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The Impact of Reduced Licorice Levels on Performance, Carcass Characteristics, Blood Constituents, Immunity, Intestinal Microflora, Intestinal Morphology, and Breast Muscle Fatty Acid Profile in Broilers

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ABSTRACT

Background: Phytogenic feed additives derived from plants have gained special attention in poultry farming. This research was conducted to investigate the effects of low doses of licorice on broiler productivity.

Methods: This experiment was conducted using 120 commercially bred Ross 308 broiler chickens at one day of age to investigate the effects of three different levels of licorice (0, 100, and 200 mg/kg) in their feed.

Results: The results showed that chickens fed with a diet supplemented with 200 mg/kg of licorice (*Glycyrrhizaglabra*) had the highest feed intake (94.27 g/d) and weight gain (57.67 g/d), along with the most favorable feed conversion ratio (1.63 g/g). Moreover, this treatment had the highest economic returns (34.99 $/m^2$) and demonstrated the best European production index (359.81) compared to other treatments. It also led to a significant increase in certain carcass characteristics compared to the control group ($P < 0.05$). The effect of two different levels of licorice on some blood parameters (LDL/HDL ratio, LDL, VLDL, triglyceride, and glucose) was statistically significant ($P < 0.05$). The use of the licorice had no significant effect on the humoral immune system ($P \geq 0.05$). Also, the inclusion of 200 mg/kg of licorice resulted in the lowest population of *Escherichia coli* (9.18 CFU/g) the highest *Bifidobacterium* count (9.673 CFU/g), and the lowest ratio of saturated fatty acids to unsaturated fatty acids (1.27).

Conclusion: The use of 200 mg/kg of licorice (*Glycyrrhizaglabra*) as a dietary additive is recommended for Ross 308 broiler chickens. The use of licorice can improve feed intake, weight gain, feed conversion ratio, cost per kilogram of live chicken, and production index at the end of the period.

1. Introduction

The use of various additives, including prebiotics, probiotics, organic acids, and plant extracts, in bird diets has been on the rise because of their efficiency in increasing production performance and maintaining bird health (Dhama et al., 2015). Thus, phytogenic feed additives derived from plants have gained special attention in this regard. The term "phytogenic compounds" refers to parts of plants or plant extracts such as essential oils, alkaloids, and flavonoids, including those derived from medicinal plants (Brenes et al., 2010; Mountzouris et al., 2011). Incorporating feed additives in poultry farming is expected to improve the feed conversion ratio, enhance product quality by reducing cholesterol levels in meat and eggs, promote animal health, reduce oxidative stress, improve the immune system and overall poultry health, and increase production and profitability (Krishan & Narang, 2014). Therefore, the use of medicinal plants has become very popular in animals, poultry, and humans (Dhama et al., 2015). Among phytogenics, substances derived from licorice (extracts and powders) are widely used as flavoring agents, feed

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preservatives, and for commercial purposes in human and animal nutrition (Pastorino et al., 2018). The pharmacological effects of licorice extract and its active substance are observed in both humans and animals. For example, licorice extract and its active compounds have antimicrobial, antiviral, antioxidant, anti-inflammatory, and anti-diabetic properties. Due to its antiviral, antiseptic, and free radical inhibitory effects, licorice can serve as a suitable additive in broiler diets (Karkanis et al., 2018). Previous studies have reported that the addition of licorice in broiler diets had no adverse effects on body weight (Sedghi et al., 2010; Abo-Samaha et al., 2022). In fact, adding licorice to drinking water leads to an increase in body weight (Hosseini et al., 2022). The inclusion of 2.5 g/kg of licorice in broiler diets resulted in optimal growth performance (Salary et al., 2014). In addition, the inclusion of licorice in the drinking water of broiler chickens at a rate of 450 mg/L under heat stress conditions improved feed conversion ratio and economic efficiency (Al-Daraji, 2012). In another study, licorice flavonoids were found to reduce human abdominal fat by increasing fatty acid oxidation and reducing their biosynthesis. Mice fed with licorice showed that licorice can change the oxidation of some fatty acids and fatty acid synthesis pathways (Tominaga et al., 2006). Additionally, medicinal plants have been reported to enhance fat availability for lipolysis in birds (Chowdhury et al., 2002). As a result, incorporating licorice into poultry diets may improve performance by reducing the number of harmful microorganisms in the digestive tract of poultry. Additions of 0.2% and 0.4% licorice extract to broiler drinking water had no significant effect on plasma albumin (Salary et al., 2014). Similarly, no effect on broiler blood globulin and total protein levels was observed. However, a mixture of thyme, licorice, and hyperanozyme led to an increased serum globulin concentration (Hamidi et al., 2018). Another study reported that chicks fed a diet containing 0.1% licorice extract exhibited increased antibody titers against the Newcastle disease virus at 42 days of age (Jagadeeswaran & Selvasubramanian, 2014). Moreover, adding 3 or 6 g of licorice extract per kg of broiler diet containing 0.5 mg of aflatoxin B1 improves the immune system and neutralizes the negative effects of aflatoxin on weight gain (Rashidi et al., 2020). Needless to say, there are conflicting reports on the effect of licorice compounds in broiler farming. However, considering their positive medicinal effects on humans and animals (Karkanis et al., 2018), we believe that licorice can be used as a suitable additive in broiler diets. Therefore, this research was conducted to investigate the effects of low doses of licorice on performance, carcass characteristics, blood parameters, immunity, intestinal microbial flora, intestinal morphology, and breast muscle fatty acid profile in broilers.

2. Materials and Methods

2.1 Animals and diets

This research was conducted in 2023 at a broiler farm located in Rasht, Iran. A total of 120 one-day-old broilers of the Ross 308 strain with an average weight of 43 ± 1 g were used for the experiment. The broilers were divided into 3 treatments with 4 replications, and 10 chickens per pen for 42 days. The experiment consisted of 3 observation periods: day 1 to 10, day 11 to 21, and day 22 to 42. Based on previous unpublished experiments, the three treatments included: treatment 1 (0 mg/kg licorice), treatment 2 (100 mg/kg licorice) and treatment 3 (200 mg/kg licorice) added to the basal diet. A ready-to-use powder form of licorice was obtained from Darvash Giah-Khazar Co (Rasht, Iran). The mash rations were formulated based on the Ross 308 strain feeding guide (Manual, 2012) (Table 1). The bedding, stock density, lighting, vaccination, temperature, and drinking and feeding regime were the same in all studied treatments.

Table 1. Ingredients, chemical composition, and energy of the diets used (from the 1st to the 42nd day of age)

Ingredients (% as-fed)	Starter diet (1st - 10th days of age)	Grower diet (11th - 21st days of age)	Finisher diet (22nd - 42nd days of age)
Wheat	5.58	5.00	5.00
Corn	47.03	59.60	65.99
Corn gluten	10.00	11.48	11.50
Soybean meal (44% Crude protein)	29.02	16.15	10.28
Limestone	1.45	1.23	1.00
soy oil	3.50	3.40	3.09
Salt	0.20	0.20	0.20
Di-calcium phosphate	1.95	1.80	1.83
L-lysine hydrochloride	0.25	0.06	0.04
DL-methionine	0.52	0.58	0.57
Vitamin and mineral supplements	0.50	0.50	0.50
Calculated compounds			
Metabolizable energy (kcal/kg)	2950	3000	3050
Crude protein (%)	22	20	19
Lysine (%)	1.3	1.2	1.1
Methionine (%)	0.56	0.54	0.52
Met+Cys (%)	0.92	0.90	0.88
Calcium (%) Available phosphorus	1.04 0.52	0.95 0.47	0.92 0.41

2.2 Growth performance and economic efficiency

The weights of all chickens in each pen we Broilers rerecorded with an accuracy of ±10 g in each period and measured with a digital scale (Kala weighing, Iran). At the end of each period (starting from 1 to 10, increasing from 11 to 21, and ending from 22 to 42), the remaining amount of feed in each feeder was weighed and the amount of feed consumed was calculated by subtracting the amount of feed given at the beginning of each period. The feed conversion ratio was observed and calculated by dividing the amount of feed consumed by the weight gained during the three observation periods (Sigolo et al., 2019). The following formula was used to calculate the European production index as a valuable parameter for evaluating broiler farming:

European production index = (10 × number of rearing days × feed conversion ratio) / (percentage of survival × average live weight).

2.3 Carcass characteristics and digestive organs

After a two-hour starvation period, at the end of the study, two birds from each replication, with weights close to the average were selected. The measurements included their dead and alive weights, cookable carcass weight, thigh weight, breast weight, neck weight, wing weight, and the weight of internal organs (spleen, heart, liver, bursa of Fabricius, abdominal fat) were measured (Shabani et al., 2015).

2.4 Parameters of blood plasma components

In order to calculate the blood factors, 5 mL of blood was collected from the wing vein of two birds per replicate at 42 days of age. The blood samples were centrifuged at room temperature (5000 rpm for 3min) (Eppendorf 5702, Germany). The resulting serum was separated, transferred to microtubes, and transported to the laboratory. After thawing, the serum samples were subjected to measurements of glucose, total protein, triglyceride, albumin, cholesterol, globulin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) using commercial kits (Pars Azman, Iran) and an autoanalyzer (Hitachi 917, Japan) (Golrokh et al., 2016).

2.5 Immune responses

Humoral immunity was assessed by immunizing broiler chickens were immunized against sheep red blood cells (SRBC) using Lemmer's method (Lerner et al., 1971). On days 28 and 36, SRBC injections were given to two birds in each pen. Blood samples were drawn 7 and 14 days after the first and second injections, as well as on days 35 and 42 (Gore & Qureshi, 1997). Antibody level against SRBC was measured by the hemagglutination inhibition method. Antibody titers were determined using the microhemagglutination pellet image method and the Van der Zipp method. In addition, the weight of the bursa of Fabricius and the spleen was measured for two birds with weights close to the average from each repetition (Dibaji et al., 2014). The titers of Newcastle disease virus (NDV) and avian influenza virus were determined on days 28 and 42 by hemagglutination inhibition (HI) test. Blood serum from two birds within each pen was combined for testing in a 96-well microplate. The titers were based on Log2.

2.6 Microbial flora

On the 42nd day of age, two birds from each treatment were slaughtered to measure the microbial flora of the cecum. The right and left cecum were separated, and their contents were collected in sterile microtubes. The populations of *Escherichia coli*, *Lactobacillus acidophilus*, and *Bifidobacterium* were calculated using the serial dilution method (ratio 1 to 10) in distilled water (Dibaji et al., 2014). Therefore, 1 g of each frozen sample was added to 9 mL of distilled water to form a dilution series ranging from 10^{-6} to 10^{-10}. Then 300 µL of each dilution series of 10^{-3}, 10^{-4}, and 10^{-5} were taken and inoculated on the plates containing the culture medium and spread completely on the culture surface using a loop. The inoculated samples were incubated at 37°C for 24h for the growth of *Escherichia coli* and *Lactobacillus acidophilus* in Eosin Methylene Blue Agar (EMB) and *Bifidobacterium* on blood agar (Jang et al., 2007). Finally, to determine Colony Forming Units (CFU), the colonies formed in the most appropriate dilution (4-10) were counted. The number of colonies in each culture medium was multiplied by the dilution ratio after counting. With accuracy to the magnitude of the numbers obtained from counting bacteria, for ease of calculations, the logarithm of the numbers mentioned in base 10 was calculated and then used for data analysis (Dibaji et al., 2014).

2.7 Intestinal morphology

On the 42nd day, intestinal samples were quickly fixed in a 10% buffered formalin solution after slaughtering followed by tissue sections. Dehydration, clarification, and paraffin embedding were performed by a tissue processor (Auto-Technicon tissue processor model 2A, The Technicon Company, Tarrytown, NY, USA), which is called tissue passage. The tissue processing device (tissue processor) used for tissue preparation was the TISSUE PROCESSOR (Tehsab Co, Iran). Following the completion of the processing steps, the blocking stage was done. Tissue sections, with a thickness of 5-7 µm, were obtained using a rotary microtome (DS4055) (Didsabz Co, Iran). Hematoxylin-eosin dyes were used for tissue staining (Kalantar et al., 2017). Azaosin was used to stain the cytoplasm, while hematoxylin was used for nuclear staining. Staining procedures involved the use of materials such as gasyl, and ethyl alcohol with concentrations of 70% to absolute, alcoholic acid, hematoxylin dye, eosin dye, and saturated lithium carbonate solution. Finally, the slides were examined in terms of villus length, villus diameter, crypt depth, and crypt diameter using an optical microscope. The microscope used in this research was the MEDIC M-107 BN (Wincom Co, China). Histomorphometric studies were performed by Dino-Light digital camera and Dino-capture 2 software (Kalantar et al., 2017).

2.8 Fatty acid profile

On the 42nd day of age, one bird/treatment was slaughtered, and their breast meat was used to measure the fatty acid profile. The breast muscle was separated and stored at -20°C to preserve its texture and composition. The fat content was extracted from the samples by the mixture of two strong solvents of chloroform and methanol (at a ratio of 2:1), respectively. The amount of 1 g of the mixed samples was weighed and poured into closed test tubes. Then, 15 cc of the prepared solvent was added and thoroughly mixed, followed by refrigeration for 24h. After the mentioned duration, 5 cc of distilled water was added to the samples to create 3 phases in the test tube. The bottom phase, consisting of chloroform and dissolved fat, was separated and

transferred to special centrifugal tubes. Centrifugation was performed at 25 °C at 3000 rpm for 15min until the phases of chloroform and fat were completely separated. Finally, the fat content of the samples was analyzed (Folch et al., 1957).

2.9 Statistical analysis

Statistical analysis of all data was done using statistical software (SAS, 2001). The data were analyzed in a completely randomized design with three treatment sets and four replicates/treatments. The GLM model consisted of licorice as the main effect. Differences among treatment means were analyzed using Duncan's multiple-range test. Statistical significance was determined at $P < 0.05$.

3. Results and Discussion

3.1 Growth performance

The results of using different levels of licorice on the performance of broilers are shown in Tables 2, 3, and 4. In all periods, the highest weight gain was related to the level of 200 licorice. Although the Feed Conversion Ratio (FCR) was not significant in any of the periods, the best FCR was related to the level of 200 mg/kg licorice. Previous researchers have suggested that the aqueous-alcoholic extract of licorice leaves increases feed digestion (Ghalib et al., 2011). Licorice extract seems to reduce the speed of feed passage and improve nutrient digestion and absorption, consequently leading to an enhanced feed conversion ratio. The findings suggest that medicinal plants can improve the digestive process (Mellor, 2000). As a result, better consumption of nutrients by increasing internal digestive enzymes in the diet containing medicinal plants can have a direct effect on improving the feed conversion ratio. These results are consistent with previous studies (Dalié et al., 2010; Haskard et al., 2000; Lahtinen et al., 2004), that have indicated licorice extract's ability to balance the intestinal bacterial population, prompt the growth of beneficial bacteria, and improve feed digestion and absorption by stimulating digestive enzyme production. Previous studies (Sedghi et al., 2010; Ocampo et al., 2016; Attia et al., 2017), have also demonstrated the positive effects of licorice extract on broiler chicken growth performance (Attia et al., 2017; Sedghi et al., 2010; Ocampo et al., 2016). According to the obtained results, the weight of one chicken at the end of the research and also the European production index was significant in two different levels of licorice ($P < 0.05$) (Table 4). In addition, the lowest price of live chicken and the best European index was related to the level of 200 licorice. In fact, licorice affects the metabolism of arachidonic acid through the flavonoids hypoglobin B and hypoglobin A and formontin contained in it and reduces the production of free radicals. These compounds also have antioxidant, anti-platelet, and anti-inflammatory properties (Ocampo et al., 2016; Somjen et al., 2004) and our results confirm these observations. Polyphenolic flavonoids of licorice root are strong antioxidant compounds and glabrin (isoflavonoid) of licorice reduces kidney infection through its anti-nephrotic effects and eliminating free radicals, thus leading to improved performance. In broiler chickens, the addition of licorice to the diet of broiler chickens improves performance and production (Aoki et al., 2007).

Table 2. Growth performance of broilers at three stages of growth-fed diets containing different levels of licorice powder (Glycyrrhizaglabra)

Licorice (mg/kg)	1st to 10th days of age			11th to 21st days of age			22nd to 42nd days of age		
	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio
0	24.125 [a]	21.600 [a]	1.118 [a]	69.825 [a]	48.932 [a]	1.427 [a]	130.437 [a]	70.963 [b]	1.839 [a]
100	23.575 [a]	21.125 [a]	1.117 [a]	69.900 [a]	49.364 [a]	1.416 [a]	138.112 [a]	74.550 [b]	1.854 [a]
200	24.725 [a]	22.650 [a]	1.092 [a]	73.000 [a]	51.091 [a]	1.429 [a]	140.389 [a]	78.800 [a]	1.782 [a]
P-value	0.540	0.436	0.258	0.465	0.503	0.478	0.093	0.004	0.481
Standard error of means	0.708	0.817	0.012	1.982	1.326	0.008	2.949	1.205	0.043

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P > 0.05$).

Table 3. Growth performance of broilers fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

Licorice (mg/kg)	1st to 42nd days of age		
	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio
0	88.416 [a]	53.012 [b]	1.668 [a]
100	92.046 [a]	54.762 [b]	1.681 [a]
200	94.276 [a]	57.671 [a]	1.635 [a]
P-value	0.161	0.002	0.473
Standard error of means	1.969	0.663	0.026

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P < 0.05$).

3.2 Characteristics of carcass, carcass fat, and digestive organs

Table 5 shows the effect of experimental treatments on carcass characteristics. Except for breast weight, the consumption of two levels of licorice had no statistically significant effect on live weight, featherless weight, wing percentage, heart percentage, thigh percentage, neck percentage, and abdominal fat ($P \geq 0.05$). However, the lowest level of abdominal fat was observed in the group receiving 100 mg/kg of licorices. Possible mechanisms for abdominal fat reduction include increased fatty acid oxidation, decreased fatty acid biosynthesis (Murase et al., 2001), reduced calorie intake (Van Gaal et al., 2005), and

inhibited fat absorption (Nakai et al., 2005; Tominaga et al., 2006; Aoki et al., 2007). The authors of these studies indicated that increased fatty acid oxidation and reduced biosynthesis are among the potential mechanisms by which licorice reduces abdominal fat. This action occurs in the liver, where licorice extract induces the expression of genes involved in fatty acid oxidation pathways and reduces fatty acid synthesis (Li et al., 2019). Phenolic compounds present in medical plants have been found to reduce harmful intestinal microbes; as a result, prevent nutrient loss and improve performance, protein synthesis, carcass weight, and breast muscle in broilers (Attia et al., 2017). These findings are consistent with the findings of the present study.

Table 4. Economical performance of broilers at 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

Licorice (mg/kg)	Weight 1 chick at 42th days of age (gr/chick)	European production index	Total cost ($/m²)	Total income ($/m²)	Total profit ($/m²)
0	2220.000 [b]	324.777 [b]	24.958 [a]	32.225 [b]	7.268 [a]
100	2291.750 [b]	333.051 [b]	25.985 [a]	33.270 [b]	7.280 [a]
200	2411.000 [a]	359.816 [a]	26.630 [a]	34.997 [a]	8.370 [a]
P-value	0.002	0.006	0.156	0.002	0.135
Standard error of means	27.175	5.883	0.556	0.394	0.399

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P < 0.05$).

3.3 Blood parameters

Table 6 illustrates the statistically significant impact of two different levels of licorice on various blood factors, including LDL/HDL ratio, LDL, VLDL, triglyceride, HDL, and glucose ($P < 0.05$). The highest level of HDL was observed in the group receiving 200 mg/kg of licorice. It has been reported that licorice consumption does not have a significant effect on serum cholesterol concentration but decreases VLDL, LDL, and total protein (Sedghi et al., 2010). Studies conducted on other animal species have shown that the consumption of licorice leads to a significant decrease in serum glucose levels. Furthermore, the consumption of powdered licorice has been found to inhibit LDL oxidation in humans and rats, which is consistent with our result (Takii et al., 2001). The reduction in LDL concentration in response to the consumption of licorice can be caused by the plant's action as a protective agent against LDL cholesterol against oxidation, an inhibitor of cyclooxygenase and lipoxinase activities, and an inhibitory agent peroxidation of lipids. Certain isoflavones in licorice bind to the structure of LDL and prevent its oxidation (Craig, 1999). Licorice extract can reduce the secretion of hormone secretion from adipose tissue by decreasing adrenal gland hormone levels and promoting fat oxidation, which leads to a decline in the level of fatty acids, including cholesterol (Karimi et al., 2015). The decline of cholesterol and triglyceride levels in the serum can be attributed to the active substances present in licorice. The hydrophobic extract of licorice reduces the activity of lipase and also prevents cholesterol absorption (Nakagawa et al., 2004).

Table 5. Means weight and relative weight of invaluable body parts of broilers at 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

Licorice (mg/kg)	Live body weight (gr)	Defeather body weight (gr)	Relative weight of breast (%)	Relative weight of drumsticks (%)	Relative weight of wings (%)	Relative weight of abdominal fat (%)	Relative weight of heart (%)	Relative weight of neck (%)
0	2411.250 [a]	1540.000 [a]	25.750 [a]	19.750 [a]	4.455 [a]	0.920 [a]	0.390 [a]	2.472 [a]
100	2538.750 [a]	1572.500 [a]	24.000 [b]	19.500 [a]	5.152 [a]	0.722 [a]	0.415 [a]	2.522 [a]
200	2537.500 [a]	1635.000 [a]	26.250 [a]	19.250 [a]	5.225 [a]	1.160 [a]	0.402 [a]	2.500 [a]
P-value	0.450	0.450	0.017	0.716	0.204	0.205	0.707	0.931
Standard error of means	78.406	51.673	0.456	0.425	0.308	0.159	0.021	0.093

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P < 0.05$).

Table 6. Means of blood constitutes of broilers at 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

Licorice (mg/kg)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	LDL/HDL ratio	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
0	241.250 [a]	122.500 [a]	72.825 [a]	14.565 [a]	38.975 [b]	64.300 [a]	1.652 [a]	3.150 [a]	1.155 [a]	1.995 [a]
100	233.000 [b]	122.000 [a]	68.825 [ab]	13.773 [ab]	41.700 [a]	61.800 [ab]	1.480 [b]	3.055 [a]	1.100 [a]	1.955 [a]
200	228.500 [b]	123.500 [a]	64.775 [b]	12.963 [b]	42.625 [a]	60.750 [b]	1.422 [b]	3.010 [a]	1.088 [a]	1.922 [a]
P-value	0.001	0.800	0.014	0.015	0.011	0.031	0.000	0.210	0.168	0.258
Standard error of means	1.470	1.599	1.500	0.304	0.681	0.797	0.025	0.052	0.024	0.029

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P < 0.05$); VLDL: Very low-density lipoprotein; HDL: High-density lipoprotein; LDL: Low density lipoprotein.

3.4 Immune system

Table 7 presents the results of consuming two different levels of licorice for the 42-day period, showing no significant effect on the humoral immune system's response to SRBC antigen injection and the antibody titer against Newcastle virus and influenza ($P \geq 0.05$). At the same time, the highest antibody titer was observed at the concentration of 200 mg/kg. Another study also reported an improvement in the antibody titer against Newcastle disease when licorice

was incorporated into the diet of broiler chickens (Wang et al., 2000). This effect was attributed to the saponins present in licorice roots (Khaligh et al., 2011). These compounds can prevent the penetration of other stimuli into the deeper layers of the damaged mucosa by depositing protein in the mucous membrane and creating an impermeable layer (Hostettmann & Marston, 1995). Moreover, the use of licorice polysaccharides has been shown to enhance the antibody titer against the Newcastle vaccine in broiler chickens (Alagawany et al., 2019). Additionally, licorice had a significant effect on antibody titer of influenza at 42 days of age.

Table 7. Mean immune response of broilers at 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

Licorice (mg/kg)	Newcastle disease (log2)		Influenza disease (log2)		Sheep red blood cell (log2)	
	Step one	Step two	Step one	Step two	Step one	Step two
0	3.000 [a]	3.250 [a]	2.500 [a]	4.500 [b]	2.500 [a]	1.000 [a]
100	2.000 [a]	3.000 [a]	2.750 [a]	5.250 [a]	2.750 [a]	1.250 [a]
200	3.750 [a]	3.500 [a]	2.500 [a]	4.000 [b]	3.750 [a]	1.250 [a]
P-value	0.244	0.869	0.767	0.010	0.594	0.622
Standard error of means	0.682	0.661	0.276	0.220	0.890	0.204

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P < 0.05$).

3.5 Relative weight of lymphatic organs

Table 8 shows the effect of adding different levels of licorice into the diet on the weight of lymphatic organs. The bursa of Fabricius and spleen weights were not affected by experimental treatments ($P \geq 0.05$). However, the use of an oral additive caused a slight and non-significant increase in the relative weight of the bursa of Fabricius and the spleen ($P \geq 0.05$). Another study reported no significant effect on the relative weight of the bursa and spleen with 0.5, 1, and 2 g/kg of licorice extract (Sedghi et al., 2010). These findings are inconsistent with another study that reported significant increases in the relative weight of the spleen in laying hens due to the use of dietary licorice seed powder (Awadein et al., 2019).

Table 8. Mean relative weight of organs related to the immune system of broilers at 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

Licorice (mg/kg)	Relative weight of liver (%)	Relative weight of spleen (%)	Relative weight of bursa of fabricius (%)
0	2.090 [a]	0.135 [a]	0.103 [a]
100	2.407 [a]	0.124 [a]	0.095 [a]
200	2.497 [a]	0.168 [a]	0.162 [a]
P-value	0.198	0.300	0.317
Standard error of means	0.153	0.019	0.032

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P < 0.05$).

3.6 Intestinal microbial flora

Table 9 displays the effect of licorice consumption on *Escherichia coli*. The population of *Escherichia coli* showed a statistically significant difference compared to the control group ($P < 0.05$), with the lowest population observed with the use of 200 mg/kg licorice. However, the number of *Lactobacillus acidophilus* and *Bifidobacterium* did not show a statistically significant difference. By changing the microbial load of the digestive tract and their metabolites, medicinal plants also affect the morphology of the digestive tract tissues (Xu et al., 2003). In fact, their phenolic components can reduce the number of harmful microbes. Useful microbes prevent the loss of nutrients and thus improve the function and increase protein efficiency in body tissues. Licorice has ten known antioxidant compounds, six analgesic compounds, eight antiviral substances, and twenty antibacterial active substances (Mohiti et al., 2010). Therefore, the phenolic components in licorice extract have probably led to improved feed efficiency and increased carcass and breast muscle weight in broiler chickens (Attia et al., 2017). Licorice root also has an antibacterial effect. It is shown that all studied bacteria are sensitive to licorice extract (Nitalikar et al., 2010).

Table 9. Microbial population in the intestine of broilers at 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

Licorice (mg/kg)	*Escherichia coli* (CFU/g)	*Lactobacillus acidophilus* (CFU/g)	Bifidobacterium (CFU/g)
0	9.767 [a]	10.297 [a]	9.350 [a]
100	9.700 [a]	10.003 [a]	9.673 [a]
200	9.180 [b]	10.113 [a]	9.673 [a]
P-value	0.003	0.907	0.687
Standard error of means	0.075	0.470	0.295

[a, b] Within columns, means followed by at least one same superscript are not significantly different ($P < 0.05$).

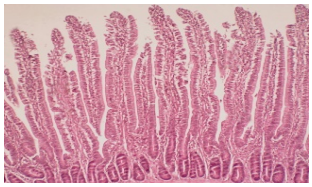
3.7 Intestinal morphology

According to the data in Table 10 and Figure 1, the maximum length of the villi was observed with the consumption of 200 mg/kg licorice. At the same dose of licorice, the width of the villi and the depth of the crypt increased compared to the control group, and the ratio of the length of the villi to the depth of the crypt decreased. Researchers believe that beneficial intestinal bacteria play an effective role in improving the function of the mucosal barrier, the maturity and health of the intestine, and the growth of lymphatic tissue, and their presence is necessary for the function of the intestinal barrier considering that the studied plant preserves the activity of beneficial intestinal bacteria and removes harmful bacteria, so it can be said that licorice powder prevents the production of toxic and destructive substances resulting from the activity of bacteria in the intestinal environment through its antimicrobial and antioxidant properties (Klaver & Van der Meer, 1993). In this

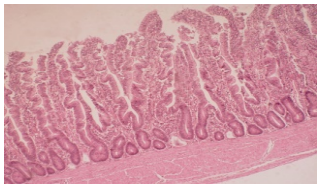
way, the health of the intestinal morphological indicators has been maintained.

Table 10. Intestinal morphological indices of Ross 308 broilers in 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)* from 1st-42nd days of age

Licorice (mg/kg)	The length of the villi (µm)	The width of the villi (µm)	Crypt depth (µm)	The length of the villi/crypt depth
0	734	93	148	8.41
100	444	132	161	9.96
200	924	135	168	6.39



Control (0 mg/kg)



Licorice (100 mg/kg)

Licorice (200 mg/kg)

Figure 1. Morphological image of jejunum of Ross 308 broilers on day 42 of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)* from 1st-42nd days of age

3.8 Fatty acid profile of breast meat

The amount of saturated fatty acids such as stearic acid, palmitic acid, and myristic acid decreased with increasing levels of licorice (Table 11). In addition, the results showed a positive effect of high licorice levels on the percentage of unsaturated fatty acids, particularly oleic acid, and palmitoleic acid, with the 200 mg/kg licorice level. Storage of n-3 unsaturated fatty acids in the breast muscle was higher than in the thigh muscle (Wang et al., 2012; Huang et al., 2011). Licorice protects cell membranes and unsaturated fatty acids from free radical oxidation. It exhibits various beneficial properties, such as preventing liver aldosterone metabolism, inhibiting cyclooxygenase activity, reducing prostaglandin production, producing active oxygen radicals, stopping 5-β-reductase activity, showing steroid-like anti-inflammatory activities, and protecting the liver (Menegazzi et al., 2008).

4. Conclusion

The use of licorice in the diet of Ross 308 broiler chickens improved feed intake, weight gain, feed conversion ratio, cost per kilogram of live chicken, and production index at the end of the period. In addition, licorice had positive effects on certain blood parameters, including LDL/HDL ratio, LDL, VLDL, triglyceride, and glucose, as well as on the immune system and reduction of abdominal fat, leading to improved carcass meat quality. Furthermore, high levels of licorice were associated with a decrease in the ratio of saturated fatty acids to unsaturated fatty acids, which is beneficial for human health. The study also reported the lowest population of *Escherichia coli* and the highest population of *Bifidobacterium*. Therefore, based on these findings, it is recommended to use a level of 200 mg/kg licorice as a potential growth promoter. Future studies should consider replicating this experiment with a larger sample size and conducting a pre-analysis of the *Glycyrrhizaglabra*.

Table 11. Profile of breast fatty acids of broilers at 42nd day of age fed diets with different levels of licorice powder *(Glycyrrhizaglabra)*

licorice (mg/kg)	Myristic Acid Methyl Ester C14:0 (%)	Palmitic Acid Methyl Ester C16:0 (%)	Palmitoleic Acid Methyl Ester C16:1c (%)	Stearic Acid Methyl Ester C18:0 (%)	Oleic Acid Methyl Ester C18:1n9c (%)	Linoleic Acid Methyl Ester C18:2n6c (%)	Linolenic Acid Methyl Ester C18:3n3 (%)	The ratio of saturated to unsaturated fatty acids
0	2.02	37.48	3.14	11.40	22.57	17.65	0.50	1.33
100	2.85	37.78	2.57	13.14	22.12	15.73	0.37	1.47
200	1.71	37.11	3.28	10.61	23.81	17.31	0.43	1.27

Authors' Contributions

Eisa Shaban: Data curation; Formal analysis; Investigation; Writing-original draft. Mehrdad Bouyeh: Conceptualization; Project administration; Validation; Writing-review & editing. Alireza Seidavi: Conceptualization; Project administration; Supervision; Validation; Writing-review & editing.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Ethical considerations

The usage and care of birds in this experiment received ethical approval from the Rasht Branch, Islamic Azad University, Rasht, Iran. All experimental procedures explained here were also approved by the same institution (11748247073098814001624938 53). Care was taken to minimize the number of birds used.

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