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Article

Dietary supplementation of fenugreek (*Trigonella foenum-graecum*) seed as an alternative to antibiotics on growth performance and health of broiler chickens

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Abstract: Growing concerns over the use of antibiotics in poultry production have led to increased exploration of natural feed additives with health-promoting benefits. This study aimed to assess the impact of fenugreek seed (*Trigonella foenum-graecum*) supplementation in broiler diets as a potential substitute for antibiotic growth promoters. Conducted over a 28-day period (May 8 to June 5, 2019) at the Sher-e-Bangla Agricultural University Poultry Farm in Dhaka, the trial involved 150 Cobb 500 day-old broiler chicks. The birds were distributed randomly across five dietary groups, each with three replicates containing 10 chicks. The treatment groups included a control (no additive), an antibiotic-supplemented diet, and diets supplemented with fenugreek seed at 1%, 1.5%, and 2% inclusion levels. The findings revealed a significant (P<0.05) enhancement in final body weight and dressing percentage in birds fed fenugreek, with the 1.5% group achieving the highest body weight (1528.33 ± 57.47 g). Although feed conversion ratio (FCR) did not show significant variation across treatments, birds receiving the 1% fenugreek diet consumed the most feed (2289.67 ± 2.60 g). No significant differences (P>0.05) were observed in the relative weights of internal organs or immune tissues. Blood cholesterol levels significantly decreased in the fenugreek-fed groups, while glucose levels remained unchanged. Hematological profiles improved notably in treated birds, particularly in hemoglobin concentration, red and white blood cell counts, and lymphocyte percentages. Microbial analysis showed that fenugreek supplementation reduced pathogenic bacteria such as *E. coli* and *Salmonella*, while promoting beneficial *Lactobacillus* populations. Additionally, antibody titers against Newcastle disease were significantly higher (P<0.05) in fenugreek-treated groups. Incorporating fenugreek seed into broiler diets positively influenced growth metrics, enhanced immune function, improved gut microbial balance, and supported key hematological parameters. These outcomes highlight fenugreek seed as a promising natural alternative to synthetic antibiotics in broiler production systems.

Keywords: phytogenic additives; hematological parameters; immune response; gut microbiota; natural growth promoter

1. Introduction

Poultry meat serves as a crucial foundation of animal-based protein worldwide and is especially important for enhancing nutrition in developing nations, including Bangladesh. In the Bangladeshi context, poultry accounts for nearly 37% of the country's total meat output and contributes around 22–27% to the overall supply of animal-derived protein (Hamid *et al.*, 2016; Mottet and Tempio, 2017; Castro *et al.*, 2023). As demand for animal protein continues to increase with population growth and urbanization, there is growing emphasis on enhancing the efficiency and sustainability of poultry production systems. However, this sector has traditionally relied heavily on the use of antibiotic growth promoters (AGPs) to maintain gut health, enhance growth, and prevent disease outbreaks in poultry. Although AGPs have proven effective in improving productivity, their use has come under scrutiny due to emerging public health concerns (Rafiq *et al.*, 2022; Obianwuna *et al.*, 2024).

Growth-promoting antibiotics are often used in poultry diets at low doses to enhance feed efficiency and accelerate weight gain by altering the composition of intestinal microflora in birds (Miyakawa *et al.*, 2024). Although AGPs have contributed to improved livestock productivity, their prolonged and unregulated use has contributed to the emergence of antibiotic-resistant bacteria, which poses serious risks to animal welfare and public health (Ma *et al.*, 2021). In response to these concerns, numerous countries have enforced stringent regulations or have completely prohibited the use of antibiotics in livestock feed to curb the rise of antimicrobial resistance and protect public health (Giubilini *et al.*, 2017; Da Silva *et al.*, 2023).

Natural growth promoters (NGPs), including acidifiers, prebiotics, probiotics, feed enzymes, phytobiotics, immune enhancers, and antioxidants, have emerged as effective and sustainable alternatives to antibiotic growth promoters in poultry production (Obianwuna *et al.*, 2024; Peng *et al.*, 2024). Among these, phytogenic feed additives—plant-derived bioactive compounds including herbs, seeds, and spices—have drawn considerable interest due to their antimicrobial, anti-inflammatory, and immune-stimulating properties. These natural substances have been used for centuries in traditional medicine, and their potential application in animal nutrition has garnered increasing research focus (Valdez *et al.*, 2023; Wang *et al.*, 2024).

Phytobiotics, or phytogenic feed additives, are widely recognized for their positive impact on animal growth and health. These bioactive compounds may enhance palatability, stimulate appetite, improve digestive enzyme secretion, enhance immune function, and suppress harmful microbial populations in the gastrointestinal tract (Valenzuela-Grijalva *et al.*, 2017; Wang *et al.*, 2024). Moreover, their natural origin reduces the risk of antibiotic resistance, and consumers generally perceive poultry raised on herbal additives as healthier and safer (Abd El-Hack *et al.*, 2022).

One such promising phytogenic additive is fenugreek (*Trigonella foenum-graecum*) seed. Fenugreek is an annual herb native to the Mediterranean and South Asia, widely used in culinary and medicinal applications. Fenugreek seeds contain a variety of bioactive components, including alkaloids, flavonoids, saponins, mucilage, and essential oils, which are accountable for their medicinal characters such as blood sugar regulation, anti-inflammatory, antiparasitic, and antimicrobial activities (Singh *et al.*, 2022; Hina *et al.*, 2025). Fenugreek is also known to improve lipid metabolism, stimulate appetite, and enhance immune responses (Faisal *et al.*, 2024).

Multiple investigations have indicated that incorporating fenugreek seeds into poultry diets can enhance overall performance, particularly by increasing feed intake and improving the efficiency of energy use in broiler chickens (Yang *et al.*, 2022). Enzyme supplementation in poultry diets, including those based on fenugreek, can enhance nutrient absorption and gut morphology, leading to better growth performance (Qureshi *et al.*, 2016; Paneru *et al.*, 2023). Furthermore, fenugreek supplementation has been linked to improvements in hematological parameters, such as increased hemoglobin concentration, RBC and WBC counts, and enhanced immune responses (Awad *et al.*, 2015; Moustafa *et al.*, 2020). Despite these promising findings, there remains a need for comprehensive studies to determine the efficacy and optimal inclusion level of fenugreek seed (FS) as a dietary supplement in broiler chicken feed, particularly in local contexts such as Bangladesh. The current study addresses this gap by systematically evaluating the impact of FS on broiler performance, health status, and immune responses.

The ongoing inclusion of antibiotic growth promoters in poultry diets raises major concerns, primarily due to the emergence of antibiotic-resistant bacteria. Although the demand for safer, natural alternatives is growing, there remains a lack of comprehensive research in Bangladesh on the potential of locally accessible herbs, such as fenugreek seeds, to serve as substitutes for antibiotics in broiler nutrition. Without sufficient evidence, poultry producers lack viable alternatives that ensure animal health and productivity while maintaining food safety.

Considering the problem statement we formed research questions such as, can FS (*T. foenum-graecum*) serve as an effective alternative to antibiotics in broiler chicken feed to improve growth performance, hematological parameters, gut microflora balance, and immune response? We hypothesized that dietary supplementation of FS at appropriate inclusion levels will improve the growth performance, hematological indices, microbial health, and immune response of broiler chickens, comparable to or better than those fed antibiotic-supplemented diets. This Investigation was designed to assess the potential of FS supplementation as a natural alternative to antibiotic growth promoters in broiler chicken diets by assessing its effects on growth performance, hematological parameters, intestinal microflora (including *E. coli*, *Salmonella*, and *Lactobacillus* sp.), and immune response, particularly antibody titres, in order to determine the optimal inclusion level for promoting health and productivity. The results of the study suggest that FS could serve as a natural substitute for antibiotic growth promoters in broiler feed, contributing to more sustainable poultry farming by enhancing both health and productivity.

2. Materials and Methods

2.1. Ethical approval

No ethical approval was required to conduct the research.

2.2. Study site, periods and experimental animal

The study was conducted at the poultry farm of Sher-e-Bangla Agricultural University (SAU), Dhaka, using 150 straight-run day-old commercial broiler chicks (Cobb 500) over a 28-day experimental period from May 8 to June 5, 2019 (Figure 1). The chicks were sourced from Kazi Hatchery, Gazipur, Dhaka, Bangladesh.

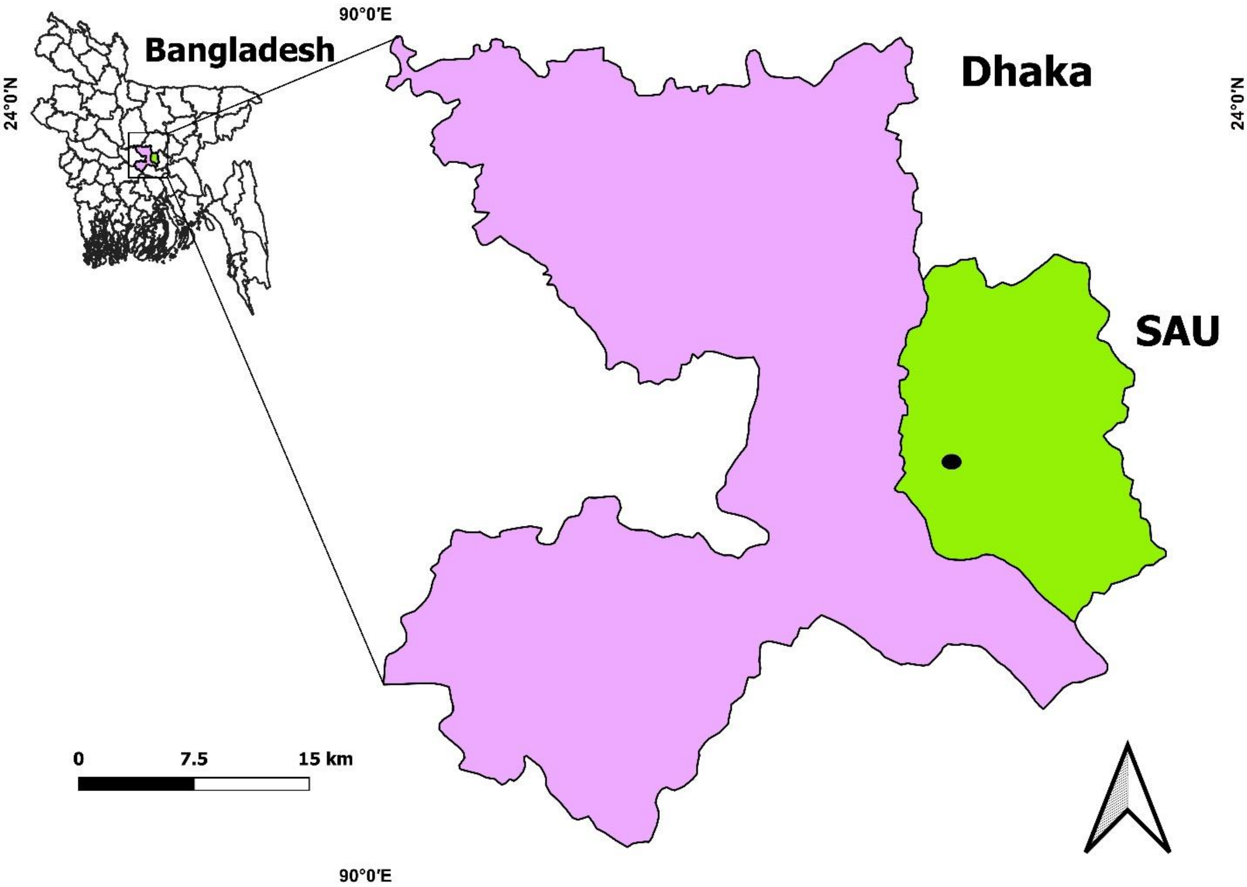

Figure 1. The map of the study site.

2.3. Experimental materials and treatments

Day-old chicks were collected in the morning and transported to the university poultry farm, where they were placed under electric brooders for two days following standard brooding practices. Throughout this period, the chicks were provided with a basal diet without FS supplementation. After the brooding phase, 90 healthy chicks were randomly selected and divided into three groups, each receiving a diet supplemented with different levels of FS. The remaining 60 chicks were randomly divided between two additional treatments—one with an antibiotic-supplemented diet and the other as a control group receiving only the basal diet. Each of the five treatment groups had three replications, with 10 birds per replication, totaling 15 replications and 150 birds (Table 1). The dietary treatments were as follows,

T1: Basal diet (control)
T2: Basal diet + antibiotic (Doxivet)
T3: Basal diet + 1% FS (1 kg FS per 100 kg feed)
T4: Basal diet + 1.5% FS (1.5 kg FS per 100 kg feed)
T5: Basal diet + 2% FS (2 kg FS per 100 kg feed)

Table 1. Arrangement of the experimental setup.

Treatments with replications			Number of birds
T_4R_2	T_3R_1	T_1R_3	30
T_1R_1	T_2R_2	T_5R_2	30
T_3R_3	T_4R_1	T_3R_2	30
T_5R_1	T_2R_1	T_1R_2	30
T_2R_3	T_5R_3	T_4R_3	30
	Total		150

*n=10 in each replications

2.4. Preparation of experimental house

The experimental room was meticulously cleaned with tap water, followed by disinfection of all walls, ceiling, and floor surfaces using a diluted iodophor solution (3 ml per liter of water). After the room dried, it was divided into 15 equal pens constructed from wood and wire mesh, with the mesh standing 36 cm high. Ten birds were randomly allocated to each pen, serving as replicates for the five treatment groups, with a stocking density maintained at 10 birds per square meter.

2.5. Diets for experimental animals

Starter and grower commercial broiler feeds were purchased from Kazi to be used in the experiment. The starter ration contained **a** minimum of 21% protein, 6% fat, 5% fiber, and essential amino acids like lysine (1.20%) and methionine (0.49%). The grower ration had slightly lower protein at 19%, with similar levels of fat, fiber, and amino acids to support continued growth (Table 2).

Table 2. Minimum ingredient composition (%) of starter and grower rations.

Ingredients in starter ration	**Minimum percentage present**
Protein	21.0 %
Fat	6.0%
Fiber	5.0%
Ash	8.0%
Tryptophan	0.19%
Lysine	1.20%
Methionine	0.49%
Cystine	0.40%
Threonine	0.79%
Arginine	1.26%
Ingredients in grower ration	**Minimum percentage present**
Protein	19.0 %
Fat	6.0%
Fiber	5.0%
Ash	8.0%
Lysine	1.10%
Methionine	0.47%
Cystine	0.39%
Tryptophan	0.18%
Threonine	0.75%
Arginine	1.18%

2.6. Collection of *T. foenum-graecum*

The FS were sourced from local markets to serve as phytobiotic feed additives in broiler diets. The seeds were carefully washed with tap water and dried in the shade under natural sunlight. After drying, they were stored in polyethylene bags until incorporated into the experimental feed formulations.

2.7. Management procedures

2.7.1. Brooding and environmental management

The experiment took place from May 8 to June 5, 2019, with an average house temperature of 31.5 °C and relative humidity of 80%. During the first week, chicks were brooded together, after which they were randomly allocated into pens at a stocking density of 10 chicks/m^2. Brooding temperature was carefully regulated according to requirements and adjusted based on ambient conditions, with no additional heating provided on warmer days. Electric bulbs were used during daylight hours to provide stimulation, while fans were operated as needed to alleviate heat stress. Temperature and humidity levels were monitored every six hours using standard measuring instruments.

2.7.2. Litter, feeding, and watering management

Rice husk was used as the bedding material at a depth of 6 cm and was stirred daily to reduce gas accumulation and lower the risk of parasites. At three weeks, surface droppings were removed, and fresh litter was added as needed. All birds were fed commercial mash feed and clean water ad libitum. Each pen had one feeder and one round drinker per four birds. Drinkers were washed daily, and feeders cleaned weekly.

2.7.3. Lighting, ventilation, and sanitation

Lighting was maintained continuously for the first two weeks (24 hours daily), after which it was reduced to 18 hours of light followed by 6 hours of darkness. The open-sided, south-facing shed allowed natural ventilation through wire mesh. Additional regulation was done using polythene screens. Disinfectants (e.g., Virkon) were applied to clean the shed and equipment, and strict sanitary practices were maintained.

2.7.4. Preventive health and vaccination

To maintain flock health, all birds received vitamin and electrolyte supplementation (Vitamin B-Complex, A-D-E-K, C, and calcium). Vaccines were sourced from Ceva and administered according to a set vaccination schedule (Table 3). Preventive measures and proper biosecurity were implemented throughout the experimental period.

Table 3. Vaccination schedule.

Age of birds	Name of disease	Name of vaccine	Route of administration
3 days	IB + ND	MA-5 + Clone-30	One drop in each eye
9 days	Gumboro	G-228E (inactivated)	Drinking Water
17days	Gumboro	G-228E (inactivated) booster dose	Drinking Water
21 days	IB + ND	MA-5 + Clone-30	Drinking Water

2.8. Data collection

We have collected the following parameters for further statistical analysis,

Live weight: The initial weight of day-old chicks was recorded at the start of the trial. Weekly live weights were measured for each replicate to track growth and to determine the final live weight per bird at the end of the 28-day experiment.

Feed consumption: Daily feed intake was recorded for each replication. These records were used to calculate the weekly and total feed consumption per bird throughout the experiment.

Mortality: Daily mortality was recorded for each replication throughout the 28-day period. These records were used to determine the mortality rate of the birds in each treatment group.

Dressing yield: Dressing yield was calculated using the formula,

Dressing yield = Live weight − (blood + feathers + head + shank + digestive system + liver + heart)

2.8.1. Dressing procedures

At the conclusion of the 28-day trial, three birds were randomly selected from each replicate for slaughter to determine dressing percentage. These birds were fasted for 12 hours with free access to water before slaughter. Their live weights were recorded immediately prior to processing.

Slaughtering was performed by making a single incision to sever the jugular vein, carotid artery, and trachea, allowing the birds to bleed out thoroughly for at least two minutes. The carcasses were then manually defeathered, skinned, cleaned, and washed to remove feathers and any foreign matter. Evisceration was carried out consistent with the procedure outlined by Handley *et al.* (2018). The heart and liver were separated from the viscera, with the gallbladder removed from the liver. The gizzard was excised by separating it at both the proventricular and intestinal junctions. Dressing yield was calculated by subtracting the inedible portions from the live weight.

2.8.2. Blood sample analysis

Blood samples (1 ml per bird) were drawn from the wing vein into EDTA tubes and delivered to the laboratory within one hour. Hematological and biochemical parameters, including glucose and cholesterol levels, as well as complete blood counts (CBC), were analyzed at Rainbow Diagnostic Centre, Dhanmondi, Dhaka, following standard laboratory protocols.

2.9. Statistical analysis and calculations

All collected data were analyzed using one-way analysis of variance (ANOVA) with SPSS version 26.0. Differences between treatment means were assessed using Duncan's multiple range test (DMRT), with significance set at $P < 0.05$. Additional calculations were performed using the following formulas,

Live weight gain: Average weight gain per replication was calculated by subtracting the initial weight from the final live weight,

Body weight gain = Final weight − Initial weight

Feed intake: Feed intake was calculated as the total feed consumed in a replication divided by the number of birds in that replicate.

Feed Conversion Ratio (FCR): FCR was determined using the formula,

FCR = Total feed intake ÷ Total body weight gain

3. Results and Discussion

3.1. Production performances of broiler chicken

3.1.1. Final live weight

The dietary inclusion of FS had a significant effect (P<0.05) on the production performance of broiler chickens, with noticeable variation among the treatment groups (Table 4). The final live weight (g/bird) was significantly influenced by the treatments (P<0.05). The recorded final live weights for groups T_1, T_2, T_3, T_4, and T_5 were 1344.22 ± 70.43, 1312.44 ± 45.82, 1527.11 ± 7.33, 1528.33 ± 57.47, and 1302.07 ± 3.77 respectively. The highest weights were observed in T_3 and T_4, suggesting improved growth at 1% and 1.5% fenugreek supplementation levels. In contrast, T_5 showed a comparatively lower weight, potentially due to the higher saponin content in fenugreek, which may impair nutrient digestion and utilization. Overall, broilers fed fenugreek-supplemented diets showed significantly better final body weights than those in the control and antibiotic-treated groups.

The present findings align with those of previous studies reporting significant improvements (P<0.05) in live body weight with dietary supplementation of FS powder at various levels (Ali *et al.*, 2021; Essien and Josiah, 2024). This enhancement in growth performance may be attributed to the presence of beneficial fatty acids or the stimulatory effects of fenugreek on the digestive system of broilers (Huang *et al.*, 2022; Essien and Josiah, 2024). The observed enhancement may be attributed to greater feed consumption or the bioactive constituents of fenugreek, which exhibit properties such as antibacterial, anti-inflammatory, antifungal, carminative, and antioxidant activity (Huang *et al.*, 2022). Comparable results were reported in studies where broilers receiving a diet supplemented with 1.5% fenugreek showed a significantly (P<0.05) greater increase in live body weight and weight gain than birds fed a control diet (Ali *et al.*, 2021; Yang *et al.*, 2022).

3.1.2. Feed consumption

There were statistically significant differences (P<0.05) in feed intake among the various treatment groups (Table 4). The T_3 group (1% FS) consumed the highest amount of feed (2289.67 ± 2.603 g), while the antibiotic-treated group had the lowest feed intake (2243.00 ± 25.813 g), showing a significant reduction (P<0.05). No significant differences were observed between the antibiotic group and T_1, T_4, or T_5, and varying FS levels did not significantly influence feed intake among the FS-supplemented groups.

The results demonstrate that dietary supplementation with FS, particularly at a 1% inclusion level (T_3), significantly increased feed consumption in broiler chickens compared to the antibiotic-treated and control groups. This enhanced feed intake may be attributed to the presence of galactomannan and other bioactive compounds in fenugreek, which are known to stimulate appetite and improve digestion (Paneru *et al.*, 2022). While the antibiotic group showed the lowest feed intake, likely due to its suppressive effect on gut flora, no significant differences were observed among the varying fenugreek levels (1%, 1.5%, and 2%), indicating that increasing the dose beyond 1% does not provide additional benefits in feed consumption (Kerek *et al.*, 2025). These findings support the potential of FS as a natural appetite stimulant and a viable alternative to antibiotics in broiler nutrition.

Table 4. Production performance of broiler chickens fed diets supplemented with fenugreek seed and antibiotic.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE
Final live wt. g/ broiler	1344.22±70.42^{b}	1312.44±45.81^{b}	1527.11±7.33^{a}	1528.33±57.46^{a}	1302.07±3.77^{b}	1402.83±32.47*
FC(g)	2244.67±5.04^{b}	2243.00±25.81^{b}	2289.67±2.60^{a}	2284.67±10.58^{ab}	2248.00±5.29^{ab}	2262.00±7.38*
FCR	1.51±0.02	1.47±0.02	1.38±0.00	1.45±0.03	1.50±0.08	1.46±0.02^{NS}
DP%(skinless)	57.27±4.67^{b}	61.00±0.75^{ab}	63.67±0.67^{ab}	65.55±0.32^{ab}	68.08±2.92^{a}	63.11*±1.37

*Here, T_1 = control; T_2 = antibiotic, T_3 = 1% FS; T_4 = 1.5% FS; T_5 = 2% FS. Values are mean ± SE (n=15) one way ANOVA (SPSS, Duncan method); FC= feed consumption; DP=dressing percentages; *Means with different superscripts differ significantly (P < 0.05); SE = standard error; NS = not significant.

3.1.3. Feed Conversion Ratio (FCR)

The FCR was not significantly affected (P>0.05) by the dietary treatments, with recorded values of 1.51±0.021, 1.47±0.027, 1.38±0.003, 1.45±0.037, and 1.50±0.084 for T_1 to T_5, respectively (Table 4). Although the differences were not statistically significant, birds fed FS diets generally exhibited better FCR compared to those in the antibiotic and control groups, with the T_3 group (1% FS) showing the most efficient FCR.

The results indicate that while FCR was not significantly influenced by dietary treatments, broilers fed diets supplemented with FS—particularly at T_3—exhibited more efficient feed utilization than those in the control and antibiotic groups. This suggests that fenugreek may enhance nutrient absorption or digestion, thereby improving growth efficiency, even if the differences are not statistically significant (Ali *et al.*, 2021; Paneru *et al.*, 2022; Essien and Josiah, 2024). The variation in findings compared to previous studies may stem from differences in experimental conditions, such as the type and quality of FS, environmental factors, and diet formulation (Camlica and Yaldiz, 2024).

3.1.4. Dressing percentage

The dressing percentages for the treatment groups T_1, T_2, T_3, T_4, and T_5 were 57.27±4.678, 61.00±0.752, 63.67±0.675, 65.55±0.329, and 68.08±2.923, respectively (Table 4). The group supplemented with 2% FS (T_5) exhibited the highest dressing percentage when compared to the control group (T_1), though this difference was not statistically significant (P>0.05). Additionally, the T_5 group showed no notable variation in dressing percentage compared to the antibiotic-treated group or the other fenugreek-fed groups.

The results indicate that dietary supplementation with FS, particularly at T_5, may enhance carcass yield in broiler chickens, as reflected by the highest dressing percentage in the T_5 group. While the observed difference was not statistically significant, the increasing trend indicates that fenugreek may serve as a promising natural growth-promoting additive (Elamin *et al.*, 2020). This improvement in dressing percentage may be attributed to better nutrient utilization and possibly fenugreek's bioactive compounds, which can positively influence metabolic and digestive processes (Obianwuna *et al.*, 2024; Fawaz *et al.*, 2025).

3.1.5. Weekly body weight gain

At the conclusion of the fourth week, the average body weight gains (g) recorded for the broiler chicks in treatment groups T_1, T_2, T_3, T_4, and T_5 were 600.73 ± 21.82, 448.43 ± 17.11, 352.23 ± 21.79, 440.57 ± 47.17, and 425.40 ± 62.23, respectively (Table 5). The T_1 showed the highest body weight gain, while the lowest was recorded in the T_3 group. However, no significant differences were observed among the antibiotic and FS-treated groups. During the first week, body weight gains varied significantly (P<0.05) among groups, with the T_4 group showing the highest gain.

The body weight gain results indicate that while the T_1 achieved the highest gain by the fourth week, the FS-treated groups, particularly T_3, showed comparatively lower gains. Although the early-stage performance (week 1) favored the T_4 group, the overall growth did not significantly differ among the antibiotic and FS-supplemented treatments by the end of the trial. This suggests that while fenugreek may initially stimulate growth—possibly due to its bioactive compounds—its long-term efficacy as a growth promoter at certain inclusion levels may not match that of a basal diet. Variations in results may be attributed to differences in seed inclusion rates, bird adaptability, and nutrient absorption dynamics (Paneru *et al.*, 2022; Essien and Josiah, 2024).

Table 5. Effect of dietary fenugreek seed and antibiotic levels on weekly body weight gain (g/bird) of broiler chickens.

Treatment	1st w. BWG	2nd w. BWG	3rd w. BWG	4th w. BWG
T_1	203.33 ± 2.52^{b}	293.40 ± 11.48	453.53 ± 11.08	600.73± 21.82^{a}
T_2	202.23 ±0 .67^{b}	307.63 ± 2.18	461.23 ± 17.32	448.43 ± 17.11^{b}
T_3	209.67± 2.48^{ab}	338.47 ± 18.40	495.47 ± 13.47	352.23± 21.79^{b}
T_4	215.07 ± 2.08^{a}	336.90 ± 16.54	500.03 ± 34.84	440.57± 47.17^{b}
T_5	202.40 ± 3.64^{b}	314.67 ± 15.09	488.73 ± 30.56	425.40± 62.23^{b}
Mean ± SE	206.54± 1.64*	318.21 ± 7.02^{NS}	479.80 ± 10.19^{NS}	453.47± 26.07*

**Means with different superscripts differ significantly ($P < 0.05$); SE = standard error; NS = not significant.

3.1.6. Weekly feed consumption (WFC)

Analysis of the mean weekly feed intake reveals notable variations among the treatment groups. In the first week, the lowest feed intake was recorded in the T_4 group (179.33±2.84 g), while the highest was in the T_2 group (185.00±1.15 g) (Figure 2). During the second week, birds in the T_5 group consumed the most feed (447.67±8.81 g), whereas the T_4 group again showed the lowest intake (431.00±5.77 g). In the third week, feed intake peaked in the T_4 group (768.67±6.66 g), while the T_2 group recorded the lowest (755.33±3.33 g). By the fourth week, the highest intake was observed in the T_1 group (903.00±3.21 g), and the lowest in the T_5 group (857.67±2.40 g).

The weekly feed intake pattern across the treatment groups showed inconsistent trends, with no single group maintaining the highest or lowest intake throughout the study period. Although T_4 had the lowest intake during the first two weeks, it recorded the highest intake in the third week, suggesting a possible delayed palatability or adaptation effect. Conversely, the T_2 group showed the highest intake early on but declined in the following weeks. The final week's highest feed intake was observed in the T_1, indicating that neither antibiotic nor fenugreek supplementation consistently enhanced feed intake over time. These fluctuations imply that the influence of FS on appetite may be dose-dependent and time-sensitive, and birds may require an adaptation period before benefiting from its digestive-stimulating properties (Ahmed, 2014; Clark *et al.*, 2019; Tiruneh *et al.*, 2025).

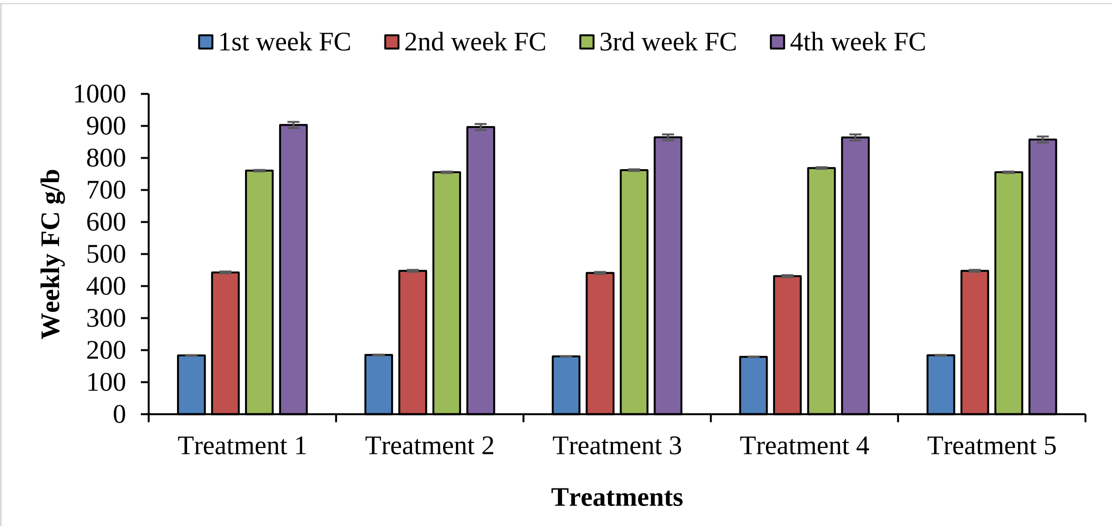

Figure 2. Effect of dietary fenugreek supplementation on weekly feed intake (g/bird) in broiler chickens.

3.1.7. Weekly feed conversion ratio

The average FCR recorded at the end of the fourth week for the treatment groups T_1, T_2, T_3, T_4, and T_5 were 1.51 ± 0.04, 2.00 ± 0.07, 2.47 ± 0.14, 1.99 ± 0.15, and 2.12 ± 0.35, respectively. Statistical evaluation revealed a significant variation (P<0.05) among the groups. The T_3 group exhibited the highest FCR, suggesting lower feed efficiency compared to the control and the antibiotic-supplemented groups (Table 6).

The notably elevated FCR in the T_3 group indicates that this supplementation level may have negatively impacted feed efficiency in broiler chickens when compared to the control and antibiotic-fed groups. While the T_1 showed the most favorable FCR, the poorer performance in T_3 may be attributed to suboptimal inclusion levels or possible antinutritional effects of fenugreek at that dose. This finding contrasts with several previous studies that reported improved FCR with fenugreek supplementation, indicating that the impact of fenugreek on feed efficiency may be dose-dependent and influenced by other factors such as feed composition, bird health, and management practices (Ali *et al.*, 2021; Arif *et al.*, 2021; Ceylan *et al.*, 2023).

Table 6. Impact of fenugreek seed and antibiotic supplementation on weekly feed conversion ratio in broiler chickens.

Treatment	1st w. FCR	2nd w. FCR	3rd w. FCR	4th w. FCR
T_1	0.90 ± 0.00^{ab}	1.51 ± 0.03	1.67 ± .04	1.51 ± 0.04^{b}
T_2	0.91± 0.00^{a}	1.46 ± 0.02	1.64 ± 0.06	2.00 ± 0.07^{ab}
T_3	0.86 ± 0.01^{bc}	1.31 ± 0.09	1.54 ± 0.05	2.47± 0.14^{a}
T_4	0.83± 0.01^{c}	1.29 ± 0.08	1.55 ± 0.09	1.99 ± 0.15^{ab}
T_5	0.91± 0.02^{a}	1.43 ± 0.07	1.56 ± 0.10	2.12 ± 0.35^{ab}
Mean ± SE	0.88± 0.01*	1.40 ± 0.03^{NS}	1.59± 0.03^{NS}	2.02± 0.10*

*Means with different superscripts differ significantly (P < 0.05); SE = standard error; NS = not significant

3.2. Glucose

No statistically significant differences (P>0.05) were found between the treatment groups. While the T_5 group showed the highest glucose concentration (10.76±0.55) and the T_4 group the lowest, these differences were not significant when compared to the control, antibiotic, or other fenugreek-supplemented groups (Table 7). No significant changes (P>0.05) in blood glucose levels were observed across the treatment groups, indicating that fenugreek seed supplementation, even at 2%, does not significantly affect glucose metabolism in broiler chickens (Essien and Josiah, 2024). Although the T_5 group showed the highest glucose values and T_4 the lowest, these differences were not statistically significant when compared to the control or antibiotic-treated groups. This indicates that FS supplementation had a neutral effect on glucose concentration, possibly due to balanced energy utilization across all dietary treatments or physiological regulation maintaining homeostasis in broiler metabolism (Paneru *et al.*, 2022).

3.3. Cholesterol

Serum total cholesterol levels varied significantly (P<0.05) among the treatment groups, ranging from 114.22±4.39 to 142.44±5.06 mg/dl (Table 7). The lowest cholesterol concentration was observed in the T_4 group (114.22±4.39), followed by T_3, T_2, and T_5, with the highest level recorded in the T_1 group (142.44±5.06). The significant reduction in serum cholesterol levels among the fenugreek-supplemented groups, particularly in T_4, suggests a positive hypocholesterolemic effect of FS in broiler diets. The T_1 exhibited the highest cholesterol concentration, while the fenugreek-fed groups consistently showed lower levels, indicating that dietary inclusion of fenugreek effectively modulates lipid metabolism. This effect is likely due to the saponins, fiber, and other bioactive compounds found in fenugreek, which are known to inhibit cholesterol absorption and promote its elimination from the body (Heshmat-Ghahdarijani *et al.*, 2020; Olayeni *et al.*, 2024).

Table 7. Impact of fenugreek seed supplementation on serum biochemical parameters in broiler chickens.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean±SE
Glucose (mmol/L)	10.07±0.41	10.08±0.53	10.18±0.37	9.37±.31	10.76±0.55	10.09±0.20^{NS}
Cholesterol (mg/dl)	142.44 ±5.06^{a}	118.89±4.04^{b}	114.56±5.73^{b}	114.22±4.39^{b}	125.22±2.21^{b}	123.07±2.46*

**Means with different superscripts differ significantly (P < 0.05); SE = standard error; NS = not significant.

3.4. Relative weight of liver, gizzard and heart

The relative liver weights (g) of broiler chicks for groups T_1, T_2, T_3, T_4, and T_5 were recorded as 36.11±1.41, 35.78±2.20, 33.56±0.94, 34.22±0.98, and 32.33±1.18, respectively (Table 8). Although the T_1 group had the highest liver weight and the T_5 group the lowest, these variations were not statistically significant (P>0.05). These results are consistent with Rahimian *et al.* (2018), who found that fenugreek supplementation did not significantly affect liver weight. Likewise, gizzard weights for groups T_1 (31.44±1.08), T_2 (34.78±1.38), T_3 (34.22±1.56), T_4 (34.56±2.14), and T_5 (31.78±1.50) showed no significant differences (P>0.05). Similarly, relative heart weights varied from 6.67±0.32 in T_5 to 7.44±0.26 in T_2, with no statistically significant variation between treatments. These findings align with earlier research indicating that fenugreek seed extract does not influence the weights of visceral organs, suggesting that its antimicrobial and bioactive compounds do not negatively impact the internal organ development of broiler chickens (Weerasingha and Atapattu, 2015).

3.5. Weight of intestine

The intestinal weights of broiler chicks across the various treatment groups ranged from 76.33 ± 5.34 to 80.33 ± 3.00 g, with no statistically significant differences detected (P>0.05) (Table 8). This indicates that fenugreek seed supplementation, at any of the tested levels, did not adversely affect intestinal development or overall organ growth in broiler chickens. This indicates that FS can be safely incorporated into broiler diets without altering the relative weight of the intestines. The findings are consistent with earlier studies, which reported no significant effects of fenugreek supplementation on intestinal or visceral organ weights, further supporting its use as a natural feed additive without adverse anatomical consequences (Elamin *et al.*, 2020; Samani *et al.*, 2020).

Table 8. Effect of dietary fenugreek seed supplementation on liver, gizzard, intestine, and heart weights across treatments.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean±SE
Liver weight (g)	36.11±1.40	35.78±2.19	33.56±0.94	34.22±.98	32.33±1.17	34.40 ±0.64^{NS}
Gizzard weight (g)	31.44±1.08	34.78±1.38	34.22±1.56	34.56±2.13	31.78±1.49	33.36 ±0.70^{NS}
Heart weight (g)	7.39±0.38	7.44±0.25	6.89±0.35	7.06±0.42	6.67±0.32	7.09 ±0.15^{NS}
Intestine (g)	77.89 ±1.73	76.33±5.34	77.89±4.26	80.33±3.00	78.11±2.23	78.11 ±1.53^{NS}

**Means with different superscripts differ significantly (P < 0.05); SE = standard error; NS = not significant.

3.6. Immune organs

The relative spleen weights (g) of broiler chicks in the dietary groups T_1, T_2, T_3, T_4, and T_5 were 1.89±0.16, 1.72±0.16, 1.94±0.25, 1.94±0.15, and 1.67±0.22, respectively. Although T_3 exhibited the highest spleen weight and T_5 the lowest, these variations were not statistically significant (P>0.05). Similarly, the bursa weights followed a comparable trend, with the highest value in T_3 (2.39±0.371) and lower weights observed in T_1 (1.72±0.188), T_2 (1.72±0.278), T_4 (1.78±0.222), and T_5 (1.78±0.278), but the differences were not significant. These findings suggest that dietary inclusion of fenugreek seed did not adversely affect the development of these immune organs (Figure 3).

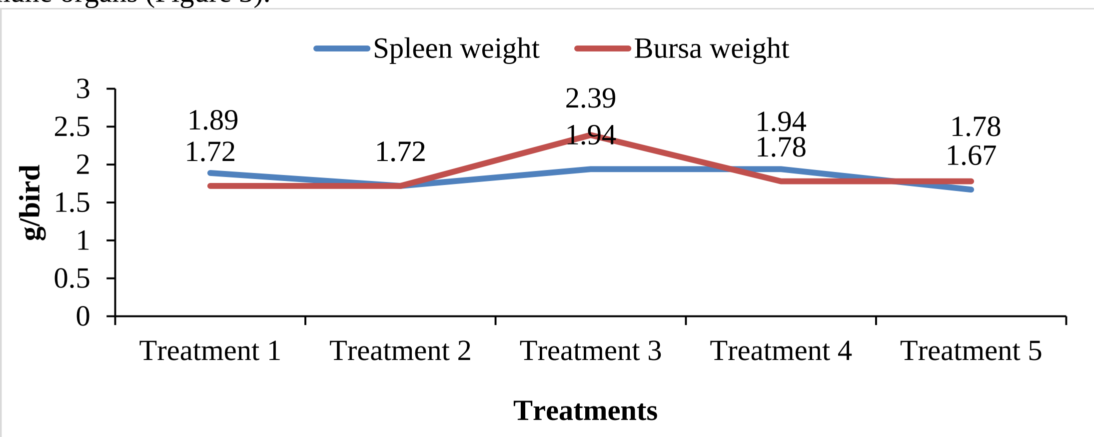

Figure 3. Effect of different levels of fenugreek seed supplementation in broiler diets on immune organ weights.

The findings indicate that dietary supplementation with FS did not significantly (*P*>0.05) influence the relative weights of immune organs, including the spleen and bursa, among the different treatment groups. Although the T3 group showed the highest values for both spleen and bursa weights, these differences were not statistically different from the control, antibiotic, or other FS-treated groups. This suggests that while fenugreek may have some influence on immune organ development, it was not strong enough to produce measurable changes under the conditions of this study (Huang *et al.*, 2022; Mow *et al.*, 2024; Olayeni *et al.*, 2024).

3.7. Hematological parameters

The findings reveal that FS supplementation had a selective impact on hematological parameters in broiler chickens. While most blood constituents remained unaffected, significant increases (P<0.05) were observed in hemoglobin, red blood cell (RBC), white blood cell (WBC), and lymphocyte counts in the FS-supplemented groups (1%, 1.5%, and 2%). This indicates a potential immunostimulatory and hematopoietic effect of FS. Although other parameters such as neutrophils, monocytes, eosinophils, PCV, MCV, MCH, and MCHC did not show statistical significance, birds in the FS groups generally exhibited numerically higher values, suggesting an overall trend toward improved hematological health (Table 9).
The findings suggest that dietary supplementation with FS positively influenced certain hematological parameters in broiler chickens. Specifically, significant improvements (*P*<0.05) were observed in hemoglobin, RBC, WBC, and lymphocyte levels in birds fed diets containing 1%, 1.5%, and 2% FS, indicating enhanced oxygen-carrying capacity and immune response (Mohammed *et al.*, 2023; Essien and Josiah, 2024). These improvements may be attributed to the bioactive compounds in fenugreek, such as antioxidants and immune-stimulating agents. However, other blood indices—including neutrophils, monocytes, eosinophils, PCV, MCV, MCH, and MCHC—remained unaffected, although most showed slightly higher numerical values in FS-treated birds. This suggests that while fenugreek does not broadly alter hematological profiles, it may selectively enhance key indicators of health and immunity (Awad *et al.*, 2015; Paneru *et al.*, 2023).

Table 9. Impact of fenugreek seed supplementation in broiler diets on blood parameters.

Parameters	T_1	T_2	T_3	T_4	T_5	Mean±SE
Hemoglobin(g/dl)	8.70±0.35^{b}	9.20±0.19^{ab}	9.78±0.11^{a}	9.25±0.24^{ab}	9.39±0.19^{ab}	9.26±0.11*
RBC	3.28±0.17^{b}	3.36±0.24^{b}	4.18±0.12^{a}	4.73±0.16^{a}	4.49±0.28^{a}	4.01±0.12*
WBC	6.78±0.70^{b}	7.44±0.33^{ab}	7.67±0.28^{ab}	8.11±0.26^{a}	8.00±0.33^{ab}	7.60±0.19*
Neutrophil	66.56±2.24	69.67±1.52	70.89±1.41	69.78±1.28	71.33±1.26	69.64±0.72^{NS}
Lymphocyte	58.67±4.24^{b}	62.33±4.06^{ab}	73.22±3.75^{a}	66.11±4.72^{ab}	70.22±4.36^{ab}	66.11±1.97*
Monocyte	1.26±0.10	1.50±0.07	1.58±0.11	1.55±0.10	1.44±0.13	1.46±0.04^{NS}
Eosinophil	1.51±0.07	1.52±0.05	1.51±0.08	1.59±0.05	1.55±0.06	1.54±0.02^{NS}
PCV	27.96±1.25	28.66±0.94	30.09±0.92	30.01±0.94	30.06±0.95	29.35±0.45^{NS}
MCV	78.89±0.44	78.46±2.77	81.70±0.93	81.81±1.49	81.52±1.330	80.48±0.71^{NS}
MCH	30.11±0.30	30.43±0.37	30.11±0.50	31.15±0.48	30.76±0.59	30.51±0.20^{NS}
MCHC	30.17±0 .63	31.53±0.33	31.20±0.32	31.14±0.44	31.27±0.36	31.06±0.20^{NS}

**Means with different superscripts differ significantly (*P* < 0.05); SE = standard error; NS = not significant.

3.8. Intestinal micro flora

The *E. coli* count was significantly (*P*<0.05) reduced in broilers fed diets containing 1%, 1.5%, and 2% FS, as well as in the antibiotic-treated group, with values of 12.66±0.59, 12.23±0.58, 12.21±0.47, and 12.12±0.35, respectively, compared to the control group (16.64±0.79). Similarly, *Salmonella* spp. were completely absent (0.00±0.00) in all fenugreek and antibiotic-treated groups, whereas the control group recorded a count of 1.33±0.89. In contrast, *Lactobacillus* counts significantly increased (*P*<0.05) in the fenugreek-supplemented groups, with the highest count observed in the T_5 group (2% fenugreek) at 19.43±0.35, and the lowest in the T_1 at 11.82±0.49 (Table 10).
The results clearly demonstrate that FS supplementation had a significant impact on gut microbial balance in broiler chickens. Birds fed diets containing 1%, 1.5%, and 2% FS, as well as those treated with antibiotics, showed a notable reduction in harmful bacteria such as *E. coli* and *Salmonella*, while exhibiting a significant increase in beneficial *Lactobacillus* spp. populations (Ali *et al.*, 2021; Yang *et al.*, 2022). The complete absence of *Salmonella* spp. in all treated groups highlights the strong antimicrobial properties of fenugreek. Moreover, the enhanced *Lactobacillus* spp. counts, particularly in the 2% fenugreek group, suggest improved gut health and potential immune modulation. These findings support previous studies suggesting that fenugreek possesses

natural antimicrobial and probiotic-promoting properties, making it a promising alternative to antibiotics in poultry nutrition (Obianwuna *et al.*, 2024; Paneru *et al.*, 2024).

Table 10. Bacterial colony counts in broiler chickens fed diets supplemented with fenugreek.

Parameters	*E. coli* $\times 10^6$ (CFU/ml)	*Salmonella* $\times 10^6$ (CFU/ml)	*Lactobacillus* $\times 10^6$ (CFU/ml)
T_1	$16.64^a \pm 0.79$	$1.33^a \pm 0.89$	$11.82^d \pm 0.49$
T_2	$12.12^b \pm 0.35$	$0.00^b \pm 0.00$	$15.02^c \pm 0.77$
T_3	$12.66^b \pm 0.59$	$0.00^b \pm 0.00$	$17.40^b \pm 0.87$
T_4	$12.23^b \pm 0.58$	$0.00^b \pm 0.00$	$18.28^{ab} \pm 0.58$
T_5	$12.21^b \pm 0.47$	$0.00^b \pm 0.00$	$19.43^a \pm 0.35$
Mean±SE	$13.17^* \pm 0.36$	$0.27^* \pm 0.18$	$16.39^* \pm 0.49$

**Means with different superscripts differ significantly ($P < 0.05$); SE = standard error; NS = not significant.

3.9. Antiviral activity

The study demonstrated a significant (P<0.05) influence of FS supplementation on haemagglutination inhibition (HI) titres against Newcastle disease. Broiler chickens in the T5 group, which received the highest FS level, showed markedly elevated ND antibody titres on day 15 (5.78), day 20 (4.00), and day 29 (6.56) compared to the control group. These findings suggest that FS inclusion in the diet may enhance the immune response in broilers (Table 11).

The findings indicate that FS supplementation, particularly at the T_5, significantly (P<0.05) enhanced the immune response of broiler chickens, as reflected by higher haemagglutination inhibition (HI) titres against Newcastle disease on days 15, 20, and 29. This improved antibody response suggests that bioactive compounds in fenugreek, such as saponins and flavonoids, may stimulate the immune system, making it a promising natural alternative to conventional immune enhancers in poultry health management (Samani *et al.*, 2020; Huang *et al.*, 2022).

Table 11. Impact of fenugreek seed supplementation on the immune response of broiler chickens.

Parameters	Day 15 (log2)	Day 20 (log2)	Day 29 (log2)
T_1	4.00 ± 0.28^b	2.89 ± 0.26^b	5.33 ± 0.28^b
T_2	4.44 ± 0.37^b	3.78 ± 0.22^a	5.89 ± 0.20^{ab}
T_3	5.44 ± 0.29^a	3.89 ± 0.26^a	6.44 ± 0.17^a
T_4	5.89 ± 0.26^a	3.89 ± 0.26^a	6.56 ± 0.29^a
T_5	5.78 ± 0.32^a	4.00 ± 0.23^a	6.56 ± 0.24^a
Mean±SE	$5.11 \pm 0.17^*$	$3.69 \pm 0.12^*$	$6.16 \pm 0.12^*$

**Means with different superscripts differ significantly ($P < 0.05$); SE = standard error; NS = not significant.

4. Conclusions

This study explored the potential of fenugreek seed as a natural substitute to antibiotic growth promoters in broiler diets. The findings revealed that incorporating fenugreek improved body weight, dressing percentage, hematological parameters, beneficial intestinal microbiota, and immune function, without adversely affecting internal organ weights or blood glucose levels. These outcomes suggest that fenugreek seed could serve as a safe and effective phytogenic feed additive to enhance broiler health and performance.

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Data availability

Data can be available upon request.

Conflict of interest

None to declare.

Authors' contribution
Sadik Ahmed: conceptualization, research design, data acquisition, data analysis, writing first draft of the manuscript, review and revise the manuscript; Noushin Angum Mow, Md. Mehedi Hasan, Mst. Nasrin Banu, Sajibul Hasan and Shanaz Parvin: data acquisition, data analysis, data interpretation, review and revise the manuscript; Md. Anwarul Haque Beg: research design, data analysis, writing first draft of the manuscript, review and revise the manuscript; Md. Zaminur Rahman: data analysis, writing first draft of the manuscript, review and revise the manuscript. All authors have read and approved the final manuscript.

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