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Effects of Fenugreek Powder on Growth Performance, Meat Quality, Immune Organ Indices, and Serum Antioxidant and Biochemical Indices of Broiler Chickens: Postprint

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Abstract

This experiment aimed to study the effects of dietary supplementation with different levels of fenugreek powder on growth performance, meat quality, immune organ indices, and serum antioxidant and biochemical indices of broiler chickens. Three hundred and twenty 1-day-old Arbor Acres broiler chickens were selected and randomly divided into 4 groups with 8 replicates per group and 10 chickens per replicate. Each group was fed a complete diet containing 0 (control), 3%, 6%, or 9% fenugreek powder with the same nutritional level for a 42-day experimental period. The results showed: 1) At 1-21 days of age, the average body weight and average daily gain of the 6% and 9% fenugreek powder groups were significantly lower than those of the control group ($P<0.05$), the average daily feed intake of the 9% fenugreek powder group was significantly lower than that of the control group ($P<0.05$), and the feed-to-gain ratio of the 3% fenugreek powder group was significantly lower than that of the control group ($P<0.05$). At 22-42 days of age and 1-42 days of age, dietary fenugreek powder level had no significant effect on any growth performance indices of broiler chickens ($P>0.05$). 2) The breast muscle drip loss rate and pressing loss rate of the 9% fenugreek powder group were significantly lower than those of the control group ($P<0.05$). Dietary fenugreek powder level had no significant effect on breast muscle pH45 min, pH24 h, shear force, and cooking loss rate ($P>0.05$). 3) The spleen index at 21 days of age in the 6% fenugreek powder group was significantly higher than that of the control group ($P<0.05$). Dietary fenugreek powder level had no significant effect on thymus index and bursa of Fabricius index at 21 days of age, nor on thymus index, spleen index, and bursa of Fabricius index at 42 days of age ($P>0.05$). 4) The serum total superoxide dismutase activity at 21 days of age in the 9% fenugreek powder group was higher than that of the control

group (P>0.05) and significantly higher than that of the 3% and 6% fenugreek powder groups (P<0.05); the serum total antioxidant capacity at 42 days of age in the 6% fenugreek powder group was significantly higher than that of all other groups (P<0.05). Dietary fenugreek powder level had no significant effect on serum total antioxidant capacity and malondialdehyde content at 21 days of age, nor on serum total superoxide dismutase activity and malondialdehyde content at 42 days of age (P>0.05). 5) The serum aspartate aminotransferase activity at 21 days of age in the 9% fenugreek powder group was significantly higher than that of the control group (P<0.05); the serum aspartate aminotransferase activity at 42 days of age in the 3% fenugreek powder group was significantly higher than that of the control group (P<0.05). Dietary fenugreek powder level had no significant effect on serum total protein, albumin, globulin, urea nitrogen content, albumin/globulin ratio, and alanine aminotransferase activity at 21 and 42 days of age (P>0.05). In conclusion, fenugreek powder can be used as a protein feed ingredient in broiler chicken production, as it has the effects of improving muscle water-holding capacity, promoting immune organ development, enhancing antioxidant capacity, and strengthening body protein metabolism. The appropriate dietary supplementation levels of fenugreek powder in broiler chickens are: not more than 3% at 1-21 days of age, and not more than 6% at 22-42 days of age.

Full Text

Effects of Fenugreek Powder on Growth Performance, Meat Quality, Immune Organ Indexes, and Serum Antioxidant and Biochemical Parameters of Broilers

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Abstract

This study was conducted to investigate the effects of dietary supplementation with different levels of fenugreek powder on growth performance, meat quality, immune organ indexes, and serum antioxidant and biochemical parameters in broilers. A total of 320 one-day-old Arbor Acres broilers were randomly allocated into 4 groups with 8 replicates per group and 10 broilers per replicate. Each group was fed a complete diet containing 0 (control), 3%, 6%, or 9% fenugreek powder at identical nutrient levels for a 42-day experimental period. The results showed: 1) During days 1-21, the average body weight and average daily gain of broilers in the 6% and 9% fenugreek powder groups were significantly lower than those in the control group (P<0.05), the average daily feed intake of the 9% fenugreek powder group was significantly lower than that of the control

group (P<0.05), and the feed-to-gain ratio of the 3% fenugreek powder group was significantly lower than that of the control group (P<0.05). During days 22-42 and 1-42, dietary fenugreek powder level did not significantly affect any growth performance indicators (P>0.05). 2) The drip loss and pressure loss of breast meat in the 9% fenugreek powder group were significantly lower than those in the control group (P<0.05). Dietary fenugreek powder level did not significantly affect pH at 45 min post-slaughter, pH at 24 h post-slaughter, shear force, or cooking loss of breast meat (P>0.05). 3) The spleen index at 21 days of age in the 6% fenugreek powder group was significantly higher than that in the control group (P<0.05). Dietary fenugreek powder level did not significantly affect thymus index or bursa of Fabricius index at 21 days of age, nor thymus index, spleen index, or bursa of Fabricius index at 42 days of age (P>0.05). 4) The serum total superoxide dismutase (T-SOD) activity at 21 days of age in the 9% fenugreek powder group was higher than that in the control group (P>0.05) and significantly higher than that in the 3% and 6% fenugreek powder groups (P<0.05). The serum total antioxidant capacity (T-AOC) at 42 days of age in the 6% fenugreek powder group was significantly higher than that in all other groups (P<0.05). Dietary fenugreek powder level did not significantly affect serum T-AOC or malondialdehyde (MDA) content at 21 days of age, nor serum T-SOD activity or MDA content at 42 days of age (P>0.05). 5) The serum glutamic-oxaloacetic transaminase (AST) activity at 21 days of age in the 9% fenugreek powder group was significantly higher than that in the control group (P<0.05). The serum AST activity at 42 days of age in the 3% fenugreek powder group was significantly higher than that in the control group (P<0.05). Dietary fenugreek powder level did not significantly affect serum total protein, albumin, globulin, urea nitrogen content, albumin-to-globulin ratio, or glutamic-pyruvic transaminase (ALT) activity at 21 and 42 days of age (P>0.05). These results indicate that fenugreek powder can be used as a protein feed ingredient in broiler production, with beneficial effects on muscle water-holding capacity, immune organ development, antioxidant capacity, and protein metabolism. The appropriate dietary inclusion levels of fenugreek powder for broilers are no more than 3% during days 1-21 and no more than 6% during days 22-42.

Keywords: fenugreek powder; broilers; growth performance; meat quality; serum biochemical parameters

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Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual leguminous herb cultivated widely as an economic crop for both medicinal and culinary purposes across Asia, Africa, America, and the Mediterranean region, with cultivation also occurring throughout China [1]. Fenugreek seeds are rich in carbohydrates (50%-60%), protein (25%-30%), and lipids (5%-10%) [2], and contain bioactive compounds including galactomannan, flavonoids, saponins, alkaloids, and 4-hydroxyisoleucine [3]. Fenugreek powder is a byproduct obtained after removing the endosperm from fenugreek seeds for galactomannan extraction, followed by grinding the seed coat and embryo. With crude protein content of 38.2% and true protein content of 28.9%, fenugreek powder has potential as an unconventional protein feed ingredient and has been included in the "China Feed Ingredient Catalogue No. 1773" [4]. Previous studies in poultry and swine have demonstrated that dietary fenugreek powder supplementation significantly improves growth performance and health status [5-6]. Furthermore, research indicates that flavonoids in fenugreek powder possess antioxidant and cholesterol-lowering properties [7], saponins can enhance poultry growth performance [8], and 4-hydroxyisoleucine promotes glucose uptake in skeletal muscle cells [9]. However, the utilization of fenugreek powder as a protein feed ingredient remains underdeveloped, with limited research evaluating its feeding value in broilers and no reports on optimal inclusion levels. Additionally, the potential for fenugreek powder to improve animal health and product quality through its bioactive compounds warrants investigation. Therefore, this study examined the effects of dietary fenugreek powder at different inclusion levels on growth performance, meat quality, immune organ indexes, and serum antioxidant and biochemical parameters in broilers to provide reference and theoretical basis for its application in broiler production.

1. Materials and Methods

1.1 Experimental Material

Fenugreek powder was provided by Yangling Shanghe Plant Science and Technology Industry Co., Ltd. Nutritional composition analysis revealed: energy 20.10 MJ/kg, crude protein 38.30%, total amino acids 26.43%, crude fat 6.98%, calcium 0.10%, and phosphorus 0.06%.

1.2 Experimental Animals and Design

A single-factor experimental design was employed. A total of 320 healthy one-day-old Arbor Acres (AA) broilers with similar body weight were randomly divided into 4 groups with 8 replicates per group and 10 broilers per replicate.

Each group was fed a complete diet containing 0 (control), 3%, 6%, or 9% fenugreek powder at identical nutrient levels. Broilers were raised in a 4-tier cage system with ad libitum access to feed and water, and were vaccinated according to standard broiler immunization protocols throughout the 42-day experimental period.

1.3 Experimental Diets

Diets were formulated according to NRC (1994), Chinese "Feeding Standard of Chicken" (NY/T 33—2004), and the "AA Broiler Feeding Manual." Dietary composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (air-dry basis), %

Items	1 to 21 days of age	22 to 42 days of age
Fenugreek powder level/%	0, 3, 6, 9	0, 3, 6, 9
Ingredients		
Corn	[values]	[values]
Soybean meal	[values]	[values]
Cottonseed meal	[values]	[values]
Fenugreek powder	[values]	[values]
Soybean oil	[values]	[values]
Limestone	[values]	[values]
CaHPO4	[values]	[values]
Premix1)	[values]	[values]
NaCl	[values]	[values]
L-Lys	[values]	[values]
L-Thr	[values]	[values]
DL-Met	[values]	[values]
Phytase	[values]	[values]
Total	100.00	100.00
Nutrient levels2)		
ME/(MJ/kg)	[values]	[values]
CP	[values]	[values]
Ca	[values]	[values]
TP	[values]	[values]
AP	[values]	[values]
Lys	[values]	[values]
Met	[values]	[values]
Thr	[values]	[values]

1) The premix provided the following per kg of diets: VA 8,000 IU, VB1 2 mg, VB2 6 mg, VB5 40 mg, VB6 3.5 mg, VB12 0.02 mg, VD3 3,000 IU, VE 25 IU, VK3 0.5 mg, biotin 0.15 mg, folic acid 1.25 mg, D-pantothenic acid 12 mg,

nicotinic acid 10 mg, Cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 100 mg, Zn (as zinc sulfate) 75 mg, I (as potassium iodide) 0.35 mg, Se (as sodium selenite) 0.15 mg.

2) Nutrient levels were calculated values.

1.4 Measurement Indicators

1.4.1 Growth Performance On a per-replicate basis, fasting body weight and feed consumption were measured at 21 and 42 days of age to calculate average body weight (ABW), average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.2 Meat Quality At 21 and 42 days of age, one broiler with body weight closest to the replicate average was randomly selected from each replicate, fasted for 12 hours, weighed, and slaughtered by jugular exsanguination. At 42 days of age, the right breast muscle was excised to determine muscle pH (pH45 min and pH24 h), drip loss, cooking loss, pressure loss, and shear force values using methods described by Zhang et al. [10].

1.4.3 Immune Organ Indexes After slaughter at 21 and 42 days of age, the spleen, thymus, and bursa of Fabricius were completely removed and weighed to calculate immune organ indexes using the formula: Immune organ index (g/kg) = fresh immune organ weight / live body weight.

1.4.4 Serum Antioxidant Parameters Before slaughter at 21 and 42 days of age, blood samples were collected from the wing vein. Serum was separated after clotting at 37°C, centrifuged at 3,000 r/min for 5 minutes, and stored at -80°C. Serum total superoxide dismutase (T-SOD) activity, malondialdehyde (MDA) content, and total antioxidant capacity (T-AOC) were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.5 Serum Biochemical Parameters Serum total protein (TP), albumin (ALB), globulin (GLO), and urea nitrogen (UN) contents, as well as glutamic-pyruvic transaminase (ALT) and glutamic-oxaloacetic transaminase (AST) activities, were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Data are expressed as means ± standard error. One-way ANOVA was performed using SPSS 16.0 software, followed by Duncan' s multiple comparison test. Differences were considered significant at $P<0.05$.

2. Results

2.1 Effects of Dietary Fenugreek Powder Level on Growth Performance of Broilers

As shown in Table 2 , during the starter phase (days 1-21), the average body weight of broilers in the 3% fenugreek powder group did not differ significantly from the control group (P>0.05), while the 6% and 9% groups showed significantly lower values (P<0.05). The average daily gain was significantly reduced in the 6% and 9% groups compared to the control (P<0.05), but not in the 3% group (P>0.05). Average daily feed intake decreased to varying degrees across all fenugreek powder groups, with the 9% group showing a significant reduction (P<0.05) while the 3% and 6% groups remained non-significant (P>0.05). The feed-to-gain ratio was significantly lower in the 3% group compared to the control (P<0.05), but no significant differences were observed in the 6% and 9% groups (P>0.05).

During the grower phase (days 22-42), dietary fenugreek powder level did not significantly affect any growth performance parameters (P>0.05). However, it is noteworthy that the 9% group exhibited numerically lower average body weight and higher feed-to-gain ratio compared to other groups. Over the entire experimental period (days 1-42), dietary fenugreek powder level had no significant effect on growth performance indicators (P>0.05).

These results suggest that 3% dietary fenugreek powder during the starter phase reduced feed-to-gain ratio without negatively affecting growth, while levels exceeding this threshold adversely impacted feed intake and weight gain. During the grower phase, 3%, 6%, and 9% fenugreek powder did not significantly affect growth performance, though the 9% level posed potential risks for reduced performance. Overall, fenugreek powder can replace portions of corn and soybean meal in broiler diets, with appropriate inclusion levels not exceeding 3% in the starter phase and 6% in the grower phase.

Table 2 Effects of dietary fenugreek powder level on growth performance of broilers

Items	Fenugreek powder level/%	P-value
	0	3
Days 1-21		
ABW/g	647.7$±11.3a|641.6±13.2a|566.6±12.8b|614.3±13.2a|9<	
	$0.001||ADG/g|29.7±0.5a|29.4±0.5a|27.0±0.8b|25.4±0.4b| <	
	$0.001||ADFI/g|47.0±1.1a|43.6±1.2ab|44.3±1.6ab|41.3±1.1b|0.015||F/G|1.59±0.37a|1.47±0.19b	
	$*Days22-42*	
	$*|||||||ABW/g|1,632.4±28.5|1,630.6±50.5|1,642.5±72.6|1,488.3±44.7|0.112||ADG/g|53.5±1.7|5	
	$*Days1-42*	
	$*|||||||ADG/g|41.6±0.7|42.2±1.3|42.6±1.7|38.9±1.2|0.105||ADFI/g|73.0±1.7|72.3±2.1|74.7±2.7	

In the same row, values with different small letter superscripts differ significantly (P<0.05), while those with the same or no superscripts do not differ significantly (P>0.05). The same applies to subsequent tables.

2.2 Effects of Dietary Fenugreek Powder Level on Breast Meat Quality of Broilers

As shown in Table 3 , breast muscle drip loss decreased progressively with increasing dietary fenugreek powder level, with the 9% group being significantly lower than the control (P<0.05). The pressure loss of breast muscle in the 9% group was also significantly lower than that in the control group (P<0.05). Dietary fenugreek powder level did not significantly affect pH45 min, pH24 h, shear force, or cooking loss of breast muscle (P>0.05).

Table 3 Effects of dietary fenugreek powder level on meat quality of breast muscle of broilers

Items	Fenugreek powder level/%				P-value
	0			3	
pH45 min	6.20$±0.06	6.28±0.08	6.34±0.10	6.26±0.04	0.652
	0.001	*Cookingloss*/±1.77	23.17±0.80	19.68±1.50	17.76±1.78	0.143		*Pressureloss*/±2.36*a*	37.4

2.3 Effects of Dietary Fenugreek Powder Level on Immune Organ Indexes of Broilers

As shown in Table 4 , the spleen index at 21 days of age in the 6% fenugreek powder group was significantly higher than that in all other groups (P<0.05). Dietary fenugreek powder level did not significantly affect thymus index or bursa of Fabricius index at 21 days of age, nor thymus index, spleen index, or bursa of Fabricius index at 42 days of age (P>0.05).

Table 4 Effects of dietary fenugreek powder level on immune organ indexes of broilers (g/kg)

Items	Days of age	Fenugreek powder level/%				P-value
		0			3	
Thymus index	21	3.83$±0.55	4.24±0.93	4.00±1.17	3.76±0.53	0.912		*Spleenindex*	21	0.70±0.22*b*	0

2.4 Effects of Dietary Fenugreek Powder Level on Serum Antioxidant Parameters of Broilers

As shown in Table 5 , the serum T-SOD activity at 21 days of age in the 9% fenugreek powder group was significantly higher than that in the 3% and 6% groups (P<0.05) and tended to be higher than the control (P>0.05). Dietary

fenugreek powder level did not significantly affect serum T-AOC or MDA content at 21 days of age (P>0.05). The serum T-AOC at 42 days of age in the 6% fenugreek powder group was significantly higher than that in all other groups (P<0.05). Dietary fenugreek powder level did not significantly affect serum T-SOD activity or MDA content at 42 days of age (P>0.05).

Table 5 Effects of dietary fenugreek powder level on serum antioxidant parameters of broilers

Items	Days of age	Fenugreek powder level/%	P-value
		0	3
T-AOC/(μmol/L)	21	6.82$±1.15\|6.24±0.62\|6.00±0.61\|5.29±0.67\|0.632\|\|$T-SOD/(U/mL)$\|21\|97.77±9.25ab\|76.41±9.16b\|87.97±7.11b\|115.45±6.24a\|0.018\|\|$AOC/(mol/L)$\|42\|6.41±1.03b\|7.93±0.84b\|11.84±0.75a\|6.95±1.18b\|0.003\|\|$T-SOD/(U/mL)$\|42\|111.75±1.53\|107.40±2.30\|108.29±4.07\|106.15±3.16\|0.456\|\|M$	

2.5 Effects of Dietary Fenugreek Powder Level on Serum Biochemical Parameters of Broilers

As shown in Table 6 , serum AST activity at 21 days of age was elevated in all fenugreek powder groups compared to the control, with the 9% group being significantly higher (P<0.05). At 42 days of age, serum AST activity was also higher in all fenugreek powder groups, with the 3% group significantly exceeding the control (P<0.05). Dietary fenugreek powder level did not significantly affect serum TP, ALB, GLO, UN content, albumin-to-globulin ratio, or ALT activity at 21 and 42 days of age (P>0.05).

Table 6 Effects of dietary fenugreek powder level on serum biochemical parameters of broilers

Items	Days of age	Fenugreek powder level/%	P-value
		0	3
TP/(g/L)	21	28.16$±0.82\|28.88±0.52\|27.77±0.73\|29.32±0.92\|0.456\|\|$ALB/(g/L)$\|21\|15.19±0.$	

3. Discussion

3.1 Effects of Dietary Fenugreek Powder Level on Growth Performance of Broilers

Current research on fenugreek powder primarily focuses on its use as a non-nutritive feed additive at low inclusion levels, with limited studies investigating its application as a protein feed ingredient. The present results demonstrate that during the starter phase (days 1-21), dietary inclusion of 3% and 6% fenugreek powder only tended to reduce average daily feed intake without significant

effects. However, when inclusion level increased to 9%, feed intake was significantly reduced. This aligns with findings by Xu et al. [11], who reported that fenugreek powder has poor palatability for poultry, with intake decreasing as inclusion levels exceed 5%. The significant reductions in average body weight and average daily gain at 6% and 9% inclusion levels during the starter phase likely resulted from decreased feed intake due to poor palatability. Regarding feed efficiency, all fenugreek powder groups showed reduced feed-to-gain ratio during the starter phase, with the 3% group achieving statistical significance. During the grower phase and overall experimental period, 3%, 6%, and 9% fenugreek powder did not significantly affect growth performance. However, given the numerically adverse effects on body weight and feed efficiency observed in the 9% group at 42 days of age, it is recommended that dietary fenugreek powder level should not exceed 6% during the grower phase.

3.2 Effects of Dietary Fenugreek Powder Level on Breast Meat Quality of Broilers

Post-slaughter muscle pH changes primarily result from glycogen glycolysis [12]. Poultry muscle pH is positively correlated with water-holding capacity and influences microbial proliferation, thereby determining meat shelf life, making it a crucial meat quality indicator [13-14]. In meat quality assessment, pH45 min below 5.8 is a criterion for pale, soft, exudative (PSE) meat, while pH24 h above 6.0 indicates dark, firm, dry (DFD) meat [15]. The current findings show that dietary fenugreek powder did not significantly affect muscle pH, with all groups maintaining pH45 min and pH24 h within normal ranges, indicating no detrimental effects on muscle pH. Shear force, an important indicator of meat tenderness, is fundamentally affected by muscle composition and chemical changes [12]. Dietary fenugreek powder level did not significantly affect muscle tenderness in this study.

Drip loss, cooking loss, and pressure loss are indicators of muscle water-holding capacity. Lower drip loss and cooking loss correspond to higher water-holding capacity, while pressure loss is negatively correlated with water-holding capacity [16]. In this study, drip loss decreased with increasing dietary fenugreek powder level, with the 9% group significantly lower than the control. Similarly, pressure loss was significantly reduced in the 9% group compared to the control. These results suggest that fenugreek powder can improve muscle water-holding capacity in broilers, with a dose-response relationship evident. Regression analysis yielded the equation: $y = 10.950 - 0.574x$ ($R^2 = 0.374$, $P<0.001$), where x represents dietary fenugreek powder level and y represents drip loss, indicating that 9% fenugreek powder provided optimal meat quality improvement among the tested levels.

3.3 Effects of Dietary Fenugreek Powder Level on Immune Organ Indexes of Broilers

The thymus, spleen, and bursa of Fabricius are important avian immune organs where immune cells are produced, differentiated, and function [17]. Changes in immune organ weight within normal ranges generally correlate with immune function status [18], making immune organ indexes preliminary indicators of immune system development and status. Research has shown that feeding mice fenugreek seed extract at 100 mg/kg body weight for 10 days significantly increased thymus relative weight, phagocyte count, and macrophage phagocytic capacity, demonstrating immunostimulatory effects [19]. The present study found that 6% dietary fenugreek powder significantly increased spleen index at 21 days of age, though no effects were observed on immune organ indexes at 42 days. As the largest peripheral immune organ in poultry, the spleen is crucial for B and T lymphocyte production and maturation, playing important roles in humoral and cellular immunity [20]. These results suggest that 6% dietary fenugreek powder may provide better immunomodulatory effects during the starter phase, possibly attributable to flavonoids and other immunostimulatory compounds reported in previous studies [21-22], though the exact mechanisms require further investigation.

3.4 Effects of Dietary Fenugreek Powder Level on Serum Antioxidant Parameters of Broilers

Normal metabolic activities continuously generate highly oxidative free radicals, which are cleared by the inherent antioxidant system to maintain dynamic equilibrium and protect tissues from oxidative damage [23]. Antioxidant capacity can be evaluated through T-AOC, T-SOD, and MDA indicators [24]. T-AOC reflects the combined action of various oxidative and antioxidant substances, serving as a comprehensive index of overall antioxidant capacity. Superoxide dismutase (SOD) is a ubiquitous endogenous antioxidant enzyme that scavenges free radicals. MDA is a lipid peroxidation product and important indicator of lipid oxidation [25].

Numerous studies have demonstrated antioxidant effects of fenugreek powder and its extracts both in vitro and in vivo. Kaviarasan et al. [26] found that polyphenol extracts from fenugreek seeds prevented erythrocyte lipid peroxidation and hemolysis in a dose-dependent manner. Ravikumar et al. [27] reported that dietary fenugreek powder reduced lipid peroxidation and restored antioxidant systems in diabetic mice. The current study' s observation that 6% fenugreek powder increased serum T-AOC at 42 days of age aligns with these findings.

Interestingly, 3% and 6% fenugreek powder reduced serum T-SOD activity at 21 days of age, while the 9% group showed increased activity compared to the control and significantly higher values than the 3% and 6% groups. Fenugreek powder contains high levels of fat (8.39%), predominantly polyunsaturated fatty

acids (PUFA) comprising 80% of total fatty acids [28]. PUFA are prone to peroxidation, potentially increasing free radical content and MDA levels [29]. During the starter phase when the antioxidant system is not fully developed, this may explain the reduced T-SOD activity at 3% and 6% inclusion levels. At 9% inclusion, the higher PUFA content may have reached the threshold needed to stimulate compensatory antioxidant responses, restoring T-SOD activity to normal levels [30].

3.5 Effects of Dietary Fenugreek Powder Level on Serum Biochemical Parameters of Broilers

Serum TP, ALB, and UN contents are closely related to protein metabolism. TP content, as the sum of ALB and GLO, reflects overall protein synthesis metabolism, while ALB synthesized by the liver indicates nutritional status and protein metabolism [31]. Elevated TP and ALB contents signify enhanced protein synthesis. UN, a protein catabolism product, reflects protein breakdown levels [32]. Dietary fenugreek powder did not significantly affect serum TP, ALB, or UN contents, indicating no adverse effects on protein metabolism. Serum GLO participates in immune activities and serves as an important indicator of humoral immunity, though dietary fenugreek powder did not significantly affect GLO content.

ALT and AST are synthesized in the liver and participate in amino acid metabolism under normal conditions. When hepatocytes are damaged or liver permeability increases, ALT and AST diffuse into blood, causing sharp serum activity increases [33]. Therefore, serum ALT and AST activities reflect both amino acid metabolism levels and liver tissue integrity. At 21 days of age, dietary fenugreek powder increased serum AST activity to varying degrees without significantly affecting ALT activity, suggesting no obvious hepatocellular damage. The increased AST activity likely resulted from enhanced hepatic AST synthesis, reflecting elevated protein and amino acid metabolism. Protein turnover and deposition are crucial physiological processes for weight gain [34]. Combined with the observed changes in feed efficiency and serum AST activity during the starter phase, altered protein metabolism may have contributed to the improved feed conversion at 3% inclusion, though the relationship requires further investigation due to confounding effects on feed intake. Similar patterns at 42 days of age suggest fenugreek powder may enhance amino acid turnover metabolism, though the specific mechanisms need further study.

3.6 Feasibility of Fenugreek Powder as a Protein Feed Ingredient in Broiler Production

The analyzed nutrient composition of fenugreek powder was: energy 20.10 MJ/kg, crude protein 38.30%, total amino acids 26.43%, crude fat 6.98%, calcium 0.10%, phosphorus 0.06%, and apparent metabolizable energy for poultry 8.518 MJ/kg, with main nutrient levels comparable to soybean meal. As a byproduct of galactomannan extraction, fenugreek powder offers clear price ad-

vantages over soybean meal and has been included in the China Feed Ingredient Catalogue, demonstrating potential as an excellent protein feed ingredient.

This study demonstrated that 3% dietary fenugreek powder during the starter phase significantly reduced feed-to-gain ratio without compromising growth performance, while 6% and 9% levels negatively affected starter phase performance. During the grower phase and overall period, 3%, 6%, and 9% fenugreek powder did not significantly impair growth performance. Importantly, these inclusion levels showed no adverse effects on immune organ development, meat quality, or serum antioxidant and biochemical parameters. Furthermore, 3% fenugreek powder enhanced protein metabolism at 42 days, 6% promoted spleen development during the starter phase and increased serum T-AOC at 42 days, and 9% improved muscle water-holding capacity. In conclusion, fenugreek powder can be utilized as a protein feed ingredient in broiler production, with recommended inclusion levels not exceeding 3% during days 1-21 and 6% during days 22-42.

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