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Effects of Different Dietary Rosmarinus Officinalis Powder and Vitamin E Levels on the Performance and Gut Gross Morphometry of Broiler Chickens

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ABSTRACT

The main objective of the present study was to evaluate the effects of the dietary addition of different levels of rosemary powder (RP) and vitamin E (VitE) on broiler performance and gut gross morphometry. A total of 270 one-day-old Ross 308 male chicks were randomly assigned to nine dietary treatments with three replicates of10 birds each. Treatments consisted of diets were supplemented with 0, 0.5 or 1.0% RP and 0, 100 or 200 mg/kg VitE (alpha-tocopherol acetate). Feed intake and weight gain were recorded weekly. On day 42, one bird per replicate was euthanized after blood sampling. Gastrointestinal tract segments were measured and/or weighed. Means were compared by least significant difference. Overall, broilers fed1.0% RP presented lower ($p< 0.05$) feed intake, weight gain, and final weight body than those fed 0.5% RP. Broilers fed the 0.5% RP plus 200 mg/kg VitE diet presented higher weight gain ($p<0.05$) in than those in the control group (0% RP and 0 mg VitE). Jejunum length and weight, colon length and width, and right cecum weight were also negatively affected ($p<0.05$) by the 1.0% RP diet when compared with the 0.5% RP diet. We suggest that the dietary supplementation of 0.5% RP plus 200mg/kg of vitE improves broiler performance and does not have significant adverse effects on gross gut morphometry. Dietary VitE may play a potential protective role against the negative effects of high levels of RP.

INTRODUCTION

During the last decades several additives with probiotic, prebiotic or symbiotic activity have been evaluated as a replacement of antimicrobials as growth promoters in intensive poultry production. Moreover, research on the use of natural plants with medicinal and/or aromatic proprieties as phytogenic feed additives have been carried out. These studies have shown that not only they improve broiler performance and health, and consequently animal welfare, but also to benefits food quality for human consumption, the environment, and public health (Windisch *et al*., 2008; Franz *et al*., 2010; Hashemi & Davoodi, 2011; Charles, 2013).

Rosemary (*Rosmarinus officinalis* L.) is an aromatic has been studied due to the antioxidant effects (Moreno *et al*., 2006) of some of its active compounds, such as carnosol, rosmanol and their acid forms or flavonoids (Ibañez *et al*., 2003), on bird physiology. Several studies reported beneficial effects of rosemary powder (RP) or their extracts (essential oils) on broilers performance, hematological and biochemical parameters, immune status, and meat or egg quality (Hernández *et al*., 2004; Ghazalah & Ali, 2008; Polat *et al*., 2011; Yesilbag *et al*., 2011, 2012, 2013). Some of these studies (Ghazalah & Ali, 2008; Polat *et al*., 2011; Yesilbag *et al*., 2011) also describe negative effects on performance, at least during short starter or finisher periods, and on hematological parameters during the 6-7 weeks production cycle, when the RP increases in diet from low to high levels. Nonetheless, due to potential dual effects on broiler performance, more detailed evaluation, using different inclusion levels, is essential for the effective use of RP as feed additive in broilers.

Although no effects of RP on intestinal weight or their annex glands were observed by Hernández *et al*. (2004), Ghazalah & Ali (2008) reported a negative effect on broiler gizzard weight. However, effects of other feed additives, such as garlic, oregano, cinnamon, pepper, thyme and fermented plant products (rice bran and fermented fruit and vegetables) on intestinal histology were observed (García *et al*., 2007; Abdullah *et al*., 2010; Lokaewmanee *et al*., 2012; Giannenas *et al*., 2014). In fact, more research is need to determine if RP as a feed additive causes gross morphometric changes in the digestive system, particularly on weight, villus density, and on the enterocytes (Applegate *et al*., 1999).

On other hand, Vitamin E (VitE) is recognized as an elective and natural antioxidant at cell membrane level. It is essential for the health of animals submitted to stressful rearing conditions, and it used as a feed additive in broilers diets (Khan *et al*., 2012; Lu *et al*. 2014a, 2014b). However, Xiao *et al*. (2013) suggested that there are functional redundancies between VitE and other natural compounds in broiler diets. Therefore is central to detect the effects of the use of different levels of RP and VitE and their interactions as feed additive on broiler performance and development.

The main objective of this study was to determine the effect of different dietary levels of RP and vitE on the performance to broilers during a 6-week period and on their digestive system developmentat42 days of age.

MATERIALS AND METHODS

Birds and housing

The experiment was conducted for 42 days in a poultry house of Agricultural and Natural Resources Research Centerof Guilan, Guilan, Rasht, Iran. Before and after to the experiment, the facility was thoroughly cleaned and disinfected, including all drinkers and feeders. Care was taken to minimize the number of animals used. All procedures have been approved by the authors' Institution Ethic Committee.

In total, 270 one-day-old Ross 308 male chicks (Aviagen, Newbridge, Scotland, UK 35805) were housed in a 5 x 20mbroiler house equipped with a potent fan (1400 m^3 per hour) and five smaller fans (total of 3500m^3 per hour). Heat was generated by a heater programed according to the Ross 308 manual (Aviagen, Newbridge, Scotland, UK 35805; infoworldwide@aviagen.com). Air humidity was maintained at 55 to 65 % during the starter growing period by spraying water on the floor. Artificial lighting was provided by 20-watt lamps were installed 2.2 m above the floor for three hours per day, between 19:00 and 22:00, until day 42.

Sanitation principles and health measures for chicken production were applied. Drinkers were cleaned and washed daily. Birds were vaccinated against infectious bursal disease, avian influenza, infectious bronchitis, and Newcastle disease. After each vaccination, 1:1000 multivitamin + electrolytes solution was mixed in the drinking water for 24 hours.

Treatments and diet composition

Nine treatments with three replicates of 10 birds, with similar body weight (p>0.05), were applied. All birds were fed according to the producer's feeding instructions. The mean composition of basal diets and their nutrient composition for the starter (1-21 days of age) and finisher (22-42 days of age) periods are given in Table 1.

Rosemary powder (RP) was included at levels of 0.5 or 1.0% in the basal diet, and VitE at levels of and 0, 100, or 200 mg/kg of VitE, according to each treatment: control group T1 (RP0 + VitE0), T2 (RP0 + VitE100), T3 (RP0 + VitE200), T4 (RP0.5 + VitE0), T5 (RP0.5 + VitE100), T6 (RP0.5 + VitE200), T7 (RP1.0 + VitE0), T8 (RP1.0 + VitE100), and T9 (RP1.0 + VitE200).

Rosemary nutritional levels were analyzed according to AOAC methods (AOAC, 2005), and presented was 3124 kcal gross energy/kg, 2511 kcal metabolizable energy/kg (ME = 0.80378* GE), 4.36% crude protein, and 20.73% crude fiber. However, due to relatively low doses of RP, of up to 10 g per kg of feed, RP nutritional levels were not considered during basal diet formulation.

Feed remaining in feeders was weighed and removed at the end of each week. This feed was not taken in account for intake calculation.

Performance, hematology and carcass parameters

Feed intake (g) and weight gain (g) were recorded weekly. Feed conversion ratio (FCR = total feed intake / total body weight gain) and production index [PI = total body weight x livability (%) / (age days x feed conversion ratio x10)] were calculated.

Table 1 – Feed ingredients and calculated nutrient composition of the basal starter (1-21 days of age) and finisher (22-42 days of age) diets.

Diet					
Ingredient (g/kg)	Starter diet	Finisher diet	Calculated composition	Starter diet	Finisher diet
Corn	556.3	585.9	Phosphorus (%)	0.74	0.69
Soybean oil	27.4	43.7	Available Phosphorus (%)	0.50	0.45
$NaHCO_3$	1.9	1.2	Potassium (%)	0.90	0.84
Soybean meal	355.3	322.3	Manganese (mg/kg)	120.00	120.00
Gluten meal	10	0	Sodium (%)	0.16	0.16
Wheat bran	7	10	Glycine (%)	0.93	0.86
CaCO3	12.9	10.5	Serine (%)	1.10	1.02
Ca%22P%18	18.9	16.7	Gly+Ser (%)	2.40	2.21
NaCl	2.3	2.7	Histidine (%)	0.59	0.55
Mineral Mixture[1]	3	3	Isoleucine (%)	0.85	0.85
Vitamin Mixture[2]	3	3	Leucine (%)	1.90	1.80
DL-Methionine	1.2	1	Lysine (%)	1.27	1.11
Lysine-Hydro-Chloride	0.8	0	Methionine (%)	0.47	0.42
Total	1000	1000	Met+Cys (%)	0.85	0.76
Calculated composition			Phenylalanine (%)	1.08	0.98
Dry Matter (%)	86.47	87.08	Tyrosine (%)	0.89	0.81
Energy (ME) (kcal/kg)	3025	3150	Phe+Tyr (%)	1.97	1.79
Crude Protein (%)	23.00	21.00	Threonine (%)	0.83	0.78
Ether Extract (%)	5.25	6.95	Tryptophan (%)	0.3	0.28
Linoleic Acid (%)	2.77	3.66	Valine (%)	1.00	0.96
Crude Fiber (%)	2.69	2.66	Arginine (%)	1.30	1.30
Calcium (%)	1.05	0.90			

[1] Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g

[2] Vitamin A: 5000 IU/g; Vitamin D3: 500 IU/g; Vitamin E: 3 mg/g; Vitamin K3: 1.5 mg/g; Vitamin B6: 13 mg/g; Vitamin B2: 1 mg/g; Calcium Pantothenate: 4 mg/g; Niacin: 15 mg/g

Before blood collection, feed was removed from feeders for a period of four hours in an attempt to allow stabilization of the various plasma components. Blood was collected in the morning to further reduce the variability of the plasma components to be measured. At 42 days of age, a 5 mL volume of venous blood was collected from the ulnar vein of the wing of one bird per replicate. Care was taken to choose the most representative birds with respect to body weight compared with the average body weight of their group. The whole blood sample was transferred from the syringe into a tube coated with 10 mg of the anticoagulante thylene diaminetetra acetic acid (EDTA). Blood samples werecentrifuged at 3000 rpm for 20 min to ensure the separation of blood cells from plasma. Plasma was collected and stored at -20°C until further analyses. Plasma component analyses were based on standard protocols, using the Roche Cobas Integra 400 Plus autoanalyzer (Roche Diagnostics, GmbH, Mannheim, Germany). Uric acid, total protein, total cholesterol, triglycerides, low density lipoprotein (LDL) cholesterol, high density lipoprotein(HDL) cholesterol, very high density lipoproteins (VLDL), aspartate amino transferase (AST) and alanine amino transferase (ALT) were assayed using commercial kits (Teif Azmoon Pars, Co., Tehran, Iran).

On day 42, the most representative bird from each replicate was euthanized after 4 hours of fasting for complete evacuation of the gut. After blood sample collection, these birds were used for measuring gastrointestinal tract characteristics and carcass yield. Birds were fully plucked by dry plucking method. Feet were separated from the carcass by the tibiotarsal joint. Neck, wingtips, gut, and liver were removed and the empty or edible carcass was weighed and intestinal segments dimensions were recorded.

Carcass parts were dissected and separately weighed. Parts included the head, breast, wings, femurs, abdominal fat, pancreas, gizzard, crop, lungs, heart, liver, kidneys, and digestive tract. Length, width, and wall thickness of the different gut segments were also recorded. Head, breast, abdominal fat, pancreas, full gizzard, full crop, lung, heart, liver, kidney, brain, testicles, duodenum, ileum, jejunum, colon, left and right cecum, vertebral column with neck and proventriculus were weighed.

All abdominal fat, including that around the rectum, gizzard and proventriculus, was collected and weighed.

The length (cm), width (mm) and wall thickness (mm) of duodenum, ileum, jejunum, left and right cecum, and colon were recorded.

Total weight of all dissected parts and the weights of various segments of the digestive tract were calculated relative to eviscerated carcass weight according to the following formula: [(weight of component/eviscerated carcass weight)×100].

Statistical analysis

Data were submitted to two-way analysis of variance, according to a 3×3 factorial arrangement, with three RP inclusion levels (0, 0.5, and 1.0 % in diet) and three vitE inclusion levels (0, 100, and 20 mg/kg in diet). Data were analyzed using the GLM procedure of SPSS (1997) statistical software. The means (± SEM) were compared by least significant difference (LSD) test. The results were considered different at 5% significance level.

RESULTS

Performance

Performance results are reported in Tables 2, 3, and 4 for the starter (1-21 days of age), finisher (22-42 days of age) and entire (1-42 days of age) experimental periods, respectively.

Overall, a deterioration effect ($p<0.05$) on the feed intake, weight gain, feed conversion ratio or production index were observed between groups when rosemary inclusion levels increased from 0 or 0.5 to 1.0%. These findings were obvious during both feed periods. During the starter period, adverse effects of the supplementation of 1% RP alone on FCR, weight gain, and production index were observed ($p<0.05$). During the finisher period, this deleterious effect was observed on feed intake and weight gain as RP increased ($p<0.05$) from 0.5 to 1%.

Average weight gain during the entire rearing period was negatively affected ($p<0.05$) by 1% RP addition compared with the level of 0.5%.The supplementation of 200 mg VitE alone increased weight gain during finisher period ($p<0.05$), but not during the starter period ($p>0.05$).

There was no effect of the supplementation of different combinations of RP and VitE on the evaluated performance parameters.

Hematological parameters

Uric acid and total protein levels were significantly affected ($p<0.05$) by RP and VitE additives (Table 5). However, total cholesterol (129.0 ± 8.9 mg/dL), triglyceride (68.0 ± 19.4 mg/dL), VLDL (13.7 ± 3.9 mg/dL), HDL cholesterol (82.3 ± 11.2 mg/dL), and LDL cholesterol (33.0 ± 7.8 mg/dL) levels, LDL/HDL ratio (0.49 ± 0.12), and AST (280.0 ± 41.2 UI/L) and ALT(2.7 ± 0.6 UI/L) were similar ($p>0.05$) among groups.

Gut gross morphometry and carcass characteristics

All significant differences in duodenal, jejunal, and colonic morphometry are reported in Table 6.

Table 2 – Performance (mean) of Ross 308 broilers from 1-21 days of age (starter period).

Treatment		Trait: Feed intake (g)	Feed conversion ratio	Weight gain (g)	Production index
Rosemary (% in diet)	0	1199.1	1.37^{a}	877.8^{a}	301.4^{a}
	0.5	1188.6	1.39^{a,b}	857.7^{a,b}	296.1^{a,b}
	1.0	1190.7	1.42^{b}	837.9^{b}	274.5^{b}
Vitamin E (mg/kg)	0	1188.6	1.36	871.5	298.0
	100	1188.6	1.38	863.1	289.1
	200	1203.3	1.40	858.9	285.0
RP X VitE interaction		p<0.05	N.S.	N.S.	N.S.
T1: RP0 + VitE0		1201.2^{ab}	1.33	900.9	315.4
T2: RP0 + VitE100		1197.0^{ab}	1.38	865.8	294.1
T3: RP0 + VitE200		1203.3^{ab}	1.39	867.7	294.7
T4: RP0.5 + VitE0		1194.9^{ab}	1.37	869.4	298.6
T5: RP0.5 + VitE100		1184.4^{ab}	1.35	879.1	295.3
T6: RP0.5 + VitE200		1188.6^{ab}	1.35	880.7	294.2
T7: RP1.0 + VitE0		1167.6^{a}	1.39	840.8	280.0
T8: RP1.0 + VitE100		1186.5^{ab}	1.41	842.3	277.8
T9: RP1.0 + VitE200		1220.1^{b}	1.47	827.6	265.7

a,b: Different letters within the same column indicate significant differences among treatment groups (p<0.05).

N.S. – Not significant.

Table 3 – Performance (mean) of Ross 308 broilers from 22-42 days of age (finisher period).

Treatment		Trait			
		Feed intake (g)	Feed conversion ratio	Weight gain (g)	Production index
Rosemary (% in diet)	0	3410.4[ab]	2.00	1706.3[ab]	385.6
	0.5	3555.3[a]	1.95	1826.1[a]	442.9
	1.0	3355.8[b]	2.09	1701.8[b]	401.7
Vitamin E (mg/kg)	0	3393.6	2.02	1684.0[a]	384.4[a]
	100	3427.2	2.00	1717.2[ab]	393.8[ab]
	200	3504.9	1.91	1832.3[b]	452.1[b]
RP X VitE interaction		N.S.	N.S.	N.S.	N.S.
T1: RP0 + VitE0		3389.4	2.03	1673.7	368.9
T2: RP0 + VitE100		3347.4	2.07	1618.0	341.6
T3: RP0 + VitE200		3498.6	1.91	1827.7	446.3
T4: RP0.5 + VitE0		3502.8	2.00	1753.6	413.5
T5: RP0.5 + VitE100		3559.5	1.96	1814.8	436.3
T6: RP0.5 + VitE200		3605.7	1.89	1907.0	479.0
T7: RP1.0 + VitE0		3282.5	2.02	1622.5	370.7
T8: RP1.0 + VitE100		3372.6	1.96	1720.5	403.5
T9: RP1.0 + VitE200		3410.4	1.94	1762.2	431.0

a,b: Different letters within the same column indicate significant differences among treatment groups (p<0.05).

N.S. – Not significant.

Table 4 – Performance (mean) of Ross 308 broilers from 1-42 days of age fed diets containing the different levels of rosemary powder and vitamin E.

Treatment		Trait				
		Feed intake (g)	Feed conversion ratio	Weight gain (g)	Weight chick at 42nd day (g)	Production index
Rosemary (% in diet)	0	4611.6[ab]	1.79	2583.0[ab]	2638.9[ab]	357.4
	0.5	4746.0[a]	1.76	2704.8[a]	2751.1[a]	388.0
	1.0	4548.6[b]	1.79	2536.8[b]	2586.9[b]	357.3
Vitamin E (mg/kg)	0	4582.2	1.80	2543.6[a]	2608.3[a]	355.8
	100	4615.8	1.79	2578.8[ab]	2628.6[ab]	356.7
	200	4708.2	1.75	2692.2[b]	2740.0[b]	387.2
RP X VitE interaction		N.S.	N.S.	N.S.	N.S.	N.S.
T1: RP0 + VitE0		4590.6	1.78	2574.6	2640.3	354.5
T2: RP0 + VitE100		4544.4	1.83	2482.2	2532.3	328.6
T3: RP0 + VitE200		4699.8	1.74	2696.4	2744.0	389.0
T4: RP0.5 + VitE0		4741.8	1.81	2625.0	2674.2	373.3
T5: RP0.5 + VitE100		4792.2	1.78	2692.2	2742.7	384.0
T6: RP0.5 + VitE200		4456.2	1.60	2788.8	2836.3	406.6
T7: RP1.0 + VitE0		4561.2	1.85	2465.4	2510.4	339.7
T8: RP1.0 + VitE100		4628.4	1.81	2562.0	2610.8	357.4
T9: RP1.0 + VitE200		4741.8	1.83	2591.4	2639.6	366.0

a,b: Different letters within the same column indicate significant differences among treatment groups (p<0.05).

N.S. – Not significant.

Dietary RP addition influenced (p<0.05) jejunum weight (0% RP: 75.2 g vs. 0.5% RP: 54.3 g or 1.0% RP: 55.9 ±5.0 g), jejunum relative weight (0% RP: 3.00% mm vs. 0.5% RP: 2.19 ±0.20 g), jejunum length (0% RP: 1291.1 cm vs. 1.0% RP: 1112.2 ± 45.5 cm), colon length (0% RP: 94.4 cm vs. 1.0% RP: 76.1 ± 3.2 cm), colon width (0% RP: 6.3 mm vs. 1.0% RP: 4.7 ± 0.4 mm), right cecum absolute weight (0.5% RP: 8.3 g vs. 1.0% RP: 6.3 ± 0.5 g; p<0.05) and relative weight (0% RP: 0.26% vs. 0.5% RP: 0.34 ± 0.02%), but no significant RP x VitE interactions were observed for these traits.

The duodenum width was influenced by RP (0% RP: 5.6 mm or 0.5% RP: 5.3 mm vs. 1.0% RP: 6.6 ± 0.3 mm; p<0.05), VitE (100g VitE: 5.2 mm vs. 200g VitE: 6.6 ± 0.3 mm; p<0.05) and by RP x VitE interactions (p<0.01). Higher duodenum width (p<0.01) was observed in control group (T1; 7.0 ± 0.5 mm) compared with T2 (4.2 mm) and T4 (4.6 mm).

Table 5 – Plasma uric acid and total protein levels (mean) of 42-d-old Ross 308 broilers fed diets containing the different levels of rosemary powder and Vitamin E.

Treatment		Trait	
		Uric acid (mg/dL)	Total protein (g/dL)
Rosemary (% in diet)	0	4.4^{ab}	3.8
	0.5	3.9^{a}	3.6
	1.0	5.8^{b}	4.0
Vitamin E (mg/kg)	0	5.7^{a}	4.0
	100	3.9^{b}	3.7
	200	4.6^{ab}	3.7
RP X VitE interaction		N.S.	p = 0.05
T1: RP0 + VitE0		5.3	3.9^{ab}
T2: RP0 + VitE100		3.7	4.1^{ab}
T3: RP0 + VitE200		4.3	3.4^{a}
T4: RP0.5 + VitE0		4.3	3.8^{ab}
T5: RP0.5 + VitE100		3.8	3.3^{a}
T6: RP0.5 + VitE200		3.7	3.8^{ab}
T7: RP1.0 + VitE0		7.6	4.4^{b}
T8: RP1.0 + VitE100		4.1	3.6^{ab}
T9: RP1.0 + VitE200		5.7	4.0^{ab}

a,b: Different letters within the same column indicate significant differences among treatment groups (p<0.05).

N.S. – Not significant.

The weight of the remaining carcass parts and of other organs or tissues evaluated in the present study was not influenced (p>0.05) by the treatments and no significant RP x VitE interactions were observed either (p>0.05).

DISCUSSION

In the present study, there were both positive and negative effects of RP and VitE feed additives on several performance parameters, according the added levels or their interactions. Overall, the dietary addition of 0.5% RP promoted in higher feed intake and weight gain at 42 days compared with 1.0% RP; however, the obtained values were not different from those obtained in the control group. This indicates that the dietary addition of 0.5% RP did not improve broiler performance and that the level of 1.0% RP negatively affected performance. Ghazalah & Ali (2008) observed positive effects of the dietary addition of 0.5% rosemary leaves on the weight gain, body weight, and feed conversion ratio of 49-d-old broilers compared with the control group. However, those authors also observed lower live body weight and feed intake when 1 and 2% of rosemary leaves were added to the diet and attributed this effect to a reduction in feed palatability for young chicks.

On other hand, the supplementation of 200 mg VitE/kg had positive effect on the weight gain and feed intake on day 42. This suggests that 200 mg VitE/kg diet may improve broiler performance. In addition, the significant interaction between that VitE level counteracted the negative effects of aflatoxin when added in combination with 1.0% RP: during

Table 6 – Duodenal, jejunal, and colonic width, weight, and length (mean) of 42-d-old Ross 308 broilers fed diets containing the different levels of rosemary powder and Vitamin E.

Treatment		Trait							
		Duodenum width (mm)	Jejunum weight (g)	Jejunum Relative weight (%)	Jejunum length (cm)	Colon length (cm)	Colon width (mm)	Right cecum weight (g)	Right cecum relative weight (%)
Rosemary (% in diet)	0	5.6^{a}	75.2^{a}	3.00^{a}	1291.1^{a}	94.4^{a}	6.3^{a}	6.5	0.26^{a}
	0.5	5.3^{a}	54.3^{b}	2.19^{b}	1183.3^{ab}	87.2^{ab}	4.9^{ab}	8.3^{a}	0.34^{b}
	1.0	6.6^{b}	55.9^{b}	2.41^{ab}	1112.2^{b}	76.1^{b}	4.7^{b}	6.3^{b}	0.27
Vitamin E (mg/kg)	0	6.0^{ab}	66.7	2.80	1225.6	85.6	5.9	6.9	0.29
	100	5.2^{a}	61.1	2.49	1251.1	87.2	4.8	7.7	0.31
	200	6.3^{b}	57.5	2.35	1110.0	85.0	5.1	6.4	0.27
RP X VitE interaction		p<0.01	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
T1: RP0 + VitE0		7.0^{a}	81.5	3.37	1283.3	90.0	7.2	6.1	0.25
T2: RP0 + VitE100		4.2^{b}	69.3	2.87	1366.7	100.0	5.6	7.9	0.33
T3: RP0 + VitE200		5.6^{abc}	74.7	2.90	1223.3	93.3	6.0	5.4	0.21
T4: RP0.5 + VitE0		4.6^{b,c}	52.5	2.17	1176.7	83.3	5.4	9.4	0.39
T5: RP0.5 + VitE100		4.8^{abc}	58.0	2.26	1256.7	85.0	4.7	6.8	0.26
T6: RP0.5 + VitE200		6.4^{abc}	52.2	2.12	1116.7	93.3	4.8	8.7	0.36
T7: RP1.0 + VitE0		6.4^{abc}	66.1	2.86	1216.7	83.3	5.3	5.3	0.23
T8: RP1.0 + VitE100		6.5^{abc}	55.8	2.39	1130.0	76.7	4.3	8.3	0.35
T9: RP1.0 + VitE200		6.9^{a,c}	45.7	2.03	990.0	68.3	4.4	5.3	0.24

a,b,c: Different letters within the same column indicate significant differences among treatment groups (p<0.05).

N.S. – Not significant.

the starter period (weeks 1-3), representing the early adaptation period of the immature gut, a significant RP X VitE interaction was observed, as confirmed by the differences in feed intake between T7 and T9 groups. This period is very important because small intestine growth peaks around the final of first week up to the early 3rd week post-hatch (Uni *et al.*, 1999; Noy *et al*., 2001). It was shown that high VitE levels in excess of nutritional requirements may reduce oxidative stress (McIlroy *et al*., 1993), which may explain the RP X VitE interactions observed in our study, when high RP levels presumably caused stress in broilers.

In present study, both RP and VitE additives influenced plasma uric acid concentrations: 0.5% RP reduced uric acid levels compared with 1.0% RP (3.9*vs.* 5.8 ± 0.5 mg/dL, respectively).This is in agreement with Ghazalah & Ali (2008), who observed reduction in blood uric acid level in broilers fed 0.5% rosemary leaves; however, VitE was not tested in their study. In our case, the supplementation of 100 mg of VitE reduced plasma uric acid level to 3.9 ± 0.5 mg/dL, but further research with a higher number of birds is need in order to confirm this result.

In the present study, gut gross morphology was also affected RP and VitE additives. Duodenal width was positively affected both by 1.0% RP and 200mg VitE levels, as well as their interaction. Additionally, poor development of the jejunum was observed at dietary 0.5 and 1.0% RP levels. Groups T2 and T4 showed lower jejunal width (4.2 mm and 4.6 mm) than group T1 (7.0 mm), but higher values were obtained group T9 (6.9 ± 0.5 mm) than group T4. These differences evidence an interaction between both additives added at high levels, suggesting the protective role of VitE at 200 mg level when 1% of RP was added. In fact, a positive effect of VitE and glutamine, supplemented until one week post hatch, on duodenum development was observed by Murakami *et al.* (2007).

In our study, large intestine (cecum and colon) morphometry was negatively affected only by 1.0% RP, whereas 0.5% RP increased right cecum relative weight. The cecum is an important site of bacterial digestion, and the colon physiology is influenced by dietary fiber contents. The positive effect of 0.5% RP on broiler performance observed in the present study may be justified by a positive effect on cecal bacteria, similar to that obtained with subtherapeutic doses of antibiotics (Mathlouthi *et al.*, 2012). Moreover, the negative influence of RP on small intestine, particularly at 1.0%, may also partially explain the variations in plasma protein and uric acid levels. In fact, the duodenum, jejunum and ileum are the main organs responsible for nutrient digestion and absorption (Murakami *et al.*, 2007). Unfortunately, the intestinal microflora and histological traits were not evaluated in the present study and further research is needed to elucidate this effect.

Ghazalah & Ali (2008) did not observe any effects of feeding broilers with rosemary leaves on broiler carcass, heart, liver, and abdominal fat relative weights, except for the gizzard, which was lighter compared with the control group. Although the gut morphometry was affected by both additives, in the present study, the other evaluated organs and tissues, as well as carcass parts, were not influenced by the treatments.

We conclude that the dietary supplementation levels 0.5% RP and 200 mg/kg VitE improved broiler performance, and did not cause any evident adverse effects. Gut gross morphometry was negatively affected by the dietary supplementation of 1.0% RP. High rosemary powder levels in the diet may impair broiler performance, despite the potential protective role of vitamin E.

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