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Original Article

Evaluation of the Effects of the Addition of Acacia Gum to the Diet on Productive Performance, Duodenal Viscosity, and Gut Health Indices of Broilers over 35 Days

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ABSTRACT

Acacia gum is a potent prebiotic, which is fermented by the microbiota and increases beneficial bacteria and short-chain fatty acids (SCFA). This experiment aimed to determine the impact of acacia gum on broiler performance, intestinal viscosity, quantitative microbiota, and immune function in broilers. A total of 504 male broilers (43.91±0.03 g, 0 day of age) were randomly divided into six acacia gum groups (A=0, B=125, C=250, D=500, E=750, and F=1000 mg/kg basal diet), with 14 cages each (6 broilers/cage). Performance was measured for 35 days. A total of 14 broilers per group were randomly selected for slaughter to collect samples for analysis of targeted tests at 35 days of age. The results showed that acacia gum improved the performance indicators of broilers (p<0.05). The viscosity of duodenal contents was higher for acacia gum levels from 500 to 1000 mg/kg (p<0.05). Total SCFA, pro-inflammatory cytokines (*IL-1*β, *IL-4*, *IL-6*, and *IL-12*; *TNF*-α; *INF*-γ), and mucosal immune factors (*MUC-2* and *sIg A*) increased with acacia gum supplementation (p<0.05). *IL-10* was lower with 250 to 1000 mg/kg of acacia gum (p<0.05). Furthermore, acacia gum increased the beneficial microbiota (*Lactobacillus* spp., *Bifidobacterial* spp., and *Bacteroides* spp.), while *Clostridium perfringens*. was reduced. The results suggest that adding acacia gum to the diet, especially at a level of 1000 mg/kg, can positively influence growth performance, improve gut health, and enhance immune function in broilers.

INTRODUCTION

In poultry production, the gastrointestinal tract plays an essential role in maintaining optimal growth and performance, especially in broilers (El Sabry & Yalcin, 2023). Historically, antibiotics have been widely used as growth promoters to control pathogenic bacteria and enhance gut health (Lee *et al.*, 2012; Smith, 2019). However, concerns regarding the emergence of antibiotic-resistant bacteria, alterations in the gut microbiota, and increasing consumer demand for antibiotic-free products have led to restrictions on their use in many countries (Belal *et al.*, 2018; Xiong *et al.*, 2018). As a result, in recent years, there has been growing interest in exploring natural feed additives as alternatives to antibiotics to improve gut health and reduce antibiotic dependence (Lockyer & Stanner, 2019). These additives, including prebiotics and natural compounds, modulate the microbiota by promoting the growth of beneficial bacteria - such as *Lactobacillus* spp. and *Bifidobacterium* spp. - and inhibiting harmful bacteria (Ramlucken *et al.*, 2020). This modulation enhances gut health, increase nutrient digestibility, and strengthens the immune system, ultimately improving broiler performance and overall health (Amein *et al.*, 2019; Angwech *et al.*, 2019; Kim & Lillehoj, 2019).

Acacia gum (*Acacia senegal*) is considered a natural prebiotic due to its content of indigestible and soluble fiber, such as rhamnose, arabinose, galactose and glucuronic acid (Ali *et al.*, 2009). It is also

recognized by the US Food and Drug Administration as one of the safest sources of soluble fiber for humans (Al-Fadil *et al.*, 2013; Ahmadi *et al.*, 2017; Korcz *et al.*, 2018). Qiu *et al.* (2022) showed that soluble fibers such as acacia gum can stimulate commensal bacteria to ferment and produce SCFAs, which promote growth and benefit host health. The addition of acacia gum to the diet has shown a strong immunomodulatory effect by altering the function of dendritic cells in the gut (Xuan *et al.*, 2010). These antigen-presenting cells interact directly with intestinal cells, facilitating the production of cytokines such as Tumor necrosis factor-α (*TNF-α*), interleukin-6, 10 and 12, (*IL-6, IL-10* and *IL-12*). Furthermore, acacia gum promotes the proliferation of CD4+ T-cells, which help regulate the immune response (Barkeer *et al.*, 2024). In addition, acacia gum demonstrates antibacterial activity against *Salmonella enteritidis*, as reported by Hu *et al.* (2016). However, research on the effectiveness of adding acacia gum to the diet of broiler chickens is limited. Specifically, the effects on the innate immunity and gut microbiota in broiler chickens remain unclear.

The current experiment hypothesized that the addition of acacia gum to the diet could enhance the gut health of broiler chickens by modulating the gut microbiota, improving gut viscosity, and promoting the immune response. However, the aim of this experiment was to investigate the potential benefits of acacia gum on growth performance and three important aspects of gut health. Firstly, whether acacia gum affects the viscosity of gut contents, which could potentially affect nutrient absorption and gut motility. Secondly, evaluate the effects of acacia gum on microbiota activity and SCFA production, which are critical for digestion and immune function. Finally, investigate the effects of acacia gum addition on the expression of genes related to the immune response in the gut, potentially increasing the resistance of chickens to pathogens. By investigating these factors, this experiment could contribute to the development of effective strategies to improve broiler health and productivity.

MATERIALS AND METHODS

Ethical Statement

Based on the recommendation for the present experiment design, which minimizes the risks for the chickens, the Ethics Committee for Scientific Research of the King Saud University in Saudi Arabia accepted the experiment (No. KSU-SE-20-39).

Preparation of Acacia Gum Powder

Acacia gum (*Acacia Senegal*) was sourced through an authorized export company (Elobied Agro. Export.) from the state of Khartoum, Sudan. Subsequently, purified and finely ground acacia gum was included at various levels in the diets of broilers during the feeding stages.

Design, Broilers and Diets

The experiment involved 504 (0 day of age) male broiler chickens (Ross 308 breed). The broilers were randomly divided into 84 cages (80 cm x 70 cm), considered the experimental units, with 14 replicates per group and 6 broilers per replicate, with an approximately equal average weight (43.91 ± 0.03 g), using a completely randomized design. Six dietary groups were supplemented with acacia gum at levels of 0, 125, 250, 500, 750 and 1000 mg/kg (A, B, C, D, E, and F; respectively) for 35 days. Corn-soybean meal-based mash diets were formulated to meet the nutritional requirements of the Ross 308 strain (Aviagen, 2022) at each of the three feeding stages: starter (0-10 days of age), grower (10-24 days of age), and finisher (24-35 days of age), as indicated in Table 1.

Table 1 – Ingredients and nutritional value of the base diet provided to the broilers at different growth stages (starter, grower, finisher).

Diet components (g/kg)	Different feeding stages		
	0–10 days of age	10–24 days of age	24–35 days of age
Corn	536.7	571.3	617.0
Soybean meal 48% CP	385.5	356	291.0
Corn oil	28.7	33.2	53.0
Dicalcium phosphate	17.1	13.7	15.0
Calcium Carbonate	14.7	11.1	10.2
Sodium chloride	4.3	4.3	4.3
DL-Methionine	4.4	3.8	3.0
L-Lysine HCL	3.6	2.7	2.0
L-Threonine	1.6	1.1	1.0
Valine	1.2	0.7	0.7
Choline CL 60%	0.2	0.1	0.8
Premix [a]	2.0	2.0	2.0
Total	1000	1000	1000
Nutrient analysis (g/kg)			
Metabolizable energy (ME), Kcal/kg	3070	3160	3250
Crude protein (CP)	222.0	206.0	192.0
Lysine	13.0	12.0	11.0
Methionine	7.0	6.0	6.0
Threonine	9.0	8.0	7.0
Ether extract	72.0	87.0	93.0
Ash	61.0	59.0	50.0
Calcium	11.0	11.0	9.0
Total phosphate	8.0	8.0	7.0
Zinc	0.097	0.109	0.097

[a] The composition of the premix /kg includes: 250,000 IU vitamin A; 110,000 IU vitamin D; 16,500 IU vitamin E; 800 mg vitamin K; 600 mg vitamin B1; 1,700 mg vitamin B2; 1,000 mg vitamin B6; 1,000 mg vitamin B12; 40 mg biotin; 400 mg folic acid; 2,000 mg copper; 80 mg cobalt; 60 mg selenium; 1,200 mg iron; 18,000 mg manganese; 400 mg iodine; and 14,000 mg zinc.

All broilers had *ad libitum* access to feed and water throughout the experimental period. Nutrient analyses of diets were carried out in duplicate according to the method described by AOAC (2012).

Housing and Care

The broilers were kept in a temperature-controlled room with automatic cages. The growth stage (24 days of age) ended at a temperature of 22°C and a humidity of 60%, after being gradually reduced from the first day of age (initially 33°C and 50%). Lighting started with continuous light (3-foot candles) for the first week, and was then changed to 20 hours of light (1-foot candles) and 4 hours of darkness for the remainder of the experiment. Standard vaccinations such as Newcastle disease and infectious bronchitis were administered after directly hatching in commercial hatchery by spraying.

Growth Performance

According to established protocols for the evaluation of growth performance during the experimental phases (different feeding stages) from 1 to 35 days of age (Chen *et al.*, 2023; Yesuf *et al.*, 2023), each cage (six broilers/cage) in all dietary groups was weighed to determine daily body weight gain (final body weight - initial body weight/day). Feed intake (feed offered - feed refused/day) was recorded per cage. Finally, the feed conversion ratio (feed intake / body weight gain) was calculated.

Sample Collection

At 35 days of age, 14 broilers from each dietary group (one broiler per cage) were randomly selected for further analysis. After euthanasia, the broilers were carefully dissected to quickly remove their gastrointestinal tract. The contents of the duodenum were collected to measure their viscosity. Cecum contents were collected from each broiler in a sterile container to analyze the SCFAs and bacterial species present. These samples were stored at -80°C until analysis. For the analysis of gene expression, tissue samples were taken from the middle section of the jejunum of each broiler. These samples were placed in sterile tubes and frozen in a special solution for RNA preservation (Qiagen, Germany) and stored at -80°C until further analysis.

Duodenal Digesta Viscosity Measurements

A sample of approximately 15 g of duodenal contents from one broiler per replicate (14 broilers per dietary group) was centrifuged at 12,000 x g for 5 minutes to produce a supernatant. The viscosity of the supernatant was determined using a Brookfield digital viscometer (Model DV-II, Brookfield Engineering Laboratories Inc., Stoughton, MA 02072, USA) as described by Al-Zawqari *et al.* (2016).

Cecal SCFAs Measurement

A sample of cecum contents (0.2 g) from one broiler per replicate (14 broilers per dietary group) was mixed with 5 ml of double distilled water in a polypropylene tube and then homogenized by shaking for 1 min. The pH of the suspension was adjusted to about 2.5 with 5M hydrochloric acid. After the addition of 1 mL acetonitrile and gentle shaking for 10 min at room temperature, the mixture was centrifuged at 12,000 x g for 5 min at 5°C. The supernatant was filtered through a 0.2 µm PTFE syringe filter and 1 mL was transferred to a 1.5 mL glass chromatography vial. The samples were dried at 70°C for approximately 24 hours and reconstituted in 0.25 mL acetonitrile. The concentrations of SCFAs (acetic, propionic, and butyric acids) were determined by gas chromatography-mass spectrometry (Agilent Technologies, Series 1260, Alto, CA) using a SCFA mixture (1000 ppm) as an internal standard (Augsburg, Germany). The peak areas of the chromatograms were used to calculate the amounts of acetate, propionate, and butyrate expressed as a percentage of total SCFA (Zhao *et al.*, 2022; Aljumaah *et al.*, 2020).

Caecal Bacterial Quantification by Real-Time PCR

Total bacterial DNA was extracted from the caecal samples of one broiler per replicate (14 broilers per dietary group) using the QIAamp DNA kit (Qiagen, Hilden, Germany). The concentration and purity of DNA was determined using a Nanodrop 2000 spectrophotometer (Thermo, DE, USA). Quantitative PCR (qPCR) was performed using an Applied Biosystems 7300 Real-Time PCR System. The target primers were added to the Power SYBR® Green PCR Master Mix (Applied Biosystems, Waltham, MA, USA) for the quantification of *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroidetes* spp., *Clostridium perfringens*, and *Escherichia coli* in the caecal content (Table 2). A standard curve was generated using serially diluted pooled DNA (from 10^2 to 10^{12} copies/g of caecal contents) to quantify the bacterial load (Kheravii *et al.*, 2018; Mushtaq *et al.*, 2019). The qPCR results were expressed as $\log_{10}$ copies/g of caecal content.

Table 2 – Primers used for the determination of some bacterial quantifications and gene expression related to the immune response.

Specific primers	Sequences (5′ → 3′)	Product size, bp	Reference
Lactobacillus spp.	F: CACCGCTACACATGGAG R: AGCAGTAGGGAATCTTCCA	93	Kheravii *et al.* (2018)
Bifidobacterium spp.	F: GCGTCCGCTGTGGGC R: CTTCTCCGGCATGGTGTTG	136	
Bacteroides spp.	F: GAGAGGAAGGTCCCCCAC R: CGCTACTTGGCTGGTTCAG	71	
Clostridium perfringens	F: CGGTACCTGACTAAGAAGC R: AGTTTGATTCTTGCGAACG	174	Mushtaq *et al.* (2019)
Escherichia coli	F: CATTGACGTTACCGCAGAAGAAGC R: CTCTACGAGACTCAAGCTTGC	95	
*IL-1*β	F: ACAAGCCGAACAAAGCACAC R: CTCCACATCTGGCTCACGTT	105	Al-Baadani *et al.* (2024)
IL-4	F: GCAGCTGATCCGATTCCTGA R: TCCAACGTACTCTGGTTGGC	100	
IL-6	F: GCTCGCCGGCTTCGA R: GGTAGGTCTGAAAGGCGAACAG	110	Elnagar *et al.* (2021)
IL-10	F: CATGCTGCTGGGCCTGAA R: CGTCTCCTTGATCTGCTTGATG	123	
IL-12	F: ATCCACTGGACCTCAGACCA R: CTCAGAGTCTCGCCTCCTCT	122	Al-Baadani *et al.* (2024)
TNF -α	F: GGGAGTGTGAGGGGTATCCT R: CTGCACCTTCTGTCTCGGTT	93	
INF-γ	F: TCCCAGAAGCTATCTGAGCAT R: CCACCGTCAGTACATCTGAAT	200	
Secretory MUC-2	F: CGGTGATGACAACGACTCCA R: AAGTTTGCACAGTCGTTCGC	162	
sIg A	F: TTCCTGAGTTGCCGAGTGAC R: AGGGATTTCTTGCTGGGAGC	106	
β - *actin*	F: CCTTCCTGGGTAGGTGTCG R: TGGCGTAGAGGTCCTTCCTG	188	

IL= interleukins, *TNF*-α= tumor necrosis factor-α, *INF*-γ= interferon-Y, *MUC-2*= secretory mucin-2, and *sIg A*= secretory immunoglobulin A.

Jejunal Gene Expression of Immune Response

Total mRNA was extracted from tissue samples of one broiler per replicate (14 broilers per dietary group) using the Zymo Quick-RNA Miniprep Kit (Zymo, Irvine, CA, USA), according to the manufacturer's protocol. RNA concentration and purity were determined using a Nanodrop 2000 spectrophotometer (Thermo, DE, USA). Complementary DNA was synthesized from mRNA using the Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA). Quantitative PCR (qPCR) was performed using an Applied Biosystems 7300 Real-Time PCR System. The primers used for the determination of interleukins (*IL-1*β, I*L-4, IL-6, IL-2*, and *IL-10*), *TNF*-α, *INF*-γ, *secretory MUC-2, sIg A*, and β-*actin* (as the housekeeper gene) for *Gallus gallus* are listed in Table 2. Each qPCR reaction was performed in triplicate. Relative gene expression was quantified using the comparative cycle threshold (2^-ΔΔCt) compared to the control group (group A), as previously described by Livak & Schmittgen (2001).

Statistical Analysis

Using general linear models integrated into the SAS software (SAS, 2008), the data were analyzed with a one-way ANOVA. Tukey's test (p<0.05) was used to identify specific differences between dietary groups. Additionally, regression analysis was also performed to determine whether the effect to increasing acacia gum levels was linear or quadratic. Each result is displayed as the mean ± standard error of the mean (mean ± SEM) to indicate the average effect and its variability.

RESULTS

Growth Performance

The effects of the addition of acacia gum on the growth performance parameters of male broilers are shown in Table 3. All groups showed no significant differences in initial body weight at 0 days of age (p>0.05). At 10 days of age, group B had higher body weight than group A (p<0.05), while other groups showed no significant differences. Broilers fed acacia gum (B-F) had a higher body weights at 24 and 35 days of age than those in group A (p<0.05). In addition, broilers fed group B had higher daily weight gain at 0-10, 25-35, and 0-35 days of age than those in group A (p<0.05). In contrast, broilers fed acacia gum (B-F) had a higher daily weight gain at 11-24 days of age than those in group A (p<0.05). Daily feed intake was lower in broilers fed acacia gum (B and C) than in group A at 11-24 and 0-35 days of age (p<0.05). Daily feed intake was not affected (p>0.05) by dietary groups at 0-10 and 25-35 days of age. Feed conversion ratio improved in broilers fed acacia gum (B-F) at all stages in broilers fed groups B and C compared to group A (p<0.05), except at 25-35 days of age. Body weight, daily weight gain and feed conversion ratio showed a quadratic effect to the addition of acacia gum (p<0.05). In addition, daily feed intake showed a linear effect to the addition of acacia gum from 0-35 days of age, while the effect was quadratic between 11-24 days of age (p<0.05).

Table 3 – Effect of acacia gum addition on growth performance parameters of male broilers.

Parameters	Dietary groups (DG)[1]						SEM[2]	*p*-value[3]		
	A	B	C	D	E	F		DG	L	Q
Body weight (g)										
0 day of age	43.93	43.92	43.92	43.95	43.89	43.88	0.04	0.865	0.332	0.535
10 days of age	259^{b}	275^{a}	268^{ab}	272^{ab}	269^{ab}	264^{ab}	3.41	0.019	0.125	0.008
24 days of age	1145^{b}	1233^{a}	1221^{a}	1222^{a}	1236^{a}	1229^{a}	14.5	0.002	0.008	0.009
35 days of age	2180^{b}	2382^{a}	2341^{a}	2345^{a}	2336^{a}	2290^{a}	23.4	<0.001	0.001	<0.001
Daily weight gain (g/day)										
0-10 days of age	21.47^{b}	23.14^{a}	22.36^{ab}	22.75^{ab}	22.54^{ab}	22.02^{ab}	0.34	0.020	0.122	0.008
11-24 days of age	63.31^{b}	68.41^{a}	68.10^{a}	67.89^{a}	69.05^{a}	68.93^{a}	1.01	0.001	0.018	0.037
25-35 days of age	94.12^{b}	104.49^{a}	101.87^{ab}	102.12^{ab}	99.93^{ab}	96.45^{ab}	1.95	0.003	0.003	0.004
0-35 days of age	59.63^{b}	65.35^{a}	64.11^{ab}	64.26^{ab}	63.86^{ab}	62.47^{ab}	0.68	<0.001	0.001	<0.001
Daily feed intake (g/day)										
0-10 days of age	25.82	24.81	24.96	25.68	25.31	25.10	0.26	0.052	0.054	0.404
11-24 days of age	90.67^{a}	86.33^{b}	86.13^{b}	89.10^{ab}	88.76^{ab}	88.19^{ab}	0.87	0.003	0.004	0.038
25-35 days of age	155.78	153.37	153.88	156.32	157.07	154.89	1.24	0.278	0.541	0.851
0-35 days of age	90.76^{a}	88.17^{b}	88.33^{b}	90.35^{ab}	90.45^{ab}	89.39^{ab}	0.56	0.002	0.003	0.178
Feed conversion ratio (g/g)										
0-10 days of age	1.20^{a}	1.07^{c}	1.12^{bc}	1.13^{bc}	1.12^{bc}	1.14^{b}	0.01	<0.001	0.001	0.007
11-24 days of age	1.43^{a}	1.26^{b}	1.26^{b}	1.32^{b}	1.29^{b}	1.28^{b}	0.02	<0.001	0.001	0.002
25-35 days of age	1.66^{a}	1.47^{b}	1.51^{bc}	1.53^{abc}	1.58^{ab}	1.61^{ab}	0.03	0.001	0.001	0.005
0-35 days of age	1.52^{a}	1.35^{c}	1.38^{bc}	1.40^{bc}	1.41^{b}	1.43^{b}	0.01	<0.001	0.001	<0.001

^{a,c} Superscripts above the values within a row suggest a significant difference (p<0.05) for each parameter. [1] Dietary groups = acacia gum was added to the diets at levels of 0, 125, 250, 500, 750 and 1000 mg/kg (A, B, C, D, E, and F; respectively). [2] SEM= Standard error of means for groups effect. [3] DG= Dietary groups effect; L= linear effect; Q= quadratic effect.

Duodenal Digesta Viscosity

The effects of the addition of acacia gum on the viscosity values of the digesta in the duodenum of male broilers are shown in Figure 1. Duodenal viscosity significantly increased in groups supplemented with 500 to 1000 mg/kg of acacia gum (p<0.05). Remarkably, the increase in viscosity was not linear, but followed a curved pattern (quadratic effect) when the acacia gum addition to the diet was increased (p<0.05).

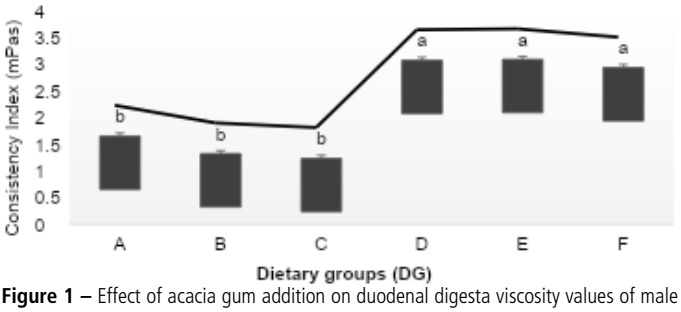

Figure 1 – Effect of acacia gum addition on duodenal digesta viscosity values of male broilers (p-value: DG=0.001; L=0.001; Q=0.001).

Cecal SCFAs Analysis

The effects of the addition of acacia gum on SCFA in the caecum of male broiler chickens are shown in Table 4. The concentrations of acetate, butyrate and total SCFAs were higher in broilers fed acacia gum (groups B-F) than in group A (p<0.05). In addition, propionate concentrations were lower in broilers from groups B and C, but higher when acacia gum was added at 500 to 1000 mg/kg (groups D-F) than in group A (p<0.05). A linear effect from the increasing addition of acacia gum was observed for the concentrations of acetate, butyrate and total SCFAs, and a quadratic effect was observed for propionate concentration to increasing addition of acacia gum (p<0.05).

Table 4 – Effect of acacia gum addition on SCFA (mg/g) in the caecum of male broilers.

Parameters	Dietary groups (DG)[1]						SEM[2]	*p*-value[3]		
	A	B	C	D	E	F		DG	L	Q
Acetate	33.15^{d}	62.33^{ab}	49.37^{c}	59.64^{ab}	54.76^{bc}	66.07^{a}	2.23	<0.001	0.038	0.180
Propionate	6.01^{c}	5.22^{d}	5.28^{d}	10.33^{a}	8.72^{b}	9.71^{a}	0.15	<0.001	0.193	0.041
Butyrate	13.52^{e}	24.42^{b}	23.29^{c}	22.16^{d}	24.13^{b}	31.72^{a}	1.19	<0.001	0.011	0.833
Total SCFA	52.67^{d}	91.96^{b}	77.95^{c}	92.13^{b}	87.61^{bc}	107.51^{a}	2.21	<0.001	0.010	0.585

^{a,e} Superscripts above the values within a row suggest a significant difference (p<0.05) for each parameter. [1] Dietary groups = acacia gum was added to the diets at levels of 0, 125, 250, 500, 750 and 1000 mg/kg (A, B, C, D, E, and F; respectively). [2] SEM= Standard error of means for groups effect. [3] DG= Dietary groups effect; L= linear effect; Q= quadratic effect.

Caecal Bacterial Quantification

The effects of the addition of acacia gum on the quantification of the microbiota in the caecum of male broilers are shown in Table 5. The quantity of *Lactobacillus* spp. and *Bacteroides* spp. was higher in broilers receiving acacia gum than in group A (p<0.05). Broilers from groups E and F had higher quantity of *Bifidobacterium* spp. than those in group A (p<0.05), but this did not affect the other groups (B-D). On the other hand, the quantification of *Clostridium perfringens* was lower in broilers fed acacia gum (B-F) than in group A (p<0.05). *Escherichia coli* showed no effect in broilers fed acacia gum as compared to group A (p>0.05). In addition, there was a linear effect with increasing addition of acacia gum for *Lactobacillus* spp., *Bifidobacteria* spp. and *Bacteroides* spp. (p<0.05). *Clostridium perfringens* showed a quadratic effect from the increasing addition of acacia gum (p<0.05).

Table 5 – Effect of acacia gum addition on the quantification of the microbiota (log_{10} CFU/g) in the caecum of male broilers.

Parameters	Dietary groups (DG)[1]						SEM[2]	*p*-value[3]		
	A	B	C	D	E	F		DG	L	Q
Lactobacillus spp.	2.98^{b}	4.74^{a}	4.19^{a}	4.23a	4.23^{a}	5.05^{a}	0.39	0.027	0.002	0.539
Bifidobacterium spp.	2.71^{b}	3.52^{ab}	3.39^{ab}	3.51^{ab}	4.23^{a}	4.01^{a}	0.27	0.017	0.003	0.495
Bacteroides spp.	4.78^{b}	7.07^{a}	6.97^{a}	6.62^{a}	6.22^{a}	6.94^{a}	0.37	0.002	0.001	0.052
Clostridium perfringen	2.18^{a}	1.55^{b}	1.43^{b}	1.26^{b}	1.24^{b}	1.49^{b}	0.13	0.001	0.009	0.049
Escherichia coli	5.10	4.28	4.75	4.57	4.84	4.32	0.38	0.632	0.206	0.847

^{a,c} Superscripts above the values within a row suggest a significant difference (p<0.05) for each parameter. [1] Dietary groups = acacia gum was added to the diets at levels of 0, 125, 250, 500, 750 and 1000 mg/kg (A, B, C, D, E, and F; respectively). [2] SEM= Standard error of means for groups effect. [3] DG= Dietary groups effect; L= linear effect; Q= quadratic effect.

Gene Expression of Immune Response in Jejunum

The effects of the addition of acacia gum on the expression of immune genes in the intestine of male broiler chickens are shown in Table 6. The expressions of *IL-1*β, *IL-4, IL-12, TNF*-α, *secretory MUC-2*, and *sIg A* were increased in broilers fed with acacia gum as compared to group A (p<0.05). The expression of *INF*-γ was increased in broilers fed acacia gum (p<0.05), with the exception of group D, which was not affected compared to group A. In broilers from groups C to F (250-1000 mg/kg), the expression of *IL-6* was increased and the expression of *IL-10* was decreased in comparison to groups A and B (p<0.05). In addition, the expression of *IL-1*β, *IL-6, IL-10*, and *IL-12* showed a linear effect with the addition of acacia gum (p<0.05). In contrast, the expression of *TNF*-α, *INF*-γ, *secretory MUC-2*, and *sIg A* showed a quadratic effect with the addition of acacia gum (p<0.05).

Table 6 – Effect of acacia gum addition on the relative expression of immune-related genes in the intestine of male broilers.

Parameters[1]	Dietary groups (DG)[2]						SEM[3]	*p*-value[4]		
	A	B	C	D	E	F		DG	L	Q
*IL-1*β	1.00^{c}	1.99^{b}	2.62^{a}	2.77^{a}	1.84^{b}	2.68^{a}	0.08	<0.001	0.016	0.282
IL-4	1.00^{c}	1.45^{b}	1.59^{ab}	1.51^{b}	1.44^{b}	1.90^{a}	0.07	<0.001	0.881	0.187
IL-6	1.00^{b}	1.19^{b}	1.59^{a}	1.66^{a}	1.72^{a}	1.65^{a}	0.06	<0.001	0.036	0.542
IL-10	1.00^{a}	0.98^{a}	0.72^{b}	0.70^{b}	0.69^{b}	0.56^{b}	0.06	<0.001	0.020	0.231
IL-12	1.00^{d}	2.93^{a}	1.73^{c}	1.56^{c}	1.93^{bc}	2.29^{b}	0.11	<0.001	0.005	0.326
TNF -α	1.00^{c}	1.89^{b}	2.27^{ab}	2.23^{ab}	1.94^{ab}	2.61^{a}	0.08	<0.001	0.274	0.030
INF-γ	1.00^{c}	1.88^{ab}	1.68^{b}	1.39^{c}	2.12^{ab}	2.38^{a}	0.12	<0.001	0.052	0.031
MUC-2	1.00^{c}	2.34^{b}	2.94^{a}	2.29^{b}	2.58^{ab}	2.24^{b}	0.11	<0.001	0.031	0.001
sIg A	1.00^{d}	2.70^{c}	3.00^{bc}	3.33^{b}	3.34^{b}	3.77^{a}	0.14	<0.001	0.021	0.007

^{a,d} Superscripts above the values within a row suggest a significant difference (p<0.05) for each parameter. [1]IL= interleukins, *TNF*-α= tumor necrosis factor-α, *INF*-γ = interferon-Y, *MUC-2*= secretory mucin-2, and *sIg A*= secretory immunoglobulin A. [2] Dietary groups = acacia gum was added to the diets at levels of 0, 125, 250, 500, 750 and 1000 mg/kg (A, B, C, D, E, and F; respectively). [3] SEM= Standard error of means for groups effect. [4] DG= Dietary groups effect; L= linear effect; Q= quadratic effect.

DISCUSSION

The gut microbiota plays a crucial role in the health and well-being of the host chicken by influencing the host's physiology and metabolism in various ways (Elnagar *et al.*, 2021). Several studies have reported that acacia gum can be used as a natural prebiotic in broiler diets (Ali *et al.*, 2009; Al-Fadil *et al.*, 2013). Acacia gum contains a high level (up to 80%) of soluble fibers such as galactose, rhamnose and arabinose, which cannot be digested by the chickens themselves (Khalid *et*

al., 2014). Normally, acacia gum is used in traditional human medicine to treat various ailments such as intestinal infections, maintenance of kidney function, and inflammation (Fedail *et al.*, 2016). Research by Abd Al-Fahad & Al-Mashhdani (2023) suggests that the addition of acacia gum can improve the intestinal health of poultry by promoting the fermentation process of beneficial bacteria. Our previous studies (Al-Baadani *et al.*, 2022; 2024) investigated the mechanism of action of acacia gum when fed in high doses to broilers. It has been found that acacia gum remains undigested in the gastrointestinal tract and is then fermented by the intestinal microbiota. This fermentation process promotes the growth and activity of beneficial bacteria and the production of their beneficial by-products such as SCFAs, ultimately leading to a healthier gut environment (Zhou *et al.*, 2024). The current experiment aimed to gain a more nuanced understanding of the effects of acacia gum on broiler performance and refine recommendations for its optimal use in broiler diets until 35 days of age.

In the present experiment, positive effects on body weight were observed in broilers fed 125 mg acacia gum/kg (10 days of age). In addition, 125 mg/kg of acacia gum resulted in higher daily weight gain between 0-10, 25-35, and 0-35 days of age. Interestingly, acacia gum supplementation led to higher body weights at 24 and 35 days of age. This indicates a broader positive effect on overall growth at different acacia gum levels. In addition, a consistent improvement in daily weight gain was observed in all acacia gum-fed groups (11-24 days of age). Overall, these results suggest that acacia gum promotes growth in broilers, with lower levels of acacia gum (125 mg/kg) often showing a more consistent positive effect across different growth stages, while higher levels contributed more to body weight at later stages. Our results are consistent with those of Siham *et al.* (2015), who reported similar beneficial effects of acacia gum administration on overall performance of broilers. They observed increased body weight gain and improved feed conversion ratio when acacia gum was added (250, 500, and 750 mg/kg). Contrary to the assumption that daily feed intake remained unaffected, we observed a significant decrease in feed intake at 11-24 days and 0-35 days of age in broilers fed acacia gum at lower doses (125 and 250 mg/kg). This decrease was not observed with other levels of acacia gum, suggesting a dose-dependent effect on feed intake. This selective reduction in feed intake for specific groups and time periods contrasts with the report by Tabidi & Ekram (2015), who found no effects of acacia gum on daily feed intake of broilers. These discrepancies could be due to differences in acacia gum source, acacia gum levels, broiler strain, or experimental conditions. Despite the observed reduction in feed intake in some acacia gum groups, the feed conversion ratio generally improved at almost all stages with the addition of acacia gum, especially between 11-24 and 0-35 days of age, even with reduced feed intake in broilers fed acacia gum (125 and 250 mg/kg); suggesting that acacia gum increases the efficiency of feed utilization, possibly due to improved nutrient digestibility, gut health, or metabolic efficiency.

The results now clearly state that higher levels of acacia gum (500 to 1000 mg/kg) led to a higher viscosity of duodenal contents. This result is consistent with studies by Williams & Phillips (2021), who explained that the highly branched structure and compact molecules of acacia gum lead to a viscous solution at higher concentrations. Increased viscosity can slow down the passage of digestive fluid through the intestine, which can prolong the time of nutrient absorption and improve nutrient utilization (Ayres *et al.*, 2019). However, it appears that high viscosity has no effect on the growth performance of broilers fed high levels of acacia gum, which may be due to stimulation of the growth of beneficial bacteria in the gut. These bacteria can contribute to improved nutrient digestion and absorption, possibly offsetting the negative effects of increased viscosity.

In the present experiment, broilers fed acacia gum (125-1000 mg/kg) showed higher concentrations of acetate, butyrate, and total SCFAs. The propionate concentrations were lower in groups B (125 mg/kg) and C (250 mg/kg), but significantly higher with doses of 500 to 1000 mg/kg. The results also explicitly state that acetate, butyrate, and total SCFAs showed a linear effect, while propionate had a quadratic effect. These results indicate that acacia gum may be fermented by intestinal bacteria, leading to the production of SCFAs. This is a positive sign, as SCFAs are important energy sources for the host and play a crucial role in intestinal health. Jiménez-Moreno *et al.* (2009) reported that concentrations of SCFAs are influenced by factors such as age, fiber type, and the extent of fermentation in broilers. SCFAs such as acetate, propionate, and butyrate are important by-products of the fermentation of gut microbiota. They play an important role in maintaining the structural and functional integrity of the gut (Sobczak & Kozlowski, 2016). Butyrate is the preferred energy source for the intestinal mucosa;

propionate contributes to gluconeogenesis in the liver, and acetate is used by the host for the biosynthesis of cholesterol and fatty acids (Louis & Flint, 2009). The result obtained is in line with Liu *et al.* (2019), who indicated that the highest butyric acid and total SCFA concentrations were associated with an improvement in mucosal structure and immunomodulation. Furthermore, the experiment results support the prebiotic potential of acacia gum (Alvarez-Sieiro *et al.*, 2016).

The intestinal microbiota in the cecum of broiler chickens can change due to fermentation and the breakdown of indigestible fibers (Qiu *et al.*, 2022). In the experiment, an increase in beneficial bacteria such as *Lactobacillus* spp., *Bifidobacteria* spp., and *Bacteroides* spp. was observed in chickens fed different levels of acacia gum (125-1000 mg/kg). Conversely, *Clostridium perfringens* decreased with acacia gum. These results are consistent with the established concept that prebiotics promote the growth of beneficial bacteria. Studies suggest a prebiotic effect of acacia gum on the growth of *Bifidobacteria* spp., which is consistent with observations in the human gut (Sasaki *et al.*, 2022). For *Bacteroides* spp., the picture is less clear. Although some *Bacteroides* spp. have been shown to utilize sugars from acacia gum in the laboratory (Cartmell *et al.*, 2018), other studies have reported an increase in *Bifidobacteria* spp. and *Bacteroides* spp. in the human gut or in simulated models following acacia gum administration (Calame *et al.*, 2008). SCFAs are also produced by intestinal bacteria that ferment oligosaccharides, which may have antimicrobial properties. These SCFAs can disrupt the cell membranes of harmful Gram-negative bacteria such as *Clostridium perfringens*, and alter the pH of the intestinal environment, ultimately killing these bacteria (Faber *et al.*, 2012). Acetic acid, butyric acid, and propionic acid all exhibit antimicrobial properties and likely play a critical role in controlling populations of pathogenic bacteria (Menconi *et al.*, 2014). This control is likely achieved by lowering the pH of the gut through the production of these acids (Suiryanrayna & Ramana, 2015). According to Al-Alawi *et al.* (2018), the high concentration of non-polar components in acacia gum could be related to its antibacterial effect. In addition, Lawrence *et al.* (2015) investigated the antibacterial effect of acacia gum extracts against various bacteria. They attributed the observed activity to secondary metabolites such as tannins, flavonoids, and other compounds contained in these extracts. It is important to know that some bacteria, such as those of the genus *Lactobacillus* spp., can lower the pH in the gut by producing lactic acid. These bacteria are considered beneficial as they help control pathogens (Baldwin *et al.*, 2018).

The current experiment also found that the expression of *IL-1*β, *IL-4, IL-12*; *TNF*-α; and *INF*-γ was increased in broilers receiving acacia gum from 125 to 1000 mg/kg in comparison to the control group. The relative expression of *IL-6* was increased, and the relative expression of *IL-10* was decreased in broilers receiving acacia gum (250 to 1000 mg/kg) supplementation to their basal diets (groups C to F) as compared to groups A and B. The experiment suggests that acacia gum itself may act as an antigen that is recognized by immune cell receptors and may have a positive effect on immunity. The observed cytokine expression patterns differ from the findings of Kamal *et al.* (2018), who reported reduced expression of *IL-4* and *IL-6* in acacia gum in humans. This suggests species-specific responses. In addition, acacia gum decreased the expression of *TNF*-α in rats (Ali *et al.*, 2013). In a previous experiment, a dietary group with prebiotic (MOS, 200 mg/kg) increased the gene expression of *IL-12* and *INF*-γ in broilers (Yitbarek *et al.*, 2012). Adhikari & Kim (2017) associated lactic acid-producing bacteria with increased *INF*-γ production, which could explain the observed increase when acacia gum was added (as acacia gum is fermented by these bacteria). Cytokines such as *IL-12* and *INF*-γ are important for cell-mediated responses against intracellular pathogens and the activation of macrophages (Palamidi *et al.*, 2016). On the other hand, the effect of acacia gum on cytokine expression in this experiment could be due to its fermentation ability, alteration of the microbiota, or epithelial integrity of the small intestine. Sivaprakasam *et al.* (2016) reported that acacia gum regulates the expression of proinflammatory cytokines (*TNF*-α, *INF*-γ and *IL-6*) both directly and indirectly by modulating the host immune response via the microbiota. The expression of *MUC-2* and *sIg A* was increased in broilers receiving acacia gum from 125 to 1000 mg/kg in comparison to the control group. The gene for *sIg A* is a protein secreted by plasma cells that helps prevent pathogens from attaching to the intestinal mucosa, thus enhancing the immune response, while the gene for *MUC-2* is associated with mucin secretion, which acts as the first line of defense in the intestine (Liu *et al.*, 2019). Dietary supplementation with prebiotics can strengthen the function of the intestinal barrier by increasing the number of goblet cells and *sIg A* -secreting cells (Shao *et al.*, 2013). These findings

are consistent with research by Rajani *et al.* (2016), who reported that prebiotics can increase *MUC-2* gene expression, further supporting the potential of acacia gum as a prebiotic to improve intestinal barrier function.

Our results are consistent with previous studies emphasizing the prebiotic and immunomodulatory properties of acacia gum. However, discrepancies with other studies could be due to different experimental conditions, such as the type of diet, the duration of supplementation, or the specific dosages used. While higher concentrations may have been used in some studies, our results suggest that even moderate doses of acacia gum may have significant effects on gut health and immune responses in broilers.

CONCLUSION

Overall, these results indicate that the addition of acacia gum to the diet has a positive effect on growth performance, gut health, and broiler immune function. The observed improvements in growth parameters, SCFA production, beneficial microbiota (*Lactobacillus* spp., *Bifidobacterial* spp., and *Bacteroides* spp.) and the expression of immune genes (pro-inflammatory cytokines and mucosal immune factors) indicate that acacia gum can be a valuable feed additive as a natural prebiotic to improve the health and productivity of broilers, especially at the dosage of 1000 mg/kg of the basal diet.

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AUTHOR CONTRIBUTIONS

Conceptualization and writing of the original draft by H.H. Al-Baadani. Methodology, investigation, formal analysis, review and editing by A.S. Alharthi, M.M. Azzam and A.A. Aboragah. Conceptualization, data curation, visualization and project management by R.A. Alhotan and I. Alhidary. All authors reviewed and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The corresponding author (H.H. Al-Baadani) can provide the data supporting the conclusions of the experiment upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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