

Effect of dietary supplementation of licorice extract and a prebiotic on performance and blood metabolites of broilers

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Abstract

Six hundred Ross 308 male broiler chickens were used to study the effect of licorice extract and the prebiotic, fermacto, on performance, blood metabolites and gastro-intestinal transit time (GTT) of feed in the birds. The birds were fed according to a three phase feeding programme on a starter, grower and finisher diet during the ages of 1 - 14 day, 15 – 35 days and 35 – 49 days of age, respectively. The basic diets during each phase contained either 100 or 95% of recommended digestible amino acid (RDAA) concentrations. The two basic starter diets were divided into five treatment diets: No supplement (control); and supplemented with 2.0 g fermacto/kg; and 2.0 (high); 1.0 (medium) and 0.5 (low) g licorice extract/kg diet. In the grower diets half the levels of these supplements were included, while the two finisher diets were fed without containing any of the experimental supplements. There was not a significant difference in body weight, feed intake and feed conversion ratio between the birds fed the control and the diets supplemented with the prebiotic or the different levels of licorice. However, birds receiving diets containing licorice extract had lighter abdominal fat pads compared to those fed the prebiotic or control diets. Blood cholesterol concentrations decreased significantly in birds receiving the high level of licorice in their starter and grower diets as compared to the control. The GTT of feed in the birds fed diets containing the prebiotic or licorice extract did not differ from that of the birds in the control. A 5% reduction in dietary RDAA concentration caused an increase in feed conversion ratio of chickens on the starter and grower diets and for the total duration of the experiment.

Keywords: Fermacto, performance, Ross 308, *Glycyrrhiza glabra*

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Introduction

Herbs' spices and various plant extracts have proven to be possible candidates to replace antibiotic growth promoters in animal diets. Licorice, the root of the leguminous *glycyrrhiza* plant species, *Glycyrrhiza glabra*, has been used as a medicinal product for over 4000 years (Shibata, 2000). It has been reported that licorice has anti-microbial (Fukai *et al.*, 2002a), anti-helicobacter (Fukai *et al.*, 2002b), anti-atherosclerotic (Fuhrman, 2002), anti-oxidative (Vaya *et al.*, 1997), anti-inflammatory (Yokota *et al.*, 1998), anti-fungal (Sato *et al.*, 2000), oestrogen-like (Somjen *et al.*, 2004), anti-viral (Fiore, 2007), anti-infective (Nowakowska, 2006), anti-nephritic and radical scavenging activities (Fukai *et al.*, 2003). Human studies suggested that licorice flavonoid oil (LFO) is a safe functional food that can be consumed for extended periods or at excessive intakes with a potential weight-reducing effect (Tominaga *et al.*, 2006). They indicated that LFO reduced body weight by decreasing body fat mass in humans. Aoki *et al.* (2007) showed that LFO reduced body weight gain and abdominal white adipose tissues in mice fed high-fat diets. Furthermore, in other studies licorice hydrophobic flavonoids decreased abdominal fat in humans and mice (Armanini *et al.*, 2003; Nakagawa *et al.*, 2004). Plant herbs or their extracted oils containing terpenoids, may improve poultry health and production. The effects of licorice extract have been evaluated *in vitro* and in laboratory animals but its influence on poultry performance has not been documented.

Fermentation products such as prebiotics have been used in poultry diets. Prebiotics enhance gut development, digestive efficiency, gut beneficial bacteria, and duodenal and jejunal villi height (Harms & Miles, 1988; Hirayama *et al.*, 2000), and increase body weight and improve the feed conversion ratio (FCR) (Khaksar *et al.*, 2008). High prices of protein and environmental concerns have forced the poultry industry to reduce dietary protein levels. Moreover, recommended levels of nutrients for animals focus on maximizing performance, and many of the currently used feed additives in animal feeds aim at enhancing nutrient utilization by means of diverse mechanisms. It seems as if birds may compensate for a marginal decrease in

digestible amino acid levels in their diets through the actions of feed additives such as prebiotics and licorice extract. Furthermore, reducing the level of protein and amino acids in diets below recommended levels has potential environmental benefits (Rodriguez *et al.*, 2005). The objective of this experiment was to determine the effects of dietary supplementation of licorice extract derived from wild growing herb in Iran and the prebiotic, fermacto, on performance, blood metabolites, fat digestibility and digestive organ size of broiler chickens fed diets containing different levels of digestible amino acids.

Material and Methods

This experiment was reviewed and approved by the Committee on Animal Research of the Ferdowsi University of Mashhad, Mashhad, Iran. Six hundred day-old male chicks (Ross-308) were obtained from a commercial hatchery, weighed on arrival and randomly assigned to 50 floor pens of 12 birds each.

Table 1 The composition and calculated nutrient content of the basal diets contained 100 or 95% recommended digestible amino acids (RDAA) concentrations

Composition	Starter ¹ (g/kg)		Grower ² (g/kg)		Finisher ³ (g/kg)	
	100% RDAA	95% RDAA	100% RDAA	95% RDAA	100% RDAA	95% RDAA
Maize	507	534.6	543	580	609	633
Wheat bran	35	40	24	21	23	28.9
Soyabean meal	375.5	345	332	302.7	270	241.6
Vegetable oil	38.5	36	60.5	55	59	57
Limestone	18.5	18.5	16.6	16.5	16	16.5
Dicalcium phosphate	14.5	15	12.8	13.4	12	12
NaCl	2.9	3	2.9	3	3.1	3
Methionine	2.1	1.9	2.2	2.1	1.9	1.7
L-Lysine	0	0	0	0.3	0	0.3
Vitamin premix ⁴	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix ⁴	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin E	1	1	1	1	1	1
Calculated nutrients						
AME (KJ/kg) ⁵	12598	12606	13297	13297	13502	13506
Crude protein	230.1	218.48	210	199.4	186.12	175.62
Calcium	10.09	10.15	8.99	9	8.5	8.61
Available phosphorus	5.02	5.09	4.51	4.56	4.23	4.2
Dig Lysine	11.73	10.92	10.4	9.93	8.86	8.35
Dig Methionine	5.4	5.06	5.27	5.06	4.7	4.38
Dig Methionine+Cystine	8.155	7.68	7.81	7.47	6.97	6.52
Linoleic acid	30.98	30.21	42.3	40	42.54	41.98

¹The levels of 0.0 (control), 2.0 g/kg fermacto and 2.0, 1.0 and 0.5 g licorice extract/kg were added to starter diets at both RDAA levels.

²The grower diets contained half of the same supplementation of the starter diets.

³The finisher diets did not contain any experimental supplements.

⁴Supplied per kilogram of diet: 10,000 IU vitamin A; 9800 IU vitamin D₃; 121 IU vitamin E; 20 µg vitamin B₁₂; 4.4 mg riboflavin; 40 mg calcium pantothenate; 22 mg niacin; 840 mg choline chloride; 30 µg biotin; 4 mg thiamine; 60 mg zinc sulphate; 60 mg manganese.

⁵Apparent metabolizable energy.

Housing temperature started at 30 - 32 °C for the day-old chicks and decreased at 2.5 °C per week to reach 20 - 22 °C at d 28 from when the temperature was kept constant to the end of experiment (49 d). The lighting cycle was 24 h from days 1 to 3 and a 23 : 1 h light : dark cycle until the end of the experiment.

Maize-soyabean meal-based broiler diets were prepared following the instructions of the Aviagen Company (2007) and the ingredient composition recommended by Leeson & Summers (2005). Two basic diets per phase were prepared to meet 100 and 95% of the recommended digestible amino acid (RDAA) allowances for the starter (1 - 14 d of age), grower (15 - 35 d) and finisher (36 - 49 d) phases of the study. Each starter diet was subdivided into five dietary treatments: The control with zero supplements; and diets supplemented with 2.0 g fermacto/kg or 2.0, 1.0 or 0.5 g licorice extract/kg diet. In the second phase the grower diets contained half the level of the same supplements as the starter diets. In the finisher phase the diets did not contain any of the experimental supplements. Each dietary treatment was randomly allocated to five pens of 12 birds each. Licorice extract and the prebiotic were obtained from Zagros (<http://www.Zagros-licorice.com>) and Javaneh Khorasan Companies (<http://www.javanehkhorasan.com>), respectively. No anti-microbial, anti-coccidial or in-feed enzymes were used in any of the experimental diets. Feed and water were provided *ad libitum*. All diets for each period were isoenergetic, but the 95% RDAA diet had *ca.* 5% less nitrogen as compared to the 100% RDAA diets. The ingredient and calculated chemical composition of the experimental starter, grower and finisher diets are presented in Table 1.

Body weight (BW) and feed intake (FI) per group of birds were determined four hours after feed removal. The feed : gain ratios were calculated for day 1 to 14, 15 to 35 and 36 to 49. Daily mortalities were recorded and used to correct performance criteria. Gastrointestinal transit time (GTT) of diets was measured at d 16 by covering the pen floors with clean paper 4 h after feed withdrawal and measuring the time between the offering of the diets containing 0.3% chromic oxide (Cr_2O_3) and the appearance of on average three spotted green excreta in each pens. The floors were covered with clean papers and excreta were collected for lipid digestibility determination after the birds consumed the chromic oxide diets for 48 h. A sample of the diets and excreta from each pen was stored at -20 °C for further analysis. Lipid concentration of the diets and excreta samples was determined by Soxhlet extraction (Soxtec System HT 1043 Extraction unit) according to the standard procedure of analysis (AOAC, 1996). Chromium in the diets and excreta was determined using the dry ashing procedure of Miller-Ihli & Greene (1992). At days 21 and 49 one chick close to the average replicate weight from each pen fed 100% RDAA was selected, weighed and killed by cervical dislocation after 4 h fasting to determine the weights of the breast, legs, abdominal fat pad, liver (with gall bladder), heart, spleen, bursa and total tract. At 21 d of age blood samples were taken from the wing vein of chickens fed 100% RDAA. Glucose, triglyceride, cholesterol, HDL, LDL and VLDL concentrations in serum were measured enzymatically using an auto-analyzer (Selectra E, Vital Scientific, Netherland). White blood cell (WBC), red blood cell (RBC), lymphocyte, monocyte, heterophil and eosinophile counts were determined based on the procedure of Gross & Siegel (1983). One hundred leukocytes (WBC) were counted on one slide prepared from the blood of each bird to calculate the proportions of heterophil and monocyte and heterophil : lymphocyte ratios in blood plasma.

Two methods of statistical analysis were used: 1) Regression analysis to evaluate the linear and quadratic effects of dietary licorice extract for starter and grower periods, as described by Morris (1999) and 2) ANOVA with treatment means separated by the least significance comparison. The data were subjected to ANOVA as a completely randomized design using the GLM procedures of SAS 9.1 software (SAS, 2004). The model used, was:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where μ = the common mean, A_i = the effect of feed additive and e_{ij} = the random error. Tukey's test was applied to compare the treatment means when the treatment effect was significant at $P \leq 0.05$. Orthogonal contrasts were used to compare the RDAA level, prebiotic and licorice extract effects on all criteria. All data were tested for normality prior to analysis. Abnormal data were transformed and then analyzed.

Results and Discussion

The linear and quadratic effects of licorice on performance, blood chemistry, carcass cuts, cell blood counts and gastrointestinal transit times were not significantly different during both the starter and grower periods (data not shown). Thus, only the results of ANOVA analyses for all criteria are shown and

Table 2 The mean body weight, feed conversion ratio and gastrointestinal transit time of feed for broiler chickens fed licorice extract and prebiotic supplemented diets which contained 100 and 95% of recommended digestible amino acids (RDAA) concentrations

Dietary treatments											
100% RDAA											
Control	Prebiotic (g/kg) ¹	Licorice extract (g/kg) ¹			95% RDAA						s.e.m.
		High	Medium	Low	Control	Prebiotic (g/kg) ¹	Licorice extract (g/kg) ¹	High	Medium	Low	
0	2	2	1	0.5	0	2	2	1	0.5		
Age (d)											
Body weight (g/bird)											
14	265	274	270	269	260	263	257	248	267	252	7.373
35	1398	1423	1450	1463	1472	1400	1385	1369	1392	1395	46
49	2542	2557	2661	2563	2620	2503	2546	2483	2484	2517	67
Period (d)											
Feed conversion ratio (g feed/g gain)											
1-14	1.59	1.59	1.47	1.49	1.59	1.60	1.62	1.65	1.75	1.71	0.065
15-35	1.92	1.87	1.92	1.89	1.89	1.93	1.87	1.89	1.93	1.91	0.046
36-49	2.25	2.21	2.25	2.24	2.27	2.30	2.22	2.31	2.29	2.29	0.132
1-49	1.90	1.85	1.92	1.82	1.96	1.96	1.94	2.02	2.0	1.99	0.060
Age (d)											
Gastrointestinal transit time of feed (min)											
16	141	169	174	166	168	166	168	190	169	172	12.19
42	198	199	217	190	214	194	210	219	210	205	16.91

¹ Level of supplements in starter diets. Grower diets contained 50% of these levels of supplements. No supplement was included in finisher diets.

s.e.m. - standard error of the mean.

discussed. The effects of different levels of the licorice extract and the prebiotic on performance parameters, GTT of feed and their orthogonal contrasts on broiler chickens fed diets with 100 and 95% RDAA are shown in Tables 2 and 3, respectively. The additions of licorice extract or the prebiotic did not have a significant effect on BW and FCR during the starter, grower and finisher periods. However, previous studies with other species of animals have shown that licorice flavonoids suppress BW by reducing body fat mass (Armanini *et al.*, 2003; Nakagawa *et al.*, 2004; Tominaga *et al.*, 2006; Aoki *et al.*, 2007). They suggested enhancement of fatty acid oxidation and reduction in biosynthesis of fatty acids are possible mechanisms for the reduction of abdominal fat and lower body weight gains (BWG). Lee *et al.* (2003) investigated the effect of thymol and carvacrol on the performance of female broiler chicks. Dietary carvacrol reduced BWG, while FCR improved as compared to control or thymol fed birds. The result of other studies using prebiotics indicated improved BWG and FCR in broiler chickens (Rodriguez *et al.*, 2005; Khaksar *et al.*, 2008).

Mean feed GTTs (minutes) were not influenced by fermacto and licorice extract concentration ($P > 0.05$) at d 16 and 42, though the GTT of feed in diets containing the licorice extract was higher than in the control diets ($P < 0.054$). Mean GTT of feed at 42 days of age was longer ($P < 0.01$) than in the chickens at 16 d of age (205 vs. 168 min). That may be due to improvement in gastro intestinal track function and

Table 3 The orthogonal contrast for the effects of diets containing licorice extract¹ or a prebiotic¹ in diets containing 100 and 95% of recommended digestible amino acids (RDAA) concentrations on body weight, feed intake, feed conversion ratio and gastrointestinal transit time of feed

Contrasts	Age (d)			
	1 - 14	15 - 35	36 - 49	1 - 49
	P values			
Prebiotic <i>vs.</i> Control	0.450	0.872	0.929	0.664
Licorice <i>vs.</i> Control	0.298	0.589	0.525	0.561
Licorice <i>vs.</i> Prebiotic	0.905	0.462	0.597	0.960
100% RDAA <i>vs.</i> 95% RDAA	0.408	0.034	0.08	0.06
Contrasts	Body weight			
	0.974	0.506	0.846	0.980
Prebiotic <i>vs.</i> Control	0.908	0.687	0.670	0.918
Licorice <i>vs.</i> Control	0.877	0.227	0.850	0.941
100% RDAA <i>vs.</i> 95% RDAA	0.315	0.002	0.197	0.0008
Contrasts	Feed intake			
	0.676	0.565	0.554	0.525
Prebiotic <i>vs.</i> Control	0.676	0.641	0.692	0.682
Licorice <i>vs.</i> Control	0.945	0.811	0.265	0.238
100% RDAA <i>vs.</i> 95% RDAA	0.006	0.007	0.315	0.020
Contrasts	Feed conversion ratio			
	16 day old	42 day old		
Prebiotic <i>vs.</i> Control	0.226	0.628		
Licorice <i>vs.</i> Control	0.054	0.358		
Licorice <i>vs.</i> Prebiotic	0.636	0.740		
100% RDAA <i>vs.</i> 95% RDAA	0.249	0.729		
Gastrointestinal transit time of feed (min)				
Contrasts	16 day old		42 day old	
	0.226		0.628	
Prebiotic <i>vs.</i> Control	0.054		0.358	
Licorice <i>vs.</i> Control	0.636		0.740	
100% RDAA <i>vs.</i> 95% RDAA	0.249		0.729	

¹ Levels of 0 (control), 2.0 g/kg fermacto and 2.0, 1.0 and 0.5 g licorice extract/kg were added to starter diets at both RDAA levels. The grower diets contained 50% of these levels of supplements; No supplement was included in finisher diets.

movements. The results of GTT were contrary to the report by Khaksar *et al.* (2008) who found that the addition of a prebiotic to broiler diets decreased the GTT of the feed. A 5% decrease in dietary RDAA concentration suppressed ($P < 0.05$) BWG during the 15 - 35 d period and numerically decreased BWG during 1 - 14, 35 - 49 and 1 - 49 d of age. Birds fed diet with 100% as compared to 95% of RDAA had a lower FCR during the starting (1.54 vs. 1.66), growing (1.89 vs. 1.96) and total (1.89 vs. 1.98) periods of the experiment ($P < 0.05$), although the FCR of broilers fed the diet with 95% of RDAA was unaffected in the finisher diets (Table 3). This result suggests that a finisher diet containing 95% RDAA has no negative effect on the FCR of broiler chickens.

The relative weight of organs and body parts (21 d) and fat digestibility (19 d) of broiler chickens fed licorice extract and fermacto supplemented diets with 100% RDAA are shown in Table 4. At 21 d of age there were no significant differences in breast, femur, liver, heart, spleen, bursa or whole gastrointestinal track weights of the birds fed the control or diets containing the prebiotic or the licorice extract. The breast meat yield clearly responded to the diets with different digestible amino acid concentrations (Rostagno *et al.*, 1995). Miorka *et al.* (2001) added prebiotics to broiler diets and did not find any effect on carcass, legs and breast yields which are corroborated by the results of the present study. However, according to Khaksar *et al.* (2008), breast yield was increased ($P < 0.05$) with fermacto supplementation of diets at either level of 100, 95 and/or 90% RDAA.

Table 4 Relative weights of organs and body parts (21 d), and fat digestibility (19 d) of broiler chickens fed licorice extract and fermacto supplemented diets containing 100% of recommended digestible amino acids (RDAA) concentrations

	Dietary treatment (100% RDAA)					s.e.m.	
	Control	Prebiotic (g/kg) ¹	Licorice extract (g/kg) ¹				
	0	2	2	1	0.5		
Relative weights of organs and body parts (% of live body weight)							
Femurs	14.7	16.5	17.9	17.0	16.4	0.885	
Breast	17.5	17.7	16.7	18.2	17.9	0.416	
GIT	15.4	15.3	14.2	14.7	14.3	0.616	
Liver	3.214	3.41	3.64	3.41	3.51	0.278	
Spleen	0.103	0.121	0.094	0.114	0.140	0.015	
Heart	0.86	0.91	0.95	0.86	0.86	0.108	
Bursa ²	5.7	5.3	5.0	4.0	3.0	0.079	
Fat digestibility (%)							
19 d old	85.7	87.2	88.6	87.7	87.7	1.11	

¹Level of supplements included in starter diet. Grower diets contained 50% of these supplements.

²Bursa size was determined using a special ruler with round holes with diameters of 3, 6, 9, 13, 16, 19, 21 and 26 mm.

GIT - gastro-intestinal tract.

At day 19 fat digestibility was not influenced by the addition of prebiotic or licorice extract ($P > 0.05$). Similarly, Cross *et al.* (2007) found that the supplementation of broiler diets with some medicinal herbs (oregano, rosemary, thyme and marjoram) and their essential oils did not have any effect on nitrogen-corrected apparent metabolizable energy and digestibility of organic and dry matter. However, Hernandez *et al.* (2004) showed that a mixture of plant essential oils or plant extracts improved fat and dry matter digestibility. At day 49 birds fed the diets supplemented with licorice showed lighter ($P < 0.05$) abdominal

Table 5 Effect of licorice extract and fermacto (g/kg) on relative weights of organs and body parts in all groups of broiler chickens fed diets containing 100 or 95% of recommended digestible amino acids (RDAA) concentrations at 49 days of age

	Dietary treatment											s.e.m.	
	100% RDAA					95% RDAA							
	Control	Prebiotic (g/kg) ¹	Licorice extract (g/kg) ¹			Control	Prebiotic (g/kg) ¹	Licorice extract (g/kg) ¹					
			High	Medium	Low			High	Medium	Low			
% of body weight													
Femurs	19.6	20.2	20.1	20.5	20.7	19.8	20.4	20.4	20.2	20.0	0.477		
Breast	18.6	18.5	19.2	19.1	19.6	19.0	19.7	19.1	19.7	20.8	0.646		
GIT	8.63	8.87	8.51	9.08	8.07	8.33	7.67	8.30	8.29	7.95	0.361		
Abdominal fat	2.30 ^a	1.98 ^{abc}	1.50 ^{bc}	1.44 ^c	1.54 ^{bc}	2.17 ^{ab}	2.03 ^{abc}	1.54 ^{bc}	1.49 ^{bc}	1.43 ^c	0.143		
Heart	0.498	0.532	0.504	0.563	0.550	0.544	0.508	0.475	0.495	0.524	0.040		
Liver	1.71	2.0	1.78	1.80	1.83	1.73	1.66	1.80	1.84	1.74	0.078		
Spleen	0.134	0.138	0.111	0.105	0.111	0.117	0.126	0.128	0.121	0.132	0.013		

¹Level of supplements in starter diets. Grower diets contained 50% of these levels of supplements. No supplement was included in finisher diets.

^{2 a,b} Means within a row and dietary treatment heading with no common superscript differ significantly ($P < 0.05$).

GIT - gastro-intestinal tract.

s.e.m. - standard error of the mean.

fat pads as compared to those fed the control diet (Table 5). Previous studies also showed that licorice flavonoids reduced the abdominal fat in other species (Armanini *et al.*, 2003; Nakagawa *et al.*, 2004; Tominaga *et al.*, 2006; Aoki *et al.*, 2007). Tominaga *et al.* (2006) indicated that the effect of licorice on reduction of abdominal fat could be due to factors such as suppression in energy intake, reduction of lipid absorption, enhancement of fatty acid oxidation or reduction in biosynthesis of fatty acids. There was not a significant change in energy intake since feed intake between treatments in the present study did not differ (Table 2). Also, lipid absorption and serum triglyceride concentration were not affected, as shown in Tables 4 and 6, respectively. Therefore, the enhancement of fatty acid oxidation and reduction in biosynthesis of fatty acids could be a possible mechanism for the decreased abdominal fat. A study on mice fed licorice showed that licorice altered some fatty acid oxidation and synthesis pathways (Tominaga *et al.*, 2006). The abdominal fat pad was unaffected by dietary fermacto supplementation, which is contrary to results reported by Khaksar *et al.* (2008).

Table 6 Blood chemistry (mg/dL) at day 21 of broiler chickens fed licorice extract and prebiotic supplemented diets containing 100% of recommended digestible amino acid (RDAA) concentrations

	Dietary treatment (100% RDAA)					s.e.m.
	Control	Prebiotic (g/kg) ¹		Licorice extract (g/kg) ¹		
		0	2	2	1	0.5
Glucose	290	280	275	260	261	24.5
Cholesterol ²	207 ^a	175 ^{ab}	139 ^b	161 ^{ab}	160 ^{ab}	10.94
Triglyceride	68.0	44.6	54.3	60.0	54.0	8.91
HDL ³	69.8	83.0	82.0	82.3	82.0	5.40
LDL ⁴	82.3	72.6	50.6	57.0	58.6	6.92
VLDL ⁵	13.6	9.0	10.6	11.6	10.6	1.73

¹ Level of supplements in starter diets. Grower diets contained 50% of these levels of supplements.
No supplement was included in finisher diets.

² Means within a row and dietary treatment heading with no common superscript differ significantly ($P < 0.05$).

³ High density lipoprotein; ⁴ Low density lipoprotein; ⁵ Very low density lipoprotein.

Table 7 Blood immune parameters of broiler chickens fed licorice extract and prebiotic supplemented diet containing 100% of recommended digestible amino acids (RDAA) concentrations at day 21

	Dietary treatment (100% RDAA)					s.e.m.
	Control	Prebiotic (g/kg) ¹		Licorice extract (g/kg) ¹		
		0	2	2	1	0.5
WBC ² (N/d l)	210000 ^{ab}	206667 ^b	209333 ^{ab}	217333 ^a	210667 ^{ab}	1813.53
RBC (N/d l)	22383333	22250000	2211667	22683333	22536667	170496
Heterophil ³	13.6	30.0	15.6	25.3	14.6	5.88
Lymphocyte ³	85.3	69.0	84.3	77.0	84.0	4.76
Monocyte ³	1.0	1.0	0.0	1.0	1.0	0.471
H/L ⁴	0.16	0.46	0.187	0.36	0.18	0.103

¹ Level of supplements in starter diets. Grower diets contained 50% of these levels of supplements.

No supplement was included in finisher diets.

² Means within a row and dietary treatment heading with no common superscript differ significantly ($P < 0.05$).

³ Expressed as a percent of total white blood cell; ⁴ Percent of heterophil : lymphocyte.

The feeding of licorice and the prebiotic did not have a significant effect on serum glucose, triglyceride, VLDL and HDL concentrations, whereas cholesterol ($P < 0.05$) and LDL ($P < 0.07$) concentrations decreased in birds fed licorice compared to the control (Table 6). This could be due to actions of licorice such as protecting of LDL cholesterol from oxidation, inhibiting cyclooxygenase and lipoxygenase enzymes and inhibiting lipid peroxidation (Craig, 1999). White blood cell numbers increased ($P < 0.05$) in birds fed diets containing 1.0 and 0.5 g licorice/kg in the starter and grower stages, respectively, compared to those fed the prebiotic containing diet (Table 7). The two dietary additives did not have significant effects on heterophil, monocyte and lymphocyte percentages, the heterophil : lymphocyte (H/L) ratio or red blood cell proliferation.

Conclusion

The addition of licorice extract to broiler diets may reduce abdominal fat content and serum cholesterol and LDL concentrations as compared to the control. Dietary licorice extract supplementation did not have any negative effects on body weight or FCR of broiler chickens.

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