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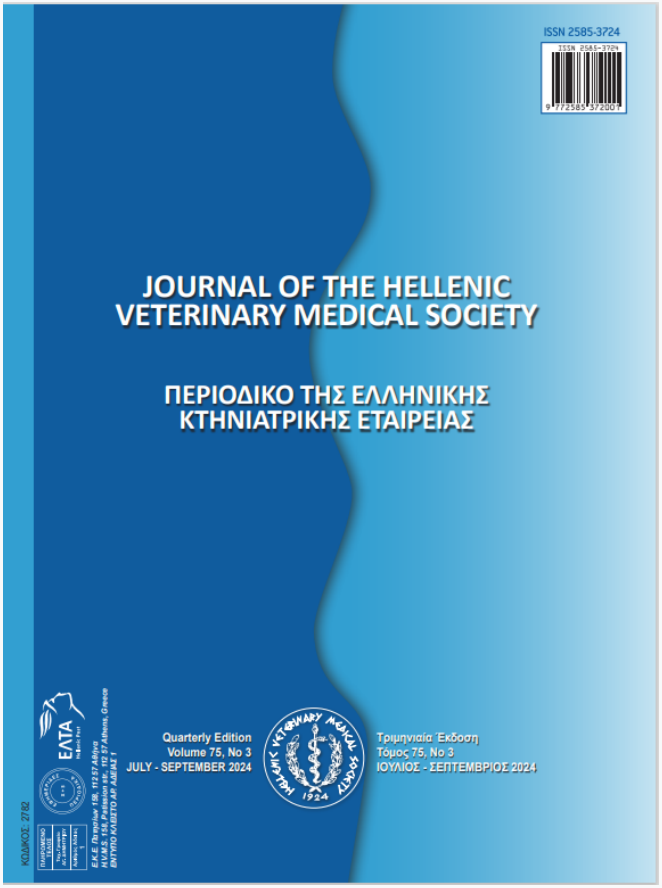

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The effect of high levels of *Glycyrrhiza glabra* powder on performance, carcass characteristics, blood parameters, immunity, intestinal microbial flora, intestinal morphology and breast muscle fatty acid profile in broilers

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ABSTRACT: We investigated the effect of a medicinal plant, namely licorice (*Glycyrrhiza glabra*) powder, on the performance, carcass characteristics, fatty acid profile of breast muscle, intestinal microbial flora, blood constitutes and immunity of broiler chickens. Using a completely randomized design, an experiment was done in which 3 treatments, 4 replications, and 10 chicks (45±2 g) per replication (total of 120 one-day-old male broiler chicks of commercial strain Ross 308) were used. Experimental treatments included three different levels of licorice (0, 300 and 400 mg/kg feed). The results showed that although variation in feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) was not significant in any of the rearing periods ($P \geq 0.05$), the highest FI, WG and the best FCR was observed in the final period of chickens were fed with Diet containing 400mg/kg licorice. According to the data, the best European index was related to this treatment. In addition, the use of two levels of licorice had no significant effect on carcass characteristics or intestinal microbial flora; however, the highest increase in body weight and the lowest number of *Lactobacillus acidophilus* bacteria were observed at the higher level of licorice. However, these high levels did not lead to a decrease in abdominal fat. According to the results obtained, different levels of licorice led to a significant reduction of harmful fats such as cholesterol, triglycerides, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) ($P<0.05$). Furthermore, although the level of high-density lipoprotein HDL was not significant ($P \geq 0.05$), its highest amount was seen at high levels of licorice. In addition, the lowest amount of total protein, albumin and globulin was observed at the level of 400 mg/kg of licorice. Although the amount of glucose was the lowest, its level was not significant ($P \geq 0.05$). The use of this same level of licorice had no significant effect on the function of the humoral immune system in response to antigen injection i.e. antibody against sheep red blood cell (SRBC) or antibody titer against Newcastle virus and influenza ($P \geq 0.05$). Nevertheless, according to the data, the highest antibody titer against Newcastle virus and SRBC antigen was associated with this treatment. These results also showed that high levels of licorice led to an increase in the number of Bifidobacterium. Villi width and crypt depth decreased at the high dose of licorice, but the ratio of villi length to crypt depth increased. In addition, the use of different levels of licorice could not reduce the ratio of saturated to unsaturated fatty acids compared to the control group. Therefore, based on the results of this research, it is recommended to use the level of 400 mg/kg of licorice to supplement the diet of broilers

Keywords: antibody; blood parameters; broiler; fatty acids; licorice

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INTRODUCTION

Medicinal plants have played an important role in maintaining health and improving the quality of human life for thousands of years. These therapeutic compounds include plant essential oils and their active compounds, which are recognized by consumers as natural and safe (Seidavi *et al*., 2022). Plant oils and essential oils are liquid aromatic compounds obtained from different parts of the plant (Besharati *et al*., 2020). In recent years, the use of medicinal plants as growth stimulants to replace antibiotic feed additives has increased (Kostadinović *et al.,* 2015). In general, the growth stimulating effects of these feed additives are due to their beneficial effects on the microbial ecosystem of the digestive system, through the control of pathogenic agents (Windisch, 2008). The use of medicinal plants and their secondary metabolites as antimicrobial and antiparasitic agents goes back centuries (Idris *et al.,* 1997).

There are novel reports on positive effects of feed additives in birds (Ghoreyshi *et al*., 2019; Seidavi *et al*., 2019; Vase-Khavari *et al*., 2019) and human (Chuai *et al*., 2023; Liu *et al*., 2023; Liang *et al*., 2024; Zhen *et al*., 2024).

Licorice is one of the oldest medicinal plants. More than four thousand years ago, the natives of the valleys and plains of Mesopotamia, Asia Minor and South Asia offered licorice root to their rulers as a sign of their servitude. The scientific name of the licorice plant is *Glycyrrhiza glabra* L, its English name is Liquorice (Khan Ahmadi, 2012). This plant has thick and abundant leaves (Omidbaigi, 2010), the root has many potential bioactive compounds including a variety of sugars, triterpenes, flavonoids, isoflavonoids, sterols, amino acids, gum and starch, coumarin, essential oils and saponins (Blumenthal *et al.,* 2000). The licorice plant has been reported to contain substances, which have antibacterial, antiviral, anti-inflammatory, antifungal, anti-protozoal and anti-allergy properties. In addition it has the ability to strengthen the immune and nervous systems, improve the activity of the hormonal system, prevent cardiovascular diseases, inhibit the activity of some enzymes, and have anti-cancer effects (Jatav *et al.,* 2011).

In a study conducted by Sedghi *et al*., (2010), the addition of 0.5, 1 and 2 grams per kilogram of licorice and 2 grams of probiotics to the diet of broiler chickens had no significant effect on body weight, FI or FCR. However, treatments containing licorice resulted in lower abdominal fat content, and a significant decrease in blood cholesterol concentration compared to the control group. A later report by Abe El-Hakim and Abd El-Magied (2009) found that the addition of licorice extract to the diet of broiler chickens in hot weather conditions had positive effects on body WG and FCR. In another experiment (Moradi *et al.,* 2017) levels of 0.1, 0.2, and 0.3 g/L of licorice extract were used as a substitute for growth-promoting antibiotics in chicken water. The results of this study showed that body weight, FI and FCR were not significantly different between experimental treatments. However, the concentration of 0.3 g/L significantly reduced the fat in the ventricular area. In fact, medicinal plants can affect the availability of fat for lipogenesis (decomposition of lipids) in birds (Seidavi *et al.,* 2021). On the other hand, medicinal plants reduce the conversion of protein into fat by increasing beneficial bacteria (lactobacilli) in the intestine and by reducing the rate of protein and amino acid breakdown in the digestive system, which results in less fat accumulation in the body. Madamanchi *et al*., (2005) stated that since inappropriate environmental conditions cause the oxidation and destruction of biological cells due to increase of free radicals; they could cause several disorders in the intestinal tissue (Ocak *et al.,* 2008; Sahin *et al.,* 2013). However, some studies show that the licorice plant has antibacterial properties, which have an inhibitory effect on some bacteria that cause problems in the intestines (Wang *et al.,* 2015; Abd El-Hack *et al*., 2020). Moreover, these researchers stated that the root and leaves of licorice plant contain effective substances such as glycyrrhizin, β-18 glycyrrhetinic acid, licoricetin, licochalcone A, licochalcone E, and glabridin, which are involved in the prevention of viral and bacterial diseases. Hamidi *et al*., (2018) reported that the mixture of thyme, licorice and hyperozyme led to an increase in serum globulin concentration. In another study on rats, it was reported that licorice extract has a protective effect against acute liver damage induced by carbon tetrachloride by reducing LDL and total cholesterol (Sadiq Layl., 2016). According to these researchers, the reduction of cholesterol and LDL cholesterol is related to the saponins and fiber present in licorice, which causes an increase in liver LDL receptors, followed by an increase in the removal of LDL from the cycle and an increase in the transfer rate of cholesterol to bile acids (Moradi *et al.,* 2017). The results of several studies indicate the positive effects of adding licorice and its effective ingredients on improving immunity and increasing antibody titers against Newcastle disease

and influenza (Yong-june *et al.,* 2008; Khaligh *et al.,* 2011). According to Yong-june *et al*., (2008), the use of polysaccharides extracted from licorice during the boiling process with water improves the antibody titer against Newcastle vaccine in broilers. Also, in a 2017 experiment (Kalantar *et al.,* 2017), it was stated that the relative weight of immune organs such as bursa and spleen increased in diet containing a mixture of thyme and licorice.

While we know positive effect of this substance in animal models, studies are limited in broiler raising period. Thus, the present experiments were conducted to evaluate high doses of licorice on performance, carcass characteristics, blood parameters, immunity, intestinal microbial flora, intestinal morphology and breast muscle fatty acid profiles in broiler chickens.

MATERIALS AND METHODS

This study was conducted in a broiler farm located in Rasht city using 120 one-day-old broiler chickens of commercial strain Ross 308 with an average weight of 43 ± 1.1 grams. A completely randomized design was used with 3 treatments and 4 replications containing 10 chickens per pen for 42 days. Three treatments were studied with increasing levels of liquorice added to the basic diet: treatment 1 (liquorice 0 mg/kg), treatment 2 (liquorice 300 mg/kg) and treatment 3 (liquorice 400 mg/kg). Mash diets were formulated according to the recommendations of the Ross 308 rearing guide table (Manual, 2012, Table 1). Chickens were reared in 1×1 cages on beds of cellulose rolls for 42 days. The temperature of the rearing hall was 33^{o}C in the first week but was gradually decreased to 23^{o}C on the 18th day of rearing and then continued until the end of the raising period. The environmental conditions for all chickens were similar and included 23 hours of light exposure and one hour of darkness at a humidity level of 65 to 70%. Chickens were given free access to water and feed during the rearing. In addition, birds were vaccinated against infectious bronchitis (10th day of age), Newcastle (4th, 21st and 35th days of age) and Infectious Bursal disease (12nd day of age).

Growth performance and economic efficiency

In each of the initial, growing and final periods (1 to 10, 11 to 21 and 22 to 42 days old), the WG of all chickens in each pen was calculated with a digital scale (Tozin Kala, Iran) with an accuracy of ± 10 grams. At the end of each period, the amount of feed remaining in each feeder was weighed and the amount

Table 1. Diet ingredients, chemical composition, and energy content (from 1st to 42nd of age).

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

1. The amount of vitamins and minerals per kg of the final diet: vitamin A, 9000 IU; vitamin D3, 3000 IU; vitamin E, 18 IU; vitamin K3, 3 mg; vitamin B1(Thiamine), 1/8 mg;vitamin B2(Riboflavin),6 mg; vitamin B6(Pyridoxine),3 mg; vitamin B12(Cyanocobalamin), 0/012 mg;vitamin B3(Niacin),30 mg; vitamin B9(Folic acid),1 mg;vitamin H3(Biotin),0/24mg;vitamin B5(Pantothenic acid),10 mg; 500 mg;Choline,100 mg;Mn,100 mg;Zinc,80 mg;Iron,10 mg; Cu,1 mg;I,0/2 mg;Selenio

of feed consumed was calculated by deducting from the amount of feed given at the beginning of each period. The FCR was calculated by dividing the amount of FI by the WG for days 1 to 10, 11 to 21, 22 to 42 and the whole period (Sigolo *et al.,* 2019).

The following formula was used to measure the European production index (Belali *et al*., 2021):

10 × number of rearing days × FCR / retention percentage × average live weight (grams) = European production index

In order to measure the cost of FI per kilogram of live chicken, the following formula was used.

Cost of feed consumed per kilogram of live chicken = Weight of a chicken in 42 days in kilograms/price of FI during 42 days for each chicken in Rials. Note that the daily price of licorice used was calculated separately for each ration and inserted into the formula.

Carcass characteristics and digestive organs

After two hours of starvation at the end of the breeding period, 2 birds from each replication with a weight close to the average were selected, killed and weighed with a digital scale (GF-300 digital scale balance, A&D Weighing Design and Manufacture, San Jose, CA) with an accuracy of 0.01g. Live weight, carcass weight, breast weight, thigh weight, wing weight, neck weight, as well as the weight of internal organs (heart, spleen, bursa of Fabricius, liver, and fat area ventricula) were measured (Shabani *et al.,* 2015).

Blood parameters

At the end of the experiment (42 days old), 2 birds with close to average weight were randomly selected from each pen and 5 ml of blood was taken from the wing vein into coagulant tubes to measure blood parameters. The samples were pooled and stored for 12 h, then were centrifuged (Eppendorf 5702, Germany) for 3 minutes at room temperature, and the serum was separated and transferred to microtubes and transported to the laboratory, where it was stored at minus 20 degrees Celsius until measurement of blood metabolites. Serum samples were thawed at room temperature and then glucose, triglyceride, cholesterol, total protein, albumin, globulin, (Very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL) and LDL (low-density lipoprotein) were determined with Pars Azmoun commercial kits or by an autoanalyzer (Hitachi 917, Japan) based on the method of Golrokh *et al.,*(2016).

Immune responses

In order to check the humoral immune response, the antibody titer against sheep red blood cells (SRBC) was determined by a hemagglutination method (Thayer and Beard., 1998). To prepare an injectable suspension of SRBC, blood was taken from 3 sheep and placed in a glass containing EDTA. The blood cells were washed three times in PBS saline phosphate buffer and finally a 2% suspension of SRBC in PBS was prepared. All the above steps were done in sterile conditions. The SRBC suspension (0.1mL) was injected into the wing vein of each pen on days 28 and 36. Blood was taken 7 and 14 days and on days 35 and 42 after the first and second injections (Gore and Qureshi, 1997). Injections were followed by measurement of the antibody titer against SRBC by the hemagglutination method. An image was prepared to measure the antibody titer of V microhemagglutination pellets. The Van derzipp method was used to determine the total antibody as follows. Fifty ul of the serum sample was mixed with 50 ul of phosphate buffered saline (PBS) in a microtiter plate, and then serial dilutions from 1.2 to 1.256 were prepared from the serum. In the next step, 50 ul of the suspension solution at 2% SRBC was added to each well, which was then placed at room temperature for 4-5 hours. Titers were expressed based on Log2, the highest dilution that showed complete agglutination (Pourhossein *et al.,* 2015). In order to check the titers of Newcastle (NDV) and influenza on days 28 and 42 from each pen, blood serum from 2 birds was mixed together followed by hemagglutination inhibition (HI) test (Office International des Epizooties) using a 96-well microplate. First, 25 microliters of PBS (Peripheral blood smear) were poured into all the wells and then 25 microliters of bird serum were added to the first well and its dilution until the last well. In the next step, 25 microliters of Newcastle and influenza antigen were added to all the wells, the microplate was placed on a mechanical shaker for 1 minute, and the microplate was placed at a temperature of 25°C for 30 minutes. In the next step, 25 microliters of 1% red blood cells were added to all wells and the microplate was again placed on a mechanical shaker for 15 seconds, and then the microplate was placed for 30 minutes at a temperature of 25˚C and the results were recorded. 4-unit antigen (Pasouk, Iran) was used to perform an HI test. The titers were diluted based on Log2. A solution of 1% red blood cells used was also prepared from SPF (Sun Protection Factor) chickens. At the end of the experiment, after two hours of star-

vation, 2 birds from each replicate were killed and weighed using a digital scale (GF-300 digital scale balance with an accuracy of 0.01g, and the weights of spleen and bursa of Fabricius were recorded (Shabani *et al.,* 2015).

Microbial flora

To check the microbial flora of the cecum, on the 42nd day of each treatment two birds were slaughtered and after opening the abdominal cavity, the right and left cecum were separated with sterile scissors and the contents were emptied into sterile microtubes. Then, samples were kept at -20°C until microbial populations of *Escherichia coli*, *Lactobacillus acidophilus*, Bifidobacterium were checked by culturing successive 1 to 10 dilutions in distilled water which were then autoclaved at 120 degrees' atmospheric pressure (Dibaji *et al.,* 2014). For this purpose, after thawing one gram of each of the frozen samples was added to 9 ml of distilled water; then 300 microliters of each dilution series of 10^{-3}, 10^{-4}, and 10^{-5} were inoculated on plates containing culture medium by completely spreading on the culture surface by means of a lap. Samples were grown at 37˚C for 24 hours in Eosin methylene blue (EMB) Agar for *E. coli* and *L. acidophilus* bacteria and in Blood Agar (BA) culture medium for Bifidobacterium bacteria (Jang *et al.,* 2007). Then, in order to determine CFU (Colony Forming Units), the colonies formed were counted (4-10) in the most appropriate dilution followed by multiplying the number of colonies on each culture medium by the dilution ratio. Due to the magnitude of the numbers obtained from the bacterial counts, in order to make calculations easier, the base 10 logarithms of these values were calculated and used for data analysis (Dibaji *et al.,* 2014).

Intestinal morphology

In order to investigate intestinal morphology, on the 42nd day immediately after slaughter, tissue sections were prepared by fixing intestinal samples in a buffered 10% formalin solution and dehydration, clarification and paraffin embedding were performed by a tissue processor (Autotechnicon; model TISSUE PROCESSOR (DS 9602 manufactured by Didehsabz). After tissue processing, the blocking stage was performed, and tissue was cut to a thickness of 5-7 micrometers using a Rotary Microtome (Didehsabz Co, Iran; model DS4055). Sections were then treated with hematoxylin-eosin dyes (Kalantar *et al*., 2017) to stain nuclei and cytoplasm. To perform this staining, substances such as gazyl, ethyl alcohol with concentrations of 70% to absolute, alcohol acid, hematoxylin dye, eosin dye and lithium carbonate saturated solutions were used. Hematoxylin was dissolved in alcohol and aluminum alum in distilled water with slight heating, followed by mixing the two solutions together and bringing the final solution to a rapid boil. Then mercuric acid was added, followed by quickly cooling the solution by placing it in water (Kalantar *et al*., 2017). Dehydrating was then done by placement of the samples in ascending concentrations of 70%, 80%, 90% and absolute ethyl alcohol for three minutes each. The slides were then placed in hematoxylin dye for 15 min, followed by washing in running water for 3-5 min. In order to remove the extra color and differentiation, slides were immersed in alcohol acid for 3 sec, followed by washing in running water for 3-5 min. To fix the color of the nuclei, the slides were placed in a container containing lithium carbonate for 3 min and then washed in running water.

Samples were then placed in a container containing eosin for 5-10 min, followed by washing in running water for 2-3 min. At this stage, the baskets containing slides were placed in a container with ascending concentrations of ethyl alcohol (70%, 80%, 90% and absolute alcohol) for 3 min each. After drying, the slides were placed in three xylol containers for 3 min each. At the last stage, stained slides were removed from the basket and cleaned around the sample. A drop of Antlan glue was placed on the slide and a coverslip was gently placed at an angle of 45 degrees to expel air remaining between the slide and the coverslip. Slight pressure with a pair of tweezers helped remove any remaining air. Samples were ready for study after drying. Staining yielded dark blue to black nuclei and pink cytoplasm I (Kalantar *et al*., 2017).

The slides were examined in terms of villus length, villus diameter, crypt depth, and crypt diameter using an optical microscope (MEDIC M-107 BN, Wincom Company Ltd., China). Histomorphometric studies were carried out using a Dino-Lite lens digital camera and Dino-capture 2 software (Kalantar *et al*., 2017).

Fatty acid profile

After 42 days of each treatment, one bird was slaughtered and, to determine the profile of breast fatty acids, a breast meat sample was taken to the laboratory for fat extraction. For this purpose, the breast muscle was first separated and then minced and stored in a freezer at -20°C to protect the texture and com-

position of the meat. The fat content of the samples was extracted by the method of Folch *et al*., (1957). Briefly, first, a mixture of chloroform and methanol was prepared in a ratio of 2 to 1. Then one gram of the mixed samples was transferred to capped test tubes, and 15 cc of the prepared solvent was added, mixed completely, and then kept in a refrigerator for 24 hours. Subsequently, 5 cc of distilled water was added to the samples to create 3 phases in the test tube. The bottom phase containing chloroform and dissolved fat was poured into special centrifuge tubes followed by centrifugation at 0.300 rpm for 15 min until the phase containing chloroform and dissolved fat separated. The solution was then poured into another test tube, which was placed under the hood in a 70°C water bath and nitrogen gas was blown on it to evaporate the chloroform. Then 50 mg of extracted pure fat was separated and treated by basic and acidic methylation in two stages. First, the fat sample was exposed to 0.5 M methoxide solution in methanol at a temperature of 50˚C for 30 min and then exposed to a 1:1 ratio of hydrochloric acid solution in methanol for 30 minutes at a temperature of 50˚C. Hexane was then added to the solution to produce methyl esters of the dissolved fatty acids. The solution was dehydrated by adding dry sodium sulfate to it, and the methyl esters of fatty acids dissolved in the hexane were passed through a special filter, which was prepared for injection into a gas chromatography column (GC7890 Series B, Agilent America). Cl3 fat was used as a standard. Pure nitrogen was used as a carrier gas for injection into the gas chromatography device in a ratio of 1:50. The temperature program used for the column was such that first, the temperature of the oven was kept constant at 100 degrees Celsius for 4 minutes. Then the temperature was increased at a rate of 3°C per minute until it reached a temperature of 240°C, followed by maintaining a constant temperature of 240°C for 20 min. The temperature of the sample injection site (injector) was 225°C and the temperature of the detector was 250˚C. The analysis time of each sample was 71 minutes.

Statistical analysis

Statistical analysis of all data was done using statistical software (SAS., 2001). The data were analyzed in a completely randomized design with 3 treatments set. The model consisted of licorice as the main effect. The differences among treatment means were analyzed using the Duncan's multiple range test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance

The effects of using different levels of licorice on the performance of broiler chickens are presented in Tables 2 and 3. The data showed that different levels of licorice had no significant effect on FI, WG or FCR in any of the rearing periods ($P \geq 0.05$). However, compared to the control group, the highest FI and WG and the best FCR were observed at high levels of licorice (400 mg/kg). Part of the WG in this group may have been due to the increase in FI (Vieira *et al.,* 2008; Rawling, 2009). The efficiency of FI has been reported by other researchers (Waran and Selvasubramanian, 2014). The use of licorice essential oil at a rate of 200 mg/kg of diet increased the daily weight and FI (Gravand *et al.,* 2017), whereas similar results were observed in the present experiment at a dose of 400 mg/kg. In addition, the use of 0.4% licorice extract in the drinking water of chickens led to an increase in FI and an increase in FCR after 21 days (Salary *et al.,* 2014). It has been found that medicinal plants containing flavonoids such as licorice protect feed tryptophan from enzymatic degradation and increase the digestibility of this amino acid. Therefore, it can be said that treatments containing licorice ex-

Table 2: Growth performance of broilers at three stages of growth fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

	1st to 10th days of age			11st to 21st days of age			22nd to 42nd days of age		
Licorice (mg/kg)	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 ^{a}	1.839 ^{a}
300	23.350 ^{a}	20.925 ^{a}	1.117 ^{a}	69.450 ^{a}	47.818 ^{a}	1.454 ^{a}	134.961 ^{a}	75.450 ^{a}	1.790 ^{a}
400	23.600 ^{a}	21.375 ^{a}	1.104 ^{a}	71.450 ^{a}	50.023 ^{a}	1.428 ^{a}	136.003 ^{a}	75.038 ^{a}	1.813 ^{a}
P-value	0.752	0.825	0.684	0.749	0.552	0.450	0.155	0.137	0.444
SEM	0.729	0.776	0.012	1.946	1.383	0.017	1.947	1.569	0.026

^{a,b}Within columns, means followed by at least one identical superscript are not significantly different ($P<0.05$); SEM: Standard error of means

Table 3: Total growth performance of broilers fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

Licorice (mg/kg)	Days 1st to 42nd		
	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed conversion ratio
0	88.416 [a]	53.012 [a]	1.668 [a]
300	90.332 [a]	54.738 [a]	1.650 [a]
400	91.443 [a]	55.238 [a]	1.656 [a]
P-value	0.247	0.082	0.652
SEM	1.195	0.639	0.013

[a,b]Within columns, means followed by identical superscripts are not significantly different (P<0.05); SEM: Standard error of means

tract, especially at high levels, have more tryptophan in the intestine. As a result, tryptophan increases the synthesis of serotonin, which also affects the central part of FI regulation (Gudev *et al.,* 2004) which is in line with the results of the present study.

There are many reports about effects of various agents on poultry performance (Sayehban *et al*., 2016; Sateri *et al*., 2017; Movahhedkhah *et al*., 2019; Hamidi *et al*., 2022; Mokhtarzadeh *et al*., 2022; Besharati *et al*., 2023). However, similar to the results of this study, Sedghi *et al*., (2010) and Medhi *et al.,* (2010) observed the lack of significant effect of licorice extract supplement in the diet of broiler chickens on the digestibility of nutrients. According to the results obtained here (Table 4), although the weight of 1 chick at 42 days of age, feed cost per kg live weight ($/kg) and European index did not vary significantly from the control group in two different levels of licorice, these variables showed a statistically significant difference (P<0.05) from control chicks. The lowest price of live chicken and the best European index was related to the level of 400 mg/kg licorice. AL-Daraji's (2013) studies also showed that adding licorice extract to the drinking water of broiler chickens improved the average body weight, FCR, water consumption and production index.

Characteristics of carcass, carcass fat and digestive organs

The effect of experimental treatments on carcass characteristics is shown in Tables 5. The use of two levels of licorice did not have a statistically significant difference on live weight, featherless weight, breast percentage, thigh percentage, wing percentage,

Table 4: Economical performance of broilers at 42nd day of age fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

Licorice (mg/kg)	Weight 1 chick at 42th days of age (gr/chick)	European production index	Total cost ($/m²)	Income ($/m²)	Profit ($/m²)
0	2220.000 [a]	324.777 [a]	24.958 [a]	32.225 [a]	7.267 [a]
300	2290.750 [a]	338.572 [a]	25.520 [a]	33.255 [a]	7.732 [a]
400	2311.250 [a]	340.578 [a]	25.850 [a]	33.550 [a]	7.702 [a]
P-value	0.082	0.103	0.222	0.081	0.335
SEM	26.184	4.997	0.337	0.379	0.234

[a,b]Within columns, means followed by at least one same superscript are not significantly different (P<0.05); SEM: Standard error of means

Table 5: Means weight and relative weight of invaluable body parts of broilers at 42nd day of age fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

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]	2.473 [a]
300	2438.750 [a]	1547.500 [a]	26.000 [a]	18.750 [a]	5.627 [a]	0.815 [a]	0.445 [a]	2.677 [a]
400	2517.500 [a]	1556.250 [a]	25.000 [a]	19.250 [a]	5.078 [a]	0.905 [a]	0.395 [a]	2.582 [a]
P-value	0.407	0.959	0.685	0.377	0.096	0.653	0.412	0.508
SEM	55.305	39.684	0.829	0.479	0.334	0.815	0.031	0.120

[a,b]Within columns, means followed by the same superscripts are not significantly different (P<0.05); SEM: Standard error of means

neck percentage or abdominal area fat, but they were significant compared to the control group (P <0.05). In the present study, the use of 400 mg/kg of licorice reduced the relative weight of the chickens' breasts. In vitro, the phenolic compounds present in licorice extract strongly bind to proteins and as a result prevent the absorption of the remaining amino acids tryptophan, lysine and cystine and reduce their biological value. This could explain breast weight reduction. Contrary to this observation, Gooderzi *et al*., (2011) reported that the percentage of thighs and breasts of chickens fed with licorice increased compared to the control group. Khamisabadi *et al*., (2015) also reported no significant effect of licorice extract on the relative weight of the liver. However, carcass weight, thigh muscles, chest and pancreas were negatively affected in that study. Hamidi *et al.,* (2018) reported that a mixture of thyme, licorice and hyperozyme had no significant effect on carcass weight, breast, liver and abdominal fat, which is consistent with the results of the present study. But the lowest abdominal fat was observed at the level of 300 mg/kg licorice. Sedghi *et al*., (2010) declared low abdominal fat percentage was observed when feeding high levels of licorice. Naser *et al.,* (2017) reported that the amount of abdominal fat in chickens fed with water containing 0.3 g/L licorice was significantly reduced, and hydrophobic flavonoids were responsible for reducing abdominal fat (Nakagawa *et al.,* 2004). The effect of licorice on reducing abdominal fat can be caused by factors such as suppressing energy intake, reducing fat absorption, increasing fatty acid oxidation or reducing abdominal fat biosynthesis (Tominaga *et al.,* 2006).

Blood parameters

The results of using two different levels of licorice on blood parameters are shown in Table 6. In this study, different levels of licorice had a significant effect on the amount of total protein, albumin and globulin (P<0.05), that is, with increasing intake levels, and the greatest decrease was observed at the level of 400 mg/kg. Hamidi *et al* (2018) reported that a mixture of thyme, licorice and hyperozyme led to an increase in serum globulin concentration. In an experimental design similar to the present study Rashidi *et al.,* (2020) observed a lack of effect of 3 and 6 grams of licorice root extract on the serum albumin of broilers consuming aflatoxin-contaminated diet, which is contrary to the results of the present study. Based on the results presented here, except for the content of cholesterol, triglyceride, VLDL, and LDL, different experimental treatments had no effect on the average concentration of other parameters (P≥0.05). In accordance with the present experiment, Saleem *et al.,* (2011) observed the effect of licorice root extract in rats. According to our results, the use of 3g/kg of licorice extract caused a significant reduction in LDL. This is consistent with the results of Attia *et al. (*2017), which reported that the consumption of licorice extract in broiler diets did not have a significant effect on the concentrations of triglycerides, HDL and cholesterol, while the concentrations of total cholesterol and LDL cholesterol decreased compared to the control treatment. Sedghi *et al.,* (2010) showed that consumption of licorice extract had no significant effect on serum glucose concentration, which is in agreement with the results of the present experiment. But the use of a dose of 400 mg/kg of licorice was able to reduce the blood glucose level. Therefore, it can be concluded that epinephrine stimulates the release of insulin and the subsequent reduction of blood sugar. The level of glucocorticoids in the blood increases. And considering that glucocorticoids stimulate insulin secretion, it may be concluded that licorice reduces blood sugar through this mechanism. The beneficial effects of licorice ethanol extract in the treatment of people with arteriosclerosis have also been proven. It is believed that licorice prevents LDL oxidation. Licorice root has antioxidant compounds including isoprenyl chalcone, glabridin and formonontin. Glabridin is the major part of licorice extract. Glabridin prevents the oxidation of LDL cholesterol by reducing the activity

Table 6: Means of blood constituents of broilers at 42nd day of age fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

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 ^{ab}	72.825 ^{b}	14.565 ^{b}	38.975 ^{a}	64.300 ^{a}	1.653 ^{a}	3.150 ^{a}	1.155 ^{a}	1.995 ^{a}
300	242.500 ^{a}	125.750 ^{a}	80.175 ^{a}	16.035 ^{a}	38.175 ^{a}	64.575 ^{a}	1.693 ^{a}	2.950 ^{ab}	1.085 ^{a}	1.865 ^{ab}
400	237.250 ^{a}	117.500 ^{b}	71.725 ^{b}	14.345 ^{b}	39.250 ^{a}	59.275 ^{b}	1.510 ^{b}	2.720 ^{b}	1.002 ^{b}	1.718 ^{b}
P-value	0.154	0.031	0.019	0.019	0.715	0.003	0.005	0.008	0.003	0.014
SEM	1.799	1.816	1.821	0.364	0.946	0.872	0.030	0.074	0.022	0.052

^{a,b}Within columns, means followed by the same superscripts are not significantly different (P<0.05); SEM: Standard error of means; VLDL: Very low density lipoprotein; HDL: High density lipoprotein; LDL: Low density lipoprotein

of NADPH oxidase, preventing the activity of cyclooxygenase and lipoxygenase enzymes.

Immune system

The effect of different levels of licorice on the humoral immune system function (in response to SRBC antigen injection and antibody titer against Newcastle virus and influenza) is shown in Table 7. The results of using two levels of licorice showed that it had no significant effect on the function of the humoral immune system in response to SRBC antigen injection or antibody titer against Newcastle virus and influenza ($P \geq 0.05$). However, the highest antibody titer an antibody against Newcastle virus was observed in 28 and 42 days at the level of 400 mg/kg. In addition, the highest titer of the effect of licorice plant on the immune system of poultry is still largely unknown. But in general, it can be said that licorice contains effective compounds for control of the humoral immune response. This medicinal plant can help the immune system by improving the digestion and absorption of feed and also reducing subclinical infections in poultry. It is believed that plants containing flavonoids have the property of regulating the immune system. and it seems that the most important pharmacological properties of the active substances in licorice are immune system modulation. (Iskender *et al.,* 2016). According to the results of this experiment, other researchers also reported that licorice increases its antioxidant impact by strengthening humoral immunity (Ocampo *et al.,* 2016; Attia *et al.,* 2017). The results of several studies indicate the positive effects of adding licorice and its effective ingredients on improving immunity and increasing antibody titers against Newcastle disease and influenza viruses. (Yong-june *et al.,* 2008; Khaligh *et al.,* 2011). According to (2008) Yong-june *et al.,* the use of polysaccharides extracted from licorice during the boiling process with water improves antibody titer against Newcastle vaccine in broilers. The presence of saponin in licorice root can also be another reason for the improvement of antibody titer in broiler chickens (Khaligh *et al.,* 2011).

Relative weight of lymphatic organs

In Table 8, the effect of different treatments on the mean relative weight of lymphatic organs is presented. Based on these results, the weight of none of the immune organs, including bursa of Fabricius and spleen were affected by the experimental treatments ($P \geq 0.05$); however, the use of licorice powder caused a slight and insignificant increase in the relative weight of bursa of Fabricius ($P<0.05$). It has also been reported that licorice root has anti-inflammatory properties, detoxification and protection of liver tissue, lymphatic organs and other visceral organs (Iskender *et al.,* 2016). However, In the present exper-

Table 7: Mean immune response of broilers at 42nd day of age fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

	Newcastle disease (log2)		Influenza disease (log2)		Sheep red blood cell (log2)	
Licorice (mg/kg)	Step one	Step two	Step one	Step two	Step one	Step two
0	3.000 ^{a}	3.250 ^{a}	2.500 ^{a}	4.500 ^{a}	2.500 ^{a}	1.000 ^{a}
300	3.250 ^{a}	3.500 ^{a}	3.000 ^{a}	5.000 ^{a}	3.750 ^{a}	1.000 ^{a}
400	3.500 ^{a}	3.750 ^{a}	2.750 ^{a}	4.500 ^{a}	4.500 ^{a}	1.750 ^{a}
P-value	0.829	0.773	0.323	0.500	0.079	0.141
SEM	0.571	0.486	0.220	0.333	0.546	0.276

^{a,b}Within columns, means followed by the same superscripts are not significantly different ($P<0.05$); SEM: Standard error of means

Table 8: Mean relative weight of organs related to the immune system of broilers at 42nd day of age fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

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}
300	2.168 ^{a}	0.178 ^{a}	0.120 ^{a}
400	2.143 ^{a}	0.130 ^{a}	0.120 ^{a}
P-value	0.855	0.259	0.919
SEM	0.099	0.021	0.035

^{a,b}Within columns, means followed by the same superscripts are not significantly different ($P<0.05$); SEM: Standard error of means

iment, there was no statistically significant difference in the relative weight of the lymphatic organs of the bursa and spleen among the experimental treatments, which is in accordance with the results obtained from the experiments of Sedghi *et al*., (2010); Moradi *et al*., (2014).

Microbial flora

The effect of using licorice on the bacterial population is shown in Table 9. The data showed that the use of different levels of licorice had no significant effect on the bacterial population (P≥0.05) nor could it reduce the population of *E coli* bacteria. This could be related to the high pollution of the breeding hall. The results also showed that the use of high levels of licorice led to an increase in the number of Bifidobacterium. It can be said that treatments containing licorice extract, especially at high levels, have a greater amount of tryptophan in the intestine. It is reported that tryptophan can increase the synthesis of serotonin, which also affects the central part of FI regulation (Gudev *et al.,* 2004). In addition, the use of licorice extract may lead to the growth of desirable bacteria in the intestine and, as a result, the bacterial population is more balanced, which improves the digestion and absorption of feed because of the production of digestive enzymes by these bacteria. The improvement of growth performance when using licorice extract has been reported in previous studies (Sedghi *et al.,* 2010; Ocampo *et al.,* 2016; Attie *et al.,* 2017). In addition, researchers believe that the phenolic compounds found in medicinal plants can lead to an increase in beneficial intestinal bacteria (lactobacillus and bifidobacteria) and the reduction of harmful intestinal bacteria (clostridium), which is consistent with the findings in the present experiment.

Intestinal morphology

The effect of experimental treatments on intestinal morphology is shown in Table 10. The maximum length of villi was observed with the consumption of 400 mg/kg licorice. Villi width and crypt depth decreased at a high dose of licorice, but the ratio of villi length to crypt depth increased. The phenolic components present in medicinal plants can lead to the reduction of harmful microbes, resulting in an increase of beneficial intestinal microbes, preventing the loss of nutrients and improving the intestinal tissue (Attie *et al.,* 2017). Researchers believe that beneficial intestinal bacteria play an important role in improving the function and health of the intestine and the development of lymphatic tissue, and their existence is necessary for proper intestinal function. Considering that the plant studied maintained the activity of beneficial intestinal bacteria and reduced harmful bacteria, it can be concluded 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, thereby improving intestinal morphology.

Table 9: Intestinal microbial population of broilers at 42nd day of age fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

Licorice (mg/kg)	*E. coli* (CFU/g)	*Lactobacillus acidophilus* (CFU/g)	*Bifidobacterium sp.* (CFU/g)
0	9.767 [a]	10.297 [a]	9.350 [a]
300	9.987 [a]	11.153 [a]	9.930 [a]
400	10.097 [a]	10.200 [a]	10.353 [a]
P-value	0.720	0.306	0.170
SEM	0.285	0.435	0.324

[a,b]Within columns, means followed by the same superscripts are not significantly different (P<0.05); SEM: Standard error of means

Table 10: Morphological indices of jejunum Ross 308 broilers in 42nd-day diets containing different levels of licorice powder *(Glycyrrhiza glabra)* from 1st-42nd days of age

	The length of the villi (μm)	The width of the vill (μm)	Crypt depth (μm)	The length of the villi/ crypt depth (Ratio)
Licorice (0 mg/kg)	734	93	148	8.41
Licorice (300 mg/kg)	577	184	215	7.14
Licorice (400 mg/kg)	726	123	173	7.15

Table 11: Profile of breast fatty acids of broilers at 42nd day of age fed diets containing different levels of licorice powder *(Glycyrrhiza glabra)*

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
300	0.61	35.17	2.92	9.4	23.69	21.91	0.54	1.47
400	1.44	36.43	2.64	10.37	23.71	20.14	0.59	1.27

Fatty acid profile of breast meat

The effect of licorice treatments on the percentage of fatty acids in breast muscle tissue is shown in Table 11. The amount of saturated fatty acids such as myristic acid and palmitic acid decreased with increasing licorice levels. Also, the results showed a positive effect of high levels of licorice on the percentage of unsaturated fatty acids such as palmitoleic acid and oleic acid; the highest increase was related to the level of 400mg/kg of licorice. If serum concentration of antioxidants decreases for any reason, active oxidizing compounds that remain free are not neutralized resulting in damage to the cells. In immune cells, during aerobic metabolism and also during specific functions of some of these cells such as phagocytosis, active oxidant compounds are created because the membrane of these cells contain many unsaturated fatty acids. And immune cells are very sensitive to oxidation damage because fatty acids from the carbon adjacent to the double bond undergo oxidation quickly. As a result of the oxidation of these fatty acids, the fluidity of the cell membrane decreases and many of the vital functions of the cells of this system are disturbed. In addition, protein molecules inside the membrane, membrane receptors, intracellular enzymes and also cellular DNA suffer oxidative damage (Dorhoi *et al.,* 2006). Therefore, the use of natural antioxidants prevents the activity of these oxidants and improves the immune status of the bird's body. Murica *et al.,* (2004) showed that licorice has acceptable antioxidant potential compared to common antioxidant compounds used in food products and suggested it as a natural antioxidant.

CONCLUSION

In general, licorice plays an important role in the preparation of several medicinal compounds for use in the poultry industry. In addition, this plant contains bioactive components such as flavonoids and glycyrrhizin, which have medicinal properties and medicinal applications. We found it have antibacterial properties, in addition to the ability to strengthen the immune, improve the activity of the hormonal system, promote rearing efficiency. It has also been determined in the current research that licorice extract, due to its immunogenic and antioxidant activity, was able to improve WG and FCR in the final period of FI. However, although a high dose did not reduce abdominal fat, it was able to reduce harmful fats such as cholesterol, triglycerides, VLDL and LDL. In addition, the highest antibody titer against Newcastle virus and SRBC antigen was related to this treatment. Therefore, based on the results of this research, it is recommended to use the level of 400 mg/kg of licorice to supplement the diet of broilers.

CONFLICT OF INTEREST

None declared

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