

Original research article

UDC 636.5.084:616.15:[582.739

DOI:<https://doi.org/10.46784/e-avm.v18i1.449>

THE INFLUENCE OF SUPPLEMENTATION OF LICORICE POWDER (GLYCYRRHIZA GLABRA) TO BROILER DIETS ON GROWTH PERFORMANCE, HEMATOLOGICAL PARAMETERS AND SERUM LIPIDS

Majdi Abdelfaraj Kairalla¹, Mohamed Idris Alshelmani^{2*}

¹Department of Animal Production, Faculty of
Agriculture, University of Sebha, Libya

²Department of Animal Production, Faculty of
Agriculture, University of Benghazi, Libya

Abstract

This study was designed to investigate the impact of dietary supplementation of licorice on growth performance, hematological, and biochemical parameters of broiler chickens. A local hatchery provided 192 one-day-old broiler chicks (Cobb500), which were randomly allocated into four dietary treatments. Each treatment was divided into four replicates, with each replicate containing 12 chicks housed in ground pens. The diet was supplemented with licorice powder (*Glycyrrhiza galabra*) at 0.0%, 0.25%, 0.5%, and 0.75%. The birds were provided with unrestricted access to food and water throughout the experiment. On day 42nd, blood samples were collected randomly from one bird in each replicate of dietary treatments. The results showed that broilers fed a diet supplemented with licorice powder experienced a significant improvement ($p < 0.05$) in body weight gain and feed conversion ratio compared to the control group. Nevertheless, the results also revealed that supplementation of licorice powder considerably decreased ($p < 0.05$) glucose, total cholesterol, triglyceride, low density lipoprotein and elevated high density lipoprotein, total protein, globulin and lowered albumin/globulin ratio, aspartate transaminase and alanine transaminase, in comparison with the control group. In conclusion, the proportion of 0.75% licorice powder in the diet resulted in an improvement in growth performance and health status.

^{1*} Corresponding Author: mohammed.alshelmani@uob.edu.ly

Key words: Chicken, licorice, medicinal plant, hematology, serum lipid profile

UTICAJ SUPLEMENTACIJE PRAHA SLADIĆA (GLYCYRRHIZA GLABRA) U ISHRANI BROJLERA NA RAST, HEMATOLOŠKE PARAMETRE I LIPIDE U KRVI

Majdi Abdelfaraj Kairalla¹, Mohamed Idris Alshelmani^{2*}

¹ Katedra za animalnu proizvodnju, Poljoprivredni fakultet, Univerzitet Sebha, Libija

² Katedra za animalnu proizvodnju, Poljoprivredni fakultet, Univerzitet Benghazi, Libija

Kratak sadržaj

Ova studija se bavi istraživanjem uticaja supletmentacije sladićem (*Glycyrrhiza glabra*) u ishrani brojlera na njihov rast, hematološke i biohemijske parametre. Lokalna inkubatorska stanica je obezbedila 192 jednodnevna brojlerska pileta (hybrid Cobb500), koji su nasumično raspoređeni u grupe koje su imale četiri režima ishrane. Svaki režim je bio podeljen na četiri ponavljanja, pri čemu je u svakom ponavljanju bilo 12 pilića smeštenih u boksove na podu. U hranu je dodat prah sladića u količini od 0.0%, 0.25%, 0.5%, i 0.75%. Kokoške su imale neograničen pristup vodi i hrani tokom eksperimenta. Uzorci su uzeti 42. dana nasumično od po jedne kokoške, svake iz različitog režima ishrane. Rezultati su pokazali da su brojleri koji su hranjeni suplementiranom hranom značajno napredovali ($p < 0.05$) u dobijanju telesne mase i imali bolju stopu konverzije hrane u poređenju sa kontrolnom grupom. Pored toga, rezultati su takođe pokazali da je suplementacija prahom sladića znatno umanjila ($p < 0.05$) glukozu, ukupni holesterol lipoprotein niske gustine, ukupni protein, globulin i smanjila albuminsko/globulinski odnos, aktivnosti aspartat transaminaze i alanin transaminaze u poređenju sa kontrolnom grupom. Može se zaključiti da je dodavanje 0.75% praha sladića u ishranu brojlera dovelo do poboljšanja rasta pilića i njihovog opšteg zdravstvenog stanja.

Ključne reči: pilići, sladić, lekovito bilje, hematologija, serumski lipidni profil

INTRODUCTION

The advancements in poultry production over the recent years, particularly in broiler production, can be largely attributed to the modifications resulting from a range of research investigations conducted in different areas such as genetics, facility adaptations, automation of equipment, health, and nutrition.

The use of natural alternatives with similar beneficial effects like synthetic antibiotics has been intensified. The search for alternatives to antibiotics has led to the development of safe, naturally derived substances whose effects are influenced by the intestinal microflora. These alternatives include medicinal plants (Kairalla et al., 2022a; Kairalla et al., 2022b; Kairalla et al., 2023) and the supplementation of exogenous enzymes (Zamani et al., 2016; Zamani et al., 2017). Recently, various medicinal plants have been utilized to in order to enhance the growth, health, and physiological aspects of poultry (Grashorn, 2010). Addition of certain antibiotics to the diet has demonstrated an improvement in nutrient availability. However, in recent years, their routine usage has been banned in many countries (Hafez and Attia, 2020). One possible alternative to antibiotics is plant-derived substances such as essential oils (Abdulla et al., 2019), flavonoids, polyphenols, alkaloids, and tannins. The increase in the use of medicinal plants in poultry feed was a result of their beneficial properties, such as anti-inflammatory, antiseptic, soothing, antibactericidal, antifungicidal, antiviral, antioxidant capacity, growth-promoting effects, dietary palatability, intestinal function, immunostimulatory effects, stimulation of secretion of digestive enzymes, and nutrient absorption (Kairalla et al., 2022a; Kairalla et al., 2022b; Kairalla et al., 2023). Among these medicinal plants, licorice (*Glycyrrhiza glabra*) has been recognized for its numerous health-promoting properties. Moreover, licorice plays an important role due to its diverse antioxidant, anti-inflammatory, antidiabetic, anti-depressant, anti-obesity, anti-allergic, anti-carcinogenic, antiulcer, and liver protective properties. The chemical constituents of licorice possess potential therapeutic properties that may help prevent and manage a wide range of diseases and emerging health conditions in humans (Noreen et al., 2021). Therefore, the objective of the this study was to investigate the impact of dietary supplementation of licorice on growth performance, blood hematology, and blood lipids of broiler chickens.

MATERIAL AND METHODS

Ethical approval

The research involving animal use was conducted in accordance with all applicable national regulations and institutional policies, as approved by the

Sebha University Ethics Committee, 3751-SUEC, 2024. The current study was conducted at the Poultry Production Unit, Department of Animal Production.

Birds and experimental diets

In the present study, the total of 192 one-day-old broiler chicks (Cobb 500) were obtained from a local hatchery. The chicks were weighed individually and subsequently distributed into four equal groups, with 48 birds in each treatment. These groups were further arranged into four replicates, with 12 birds in each. The chicks were housed in ground pens (1.5 × 1.5 meters) and provided with wood shavings as bedding material. The birds were fed starter and finisher diets from 1 to 21 and 22 to 42 days of age, respectively (Table 1.). Food and water were *ad libitum* during the feeding trial. The birds were weighed individually, and the quantity of food consumed was recorded every week for all replicates. Feed conversion ratio (FCR) was calculated based on body weight gain (BWG). All chicks were vaccinated against infectious bronchitis disease and Newcastle disease, and Gumboro (infectious bursal) disease at 7 and 21 days, respectively. The diets were formulated based on (NRC, 1994). The experimental diets were supplemented with licorice powder as follows: 0%, 0.25%, 0.5%, and 0.75%.

Table 1. Ingredients and nutrient content of broiler meals (% as fed)

Ingredients (kg)	Starter 1 to 21 Day	Finisher 22 to 42 day
Yellow Corn	50.30	57.90
Soybean Meal 44%	31.10	28.15
Corn Gluten Meal	10.00	6.19
Di-calcium phosphate	2.20	2.05
Lime stone	1.10	1.00
Salt (NaCl)	0.25	0.25
Veg. oil	4.55	4.00
L-lysine	0.15	0.15
DL-Methionine	0.05	0.01
Vitamin and Mineral Premix*	0.30	0.30
Total	100	100

Ingredients (kg)	Starter 1 to 21 Day	Finisher 22 to 42 day
Calculated analysis		
Crude protein (%)	22.12	18.07
M.E (kcal/ kg)	2,980	3,158
C/P	134.71	175.76
Fat (%)	3.80	6.29
Crude fiber (%)	2.40	2.58
Calcium (%)	1.04	0.98
Available phosphorus (%)	0.44	0.38
Methionine (%)	0.53	0.42
Lysine (%)	1.15	0.99

* Each kilogram of Premix contained: Vit A 3350000 IU; Vit D3 760 000 IU; Vit E 6700 IU; Vit K3 335 mg; Vit B1 334 mg; Vit B2 1670 mg; Vit B6 500 mg; Vit B12 3.4 mg; Niacin 10000 mg; Ca.D. Pantothenate 3334 mg; Biotin 16.7 mg; Folic acid 334 mg; Trace minerals: Iron 13350 mg; Copper 3335 mg; Zinc 16700 mg; Manganese 25000 mg; Iodine 500 mg; Cobalt 84 mg; Selenium 100 mg; Additives: Ethoxyquine 600 mg; and Carrier (CaCO₃) up to 1 kg.

Blood parameters

On day 42, one bird was randomly chosen from each replicate in each dietary treatment. Blood samples were obtained as described by (Kairalla et al., 2023). These tubes were employed for the determination of packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), and hemoglobin (HB). Subsequently, all samples were subjected to centrifugation at 3000 g for 15 minutes. The resulting plasma was then transferred into Eppendorf tubes and kept at -20 °C for further analysis. The total protein, albumin, total cholesterol (Khatun et al., 2020), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride, and glucose, as well as the enzymatic activities of alanine amino transferase (ALT) and aspartate amino transferase (AST) were measured by colorimetric methods using commercial kits (Kairalla et al., 2023).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA). The collected data was subsequently analyzed using the general linear model procedure of the statistical analysis system (SAS 2003). In order to contrast the means of treatment at ($p < 0.05$), Tukey's test was performed.

RESULTS

Growth performance

Table 2 presents the impact of supplementation of licorice powder on the production efficiency of broiler chickens. The outcomes indicated that the inclusion of 0.5% and 0.75% licorice powder in the diets significantly enhanced ($p < 0.0001$) BWG, and FCR compared to the birds fed 0% or 0.25% licorice powder in their diets. Feed intake did not show any considerable differences among the dietary treatments or the control group at 0 - 21 days of age. Similarly, there were no significant variations in feed intake, BWG, or FCR among the dietary treatments and control group during the 22-42-day period. However, at the end of the experiment, broiler chickens that were fed diets supplemented with 0.5% and 0.75% licorice powder exhibited significantly improved BWG and FCR ($p < 0.0001$) in comparison with the control group or those fed 0.25% licorice powder.

Table 2. The effect of dietary supplementation of licorice powder on broiler performance

Traits	Licorice powder Level (%)				SEM ¹	p-value
	0	0.25	0.5	0.75		
<i>0 - 21 days</i>						
Body weight gain (g/bird)	697.04 ^c	708.85 ^c	830.79 ^b	873.25 ^a	15.221	<0.0001
Feed intake (g/bird)	988.25	990.79	972.21	990.81	20.581	0.8341
FCR	1.41 ^a	1.40 ^a	1.17 ^b	1.13 ^b	0.0347	0.0001
<i>22 - 42 days</i>						
Body weight gain (g/bird)	1706.15	1704.11	1690.31	1715.22	17.192	0.725
Feed intake (g/bird)	3073.26	3079.12	3026.05	3096.79	28.729	0.377

Traits	Licorice powder Level (%)				SEM ¹	p-value
	0	0.25	0.5	0.75		
FCR	1.80	1.80	1.79	1.80	0.04182	0.754
Overall (0 - 42 days)						
Body weight gain (g/bird)	2403.04 ^c	2412.96 ^c	2521.10 ^b	2588.47 ^a	15.206	<0.0001
Feed intake (g/bird)	4061.50	4069.91	3998.26	4087.60	39.937	0.3900
FCR	1.69 ^a	1.68 ^{a,b}	1.58 ^{a,b}	1.57 ^b	0.02319	0.0149
Overall Live Body weight	2445.12 ^c	2455.59 ^c	2563.35 ^b	2631.80 ^a	15.212	<0.0001

^{a, b, c} Means within the same row with different superscript letters are significantly different at ($p < 0.05$).

¹SEM: Pooled standard error.

Hematological parameters

Hematological parameters analyses are presented in Table 3. Feeding a diet supplemented with licorice powder significantly reduced glucose levels ($p < 0.0001$) in comparison with control group. Birds consumed a diet incorporated with licorice powder did not show a considerable elevated levels of ($p > 0.05$) in WBC, RBC, hemoglobin, PCV and lymphocyte.

Table 3. Effect of licorice powder supplementation on hematological parameters

Parameters	Licorice powder Level (%)				SEM ¹	P-value
	0	0.25	0.5	0.75		
WBC's, 10 ⁶ /mm ³	20.03	20.62	20.24	20.26	0.4971	0.882
RBC's, 10 ⁶ /mm ³	2.92	2.94	2.90	2.84	0.0532	0.600
Hemoglobin, g/dL	8.29	8.32	8.30	8.30	0.0985	0.997
PCV, %	34.49	34.85	34.22	34.53	0.7409	0.945
Lymphocytes, %	54.10	53.98	54.76	54.42	1.1084	0.956
Glucose, mg/dL	116.88 ^a	93.30 ^b	95.33 ^b	94.65 ^b	1.4129	<0.0001

^{a, b} Means within the same row with different superscript letters are significantly different at ($p < 0.05$).

¹SEM: Pooled standard error.

Blood biochemistry

Table 4 indicates the impact of licorice powder supplementation on blood biochemistry. It is obvious that significant differences were found due to the experimental diets of growing birds on plasma total protein, globulin, A/G ratio, triglycerides and total cholesterol with its fraction (LDL and HDL) and liver functions (ALT and AST). In contrast to the control group, feeding licorice powder at 0.5% and 0.75% resulted in a substantial ($p < 0.05$) decline in ALT, AST, total cholesterol, triglycerides, albumin, and LDL. Broilers given a diet with licorice had substantially higher HDL levels ($p < 0.05$) than the control group. The birds in the group that were fed meals enriched with 0.5% or 0.75% showed a substantial elevated levels of total protein and globulin in comparison with the control group.

Table 4. Effect of licorice powder supplementation on serum lipid of broiler chickens

Parameters	Licorice powder Level (%)				SEM ¹	P-value
	0	0.25	0.5	0.75		
Total cholesterol (mg/dL)	119.74 ^a	121.24 ^a	111.01 ^b	110.24 ^b	1.263	<0.0001
Triglycerides (mg/dL)	66.16 ^a	65.11 ^a	53.12 ^b	52.67 ^b	1.461	<0.0001
LDL-cholesterol (mg/dL)	80.09 ^a	67.56 ^b	61.97 ^c	61.20 ^c	1.150	<0.0001
HDL-cholesterol (mg/dL)	37.40 ^b	36.79 ^b	46.30 ^a	47.84 ^a	1.295	<0.0001
Total protein (g/L)	3.03 ^c	3.09 ^c	3.44 ^b	3.86 ^a	0.062	<0.0001
Albumin(g/L)	1.18 ^{ab}	1.14 ^b	1.22 ^a	1.21 ^a	0.021	0.0106
Globulin	1.85 ^c	1.95 ^{bc}	2.22 ^b	2.65 ^a	0.070	<0.0001
A/G ratio	0.64 ^a	0.58 ^a	0.55 ^a	0.46 ^b	0.031	0.0004
ALT (IU/L)	43.36 ^a	41.93 ^a	35.42 ^b	35.85 ^b	1.141	0.0004
AST(IU/L)	54.44 ^a	55.08 ^a	46.26 ^b	46.50 ^b	0.87	<0.0001

^{a, b, c} Means within the same row with different superscript letters are significantly different at ($p < 0.05$).

¹SEM: Pooled standard error.

DISCUSSION

The observed increase in BWG and FCR can be attributed to the presence of licorice extracts, which are believed to enhance digestibility, stimulate the secretion of digestive enzymes, prevent oxidation of tissue, and alter gut microflora. Consequently, these improvements contribute to enhanced growth performance in the birds. Furthermore, the positive effects on performance can also be attributed to the bioactive components present in licorice, such as flavonoids and glycyrrhizin, which possess pharmacological and medicinal properties, as well as immunogenic and antioxidant activities. The synergistic effects of these components may improve growth performance and blood biochemistry in poultry, while also providing potential support to respiratory, digestive, and immune tissues (Alagawany et al., 2019). These findings are in line with previous studies conducted by Ocampo et al. (2016); Attia et al. (2017); Dogan et al. (2018); Beski et al. (2019); Rashidi et al. (2019); Alagawany et al. (2019); Ibrahim et al. (2020); Abo-Samaha et al. (2022), who similarly reported improvements in body weight of broiler chickens supplemented with licorice in comparison with the control.

Ocampo et al. (2016) stated that supplementing licorice in drinking water can play a crucial role in enhancing broiler growth. It also can play vital role in promoting the productive performance of broiler chickens under heat stress (Lashin et al., 2017). These findings may be due to the positive impact of licorice extracts which could improve feed palatability (Salary et al., 2014). Dhama et al. (2015) claimed that herbs have demonstrated powerful immunomodulatory and antiviral effects, and licorice extracts have a favorable influence on chicken immune systems, and therefore can be utilized to increase their immunological response and productivity. In contrast, several authors reported that no significant variation in body weight was found among birds that were given licorice through their feed (Sedghi et al., 2010; Hosseini et al., 2014; Dogan et al., 2018) or by drinking water (Moradi et al., 2014; Naser et al., 2017). Likewise, Moradi et al. (2014) found that varied amounts of licorice extract in drinking water - 0.1, 0.2, and 0.3% - had no impact on feed consumption in broiler chickens. The outcomes are in consistent with Salary et al. (2014); Ibrahim et al. (2020); Abo-Samaha et al. (2022), who reported that drinking water supplementation with licorice extracts enhanced feed intake in broiler chickens.

Regarding the FCR, the enhancement can be attributed to the favorable impact of supplementation with licorice extracts, which may enhance digestibility and regulate the populations of gut microbes. Consequently, this leads to an

increase in feed intake, FCR, and the growth performance of the avian species (Salary et al., 2014). These findings are consistent with those of Ibrahim et al. (2020) and Abo-Samaha et al. (2022), who reported that the inclusion of licorice influenced the feed conversion ratio (FCR). Additionally, Salary et al. (2014) documented that incorporating licorice extracts in the drinking water of broiler chickens led to a significant improvement ($p < 0.05$) in FCR during the starter phase. Conversely, some researchers have reported contradictory results, such as Khamisabadi et al. (2015) and Beski et al. (2019) carcass cuts and intestinal histomorphology of broiler chickens</title><secondary-title>Iraqi Journal of Agricultural Sciences</secondary-title></titles><periodical><full-title>Iraqi Journal of Agricultural Sciences</full-title></periodical><pages>842-849</pages><volume>50</volume><number>3</number><dates><year>2019</year></dates><isbn>0075-0530</isbn><urls></urls><electronic-resource-num>https://doi.org/10.36103/ijas.v50i3.701</electronic-resource-num></record></Cite></EndNote>, who stated that the addition of licorice (*Glycyrrhiza glabra*) to broiler chicks had no impact on FCR.

Meanwhile, these values fall within the normal range for farm animals. The results were in line with the observations of Moradi et al. (2014); Naser et al. (2017), who argued that total glucose levels were lower in birds fed a diet supplemented with licorice powder compared to the control group. Sedghi et al. (2010) suggested that the prebiotic incorporated with licorice in the feed had no considerable influence on the percentages of lymphocytes, heterophils, and monocytes, as well as the heterophil to lymphocyte ratio and red blood cell proliferation in broiler chickens. Additionally, the administration of 0.5, 1, and 2 g/kg of licorice in the diet did not affect the levels of monocytes, lymphocytes, and heterophils in the bloodstream, nor did it influence the heterophil to lymphocyte ratio or red blood cell proliferation (Sedghi et al., 2010). In addition, Abo-Samaha et al. (2022) mentioned that licorice contains active compounds which have antioxidant and anti-inflammatory properties. As a result, it has a beneficial impact on their growth performance. In contrast, Al-Daraji (2012) showed that high amounts of licorice (150 to 450 mg/L in drinking water) boosted broiler blood glucose levels. Blood indices, which include responsiveness to internal and external stimuli and stressors, are generally reliable indicators of overall health. None of the blood markers tested was abnormal. Furthermore, licorice supplements caused no toxicity or damage to the animals.

The outcomes indicated that the consumption of diets supplemented with licorice powder (0.75%) led to a noteworthy ($p < 0.05$) decrease in A/G ratio against the control group. These findings indicate an improved blood condi-

tion for the groups of birds fed diets enriched with licorice powder.

The findings align with the research by Sharifi et al. (2013), who demonstrated that the addition of licorice root (2 mg/kg diet) to broiler diets decreased blood components such as triglycerides, cholesterol, and LDL, while increasing HDL levels. This relation may be attributed to licorice's ability to suppress lipid peroxidation, lipoxygenase, and cyclooxygenase enzyme activity, as well as its alleviation of LDL oxidation. Higher concentration of total protein may also indicate the good health of the broiler chicks, which enhances their humoral immunological condition (Rezaei et al., 2014). Broiler chicks that consumed licorice in drinking water (0.1, 0.2, or 0.3 g/L) exhibited lower levels of LDL cholesterol and total cholesterol (Naser et al., 2017). Furthermore, the current results consistent with those of Salary et al. (2014), who reported a significant decrease in AST and ALT levels following the inclusion of licorice in broiler feed. The decline in the concentration of liver enzymes may be attributed to the mechanism of glycyrrhizin, which inhibits the progression of liver damage.

As a result of its high portions of flavonoids and ascorbate, dietary treatments with licorice powder enhanced HDL concentration and HDL/LDL ratio in blood (Naser et al., 2017). Furthermore, the addition of 0.4% licorice powder to broiler drinking water increased plasma HDL levels while simultaneously decreasing ALT levels (Salary et al., 2014). Pastorino et al. (2018) suggested that licorice had hepatoprotective qualities in terms of liver function enzymes (ALT and AST). The groups of birds fed licorice powder had the lowest levels of ALT and AST. The bioactive components of licorice may be responsible for improvements in the immunological and oxidative status of broiler chickens. As a result, the presence of saponins and phytosteroids in licorice powder may play a key role in reducing cholesterol levels and enhancing hepatic bile salt production in animals fed diets containing licorice powder (Shahryar et al., 2018).

CONCLUSION

In conclusion, supplementing the diet with 0.75% licorice powder is recommended for improving both the production efficiency and overall health status of broiler chickens.

Author's Contribution:

MAK contributed to the study design, conducted the feeding trial and sampling, and drafted the manuscript. MIA performed the statistical analysis and participated in writing and editing the manuscript. All authors have read and approved the final version of the manuscript.

Competing interest

The authors declare no conflict of interest related to the present study.

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Received: 16.03.2025.

Accepted: 18.04.2025.