0.14
	L-lysine	0.03	0.00	0.00
	L-threonine	0.10	0.00	0.00
	Vitamin premix[1]	0.25	0.25	0.25
	Mineral premix[2]	0.25	0.25	0.25
	Sodium chloride	0.30	0.30	0.25
	Toxin binder	0.05	0.05	0.05
Calculated composition	Metabolizable energy (kcal/kg)	2900.00	2980.00	3000.00
	Crude protein (%)	22.20	20.70	19.10
	Lysine (%)	1.22	0.92	0.92
	Methionine + cysteine (%)	0.90	0.72	0.72
	Calcium (%)	1.00	0.85	0.80
	Available phosphorous (%)	0.48	0.42	0.40
	L-threonine (%)	0.75	0.62	0.62

[1]: Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg.
[2]: Mineral premix per kg of diet: Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10 mg; I (KI, 58% I), 1 mg rice hull; Se ($NaSeO_3$, 45.56% Se), 0.2 mg.

2.3. Data collection and sampling

Body weight of broilers was determined on 1, 14, 28 and 42 days of age. Daily feed intake and weight gain of chicks in each pen was recorded at different phases of the experiment. The feed conversion ratio was calculated (daily feed intake/weight gain).

At the final day of experiment, two birds close to the mean body weight of the pen were individually weighed and slaughtered. Carcass, abdominal fat, heart, liver, bursa of Fabricius and spleen were excised, weighed and finally calculated as percentage of live body weight.

In order to evaluate digestive organs, proportions of gizzard, proventriculus, pancreas, duodenum, jejunum, ileum and cecum to live body weight were calculated at the end of the experiment. The length of intestinal segments including duodenum, jejunum, ileum and cecum were measured separately.

2.4. Immune parameters and total serum antioxidant activity

At 9 days of age, Newcastle and influenza antigens were injected to chicks with dual vaccine of Newcastle-influenza. Also, chicks were orally vaccinated against Newcastle disease (Lasota) at 17 days

of age. Two chicks per pen were selected randomly for intraperitonal injection with a 1.0 mL of sheep red blood cells (SRBC) suspension diluted with phosphate buffer saline on Day 27. Five days later, the same wing-banded birds were bled to determine antibody titer against SRBC and also against influenza disease virus and Newcastle disease virus. Subsequently antibody titer against SRBC was measured by hemagglutination assay method and also antibody titer against influenza disease virus and Newcastle disease virus separately were measured by hemagglutination inhibition method. Hemagglutination inhibition antibodies were then converted to log2. Antibody titers against SRBC were measured by the microtiter procedure described by Wegmann and Smithies[10].

Heterophil to lymphocyte ratio was obtained by blood sampling on Day 32. In this process syringes containing heparin were used to avoid blood clot formation. Blood smears were stained by May-Greenwald-Giemsa stain[11]. A hundred leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope (Nikon, Japan) with 100× oil immersion lens and heterophil to lymphocyte ratio was calculated and recorded[12].

On Day 42 of age, two randomly selected birds from each pen were bled from wing vein and then sera samples were separated by centrifuge. Total serum antioxidant activity was determined by spectrophotometer system based on Koracevic *et al.*[13].

2.5. Statistical analysis

Data were subjected to the analysis of variance appropriate for a completely randomized design using general linear model procedure of statistical analysis system 9.2 (SAS Institute Inc., Cary, North Carolina, USA). If a significant effect was detected, differences between treatments were separated using least significant difference test. Statements of statistical significance were based on a probability of $P < 0.05$. Means were presented with their SEM.

3. Results

3.1. Growth performance, carcass traits and digestive organs

Data for growth performance are present in Table 2. Although there were no significant effects of dietary treatments on daily feed intake in broiler chicks during starter, finisher and whole production period of trial, chicks given RP1 tended to have lower daily feed intake than other groups across the entire rearing period ($P > 0.05$).

Broilers receiving RP1 had significantly lower body weight than control, antibiotic and vitamin E on Day 14 post-hatch ($P < 0.05$). Moreover, body weight was lower in broilers given RP1 as compared with control on Day 28 of age ($P < 0.05$). Similarly, inferior body weight was observed for chickens receiving RE1 than the control on Days 28 and 42 of age ($P < 0.05$). Dietary treatments failed to induce any substantial impact on feed conversion ratio across different rearing periods of the experiment.

Although broilers fed on rosemary powder or rosemary ethanolic extract had significantly lower carcass yield than control ($P < 0.05$), other carcass components such as abdominal fat, heart and liver were unaffected by experimental treatments (Table 3).

Table 3

Effect of dietary treatments on carcass traits on Day 42 of age. %.

Treatment	Carcass traits			
	Carcass	Abdominal fat	Heart	Liver
Control	72.73^{a}	1.23	0.52	2.27
FPL	71.45^{ab}	1.6	0.54	2.32
Vitamin E	71.38^{ab}	1.6	0.49	2.32
RP1	69.78^{b}	1.42	0.52	2.39
RP2	69.73^{b}	1.16	0.5	2.31
RE1	69.29^{b}	1.39	0.48	2.34
RE2	68.72^{b}	1.45	0.49	2.23
SEM	0.75	0.07	0.01	0.03

Values in the same column not sharing a common superscript differ significantly ($P < 0.05$). FPL: Flavophospholipol.

Broilers supplemented with vitamin E had remarkably higher duodenum relative weight than control, RP1, RP2 and RE2 ($P < 0.05$; Table 4). On the other hand, jejunal length was higher in broilers received RP1 than the other experimental treatments ($P < 0.05$).

3.2. Immune related parameters and total serum antioxidant activity

The effect of experimental treatments on immune related parameters was shown in Tables 5 and 6. None of humoral immunity related parameters were significantly affected by experimental treatments. However, antibody titer against Newcastle disease virus was slightly greater in birds receiving incremental level of rosemary compared with control and vitamin E ($P > 0.05$). Heterophil to lymphocyte ratio was unaffected by treatments. Total serum antioxidant activity was significantly higher in birds supplemented with RP2 and RE1 than those fed on control, antibiotic or vitamin E ($P < 0.05$). Moreover there was no effect of dietary treatments on lymphoid organs such as spleen and bursa of Fabricius.

Table 2

Effect of dietary treatments on performance of broilers.

Treatment	Daily feed intake (g/d)				Body weight (g)			feed conversion ratio			
	1–14 days	14–28 days	28–42 days	1–42 days	Day 14	Day 28	Day 42	1–14 days	14–28 days	28–42 days	1–42 days
Control	25.80	80.00	194.50	100.20	321.700^{a}	935.100^{a}	2261.300^{ab}	1.420	1.830	2.030	1.900
FPL	26.70	80.00	190.90	96.40	311.900^{a}	896.900^{ab}	2241.700^{ab}	1.480	1.700	2.110	1.990
Vitamin E	26.50	79.90	187.90	98.80	310.500^{a}	890.900^{ab}	2230.800^{ab}	1.460	1.850	2.080	1.950
RP1	26.90	78.80	203.70	102.60	306.500^{b}	871.100^{bc}	2217.000^{abc}	1.470	1.860	2.120	1.980
RP2	26.50	78.70	192.10	99.10	307.800^{ab}	924.100^{ab}	2253.000^{abc}	1.460	1.790	2.030	1.920
RE1	26.80	78.20	193.30	99.90	308.000^{ab}	868.560^{bc}	2183.700^{bc}	1.460	1.950	2.060	1.960
RE2	25.60	77.70	189.60	97.60	311.010^{ab}	893.130^{ab}	2215.500^{a}	1.500	1.880	2.010	1.900
SEM	0.16	0.99	1.53	0.56	2.010	9.810	15.600	0.011	0.021	0.013	0.010

Values in the same column not sharing a common superscript differ significantly ($P < 0.05$). FPL: Flavophospholipol.

Table 4
Effect of dietary treatments on digestive organs on day 42 of age.

Treatment	Digestive organs (%)							Length of intestine (cm)			
	Gizzard	Pancreas	Proventriculus	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum
Control	1.53	0.23	0.35	0.45^{b}	1.08	0.88	0.33	30.4	76.70^{b}	76.2	37.7
FPL	1.6	0.24	0.38	0.52^{ab}	1.08	0.84	0.35	30.6	74.44^{b}	72.11	36.88
Vitamin E	1.58	0.25	0.42	0.58^{a}	1.16	0.93	0.34	30.9	76.55^{b}	79.3	37.7
RP1	1.52	0.24	0.36	0.49^{b}	1.11	0.85	0.34	32.8	82.80^{a}	83.3	39.3
RP2	1.56	0.25	0.35	0.47^{b}	1.1	0.81	0.34	30.9	76.70^{b}	77.2	37.9
RE1	1.62	0.24	0.39	0.53^{ab}	1.14	0.87	0.39	31.4	74.30^{b}	77	40.1
RE2	1.46	0.24	0.38	0.47^{b}	1.07	0.91	0.36	29.8	76.70^{b}	76.2	37.8
SEM	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.33	2.95	0.96	0.41

Values in the same column not sharing a common superscript differ significantly ($P < 0.05$). FPL: Flavophospholipol.

Table 5
Effect of dietary treatments on humoral immunity, heterophil to lymphocyte ratio and total serum antioxidant activity.

Treatment	Influenza disease virus (log2)	Newcastle disease virus (log2)	SRBC (log2)	Heterophil to lymphocyte ratio	Total serum antioxidant activity
Control	7.50	50.00	6.00	0.170	0.440^{c}
FPL	5.80	6.60	7.50	0.190	0.520^{bc}
Vitamin E	5.00	3.90	6.70	0.170	0.650^{b}
RP1	7.40	5.80	6.00	0.190	0.550^{bc}
RP2	6.00	6.10	7.10	0.180	0.990^{a}
RE1	5.70	3.30	7.60	0.170	0.960^{a}
RE2	6.30	5.40	5.50	0.170	0.510^{bc}
SEM	0.23	0.27	0.21	0.005	0.13

Values in the same column not sharing a common superscript differ significantly ($P < 0.05$). FPL: Flavophospholipol.

Table 6
Effect of dietary treatments on lymphoid organs. %.

Treatment	Lymphoid organs	
	Bursa of Fabricius	Spleen
Control	0.22	0.11
FPL	0.20	0.12
Vitamin E	0.18	0.10
RP1	0.22	0.12
RP2	0.20	0.11
RE1	0.21	0.13
RE2	0.20	0.11
SEM	0.01	0.01

FPL: Flavophospholipol.

4. Discussion

When diets are supplemented by herbs, it is of crucial importance to expect possibility of suboptimal performance[14]. Likewise, similar daily feed intake but deteriorated body weight was observed in broilers received RP1 and RE1 during different periods of current trial. It is likely because strong flavor of rosemary that shows a need to adaptation period. Moreover, cell walls of rosemary leaves contain high crude fiber particularly, cellulose which may have hampered nutrient utilization by chickens[6]. Effects of rosemary on growth performance of broilers have shown contradictory results. In this respect, Ghazalah and Ali[6] indicated a reduction in growth of broilers,when rosemary ground leaves increased from 5 to 20 g/kg diet. Yesilbag *et al.*[7] reported that live weight gain impaired in birds fed on 5.7, 8.6 and 11.5 g/kg rosemary leaves while daily feed intake was unaffected by dietary treatments. Moreover, supplementing 25 g/kg rosemary ground leaves decreased weight gain and reduced daily feed intake of broilers at their second and third weeks post-hatch[14]. Otherwise, Mathlouthi *et al.*[15] showed that 100 mg/kg rosemary essential oil improved weight gain and daily feed intake of broilers. Thereby, it seems that some factors such as physiological condition of animals, rearing environment, diet composition and various experimental approaches have resulted in those discrepancies.

Generally, little information about the effects of rosemary on carcass yield is available. In the current trial, carcass relative weight impaired, supplementing different levels of rosemary powder and ethanolic extract. In accord to our results, Loetscher *et al.*[14] reported that 25 g/kg rosemary leaves tended to reduce carcass proportional weight. Nevertheless, Yesilbag *et al.*[7] indicated that rosemary oil improved carcass yield as compared with rosemary ground leaves and vitamin E. Furthermore, Ghazalah and Ali[6] observed no effect of rosemary ground leaves in different levels on carcass yield. These discrepancies may be explained in part by different properties of applied rosemary. Therefore, more research in this field is warranted.

Broilers given RP1 exhibited lower jejunal length than those received other dietary treatments. Otherwise, vitamin E significantly increased duodenum weight compared with control group. On the contrary, Khaligh *et al.*[16] indicated no effect of herbal blend contained rosemary on jejunal length as compared with control. Furthermore, Hernández *et al.*[17] observed that an extract contained rosemary did not affect relative weight of small intestine which is in consistent with the results of this study.

Although rosemary powder and extract failed to show significant effects on antibody production against Newcastle disease virus, SRBC and influenza disease virus, supplementing RE2 and RP1 tended to improve antibody titer against Newcastle disease virus. In contrast to our results, Khaligh *et al.*[16] observed that antibody titer against Newcastle disease virus increased in broilers supplemented with blend of garlic, thyme, cinnamon, rosemary and anise. Otherwise, rosemary oil has been demonstrated to have no substantial effect on antibody titers against Newcastle disease virus and infectious bursal disease[18]. Additionally, antibody titer against SRBC was not changed using up to 20 g/kg of rosemary leaf meal[6] which is in agreement with our obtained results in the current study.

According to antioxidant and antimicrobial properties of rosemary, antibody titers were expected to be elevated. It is likely that higher levels of rosemary powder or extract was needed to significantly affect antibody production.

Supplementing RP2 and RE1 considerably improved total serum antioxidant activity as compared with other dietary treatments. It has been demonstrated that antioxidant activity and total phenolic content of plants are positively correlated[7]. In addition, antioxidant properties of aromatic plants originate from the presence of hydroxyl groups in their phenolic compounds[19]. The rosemary contains high phenol compounds such as polyphenols that probably hamper free radical formation[8]. There is dearth of reports pointing out the effect of rosemary on total serum antioxidant activity in broiler chicks. Yesilbag *et al*.[7] indicated that 11.5 g/kg rosemary ground plant or 200 mg/kg of rosemary essential oil decreased thiobarbituric acid values compared with α-tocopherol acetate in the breast meat of broiler chicks. Furthermore, the greatest activity of superoxide dismutase observed through 100 mg/kg supplementation of rosemary oil. Moreover, Yesilbag *et al*.[1] exhibited a reduction in thiobarbituric acid values, when quails received different levels of rosemary and oregano volatile oil on Days 7, 15 and 30 post-hatch. The reported results show high antioxidative potential of rosemary as observed in our study. On the other hand, Loetscher *et al*.[14] indicated that although 25 g/kg rosemary ground plant improved the oxidative stability of meat, the effect was smaller than that of applied α-tocopherol acetate. Similar results observed by Cardinali *et al*.[20] which is in contrast to obtained data in this experiment.

A clear conclusion to emerge from this study is that RP1 and RE1 impaired body weight of chickens on 28 days of age which might be due to strong flavor and also high cellulose content of rosemary. Furthermore, carcass yield was deteriorated using dietary rosemary powder and extract as compared with control, antibiotic and vitamin E. Although supplementing rosemary powder and extract failed to show any significant impact on antibody titers against Newcastle disease virus, SRBC and influenza disease virus, inclusion of RP2 and RE1 remarkably improved total serum antioxidant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Yesilbag D, Gezen SS, Biricik H, Bulbul T. Effect of a rosemary and oregano volatile oil mixture on performance, lipid oxidation of meat and haematological parameters in Pharaoh quails. *Br Poult Sci* 2012; **53**(1): 89-97.

[2] Huyghebaert G, Ducatelle R, Van Immerseel F. An update on alternatives to antimicrobial growth promoters for broilers. *Vet J* 2011; **187**(2): 182-8.

[3] Charles DJ. *Antioxidant properties of spices, herbs and other sources.* New York: Springer; 2013.

[4] Okoh OO, Sadimenko AP, Afolayan AJ. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. *Food Chem* 2010; **120**(1): 308-12.

[5] Benincá JP, Dalmarco JB, Pizzolatti MG, Fröde TS. Analysis of the anti-inflammatory properties of *Rosmarinus officinalis* L. in mice. *Food Chem* 2011; **124**(2): 468-75.

[6] Ghazalah AA, Ali AM. Rosemary leaves as a dietary supplement for growth in broiler chickens. *Int J Poult Sci* 2008; **7**(3): 234-9.

[7] Yesilbag D, Eren M, Agel H, Kovanlikaya A, Balci F. Effects of dietary rosemary, rosemary volatile oil and vitamin E on broiler performance, meat quality and serum SOD activity. *Br Poult Sci* 2011; **52**(4): 472-82.

[8] Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract: part V. *J Ethnopharmacol* 1999; **67**(3): 373-6.

[9] Aviagen. Ross nutrition supplement. Newbridge: Ross Breeders Limited; 2009. [Online] Available from: http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross_Nutrition_Supplement.pdf [Accessed on 15th February, 2016]

[10] Wegmann TG, Smithies O. A simple hemagglutination system requiring small amounts of red cells and antibodies. *Transfusion* 1966; **6**(1): 67-73.

[11] Lucas AM, Jamroz C. *Atlas of avian hematology*. Washington, DC: United States Department of Agriculture; 1961.

[12] Gross WB, Siegel HS. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis* 1983; **27**: 972-9.

[13] Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 2001; **54**(5): 356-61.

[14] Loetscher Y, Kreuzer M, Messikommer RE. Oxidative stability of the meat of broilers supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle, and effects on performance and meat quality. *Poult Sci* 2013; **92**(11): 2938-48.

[15] Mathlouthi N, Bouzaienne T, Oueslati I, Recoquillay F, Hamdi M, Urdaci M, et al. Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: *in vitro* antimicrobial activities and effects on growth performance. *J Anim Sci* 2012; **90**(3): 813-23.

[16] Khaligh F, Sadeghi G, Karimi A, Vaziry A. Evaluation of different medicinal plants blends in diets for broiler chickens. *J Med Plants Res* 2011; **5**(10): 1971-7.

[17] Hernández F, Madrid J, García V, Orengo J, Megías MD. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poult Sci* 2004; **83**(2): 169-74.

[18] El-Latif ASA, Saleh NS, Allam TS, Ghazy EW. The effects of rosemary (*Rosemarinus afficinalis*) and garlic (*Allium sativum*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. *Br J Poult Sci* 2013; **2**(2): 16-24.

[19] Shahidi F, Wanasundara PK. Phenolic antioxidants. *Crit Rev Food Sci Nutr* 1992; **32**(1): 67-103.

[20] Cardinali R, Cullere M, Dal Bosco A, Mugnai C, Ruggeri S, Mattioli S, et al. Oregano, rosemary and vitamin E dietary supplementation in growing rabbits: effect on growth performance, carcass traits, bone development and meat chemical composition. *Livest Sci* 2015; **175**: 83-9.