Guava Leaf Meal (*Psidium guajava* L.) in Broiler Diets: Effects on Performance, Nutrient digestibilty, and Intestinal Morphology

Harina de hojas de guayaba (*Psidium guajava* L.) en dietas para pollos de engorde: efectos sobre el rendimiento, la digestibilidad de los nutrientes y la morfología intestinal

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Abstract

This study investigated the effect of guava leaf meal (GLM) as a phytobiotic in broilers, focusing on its chemical properties and potential physiological benefits. 135 one-day-old male Cobb broilers were randomly allocated to five treatments (nine replicates per treatment and three birds per replicate): a basal diet with regulated commercial antibiotic (T1), without regulated commercial antibiotic or growth promoters (T2), 1% GLM (T3), 1.5% GLM (T4), and 2% GLM (T5) for 38 d. T2, T3, T4, and T5 reduced feed intake (FI) during the finishing phase (days 20–38, $P < 0.0001$), but there were no statistical differences in accumulated feed intake (AFI) between treatments. GLM groups had lower ADG during the starter phase (days 3-20, $P < 0.05$), but there were no statistical differences in accumulated gain. Accumulated feed conversión rate (FCR) was better in T2 to T5 compared to T1 ($P < 0.05$). GLM groups (T3, T4 and T5) showed significantly higher values of nutrient digestibility ($P < 0.05$). Duodenum morphology showed that number of villi ($P=0.02$) and the villus height ($P= 0.03$) increased with GLM supplementation with respect to control groups (T1 and T2). In conclusion, GLM-based diets enhanced nutrient digestibility and improved intestinal architecture, thereby supporting their inclusion in broiler chicken diets to optimize production efficiency.

Keywords
Intestinal architecture • phytobiotics • poultry • plant extracts • additives

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Resumen

Se estudió el efecto de la harina de hojas de guayaba como fitobiótico en pollos de engorde, centrándose en sus propiedades químicas y sus posibles beneficios fisiológicos. 135 pollos Cobb machos de un día de edad se aleatorizaron en 5 tratamientos (9 réplicas por tratamiento y 3 aves por réplica): una dieta basal con antibiótico comercial (T1), sin antibiótico comercial ni promotores de crecimiento (T2), 1% GLM (T3), 1,5% GLM (T4) y 2% GLM (T5) durante 38 días. T2, T3, T4 y T5 redujeron el consumo de alimento (CA) en fase de finalización (día 20-38, $P < 0,0001$), pero en consumo de alimento acumulado (CAA), no hubo diferencias estadísticas entre tratamientos. Los grupos GLM tuvieron menor ganancia media diaria (GMD) en fase de inicio (día 3-20, $P < 0,05$), pero en ganancia acumulada no hubo diferencias estadísticas. La tasa de conversión alimenticia acumulada (TCA) fue mejor en los grupos T2 a T5 en comparación con T1 ($P < 0,05$). Los grupos GLM (T3, T4 y T5) mostraron valores significativamente mayores de digestibilidad de nutrientes ($P < 0,05$). La morfología del duodeno mostró que el número de vellosidades ($P = 0,02$) y altura de vellosidades ($P = 0,03$) aumentaron con la adición de GLM en comparación con los grupos control (T1 y T2). En conclusión, las dietas adicionadas con GLM mejoraron la digestibilidad de los nutrientes y la arquitectura intestinal, lo que justifica su inclusión en las dietas de pollos de engorde, para optimizar la eficiencia productiva.

Palabras clave
Arquitectura intestinal • fitobióticos • aves de corral • extractos vegetales • aditivos

Introduction

Using technological or food additives is an effective strategy to enhance animal productivity while providing a natural approach to reducing production costs. Plant extracts, also known as phytobiotics or phytogenics, have been used for medicinal purposes since ancient times and are widely employed in traditional and alternative veterinary medicine (6). In recent years, the use of herbal medicines and plant-based extracts in livestock production has gained popularity, driven by concerns over the side effects of conventional drugs, high input costs, toxic residues in feed, microbial resistance, and the growing demand for organic and sustainable livestock production systems (16, 20). For this reason, plant-based additives have been widely investigated as alternatives to antibiotics and growth promoters for use in animal health and production, since they perform multiple beneficial functions in the gastrointestinal tract and are less likely to induce the development of microbial resistance (34).

Guava leaf, characterized by its unique chemical composition (28), has been demonstrated to exert various physiological effects in humans and animals. It serves as an antimicrobial agent (8, 10), provides health-promoting bioactive compounds (15, 19), offers protective effects on the gastrointestinal tract (33), and exhibits potential nutraceutical benefits (13), among other advantages.

Adding guava leaf extracts has been shown to enhance productive performance and reduce the incidence of diarrhea in weaned piglets (33). In broiler chickens, incorporating guava fruit by-products during the starter phase (7-21 days) improved productive performance and meat quality, with a linear increase in daily weight gain (DWG) as the by-product inclusion increased. Although no changes were observed in villus height or crypt depth, the villus height-to-crypt depth ratio increased with higher levels of the by-product (23). Similarly, diets containing guava leaf meal combined with olive oil in chickens led to improved weight gain, better feed conversion rates, reduced fat content in breast and thigh muscles, and lower total blood lipid levels (22).

The gastrointestinal tract plays a critical role in digestion and nutrient absorption, essential for proper animal growth and development, ultimately enhancing productive performance. Specifically, the morphology and histological structure of the small intestine, particularly the duodenum, are vital. The duodenal mucosa with its villi and microvilli, facilitates efficient nutrient assimilation. These structures significantly amplify nutrient

absorption, increasing it by approximately 10 and 20 times, respectively. Moreover, the quantity and length of villi and microvilli can further expand the intestinal mucosa's surface area, enhancing absorption capacity (11, 14, 31).

Research indicates that guava leaf meal and its extracts, when included in animal diets, positively influence intestinal morphology, immune response, and productive parameters. Specifically, these supplements enhance intestinal morphology, improving nutrient absorption (12, 16, 31). They also strengthen immune response, reducing microbial load and supporting better physiological outcomes (8, 10, 13, 18). Additionally, they contribute to improved productive parameters, such as growth and feed efficiency (1, 2). However, no prior studies have specifically investigated these effects in chickens, particularly regarding nutrient digestibility. Therefore, this study aims to evaluate the impact of adding guava leaf meal to chicken diets on intestinal morphology, nutrient digestibility, and productive parameters.

Materials and methods

The trial was conducted at the Experimental Farm of the Facultad de Ciencias Agrarias de la Universidad Politécnico Colombiano JIC (Jaime Isaza Cadavid). The experimental procedures in this trial were approved by the Ethical Committee Animal Care and Use of the University under filed protocol number 20610801-202301004049.

Experimental Design and Diets

A total of 135 male day-old broiler chickens (Avian Male Cobb) were purchased from a local authorized distribution company. The broiler chickens were randomly assigned to five groups in a completely randomized design. Each group consisted of nine replicate cages, with three birds housed per cage in an appropriate housing facility. The five dietary treatments consisted of a basal diet with a regulated commercial antibiotic (Zinc bacitracin 15%, 500 g/t and Halquinol 60, 100g/t) (T1), a basal diet without antibiotics or growth promoters (T2), and diets added with1% (T3), 1.5% (T4), or 2% (T5) guava leaf meal (GLM) in both starter (days 3-20) and finisher (days 21-38) phases. The basal diet, a corn-soybean meal-based commercial crumble feed, was formulated to meet broiler nutritional requirements per NRC (1994) and purchased from a recognized local feed company. Diets were isoproteic and isoenergetic, with their chemical composition analyzed following AOAC (2005) methods, as presented in table 1 (page 168). Broiler chickens were fed a basal diet during the first 2 days of acclimatization. The groups were provided a basal diet, either added with guava leaf meal (GLM) or unadded based on the treatment. The birds had ad libitum access to feed and water throughout the experimental period. Lighting was 24 hours per day during the first week and 16 hours of light and 8 hours of darkness in subsequent weeks. All birds were subjected to consistent environmental and management conditions.

GLM Collection

To prepare guava leaf meal (GLM), leaves were collected from the northern region of Antioquia in the Colombian tropics, located at 1,100 meters above sea level with an average temperature of 26°C. The leaves were cleaned, dried in a forced-air oven, and ground into a fine powder using a laboratory mill for incorporation into experimental diets. The composition of GLM is presented in table 2 (page 168).

Growth Performance

Initial body weight (BWi) was determined at the beginning of the experiment. Body weight was recorded weekly for each cage and subsequently analyzed by period (starter: days 3-20; finisher: days 21-38). In addition, the feed offered and the feed refused were weighed daily. The recorded data was used to calculate average daily gain (ADG, $g.chick^{-1}$), feed intake (FI, $g.chick^{-1}$), feed conversion ratio (FCR, $FI.WG^{-1}$) and weight gain (WG, $g.chick^{-1}$) for the starter, finisher and total periods.

Table 1. Composition and nutritional value of basal diets for starter and finisher periods ($g.kg^{-1}$).

Tabla 1. Composición y valor nutritivo de las dietas basales para periodo iniciador y terminador ($g.kg^{-1}$).

Diet ($g.kg^{-1}$)		
	starter	**Finisher**
Ingredients		
Corn meal (*Zea mays*)	522	540
Soybean meal (46% CP)	218	193
Extruded soybean meal	200	200
Soy oil	21.5	31.8
Salt	3.3	4.0
$CaCO_3$	9.0	8.2
PO_4H_2Ca	19.8	17.5
DL-Methionine	2.4	1.4
Vitamin and mineral mixture [1]	4.0	4.0
Calculated Analysis [2]		
AME_n ($kcal.kg^{-1}$)	3,100	3.200
Total lysine ($g.kg^{-1}$)	12.1	11.5
Sulfur amino acids ($g.kg^{-1}$)	9.0	7.7
Determined Analysis		
Dry matter (DM, $g.kg^{-1}$)	880	885
Gross energy (GE, $kcal.kg^{-1}$)	4,680	4.710
Crude protein (CP, $g.kg^{-1}$)	215	191
Crude ash ($g.kg^{-1}$)	70	65
Ca (Ca, $g.kg^{-1}$)	10.5	8.9
P (total P, $g.kg^{-1}$)	7.4	6.9

[1]Provided the following per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D_3, 2,400 IU; vitamin E, 30 mg; vitamin K_3, 3 mg; vitamin B_1, 2.2 mg; vitamin B_2, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 11 mg; folic acid, 1.5 mg; biotin, 150 mg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; ethoxyquin, 150 mg. [2]FEDNA (2003).

[1]Se proporcionó lo siguiente por kilogramo de dieta completa: vitamina A, 12.000 UI; vitamina D3, 2.400 UI; vitamina E, 30 mg; vitamina K3, 3 mg; vitamina B1, 2,2 mg; vitamina B2, 8 mg; vitamina B6, 5 mg; vitamina B12, 11 mg; ácido fólico, 1,5 mg; biotina, 150 mg; pantotenato de calcio, 25 mg; ácido nicotínico, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0,33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0,15 mg; etoxiquina, 150 mg. [2]FEDNA (2003).

Table 2. Chemical Composition of Guava Leaf Meal (GLM) ($g.100^{-1}$) on a Dry Matter Basis.

Tabla 2. Composición química ($g.100^{-1}$) de la harina de hoja de guayaba en base a materia seca.

Component	Result	Analysis Method
Calcium	1.17	Atomic Abs Spectrometry
Crude Protein	11.2	Volumetric (Kjeldahl)
Ash	5.58	Gravimetric
Fat content	2.73	Gravimetric
Phosphorus	0.15	UV-VIS Spectrophotometry
Moisture and Volatile matter	9.0	Gravimetric
Gross calorific value	4817 $cal.g^{-1}$	Calorimetry

Results are expressed on a dry matter basis. Crude protein content was calculated using a conversión factor of 6.25. Results apply only to the analyzed simple (Sample Code: 78376, Request: 12247).

Los resultados se expresan en materia seca. El contenido de proteína cruda se calculó utilizando un factor de conversión de 6,25. Los resultados corresponden únicamente a la muestra analizada (Código de muestra: 78376, Solicitud: 12247).

Digestibility

To assess nutrient digestibility, feed intake was recorded, and total excreta were collected on days 34 and 35. Excreta from each experimental diet were quantitatively collected daily from five cages per treatment. Excreta samples were homogenized, and subsamples were taken per cage. Both feed and excreta samples were dried in a forced-air oven at 103°C to constant weight to determine dry matter (DM) content, following AOAC method 925.09 (4). Ash content was determined by incinerating the samples in a muffle furnace at 525°C for 7 hours, according to AOAC method 923.03 (4). Organic matter (OM) was calculated as:

$$OM\ (\%) = 100 - \%\ Ash$$

Crude protein (CP) was determined using the Kjeldahl method, multiplying nitrogen content by 6.25, as per AOAC method 984.13 (4). Calcium (Ca) and phosphorus (P) were analyzed by atomic absorption spectrophotometry for Ca (AOAC method 927.02) and colorimetry for P (AOAC method 965.17) (4). Apparent digestibility for each nutrient (dry matter, organic matter, crude protein, calcium, and phosphorus) was calculated using the formula:

$$\text{Apparent Digestibility (\%)} = ((\text{Nutrient intake} - \text{Nutrient excreted}) / \text{Nutrient intake}) \times 100.$$

Experimental Sampling and Intestinal Morphology

On day 38, six birds per treatment were randomly selected from two replicate cages (three birds per cage) to ensure representative sampling. The birds were transported to the slaughterhouse and euthanized by cervical dislocation. Slaughter weight was recorded for each bird. A 1 cm segment of the medial duodenum was collected from each bird and immediately fixed in 10% neutral buffered formalin for preservation. Tissue samples were processed using a rotary microtome to obtain 5 µm sections, stained with hematoxylin and eosin (H&E) following standard histological procedures.

The intestinal mucosa was examined under a light microscope with a Moticam® digital camera at 4× and 10× magnifications. Morphometric analysis focused on the duodenal villi and crypts. Villus height was measured in microns from the basal edge (at the junction with the crypt) to the apical edge. Crypt depth was determined by measuring the distance from the base of the crypt to the villus-crypt junction. The number of villi per visual field (villi/visual field ratio) was quantified by counting the total number of intact villi within a standardized field of view at 4× magnification, ensuring consistent measurements across samples. All measurements were performed using calibrated image analysis software coupled with the Moticam® system.

Model and Statistical Analysis

The experiment was conducted using a completely randomized design with nine replicates per treatment. For performance parameters, the experimental unit was defined as a cage containing three birds, while for intestinal morphology measurements, six birds per treatment were sampled from two replicate cages (three birds per cage). The statistical model used for analysis was:

$$Yij = \mu + Ti + eij$$

where
Yij = the observed dependent variable
µ = the overall mean
Ti i = the fixed effect of the ith treatment
eij = the random error term.

Data were analyzed using the General Linear Model (GLM) procedure (PROC GLM) in SAS (version 9.4) (2017). Differences among treatment means were evaluated using analysis of variance (ANOVA), followed by Tukey test for comparisons when significant effects were detected ($P < 0.05$).

Results

Growth Performance

All broiler chickens fed with the experimental diets remained healthy throughout the study, with no observed adverse symptoms or signs of disease. Table 3 summarizes the growth performance parameters across starter (days 3-20), finisher (days 20-38), and accumulated phases for broiler chickens fed diets supplemented with guava leaf meal (GLM). Feed intake (FI) in the finisher phase was significantly reduced in treatments T2 (negative control), T3 (1% GLM), T4 (1.5% GLM), and T5 (2% GLM) compared to T1 (positive control with commercial antibiotic) ($P < 0.0001$). However, accumulated feed intake (AFI) showed no significant differences across treatments ($P = 0.29$). Average daily gain (ADG) in the starter phase was lower in T3 and T4 than T2 ($P = 0.009$), with T1 and T5 showing intermediate values. In contrast, no significant differences were observed in ADG during the finisher phase ($P = 0.26$) or accumulated ADG ($P = 0.26$). The accumulated feed conversion ratio (FCR) was significantly improved in T2, T3, T4, and T5 compared to T1 ($P = 0.007$), with T2 and T5 exhibiting the lowest FCR values (1.55 and 1.62, respectively).

Table 3. Effect of additing guava leaf meal (GLM) in the diet of broiler chickens on the growth performance during starter, finisher and accumulated phases.

Tabla 3. Efecto de la adición de harina de hoja de guayaba (GLM) en la dieta de pollos de engorde sobre parámetros productivos en las fases de crecimiento, finalización y acumulado.

Parameter	Treatment						
	T1 contr +	T2 contr -	T3 1% GLM	T4 1.5% GLM	T5 2% GLM	SEM	p-value
Starter Phase (days 3-20)							
FIi ($g\ d^{-1}$)	92.6	94.7	97.6	100.6	97.7	32.7	0.51
BWi (g)	909.6^{ab}	925.3^{a}	851.7^{ab}	841.7^{b}	876.6^{ab}	902	0.03
ADG i ($g\ d^{-1}$)	65.8^{ab}	67.5^{a}	59.15^{b}	58.6^{b}	61,5^{ab}	7.8	0.009
FCR i (FI.BW^{-1})	1.43^{a}	1.42^{a}	1.72^{bc}	1.77^{bc}	1.62^{ac}	0.008	0.002
Finisher Phase (days 20-38)							
FIf ($g\ d^{-1}$)	156,6^{a}	139^{b}	140^{b}	141,3^{b}	141,6^{b}	7.24	<.0001
BWf (g)	2197.8	2330.1	2212.6	2204.6	2254.1	63,8	0.29
ADG f ($g\ d^{-1}$)	92	100.3	97.2	97.3	98.4	18.2	0.26
FCR f (FI.BW^{-1})	1.95^{a}	1.52^{bce}	1.48^{cd}	1.52^{bd}	1.48^{cde}	0.018	0.008
Accumulated period							
AFI ($g\ d^{-1}$)	138.3	131.8	133.8	135.9	134.8	12.28	0.29
ADG ac ($g\ d^{-1}$)	81.8	87.5	82.3	82.2	83.9	10.8	0.26
FCR ac (FI.BW^{-1})	1.76^{a}	1.55^{b}	1.65^{ab}	1.69^{ac}	1.62^{bc}	0.002	0.007

Nutrient Digestibility

Table 4 shows apparent whole-tract digestibility of nutrients. Supplementation with GLM, particularly at 1.5% (T4) and 2% (T5), significantly enhanced crude protein (CP) digestibility compared to T1, T2, and T3 (P = 0.0002). Specifically, T4 and T5 achieved CP digestibility values of 0.52 and 0.45, respectively, compared to 0.32 (T1), 0.30 (T2), and 0.35 (T3), indicating that 1% GLM (T3) did not improve CP digestibility relative to the control groups. Organic matter (OM) digestibility was significantly higher in T4 (0.64) compared to T2 (0.52) (P = 0.045), with T1, T3, and T5 showing intermediate values. Dry matter (DM) digestibility was also improved in T4 (0.58) compared to T1, T2, and T3 (P = 0.003), and was statistically similar to T5 (0.52). Calcium (Ca) digestibility was highest in T3 (0.68) (P < 0.0001), followed by T2 and T4, with T1 and T5 exhibiting the lowest values. Phosphorus (P) digestibility was significantly enhanced with increasing GLM supplementation, peaking in T5 (0.56), followed by T4 (0.40) and T3 (0.37) (P < 0.0001), compared to T1 (0.19) and T2 (0.23).

Table 4. Effect of adding guava leaf meal (GLM) in the diet of broiler chickens on nutrient digestibility.

Tabla 4. Efecto de la adición de harina de hoja de guayaba (GLM) en la dieta de pollos de engorde sobre la digestibilidad de los nutrientes.

Parameter	Treatment						
	T1 (Control+)	T2 (Control-)	T3 (1%GLM)	T4 (1.5%GM)	T5 (2%GLM)	SEM	P-value
DMDig (%)	0.47 [a]	0.46 [a]	0.47 [a]	0.58 [b]	0.52 [ab]	0.0009	0.003
OMDig (%)	0.55 [ab]	0.52 [a]	0.54 [ab]	0.64 [b]	0.58 [ab]	0.0018	0.045
CPDig (%)	0.32 [a]	0 .3 [a]	0.35 [a]	0.52 [b]	0.45 [b]	0.0016	0.0002
Ca Dig (%)	0.35 [a]	0.50 [a]	0.68 [c]	0.45 [b]	0.35 [a]	0.0004	<.0001
P Dig (%)	0.19 [a]	0.23 [a]	0.37 [b]	0.40 [c]	0.56 [d]	0.0008	<.0001

Digestibility results are presented as means of five replicates per treatment. In the same row, values with no superscript or the same superscript indicate no significant difference (P > 0.05), while different superscripts indicate significant differences (P < 0.05). DM Dig: Dry Matter Digestibility; OM Dig: Organic Matter Digestibility; CP Dig: Crude Protein Digestibility; Ca Dig: Calcium Digestibility; P Dig: Phosphorus Digestibility. T1: Positive control with commercial antibiotic; T2: Negative control without antibiotic or growth promoters; T3: 1% GLM; T4: 1.5% GLM; T5: 2% GLM.

Los resultados de digestibilidad se presentan como medias de cinco réplicas por tratamiento. En la misma fila, los valores sin superíndice o con el mismo superíndice indican que no hay diferencia significativa (P > 0,05), mientras que diferentes superíndices indican diferencias significativas (P < 0,05). Dig. MS: Digestibilidad de la materia seca; Dig. MO: Digestibilidad de la materia orgánica; Dig. PC: Digestibilidad de la proteína cruda; Dig. Ca: Digestibilidad del calcio; Dig. P: Digestibilidad del fósforo. T1: Control positivo con antibiótico comercial; T2: Control negativo sin antibiótico ni promotores de crecimiento; T3: 1 % GLM; T4: 1,5 % GLM; T5: 2% GLM.

Duodenum Morphology

The effects of GLM inclusion on the duodenum morphology are detailed in table 5 and illustrated in figure 1, page 173. The number of villi per visual field (Villi/Vis field) was significantly higher in T5 (27 villi) compared to T2 (16.3 villi) (P = 0.02), with T1, T3, and T4 showing intermediate values. Villus height was significantly increased in T3, T4, and T5 (115.1 - 120.7 ţm) compared to T2 (80 ţm) (P = 0.031), and was comparable to T1 (116.8 ţm). Crypt depth did not differ significantly among treatments (P = 0.22). Figure 1 (page 173) illustrates the morphological differences in the small intestine, with T2 (negative control, figure 1A, page 173) exhibiting the shortest villi and T4 and T5 (figures 1C and 1D, page 173) showing enhanced villus height, supporting improved nutrient absorption capacity. These findings suggest that GLM supplementation, particularly at 1.5% and 2%, enhances intestinal morphology, potentially contributing to improved nutrient digestibility.

Table 5. Effect of adding guava leaf meal (GLM) in the diet of broiler chickens on duodenum morphology.

Tabla 5. Efecto de la adición de harina de hoja de guayaba (GLM) en la dieta de pollos de engorde sobre la morfología del duodeno.

Parameter	Treatment						
	T1 (Control+)	T2 (Control-)	T3 (1%GLM)	T4 (1.5%GM)	T5 (2%GLM)	SEM	P-value
Villi per visual field (n)	20.3 ^{ab}	16.3 ^{a}	22.6 ^{ab}	25.3 ^{ab}	27 ^{b}	0.11	0.02
Villus Height (ţm)	116.8 ^{b}	80 ^{a}	115.1 ^{b}	117.1 ^{b}	120.7 ^{b}	7.2	0.031
Crypt Depth (ţm)	30	21	33.7	26.2	22	1.3	0.22

Duodenum morphology results are presented as means of two replicates (six birds per treatment). In the same row, values with no superscript or the same superscript indicate no significant difference (P > 0.05), while different superscripts indicate significant differences (P < 0.05). T1: Positive control with commercial antibiotic; T2: Negative control without antibiotic or growth promoters; T3: 1% GLM; T4: 1.5% GLM; T5: 2% GLM.

Los resultados de la morfología del duodeno se presentan como medias de dos réplicas (seis aves por tratamiento). En la misma fila, los valores sin superíndice o con el mismo superíndice indican que no hay diferencia significativa (P > 0,05), mientras que los superíndices diferentes indican diferencias significativas (P < 0,05). T1: Control positivo con antibiótico comercial; T2: Control negativo sin antibiótico ni promotores de crecimiento; T3: 1% GLM; T4: 1,5% GLM; T5: 2% GLM.

Images were obtained by hematoxylin and eosin staining and observed under 40x magnification. A: Negative control (basal diet without GLM or commercial antibiotic). B: T3 (diet with 1% GLM). C: T4 (diet with 1.5% GLM). D: T5 (diet with 2% GLM). The scale represents 200 µm. Long lines indicate villi height, while short lines indicate crypt depth. Average villi height and crypt depth values are shown in table 5, page 172.

Las imágenes se obtuvieron mediante tinción con hematoxilina y eosina y se observaron con un aumento de 40x. A: Control negativo (dieta basal sin GLM ni antibiótico comercial). B: T3 (dieta con 1% de GLM). C: T4 (dieta con 1,5% de GLM). D: T5 (dieta con 2% de GLM). La escala representa 200 µm. Las líneas largas indican la altura de las vellosidades, mientras que las cortas indican la profundidad de las criptas. Los valores promedio de la altura de las vellosidades y la profundidad de las criptas se muestran en la tabla 5, pág. 172.

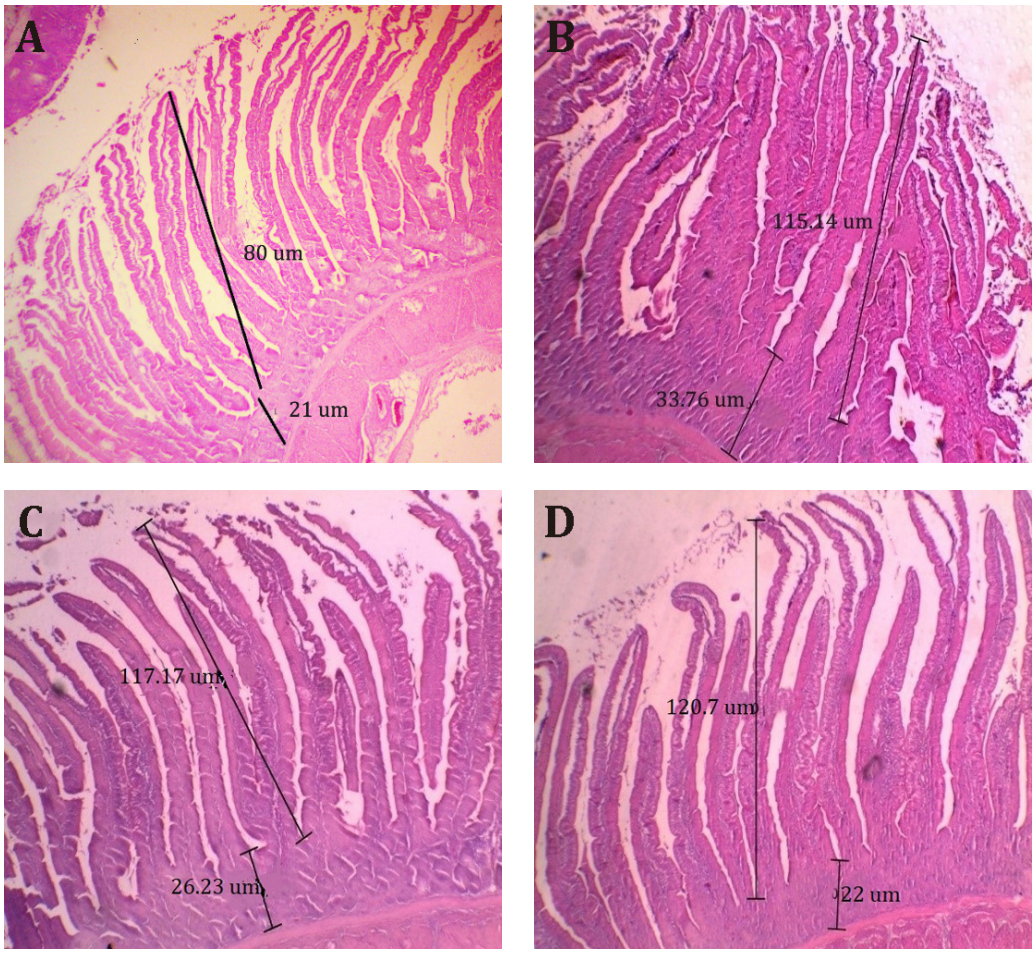

Figure 1. Small intestine (medial duodenum) morphology in broiler supplemented with guava leaf meal (GLM).

Figura 1. Morfología del intestino delgado (duodeno medial) en pollos de engorde suplementados con harina de hoja de guayaba (GLM).

Discussion

In the starter phase, feed intake (FIi) showed no significant differences across treatments (P=0.51), indicating that guava leaf meal (GLM) inclusion at 1% (T3), 1.5% (T4), and 2% (T5) did not affect early feed consumption compared to the positive (T1) and negative (T2) controls. However, body weight (BWi) and average daily gain (ADGi) varied significantly (P=0.03 and P=0.009, respectively). The negative control (T2) exhibited the highest ADGi (67.5 g), followed by T1 (65.8 g), while T3 and T4 showed lower values (59.15 g and 58.6 g, respectively), suggesting that GLM at 1% and 1.5% may not enhance early growth compared to T2. In contrast, T5 (2% GLM) displayed an intermediate ADGi (61.5 g), indicating a potential dose-dependent effect (2, 3, 26). Feed conversion ratio (FCRi) was significantly less efficient in T3 and T4 (1.72 and 1.77, respectively) compared to T1 and T2 (1.43 and 1.42, P=0.002). This suggests that lower GLM doses may reduce feed efficiency in the starter phase, possibly due to palatability issues or mild antinutritional effects (26).

In the finisher phase, feed intake (FIf) was significantly higher in T1 (156.6 g/d) compared to T2, T3, T4, and T5 (139-141.6 g/d, P<0.0001), indicating that GLM inclusion reduced feed consumption. Despite this, final body weight (BWf) and average daily gain (ADGf) showed no significant differences across treatments (P=0.29 and P=0.26, respectively), suggesting that GLM maintained overall growth despite lower feed intake. Notably, FCR in

the finisher phase (FCRf) improved significantly in T3, T4, and T5 (1.48-1.52) compared to T1 (1.95, P=0.008), with T2 showing an intermediate value (1.52). These results align with Langerudi *et al.* (2022), who reported improved FCR with guava leaf essential oil (5 mg/kg), and suggest that GLM enhances feed efficiency in later growth stages, likely due to improved nutrient utilization (1, 30).

Over the entire growth cycle, accumulated feed intake (AFI) and accumulated average daily gain (ADGac) showed no significant differences (P=0.29 and P=0.26, respectively), indicating that GLM did not affect overall feed consumption or weight gain. However, accumulated FCR (FCRac) was significantly better in T2, T3, T4, and T5 (1.55-1.69) compared to T1 (1.76, P=0.007), reinforcing the role of GLM in improving feed efficiency without compromising final body weight. These findings differ from Mahmoud *et al.* (2013), who reported significant improvements in body weight, daily gain, and FCR with 1% dried guava leaves, and Adeyemi *et al.* (2022), who noted differences in cumulative feed intake with 0.25-0.5% GLM. These discrepancies may arise from variations in GLM dosage, bird genetics, or environmental conditions (8, 27).

The GLM doses (1-2%) used in this study, compared to lower doses (0.25-0.5%) in Adeyemi *et al.* (2022), may explain the lack of consistent growth performance improvements. High GLM doses may introduce antinutritional factors, such as tannins or phenolic compounds, which can bind proteins and minerals, reducing bioavailability and negatively impacting early growth (5, 20). For example, tannins form complexes with dietary proteins, impairing digestion and absorption (20). The numerically higher FIi in T3 and T4 (97.6 and 100.6 g/d, respectively) compared to T1 (92.6 g/d) and T2 (94.7 g/d) may reflect reduced palatability or mild antinutritional effects. However, T5 (2% GLM) showed comparable FIi (97.7 g/d), suggesting that higher doses may not exacerbate these effects, possibly due to adaptive responses in gut microbiota or enzyme activity (24, 25). Excessive phenolic compounds at higher doses could also inhibit digestive enzymes or disrupt gut microbiota balance, as noted in studies on phytogenic additives (26).

Nutrient digestibility was significantly enhanced by GLM inclusion. Dry matter digestibility (DM Dig) was highest in T4 (0.58) compared to T1, T2, and T3 (0.46-0.47, P=0.003), with T5 (0.52) showing an intermediate value. Organic matter digestibility (OM Dig) followed a similar trend, with T4 (0.64) outperforming T2 (0.52, P=0.045). Crude protein digestibility (CP Dig) was markedly improved in T4 and T5 (0.52 and 0.45, respectively) compared to T1, T2, and T3 (0.30-0.35, P=0.0002). Calcium digestibility (Ca Dig) was highest in T3 (0.68) and significantly lower in T1 and T5 (0.35, P<0.0001), while phosphorus digestibility (P Dig) showed a dose-dependent increase, with T5 (0.56) outperforming all other treatments (0.19-0.40, P<0.0001). These improvements likely stem from the bioactive compounds of GLM, such as polyphenols, flavonoids, and essential oils, which stimulate digestive enzyme secretion, enhance bile acid synthesis, and modulate gut microbiota (25, 32, 35, 36). For instance, flavonoids promote villus development, increasing absorptive surface area, as evidenced by increased villi height in T3, T4, and T5 (115.1-120.7 µm) compared to T2 (80 µm, P=0.031). The dose-dependent increase in P Dig suggests that higher GLM levels (2%) enhance phosphorus absorption, possibly through improved phytase activity or reduced antinutritional interference (3). The variability in Ca Dig, with T3 showing the highest value, may reflect complex interactions between GLM bioactives and mineral metabolism, warranting further investigation (35).

Improved nutrient digestibility in GLM-supplemented groups contributed to enhanced FCR in the finisher and accumulated phases (P=0.008 and P=0.007, respectively), despite no significant differences in final body weight. This suggests that GLM enables broilers to achieve comparable growth with reduced feed intake, potentially lowering production costs (1, 30). GLM supplementation also improved intestinal morphology, with T5 showing higher villi counts (27 villi/visual field) and villi heights (115.1-120.7 µm) compared to T2 (16.3 villi/visual field and 80 µm, P=0.02 and P=0.031, respectively). Notably, T5 (2% GLM) achieved villi height and count comparable to or numerically surpassing T1 (positive control with antibiotics), suggesting that GLM can replicate the beneficial effects of antibiotics on gut health (1, 7, 18). Similar crypt depth in T5 (22 µm) and T2 (21 µm) indicates that GLM maintains mucosal integrity. These findings are consistent with what was reported by Wang *et al.* (2024), on the effect of GLM on intestinal structure.

Conclusions

GLM inclusion at 1-2% did not significantly enhance growth performance but significantly improved nutrient digestibility and feed efficiency, particularly in the finisher and accumulated phases. The dose-dependent effects on digestibility and gut morphology suggest that GLM's bioactive compounds enhance nutrient absorption and maintain intestinal integrity. These findings support further research to identify key bioactive compounds, evaluate interactions with dietary components, and determine optimal inclusion levels. Additionally, GLM's ability to replicate antibiotic effects on gut health positions it as a promising alternative to synthetic growth promoters, reducing reliance on antibiotics.

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