Dietary effect of licorice (*Glycyrrhiza glabra*) on quail performance, carcass, blood metabolites and intestinal microbiota

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ABSTRACT This study aimed to assess the impacts of licorice (*Glycyrrhiza glabra*) on the growth performance, carcass traits, intestinal microbiota, liver and kidney functions, immunity, oxidative status, and lipid profile of Japanese quails. A total of 200 one-week-old unsexed Japanese quails with an average initial body weight of 26.24 ± 0.2 g were randomly distributed into 5 equal groups of 40 birds and further subdivided into 5 replicates. The first (control) group was fed a diet without any licorice, while licorice powder was added at levels of 250, 500, 750, and 1000 mg per kg diet in the second, third, fourth, and fifth groups, respectively. At the age of 3 wk, the group of quail fed on a diet supplemented with 750 and 1000 mg licorice/kg of diet gained the highest body weight (**BW**) and daily body weight (**DBW**), while attaining the lowest feed conversion ratio (**FCR**) compared to other groups. Meanwhile, groups fed diets with licorice at levels of 0 and 250 mg/kg showed the highest feed intake. After the 5-wk feeding trial, the highest BW and DBW values, and the lowest FCR were recorded in the group fed with 750 mg licorice/kg diet. The different treatments produced no significant differences ($P > 0.05$) in quail carcass characteristics, including percentages of carcass, liver, gizzard, heart, giblets, and dressing. The blood of the group fed a 750 mg licorice diet had higher contents of total protein and GLOB, while its contents of A/G%, lactate dehydrogenase (**LDH**), total cholesterol, triglyceride **(TG)**, and low density lipoprotein **(LDL)** were lower. Apart from the high level of licorice (1000 mg/kg), the MDA level was linearly and quadratically ($P = 0.0413$ and 0.001) decreased with different licorice groups, while superoxide dismutase (**SOD**), total antioxidant capacity **(TAC)**, immunoglobulin G **(IgG)**, and M (**IgM)** were quadratically increased when compared to the control group. Licorice supplementation resulted in marked reductions in the number of total bacteria, coliforms, *E. coli,* and *Salmonella*, compared to those in the control. In conclusion, the inclusion of licorice at levels of 750 and 1000 mg/kg into the diet of Japanese quail enhances the animal's performance, immunity, antioxidant capacity, and maintains a healthy gut microbiota.

Key words: licorice, performance, blood, intestinal microbiota, quails

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INTRODUCTION

Poultry provides high-quality animal protein, and various approaches have been implemented to increase profitability in poultry production. One of these approaches, dietary modulation, can noticeably alter animal performance (Akbari et al., 2018; El-Senousey et al., 2018). Designing a balanced diet that provides all nutritional needs is the basis of any successful production (Sebola and Mlambo, 2018). When nutritional imbalances occur, natural feed additives may be used to overcome their effects.

For a long time, antibiotics have been used to treat ailments and improve growth; however, prohibitions on their use have prompted many countries to ban their use as a feed additive (Mehdi et al., 2018; Roth et al., 2019; Abd El-Hack et al., 2020a). Therefore, finding alternatives to antibiotics has been an active focus of research. Functional feed additives, such as medicinal herbs, probiotics, prebiotics, and synbiotics, are promising natural alternatives to antibiotics (Dhama et al., 2015, 2021; Alagawany et al., 2018, 2021; Elgeddawy et al., 2020; Rehman et al., 2020). Medicinal plants are remedies used in a field of complementary medicine that has existed since ancient times. In this field, all or parts of

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plants, herbs, their extracts, or other derived products are administered through various means (e.g., orally, topically, massaged or inhaled) (Ali et al., 2015; Yatoo et al., 2017). Medicinal plants possess distinctive therapeutic properties, including antimicrobial, anti-inflammatory, antistress, antioxidant, orexigenic, analgesic, and aphrodisiac properties. Thus, they have been successfully used in animal production (Abd El-Hack et al., 2020b; Alagawany et al., 2020a, 2020b; Ebrahim et al., 2020).

Licorice (*Glycyrrhiza glabra*), an herbaceous perennial belonging to the family Fabaceae, has been used since ancient times as a flavoring agent in diets, beverages, and medicinal remedies (Quirós-Sauceda et al., 2016; Tamer et al., 2019). The main issue of poultry production is the epidemiological diseases; in particular those are confined to digestive, respiratory and immune systems disease (Karkanis et al., 2016). Licorice herb offers some of bioactive molecules as glycyrrhizin and flavonoids (Tiwari et al., 2018). Licorice root contains 1 to 9% glycyrrhizin which has several nutritional roles such as its role in enhancing growth rate and pharmacological actions like immunomodulatory, antioxidant, antiviral, and anti-inflammatory properties (Alagawany et al., 2019a, 2019b; Mahmoud et al., 2021). According to phytochemical analysis, the extract of licorice consists of flavonoids (e.g., liquiritin, formononetin and isoflavonoids), triterpene saponins (e.g., glycyrrhetinic acid, glycyrrhizin and licorice acid) and starch, sugars, amino acids, tannins, ascorbic acid, coumarins, choline, phytosterols, and other molecules (Shalaby et al., 2004). Al-Zuhairy et al. (2014) used garlic and licorice in broiler diets and they found that diet containing mixture of garlic and licorice both in 0.25, 0.50 and 1% supplementation improved the production performance of broiler birds. Karimi et al. (2015) showed the beneficial effects of 1% plant extract of licorice on growth performance, immune system and blood parameters of broilers along with other plants such as Iranian caraway, German chamomile, Yarrow and garlic. Many studies have used herbal complex formulas with partial levels of *Glycyrrhiza glabra*. But, using this herb individually is very limited. Thus, in the present study, we aimed to assess the impacts of licorice on the growth performance, carcass traits, intestinal microbiota, blood chemistry, immunity and oxidative status of Japanese quails.

MATERIALS AND METHODS

Birds, Experimental Design, and Treatments

A total of 200 one-week-old unsexed Japanese quails with an average initial body weight (**BW**) of 26.24 ± 0.2 g were randomly distributed into 5 equal groups of 40 birds, subdivided into 5 replicate groups containing 8 chicks each. The first (control) group was fed a diet without any licorice, while licorice powder was added at levels of 250, 500, 750, and 1000 mg per kg diet in the second, third, fourth, and fifth groups, respectively. The licorice was purchased from the local market, Zagazig, Egypt. All groups of quail were maintained in cages measuring 90 × 40 × 40 cm, with free access to the feed and water throughout the feeding trial that lasted 5 wk. Table 1 shows the ingredients and nutrient contents of the basal diet of growing Japanese quail. This study was performed at quail unit, Waterfowl Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Egypt.

Growth Performance and Carcass Measurements

The quails' BW, weight gain, feed intake (**FI**), and feed conversion ratio (**FCR)** were evaluated at 1, 3, and 5 wk of age. At the end of the feeding trial, 25 quails were randomly selected, weighed, and slaughtered after fasting for 6 h. All the edible parts (liver, heart, gizzard, and eviscerated carcass) were weighed, and the measurements are expressed as the percentage of the preslaughter weight.

Blood Chemistry

At the end of the feeding trial, blood samples were collected from 5-week-old slaughtered quails into heparinized tubes and centrifuged for 15 min at 3000 rpm to obtain plasma. The biochemical profile of the plasma consisted of measurements of total protein (**TP**), albumin (**Alb**), globulin (**GLOB**), albumin/globulin ratio (**A/G**), aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), lactate dehydrogenase (**LDH**), total cholesterol (**TC**), triglycerides (**TG**), high density lipoprotein (**HDL**), low density lipoprotein (**LDL**), very-low-density lipoprotein (**VLDL**), immunoglobulins (**IgG** and **IgM**), creatinine, and urea. The biochemical profiles were determined using an automatic analyzer with a commercial kit from Bio-diagnostic Company (Giza, Egypt) according to the manufacturer's instructions.

Antioxidant and Lysozyme Activities

Plasma samples were examined for levels of superoxide dismutase (**SOD**), malondialdehyde (**MDA**), and total antioxidant capacity (**TAC**) using a microplate spectrophotometer with a commercial detection kit (Bio-diagnostic, Egypt), following the manufacturer's instructions.

Plasma lysosomal activity was assessed using a 96-well microplate turbidity assay as described by Lygren et al. (1999). Briefly, in each well of a 96-well microplate, 10 μL of plasma was mixed with 190 μL of a solution containing 0.2 mg of *Micrococcus lysodeikticus* per mL of PSB, pH = 7.4. The plate was shaken gently at room temperature for 1 and 5 min. After each time point, the solution's turbidity was measured at 450 nm using a microplate reader (UVM). One unit of lysozyme activity is defined as the amount of enzyme causing a 0.001/min reduction in absorption.

Microbiological Analysis

Approximately 10 g of cecal samples were obtained from 5 birds per treatment. Each sample was placed in a 250-mL Erlenmeyer flask containing 90 mL of saline solution (0.85% NaCl) containing 0.1% peptone. The mixture was thoroughly mixed, and the abundance levels (counts) of total bacteria (**TBC**), *Enterococcus spp.*, yeasts and molds (**TYMC**), lactic acid bacteria, coliforms, *E. coli*, and *Salmonella spp.* were estimated according to previously published procedures (Xia et al., 2004; Reda et al., 2020). The abundance levels of bacteria and fungi are expressed as log numbers.

Statistical Analysis

The data were analyzed using statistical analysis software (SAS). Treatment means were compared using one-way ANOVA. Orthogonal polynomial contrasts were used to test the significance (linear and quadratic) of the gradual levels of dietary licorice powder.

RESULTS

Quail Performance

The growth performance and feed utilization of Japanese quail fed the various diets are shown in Table 2. Licorice supplementation significantly impacted body weight (**BW**, g), daily body weight gain (**DBW**, g/d), daily FI, g/d), and FCR at 3 wk of the age. These effects were also observed at the end of the feeding trial (5 wk) except for FI ($P > 0.05$). Among all groups, those fed on diets supplemented with licorice at levels of 750 and 1000 mg/kg showed the highest BW and DBW, and the lowest FCR values at 3 wk of age. Meanwhile, groups fed diets with licorice at levels of 0 and 250 mg/kg showed the highest FI values. After 5 wk, the group fed the diet with 750 mg/ kg licorice showed the highest BW and DBW and the lowest FCR values, while groups fed diets with 0 and 250 mg/ kg licorice showed the lowest BW and DBW and the highest FCR values.

Table 1. Ingredients and nutrient contents of basal diet of growing Japanese quail.

Items	(g/kg)
Ingredient	
Maize 8.5%	518.0
Soybean meal 44%	367.0
Maize gluten meal 62 %	52.1
Soybean oil	29.0
Limestone	7.0
Di-calcium phosphate	16.5
Salt	3.0
Premix[1]	3.0
L-Lysine	1.3
Dl-Methionine	1.1
Choline chloride	2.0
Total	1000
Calculated composition[2]	
Metabolizable energy (MJ/kg)	12.53
Crude protein (g/kg)	240.0
Calcium (g/kg)	8.0
Nonphytate phosphorus (g/kg)	4.5
Lysine (g/kg)	13.0
Total sulphur amino acids (g/kg)	9.2

[1]Provides per kg of diet: Vitamin A, 12,000 I.U; Vitamin D3, 5000 I.U; Vitamin E, 130.0 mg; Vitamin K3, 3.605 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin), 8.0 mg; Vitamin B6, 4.950 mg; Vitamin B12, 17.0 mg; Niacin, 60.0 mg; D-Biotin, 200.0 mg; Calcium D-pantothenate, 18.333 mg; Folic acid, 2.083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.
[2]Calculated according to NRC (1994).

Carcass Traits

The effects of licorice-supplemented diets on carcass and edible organ traits of Japanese quail are presented in Table 3. The different diets did not affect ($P > 0.05$) quail carcass characteristics in terms of percentages of carcass, liver, gizzard, heart, giblets, and dressing.

Blood Chemistry

Tables 4 and 5 present the blood profiles of Japanese quail fed the different diets for 5 wk. All blood values are

Table 2. Growth performance of growing Japanese quail as affected by licorice powder.

	Licorice levels (mg/kg diet)						*P* value[2]	
Items	0	250	500	750	1000	SEM[1]	Linear	Quadratic
Body weight (g)								
1 wk	26.28	26.33	26.17	26.27	26.18	0.204	0.7041	0.9967
3 wk	92.40	90.30	94.10	102.66	101.11	1.690	0.0003	0.3860
5 wk	177.52	179.04	191.60	201.29	191.72	1.794	<0.0001	0.0059
Daily body weight gain (g/d)								
1–3 wk	4.72	4.57	4.85	5.46	5.35	0.106	<0.0001	0.3407
3–5 wk	6.08	6.34	6.96	7.05	6.47	0.039	<0.0001	<0.0001
1–5 wk	5.40	5.45	5.91	6.25	5.91	0.057	<0.0001	0.0032
Daily feed intake (g/d)								
1–3 wk	15.18	15.21	13.56	14.21	14.32	0.217	0.0048	0.0199
3–5 wk	20.73	20.22	21.43	20.51	21.75	0.343	0.0690	0.3375
1–5 wk	17.95	17.71	17.49	17.36	18.03	0.258	0.8347	0.1034
Feed conversion ratio (g / g)								
1–3 wk	3.21	3.33	2.80	2.60	2.68	0.078	<0.0001	0.4532
3–5 wk	3.41	3.19	3.08	2.91	3.36	0.054	0.0683	0.0001
1–5 wk	3.32	3.25	2.96	2.78	3.05	0.064	0.0006	0.0079

[1]Standard error means.
[2]Linear and quadratic effects.

Table 3. Carcass traits and relative organs of growing Japanese quail as affected by licorice powder.

	Licorice levels (mg/kg diet)						*P* value[2]	
Items	0	250	500	750	1000	SEM[1]	Linear	Quadratic
Carcass %	75.57	76.89	77.7	72.59	74.57	1.507	0.2423	0.4620
Liver %	2.88	1.95	2.11	2.86	3.19	0.318	0.1795	0.0336
Gizzard %	2.63	2.51	2.24	2.27	2.38	0.151	0.1949	0.2595
Heart %	1.10	1.05	1.06	0.99	0.93	0.137	0.3915	0.8597
Giblets %	6.61	5.51	5.41	6.12	6.50	0.330	0.7311	0.0177
Dressing %	82.18	82.41	83.11	78.70	81.07	1.452	0.2677	0.8911

[1]Standard error means.
[2]Linear and quadratic effects.

Table 4. Liver and kidney functions of growing Japanese quail as affected by licorice powder.

	Licorice levels (mg/kg diet)						*P* value[2]	
Items	0	250	500	750	1000	SEM[1]	Linear	Quadratic
TP (g/dL)[3]	3.24	2.78	3.57	3.72	3.61	0.087	0.0007	0.8486
ALB (g/dL)	2.00	1.98	2.30	1.92	1.93	0.038	0.1549	0.0032
GLOB (g/dL)	1.24	0.80	1.27	1.80	1.69	0.061	<.0001	0.0357
A/G (%)	1.64	2.47	1.86	1.07	1.14	0.072	<.0001	0.0009
AST (IU/L)	175	210	226	160	159	3.077	<.0001	<.0001
ALT (IU/L)	16.49	13.84	13.35	13.96	13.64	0.760	0.0647	0.1006
LDH (IU/L)	155	228	207	120	133	5.122	<.0001	<.0001
Creatinine (mg/dL)	0.36	0.33	0.33	0.36	0.40	0.016	0.0724	0.0243
Urea (mg/dL)	7.11	6.50	6.74	7.31	6.97	0.188	0.4124	0.2549

[1]Standard error means.
[2]Linear and quadratic effects.
[3]TP: total protein; Alb: albumin GLOB: globulin; A/G: albumin/ globulin ratio; AST: aspartate aminotransferase and ALT: alanine aminotransferase. LDH: lactate dehydrogenase.

Table 5. Lipid profile of growing Japanese quail as affected by licorice powder.

	Licorice levels (mg/kg diet)						*P* value[2]	
Items	0	250	500	750	1000	SEM[1]	Linear	Quadratic
TC (mg/dL)[3]	226.70	226.55	174.95	168.80	210.30	4.388	0.0002	<.0001
TG (mg/dL)	293.00	233.50	235.71	182.08	384.00	9.097	0.0012	<.0001
HDL (mg/dL)	47.50	53.02	46.53	57.48	41.13	2.682	0.3740	0.0316
LDL (mg/dL)	120.60	126.84	81.29	74.91	92.38	3.491	<.0001	0.0010
VLDL (mg/dL)	58.60	46.70	47.14	36.42	76.80	1.819	0.0012	<.0001

[1]Standard error means.
[2]Linear and quadratic effects.
[3]TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein. VLDL: very-low-density lipoprotein.

within the normal ranges. The different diets produced no significant differences in the levels of ALB, AST, ALT, creatinine, urea, HDL, and VLDL. The group fed a 750-mg licorice diet had higher blood contents of TP and GLOB and lower contents of A/G%, LDH, TC, TG, and LDL; while the group fed the 250-mg licorice diet showed the poorest state of these parameters, although values remained within normal limits.

Antioxidant and Immune Indices

The levels of antioxidants (SOD, MDA, and TAC) and immunological indices (IgG, IgM, and lysozyme) of Japanese quail fed the experimental diets are shown in Table 6. Levels of plasma lysozyme activity were similar ($P > 0.05$) in all groups. *P*-values of the quadratic model showed a significant modulation in SOD, MDA, TAC,

Table 6. Antioxidants and immunity of growing Japanese quail as affected by licorice powder.

	Licorice levels (mg/kg diet)						*P* value[2]	
Items	0	250	500	750	1000	SEM[1]	Linear	Quadratic
SOD (U/mL)[3]	0.09	0.36	0.24	0.25	0.11	0.019	0.2060	<0.0001
MDA (nmol/mL)	0.46	0.14	0.21	0.27	0.49	0.022	0.0413	<0.0001
TAC	0.10	0.21	0.21	0.16	0.12	0.037	0.7993	0.0394
IgG (mg/dL)	0.70	1.27	0.75	1.85	0.70	0.110	0.1663	0.0030
IgM (mg/dL)	0.44	0.48	1.36	0.95	0.46	0.043	0.0427	<0.0001
Lysozyme (U/mL)	0.11	0.28	0.22	0.21	0.19	0.039	0.5259	0.0525

[1]Standard error means.
[2]Linear and quadratic effects.
[3]SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; IgG and M: immunoglobulin G.

Table 7. Cecal microbiota of growing Japanese quail as affected by licorice powder.

Items	Licorice levels (mg/kg diet)					SEM[1]	*P* value[2]	
	0	250	500	750	1000		Linear	Quadratic
Microbiological count (Log CFU/g)[3]								
TBC	5.97	5.06	4.31	5.04	4.96	0.083	<.0001	<.0001
TYMC	5.08	4.15	4.87	4.85	4.64	0.125	0.6676	0.1820
Coliform	5.90	4.97	4.98	4.24	4.80	0.069	<.0001	<.0001
E. coli	5.85	4.95	4.22	4.97	4.75	0.062	<.0001	<.0001
Lactic acid bacteria	5.90	4.38	5.00	6.11	5.06	0.093	0.8860	0.0052
Enterococcus	5.13	5.06	4.88	4.91	4.87	0.142	0.1817	0.1817
Salmonella	6.25	5.03	4.88	3.72	3.92	0.124	<.0001	0.0031

[1]Standard error means.
[2]Linear and quadratic effects.
[3]TBC: total bacterial count; TYMC: total yeast and molds count.

IgG, and IgM. Meanwhile, *P*-values of the linear model for the previous parameters show variation ($P < 0.05$) only in MDA and IgM. The control group (0 mg licorice/kg diet) produced the lowest levels of SOD, TAC, IgG, and IgM; and licorice supplementation produced higher levels of these parameters.

Bacteriology

Table 7 shows the results of microbial analysis of the cecal contents of Japanese quail after 5 wk of feeding on various levels of licorice powder supplementation. The number of yeast and molds, lactic acid bacteria, and *Enterococcus* were not affected by various levels of licorice supplementation. In contrast, licorice supplementation resulted in a marked decrease in the number of total bacteria, coliforms, *E. coli,* and *Salmonella.* The control group fed a licorice-free diet produced the highest abundance levels of the microorganisms tested.

DISCUSSION

Intensive production of animals requires the use of specific techniques to maintain performance levels. Dietary modulation, including the use of feed additives such as medicinal plant products, immunostimulants, antioxidants, exogenous enzymes, acidifiers, probiotics, prebiotics, and synbiotics, is one of the current approaches (Gong et al., 2013; Mohamed et al., 2019; Puvača et al., 2019; El Basuini et al., 2020). The use of antibiotics in dietary supplements to promote growth has been banned in various countries due to antibiotic residues accumulating in tissues, transfer of antibiotic resistance to pathogens, and other environmental risks (Hojati et al., 2014). Thus, the use of natural substances as antibiotic substitutes, growth promoters, and immunostimulants has become more common in the poultry industry (Murugesan et al., 2015; Calik et al., 2019). Medicinal plants, long considered a cornerstone of medicine since ancient times (Su et al., 2016; Surai et al., 2019), have been used as promoters of growth and immunity, and as a source of antioxidants (Aroche et al., 2018; Stratev et al., 2018). Licorice, widely known as a flavoring agent in food and beverages, has been used medicinally since ancient times (Quirós-Sauceda et al., 2016; Tamer et al., 2019; Alagawany et al., 2019b).

Poultry growth and laying performance are known to be enhanced by feed additives and growth promoters (Dhama et al., 2015, 2018). The growth period of quail life is an important phase in realizing the long-term great performance (Elnesr et al., 2019). In the present study, Japanese quail fed licorice at 750 mg/kg for 5 wk had significantly higher final BW, DBW, and lower FCR compared to the control group. The lower FCR may partially explain the faster growth of groups fed on licorice diets. Furthermore, enhanced growth and lower FCR may be linked to improved digestion and appetite in broilers fed diets supplemented with licorice (2.5 g/kg), as previously reported by Alagawany et al. (2019b). These authors also reported the positive effect of licorice supplementation on internal organ development, which may be one of the reasons behind enhanced poultry growth (2019b). In a related study, Myandoab and Mansoub (2012) reported that incorporating 200 ppm of licorice root extract containing 1% probiotic supplement in the diet of Japanese quail increased the amounts of daily FI and BW gain. In 42-day-old broiler chickens, Jagadeeswaran and Selvasubramanian (2014) reported that a diet with 1% licorice resulted in higher BW and lower FCR compared to the control group. Similarly, up to 0.5% licorice supplementation during the pullet growing period resulted in enhanced performance in laying hens (Alagawany et al., 2019a).

In contrast, supplementing a broiler diet with 5 g licorice/kg xx does not affect ($P > 0.05$) BW, FI, FCR, % livability, and production index (Hosseini et al., 2014). Similarly, dietary licorice levels of 0.5%, 1.0%, 1.5%, and 2.0% had no significant effect on broiler FI (Dogan et al., 2018). Moreover, Sedghi et al. (2011) reported that 0.5, 1, and 2 g licorice/kg broiler diet had no impact on BW, FI, and FCR compared to the control group.

An alternative to feed modification is the administration of licorice in poultry drinking water. Using this approach, 0.4% licorice in drinking water increased ($P < 0.05$) broiler FI at 21 and 42 d, although this did not result in BW differences ($P > 0.05$) at different ages (Al-Daraji, 2012). However, licorice water supplementation at 60 μg/mL resulted in higher values of final BW and BW gain, better FCR, and lower mortality compared to

the control (Ocampo et al., 2016). On the other hand, Moradi et al. (2014) reported that licorice water supplementation at levels of 0.1, 0.2, and 0.3 mg/L had no effect ($P > 0.05$) on the BW, FI, and FCR of broiler chicks compared to a control group.

Blood indices are generally reliable indicators of overall health, including reactions to internal and external stimuli and stressors (Amaral et al., 2017). None of the blood indices measured indicated abnormal values. Also, licorice supplements did not significantly affect ALT and AST (liver metabolic enzymes) levels, suggesting that licorice produced no toxicity or impairment in the animals. Similarly, indicators of kidney function (creatinine and urea) were normal after licorice supplementation. Licorice supplementation at 750 mg/kg resulted in higher contents of TP, GLOB, and lower contents of A/G%, LDH, TC, TG, and LDL compared to control; these results reflect a better blood status compared to control. The elevated TP level may also indicate a healthy quail status. For example, 0.5% licorice extracts in broiler diets increases serum globulin concentrations that enhance the bird's humoral immune status (Rezaei et al., 2014). Moreover, dietary licorice levels of 0.5, 1, and 2 g/kg have no effect on the percentages of monocytes, lymphocytes (L), and heterophils (H); neither do they affect the heterophil to lymphocyte (H/L) ratio, or the proliferation of red blood cells, although they reduce the levels of cholesterol and LDL in the blood (Sedghi et al., 2011). Furthermore, Sharifi et al. (2013) demonstrated that the addition of licorice in broiler diets (2 mg/kg diet) reduced serum levels of triglycerides, cholesterol, and LDL, while increasing that of HDL. When supplemented via drinking water, licorice also significantly impacts blood health. For example, administering licorice (0.1, 0.2, or 0.3 g/L) through this means significantly reduces ($P < 0.05$) serum levels of glucose, LDL cholesterol, and TC in broiler chickens (Moradi et al., 2017). It should also be noted that licorice supplementation (0.1, 0.2, and 0.3 mg/L) in drinking water does not impact the percentages of heterophils and lymphocytes, or the H/L ratio (Moradi et al., 2014).

The immunity and well-being of poultry are positively correlated to the animal's oxidative status (Akbarian et al., 2016). Oxidative stress is the result of an imbalance between the production and disposal of reactive oxygen species (**ROS**) (Lee et al., 2019). The removal of excess ROS depends on the action of several enzymes such as SOD and CAT (Aruoma, 1998). Results of the present study suggest that the antioxidant status of Japanese quail is enhanced by the antioxidant capacity of licorice components, as previously reported (Rahal et al., 2014; Vlaisavljević et al., 2018; Yatoo et al., 2018). Japanese quail fed diets with licorice showed significantly increased levels of SOD, MDA, TAC, IgG, and IgM; and the lowest values of these parameters were observed in the control group. The improvements in the immune and oxidation statuses of quail may be linked to the bioactive components of licorice (e.g., flavonoids, saponins, sugars, coumarins, amino acids, starch, tannins, phytosterols, choline, and vitamins) (Kataria et al., 2013; Karahan et al., 2016; Pastorino et al., 2018). Thus, dietary supplementation of licorice (0.1%) can boost the humoral immunity of broilers by generating antibody titers against nonspecific and specific antigens (Jagadeeswaran et al., 2014). Furthermore, the addition of licorice (50 μg/mL) to laying hens' diet provides beneficial impacts to the animal's cellular immunity by increasing the levels of mononuclear and granulocytes cells (Dorhoi et al., 2006). Rezaei et al. (2014) also reported that adding licorice to broiler diets enhanced the weight of immune organs, such as the spleen or bursa. Meanwhile, Moradi et al. (2014) reported that the addition of licorice to broiler drinking water had no impact on the weights of the liver and lymphoid organ, and on antibody titers against Newcastle disease and avian influenza viruses.

Our microbial analysis of the cecal microbiota of Japanese quail shows that licorice supplementation resulted in marked reductions in the number of the pathogenic bacteria compared to those in the control. This result is consistent with those of previous reports showing the antimicrobial effect of licorice (Karahan et al., 2016; Alagawany et al., 2019b). The antimicrobial analysis indicated that the extract of licorice root was effective against Gram-negative and Gram-positive bacteria (Karahan et al., 2016). Our findings are in agreement with previous studies that extracts of licorice have antioxidant and antibacterial activity. Chopra et al. (2013) reported that methanolic extracts of *Glycyrrhiza glabra* roots played an important role as antibacterial agents. Also, Sultana et al. (2010) stated that licorice extracts exhibited antibacterial activity against Gram-negative (*P. aeruginosa, E. coli,* and *Salmonella paratyphi*) and Gram-positive (*Bacillus megaterium, S. aureus* and *B. subtilis*) bacteria. On the other hand, a relative increase in *E. coli* and *coliform* was observed with *Glycyrrhiza glabra* roots powder (Al-Bachir and Al-Adawi, 2014).

CONCLUSIONS

The present study suggests the use of novel feed additives such as licorice in poultry production. Results of the study showed that the inclusion of licorice at levels of 750 and 1000 mg/kg into the diet of Japanese quail enhances the animal's performance, immunity, and antioxidant capacity and declines the intestinal pathogens.

DISCLOSURES

The authors declare no conflicts of interest.

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