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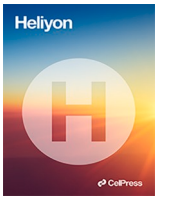

Research article

Production performance, serum lipid profile and gut health in Indian native Kadaknath chickens fed diet incorporated with liquorice root powder

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ABSTRACT

The principal constituent of liquorice root (*Glycyrrhiza glabra*) is glycyrrhizin, a triterpene saponin that is approximately many times sweeter than sucrose, the main active component. This study aimed to investigate the dietary liquorice root powder (LRP) on production performance, serum biochemical, gut health and carcass characteristics of Kadaknath (KN) birds as replacement of antibiotic growth promoter. Day-old Kadaknath chicks (n = 240) with uniform body weight were selected randomly and divided into six different treatments, each one with five replicates and eight birds per replicate, and raised in battery brooder cages for 15 weeks. Corn soya based basal diet (T_1) was prepared. In addition to the basal diet, five experimental diets were created with varying amounts of LRP i.e., T_2: T_1+ 0.1 % LRP, T_3: T_1+ 0.3 % LRP, T_4: T_1+ 0.5 % LRP, T_5: T_1+ 0.7 % LRP, and T_6: T_1+ 0.0335 % Chlortetracycline (CTC). Body weight gain and feed intake significantly ($P \leq 0.05$) increased in T_3 group on 0–5 wks and 5–9 wks of age. Significant ($P \leq 0.01$) reduction in the feed intake was noted in the T_5 group which was fed with maximum level (0.7 %) of inclusion of LRP. Dietary inclusion of liquorice in higher doses resulted in a significant ($P \leq 0.05$) decrease in serum lipids such as triglyceride, LDL, and total cholesterol concentrations and a significant increase in the HDL cholesterol. Decrease in the coliform count of caecum significantly ($P \leq 0.05$), but dose-dependent lactobacilli proliferation was seen in the caecum of treated birds ($P \leq 0.01$). Supplementation of liquorice root powder in kadaknath birds resulted in significant increase ($P \leq 0.05$) in the villus length and VH: CD ratio. Thus it may be concluded that dietary supplementation of liquorice root powder improved the bird's growth performance, serum lipid profile and gut health of Kadaknath birds.

1. Introduction

Over the last five decades, the global poultry industry has undergone a significant transformation from subsistence farming to a highly business-oriented and intensive system of farming. Antibiotic growth promoters (AGPs) are commonly used in modern poultry production systems to increase the meat-producing capacity of animals. Nonetheless, the use of AGPs in poultry production may result in human and animal antibiotic resistance. The European Union's health authorities-imposed restrictions to reduce or prohibit the use of antibiotics in animal feed in 2006. Any restrictions on the use of AGPs in the production of eggs and meat will raise production costs,

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resulting in lower benefits. Likewise, today's consumers prefer organic products, and the demand for organic production has grown. Because of the cost-effectiveness and harmful residual effects of antibiotics, the use of herbal feed additive substances is gaining popularity in animal production [1,2]. Nonetheless, the use of AGPs in poultry production may result in human and animal antibiotic resistance. Because of the prohibition on the use of anti-biotics due to cost-effectiveness and harmful residual effects, the use of herbal feed added substance is gaining importance in animal production. Probiotics, prebiotics, synbiotics, organic acids, minerals, enzymes, and herbs can all be considered as replacement of antibiotic feed additives [2].

Kadaknath (KN) is one of the important native breed of poultry to western Madhya Pradesh of India and is mainly seen in the Jhabua district, India [3]. KN breed usually known as 'Kalamasi' is famous for its black meat and it is well known for poor egg production (80–120 eggs/year), slow growth rate, frequent broodiness, smaller body size (1.2–1.5 kg) as well as late sexual maturity (25–26 week).

Despite the importance of high yielding strains from across the world, the local breed still retains preference in its native environment mainly due to its special capabilities e.g., good foraging, efficient mothers and lower cost. KN is the preferred bird over exotic counterparts for landless labourers and marginal farmers because of their greater adaptability to extreme climatic conditions and resistance to protozoans and ecto-parasites. They are comparatively hardy and need minimum health care compared to other breeds [4]. KN eggs and meat are considered rich in protein and iron sources and the diminished presence of collagen filaments in KN meat is proposed to be the contributing variable to the unrivalled meat quality of KN chickens [5,6].

Glycyrrhiza glabra, usually known as liquorice, cultivated liquorice or licorice is a traditional crop, which have health benefits and medicinal uses have been dated for centuries [7]. *Glycyrrhiza glabra* is a perennial herb, usually known as mulethi or liquorice that is native to northern Africa, western Asia and Eurasia [8]. The Glycyrrhiza genus has nearly 30 species including *Glycyrrhiza inflata, Glycyrrhiza glabra* and *Glycyrrhiza eurycapra* [9]. Biological compounds such as saponins, flavonoids and triterpenes have been isolated [10]. The principal constituent of liquorice root is glycyrrhizin. It is a triterpene saponin that is about multiple times sweeter than sucrose, which is the primary active component [11]. In addition, 49 phenolic compounds and 15 different saponins (including their glycosides) have been identified in liquorice root [12]. Liquorice is used in so many conditions like arthritis, mouth ulcers and also a potential anti-inflammatory, antineoplastic, immunomodulatory, anti-oxidant, antimicrobial, detoxifying and also having growth-promoting effects [13].

Thus, the objective of this present study was to determine effects of liquorice root powder (LRP) on growth performance, serum lipid profile, carcass traits and gut health of Kadaknath birds.

Table 1
Composition of basal diet with inclusion of liquorice root powder in different levels.

Ingredients (%)	Starter diet (0–8 weeks)	Grower diet (9–15 weeks)
Maize	56.00	57.775
[a]DORB	7.27	15.17
Soyabean	31.455	21.51
Fishmeal	2.0	2.0
Limestone	0.95	1.0
[b]DCP	1.5	1.74
Salt	0.3	0.3
DL-Methionine	0.1	0.1
Lysine	0.06	0.04
[c]TM Premix-1	0.1	0.1
[d]Vit. Premix-2	0.15	0.15
[e]Vit. B. Complex	0.015	0.015
Ch. Chloride	0.05	0.05
Toxin binders	0.05	0.05
Estimated Value		
Metabolizable energy (MJ/kg)	11.80	12.15
Crude protein (%)	20	17
Ether extract (%)	2.5	3
Total calcium (%)	0.98	1.02
Total phosphorus %)	0.46	0.48

[a] DORB = De-oiled rice bran.
[b] DCP = Di-calcium phosphate.
[c] Trace minerals (TM premix-1 (mg/kg) diet: MgSO4•5H2O, 300 mg/kg; MnSO4•H2O, 55 mg/kg; KI, 0.4 mg/kg; FeSO4•7H2O, 56 mg/kg; ZnSO4•7H2O, 30 mg/kg; CuSO4•5H2O, 4 mg/kg.
[d] Vitamin premix-2: supplied per kg diet: vitamin A (retinol), 8250 IU; vitamin D3 (cholecalciferol), 1200 IU; vitamin K (menadione), 1 mg.
[e] B complex: supplied per kg diet: vitamin B1 (thiamine), 2 mg; vitamin B2, 4 mg; vitamin B2 (riboflavin), 10 μg; niacin (nicotinic acid), 60 mg; pantothenic acid, 10 mg; choline, 500 mg.

2. Materials and methods

2.1. Ethics statement

The experimental procedures carried out in the study were approved by the Institutional Animal Ethics Committee (IAEC) following the guidelines of 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) 2012' established under the 'Prevention of Cruelty to Animals Act 1960' of Indian Penal Code.

2.2. Supplements

The antibiotic Chlortetracycline (CTC) was purchased from M/s Atulya Agro and Healthcare, Nasik, Maharashtra, India. The root of liquorice (*Glycyrrhiza glabra*) was obtained from a local market in Bareilly, Uttar Pradesh, India. The root was ground to make as a fine powder.

2.3. Composition of liquorice root powder (LRP)

The estimated compositions of LRP are as follows: moisture (4.94 %), ash (4.95 %), protein (9.41 %), crude fibre (0.1 %), crude fat (0.54 %), calcium (1.98 %) and phosphorus (0.074 %) respectively.

2.4. Birds, experimental design, diets and management

The experiment was carried out in a completely randomized design (CRD). Day-old Kadaknath chicks (n = 240) with uniform body weight were selected randomly, weighed individually, and divided into six different treatments, each one with five replicates and eight birds per replicate, and raised in battery brooder cages for 15 weeks. Corn soya based basal diet (T_1) was prepared. In addition to the basal diet, five experimental diets were created with varying amounts of LRP i.e., T_2: T_1+ 0.1 % LRP, T_3: T_1+ 0.3 % LRP, T_4: T_1+ 0.5 % LRP, T_5: T_1+ 0.7 % LRP, and T_6: T_1+ 0.0335 % CTC. Table-1 shows the components and nutritional composition of the starter (0–8 weeks) and grower (9–15 weeks) phases of the basal diet.

2.5. Growth performance

Each dietary treatment provided a weighed quantity of the appropriate diet *ad lib* twice daily, with the residue weighed weekly to estimate feed consumption. Individual bird body weights were measured on day one and then every four weeks for the duration of the experiment to determine both the periodic and overall body weight growth. At the end of each trial period, the feed conversion ratio was determined as feed intake (FI) divided by body weight gain (BWG).

2.6. Carcass characteristics

At 15 weeks of age, six birds were chosen at random from each dietary treatment (n = 36) and slaughtered for evaluation of carcass characteristics such as carcass weight (%), blood weight (%), evisceration weight (%) and defeathered weight (%). The weight of each giblet, such as the gizzard, heart, and liver, was recorded and represented as a percentage of the pre-slaughter weight. In addition, the abdominal fat was recorded and reported as a percentage of the eviscerated weight.

2.7. Serum biochemical parameters

Blood samples were obtained at 15 weeks after hatching from six birds in each treatment to estimate serum biochemical such as serum enzymes, including aspartate aminotransaminase (AST) by modified Kind and King's [14] method; alanine amino-transaminase (ALT); Total cholesterol (CHOD/PAP procedure), HDL and LDL cholesterol, triglycerides (GPO/PAP method), and serum protein level (Biuret method) were estimated by Coral Clinical System Diagnostic ready-to-use kits.

2.8. Microbiological quality analysis of the intestinal

Microbial contents in the caecum, such as total coliform count and *Lactobacillus* spp. counts were determined by plating dilutions for coliform and lactobacillus counts on MacConkey and MRS agar, respectively [15]. The contents of each caecum were taken aseptically from six birds in each treatment group 16 weeks after hatching. After proper mixing of the contents, serial ten-fold dilutions were made, and duplicate plating was performed in a Class II biosafety cabinet. Colony counts were observed from countable dilutions and then reported as $\log_{10}$ value of colony-forming units per gram of intestinal contents ($\log_{10}$ cfu/g) after 24 and 48 h of incubation at 37 °C for coliforms and Lactobacillus count, respectively.

2.9. Intestinal morphometry

A portion of the jejunum (0.5–1 cm) was taken from six healthy birds per treatment at 16 weeks post-hatch after identification using

Meckel's diverticulum at the jejunoileal junction. After extracting the intestinal contents without destroying the mucosa, the collected piece was stored in a 10 % formalin solution until it was processed for histology slide preparation. The mounted slides were also examined under a microscope at low magnification (10xs). Zeiss zen microscope software was used to determine the height and average width of jejunal villi.

2.10. Statistical analysis

The data from several studies were submitted to a one-way ANOVA test of significance using SPSS20 software and the Snedecor and Cochran methods [16]. Tukey's [17] multiple comparison tests were used to separate means in all statistical analyses, and significance was indicated at $P \leq 0.05$.

3. Results

3.1. Growth performance

Significant ($P \leq 0.05$) increase in the body weight gain was noted in LRP supplemented group i.e., T_3 (0.3 % LRP) at 0–5 and 6–9 wks, compared to the control group (Table-2). There was a significant ($P \leq 0.01$) increase in the feed intake of birds which was fed with 0.3 % LRP (T_3) compared to the other treated group whereas, significant ($P \leq 0.01$) reduction in the feed intake was noted in the T_5 (0.7 % LRP) group. No significant difference in feed conversion ratio (FCR) was noted among control and LRP treated birds over the experimental study.

3.2. Carcass characteristics

Table 3 displays data on carcass characteristics and organ weights. Giblet organ weights, such as the liver, heart, and gizzard did not show a significant ($P > 0.01$) effect. There was no discernible effect in the heart or liver. The liver weights did not differ significantly, but almost all treatment birds had slightly larger liver weights than control (T_1) birds, with T_4 having the largest, followed by T_2 and T_5. There was no effect of dietary treatments on carcass yield weight. The control birds (T_1) had the most abdominal fat ($P \leq 0.05$), while the T_2 (0.1 % LRP) had the least.

3.3. Serum biochemical parameters

The effect of supplementing with liquorice root powder on the serum lipid profile of Kadaknath birds at 15 weeks is shown in Table 4. Serum triglyceride levels, total cholesterol levels, and low-density lipoprotein (LDL) levels all significantly decreased across treatment groups ($P \leq 0.01$), with group T_5 (0.7 % LRP) experiencing the largest decreases. On the other hand, when compared to the control group and the antibiotic-treated group, the high-density lipoprotein (HDL) level in the LRP-treated groups significantly ($P \leq 0.01$) rose. The T_5 (0.7 % LRP) had the highest level of HDL improvement. The total and LDL concentrations were higher in the control (T_1) and treatment (T_6) groups of birds than in the treatment groups. Increased LRP inclusion (T_4 and T_5) may result in lower levels of LDL and total cholesterol in the blood. No differences in serum total protein, albumin, or globulin concentrations were found between Kadaknath fed a control diet or a diet supplemented with varying doses of liquorice root powder ($P > 0.05$). The levels of the serum enzymes ALT and AST did not significantly differ between the treatment groups and the control groups. A higher level of serum

Table 2
Effects of dietary supplementation of Liquorice root powder on growth performance of Kadaknath chickens.

Weeks	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p*-value
Body weight gain (g)								
0–5 wk	125.12^{ab}	136.04^{c}	136.20^{c}	121.50^{a}	124.80^{ab}	130.12^{b}	3.95	0.023
5–10wk	289.80^{a}	304.20^{b}	322.92^{b}	289.22^{a}	286.08^{a}	304.16^{ab}	9.45	0.047
10–15 wk	291.58	294.12	321.10	290.48	272.26	293.35	13.55	0.217
0–15 wk	959.49^{ab}	995.70^{b}	1070.51^{c}	973.36^{b}	935.69^{a}	984.77^{b}	29.17	0.038
Feed intake (g)								
0–5 wk	338.22	341.96	349.04	340.92	339.18	347.42	1.45	0.073
5–10 wk	871.60^{b}	913.40^{ab}	938.04^{a}	869.40^{b}	874.96^{b}	905.35^{ab}	4.82	0.001
10–15wk	1314.5^{b}	1383.7^{a}	1456.7^{a}	1273.2^{c}	1264.3^{c}	1363.1^{b}	9.56	0.001
0–15 wk	4179.97^{b}	4286.30^{ab}	4515.79^{a}	4085.72^{b}	3996.58^{c}	4288.62^{ab}	36.25	0.001
Feed conversion ratio (FCR)								
0–5 wk	2.87	2.64	2.65	2.93	2.82	2.76	0.07	0.131
5–10 wk	3.25	3.12	2.98	3.18	3.23	3.17	0.15	0.672
10–15wk	5.29	4.94	4.81	4.85	5.48	5.29	0.32	0.709
0–15 wk	4.56	4.42	4.33	4.34	4.41	4.47	0.14	0.799

Mean values bearing the same superscript in a row did not differ significantly ($P \geq 0.05$ and $P \geq 0.01$).
T1 = Control, T2 = T1+0.1 % LRP, T3 = T1+ 0.3 % LRP, T4 = T1+ 0.5 % LRP, T5 = T1+ 0.7 % LRP, T6 = T1+ + 0.0335 % CTC.
SEM= Standard error of mean.

Table 3
Effects of dietary supplementation of *Liquorice root powder* on carcass characteristics and organ yield (% of live weight) yield of Kadaknath chickens.

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p*-value
Carcass weight	96.98	96.97	96.96	96.99	96.93	96.98	0.22	0.861
Blood loss	3.02	3.03	3.03	3.01	3.07	3.02	0.03	0.861
De-feathered weight	80.75	80.94	80.90	80.63	79.75	80.54	4.57	0.253
Eviscerated weight	67.16	67.36	67.612	67.44	67.19	67.43	2.86	0.507
Abdominal fat	1.56	1.40	1.41	1.42	1.41	1.54	0.05	0.044
Heart	0.42	0.40	0.40	0.40	0.42	0.41	0.05	0.915
Liver	1.52	1.62	1.56	1.63	1.62	1.61	0.12	0.617
Gizzard	2.44	2.56	2.39	2.27	2.35	2.48	0.32	0.693

Mean values bearing the same superscript in a row did not differ significantly ($P \geq 0.05$ and $P \geq 0.01$).
T1 = Control, T2 = T1+0.1 % LRP, T3 = T1+ 0.3 % LRP, T4 = T1+ 0.5 % LRP, T5 = T1+ 0.7 % LRP, T6 = T1+ + 0.0335 % CTC.
SEM= Standard error of mean.

Table 4
Effects of dietary supplementation of Liquorice root powder on biochemical profile of Kadaknath chickens.

	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p*-value
Serum Proteins (g/dl)								
Total protein	4.24	4.14	4.15	4.17	4.21	4.21	0.42	0.058
Albumin	2.52	2.72	2.64	2.78	2.71	2.62	0.15	0.078
Globulin	1.71	1.41	1.50	1.38	1.50	1.58	0.18	0.071
Serum lipids (mg/dl)								
Triglyceride	161.31^{a}	151.11^{ab}	153.81^{ab}	149.11^{b}	130.61^{c}	155.91^{ab}	2.25	0.024
Totalcholesterol	203.10^{a}	196.89^{a}	193.79^{a}	193.02^{a}	171.31^{b}	204.65^{a}	3.22	0.001
HDL	44.43^{c}	51.23^{b}	55.83^{ab}	58.03^{ab}	64.93^{a}	46.73^{bc}	2.50	0.042
LDL	79.96^{a}	70.73^{c}	72.11^{b}	74.27^{ab}	73.50^{b}	79.19^{a}	2.30	0.035
Serum enzymes (U/L)								
AST	165.89	167.02	165.54	169.50	168.79	166.46	2.06	0.74
ALT	4.14	4.37	4.37	4.29	4.29	4.06	0.36	0.99

Mean values bearing the same superscript in a row did not differ significantly ($P \geq 0.05$ and $P \geq 0.01$).
T1 = Control, T2 = T1+0.1 % LRP, T3 = T1+ 0.3 % LRP, T4 = T1+ 0.5 % LRP, T5 = T1+ 0.7 % LRP, T6 = T1+ + 0.0335 % CTC.
SEM= Standard error of mean.

enzymes was found when values were quantitatively compared between the LRP included diet and the two control groups, though this difference was not statistically significant.

3.4. Enumeration of bacterial contents

Table 5 shows the bacterial count (*lactobacillus*) and total coliforms count, both expressed in colony-forming units (CFU). However, a significant ($P \leq 0.01$) dose-dependent linear rise in Lactobacillus count was noticed in the caecum of treated birds. The highest *lactobacillus* count in the caecum is found in T_5 (0.7 % LRP), followed by T_4 (0.5 % LRP). Contrarily, the control (T_1) showed a low level of *lactobacillus*. The total coliform count in the caecum of birds treated with LRP decreased significantly ($P \leq 0.05$) and exhibited an inverse relationship with the level of LRP inclusion (Table$_5$). LRP had a dose-dependent antibacterial effect. Out of all the groups, T_3 had the fewest colonies (0.3 %). Comparatively to LRP-treated groups, the control group (T_1) showed a higher coliform count.

3.5. Intestinal histo-morphometry

The histo-morphometry of the jejunum is displayed in Table-5. Dietary interventions had a significant ($P \leq 0.01$) impact on the

Table 5
Effects of dietary supplementation of LRP on jejunal histomorphometry and caecal gut microbiology of Kadaknath chicken.

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p*-value
VH (μm)	1103.05^{b}	1220.09^{a}	1224.08^{a}	1223.55^{a}	1159.41^{ab}	1172.28^{ab}	19.33	0.001
VW *(μm)*	187.24^{b}	189.25^{ab}	196.10^{a}	203.11^{a}	195.54^{a}	192.30^{ab}	5.36	0.037
CD *(μm)*	138.78	135.22	134.27	139.00	136.26	138.35	4.76	0.96
VH: CD	7.96^{b}	9.05^{ab}	9.16^{a}	8.83^{ab}	8.54^{ab}	8.59^{ab}	0.45	0.048
Lactobacillus *(log_{10} cfu/g)*	5.20^{c}	5.22^{bc}	5.34^{b}	5.56^{a}	5.68^{a}	5.52^{ab}	0.05	0.001
Coliforms *(log_{10} cfu/g)*	6.37^{a}	5.91^{ab}	5.65^{b}	6.08^{ab}	6.03^{ab}	5.99^{ab}	0.13	0.033

Mean values bearing the same superscript in a row did not differ significantly ($P \geq 0.05$ and $P \geq 0.01$).
T1 = Control, T2 = T1+0.1 % LRP, T3 = T1+ 0.3 % LRP, T4 = T1+ 0.5 % LRP, T5 = T1+ 0.7 % LRP, T6 = T1+ + 0.0335 % CTC.
SEM= Standard error of mean.

villus height and other histomorphological features of the jejuna. The depth of the crypt, however, did not differ significantly. Villus height and the ratio of the depth of the crypts in the jejunal mucosa were statistically ($P \leq 0.05$) higher in the T_3 (0.3 % LRP) group than in the control group. The minimum villus height and crypt depth in control birds (T_1) were measured.

4. Discussion

Dietary inclusion of liquorice root powder could produce significant change in feed intake and weight gain over the experimental period (0–15 weeks). The feed conversion ratio, on the other hand, remained unaltered. The above finding is similar with the early findings of Nimje et al. [18] who reported that significant increase in the body weight gain in broilers which were fed with 0.25 % liquorice root powder. Similarly, Myandoab and Hosseini [19] concluded that feed conversion ratio (FCR) decreased while there was an increase in average body weight and feed intake in quails fed containing 200 parts per million of liquorice root extract and 1 % probiotic in their diet. In contrast to the above results, Al-Sofee [20] concluded that the basal diet for Japanese quails supplemented with 0.5 %, 1 %, 1.5 % of crushed liquorice root/kg ration and fed *ad libitum* ration and water showed no significant differences in average total weight gain. The increase in body weight gain at a lower level of liquorice inclusion may be attributed to plant extracts having digestion and hunger-stimulating characteristics, as well as growth boosting due to increased feed utilisation efficiency [21]. Upregulation of growth hormone and hepatic growth hormone receptor, which increases the concentration of insulin-like growth factor-1, may explain the beneficial effect of *G. glabra* flavonoids such as glabridin, liquiritigenin, and isoliquiritoside on broiler growth performance thereby increasing growth rate [22]. Addition of liquorice root powder above 3 g/kg decreased the feed intake and bodyweight gain which could be due to the aromatic odour of liquorice root powder [23]. Studies also revealed that liquorice flavonoids can reduce the content of fat in the body and contribute to the loss in body weight [24]. This may be the reason behind the reduction in the body weight gain when liquorice is fed in higher levels.

In different treatment groups, significantly higher feed intake is noted in the T3 treatment group (0.3 % LRP), and significantly lesser feed intake is noted in the T_5 (0.7 % LRP). The above findings are similar to Salary et al. [25] who reported that feed consumption was increased it may be due to stimulation of appetite by a change in feed taste. These results are in contrast with Sedghi et al. [26] who showed that diets supplemented with 0, 2, 4, or 6 gm/kg of liquorice extract did not influence feed intake and FCR in broilers. The present study showed that there is no significant difference in the FCR and the result of the present study are in agreement with Dogan et al. [20]; Al-Sofee [23].

In the current study, the carcass percentages have no significant difference between LRP supplemented diets and control diets. These results are similar to Hosny et al. [27] who concluded that liquorice extract supplementation in Japanese quails had no significant effect on carcass percentage of both females and males compared to control. The above results are contrasted with Myandoab and Hosseini [19] who reported that supplementation of 200 ppm of the liquorice root extract in Japanese quail resulted in a significant increase in the carcass percentages. In the current study, the giblet organs such as liver, heart, and gizzard have no significant difference between the control group and liquorice supplemented groups. These results are similar to Sedghi et al. [26] and Moradi et al. [28]. They reported that the weight of the liver had no significant difference by supplementing liquorice extract along with drinking water in broilers. On the other study, Myandoab and Hosseini [19] revealed that liquorice extract supplementation of 200 ppm to Japanese quails was no huge difference in gizzard weight and announced significant elevation in the liver weight contrasted with control.

In the present study, there is a significant difference in the abdominal fat (AF) compared to the control group. Similar kinds of results were obtained by Moradi et al. [29] who stated that AF showed a significant decrease when broilers were given water that contains 0.3 g/l liquorice. The decline in AF was may be attributed to the presence of hydrophobic flavonoids found in liquorice [30]. In the present study, the cholesterol level, triglycerides, and LDL are significantly reduced and the serum HDL level is significantly increased in liquorice-fed treatments comparing to the control group. The above results are similar to Moradi et al. [29] who concluded that chickens are given drinking water and Liquorice Extract (0.1, 0.2, or 0.3 g/L) resulted in a significant reduction in serum total cholesterol and LDL. Myandoab and Hosseini Mansoub [19], Al-Sofee [20] and Dogan et al. [23] also reported the similar kind of results. They concluded that significant increase in the serum HDL and a significant decrease in the serum LDL. In contrast to the present results, no significant difference in plasma triglyceride was seen in broiler chickens that were fed with liquorice root powder (0 %, 0.5 %, 1 %, 2 %) [23]. According to Visavadiya and Narasimhacharya [31] the cholesterol-lowering actions of liquorice extract in rats are due to greater cholesterol and bile acid elimination, as well as an increase in hepatic bile acid levels. Phytosterols, saponins, and fibre in liquorice extract may play a function in cholesterol clearance and an increase in hepatic bile acid concentration in liquorice extract given animals in this context. As a result, these substances can selectively displace cholesterol from micelles in the intestinal lumen, lowering intestinal cholesterol absorption and blood cholesterol levels [32]. Saponins, on the other hand, have been found to form insoluble complexes with cholesterol in the digestive tract and to alter bile acid circulation in the enterohepatic system, rendering them inaccessible for intestinal absorption [33]. Dietary fibres also appear to obstruct cholesterol absorption and enterohepatic bile circulation, resulting in a depletion of hepatic cholesterol stores and an increase in the rate of cholesterol clearance from the bloodstream. Furthermore, fiber's cholesterol-lowering ability appears to be mostly due to increased cholesterol and bile acid excretion [34]. In birds, a significant reduction of serum LDL correlated to the saponin and fiber content of liquorice. the hepatic LDL receptor levels increased by the saponins and fiber content of liquorice, increases the rate of cholesterol translation to bile acids, and improve the hepatic clearance of LDL cholesterol from circulation Although dietary saponins and fibers have not been shown to enhance HDL cholesterol levels [33,34]. Ascorbic acid and flavonoids [35,36] have been increasing the HDL concentration level in serum. In the current investigation, the liquorice extract included both ascorbic acid and flavonoids, which might have led to an elevation in the HDL to LDL ratio. One of the root's main components, glabridin, is said to be a powerful antioxidant that inhibits LDL

oxidation [37]. In the current study, the triglycerides level has a significant difference among the different groups fed with liquorice supplementation. These results are similar to Abdul-Majeed [38] who concluded that in the Japanese quail ration, 500 mg, 1000 mg, 1500 mg of liquorice root powder/kg was supplemented and it showed a significant decrease in triglyceride level compared to the control group. The results are in contrast to Dogan et al. [23] who concluded that there is no significant difference in plasma triglyceride in broiler chickens that were fed with liquorice root powder (0 %, 0.5 %, 1 %, 2 %).

In the current study, no significant difference was observed in total protein in the dietary treatment groups which was fed with liquorice different level. The above study is similar to Abdul-Majeed[38] who also reported that there was no change among the groups in serum protein levels that were fed with liquorice root powder (500 mg, 1000 mg, 1500 mg/kg) in Japanese quails. Result of the present study differ from those of Rezaei et al. [39] who concluded that the diets containing *G. glabra* showed a significant increase in total protein content. In the current study, the serum enzymes like AST and ALT have no significant difference between various groups fed with liquorice supplementation comparing to control groups. The above results were similar to Reda et al. [40] and Guo et al. [41] and they concluded that there were no aberrant results in any of the blood indicators that were tested. Furthermore, liquorice supplementation did not affect the levels of ALT and AST (liver metabolic enzymes), suggesting that liquorice had neither toxicity nor damage in the animals.

In the current study, the *lactobacillus* count and coliform counts in the caecum have significantly increasing and decreasing respectively. The above finding is similar to Jyotsana and Berwal [42] who reported the supplementation of liquorice supplementation reduced then coliform counts and increasing the lactobacillus count. The reduction in the coliform count may be attributed to the antimicrobial activity of liquorice. The liquorice extracts showed antibacterial activities against 2 g-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and 2 g-positive (*Staphylococcus aureus* and *Bacillus subtili*) bacteria [43]. Another study found that including the plant extract mixed had a significant effect on caecal microflora count (raised *lactobacilli* count and reduced coliforms) [44].

The small intestine is an important digestive organ that helps the body absorbs nutrients. As a result, any enhancements to this component are essential to broiler health and performance [45]. Increases in VH and VH: CD ratio lead to improved nutrient absorption, which has a beneficial impact on growth performance [46]. In the present study, the villus height and VH: CD shows a significant increase in the liquorice supplemented diet compared to control. The above results are similar to Jyotsana and Berwal [42]. Maybe it's because of using phytogenic extracts in bird's causes an increase in villus height due to a decrease in harmful bacteria in the intestinal wall, thereby reducing the by-products of these bacteria, such as toxic compounds, which have a negative effect on the epithelial cells of the intestine, and finally inhibiting villus destruction and minimizing lumen repair [47].

It is concluded here that the dietary inclusion of liquorice root powder, especially in T_3 (0.3 %) group has an instrumental role to improve serum lipids as well as gut health of Kadaknath birds over control and antibiotic supplemented birds without affecting the growth performance adversely.

CRediT authorship contribution statement

Gowthaman V.: Investigation, Data curation. **Divya Sharma:** Project administration, Methodology, Formal analysis, Conceptualization. **Chandra Deo:** Writing – original draft, Supervision, Formal analysis. **Tiwari A.K.:** Writing – review & editing, Supervision. **Avishek Biswas:** Writing – review & editing, Conceptualization.

Data and code availability statement

Data will be made available on request.

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Declaration of competing interest

All the authors have read the manuscript and have agreed to submit it in its current form forconsideration for publication in the journal.

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