EFFECTS OF THE ADDITION OF AQUEOUS LIQUORICE (Glycyrrhiza glabra) EXTRACT TO DRINKING WATER IN THE PRODUCTION PERFORMANCE, CARCASS CUTS AND INTESTINAL HISTOMORPHOLOGY OF BROILER CHICKENS

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ABSTRACT

This study was undertaken to investigate the effect of aqueous liquorice extract (ALE) on the growth performance, carcass cuts and intestinal histomorphology of broiler chickens between hatch and 35 days of age. A total of 160-day-old (Ross 308) broiler chicks were randomly assigned to 4 treatments, each with 4 replicates, 10 birds per replicate. Four different levels of aqueous liquorice (0, 0.5, 0.7 and 0.9g) were administrated to a liter of drinking water and offered to the birds throughout the entire experimental period. Across the 35 days of trial, administration of ALE to the drinking water resulted in higher (P<0.05) body weight and weight gain. While, feed intake, water intake and FCR did not influence by ALE administration. On the other hand, there was no significant effect of the ALE on carcass cuts, visceral organs and intestinal histomorphology. The study demonstrated that ALE could be administrated to the drinking water of broilers between 0.5 and 0.9g/ liter. However, it would be more economical to use the medium level of ALE (0.7g/ liter) to achieve better results.

Keywords: Liquorice, Performance, Carcass, Histomorphology, Broilers.

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تأثر اضافة مستخلص مسحوق عرق السوس على الاداء الانتاجي وقطعيات الذبيحة و الصفات النسيجية لامعاء فروج اللحم.

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تُجري هذه التجربة لمعرفة مدى تأثير اضافة مستخلص عرق السوس إلى ماء الشرب في الاداء الانتاجي و قطعيات الذبيحة و الصفات النسيجية للامعاء لفروج اللحم خلال الفترة ما بعد الفقس إلى عمر 35 يوم.

160 فروج اللحم سلالة Ross 308 ووزنها 160 غرام تم توزيعها على 4 عواملات بواقع 4 نماذج لكل عاملة، حيث تم إضافة مستخلص عرق السوس بكميات متفاوتة (0، 0.5، 0.7 و 0.9 غرام لكل لتر من ماء الشرب) خلال الفترة ما بعد الفقس إلى عمر 35 يوما.

أظهرت النتائج أن إضافة مستخلص عرق السوس إلى ماء الشرب كان له تأثير إيجابي على الاداء الانتاجي، حيث أن الوزن最終ي وزن النمو اليومي وكمية الطاقة السائبة للفروج والكبدخدودية والدمرياح ووزن أجزاء الجسم الأخرى، بينما لم يتأثر معدل استهلاك العلف والماء والأداء الغذائي بشكل ملحوظ.

استنتجت هذه التجربة أن مستخلص عرق السوس يمكن أضافةه إلى ماء الشرب لفروج اللحم بين 0.5 إلى 0.9 غرام لكل لتر من الماء وفقاً لنتائج الدراسة.

كلمات مفتاحية: الانتاجية، عمر الافراخ، فروج اللحم، القيادة اليومية، التحويل الغذائي.

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INTRODUCTION
Ross 308 Herbal extracts have got growing attention as possible feed additives for animal production (36). Subsequently, following the restriction use of antibiotic, feed additives particularly that of plant origin (phytogenic) have gained the interest to be used in poultry nutrition. Numerous herbal plants have been intensively studied to be used as possible natural growth and health promotors in poultry nutrition in lieu of antibiotic due to its content of biologically active compounds (21).

Liquorice (Glycyrrhiza glabra) is a perennial plant or sub-shrub rising to a height of 2 m with horizontal underground stem. Liquorice contains numerous active compounds including saponin triterpenes (glycyrrhizin, glycyrrhetic acid and liquorice acid), flavonoids (liquiritin, isoflavonoids and formononetin) and other components such as coumarins, sugars, amino acids, tannins, starch, choline, ascorbic acid, phytosterols and bitter principles (4, 12, 33). It has been used as a pharmaceutical product in ancient Asia (37). The pharmacological impacts of liquorice and its isolated active compounds on animals have been confirmed by many workers: antimicrobial (14), antihelicobacter (13), antiatherosclerotic (10), antioxidative (35), antifungal (30), antiviral (9), antiinfective (26), and immune stimulator impacts (11). The administration of liquorice extract (LE) to the drinking water, improved the productive performance of broiler chickens challenged with heat stress (2). Nevertheless, the addition of liquorice powder significantly decreased the poisonous effect of aflatoxins contaminated diets on broiler performance (2). However, the impacts of LE supplementation on the Intestinal histomorphological changes of broilers have not been well investigated. Therefore, this study was accomplished to clarify the impact of aqueous extract of liquorice administration through drinking water on the performance, carcass traits and intestinal histomorhology of broiler chickens.

MATERIALS AND METHODS
The study was carried out at the poultry houses of Dept. of Animal Production, College of Agriculture, University of Duhok. One hundred sixty Ross 308 broilers were collected from a local hatchery. Ten birds were selected at random and allocated to each of the 4 single floor-pen replicates of each of the 4 treatments. Replicates of the treatments were randomly assigned to 16 floor pens bedded with softwood shavings. The room and equipment used for the study were thoroughly cleaned and disinfected before the in vivo study commencement. Treatments were allocated to each of the starter, grower and finisher basal diets comprising mainly of wheat, and soybean meal as shown in Table 1. Three phases of feeding were adopted, a starter diet from 1 to 10 d, grower diets from 11 to 24 d, and finisher diets from 25 to 35 d. All diets were formulated to meet the requirements for Ross 308 broiler chickens. Four levels of aqueous liquorice extract were created by soaking four different amounts (0, 0.5, 0.7 and 0.9g) of liquorice root powder into a liter of water. Thereafter, the water was filtrated and the solutions were collected into four plastic bottles and offered to the four different groups of birds for 35 days. All birds had ad libitum access to feed and water throughout the study. The room temperature was maintained at 33°C during the first 5 d and then gradually decreased to 23°C by d 24 of age. Birds received continuous light for the first 24 h, then 23L (light)/:1D (darkness) for the first weeks and were then maintained under 16L:8D for the remainder of the study. Birds and feeds in each pen were weighed by the end of each feeding phase and FCR was adjusted for mortality whenever it occurred. All the birds were monitored for general health at least twice a day. The production and maximising return index were calculated as following:

\[
\text{Production index} = \frac{\text{Average body weight (g)} \times \text{Livability} (\%)}{\text{Number of rearing days} \times \text{Feed conversion ratio}} \times 10
\]

(\text{Naji et al.}(25))

\[
\text{Maximising return index} = (M \times SR \times L \times LP) - (\text{AFC} \times FCR \times M) - (MC) - (CP)
\]

\[
\text{Age (days)}
\]

M: (Mass kg); SR: (Stocking Rate kg/m\(^2\)); L: (Livability %); LP: (Live price R/kg); AFC: (Average Feed Cost); FCR: (Feed Conversion Ratio); MC: (Medication Cost); CP: (Chick price) (20) At day 35, 2 birds were euthanized by cervical dislocation for measuring carcass characteristics and intestinal tissue collected for morphometric analyses. Approximately 1
cm of the jejunum was collected. The intestinal samples were opened and gently flushed clean with phosphate buffered saline (PBS, pH 7.4) and then fixed in 10% buffered formalin for 24 h. Formalin was subsequently replaced by 70% ethanol for storage. Each segment was embedded in paraffin. A 5 (μm) section of each sample was placed on a glass slide and stained with hematoxylin and eosin, and then examined by microscope (27).

**Statistical analysis of data**
All data were subjected to CRD (Completely Randomized Design) analysis using SAS, 2003) (29). Differences between mean values were determined using Duncan`s multiple range tests (8).

### Table 1. Proximate analysis of experimental diets (dry matter basis).

| Nutrients % | Starter | Grower | Finisher |
|-------------|---------|--------|----------|
| Crude protein (Det.) | 24.8    | 22.50  | 19.25    |
| ME, kcal/kg (Cal.)** | 3,025   | 3,100  | 3,150    |
| C:P ratio (Cal.) | 121.98  | 137.78 | 163.64   |
| Moisture (Det.) | 7.61    | 7.62   | 7.46     |
| Dry matter (Det.) | 92.39   | 92.38  | 92.54    |
| Ether extract (Det.) | 4.50    | 4.80   | 7.10     |
| Ash (Det.) | 6.90    | 5.96   | 5.40     |

* Determined, **Calculated

### RESULTS AND DISCUSSION

#### Growth performance

Over the starter period (1-10 days), the body weight, weight gain and feed intake were not affected when ALE was administrated to the drinking water of broiler chickens. However, FCR was significantly (P< 0.05) in ALE supplemented groups than those of control (Table 2). Whereas, the results were almost same among all experimental groups over the grower period of broiler age (Table 3).

**Table 2. Effect of aqueous liquorice extract on growth performance of broiler chickens (1-10) days**

| Response | Liquorice level | SEM |
|----------|----------------|-----|
| BW (g)   | 278.5          | 0.5 | 1.74 |
|          | 287.2          |     |
|          | 286.5          |     |
|          | 287.7          |     |
|          | 1.74           |     |
| WG (g)   | 238.6          | 0.5 | 1.83 |
|          | 248.0          |     |
|          | 248.2          |     |
|          | 249.0          |     |
|          | 1.83           |     |
| FI (g)   | 268.7          | 0.7 | 2.65 |
|          | 263.7          |     |
|          | 260.0          |     |
|          | 269.3          |     |
|          | 2.65           |     |
| WI (ml)  | 775.2          | 0.9 | 11.74|
|          | 760.7          |     |
|          | 766.1          |     |
|          | 825.4          |     |
|          | 11.74          |     |
| FCR (g/g) | 1.14a           | 0.7 | 0.01|
|          | 1.07b          |     |
|          | 1.08b          |     |
|          | 1.11ab         |     |

BW: body weight. BWG: body weight gain. FI: feed intake. WI: water intake. FCR: feed conversion ratio. 

Means within a row with no common superscript differ significantly (P < 0.05).

**Table 3. Effect of aqueous liquorice extract on growth performance of broiler chickens (11-24) days**

| Response | Liquorice level | SEM |
|----------|----------------|-----|
| BW (g)   | 1,109.50       | 0.5 | 11.01 |
|          | 1,138.33       |     |
|          | 1,122.96       |     |
|          | 1,099.33       |     |
|          | 11.01          |     |
| BWG (g)  | 835.17         | 0.5 | 10.14 |
|          | 851.17         |     |
|          | 841.96         |     |
|          | 817.83         |     |
|          | 10.14          |     |
| FI (g)   | 1,074.83       | 0.7 | 11.00 |
|          | 1,114.50       |     |
|          | 1,116.69       |     |
|          | 1,080.17       |     |
|          | 11.00          |     |
| WI (ml)  | 2,765.28       | 0.9 | 37.00 |
|          | 2,820.11       |     |
|          | 2,852.00       |     |
|          | 2,971.78       |     |
|          | 37.00          |     |
| FCR (g/g)| 1.35           | 0.7 | 0.01|
|          | 1.33           |     |
|          | 1.35           |     |
|          | 1.36           |     |
|          | 0.01           |     |

BW: body weight. BWG: body weight gain. FI: feed intake. WI: water intake. FCR: feed conversion ratio. 

Means within a row with no common superscript differ significantly (P < 0.05).
BWG in birds that received ALE in their drinking water (Table 5). Feed intake and FCR were not affected by the treatments. The production index (PI) was significantly higher (P < 0.05) in the experimental groups that offered ALE than the control group (Fig. 1). The highest PI (408.88) was recorded for the birds that received 0.7g/ liter of ALE compared to the other experimental groups. The maximising return index was significantly higher in birds that received 0.7 and 0.9g of ALE than those in the control group (Fig. 2).

**Table 4. Effect of aqueous liquorice extract on growth performance of broiler chickens (25-35) days**

| Response | 0          | 0.5         | 0.7          | 0.9          | SEM         |
|----------|------------|-------------|--------------|--------------|-------------|
| BW (g)   | 1,882.67 b | 2,002.00 a  | 2,036.67 a   | 1,995.00 a   | 22.19       |
| BWG (g)  | 787.67 b   | 870.70 ab   | 926.35 a     | 895.67 a     | 19.06       |
| FI (g)   | 3,366.70   | 3,380.00    | 3,328.30     | 3,533.30     | 41.70       |
| Wt (ml)  | 1.67       | 1.68        | 1.67         | 1.64         | 0.02        |

BW: body weight. BWG: body weight gain. FI: feed intake. Wt: water intake. FCR: feed conversion ratio. a,b Means within a row with no common superscript differ significantly (P < 0.05).

**Table 5. Effect of aqueous liquorice extract on the accumulative growth performance of broiler chickens (1-35) days**

| Response | 0          | 0.5         | 0.7          | 0.9          | SEM         |
|----------|------------|-------------|--------------|--------------|-------------|
| BW (g)   | 1882.7 b   | 2002.0 a    | 2036.7 a     | 1995.0 a     | 22.19       |
| WG (g)   | 1842.7 b   | 1963.0 a    | 1998.4 a     | 1956.7 a     | 22.32       |
| FI (g)   | 2667.3     | 2836.1      | 2855.4       | 2799.8       | 34.00       |
| C (ml)   | 6981.1     | 6964.4      | 7004.6       | 7370.1       | 82.54       |
| FCR (g/g)| 1.46       | 1.46        | 1.47         | 1.46         | 0.01        |

BW: body weight. BWG: body weight gain. FI: feed intake. Wt: water intake. FCR: feed conversion ratio. a,b Means within a row with no common superscript differ significantly (P < 0.05).

![Figure 1. Effects of aqueous liquorice extract on production index of broiler chickens (1-35) days. T1: Control group, T2 (0.5g LE/ liter), T3 (0.7g LE/ liter) and T4 (0.9g LE/ liter). Different letters above each column indicate significant difference between means (P < 0.05).](image1)

![Figure 2. Effects of aqueous liquorice extract on maximising return index of broiler chickens (1-35) days. T1: Control group, T2 (0.5g LE/ liter), T3 (0.7g LE/ liter) and T4 (0.9g LE/ liter). Different letters above each column indicate significant difference between means (P < 0.05).](image2)
Administration of ALE improved the FCR, BW and BWG. The positive effect of ALE on the broiler performance could be due to the improvement in the intestinal health and digestion functions. The beneficial effects of phytopgenic extracts on the growth performance of poultry, arises from its ability to promote the digestibility, improve the gut microflora and increase the secretion of endogenous digestive enzymes (28). In addition, these extracts found to stimulate appetite and digestion (6, 36). Furthermore, Grieve (17) stated that liquorice is acting as appetite and digestion stimulators. It also raised blood flow through mucous membranes of gut increasing the utilization efficiency of nutrients. The results were in line with the findings of (2, 3, 28) who found that delivering the liquorice via feed or drinking water, significantly improved the growth performance of broiler chickens. The presence of active compounds especially those belong to isoflavonoid class of chemicals, liquorice may have the ability to improve the function of immune system (1, 7, 32). This could be the only explanation of low mortality rate in ALE supplemented group in the current study. This was in accordance with the findings of (34) who showed that the survival rate increased after intraperitoneal administration of liquorice active compound (glycyrrhizin) 0.2 ml of a saline solution/mouse 1 day before infection and 1 and 4 days post infection in mice infected with 20 and 10 LD50s of influenza virus (H2 N2). In contrast (23, 24, 31) stated that using LE as a dietary supplementation or via drinking water had no significant effects on the performance and immunological parameters of broiler chickens and Japanese quails.

**Carcass cuts**

There was no significant effects of the ALE administration on the dressing percentage and the relative weight of carcass parts (Table 6). However, although not significant, the dressing percentage and the relative weight of breast percentages were numerically higher ALE supplemented birds than those in control. Similar results have been obtained by (31) when licorice extract was included to the broiler diets. The carcass yield in broilers were mainly influenced by genetic factors than nutritional ones (16). In contrast, (2, 3) stated that the addition of liquorice extract (LE) to the aflatoxin contaminated diets significantly eliminated the negative effect of aflatoxin on the carcass characteristics of broiler chickens. Furthermore, the dressing percentage significantly improved when LE was administrated into the drinking water of broiler chickens.

**Table 6. Effect of aqueous liquorice extract on carcass cuts of broiler chickens at 35 days of age**

| Response  | Liquorice level | SEM  |
|-----------|-----------------|------|
|           | 0   | 0.5 | 0.7 | 0.9 |      |
| Dressing %| 73.1| 73.7| 74.1| 75.0| 0.36 |
| Breast    | 36.5| 38.7| 38.7| 38.5| 0.43 |
| Thighs    | 15.5| 15.0| 14.6| 15.4| 0.18 |
| Drumsticks| 12.8| 12.8| 13.5| 13.2| 0.15 |

SEM=standard error of means

**The relative weight of visceral organs**

In general, the effect of ALE administration was not significant on the relative weight of internal organs (including liver, heart, bursa and pancreas) except for the relative weight of spleen which was significantly (P < 0.05) lower in ALE supplemented groups than those of control (Table 7). In this study, the relative weights of visceral organs were not affected by the administration of ALE to the drinking water of broiler chickens. Except for the spleen weight which was decreased in ALE supplemented groups. This is in agreement with (23, 24, 31) who found no influence of liquorice on these organs in broilers and quails. Also Al-Daraji (3) reported a significant decrease in the relative weight of spleen as a result of ALE administration to the broiler drinking water. However, Salary et al.(28) found a significant increasing in pancreas percentage in broilers when introduced to LE.
Intestinal histomorphology

The effect of the ALE administration on the jejunum histomorphology is presented in table 8. Villus height (VH), crypt depth (CD) and villus height/crypt depth (VH/CD) were not affected by the administration of ALE to the drinking water or broiler chickens. However, in general VH and VH/CD were higher in birds that received the medium level ALE (0.7g/liter) than other experimental groups.

Table 7. Effect of aqueous liquorice extract on visceral organs of broiler chickens at 35 days of age

| Response  | Liquorice level | SEM |
|-----------|-----------------|-----|
| Liver     | 3.63            | 0.5 | 0.7 | 0.9 |
| Heart     | 0.93            | 0.91| 0.89| 0.87| 0.03|
| Bursa     | 0.147           | 0.176| 0.179| 0.153| 0.016|
| Spleen    | 0.184<sup>a</sup> | 0.132<sup>b</sup> | 0.108<sup>b</sup> | 0.109<sup>b</sup> | 0.011|
| Pancreas  | 0.232           | 0.252| 0.229| 0.210| 0.009|

<sup>a,b</sup> Means within a row not sharing the same superscript are significantly different (P < 0.05). SEM=standard error of means

The critical digestive organ which involved in the absorption of nutrients is a small intestine. Therefore, any improvement of this part is very important for the performance and health status of broiler (19). Villus height (VH) and crypt depth (CD) became a popular measurement in supporting the impacts of nutrition on the physiology of alimentary canal. Nevertheless, the positive correlations between the improvement of performance and VH and CD have been documented (18). The increasing in VH and VH: CD ratio lead to better absorption of nutrients, consequently, has positive effect on the growth performance (5, 22). Additionally, using phytogenic extracts in poultry causes increasing in height of villus due to decline of harmful bacteria in the intestinal wall, therefore minimizing the byproducts of these bacteria such as toxic compounds which negatively effect on the epithelial cells of intestine and finally inhibit villus destruction and minimizes repairing of the lumen (15).

Table 8. Effect of aqueous liquorice extract on intestinal histomorphology of broiler chickens at 35 days of age

| Response  | Liquorice level | SEM  |
|-----------|-----------------|------|
| VH (μm)   | 1320.0          | 1207.7| 1367.6| 1325.9| 38.15|
| CD (μm)   | 206.3           | 211.4| 209.9| 215.4| 6.41|
| VH/CD ratio | 7.00         | 6.11| 7.39| 6.57| 0.26|

VH: villus height. CD: crypt depth, VH/CD= villus height/crypt depth, SEM=Standard error of means

The results of this experiment provides evidences of the positive effects of the administration of ALE to the drinking water on the performance of broiler chickens. The outcomes achieved in this study suggest that ALE would be more beneficial if used at a medium level (0.7g/liter) throughout the broiler production cycle.

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