



Research Article

Effects of Fenugreek (*Trigonella Foenum-Graecum*) Seed Meal on Growth Performance, Haematological Indices, Lipid Profile, Liver Functions, and Immunological Response in Broiler Chickens

Grace Olobumi Sodipe^{ID}, Godfrey Odeh Gabriel^{ID}, Adetunmbi Tella^{ID}, Opeyemi Ayomide Adesina^{ID}, Pelumi Priscilla Owolabi^{ID}, Daniel Timileyin Eytayo^{ID}, Oluwatimileyin Ahmed Jegede^{ID}, Janet Bosede Oke^{ID}, Adesina Samuel Adisa^{ID}, Precious Adetomiwa Ajiboye^{ID}, and Ephraim Temiloluwa Ogungbemi^{ID}

Department of Animal Production and Health, Faculty of Agriculture, Federal University Oye-Ekiti, Ekiti State, Nigeria

* **Corresponding author:** Gabriel Godfrey Odeh, Department of Animal Production and Health, Faculty of Agriculture, Federal University Oye-Ekiti, Ekiti State, Nigeria. Email: godfrey.gabriel@fuoye.edu.ng

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ABSTRACT

Introduction: Fenugreek (*Trigonella foenum-graecum*) seed meal is rich in bioactive phytochemicals that can enhance growth and health. The present study aimed to evaluate the effects of graded dietary levels of fenugreek seed meal on growth performance, carcass traits, haematology parameters, lipid profile, liver function, and thyroid hormones in broiler chickens.

Materials and methods: A total of 256 day-old broiler chickens were divided into four dietary treatments, replicated four times with 16 chickens per replicate. The groups included the control diet (T1), diet containing 100 g/kg of fenugreek seed meal (T2), 200 g/kg of fenugreek seed meal (T3), and 300 g/kg of fenugreek seed meal (T4). The study lasted 49 days, and at the end of the study, growth performance, carcass traits, and haematological parameters were measured. Serum lipid profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and liver enzymes, including alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST), were analysed. Thyroid hormones, including thyroxine (T₄) and triiodothyronine (T₃), and thyroid-stimulating hormone (TSH), were measured by enzyme-linked immunosorbent assay.

Results: Fenugreek supplementation significantly improved final body weight, weight gain, and feed conversion ratio in T3 and T4 compared to the control group. Carcass weight and defeathered weight in supplemented groups increased compared to the control group. Haematological analysis revealed the highest haemoglobin and red blood cell (RBC) counts in T2, while T3 exhibited the lowest RBC and the highest neutrophil count. Lipid profile evaluation indicated reduced levels of total cholesterol, triglycerides, and LDL, with elevated HDL in Fenugreek-supplemented groups compared to the control group. Liver function profile demonstrated decreased levels of ALP and ALT across all groups, with the lowest values observed in T4. There were no significant effects on AST among all groups. The free T₃ levels were significantly increased in T3 and T4, while free T₄ and TSH levels indicated no significant differences compared to the control group.

Conclusion: Dietary inclusion of 200-300 g/kg fenugreek seed meal effectively improved growth performance, carcass yield, haematological parameters, lipid metabolism, liver enzyme profile, and T₃ levels in broiler chickens, highlighting its potential as a functional phytochemical feed additive in poultry production.

1. Introduction

Food insecurity is a critical variable for understanding the nutritional status of low-income populations in the world¹. Undernourishment is least common in African countries, with 32% of the population, about one in three

Africans, affected by undernutrition². However, this issue persists in Nigeria, where nutrition, health, and management continue to limit animal growth and productivity³. In developing countries such as Nigeria,

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Ghana, and South Africa, these factors still play major roles in poultry outcomes³. Feed additives are essential for optimal performance and productivity in modern poultry production⁴. Sub-therapeutic levels of antibiotics have been extensively used in commercial poultry production to maintain health, improve growth performance, and feed conversion ratio (FCR)⁵.

The use of antibiotics as feed additives and as a growth promoter has been restricted in many countries, mainly in European countries, because of the increasing concerns about the persistence of antibiotic residues in animal products and the transmission of antibiotic resistance, both of which pose a potential threat to human^{6,7}. The European Union (EU) banned the use of antibiotics as growth promoters in animal production in January 2006; the industry needed alternatives. There are significant reasons to consider new strategies to sustain optimal poultry production without relying on antibiotics⁸. This led to the rise of natural growth promoters, including organic acids, prebiotics, probiotics, phytogenic feed additives, essential oils, and enzymes, as alternatives to conventional growth enhancer antibiotics^{9,4,10}.

Fenugreek (*Trigonella foenum-graecum* L.) is a Fabaceae plant with a long history of medicinal use that dates back to its early cultivation in Central Asia¹¹. Fenugreek seeds have been used in traditional medications for centuries to treat digestive problems, diabetes, and respiratory issues¹¹. Chemically, fenugreek is rich in fiber, lipids, essential fatty acids, vitamins, and bioactive compounds, including phospholipids, glycolipids, oleic, linoleic, and linolenic acids, choline, niacin, and nicotinic acid¹¹⁻¹⁴. Recent studies have highlighted fenugreek seeds' prebiotic properties attributed to their high content of soluble fibre, saponins, and antimicrobial compounds^{12,15}. The fenugreek seeds include alkaloids, steroidal saponins, flavonoids and polyphenols, amino acid derivatives, coumarins and tannins, vitamins and minerals, and volatile compounds. Fenugreek can inhibit the growth of pathogenic bacteria, support the proliferation of beneficial gut microbiota, and enhance nutrient absorption¹². Some studies have reported that fenugreek can stimulate humoral and cellular immune responses and regulate body immunity¹⁶. Recently, there has been growing interest in the use of natural feed additives as alternatives to synthetic growth promoters, driven by concerns over antibiotic resistance, chemical residues, and consumer demand for organic poultry products⁷. The present study aimed to assess the effects of fenugreek seeds on growth performance, carcass and organs traits, haematology indices, lipid profile, liver function, and thyroid hormones in broiler chickens, thereby offering poultry farmers and nutritionists a natural alternative to enhance flock performance.

2. Materials and Methods

2.1. Ethical approval

The present study was conducted with the approval of the Animal Welfare and Ethics Committee of the Department of Animal Production and Health, Federal University Oye-Ekiti, Nigeria (Approval number: APH/R-010/20/17/25), and adhered to the ethical guidelines for the use of animals in research.

2.2. Fenugreek preparation

Fenugreek seeds (*Trigonella foenum-graecum*) were obtained from local markets in Ikole Local Government Area, Ekiti State, Nigeria. The seeds were verified at the Department of Plant Science and Biotechnology, Federal University, Oye-Ekiti, Nigeria. The seeds were then carefully washed with clean water and air-dried away from sunlight at ambient temperature (25°C) and relative humidity ranging from 60 to 70% for 14 hours a day. After drying, the samples were ground into a fine powder using an electric grinder (Model BLG-450, Kenwood Ltd., Havant, UK) and sieved to ensure a uniform particle size. The powder was stored in airtight, moisture-free containers at room temperature until its further use in the experimental diets.

2.3. Experimental design

A total of 256 one-day-old female Arbor Acre broiler chickens, weighing 38-41 grams, obtained from Amo Farm Sieberer Hatchery in Oyo State, Nigeria, were used in the present study. The broiler chickens were divided into four treatment groups with four replicates of 16 chickens each. The dietary inclusion levels of fenugreek seed meal (100, 200, and 300 g/kg) were selected based on the experimental framework of Weerasingha and Atapattu¹⁷ (2013), but with critical modifications to investigate higher dosage thresholds. While the Weerasingha and Atapattu¹⁷ study evaluated levels up to 50 g/kg, the present study extended the upper limit to 300 g/kg to thoroughly assess the effects of fenugreek as a high-inclusion feed ingredient rather than a low-dose additive. Treatment groups included the control group (T1), which received a basal diet (BD) with no additives, T2 received 100g/kg of fenugreek seed meal, T3 included 200g/kg of fenugreek, and T4 received 300g/kg of fenugreek per kg diet¹⁷. The fenugreek seed meal was evaluated in broiler chickens without any adverse effects¹⁶. The experimental pens measured 1.3 m by 1.2 m and were furnished with dry wood shavings serving as bedding material. In the first week of the experiment, the room temperature was maintained at 33 ± 1°C with heating facilities. The temperature was gradually reduced every other week until it reached 24 ± 1°C. Humidity ranged from 60 to 70% during the first week and 50 to 60% thereafter⁴. The chickens were vaccinated against Newcastle disease (Nobilis ND LaSota, The Netherlands), administered orally at 7 and 21 days of age, and against Gumboro disease at 14 and 28 days of age using an intermediate live vaccine (Nobilis® Gumboro D78, The Netherlands), administered via drinking water. Vaccination programs adhered to the manufacturer's instructions and the guidelines of the WOA¹⁹. Lighting during the night was provided for feeding

using an automated Ecko 400 W solar-LED floodlight (Ecko Solar Ltd., Lagos, Nigeria). The ingredients and nutrient compositions of the basal diets during the starter (1-21

days) and finisher (22-49 days) periods are presented in Table 1, formulated to meet the nutrient requirements of broiler chickens in accordance with Aviagen²⁰.

Table 1. Ingredients and nutrient composition of the experimental basal diets for broiler chickens

Ingredients (kg)	Starter (1-21 d)	Finisher (22-49 d)
Maize	50.50	56.00
Soybean	36.00	30.00
Maize gluten meal	6.00	6.00
Fish meal	3.50	2.00
Soybean oil	1.50	3.55
Limestone	1.50	1.50
NaCl	0.25	0.25
Vitamin-mineral premix*	0.25	0.25
Lysine	0.20	0.15
Methionine	0.30	0.30
Total	100.00	100.00
Calculate composition (%)		
Crude protein	23.00	20.00
Crude fibre	4.68	3.94
Calcium	1.24	1.15
Available phosphorus	0.79	0.73
Lysine	1.27	1.08
Methionine	0.53	0.58
Metabolizable energy (kcal/kg)	12.25	13.18

*Vitamin-mineral premix (per 1kg) contains antioxidant (500 mg), biotin (300 mg), chlorine chloride (70000 mg), cobalt (80 mg), copper (1200 mg), folic acid (200 mg), iodine (400 mg), iron (8000 mg), manganese (16000 mg), niacin (8000 mg), pantothenic acid (2000 mg), selenium (80 mg), vitamin A (3400000 IU), vitamin B1 (640 mg), vitamin B12 (4 mg), vitamin B2 (16000 mg), vitamin B6 (600 mg), vitamin D3 (60000 IU), vitamin E (4000 mg), vitamin K3 (600 mg), zinc (12000 mg).

2.4. Growth performance

The feed was provided to the broiler chickens on a daily basis, and the daily feed intake was determined by subtracting the remaining feed. The broiler chickens were weighed weekly, and consequently, the body weight gain (BWG) was calculated according to the following formula²⁰. $BWG = \text{Final body weight (FBW, g)} - \text{Initial body weight (g)}$.

Based on the feed intake and BWG, the FCR was estimated by dividing the feed intake by the BWG of the broiler chickens²¹.

2.5. Carcass traits

At the end of the experiment, after a 12-hour feed withdrawal, three chickens from each replicate were selected, weighed for the live weight before being humanely slaughtered, after which the chickens were allowed approximately 180 seconds to bleed²². After bleeding, the carcasses were defeathered and eviscerated to obtain the carcass weight. The carcass percentage was calculated by dividing the carcass weight (g) by the live weight (g) and then multiplying the result by 100²¹. Other body parts, such as the breast, wing, head, neck, drumstick, thigh, shank, liver, spleen, heart, intact gizzard, empty gizzard, and kidney, were weighed and measured accordingly.

2.6. Blood sampling

On day 49, three chickens per replicate were randomly selected for blood sampling. Approximately 5 mL of a fresh blood sample was collected from the brachial vein using disposable syringes and needles, which were then promptly transferred into sample bottles containing EDTA for hematological analysis, including a complete blood cell count with a WBC differential, and into serum tubes without coagulant for serum biochemistry and thyroid hormone analysis. Haematology parameters, including packed cell

volume (PCV, %), red blood cell (RBC, $\times 10^{12}/L$) count, haemoglobin (Hb, g/dL), white blood cell (WBC, $\times 10^9/L$) count, neutrophil (%), lymphocyte (%), monocyte (%), eosinophil (%), and basophil (%)⁴ were subsequently analysed via an automated LaserCyte Haematology Analyser (IDEXX Laboratories Inc., Westbrook, Maine, USA). Blood samples from the serum tubes were centrifuged at 3500 rpm for 15 minutes to obtain clear supernatant serum. The serum was used to analyse total protein (g/dL), albumin (g/dL), globulin (g/dL), cholesterol (mg/dL), triglyceride (mg/dL), high-density lipoprotein (HDL, mg/dL), low-density lipoprotein (LDL, mg/dL), creatinine (mg/dL), aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), potassium (mmol/L), sodium (mmol/L), chloride (mmol/L), Bicarbonate (mmol/L), total calcium (mmol/L), and magnesium (mmol/L) using an automated Vet Test Chemistry Analyser (IDEXX Laboratories Pty Ltd, South Africa). Serum concentrations of triiodothyronine (T_3 , pg/mL), thyroxine (T_4 , ng/dL), and thyroid-stimulating hormone (TSH, $\mu IU/mL$) were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Shanghai, China), following the manufacturer's instructions.

2.7. Statistical analysis

All the data were subjected to one-way analysis of variance (ANOVA) using the General Linear Model procedure (PROC GLM) in SAS version 9.4. Significant treatment means ($p < 0.05$) were compared using Tukey's Honestly Significant Difference test. The standard error of the mean (SEM) was employed, and all data are presented as mean \pm SEM.

3. Results

3.1. Growth performance

Table 2 represents the effects of fenugreek seed supplementation on the growth performance of broiler chickens. The results revealed significant differences in FBW, BWG, and FCR ($p = 0.017$, 0.014 , and 0.001 , respectively). Chickens in T3 and T4 demonstrated significantly higher FBW, with T3 reaching 3030.41 g and T4 achieving 3102.22 g, as well as greater BWG, with T3 at

2989.66 g and T4 at 3061.84 g, compared to the FBW of T1 at 2748.44 g ($p = 0.017$) and BWG of T1 at 2708.10 g ($p = 0.014$). Although total feed intake was not significantly affected by dietary treatments ($p > 0.05$), FCR improved significantly across all fenugreek-supplemented groups, with T2 showing an intermediate improvement (1.69) and T3 and T4 showing the greatest improvement (1.60) compared to the control group (1.74; $p < 0.05$).

Table 2. Effect of fenugreek seeds on the growth performance in broiler chickens at eight weeks of age

Groups	T1	T2	T3	T4	p-value
Parameters					
Initial body weight (g)	40.34 ± 0.23	40.11 ± 0.23	40.75 ± 0.23	40.38 ± 0.23	0.871
Final body weight (g)	2748.44 ± 45.62 ^b	2891.39 ± 45.62 ^{ab}	3030.41 ± 45.62 ^a	3102.22 ± 45.62 ^a	0.017
Body weight gain (g)	2708.10 ± 46.18 ^b	2851.28 ± 46.18 ^{ab}	2989.66 ± 46.18 ^a	3061.84 ± 46.18 ^a	0.014
Total feed intake (g)	4703.39 ± 38.55	4811.51 ± 38.55	4798.20 ± 38.55	4889.39 ± 38.55	0.103
Feed conversion ratio	1.74 ± 0.03 ^a	1.69 ± 0.03 ^b	1.60 ± 0.03 ^c	1.60 ± 0.03 ^c	0.001

T1: Control group with basal diet only, T2: Basal diet + 100 g fenugreek per kg of feed, T3: Basal diet + 200 g fenugreek per kg of feed, T4: Basal diet + 300 g fenugreek per kg of feed. ^{a, b, and c} Means with different superscript letters within the same row differ significantly ($p < 0.05$). Data are presented as the standard error of mean.

3.2. Carcass and organ quality

The results of the effect of fenugreek indicated a significant difference in carcass weight and defeather weight ($p < 0.05$; Table 3). Broiler chickens fed fenugreek had higher carcass weight and defeather weight compared to the control group ($p < 0.05$). No significant difference was observed in other carcass and other organ parameters in

all the groups ($p > 0.05$). Organ weights, including heart, liver, gizzard, lungs, spleen, and proventriculus, were not significantly affected, indicating that fenugreek supplementation did not negatively impact the development of the internal organs ($p > 0.05$). Breast, thigh, drumstick, wing, and back weights were numerically higher in the supplemented groups, but the differences were not statistically significant ($p > 0.05$).

Table 3. Effect of fenugreek seeds on the carcass and organ quality in broiler chickens at eight weeks of age

Groups	T1	T2	T3	T4	p-value
Parameters					
Live weight (g)	2645.75 ± 37.33	2838.75 ± 37.33	2835.50 ± 37.33	2877.00 ± 37.33	0.105
Carcass weight (g)	2564.00 ± 36.94 ^b	2766.25 ± 36.94 ^a	2766.00 ± 36.94 ^a	2819.50 ± 36.94 ^a	0.049
Defeather weight (g)	2434.25 ± 39.80 ^b	2681.75 ± 39.80 ^a	2669.00 ± 39.80 ^a	2723.50 ± 39.80 ^a	0.022
Thigh (g)	171.25 ± 7.26	206.50 ± 7.26	192.50 ± 7.26	193.38 ± 7.26	0.330
Drumstick (g)	165.25 ± 6.06	202.50 ± 6.06	193.00 ± 6.06	195.75 ± 6.06	0.128
Breast (g)	790.00 ± 9.01	826.00 ± 9.01	838.25 ± 9.01	848.00 ± 9.01	0.098
Wing (g)	156.50 ± 2.43	167.25 ± 2.43	168.50 ± 2.43	166.75 ± 2.43	0.288
Back (g)	323.25 ± 9.81	352.00 ± 9.81	360.00 ± 9.81	356.25 ± 9.81	0.580
Heart (g)	11.25 ± 0.33	11.50 ± 0.33	12.50 ± 0.33	11.50 ± 0.33	0.582
Liver (g)	35.50 ± 1.30	38.25 ± 1.30	37.00 ± 1.30	39.25 ± 1.30	0.798
Intact gizzard (g)	65.50 ± 3.10	65.50 ± 3.10	60.75 ± 3.10	69.25 ± 3.10	0.847
Empty gizzard (g)	40.00 ± 1.61	41.25 ± 1.61	39.50 ± 1.61	44.75 ± 1.61	0.699
Lungs (g)	13.42 ± 0.21	12.90 ± 0.21	12.53 ± 0.21	12.88 ± 0.21	0.565
Neck (g)	70.32 ± 2.20	79.68 ± 2.20	71.64 ± 2.20	75.10 ± 2.20	0.465
Shank (g)	83.25 ± 0.67	85.00 ± 0.67	81.50 ± 0.67	84.25 ± 0.67	0.300
Head (g)	52.00 ± 2.19	56.00 ± 2.19	55.00 ± 2.19	58.75 ± 2.19	0.787
Spleen (g)	2.75 ± 0.21	2.75 ± 0.21	2.75 ± 0.21	2.75 ± 0.21	1.000
Proventriculus (g)	11.25 ± 0.50	11.00 ± 0.50	10.25 ± 0.50	10.50 ± 0.50	0.914

T1: Control group with basal diet only, T2: Basal diet + 100 g fenugreek per kg of feed, T3: Basal diet + 200 g fenugreek per kg of feed, T4: Basal diet + 300 g fenugreek per kg of feed. ^{a, b} Means with different superscript letters within the same row differ significantly ($p < 0.05$). Data are presented as the standard error of mean.

3.3. Haematological profile

The effects of fenugreek seeds on the haematological parameters of broiler chickens are presented in Table 4. No significant differences were observed in PCV, WBC count, lymphocytes, monocytes, eosinophils, and basophils across all groups ($p > 0.05$).

Broiler chickens fed T2 diets had significantly higher Hb and RBC counts than those fed T1, T3, or T4 ($p < 0.05$). The RBC was observed to be the lowest ($2.05 \times 10^{12}/L$) in broiler chickens fed the T3 diet. Neutrophil was higher in broiler chickens in T3 (42.67%) compared to other treatment groups ($p < 0.05$), while the lowest value for neutrophil was observed in T4 (39.49%).

Table 4. Effect of fenugreek seeds on the haematological parameters in broiler chickens at eight weeks of age

Groups	T1	T2	T3	T4	p-value
Parameters					
Packed cell volume (%)	29.82 ± 0.40	30.38 ± 0.40	29.27 ± 0.40	30.72 ± 0.40	0.649
White blood cell ($\times 10^9/L$)	25.73 ± 0.38	25.44 ± 0.38	27.02 ± 0.38	25.81 ± 0.38	0.515
Haemoglobin (g/dL)	11.85 ± 0.08 ^b	12.40 ± 0.08 ^a	11.90 ± 0.08 ^b	11.75 ± 0.08 ^b	0.003

Red blood cell ($\times 10^{12}/L$)	2.25 \pm 0.59 ^{bc}	2.63 \pm 0.59 ^a	2.05 \pm 0.59 ^c	2.29 \pm 0.59 ^b	<0.001
Neutrophils (%)	40.27 \pm 0.43 ^{ab}	41.79 \pm 0.43 ^{ab}	42.67 \pm 0.43 ^a	39.40 \pm 0.43 ^b	0.013
Lymphocytes (%)	32.39 \pm 0.57	33.65 \pm 0.57	32.34 \pm 0.57	30.78 \pm 0.57	0.399
Monocytes (%)	5.38 \pm 0.10	5.08 \pm 0.10	5.29 \pm 0.10	5.10 \pm 0.10	0.730
Eosinophils (%)	2.24 \pm 0.04	2.20 \pm 0.04	2.10 \pm 0.04	2.01 \pm 0.04	0.203
Basophils (%)	1.19 \pm 0.03	1.20 \pm 0.03	0.94 \pm 0.03	1.05 \pm 0.03	0.532

T1: Control group with basal diet only, T2: Basal diet + 100 g fenugreek per kg of feed, T3: Basal diet + 200 g fenugreek per kg of feed, T4: Basal diet + 300 g fenugreek per kg of feed. ^{a, b, and c} Means with different superscript letters within the same row differ significantly ($p < 0.05$). Data are presented as the standard error of mean.

3.4. Serum minerals, proteins, and creatinine

The results of the effects of fenugreek seeds on the serum biochemistry of broiler chickens are presented in Table 5. Potassium, chloride, bicarbonate, magnesium, creatinine, and albumin concentrations did not differ significantly among all groups ($p > 0.05$). However, significant differences were observed in sodium, total calcium, total protein, and globulin levels ($p < 0.05$). Broiler chickens in T3 (139.05 mmol/L) and T4 (141.10 mmol/L) had significantly

higher sodium levels compared to T1 (133.45 mmol/L). Group T4 (2.47 mmol/L) recorded the highest total calcium concentration, significantly different from groups T2 (2.19 mmol/L) and T3 (2.13 mmol/L; $p < 0.05$). Total protein levels were significantly increased in all groups, T2 (42.50 g/L), T3 (44.95 g/L), and T4 (43.90 g/L), fed fenugreek, compared to the control group, T1 (37.90 g/L; $p < 0.05$). Similarly, globulin concentrations were significantly higher in groups T2 (16.65 g/L), T3 (19.50 g/L), and T4 (17.40 g/L) compared to T1 (12.30 g/L; $p < 0.05$).

Table 5. Effect of fenugreek seeds on the serum minerals, proteins, and creatinine in broiler chickens at eight weeks of age

Groups	T1	T2	T3	T4	p-value
Parameters					
Potassium (mmol/L)	4.04 \pm 0.12	4.39 \pm 0.12	4.53 \pm 0.12	4.61 \pm 0.12	0.377
Sodium (mmol/L)	133.45 \pm 0.85 ^b	138.80 \pm 0.85 ^{ab}	139.05 \pm 0.85 ^a	141.10 \pm 0.85 ^a	0.006
Chloride (mmol/L)	104.50 \pm 0.72	107.45 \pm 0.72	108.15 \pm 0.72	109.25 \pm 0.72	0.112
Bicarbonate (mmol/L)	20.53 \pm 0.62	21.16 \pm 0.62	24.26 \pm 0.62	21.49 \pm 0.62	0.062
Total calcium (mmol/L)	2.25 \pm 0.04 ^{ab}	2.19 \pm 0.04 ^b	2.13 \pm 0.04 ^b	2.47 \pm 0.04 ^a	0.003
Magnesium (mmol/L)	1.36 \pm 0.03	1.52 \pm 0.03	1.41 \pm 0.03	1.46 \pm 0.03	0.333
Creatinine (mmol/L)	0.06 \pm 0.01	0.06 \pm 0.01	0.05 \pm 0.01	0.05 \pm 0.01	0.258
Total protein (g/L)	37.90 \pm 0.65 ^b	42.50 \pm 0.65 ^a	44.95 \pm 0.65 ^a	43.90 \pm 0.65 ^a	< 0.001
Albumin (g/L)	25.60 \pm 0.16	25.85 \pm 0.16	25.45 \pm 0.16	26.50 \pm 0.16	0.103
Globulin (g/L)	12.30 \pm 0.63 ^b	16.65 \pm 0.63 ^a	19.50 \pm 0.63 ^a	17.40 \pm 0.63 ^a	< 0.001

T1: Control group with basal diet only, T2: Basal diet + 100 g fenugreek per kg of feed, T3: Basal diet + 200 g fenugreek per kg of feed, T4: Basal diet + 300 g fenugreek per kg of feed. ^{a, b} Means with different superscript letters within the same row differ significantly ($p < 0.05$). Data are presented as the standard error of mean.

3.5. Serum lipid profile

The results of the effect of fenugreek seeds on the lipid profile of broiler chickens are presented in Table 6. Dietary supplementation with fenugreek seeds significantly lowered triglyceride concentrations in T3, T2, and T4 compared to the control group ($p < 0.05$). Additionally, T4 exhibited the most significant increase in HDL

concentration (2.15 mg/dL) compared to the other groups ($p < 0.05$). A significant decrease in total cholesterol concentration was observed from T1 (3.65 mg/dL) to T4 (3.15 mg/dL; $p < 0.05$). A significant reduction in LDL concentration was observed across T3 (1.27 mg/dL) and T4 (1.18 mg/dL) compared to T1 (1.71 mg/dL).

Table 6. Effect of fenugreek seeds on the lipid profile in broiler chickens at eight weeks of age

Groups	T1	T2	T3	T4	p-value
Parameters					
Total cholesterol (mg/dL)	3.65 \pm 0.07 ^a	3.64 \pm 0.07 ^a	3.20 \pm 0.07 ^{ab}	3.15 \pm 0.07 ^b	0.009
Triglyceride (mg/dL)	0.85 \pm 0.27 ^a	0.60 \pm 0.27 ^b	0.65 \pm 0.27 ^b	0.55 \pm 0.27 ^b	< 0.001
High-density lipoprotein (mg/dL)	1.65 \pm 0.07 ^b	1.75 \pm 0.07 ^{ab}	1.70 \pm 0.07 ^{ab}	2.15 \pm 0.07 ^a	0.038
Low-density lipoprotein (mg/dL)	1.71 \pm 0.06 ^a	1.32 \pm 0.06 ^{ab}	1.27 \pm 0.06 ^b	1.18 \pm 0.06 ^b	0.005

T1: Control group with basal diet only, T2: Basal diet + 100 g fenugreek per kg of feed, T3: Basal diet + 200 g fenugreek per kg of feed, T4: Basal diet + 300 g fenugreek per kg of feed. ^{a, b, and c} Means with different superscript letters within the same row differ significantly ($p < 0.05$). Data are presented as the standard error of mean.

3.6. Liver function profile

Table 7 shows the results of the effect of fenugreek seeds on the liver function of broiler chickens. The results indicated significant differences in ALP and ALT, but not in AST ($p < 0.05$). A significant decrease in ALP levels was observed in groups T4 and T3 compared to T1 ($p < 0.05$).

Broiler chickens fed the T4 diet had the lowest ALP levels (115.98 U/L), followed by T3 (116.38 U/L), T2 (118.14 U/L), and T1 (119.00 U/L). A significant decrease in ALT levels was observed in groups T4 (11.92 U/L) and T3 (12.27 U/L) compared to T1 (15.37 U/L; $p < 0.05$).

Table 7. Effect of fenugreek seeds on the liver function profile in broiler chickens at eight weeks of age

Groups	T1	T2	T3	T4	p-value
Parameters					
ALP (U/L)	119.00 ± 1.86 ^a	118.14 ± 1.86 ^{ab}	116.38 ± 1.86 ^{bc}	115.98 ± 1.86 ^c	0.001
AST (U/L)	326.04 ± 7.69	323.42 ± 7.69	320.63 ± 7.69	316.24 ± 7.69	0.058
ALT (U/L)	15.73 ± 2.62 ^a	14.64 ± 2.62 ^{ab}	12.27 ± 2.62 ^b	11.92 ± 2.62 ^b	0.003

T1: Control group with basal diet only, T2: Basal diet + 100 g fenugreek per kg of feed, T3: Basal diet + 200 g fenugreek per kg of feed, T4: Basal diet + 300 g fenugreek per kg of feed. ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. ^{a, b, and c} Means with different superscript letters within the same row differ significantly ($p < 0.05$). Data are presented as the standard error of mean.

3.7. Thyroid hormones profile

Table 8 presents the effects of dietary supplementation with fenugreek seeds on the thyroid hormones in broiler chickens. No significant differences were observed in the levels of FT₄ across groups; T1 (0.53 ng/dL), T2 (0.55 ng/dL), T3 (0.55 ng/dL), and T4 (0.60 ng/dL; $p > 0.05$), as

well as for TSH in T1 (0.10 µIU/mL), T2 (0.10 µIU/mL), T3 (0.10 µIU/mL), and T4 (0.10 µIU/mL). However, broiler chickens in T3 (2.70 pg/mL) and T4 (2.70 pg/mL) exhibited significantly higher concentrations of FT₃ compared to those in T1 (2.15 pg/mL) and T2 (2.20 pg/mL; $p < 0.05$).

Table 8. Effect of fenugreek seeds on the thyroid hormones in broiler chickens at eight weeks of age

Groups	T1	T2	T3	T4	p-value
Parameters					
FT ₃ (pg/mL)	2.15 ± 0.06 ^b	2.20 ± 0.06 ^b	2.75 ± 0.06 ^a	2.70 ± 0.06 ^a	0.001
FT ₄ (ng/dL)	0.53 ± 0.01	0.55 ± 0.01	0.55 ± 0.01	0.60 ± 0.01	0.175
TSH (µIU/mL)	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	1.000

T1: Control group with basal diet only, T2: Basal diet + 100 g fenugreek per kg of feed, T3: Basal diet + 200 g fenugreek per kg of feed, T4: Basal diet + 300 g fenugreek per kg of feed. FT₃: Free Triiodothyronine, FT₄: Free Thyroxine, and TSH: Thyroid-stimulating hormone. ^{a, b} Means with different superscript letters within the same row differ significantly ($p < 0.05$). Data are presented as the standard error of mean.

4. Discussion

Based on the present results, considerable improvements in FBW, BWG, and FCR were observed in broiler chickens supplemented with fenugreek seeds, with the highest effects observed at inclusion levels of 200-300 g/kg feed. The present findings align with those of Hassan et al.²³ who found that supplementing fenugreek seed meal at 1.5% remarkably increased final live weight gain and feed efficiency in broiler chickens. Additionally, Yang et al.²⁴ reported that supplementation with fenugreek seed extract resulted in improved average daily weight gain and an enhanced feed-to-gain ratio in yellow-feathered broiler chickens.

The improved growth performance that was observed during the present study could be associated with the bioactive compounds of fenugreek, including saponins, alkaloids, and flavonoids, which are reported to possess growth-promoting and digestion-enhancing properties²⁵. Fenugreek extract contains 50% polysaccharides, 10% flavonoids, 15% saponins, and 2% alkaloids, which can effectively stimulate the hypothalamic gland, improve feed intake, and activate the digestive system¹⁶. The improved FCR in the present study was similar to that reported by Park and Kim²⁶, who found that supplementing fenugreek seed extract at 0.1% improved the total tract digestibility of dry matter and nitrogen in broiler chickens. Additionally, the observed improvements in the growth and FCR of the chickens studied could be a result of the ability of the fenugreek to enhance nutrient digestibility and gut health.

The dose-response observation of fenugreek seed meal in the present study highlighted the importance of optimal inclusion levels, with 200 g/kg and 300g/kg of fenugreek seed meal indicating promising results compared to the 100

g/kg of fenugreek seed meal and the control group. Paneru et al.¹⁸ observed linear decreases in BWG and an improvement in FCR at higher inclusion levels (500-1000 g/kg) during the starter phase, which is a potential indication of an anti-nutritional effect at increased doses. The current findings indicated no effect on total feed intake and were similar to other studies that found no considerable impacts on feed consumption when laying hens were fed 0.5% of the fenugreek²⁷. The improved FCR in the treatment groups was primarily due to the more effective utilization of nutrients, rather than increased feed intake, indicating better metabolic efficiency.

The current results indicated that broiler chickens supplemented with fenugreek notably improved Hb concentration and RBC count, while the highest neutrophil count was observed in broiler chickens supplemented at 200 g/kg, and the lowest in those supplemented at 300 g/kg. The current results are in line with the findings of Paneru et al.¹⁸, who reported a linear effect on haematological indices due to the supplementation of fenugreek seeds. However, these improvements may be due to the presence of iron, protein, and B vitamins, which are essential for erythropoiesis. Moreover, bioactive compounds such as alkaloids, steroids, flavonoids, and saponins are present in fenugreek, which may aid in Hb synthesis¹⁸. Although there was an observed decrease in value at higher levels of fenugreek seed meal at 300g/kg, suggesting a negative dose-dependent response at excessive levels. The present findings are similar to those reported by Alkatan et al.²⁸, who studied sesame seeds, where their supplementation at 250 mg/kg and 500 mg/kg substationally increased RBC, Hb, and PCV when compared to control groups, supporting that phytogenic feed additives have better effects when used within specific doses. In the present study, no remarkable

differences were observed in total WBC count, lymphocytes, monocytes, eosinophils, and basophils across all treatment groups, indicating that the supplemented fenugreek at different levels did not alter the general immune cell populations. The findings of Paneru et al.¹⁸ contradicted the present study, as they reported a linear increase in WBC, heterophil, and lymphocyte counts with higher levels of fenugreek seed supplementation. This discrepancy may be attributed to the greater inclusion rates (ranging from 500 to 1000 g/kg) used in their study compared to the lower levels (100-300 g/kg) applied in the current study. In addition, a higher neutrophil percentage was observed in T3 chickens (42.67%) compared to the other treatments, which may indicate a mild inflammatory response, resulting from the presence of phytochemical compounds that can cause immune activation at moderate doses²⁹. Obianwuna et al.²⁸ reported that fenugreek seed meal suppresses the expression of proinflammatory cytokines and decreases the activity of nitric oxide synthases (NOS), such as inducible NOS (iNOS).

The present study demonstrated a substantial improvement in the carcass weight and defeathered weight of broiler chickens supplemented with 300g/kg of fenugreek seeds, with no particular effect on carcass and organ parameters. The current findings are similar to those of Hassan et al.²³, who reported that the inclusion of fenugreek seeds at 1.5% in broiler chickens' diets notably increased carcass weight compared to the control group. Similarly, Al-Homidan et al.³⁰ reported that supplementing fenugreek at 2% level resulted in higher dressed carcass, breast, thigh, and drumstick meat weight compared to chickens on lower levels of inclusion. Moreover, Weerasingha and Atapattu¹⁷ indicated that supplementing fenugreek at 1% improved FCR by 13.8% compared to the control group, which may contribute to the enhanced carcass weights observed. Therefore, the consistent improvements observed across treatments suggested that including fenugreek at 100-300 g/kg of feed effectively enhanced broiler carcass traits, aligning with the optimal dosage reported by Paneru et al.²⁴. The current findings revealed that a supplemented diet with fenugreek did not affect organ weights, including the heart, liver, gizzard, lungs, spleen, and proventriculus, across all treatments. The present findings were supported by Weerasingha and Atapattu³⁰, who reported similar findings on fenugreek seed meal, thereby indicating safe inclusion levels. Paneru et al.²⁵ reported negative effects due to increased saponin concentrations resulting from higher fenugreek seed inclusion levels (above 500 g/kg).

A remarkable improvement in the serum lipid profile of broiler chickens fed diets supplemented with fenugreek seeds was observed during the present study. Based on the present findings, the highest reductions in total cholesterol, triglycerides, and LDL cholesterol, alongside the highest increase in HDL cholesterol concentrations, were observed in chickens fed 300 g of fenugreek per kg feed. The lipid reduction observed in the present study was similar to the findings by Alloui et al.³¹, who used a similar inclusion rate (300 g/kg feed) and reported similar improvements in

broiler performance and metabolic parameters. Similarly, Hafeez et al.³² demonstrated that broiler chickens fed phytogetic mixtures containing fenugreek at rates of 1-3% exhibited improved growth performance and positive blood metabolite profiles. In the present study, a dose-dependent reduction was observed, which was in line with the clinical trial conducted by Heshmat-Ghahdarjani et al.³³. Considerable improvements were reported in all lipid profiles, including LDL, triglycerides, total cholesterol, and HDL levels of 300 g/kg of fenugreek. The observed effects in the current study may be linked to mechanisms involving its bioactive compounds, mostly galactomannan fibre, flavonoids, and alkaloids. The decrease in total cholesterol and LDL observed in the present study can be partly attributed to the inhibition of hepatic HMG-CoA reductase, the key enzyme in cholesterol biosynthesis¹⁸. This has been similarly reported, with fenugreek's bioactive compounds downregulating hepatic enzymes, thereby reducing endogenous cholesterol synthesis³⁴. Wang et al.³⁵ indicated that galactomannan from fenugreek increases intestinal viscosity, thereby inhibiting cholesterol and bile salt absorption through hydrophobic interactions, electrostatic interactions, and hydrodynamic restriction. Generally, the observed lipid improvements could be due to diosgenin-mediated effects, which is a primary steroidal saponin in fenugreek. A Study by Tak et al.³⁴ has indicated that diosgenin administration reduces hyperglycemia, hypercholesterolemia, and hypertriglyceridemia while promoting adipocyte differentiation and inhibiting inflammation in adipose tissues.

Based on the present results, supplementation with fenugreek seeds remarkably improved liver function parameters, with a reduction in ALP and ALT levels. There was a consistent decrease in liver enzymes from control to the highest inclusion level, thereby indicating a hepatoprotective effect of fenugreek supplementation. The current findings were similar to those of Tabrizian et al.³⁶, who reported improvements in the liver enzyme profiles of Ross 308 broiler chickens supplemented with fenugreek extract, likely due to the presence of bioactive compounds in fenugreek. The present study has shown the hepatoprotective effects of fenugreek. Fenugreek seeds contain approximately 4-8% saponins, with diosgenin being the main bioactive compound responsible for hepatoprotection³⁷. Fuller and Stephens³⁸ found that diosgenin can protect the liver by activating antioxidant enzymes and suppressing inflammation. Furthermore, flavonoids in fenugreek, especially vitexin and isovitexin, act as antioxidants that neutralize free radicals and prevent oxidative damage to liver cells³⁹.

The present study indicated that supplementing broiler diets with fenugreek seeds strikingly increased serum FT₃ concentrations compared to the control, while FT₄ and TSH levels remained unaffected. These findings suggested that adding fenugreek to the diet at higher levels could improve the activity of thyroid hormone. Olayeni et al.⁴⁰ found that the inclusion of fenugreek at 100 g/kg improved the weight gain compared to the inclusion at 300 g/kg. This suggested that fenugreek supplementation may affect optimal

performance and physiological responses in broiler chickens when administered at different dosages, with the greatest benefits observed at moderate levels, whereas higher doses tend to promote hormonal effects modulation. The variations observed between the present study and a study conducted by Selim et al.⁴¹ may be attributed to differences in thyroid hormone metabolism across species. Unlike mammals, poultry exhibit distinct distributions and activities of deiodinase enzymes, which may respond differently to the bioactive compounds in fenugreek⁴¹. The presence of several bioactive compounds, such as diosgenin, trigonelline, and flavonoids, in fenugreek may modulate thyroid hormone metabolism⁴². In another study conducted by Kiss et al.⁴³, it was found that fenugreek seeds can modify the T_4/T_3 ratio through interactions with hypothalamic-pituitary-thyroid axis regulatory mechanisms. There were no considerable effects on FT_4 and TSH levels, and this may demonstrate that fenugreek primarily affects peripheral thyroid hormone conversion rather than central thyroid regulation in the present study.

Based on the present study, it was found that supplementing fenugreek seed in broiler chickens' diets considerably improved serum protein metabolism and altered mineral homeostasis. There were substantial increases in total protein and globulin concentrations in the treatment groups, indicating improved protein synthesis capacity. The current study was supported by Paneru et al.¹⁸, who reported similar findings that protein increases in chickens fed fenugreek extract at 100 mg/kg diet, which may be due to the active presence of bioactive compounds in fenugreek, particularly diosgenin (0.2-0.9%) and saponins (4.8%), which were known as key modulators of protein metabolism³⁴. Moreover, serum sodium levels increased in a dose-dependent manner, reaching the highest concentrations in T_4 , suggesting that fenugreek supplementation affects electrolyte transport mechanisms. Similarly, Chrystal et al.⁴⁴ reported that dietary interventions can notably alter electrolyte balance in broiler chickens, with sodium being essential for nutrient absorption and cellular metabolism. The increase in sodium levels observed in the present study may be linked to the presence of bioactive compounds, which could affect sodium-glucose cotransporter 1 (SGLT-1) and $Na^+-K^+-ATPase$ activity. The total calcium level was substantially elevated at 300 g/kg of fenugreek seed meal compared to the levels observed at 100 g/kg and 200 g/kg of fenugreek seed meal, indicating a positive calcium metabolism. These findings are consistent with those of Navarro del Hierro et al.⁴⁵, who reported that steroidal saponins in fenugreek improve mineral bioavailability by modulating intestinal transport proteins. The dose above 200-300 g/kg of fenugreek indicated the maximum response to the protein and mineral ratios. Nevertheless, the observed dose-response pattern with calcium suggested that increased levels of fenugreek (300 g/kg) may be beneficial for mineral metabolism.

4. Conclusion

The present study indicated that fenugreek seed meal functions as a potent natural growth promoter in broiler chickens. The inclusion of 200 g/kg and 300 g/kg of fenugreek significantly improved FBW and FCR. Physiologically, the highest dose (300 g/kg) resulted in an increase in serum FT_3 levels, indicative of an elevated metabolic state driving growth. Meanwhile, 300 g/kg of fenugreek improved the serum lipid profile by increasing HDL and lowering LDL, cholesterol, and triglycerides, supporting hepatocyte health, as indicated by decreased ALT and ALP activities. Immunomodulatory effects were observed at a dose of 100 g/kg of fenugreek, resulting in a significant increase in Hb and RBC counts. These multifaceted improvements were probably due to the different bioactive compounds in fenugreek, such as saponins and flavonoids. The current findings on the phyto-genic benefits of fenugreek in poultry nutrition offered a positive alternative to synthetic growth promoters. Fenugreek seed meal supplementation can be recommended as an environmentally sustainable feed additive to enhance broiler health and productivity. However, further studies should elucidate the precise mechanisms of action of fenugreek and assess its efficacy under different production systems and stress conditions. Future studies can focus on establishing the long-term safety and overall effectiveness of fenugreek.

Declarations

Competing interests

The authors declared no competing interests.

Authors' contributions

Grace Olobumi Sodipe has contributed to the Conceptualization, methodology, project administration, supervision, and writing the original draft, review, and editing. Godfrey Odeh Gabriel was responsible for conceptualization, methodology, formal analysis, data curation, project administration, supervision, writing the original draft, review, and editing. Adetunmbi Tella and Opeyemi Ayomide Adesina contributed to the resources, supervision, writing of the review, and editing. Pelumi Priscilla Owolabi and Precious Adetomiwa Ajiboye contributed to the investigation, wrote the original draft, reviewed it, and edited it. Daniel Timileyin Eytayo and Adesina Samuel Adisa contributed to resources, investigation, writing the review, and editing. Oluwatimileyin Ahmed Jegede, Ephraim Temiloluwa Ogungbemi, Janet Bosede Oke, and Adesina Samuel Adisa contributed to the methodology, formal analysis, writing the original draft, review, and editing. All authors have read and approved the final edition of the manuscript.

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Availability of data and materials

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

Ethical considerations

The authors confirmed that the present manuscript is an original submission, prepared exclusively for the Journal of World's Poultry Science and not under consideration elsewhere. The final manuscript was thoroughly checked for plagiarism, data fabrication, and duplication to ensure scientific integrity. No AI tools have been used in generating or writing the present study.

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