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Guazuma ulmifolia L. biomass: A complementary ingredient in starter piglets diets

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ABSTRACT

Objective: To evaluate the potential of *Guazuma ulmifolia* Lam (*G. ulmifolia* L.) biomass as an alternative ingredient in feed for piglets.

Design/Methodology/Approach: The microbiological characterization of *Guazuma ulmifolia* L. fruit flour was carried out through sequencing of a 16S rRNA gene region. Based on this ingredient, a balanced feed mixture (FMIX) was formulated, consisting of *G. ulmifolia* L. flour, Sorghum, *Glycine max*, and shrimp by-products. The analytical composition of the FMIX was determined, and its nutritional effect was evaluated over a 53-day period in 15 weaned female pigs in the starter phase. The animals were randomly assigned to three experimental groups: G1 (100% commercial diet), G2 (90% commercial diet + 10% FMIX), and G3 (80% commercial diet + 20% FMIX). At 11 weeks of age, complete blood counts were performed to assess potential hematological changes associated with the experimental diets.

Results: Using 16S rRNA gene sequencing, *Bacillus subtilis*, *Cronobacter* spp., and *Enterococcus* spp. were identified in the fruit flour of *Guazuma ulmifolia* L. The bromatological analysis of this flour revealed a crude protein content of 7.12%, crude fiber of 35.40%, and digestible organic matter (DOM) of 47.81%. The analytical composition of the FMIX balanced feed was determined to be 19.98% crude protein, 6.41% crude fiber, and 55.04% DOM. Group G2 showed the highest average weight gain (33.05±8.901 kg) and maintained a hematological profile within physiological ranges, in contrast to groups G1 and G3, which exhibited alterations such as leukocytosis, lymphocytosis, and basophilia. Additionally, thrombocytopenia and neutropenia were observed in group G1. Overall, group G2 demonstrated a slight improvement in weight gain and a more favorable hematological profile, suggesting a potential immunomodulatory and nutritional benefit from the diet supplemented with 10% FMIX.

Limitations on Study/Implications: The climatic conditions (autumn and winter) and the 53-day evaluation period limit the generalization of the results to other seasons and the long term.

Findings/Conclusions: This study showed that the biomass of *G. ulmifolia* L., due to its probiotic microorganism content, is a viable alternative in pig nutrition, as it enhances nutrient absorption, improves gut health, and contributes to better productive performance.

Keywords: *Guazuma ulmifolia* L.; Biomass; Agrifood; Piglets dietary; *Bacillus* spp.; Swine supplements.

INTRODUCTION

G. ulmifolia Lam, is a tropical tree native to the Americas, including Mexico, and some tropical regions of South America [1-3]. Previous research has explored *G. ulmifolia* L. as a feed supplement for livestock, demonstrating positive effects on intake, digestibility, and nitrogen balance [4]. Studies on sheep have highlighted the nutritional importance of *G. ulmifolia* L. biomass, with higher preference rates and valuable nutritional content observed at different maturation stages [5-6]. Particularly, have demonstrated the productive response of lambs fed with *G. ulmifolia* L. fruits, emphasizing the potential of this tree species as a dietary supplement for small ruminants [6].

G. ulmifolia L. has been recognized for its nutritional value and bioactive properties [7-9], with both its leaves and fruits being considered valuable sources of protein for animal feeds, particularly in the context of swine nutrition [10]. *G. ulmifolia* L. is renowned for its rich concentration of bioactive molecules, particularly within its leaves and fruits [4,11-12]. The leaves are a reservoir of tannins [13], recognized for their antioxidant properties and potential anti-inflammatory benefits, and flavonoids like kaempferol, contributing to antioxidant and anti-inflammatory effects. Also, the essential oil extracted from leaves is predominantly composed of eugenol, a compound recognized for its antiseptic and analgesic properties. Additional key components include spathulenol, β-caryophyllene, sabinene, globulol, γ-terpinene, and α-copaene, each contributing to the oil's overall biological activity [14]. Interestingly, while the fruit lacks steroids [15], these compounds have been detected in the leaves, hinting at potential biological activities. The fruits, sweet and edible, contain mucilage, promoting digestive health, and xanthum gum, a polysaccharide known for its thickening and stabilizing properties, though its impact on gut health when consumed in larger quantities warrants further investigation.

While the inherent nutritional value of *G. ulmifolia* L. is promising, targeted interventions could further enhance its benefits. A research team reported a probiotic strategy using *L. acidophilus* and *Lactococcus lactis* [9], which improved nutrient intake, feed efficiency, gut health, immune response, stress reduction, and the safety of *Coturnix japonica* products for human consumption [10]. Unlike studies using exogenous probiotics, our research employs indigenous strains naturally present in *G. ulmifolia* L. fruit. We evaluated early-stage pigs fed a standard diet versus one enriched with *G. ulmifolia* biomass. Native probiotics such as *Bacillus subtilis*, *Cronobacter* spp, and *Enterococcus* spp., identified via 16S rRNA sequencing, combined with the fruit's nutritional value, may enhance nutrient absorption, gut health, and overall performance through a synergistic and naturally derived formulation.

MATERIALS AND METHODS

Harvesting, cleaning and processing of G. ulmifolia L. biomass

The dried fruit of *G. ulmifolia* L. was harvested in the "Huasteca" zone of the state on the northern region of Veracruz, Mexico (21° 20' 12.7" N 97° 49' 57.2" W). Disinfection of the dried fruit was carried out by washing with neutral soap and a NaClO (Hach, Mexico) solution at a concentration of $10\frac{v}{v}\%$, leaving it to react for 10 minutes to reduce the presence of microorganisms. It was then dried at 60 °C for 36 hours using a conventional oven (Memmert, Germany). Then, the disinfected dried fruit was milled (Insely, USA) until it reached a flour consistency and was stored in sterile plastic containers (Thermo Fischer Scientific, USA) at room temperature for its subsequent formulation.

Microbial isolation in the flour of *G. ulmifolia* L. fruit

From the cleaned fruit sample (WF), serial dilutions (10^{-1} to 10^{-5}) were prepared, and 100 μL of each dilution was spread on nutrient agar plates (Bioxon, Mexico). The plates were incubated at 35 °C for 24 hours (CRM Globe, Mexico). Axenic cultures were characterized by colonial morphology on nutrient agar and by cell morphology using Gram staining (Hycel, Mexico). For cryopreservation, 700 μL of microbial culture was mixed with 300 μL of sterile glycerol (Thermo Fisher Scientific, USA) in nutrient broth and stored at −80 °C in an ultra-freezer (Thermo Fisher Scientific, USA).

Identification and characterization of *G. ulmifolia* L. fruit microorganisms

PCR was carried out using colony PCR technique. A microwave-based rapid lysis protocol was used for DNA extraction. Briefly, each colony was resuspended in 100 μL of TE lysis buffer (Thermo Fisher Scientific, USA.), composed of 10 mM Tris and 1 mM EDTA, and subjected to three cycles of microwave heating (Samsung, South Korea) consisting of 1 min at maximum power of 1.4 kW, followed by a rest for 30 seconds at room temperature and finally 1 min additional at 1.4 kW wattage. The extracted DNA was used directly for PCR. PCR was carried out in a final volume of 25 μL, employing the PCR kit (Invitrogen, U. S. A.). Each reaction contained 2. 5 μL of 10X PCR-$MgCl_2$ buffer, 2 μL of 50 mM $MgCl_2$, 1 μL of 10 mM dNTPs, 1 μL of the direct oligonucleotide P1 (CGGGATC CAGAGTTTGATCCTGGCTCAGAACGAACGCT) (15 pmol/μL), 1 μL of the reverse oligonucleotide P6 (CGGGATCCTACGGCTACGGCTACCTTGTTACGACTTCAC CCC) (15 pmol/μL), 17. 2 μL of DNA extracted directly from the colony, and 0.3 μL of Taq polymerase (5 U/μL) [16]. Amplification conditions in a thermal cycler (Biometra, Germany) proceeded as follows: 94 °C for 5 min (1 cycle); 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min (30 cycles); 72 °C for 10 min (1 cycle). PCR products were analyzed by 1% agarose gel electrophoresis to confirm amplification of the expected fragment. The target band was purified using a DNA gel extraction kit (Zymo Research, USA). Purified samples were analyzed by vertical electrophoresis (Axygen, USA), quantified with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, USA), and sent for sequencing to an external laboratory (LANGEBIO-CINVESTAV, Mexico). Sequences obtained for the identification of microorganisms were analyzed by Basic Local Alignment Search Tool (BLAST), in order to find regions of similarity with sequences from the database of the National Center for Biotechnology Information (NCBI), considering a percentage identity ≥99% [17-18].

Feed formulation and balance with *G. ulmifolia* L. as ingredient

A balanced mixture was formulated using commercial flours of *Glycine max* (soybean) and sorghum, both sourced from local feed stores. Shrimp shells were obtained as waste from shrimp processing in the coastal region of the Tamiahua Lagoon, Veracruz. The shells were dehydrated at room temperature (32.5±2.5 °C) for 72 hours and ground into flour (Insely, China). *G. ulmifolia* L. fruit was processed similarly, as described previously. The nutritional composition of sorghum, soybean, and shrimp meal was verified using literature and swine feed composition tables [19-24]. Ingredients were mixed using an electric horizontal mixer (Covema, Italy).

Bromatological analysis and formulation of *G. ulmifolia* L. biomass

To determine the analytical composition of *G. ulmifolia* meal and the formulated mix, two 500 g samples were sent to the EuroNutec laboratory (Nutec, Mexico): one of *G. ulmifolia* meal and another of the mixture containing *G. ulmifolia*, sorghum, *Glycine max*, and shrimp meal. Nutritional values of reference diets were obtained from commercial products PigTech3 PTBN (BioNova, Netherlands) and Lechoncina PT (Purina, USA).

Study group of pigs and dosage of balanced diet

The effect of the FMIX formulation containing *G. ulmifolia* biomass meal was evaluated in a study involving 15 six-week-old female pigs (10-12 kg), from a genetic line of 50% Landrace, 25% Hampshire, and 25% Duroc (Landrace sow × Hampshire/Duroc boar). Pigs were randomly assigned to three groups (n=5) and housed in separate 4.0 m^2 pens with independent feeders and waterers. Group 1 (G1, control) received a commercial diet: PTBN during week one, followed by LPT. Group 2 (G2) received PTBN during week one and a mix of LPT with 10% FMIX (4 kg) in subsequent weeks. Group 3 (G3) was fed LPT mixed with 20% FMIX (8 kg) from the start. Diets were blended using an electric mixer (Covema, Italy) (Table 1).

Weight measurements in pigs

Weight and thoracic girth measurements were taken every three days during the study period. Each pig was weighed using a commercially available moving platform scale (Torrey, Mexico). To avoid animal movement and stress, the animals were placed in a rectangular plastic box, and the scale was tared and weighed.

Blood collection for swine complete blood count analysis

Fifteen blood samples were collected 23 days after the start by mean the external jugular vein punction using sterile 10 mL plastic syringes with 20G × 32 mm needles (BD, USA). Samples were transferred to 7.2 mL EDTA K2 Vacutainer tubes (purple cap; BD, USA).

Table 1. Table showing the dosage of feed portions for each group of pigs for the duration of the evaluation protocol. Abbreviations: G1, Group 1; G2, Group 2; G3, Group 3; W, Week; F-10%, Feed-10%; F-20%, Feed-20%, F-10%=36 kg of LPT feed mixed with 4 kg of FMIX formula, F-20%=32 kg of LPT feed mixed with 8 kg of FMIX formula.

Group	Experimental period								
	W1	W2	W3	W4	W5	W6	W7	W8	W9
G1	PTBN	LPT							
G2	PTBN	F-10%							
G3	F-20%								

Tubes were stored at 4 °C and sent to an external veterinary diagnostic laboratory (AXIS Laboratory, Mexico) for complete blood count analysis.

RESULTS AND DISCUSSION

Microbial identification from the fruit *G. ulmifolia* L.

Five colonies with different morphology, named WF1, WF2, WF3, WF4 and WF5, were isolated from the washed fruit pulp sample of *G. ulmifolia* L. The cell morphology of these colonies was Gram+bacilli, Gram−bacilli, and Gram+cocci (Figure 1). From the colony PCR test, amplification and purification of the amplified bands of the 16S rRNA gene of 1500 bp were performed and sent for sequencing (Figure 2).

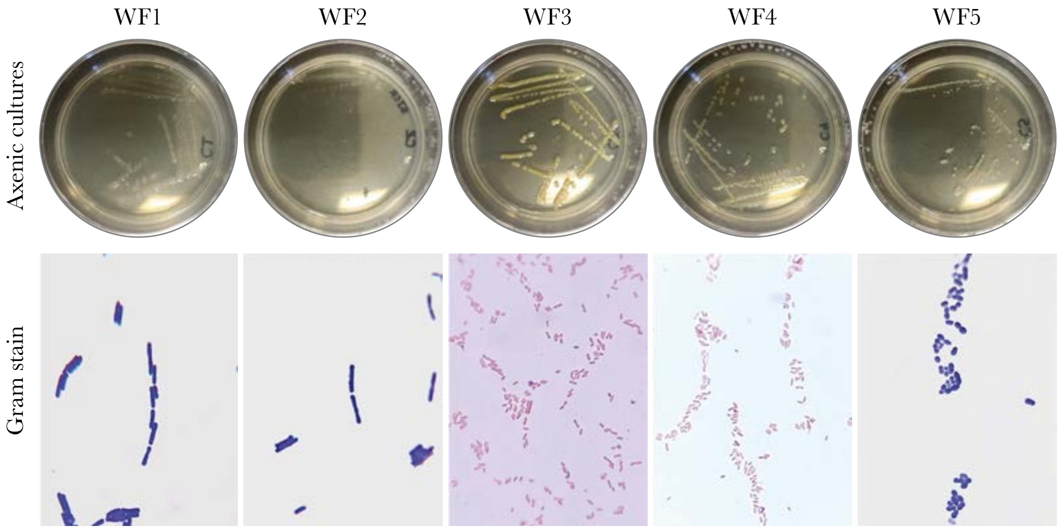

Figure 1. Axenic cultures and Gram staining obtained from the microorganisms present in the sample of *G. ulmifolia* L. WF1) Irregular colony, curled rim, bright white color, convex elevation, mucous consistency and cell morphology are associated with Gram-positive bacilli; WF2) Circular colony, entire rim, bright white color, flat elevation, smooth consistency, and cell morphology are associated with Gram-positive bacilli; WF3) Circular colony, entire rim, bright yellow color, convex elevation, mucous consistency, and the cell morphology are associated with Gram-negative bacilli; WF4) Circular colony, entire rim, translucent cream color, convex elevation, mucous consistency and; WF5) Circular colony, entire rim, bright white color, flat elevation, smooth consistency and the cell morphology are associated with Gram-positive cocci.

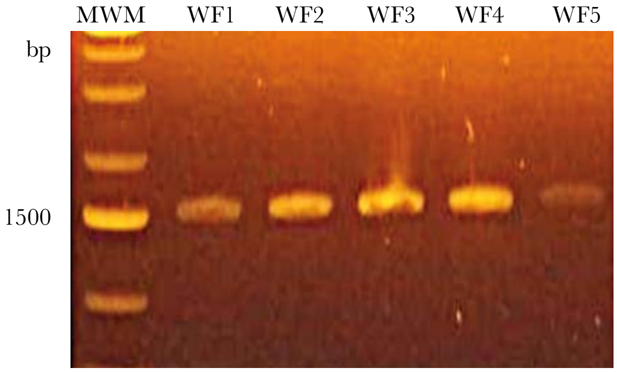

Figure 2. Purification of the amplified 16S rRNA gene band from isolated colonies WF1, WF2, WF3, WF4 and WF5. Abbreviations: MWM) Molecular Weight Marker.

Based on BLAST analysis, and using the NCBI database, samples WF1 and WF2 were identified as *Bacillus subtilis* (Table 2). *B. subtilis* is widely distributed in agrosystems, and one of its main applications is disease control in agricultural crops [25]. In addition, *B. subtilis* has been reported as probiotics, and presents antimicrobial, anticancer and antioxidant properties [26].

On the other hand, samples WF3 and WF4 were identified as *Cronobacter* sp. (Table 2). The *Cronobacter* genus are Gram-negative bacilli belonging to the Enterobacteriaceae family, and they are considered opportunistic pathogens and are frequently spread by contaminated food. This genus is made up of seven species: *C. sakazakii* and *C. malonaticus*, which constitute the majority of clinical isolates from patients, leading to severe neonatal infections and rarely to other diseases, such as conjunctivitis and urinary tract infections; *C. turicencis* and *C. universalis*, which are rarely associated with clinical cases; and *C. dublinensis*, *C. muytjensii* and *C. condimenti* (Group 3), which are environmental commensals and possibly pose little or no clinical significance [27]. Finally, sample WF5 was identified as *Enterococcus* spp. The *Enterococcus* are ubiquitous microorganisms, with Gram-positive cocci cell morphology which are part of the microbiome of terrestrial animals, since they are present in the gastrointestinal tract of these organisms. They can also be found in other environments such as plants and food; due to the fact that they act as initiators for the development of organoleptic properties. Several species such as *E. faecalis* and *E. faecium* are used as probiotics; they also lower serum cholesterol levels and strengthen the immune system. However, these bacteria are also capable of causing serious infections especially in hospitalized individuals, since they can be opportunistic pathogens and some species have acquired virulence factors and antibiotic resistance [28-29].

Bromatological comparative analysis of the proposed formulation

The ingredients that were chosen to formulate the FMIX mixture provide a variety of nutritional properties in swine feed, which were confirmed by the data obtained

Table 2. Identification of microorganisms based on the basic local alignment search tool and the National Center for Biotechnology Information.

Sample	Microorganism	E value	Percent identity	Accesion length	Accesion
WF1	*B. subtilis*	0.0	100	1514	PP917513.1
WF2	*B. subtilis*	0.0	98.69	1286	ON997083.1
WF3	*C. sakazakii*	0.0	99.3	1501	KU696331.1
	C. malonaticus	0.0	99.15	1419	FN401339.1
WF4	*C. sakazakii*	0.0	99.56	755	JQ312046.1
	C. dublinensis	0.0	99.27	4431067	CP012266.1
	C. turicensis	0.0	99.27	1439	MW013478.1
WF5	*E. faecium*	0.0	100	2824615	CP112586.1
	E. durans	0.0	100	1408	MF428861.1
	E. faecalis	0.0	100	1535	MW447606.1
	E. lactis	0.0	100	1344	ON426954.1

from different scientific reports. Sorghum contains an average crude protein of 10.6 % and an organic matter digestibility of about 81.4%, which makes sorghum an excellent alternative ingredient that can successfully substitute corn used in diets for pigs from weaning [19,22-23]. In addition, sorghum grains provide a high content of minerals, vitamins, carbohydrates, and their antioxidant activity [19,21,24]. *Glycime max* meal is commonly known as a high-quality edible oilseed for humans, however, it has a positive impact on animal feed, as it provides amino acids and high levels of vegetal protein ranging from 43-45% [20,24,30-31]. Shrimp waste flour is another ingredient that provides 43.4% crude protein and an organic matter digestibility of 48.31% [24,32]. For the case of *G. ulmifolia* L., its analytical constituents yielded a crude protein content of 7.12%, a digestible organic matter of 47.81% and a crude fiber content of 35.40%. *G. ulmifolia* L. provides an important contribution of fiber for pig nutrition, it also has the role of regulating gastrointestinal transit, helps prevent constipation, and contributes to maintain a balanced intestinal microbial community, which is essential for a healthy digestion [33]. Table 3 shows the concentration of the analytical constituents of each ingredient used for the FMIX formulation.

Taking into account the percentages of the analytical constituents of each ingredient in FMIX shown in Table 3, 20% of *G. ulmifolia* L. flour, 49% of Sorghum flour, 30% of *Glycine max* paste and 1% of shrimp waste were considered for the FMIX formulation. Thus, the theoretical estimate of the analytical constituents is described in Table 4.

The FMIX analytical constituents evaluated by the external laboratory, as well as the analytical constituents for each commercial balanced feed (PTBN and LPT), are shown in Table 5. A similarity was maintained between each parameter, with the purpose of not significantly affecting crude protein, nitrogen free extract and crude fiber, mainly. The parameters described in Table 5 were considered for the final mixtures in the dosage of the groups of pigs. The adjustment of the analytical constituents during the dosing of LPT mixed with FMIX at 10 and 20% can be seen in Table 5, which means that the dosing of 10% FMIX provided 18.19% crude protein and 53.65% digestible organic

Table 3. Comparative of analytical constituents for each ingredient of the formulated flour mixture. GUL: *G. ulmifolia* L. (Data obtained from bromatological analysis by EuroNutec laboratory), S: Sorghun [19,22-23], GMP: *Glycine max* paste [20,24,30], SW: shrimp waste [24]. Abbreviations: ELN, Extract Free Nitrogen-Digestible Organic Matter.

Parmeter	**Flour ingredient (%)**			
	GUL	**S**	**GMP**	**SW**
Crude protein	7.12	10.60	43.00	43.30
Crude fiber	35.40	2.90	9.00	7.00
ELN	47.81	80.30	22.76	48.31
Moisture	4.19	15.00	8.50	4.49
Fat	2.72	3.06	19.30	1.82
Ashes	2.76	1.60	6.30	24.25
Portions of each ingredient for FMIX				
%	20	49	30	1
g/kg	200	490	300	10

Table 4. Estimates of analytical constituents for FMIX from the percentage values for each ingredient: GUL: 20% *G. ulmifolia* L., S: 49% Sorghum meal, GMP: 30% *Glycine max* paste and SW: 1% shrimp waste. Abbreviations: ELN, Extract Free Nitrogen-Digestible Organic Matter.

Parmeter	Flour ingredient				Calculated FMIX (%)
	20%	49%	30%	1%	
	GUL	S	GMP	SW	
Crude protein	1.42	5.19	12.90	0.43	19.95
Crude fiber	7.08	1.42	2.70	0.07	11.27
ELN	9.56	39.35	6.82	0.48	56.22
Moisture	0.84	7.35	2.55	0.04	10.78
Fat	0.54	1.49	5.79	0.01	7.85
Ashes	0.55	0.78	1.89	0.24	3.46

Table 5. Analytical components of the balanced feeds used for feeding pigs in the initiation stage. Abbreviations: PTBN, PigTech3 Bio-Nova; LPT, Lechoncina PT; FMIX, Formula balanced with *G. ulmifolia* L. flour; ELN, Extract Free Nitrogen-Digestible Organic Matter.

Component	PTBN	LPT	FMIX	LPT+FMIX	
				10%	20%
Crude protein	19.00	18.00	19.98	18.19	18.39
Crude fiber	3.50	5.00	6.41	5.14	5.28
ELN	56.00	53.50	55.04	53.65	53.80
Moisture	12.00	12.00	10.50	11.85	11.70
Fat	2.50	4.00	4.35	4.03	4.07
Ashes	7.00	7.50	3.72	7.12	6.74
Phosporus	No reported	0.80	0.54	0.77	0.74

matter, and for 20% FMIX a contribution of 18.39% crude protein and 53.80% digestible organic matter.

Although the bromatological parameters of each feed show similarities, it is important to consider that in the case of FMIX, the evaluation of digestible organic matter is interesting due to the presence of microorganisms which contribute advantages to the digestive process of pigs and can be considered as probiotics of natural origin.

Weight gain analysis for each group of pigs

Over 53 days, the average weight gain (AWG) for each group of pigs was recorded and finally estimated. It was observed that the trends among the three groups maintained similar behavioral slopes until day 29. Subsequently, the G2 group showed a slight increase in weight gain in relation to the G1 and G3 groups (Figure 3A). Figure 3B shows the final average weight gain (WG) by group at day 53. For the groups that consumed FMIX in their daily ration, G2 had a weight gain of 33.05±8.90 kg and G3 of 29.10±3.61 kg, while the G1 group that did not include FMIX recorded a final weight gain of 28.34±5.00 kg. It should be noted that weight gains were recorded for the three groups, however, when comparing the data among groups, these differences were not significant ($p>0.05$); ruling

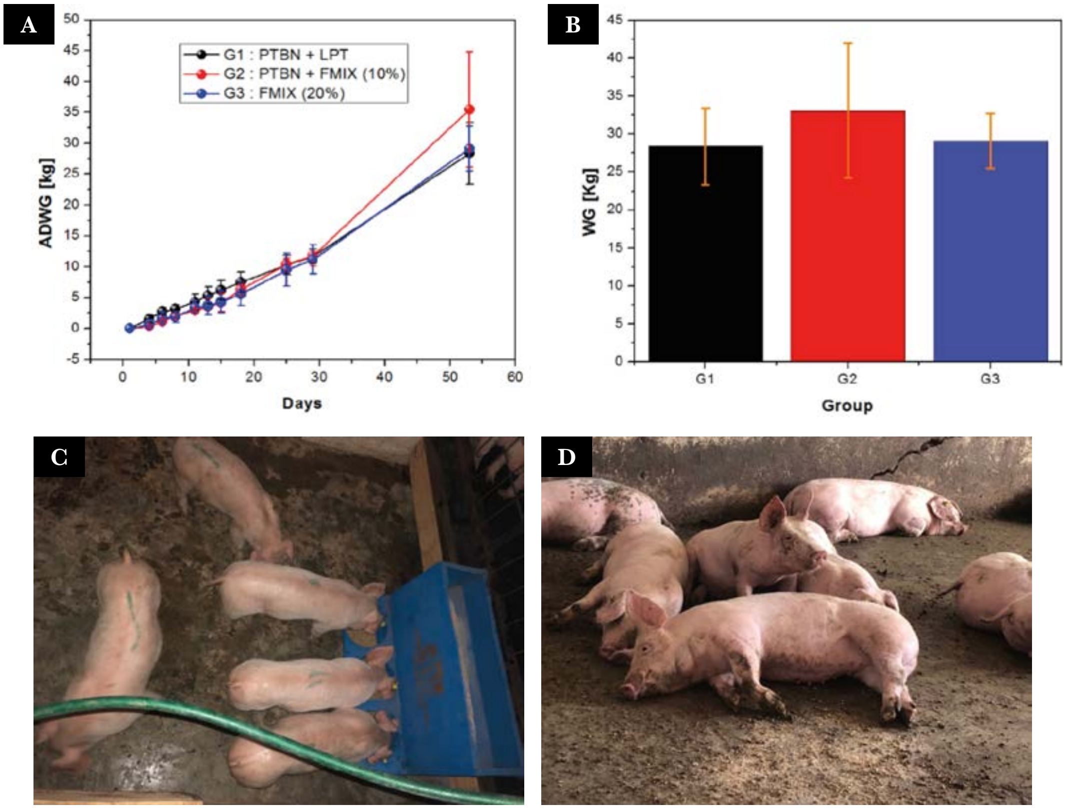

Figure 3. Comparative behavior of weight gain by group of pigs. A) Aver-age daily weight gain by group: G1, G2 and G3, B) Total average weight gain, C) Representative group (G2) of five piglets distributed in a space with water drinkers and a food container, D) Representative group of pigs in a common use space for the three groups after 53 days. Abbreviations: ADWG, Average Daily Weight Gain; WG, Weight Gain.

out any type of negative effect on weight gain, *i.e.*, the weight gain behavior was maintained in the three groups in relation to the progression of growth week by week according to their growth stage reported in different literatures [34-36].

Hemogram analysis per pig and group

Five blood analytes were monitored to assess the overall health of the study animals, such as total leukocytes, lymphocytes, neutrophils, monocytes, and thrombocytes. Table 6 shows the average complete blood count per group. High leukocytes were observed in the G1 group ($24.36\times10^9\pm7.24$/L powered by PTBN) and in group G3 ($20.58\times10^9\pm5.54$/L fed with LPT+FMIX 20%), the leukocyte level was slightly higher in group G1, as three of the five pigs had values higher than the reference level, while in group G3, only two pigs had high values in relation to the maximum reference parameter.

No cases of elevated lymphocytes were observed in group G2 (LPT+FMIX 10%). In contrast, high leukocyte counts in groups G1 and G3 were accompanied by increased lymphocytes, neutrophils, basophils, and monocytes key cells in bacterial infection response [36–37]. Lymphocyte levels exceeded reference values in both G1 ($15.58\times10^9\pm8.78$/L) and G3 ($12.66\times10^9\pm3.21$/L).

Table 6. Complete blood counts by group. Abbreviations: G1, Group1; G2, Group2; G3, Group3.

Analyte	**Experimental group**			**Reference values**	
	G1	**G2**	**G3**	**Min.**	**Max.**
Hematocrit (L/L)	0.40±0.032	0.38±0.01	0.42±0.02	0.32	0.5
Hemoglobin (g/L)	129.80±11.03	127.25±5.93	135.20±5.21	100	160
Erythrocytes ($\times 10^{12}$/L)	7.06±0.57	6.97±0.29	7.22±0.30	5	8
VGM (fL)	57.00±1.41	55.00±2.23	58.60±3.91	50	68
CGMH (g/L)	321.00±5.56	328.00±1.00	317.60±5.36	300	340
Protein (g/L)	77.20±4.65	77.75±3.89	78.20±4.32	60	90
Reticulocytes ($\times 10^{9}$/L)	-	-	-		<50
Leukocytes ($\times 10^{9}$/L)	24.36±7.24	15.50±2.70	20.58±5.54	11	22
Platelets ($\times 10^{9}$/L)	456.60±120.28	283.50±134.52	390±69.28	300	700
Neutrophils ($\times 10^{9}$/L)	7.94±3.03	6.85	6.30±4.38	4	7.5
Myelocytes ($\times 10^{9}$/L)	0	0	0		<0.5
Lymphocytes ($\times 10^{9}$/L)	15.58±8.78	7.67±2.17	12.66±3.21	3	13
Monocytes ($\times 10^{9}$/L)	0.68±0.27	0.82±0.25	1.32±1.34	0	1.5
Eosinophils ($\times 10^{9}$/L)	0.12	0.15±0.11	0.22±0.14	0	2
Basophils ($\times 10^{9}$/L)	0.04±0.08	0	0.02±0.04	0	0.03

The mucosa associated lymphoid tissue plays a key defensive role in pigs, as it is the first barrier against pathogens entering through the intestinal, respiratory, and urogenital mucosa. Elevated neutrophil counts observed in all groups suggest immune activation, likely in response to bacterial or fungal agents [38]. Notably, total protein levels and red blood parameters hemoglobin, hematocrit, and erythrocytes remained within normal ranges across all groups. The elevated leukocyte count in group G3 may be related to the presence of *Enterococci*, likely due to the higher concentration of *G. ulmifolia* flour, which increased levels of *Bacillus* spp. and *Enterococci*. Although these microorganisms are used as probiotics, some strains can be opportunistic pathogens [27]. It is also important to note that pigs in the early growth phase are more susceptible to immune disturbances due to dietary transitions involving solid feed [37]. Finally, weight gain per pig and per group remained within normal ranges according to standard growth charts [39-41].

CONCLUSIONS

Based on the bromatological, biological and nutritional properties of the flour from the fruit of *G. ulmifolia* L., it can be considered a potential ingredient in a balanced diet for feeding growing and developing pigs, particularly due to the content and benefits of probiotic microorganisms such as *B. subtilis.*

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