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Effect of Licorice Extract and Prebiotic on Laying Hen Performance and Egg Quality in the Pre and Early Laying Periods

Somaye Sabzipoor, Ali Asghar Saki, Hossein Jahanian Najafabadi, Pouya Zamani & Mohammad Houshyar

Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran

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Corresponding author
Ali Asghar Saki
alisaki34@yahoo.com /
asaki@basu.ac.ir

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Abstract

This study was conducted to investigate the effect of licorice (*Glycyrrhizaglabra*) extract, Active-mos®prebiotic, and flavomycin antibiotic on performance, egg quality, and body mass status in the pre and early laying periods. A total of 180 Leghorn pullets (Hy-line W-36), were assigned into 6 treatments (5 cages/treatment, 6 pullets/cage) in a completely randomized design. The experimental treatments included control (feed additive-free- diet), and control supplemented by licorice extract (5 and 10 g/kg of diet; as $LIEX_5$ and $LIEX_{10}$, respectively), flavomycin antibiotic (400 and 650 mg/kg of diet; as FL_{400} and FL_{650}, respectively), and Active-mos®prebiotic (1 kg/ton of diet; as ACPR). Birds were raised in a cage-layer facility. Body weight, feed intake, and feed conversion ratio were determined weekly. Body mass index was recorded before and after using the treatments. Also, the growth, egg quality, egg cholesterol, serum cholesterol, and triglyceride were tested. During weeks 18 to 19 of age, birds received control, FL_{650}, and ACPR treatments showed greater feed intake compared to $LIEX_{10}$. The body weight of birds that received FL_{400} diet was greater than $LIEX_5$ and ACPR treatments at weeks 17 to 19 of age. All treatments, except for ACPR, decreased serum cholesterol compared with the control treatment ($P < 0.05$). No significant effect on feed conversion ratio, egg production, and body mass index was observed by treatments throughout the study (17-25 wk). Furthermore, there was no significant effect of treatments on the eggs' internal and external quality status, egg cholesterol, and serum triglyceride by treatments. However, more research is needed on the use of licorice extract and prebiotics as antibiotic alternatives and their effects on the body mass index in laying hens during pre- and early-laying periods.

Introduction

Increasing knowledge related to antibiotics and antibiotic-resistant pathogens has led to the prohibition of growth-promoting antibiotics in many countries. Thus, poultry producers should use alternatives to growth-promoting antibiotics. Antibiotics help stabilize the intestinal microbial population and improve the general functions of the bird and pre prevented the establishment of certain intestinal bacterial pathogens (Roe and Pillai, 2003; Chauvin *et al.*, 2005). The researchers are working to apply prebiotics to improve productivity in the poultry industry. Prebiotics include different types of fructooligosaccharides, gluco-oligosaccharides, and mannan oligosaccharides. Research related to the effects of mannan oligosaccharides on broiler chickens has shown improved performance and feed conversion ratio by reducing the competition between host and intestinal pathogens, improving the utilization of nutrients, increasing immunity, and reducing mortality (Gurbuz *et al.*, 2011; Bozkurt *et al.*, 2012; Micciche *et al*, 2018). The main effect of prebiotics is the stimulation of growth and proliferation of probiotic bacteria in the small

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intestine (Roberfroid, 2001). The effects of prebiotics on improving the performance of laying hens have been investigated in multiple studies (Bozkurt *et al*. 2012; Yousefi and Karkoodi, 2007; Martínez *et al*., 2010).

Plants that are rich in flavonoids and terpenes indirectly improve the immune system via their antibacterial effects. Some of the most effective substances in licorice include saponin, flavonoids, and isoflavones. The most important substance in the licorice plant is *Glycyrrhizicacid*, its content in the licorice root is more pronounced than in the other parts of the plant (Wang and Nixon, 2001). The licorice extract has antibacterial properties, so it can be used as an antibacterial agent in the treatment of diseases (Nowakowska, 2007). The phytobiotic increases the metabolism of proteins, fats, and carbohydrates in the broilers and influences their performance (Habil *et al*., 2003). The beneficial effects of licorice extract as a feed additive in improving the performance of laying hens have been investigated (Rezaei *et al.* 2014; Alagawany *et al*., 2019).

Human body fat is determined by body mass index (BMI). The logic of this indicator is that the early signs of a health condition are the proper ratio of body weight and height (Mendes *et al*., 2007). Also, for farm animals that are grown for economic purposes, the use of BMI could provide information regarding body mass (Shahin and EL Azeem, 2006; Mendes *et al*., 2008). Laying hens exhibit excessive fat accumulation, and have negative effects on reproductive performance (Hocking *et al*., 2002; Xing *et al*., 2009). Therefore, it is important to use an index to detect the accumulation of fat in the laying hen's body. The BMI is an attempt to quantify the amount of tissue mass (muscle, fat, and bone) in an individual, and then categorize that person as underweight, normal weight, overweight, or obese based on that value (Blackburn and Jacobs Jr, 2014). Today, nutritional programs can produce pullets of similar weight, but with different body compositions and reproduction patterns. This suggests that proper body composition during light stimulation is more important than weight to success in reproduction (Powell, 2004).

The hypothesis of this research was based on that Licorice extract and Active-mos®prebiotic versus Flavomycin antibiotic may have better efficiency and improve the performance, egg quality, and body mass status in laying hens during the pre- and early laying periods. In this study, Active-mos®prebiotic and Licorice extract were evaluated as possible alternatives to flavomycin.

Materials and Methods

Animal and Feed

The experiment was conducted by 180 Hy-line pullets of similar weight. The lighting program was arranged according to the Hy-line recommendation (16 hours of light at peak of production) from 17 to 25 weeks of age (pre-laying 17 to 20 and early laying 21 to 25 weeks) and birds were raised in a cage-layer facility. During the experiment, all hens were kept at 21ºC temperature and 50 - 60% relative humidity. Throughout the study, birds had ad libitum access to feed and water, and diets were in mash form. Two weeks were adjusted as the adaptation period before the experiment started (15-17 weeks). Nutritional requirements were tested based on the recommendation of the Hy-line catalog 2016. Also, the amino acid pattern of edible items (corn, soybean meal) was estimated by Near Infrared Reflectance Spectroscopy (NIR) and standardized ileum digestibility coefficients.

In this experiment, six treatments were considered, control treatment (without any additive), $LIEX_5$ and $LIEX_{10}$ treatments including dry licorice (*Glycyrrhizaglabra*) extract (Produced by spray drying method in a local company) with 5 and 10 g/kg diet respectively, FL_{400} and FL_{650} treatments contained flavomycin antibiotic, 400 and 650 mg/kg diet respectively and ACPR treatment contained Active-mos® prebiotic (1 kg/ton of feed). This experiment was conducted by 180 Hy-line W-36 pullets, which were assigned to 6 treatments (5 cages/treatment, 6 pullets/cage). The basal diet was presented in Table 1.

Performance

Throughout the study, the body weight, uniformity, egg mass, production percentage, and egg weight were measured every 2 weeks and feed intake was measured at the end of each week. The feed conversion ratio was calculated from weeks 17 to 19 based on weight gain and from weeks 19 to 25 based on egg production. The uniformity percentage of the flock is calculated by the following formula (Aviagen, 2018):

Percentage of weight distribution (PWD) = (SD)/(AWF)×100

Weight uniformity percentage = 100- PWD

Where, SD and AWF are the standard deviation and the average weight of the flock, respectively. Measuring the Body Mass Index (BMI) was computed by the following formula at the beginning and end of the study (Mendes *et al*., 2007).

$$\text{BMI} = \frac{Body\ weight\ (g)}{(\text{Body length (cm)})2}$$

Table 1. Dietary ingredient and chemical composition

Ingredient (%)	Developer	Pre-lay	Lay
Corn (CP=7.5%)	64.80	63.48	57.74
Soybean meal (CP=44%)	29.32	26.50	27.41
Soybean oil	1.66	1.74	1.88
Dicalcium Phosphate	1.94	2.03	2.16
Oyster shell Powder	1.28	5.19	9.75
Salt	0.36	0.39	0.38
Mineral supplement	0.25	0.25	0.25
Vitamin Supplement	0.25	0.25	0.25
DL-Methionine	0.14	0.17	0.18
Total	100	100	100
Calculated value			
Metabolizable energy (kcal/kg)	2977	2911	2805
Crud protein (%)	17.84	16.52	16.49
Calcium (%)	1	2.50	4.15
Available phosphorus (%)	3.233	0.48	0.49
Sodium (%)	0.17	0.18	0.18
Chlorine (%)	0.25	0.26	0.26
Lysine (%)	0.88	0.80	0.87
Methionine (%)	0.40	0.41	0.42
Methionine + Cystine (%)	0.67	0.66	0.76
Threonine (%)	0.60	0.55	0.66
Tryptophan (%)	0.19	0.17	0.20
Valine (%)	0.78	0.72	0.78
Isoleucine (%)	0.71	0.65	0.68

Provided per kg of the mineral supplement: 70 mg of manganese (oxide), 60 mg of zinc (oxide), 60 mg of iron (sulfate), 8 mg of copper (sulfate), 1.1 mg of iodine (calcium iodate), 0.15 milligrams of cobalt and 0.25 milligrams of selenium. Each kilo of vitamin supplement: 10,000 units of vitamin A, 2500 international units of vitamin D_3, 20 international units of vitamin E, 3 milligrams of vitamin K_3, 2 mg of thiamine, 5 mg of riboflavin, 12 mg of pantothenic acid, 40 mg niacin 5200 mg choline chloride, 5 mg Pyridoxine, 0.015 mg of cobalamin, 0.05 grams of biotin, 400 mg choline chloride, 0.75 mg of folic acid.

Internal and external eggs characteristics

To evaluate the characteristics of eggs, two eggs were selected randomly from each replicate on the two final days of the week and transferred to the laboratory. Initially, the length and width of each egg were measured to calculate the shape index, as follows equation (width /length) × 100. In order to examine the characteristics of eggshell, the shells were washed, placed in an oven at 65°C for 24 hours, and then weighed. The thickness of the shells was measured by a digital micrometer (Insize Manufacturing Model in Taiwan) with a precision of 0.001 mm. A digital caliper with a precision of 0.1 mm was used to calculate the yolk index (YI), albumen index (AI), Haugh unit (HU), yolk diameter (YD), albumen length (AL), and albumen width (AW). A tripod micrometer was used to measure the height of albumens (AH) and yolks (YH). Also, the shell weight (SW) was obtained with an accurate scale. Yolk index (YI), albumen index (AI), haugh unit (HU), and shell ratio (SR) were calculated using the following formulas (Taskin *et al*., 2017; Sözcü *et al.*, 2021).

$$YI = (YH/YD) \times 100$$

$$AI = (AH/(AL + AW)/2)) \times 100$$

$$HU = 100 \times \log (AH + 7.57 - 1.7 \times EW0.37)$$

$$SR = (SW / EW) \times 100$$

Blood biochemical status

In the 25th week, two hens from each replicate were randomly selected and blood samples were collected for measuring serum cholesterol and triglyceride. Measurement of serum cholesterol and triglyceride concentration was carried out by conventional methods and by a kit-manufactured Pars-test company based on the enzymatic-calorimetric method (GPO-PAP).

Yolk cholesterol

At the end of the experiment in the measurement of yolk cholesterol, three eggs were selected for each replicate and their yolks were separated and mixed. The cholesterol content was measured by diagnostic commercial kits (Pars Azmoon and photometric method).

Statistical Analysis

This experiment was conducted in a completely randomized design (CRD) and analyzed via SAS software (v. 9.4, 2014). The means were compared based on Duncan's Multiple Range Test, with the significance level of 0.05 was used for the analyses.

Results

Performance

Feed intake was significantly reduced ($P < 0.05$) by $LIEX_{10}$ treatment (10 mg/kg licorice extract) and FL_{650} (650 mg/kg flavomycin) treatments than control at the 18-19 weeks age (Table 2). However, no significant difference was found in feed intake by other treatments. Furthermore, lower body weight ($P > 0.05$) was observed in birds under $LIEX_5$ (5 mg/kg licorice extract), FL_{400}, and FL_{650} than in control at the age of 17-19 weeks (Table 3). Moreover, during these weeks, body weight was greater in FL_{400} treatment compared with those of ACPR. In addition, none of the treatments had a significant effect on the feed conversion ratio and body weight uniformity (Table 4 and Table 5).

Table 2. Effect of experimental treatments on daily feed intake (g/day) from 17 to 25 wk of age

Treatments	Weeks							
	17-18	18-19	19-20	20-21	21-22	22-23	23-24	24-25
Control[1]	54.52	54.33[ab]	58.24	55.85	56.02	59.95	62.54	72.27
$LIEX_5$[2]	52.88	51.41[bc]	55.20	54.01	55.14	59.64	65.02	73.78
$LIEX_{10}$[3]	52.51	50.39[c]	54.15	55.32	55.63	60.53	63.23	72.50
FL_{400}[4]	53.83	52.04[bc]	57.43	55.42	58.70	60.12	62.88	74.57
FL_{650}[5]	53.97	55.89[a]	56.87	54.52	56.09	58.99	62.33	72.42
ACPR[6]	53.64	52.93[abc]	55.92	54.86	55.25	60.22	65.02	73.33
SEM[7]	0.817	1.076	1.330	1.210	1.456	2.041	1.839	2.231
P-value	0.548	0.016	0.303	0.904	0.551	0.996	0.818	0.972

[1]without any additive; [2] 5 g/kg licorice extract; [3] 10 g/kg licorice extract; [4] 400 mg/kg flavomycin); [5] 650 mg/kgflavomycin); [6] *Active-mos*® prebiotic, 1 kg/ton; [7] Standard error of the means. [a-c] Different letters indicate significant differences between groups ($P < 0.05$). - Each value represents the means of 5 pen replicates with 6 birds per pen.

Table 3. Effect of experimental treatments on body weight (kg) from 17 to 25 wk of age

Treatments	Weeks				
	17	17-19	19-21	21-23	23-25
Control[1]	1.10	1.16[ab]	1.24	1.27	1.45
$LIEX_5$[2]	1.06	1.11[c]	1.21	1.24	1.42
$LIEX_{10}$[3]	1.08	1.15[abc]	1.22	1.27	1.43
FL_{400}[4]	1.10	1.18[a]	1.23	1.28	1.47
FL_{650}[5]	1.10	1.16[ab]	1.23	1.27	1.44
ACPR[6]	1.07	1.14[bc]	1.20	1.24	1.44
SEM[7]	0.012	0.013	0.012	0.013	0.020
P-value	0.698	0.014	0.255	0.185	0.667

[1]without any additive; [2] 5 g/kg licorice extract; [3] 10 g/kg licorice extract; [4] 400 mg/kg flavomycin); [5] 650 mg/kgflavomycin); [6] *Active-mos*® prebiotic, 1 kg/ton; [7] Standard error of the means.
[a-c] Different letters indicate significant differences between groups ($P < 0.05$). - Each value represents the means of 5 pen replicates with 6 birds per pen.

Table 4. Effect of experimental treatments on feed conversion ratio (g:g) from 17 to 25 wk of age

Treatments	Weeks			
	17-19	19-21	21-23	23-25
Control[1]	12.86	4.08	3.41	2.68
$LIEX_5$[2]	12.55	4.85	3.43	2.5
$LIEX_{10}$[3]	9.46	4.16	3.38	2.99
FL_{400}[4]	9.45	4.56	3.56	2.49
FL_{650}[5]	12.46	4.03	3.40	2.55
ACPR[6]	13.60	4.57	3.43	3.14
SEM[7]	1.759	0.318	0.118	0.416
P-value	0.456	0.835	0.297	0.867

[1]without any additive; [2] 5 g/kg licorice extract; [3] 10 g/kg licorice extract; [4] 400 mg/kg flavomycin); [5] 650 mg/kgflavomycin); [6] *Active-mos*® prebiotic, 1 kg/ton; [7] Standard error of the means.
-The feed conversion ratio was calculated from weeks 17 to 19 based on weight gain and from weeks 21 to 25 based on egg production.

Egg characteristics

As shown in Table 6 and Table 7, egg parameters including average egg weight; egg mass; egg production, yolk index; albumen index; haugh unit; shell weight; shell thickness were not significantly affected ($P > 0.05$) by treatments.

Table 5. Effect of experimental treatments on body weight uniformity from 17 to 25 wk of age

Treatments	Weeks				
	17	17-19	19-21	21-23	23-25
Control[1]	92.05	91.78	90.18	90.18	92.65
$LIEX_5$[2]	91.12	93.61	92.05	90.52	91.79
$LIEX_{10}$[3]	91.57	91.46	91.92	91.02	90.20
FL_{400}[4]	91.59	92.42	91.86	91.86	91.88
FL_{650}[5]	91.67	93.15	91.25	91.25	92.17
ACPR[6]	91.13	91.85	90.84	90.84	91.29
SEM[7]	0.271	1.003	1.195	1.369	1.253
P-value	0.995	0.620	0.961	0.965	0.806

[1]without any additive; [2] 5 g/kg licorice extract; [3] 10 g/kg licorice extract; [4] 400 mg/kg flavomycin); [5] 650 mg/kgflavomycin); [6] *Active-mos*® prebiotic, 1 kg/ton; [7] Standard error of the means.

Table 6. Effect of experimental treatments on egg parameters from 19 to 25 wk of age

Treatments	AEW[1] (g)			EM[2] (g/day)			EP[3] (%)		
	19-21W	21-23W	23-25W	19-21W	21-23W	23-25W	19-21W	21-23W	23-25W
Control[4]	49.68	52.74	55.90	3.79	12.77	30.73	7.61	24.28	55.23
$LIEX_5$[5]	51.23	52.83	55.09	5.16	10.53	30.24	10.00	20.00	5523
$LIEX_{10}$[6]	43.93	51.49	54.73	1.53	13.45	29.53	3.33	25.71	53.80
FL_{400}[7]	48.14	51.60	55.00	4.63	16.66	32.46	9.52	32.38	59.04
FL_{650}[8]	47.77	54.92	55.81	2.80	7.11	30.10	5.71	12.85	53.80
ACPR[9]	50.50	53.24	56.66	1.44	5.56	25.59	2.85	10.47	45.23
SEM[10]	2.44	0.94	0.367	1.729	3.55	2.827	3.48	6.70	2.358
P-value	0.41	0.19	0.1	0.546	0.274	0.935	0.582	0.221	0.907

[1]AEW= Average egg weight; [2] EM= Egg mass; [3] EP= Egg production; [4]without any additive; [5] 5 g/kg licorice extract; [6] 10 g/kg licorice extract; [7] 400 mg/kg flavomycin); [8] 650 mg/kgflavomycin); [9] *Active-mos*® prebiotic, 1 kg/ton; [10] Standard error of the means.

Table 7. Effect of treatments on egg content in laying hens

Treatments	YI (%)[1]	AI (%)[2]	HU[3]	SW (g)[4]	ST (Mm)[5]
Control[6]	44.63	5.34	80.54	5.66	0.41
$LIEX_5$[7]	42.85	4.93	77.77	5.61	0.40
$LIEX_{10}$[8]	39.33	5.35	81.41	5.85	0.42
FL_{400}[9]	37.69	5.44	78.46	5.32	0.41
FL_{650}[10]	39.70	5.25	75.13	5.61	0.42
ACPR[11]	45.28	5.21	78.21	5.52	0.40
SEM[12]	2.243	0.129	2.349	0.558	0.404
P-value	0.164	0.068	0.517	0.192	0.111

[1] YI= Yolk index; [2] AI= Albumen index; [3] HU= Haugh unit; [4] SW= Shell weight; [5] ST= Shell thickness; [6] without any additive; [7] 5 g/kg licorice extract; [8] 10 g/kg licorice extract; [9] 400 mg/kg flavomycin); [10] 650 mg/kgflavomycin); [11] *Active-mos*® prebiotic, 1 kg/ton; [12] Standard error of the means.

Table 8. Effect of experimental treatments on egg yolk, serum cholesterol and triglyceride concentrations in laying hens

Treatments	EYCH (mg/dL)[1]	SCH (mg/dL)[2]	STG (mg/dL)[3]
Control[4]	178.05	133.63[a]	711.0
$LIEX_5$[5]	162.86	124.77[bc]	705.1
$LIEX_{10}$[6]	189.56	123.24[c]	543.0
FL_{400}[7]	187.85	124.91[bc]	728.9
FL_{650}[8]	187.00	125.64[bc]	786.9
ACPR[9]	184.29	130.25[ab]	764.3
SEM[10]	12.306	3.410	100.183
P-value	0.655	0.005	0.604

[1] EYCH= Egg yolk cholesterol; [2] SCH= Serum Cholesterol; [3] STG= Serum Triglyceride; [4] without any additive; [5] 5 g/kg licorice extract; [6] 10 g/kg licorice extract; [7] 400 mg/kg flavomycin); [8] 650 mg/kgflavomycin); [9] *Active-mos*® prebiotic, 1 kg/ton; [10] Standard error of the means.

[a-c] Different letters indicate significant differences between groups ($P < 0.05$). - Each value represents the means of 5 pen replicates with 6 birds per pen.

Serum characteristics

The serum cholesterol levels in treatments that contained licorice extract and flavomycin antibiotics were significantly lower ($P < 0.05$) than the control treatment (Table 8). Moreover, the use of $LIEX_{10}$ treatment decreased serum cholesterol concentration compared to the ACPR treatment. The concentration of egg yolk cholesterol and serum triglyceride was not affected by experimental treatments ($P > 0.05$).

Body mass index (BMI)

The BMI value was not significantly affected by treatments (Table 9). However, the lowest BMI was numerically found in $LIEX_{10}$ treatment at week 25 of age.

Table 9. Effect of experimental treatments on body mass index (BMI) at 17 and 25 wk of age

Treatments	Week 17	Week 25
Control[1]	0.356	0.397
$LIEX_5$[2]	0.360	0.390
$LIEX_{10}$[3]	0.358	0.385
FL_{400}[4]	0.357	0.413
FL_{650}[5]	0.361	0.420
ACPR[6]	0.360	0.410
SEM[7]	0.02	0.016
P-value	0.557	0.350

[1]without any additive; [2] 5 g/kg licorice extract; [3] 10 g/kg licorice extract; [4] 400 mg/kg flavomycin); [5] 650 mg/kgflavomycin); [6] *Active-mos*® prebiotic, 1 kg/ton; [7] Standard error of the means.

Discussion

There were no significant differences in feed intake, feed conversion ratio, egg weight, egg production, egg mass, uniformity, body weight gain, and body weight changes by experimental treatments ($P > 0.05$). The best act of additives in certain conditions such as disease, stress, high temperature, and adverse environmental conditions is achieved (Patterson and Burkholder, 2003). However, this study was conducted under normal conditions. Furthermore, Zhang *et al*., (2003) have shown that improving the activity of protease and amylase leads to better digestion and absorption of nutrients by supplementing the diet with oligosaccharides. However, Bozkurt *et al*. (2012) have noted that improved performance, by mannan oligosaccharides but no beneficial effects were found on egg characteristics. In addition, similar studies found no effect of prebiotic mannan oligosaccharides on egg weight (Yildiz *et al*., 2004; Mahdavi *et al*., 2005; Yousefi and Karkoodi, 2007; Martínez *et al*., 2010). Increasing beneficial microorganisms enhances the bioavailability of nutrients to absorb and improves performance (Yusrizal and Chen, 2003). Chen *et al*., (2005) have shown that improvement in the performance of laying hens by commercial prebiotics and egg weight was affected by the Active-mos® prebiotic ($P < 0.05$). This experiment did not demonstrate any beneficial effects of Active-mos ®prebiotics on performance. However, Li *et al*., (2007) and Shalaei *et al*., (2014) showed that egg weight, egg production, feed intake, and feed conversion ratio of layers improve by supplementing the diet with 2,000 mg/kg fructo-oligosaccharides. Habib *et al*., (2017) have indicated that the addition of flavomycin to feed did not affect egg production or feed intake ($P > 0.05$). Also, we did not find any positive effects on laying hen performance by adding flavomycin antibiotics.

This study found that licorice extract by 10 g and 5 g/kg of diets did not affect performance significantly. The cause of the various responses to the additives may be due to the bird's age, base diet composition, the type of microbial population present in the gastrointestinal tract, levels of peri-biotics in the diet, and different environmental conditions (Patterson and Burkholder, 2003). Dogan *et al*., (2018a) have indicated no significant effect on egg weight and feed conversion ratio by licorice root supplementation ($P > 0.05$). However, in this experiment weekly feed intake has shown a significantly decrease compared with control treatment by 10 g / kg licorice in the diet at 18-19 weeks of age. In contrast, no significant differences were observed after the beginning of the laying period. According to Dogan *et al*., (2018b), the highest and lowest feed intakes were obtained by control and 2.0% licorice extract, respectively. The plant's aromatic aroma may contribute to the decrease in feed intake when licorice root is added to the diet. Also, the results of Rezaei *et al*. (2014) related to a decrease in feed intake conforming to the current study.

In this study, the results highlighted that body weight decreased significantly from 17 to 19 weeks compared with control treatment by licorice extract, but no significant differences were found after the beginning of the laying period. Licorice extract may cause reduce body weight gain by increasing fatty acid oxidation and reducing fatty acid biosynthesis (Nakagawa *et al*., 2004; Tominaga *et al*., 2006; Dogan *et al*., 2018b).

In this experiment, no significant effect on egg quantity and quality was shown by different treatments. Similar findings have been reported by Bozkurt *et al*., (2012) and Shalaei *et al*., (2014). But contrary to the results of this experiment, Bozkurt *et al*. (2012) observed a significant effect on eggshell weight by adding the MOS. Egg yolk cholesterol was influenced by genetic factors, diet composition, laying intensity, layer age, and medical treatment (Vorlova *et al*., 2001). The egg cholesterol is influenced by the yolk content lipoprotein cholesterol synthesized in the liver, but there is no cholesterol density in the chicken plasma derived from the diet (Klasing, 1998). The triglyceride of serum was not affected by treatments but a significant reduction in serum cholesterol was found by licorice extract and flavomycin treatments compared with the control treatment. The decrease in serum cholesterol could be due to the functions of licorice such as protecting LDL (low-density lipoprotein) cholesterol from oxidation and, inhibiting cyclooxygenase and lipoxygenase enzymes (Craig, 1999).

According to Dogan *et al*., (2018b), plasma low-density lipoprotein (LDL) and egg yolk cholesterol decreased, while plasma high-density lipoprotein (HDL) levels increased with licorice root addition ($P < 0.05$). Also, Li *et al*., (2007) have shown that increased the thickness of egg shell, yolk color, haugh unit, and decreases yolk cholesterol concentration by adding 2000 mg/kg of fructo-oligosaccharides to the diet. It is possible that some of the organisms present in the gastro-intestinal tract for their own cellular metabolism need cholesterol for activation (Nelson and Gilliland, 1984; Gilliland *et al*., 1985). Jenkins *et al.*, (2005) have stated that metronidazole significantly reduced low-density lipoprotein cholesterol serum by increasing in Bifidobacteria. In this study, flavomycin significantly reduced serum cholesterol levels. A change in gastrointestinal flora may explain this phenomenon.

The BMI was used for the first time for the relationship between weight and height of humans (Engeland *et al*., 2007) and later on was attention for some animal species. The BMI was used by several researchers as an indicator for evaluating nutritional status and body fat since reducing this index could indicate a decrease in body fat content (Mendes *et al*., 2007). Therefore, calculating BMI for birds may provide valuable information related to body fat (Mendes *et al*., 2008). Nakagawa *et al*., (2004), Aoki *et al*., (2007), and Tominaga *et al*., (2006) have reported that significantly reduced the ventricular cavity fat by licorice essential oil in the diet of mice. Also, reduced abdominal fat content in the broilers by Licorice extract. Aoki *et al*. (2007), have shown that licorice extract reduces fat by regulating the expression of effective genes involved in the oxidation of fatty acids. Based on various studies, licorice extract may indicate lower BMI by reducing abdominal fat. Although the consumption of licorice extract at a young age may have a significant effect on BMI and further research is needed in this area.

Conclusion

Based on the findings of the present study, the performance was not affected by any of the experimental treatments. However, a significant decrease in serum cholesterol has been demonstrated with licorice extract and flavomycin supplementation in the laying period compared with the control treatment, but further studies are needed to evaluate licorice extract and Active-mos®prebiotic as antibiotic alternatives and body mass index status in the pre and early laying periods.

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