

***Original Article***

# **Effect of Licorice Essential Oil (*Glycyrrhizaglabrabra*) on Performance and Some Biochemical Parameters of Broiler Chickens**

**Dheyauldeen Salahdin, O<sup>1</sup>\*, Othman, H<sup>2</sup>, Hafsan, H<sup>3</sup>, Mohammed, F<sup>4</sup>, Ahmed Hamza, T<sup>5</sup>, Kadhim, M. M<sup>6</sup>, Aravindhana, S<sup>7</sup>, Prakaash, A. S<sup>8</sup>, Fakri Mustafa, Y<sup>9</sup>**

1. Medical Laboratory Techniques Department, Al-Maarif University College, Anbar-Ramadi, Iraq
2. INTI International University, Nilai, Malaysia
3. Biology Department, Faculty of Science and Technology, Universitas Islam Negeri Alauddin Makassar, Sultan Alauddin Street, Gowa 92118, Indonesia
4. Al-Manara College for Medical Sciences, Maysan, Iraq
5. Medical Laboratory Techniques Department, Al-Mustaqbal University College, Babylon, Iraq
6. Department of Dentistry, Kut University College, Kut, Wasit, 52001, Iraq
7. Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India
8. Department of Mathematics, Panimalar Institute of Technology Poonamallee Chennai, Chennai Tamilnadu, India
9. Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul-41001, Iraq

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Corresponding Author: jawadabed78@yahoo.com

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## **Abstract**

Due to its beneficial components, such as glycyrrhizin, licorice is regarded a medicinal and fragrant plant. This research was designed to investigate the efficacy of licorice essential oil as an alternative to chemical antibiotics on broiler production, carcass features, cellular and humoral safety, and numerous biochemical variables in broiler blood serum. A total of 160 day-old broiler chicks were assigned to four treatment groups using a totally randomized approach. Each treatment consisted of 4 replicates, with 10 chicks in each replication. The experimental treatments included a control group, a group receiving an elemental diet containing 0.1% licorice essential oil, a group receiving an elemental diet containing 0.2% licorice essential oil, and a group receiving an elemental diet containing 0.3% licorice essential oil. Broilers had ad libitum access to feed and water in accordance with a three-phase feeding schedule consisting of a starter, grower, and finisher diet. There was no statistically significant difference ( $P>0.05$ ) in body weight, feed intake, or feed conversion ratio between birds given the control or essential oil licorice at various stages of the experiment. However, birds receiving 0.1% licorice essential oil had a lower gallbladder relative weight and 0.3% licorice essential oil had less abdominal fat than the control group ( $P<0.05$ ). Blood glucose, cholesterol, and LDL concentrations all fell considerably in licorice essential oil-treated birds relative to controls ( $P<0.05$ ). The cellular immune response of birds fed licorice-containing diets did not differ from that of control birds ( $P>0.05$ ), however there was a significant difference in the humoral immune response at 0.1% licorice essential oil compared to the control group ( $P<0.05$ ). In overall, the results of this experiment demonstrated that incorporating licorice essential oil into a bird's diet improves its health and safety.

**Keywords:** Licorice Essential Oil, Performance, Carcass, Humoral immune, Broiler

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## 1. Introduction

Therapeutic usage of medicinal plants has a 5000 year history, dating back to the Sumerians (1). Medicinal plants have played a key role in sustaining human health and enhancing the quality of human existence for millennia (2). The essential oils and extracts of these plants, which contain natural chemicals with therapeutic qualities, have been utilized to treat numerous disorders since antiquity (3, 4). These chemicals altered societies' medical systems. The improper use of antibiotics in the feed of cattle and poultry has made it harder to treat infections and diseases (5). Therefore, the use of antibiotics is restricted in the European Union<sup>6</sup>, prompting researchers to seek alternatives to antibiotics for use in chicken feed.

Due to the negative side effects of synthetic medical chemicals, the usage of medicinal plants has expanded in recent years, and herbal supplements to replace antibiotics have garnered considerable attention. Since 4000 years ago, Glycyrrhizaglabraglabra, a legume, has been one of the most important medicinal herbs used to treat numerous ailments (6). Some of the active ingredients of this plant include saponin (7), triterpenes (8), flavonoids (9), and isoflavones (10). The essential substance in licorice is glycyrrhizic acid (11, 12), which is more abundant in licorice roots than in other parts of the plant. Due to the various properties of licorice, products such as licorice powder, extract, and juice have long been prepared (13). Licorice extract has antibacterial properties, so it has been suggested that this compound can be used as an antibacterial drug in treating diseases (14-16). The researchers studied the antiviral effects of licorice and its effective compound (glycerin) (17). In general, this study's results showed that the licorice plant's root extract has an active antiviral effect against a wide range of viruses.

Plants have different mechanisms to reduce the damaging effects of oxygen-free radicals (18). Plant tissues contain free radical scavenging enzymes (catalase and ascorbate peroxidase, etc.) and a network of low molecular weight antioxidants (phenolic

compounds, carotenoids, etc.) enzymatic and non-enzymatic antioxidant defense systems, respectively (19, 20). These antioxidants protect the cell against oxidative stress by removing free radicals or preventing their formation. Another advantage of this plant is its antifungal properties. Sato, Goto (21) investigated the effect of antifungal properties of several plant extracts. The results of this study showed that licorice has antifungal properties. In addition to the properties mentioned above, licorice also has properties that reduce pain (22) and asthma (23). Previous results showed that consumption of licorice in rats and human nutrition reduces weight and abdominal fat (24-26). Licorice essential oil with antibacterial, antiviral, antifungal, antioxidant properties, etc., can be a suitable plant to replace in broilers' diet. Including it in the diet can reduce the consumption of synthetic antibiotics.

However, there are few reports of using this plant in feeding broiler chickens. Due to the abundance of licorice in most parts of the world, and the medicinal properties of this plant, its use is probably essential as an additive in the diet of broilers, which is investigated in this study.

## 2. Materials and Methods

### 2.1. Plant Collection and Essential Oil Extraction

Licorice root was obtained from the local market. To ensure the minimum possible moisture content, the roots of the dried plant were placed in a vacuum oven for 1 hour at 42 ° C. First, the dried root powder and solvent were poured into a balloon, and the balloon was placed on the heater. After a while, the water inside the balloon boiled and evaporated with the essential oil and entered the refrigerant. Put the set in this state for twelve hours to ensure that all the essential oils are extracted; then, the heater is turned off.

### 2.2. Birds and Treatments

In this experiment, 160 one-day-old broiler chickens of the Ross 308 were purchased, and after weighing, they were randomly distributed in experimental cages. The test unit arrangement based on the hall's heating and ventilation facilities was designed equally. Four levels of

0, 0.1, 0.2, and 0.3% licorice essential oil were supplemented with a basal diet. Each treatment consisted of 4 replications (10 broilers in each replication). Based on UFFDA software, the soybean-based diet was adjusted to meet all broilers' nutritional needs (NRC). The basic dietary compositions used in this experiment are shown in table 1. The temperature in the first week was 33 ° C, which decreased with the growth of chickens, and finally, after 28 days, it was fixed at 24 ° C. Exposure of the hall was done using 60-watt lamps, and the duration of daily lighting of the nest was 23 hours. During the experiment, the birds had access to water and food *ad libitum*.

**Table 1.** Percentage composition and calculated of experimental diets for broilers

Ingredients (%)	Starter 1 to 10 d	Grower 11 to 28 d	Finisher 29- 42 d
Corn grain	58.80	59.90	65.30
Soybean meal	35.60	33.60	28.30
Oil	1.44	2.96	2.81
Dicalcium phosphate	1.74	1.50	1.56
Calcium carbonate	1.34	1.18	1.19
L-Lysine	0.15	**	**
DL-Methionine	0.23	0.16	0.14
Salt	0.20	0.20	0.20
Vitamins	0.25	0.25	0.25
Minerals	0.25	0.25	0.25
<b>Nutritional levels</b>			
Metabolizable energy (Kcal/Kg)	2904	3024	3072
Crude Protein (%)	21.12	20.16	18.28
Available phosphorus (%)	0.48	0.43	0.43
Calcium (%)	1.00	0.86	0.87
Lysine (%)	1.22	1.06	0.93
Methionine (%)	0.56	0.47	0.42
Methionine+Cysteine (%)	0.90	0.80	0.73
Tryptophan (%)	0.78	0.75	0.67

**Starter:** vitamin A 140,000 IU; vitamin D<sub>3</sub> 40,000 IU; vitamin E 220 IU; vitamin K<sub>3</sub> 2,622 mg; vitamin B<sub>1</sub> 3,920 mg; vitamin B<sub>2</sub> 96 mg; vitamin B<sub>6</sub> 3,920 mg; vitamin B<sub>12</sub> 200 mcg; niacin 70,460 mg; pantothenic acid 23,520 mg; folic acid 1,960 mg; biotin 0.80 mg; vitamin B<sub>8</sub> 5,928 mg; sodium 40 g; manganese 1,200 mg; zinc 1,000 mg; iron 800 mg; copper 160 mg; iodine 1,844 mg; selenium 9 mg; **Grower:** vitamin A 110,000 IU; vitamin D<sub>3</sub> 24,000 IU; vitamin E 200 IU; vitamin K<sub>3</sub> 24 mg; vitamin B<sub>1</sub> 24 mg; vitamin B<sub>2</sub> 80 mg; vitamin B<sub>6</sub> 38 mg; vitamin B<sub>12</sub> 160 mcg; niacin 560 mg; pantothenic acid 180 mg; folic acid 12 mg; vitamin B<sub>8</sub> 5,200 mg; sodium 31 g; manganese 1,200 mg; zinc 1,000 mg; iron 800 mg; copper 160 mg; iodine 1,791 mg; selenium 5 mg; **Finisher:** vitamin A 110,000 IU; vitamin D<sub>3</sub> 10,000 IU; vitamin E 110 IU; vitamin K<sub>3</sub> 11 mg; vitamin B<sub>2</sub> 40 mg; vitamin B<sub>12</sub> 100 mcg; niacin 400 mg; pantothenic acid 130 mg; vitamin B<sub>8</sub> 2,183 mg; sodium 32 g; manganese 1,200 mg; zinc 1,000 mg; iron 800 mg; copper 160 mg; iodine 1,795 mg; selenium 5 mg.

### 2.3. Performance Characteristics

The chickens were weighed at the beginning of the rearing period, and their average weight was calculated. The chickens were starved for about 3 to 4 hours before weighing. To calculate weight gain at each period. The weight difference between the beginning and end of different breeding stages was determined. The feed intake of each experimental replication was determined through the difference between the amount of feed allocated at the beginning and the remaining feed at the end of the week. The feed conversion ratio was calculated by dividing the feed intake by weight gain in each period.

### 2.4. Carcass Characteristics

On day 42 of the rearing period, two birds from each pen weighing the average weight per replicate were randomly selected, weighed, and then slaughtered. After removing feathers, carcass weight was recorded. The evaluated factors were relative carcass percentage and the relative weight of thigh, breast, liver, abdominal fat, gallbladder, bursa Fabricius, and spleen, measured by digital balance.

### 2.5. Determination of Cellular and Humoral Immune Systems

On the 28<sup>th</sup> and 35<sup>th</sup> days, two ml of each bird was injected with 0.2 ml of 0.5% lamb red blood cell suspension washed in sterile phosphate buffer into the vein of the wing, and the birds were marked. Then, about 2 ml of blood was taken from the same birds through the wing vein (7 days after the second injection) at 42 days. Blood samples were kept at room temperature to separate the serum from the blood clot. The serum was centrifuged at 4000 rpm for 15 minutes and immediately placed at 4 ° C. Microtiter hemagglutination was used to determine the titer of antibodies produced against sheep erythrocytes.

At 41 days of age, two birds from each replicate were randomly selected to determine the response to phytohaemagglutinin injection (27). First, the bird's right foot was cleaned with 70% ethanol, and then the thickness between the third and fourth toe was measured using a caliper with an accuracy of 0.01 mm. 0.1 ml of

phytohaemagglutinin solution was injected into the skin. After 24 hours, the injection site was measured again, and the response to increased sensitivity of skin re-profiles was determined by the difference in skin thickness before and 24 hours after injection.

## 2.6. Measurement of Blood Parameters

Blood samples were taken from the jugular vein (8 samples for each treatment) to determine blood metabolites at the time of slaughter (42 days old). Blood samples were poured into test tubes, and the samples were centrifuged at 3000 rpm for 7 minutes to extract serum and transferred to special microtubes at  $-20^{\circ}\text{C}$  until the main tests were kept in the freezer. Serum samples were analyzed laboratory to determine some metabolites (glucose, triglyceride, cholesterol, HDL, LDL) using the colorimetric method (commercial kits of the Spanish Biosystem Company).

## 2.7. Statistical Analysis

The experimental design was a completely randomized design (CRD), and the statistical model was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

$Y_{ij}$ : Observed value

$\mu$ : Mean

$T_i$ : The effect of experimental treatments

$e_{ij}$ : Effect of experimental error

All statistical analyses were performed using SAS software (version 9.1). Tukey's HSD (honestly significant difference) test was used at a significant level of 5% to compare the means.

## 3. Results

A comparison of the different experimental treatments on feed intake at different growth periods is shown in table 2. The feed consumption in the starter (1-10 days), growth (11-28 days), finisher (29-42 days), and the whole period was not affected by the use of different levels of licorice essential oil ( $P > 0.05$ ). Analysis of experimental data showed that the effects of different experimental treatments on the average weight gain of broilers in different experimental periods were insignificant. The relationship between feed conversion ratio, feed intake,

and body weight gain is an important indicator for evaluating the variables applied through diet or environmental conditions. The results showed that the lack of significant difference between the average feed intake and the increase in weight of broiler chickens did not significantly affect the feed conversion ratio.

Consumption of different levels of licorice essential oil had no significant effect on carcass weight, the relative weight of breast, legs, liver, spleen, and bursa Fabricius. However, it reduced gallbladder and abdominal fat ( $P < 0.05$ ). The highest gallbladder weight and abdominal fat percentages related to the control group were 0.17 and 2.29, respectively (Table 3).

Figure 1 shows the effect of different experimental groups on the response against phytohaemagglutinin (PHA-P) injection and the response against the sheep erythrocyte injection (SRBC) challenge. As can be seen, the experimental treatments in response to phytohaemagglutinin injection into the middle membrane of the third and fourth toes of broilers did not show a statistically significant difference from each other ( $P > 0.05$ ). A comparison of graph columns showed that the groups' Experiments in antibody response against the SRBC challenge were significantly different from each other ( $P < 0.05$ ). As can be seen, the groups receiving licorice essential oil had a significant difference compared to the control group and the group receiving 0.1%. Licorice essential oils have the highest response against sheep erythrocytes.

A comparison of the mean effects of different experimental groups on LDL, HDL, glucose, triglyceride concentrations, and blood cholesterol in broiler chickens is shown in table 4. Using different levels of licorice essential oil in broiler diets did not affect triglyceride and HDL concentrations ( $P > 0.05$ ). However, different levels of licorice essential oil reduced blood glucose. Among these, the lowest blood glucose content belonged to the group of 0.2 and 0.3% licorice essential oil. The quantity of LDL and cholesterol in broiler chickens decreased due to the increased levels of licorice essential oil. Therefore, the level of 0.3% of licorice essential oil. Applying different treatments did not significantly affect HDL concentration ( $P > 0.05$ ).

**Table 2.** The effect of experimental treatments on broiler performance

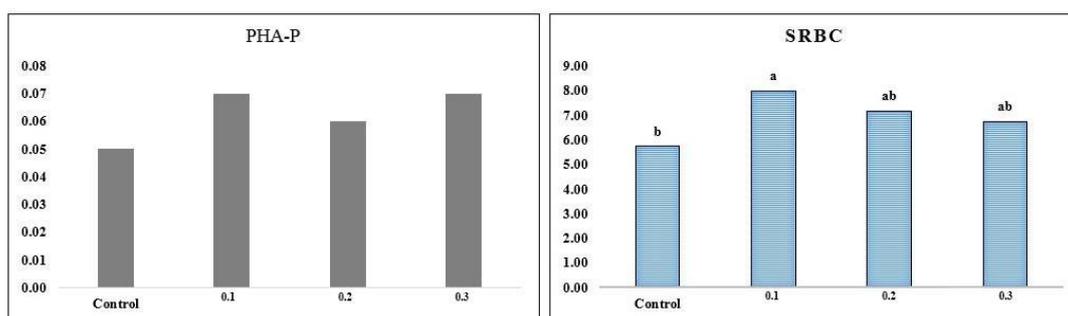
Performance	Days	Licorice Essential Oil (%)				SEM	P-Value
		Control	0.1	0.2	0.3		
Feed Intake (g)	0-10	453.500	455.250	454.500	453.250	1.060	0.530
	11-29	1310.500	1311.750	1311.000	1309.000	1.660	0.690
	29-42	2438.250	2440.000	2439.000	2441.000	1.430	0.570
	1-42	4202.250	4207.000	4204.500	4203.250	1.180	0.080
Body weight gain (g)	0-10	400.500	401.500	403.500	401.000	1.210	0.081
	11-29	828.500	831.250	829.250	830.500	1.890	0.410
	29-42	1218.000	1215.500	1214.500	1216.500	1.090	0.570
	1-42	2447.000	2448.250	2447.250	2448.000	1.650	0.930
Feed conversion	0-10	1.132	1.134	1.126	1.130	0.003	0.250
	11-29	1.582	1.578	1.581	1.576	0.003	0.450
	29-42	2.002	2.007	2.008	2.007	0.003	0.560
	1-42	1.717	1.718	1.718	1.717	0.123	0.796

a-b: dissimilar letters in each column indicate a significant difference ( $P < 0.05$ )

**Table 3.** The effect of experimental treatments on broiler carcasses characteristics

Carcass Characteristics (%)	Control	Licorice Essential Oil (%)			SEM	P-Value
		0.1	0.2	0.3		
Carcass	63.750	65.250	64.250	65.750	0.520	0.069
Breast	23.500	25.250	24.000	25.500	0.554	0.071
legs	15.000	16.250	15.120	16.500	0.594	0.227
Liver	1.750	1.750	1.730	1.740	0.005	0.082
Gallbladder	0.170 <sup>a</sup>	0.110 <sup>c</sup>	0.140 <sup>b</sup>	0.120 <sup>bc</sup>	0.008	0.001
Spleen	0.130	0.160	0.150	0.140	0.008	0.109
Bursa Fabricius	0.130	0.150	0.140	0.140	0.006	0.168
Abdominal fat	2.290 <sup>a</sup>	1.920 <sup>b</sup>	1.860 <sup>b</sup>	1.750 <sup>b</sup>	0.045	0.000

a-b: dissimilar letters in each column indicate a significant difference ( $P < 0.05$ )



**Figure 1** The effect of experimental treatments on broiler cell and humoral immune system  
a-b: dissimilar letters in each column indicate a significant difference ( $P < 0.05$ )

**Table 4.** The effect of experimental treatments on broiler blood parameters

Blood parameters	Control	Licorice Essential Oil (%)			SEM	P-Value
		0.1	0.2	0.3		
Glucose	253.500 <sup>a</sup>	210.370 <sup>b</sup>	189.120 <sup>c</sup>	178.680 <sup>c</sup>	3.622	0.000
Triglycerides	73.870	78.500	77.250	76.670	1.674	0.301
LDL	32.120 <sup>a</sup>	23.750 <sup>b</sup>	19.000 <sup>bc</sup>	12.000 <sup>c</sup>	1.782	0.000
HDL	59.370	62.870	66.000	12.660	2.248	0.166
Cholesterol	128.620 <sup>a</sup>	129.370 <sup>b</sup>	117.370 <sup>bc</sup>	110.870 <sup>c</sup>	3.822	0.000

a-b: dissimilar letters in each column indicate a significant difference ( $P < 0.05$ )

#### 4. Discussion

As shown in table 2, the effect of different groups on feed intake at different growth periods was insignificant. Sedghi, Golian (28) examined the effect of different levels of licorice extract on egg quality and performance of laying hens. They reported that the use of licorice extract had no effect on feed intake, which is consistent with the present study's findings. In another study, they examined the effects of licorice extract on broilers' performance and blood parameters, and their experimental results again showed that the use of licorice extract did not affect feed intake (28). Similar results are seen in mice (24) and rats (29), while Awadein, Eid (30) reported that using 0.5 and 1% of ground licorice powder in broiler diets significantly reduced their feed intake compared to the control group. Drinking versus food is one of the possible reasons for the differences in the results of the present study and the research of Awadein, Eid (30).

The effect of different experimental groups on the weight gain of chickens in the starter periods (1-10), grower (11-28 days), finisher (29-42 days), and the whole period were not affected by the use of different levels of licorice essential oil. Sedghi, Golian (28) reported that licorice extract did not affect the weight of broiler chickens, while Abd El-Hakim and Abd El-Magied (31) reported that using levels of 0.25 and 0.5% of licorice extract improved the weight gain of broilers raised in summer. The presence of heat stress conditions is the most critical possible reason for the discrepancy between the study's findings and the present study's results. However, the small number of

studies on licorice extract in the diet of poultry and other livestock makes it challenging to discuss and draw conclusions about the results. The results of this experiment on licorice and its effect on weight gain differed from the results of Nakagawa, Kishida (26). Weight loss in humans and laboratory animals is due to reduced tissue fat and stored fat.

Usually, more than 50% of the necessary expenses are related to the cost of produced meat. Therefore, reducing feed conversion should be a priority. Measuring the feed conversion ratio is of great economic importance. The feed conversion ratio, which determines the amount of feed consumed per kilogram of weight gain, is obtained using weight gain and feed intake data. In young birds, more feed is consumed for growth (80%) and less for maintenance (20%). So their feed efficiency is excellent. Over time, the need for maintenance increases, and efficiency deteriorates. Over the years, we have seen a decrease in the conversion rate from about 2.2 in the early 1960s to 1.75 today. This improvement in conversion ratio is due to the development of genetic potential and more food consumption for growth and less for maintenance (32). Bodyweight is due to food consumption; therefore, food consumption is the main variable in evaluating food efficiency. Previously, broilers were raised to  $45 \pm 3$  days old by receiving diets containing standard energy levels. Under such circumstances, measuring the feed conversion ratio was valuable and related to herd profit. Plant extracts have been reported to affect live weight and improve poultry health. In addition, they are effective on other functional traits

such as feed conversion ratio and feed intake (33). However, variable results of the effect of these additives on bird performance have been reported (34).

Consistent with the present study's findings, Sedghi, Golian (28) reported that the use of licorice extract did not affect broilers' feed efficiency of broilers. Awadein, Eid (30) also examined the effects of using 0.5% levels of 1% licorice seed powder in poultry feed. They reported that the best feed conversion ratios during the experimental period were 4, 8, and 12 weeks after the onset experiment belonging to groups receiving licorice, especially its 5% level. Similar findings can be seen in rats fed with licorice extract (29). Among other reasons mentioned in the previous sections, can be considered as the most important reasons for differences in the results of different experiments; with changing the habitat and harvest time, the number of effective compounds in medicinal plants changes, so to compare the results of experiments In the field of medicinal plants, it is necessary to measure the number of effective compounds of the consumable substance.

The effect of different experimental treatments on carcass percentage and relative weight of legs and breast weight was insignificant ( $P > 0.05$ ), and the results of this study are consistent with the findings of the Rashidi, Khatibjoo (35). In confirmation of the present study's findings, Sedghi, Golian (28) reported that the use of 0.5, 1, and 2% licorice extract had no effect on the relative weight of thighs and breasts of broilers. Various medicinal plants reported on broilers' carcass traits that using alfalfa leaves, licorice root, poppy root, and cinnamon did not affect their carcass percentage.

As observed, the relative weight of the spleen, liver and Fabricius bursa was not affected by using different levels of licorice essential oil ( $P > 0.05$ ). However, using different levels of licorice essential oil caused a significant difference in the gallbladder and abdominal fat percentage.  $P < 0.05$ . According to the results, the lowest gallbladder weight percentage was the group receiving 1% licorice essential oil. The group receiving

3% licorice essential oil had the lowest abdominal fat percentage. In confirming the present study's findings, Sedghi, Golian (28) reported that the use of 0.5, 1, and 2 g/kg of licorice extract in the diet of broilers reduced the relative weight of their abdominal fat. It has been observed in laboratory animals and humans that the increase in ventricular fat in broiler chickens is a negative factor that many efforts have been made to reduce this stored fat in the body. The three reasons for the decrease in feed intake are lipid uptake, biosynthesis of fatty acids, and increased oxidation of these acids. The results of the researchers' experiments showed no change in the amount of feed consumed. Also, blood triglyceride levels did not change, and it was concluded that this fat reduction could not be due to reduced fat absorption (36). Therefore, the reduction of fat deposits was probably due to the decrease in the synthesis of fatty acids and increased oxidation of these acids.

On the other hand, in the present study, using different levels of licorice essential oil reduced the relative weight of the gallbladder, which may be a reason for reducing its activity in the secretion of bile salts, followed by reduced digestion and absorption of fats. This highlights the need for further studies in this area.

Experimental treatments were not statistically significant in response to the injection of phytohaemagglutinin into the middle membrane of the third and fourth toes of broilers. Experimental groups differed significantly in antibody response against the SRBC challenge. As observed, the groups receiving licorice essential oil had a significant difference compared to the control group, so the group receiving 0.1% licorice essential oil had the highest response against sheep erythrocyte injection. Consistent with the study's findings, some researchers reported that using licorice-derived polysaccharides during the boiling process with water improved the antibody titer against the Newcastle disease vaccine in broilers (37). Khaligh, Sadeghi (38) also attributed part of the improvement in

antibody titer in broiler chickens fed with alfalfa plants, licorice root, poppy root, and cinnamon to saponins in their roots. Rashidi, Khatibjoo (35) reported that the response of antibody titer to Newcastle disease and analyze vaccine was not affected by licorice extract in broiler diets. According to research in general, medicinal plants and their products can increase the immune system and improve growth in livestock and poultry (39). Simultaneous injection of polyphenol extract of thistle and licorice seeds with thioamide reduced total bilirubin and aspartate aminotransferase activity, alanine aminotransferase, and alkaline phosphatase in comparison with thioacetamide groups (40). This means that these extracts have an excellent protective effect on liver cells against damage caused by thioacetamide.

Using different levels of licorice essential oil in broiler diets did not affect HDL triglyceride concentration. However, using different levels of licorice essential oil was significant in broiler chickens' concentration of glucose, LDL and blood cholesterol. The use of different levels of licorice essential oil reduced blood glucose concentration. As you can see, the amount of LDL and cholesterol in the blood of broilers has decreased due to the increased levels of licorice essential oil. So that the level of 0.3% licorice essential oil has the lowest LDL and blood cholesterol concentration; on the other hand, using different levels of licorice essential oil increased the concentration of triglycerides and HDL, but this increase was not statistically significant. In confirmation of our findings, Sedghi, Golian (28) also reported that the use of 0.5, 1, and 2 g/kg of licorice extract in the diet of broilers did not affect their serum triglyceride concentration.

On the other hand, in the mentioned study, unlike the present study, glucose concentration was not affected by experimental treatments. Nakagawa, Kishida (26) also examined the effects of different levels of licorice flavonoid oils on the blood parameters of obese diabetic rats<sup>26</sup>. Their findings showed that blood glucose levels remained constant after receiving a diet containing 2% of this compound. They also found that

blood glucose levels increased in groups fed 0.5 and 1% levels of licorice flavonoid oils, but this increase was significantly lower compared to the control group. They attributed the changes in glucose concentration following the compound's excretion to insulin concentration's effect.

Shahabinezhad, MR (41) reported that oral administration of 100 mg of licorice extract per day did not affect blood glucose levels in laboratory rats. However, 200 and 300 mg/kg levels caused a significant decrease in blood glucose levels of diabetic animals. While it did not affect blood glucose levels in healthy mice. Licorice contains a compound called Isoliquiritigenin, which acts as a monoamine oxidase inhibitor, increasing the levels of epinephrine and serotonin in the body. Epinephrine has a strong effect on stimulating hepatic glycogenolysis and releases blood sugar. Therefore, it can be concluded that this effect of epinephrine stimulates insulin secretion and subsequently lowers blood sugar. Kumagai, Yano (42) showed that glycerin in licorice inhibits glucocorticoid metabolism, thus increasing glucocorticoid levels in the blood. Glucocorticoids stimulate insulin secretion, suggesting that licorice by the above mechanism reduces blood sugar (42).

Consumption of licorice extract in hypercholesterolemic patients reduced LDL, cholesterol, and triglycerides. Licorice extract contains flavonoids, including formononetin, hispaglabridin A, and Hispaglabridin B, which affect arachidonic acid metabolism and reduce the production of free radicals. These compounds also showed anti-platelet, anti-inflammatory, and antioxidant properties (43).

## **5. Conclusion**

Increased feed consumption was not affected by adding different levels of licorice essential oil. According to the results, the percentage of the carcass, legs, breasts, spleen, liver, and bursa fabricius was not affected by different levels of licorice essential oil. However, the use of licorice essential oil in the diet reduced the fat content of the gallbladder and

abdominal fat compared to the control group. Blood factors showed that birds on a diet containing licorice essential oil had lower LDL and cholesterol than the control group. However, elevated levels of licorice essential oil were not affected HDL and triglyceride levels. Cellular immunity in this study was not affected by different levels of licorice essential oil, but different experimental groups influenced humoral immunity.

### Authors' Contribution

Study concept and design: O. D. S. and H. O.

Acquisition of data: O. D. S. and H. H.

Analysis and interpretation of data: M. F. and T. A. H.

Drafting of the manuscript: M. M. K. and S. A.

Critical revision of the manuscript for important intellectual content: O. D. S., A. S. P. and Y. F. M.

Statistical analysis: H. H.

Administrative, technical, and material support: O. D. S. and H. O.

### Ethics

All the study procedures were approved by the ethics committee of the Al-Maarif University College, Anbar-Ramadi, Iraq.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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