

AGRO PRODUCTIVIDAD

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in a fermented mixture of

Orange

bagasse, yeast and nitrogen
additives to enhance
nutritional quality

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Evaluation of a pilot sustainable aquaculture module

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ABSTRACT

Objective: The primary objective of this study was to evaluate the effect of an alternative feed throughout the production cycle of a sustainable aquaculture system using basa fish (*Pangasius hypophthalmus*) approximately three months of age.

Design/methodology/approach: The study was conducted during the Fall-Winter 2023-2024 period in the municipality of Paso de Ovejas, Veracruz, in collaboration with a partner aquaculture production unit that provided the necessary conditions for implementation of the experimental protocol. The assessed variables included the nutritional characterization of the formulated alternative feed and morphometric parameters (length, width, and weight) of the cultured organisms. In addition, systematic water quality monitoring was conducted, incorporating key physicochemical indicators essential for optimal species development. Three thousand fish were used per treatment and in the control group, with a stocking density of six organisms per cubic meter.

Results: The alternative feed exhibited a moderately high protein content (21.6%), which corresponded to an approximate 100% increase in body weight relative to initial biomass. Water quality parameters remained within acceptable ranges for the cultivation of this species, thereby supporting appropriate animal welfare conditions. No significant differences were observed between the treatment and control groups.

Limitations/implications: A primary limitation was the difficulty of inducing basa fish reproduction under *ex situ* conditions, as this species requires specific environmental factors that are challenging to replicate outside its natural habitat.

Conclusions: Incorporating alternative feeds into sustainable aquaculture systems can achieve performance comparable to commercial diets while reducing production operating costs by up to 25%.

Keywords: Alternative feed; sustainability; aquaculture; Veracruz.

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INTRODUCTION

The growing urgency to mitigate the environmental, economic, and social impacts associated with the excessive and indiscriminate use of agrochemicals in industrial agroecosystems underscores the need to reconfigure agricultural systems toward more resilient and sustainable models. In this context, the design of self-sufficient agroecosystems characterized by minimal dependence on synthetic inputs and grounded in diversification



through polycultures has been proposed as a viable alternative. This strategy aims to enhance functional biodiversity, thereby contributing to the reduction of multiple challenges associated with conventional agriculture, including global warming (Bianconi *et al.*, 2013; Altieri & Nicholls, 2017; Altieri, 2018). These transformations have prompted significant social and structural changes both within and beyond agroecosystems, revalorizing the strategic role of agrifood production, raw material generation, and the provision of ecosystem services from a sustainability-oriented perspective (Andersen *et al.*, 2013).

Furthermore, per capita fish consumption in Mexico is approximately 12 kg per year. The country ranks as the world's second largest importer of frozen basa fillets from Vietnam and the second largest importer of whole and filleted tilapia from China (Platas-Rosado *et al.*, 2021). Domestically, aquaculture possesses the productive capacity necessary to meet current and future demand for fish and seafood, including export potential. This capacity is partly attributable to the favorable feed conversion ratios of these species compared with other livestock species of agricultural interest (Olsen & Hasan, 2012).

Sustainable food production addresses the environmental, economic, and social challenges generated by high input chemical agriculture (Reganold & Wachter, 2016). In this context, sustainable aquaculture has emerged as an alternative approach aimed at mitigating the environmental, economic, and social limitations that conventional intensive aquaculture systems have been unable to adequately resolve (Sicuro, 2019; Ahmed *et al.*, 2020). Among the strategies applicable to sustainable aquaculture, practices such as zoning and circular economy approaches are particularly prominent. Zoning, in particular, constitutes a spatial planning tool that enables the assessment of the carrying capacity of natural resources within a defined area in order to respond in a balanced manner to the demands for goods and services of local populations. This practice has gained increasing relevance in recent years due to accelerated global population growth, which exerts mounting pressure on finite resources such as land and water. Zoning is therefore essential for anticipating and strategically managing current and future challenges facing productive systems, including food price volatility and imbalances between supply and demand. Its implementation facilitates the rational use of resources, promoting sustainability in terms of ecological balance as well as long-term economic and social viability (FAO, 2007).

The circular economy has emerged as a sustainable and economically viable alternative to the linear economic model, with the primary objective of preserving and enhancing natural capital. It fosters innovation in business models by promoting the valorization of waste and by-products for the development of value-added products. In this way, the circular economy seeks to provide a second life to by-products generated from major agroindustrial activities (Cerdá & Khalilova, 2016; Geisendorf & Pietrulla, 2018).

Some of these by-products can be used to formulate fermented and/or pre-digested feeds for aquaculture. Feed costs in aquaculture production systems account for approximately 50% to 70% of total operational expenditures (Khan *et al.*, 2018). The use of conventional compounded feeds may be reduced by approximately 20% to 50% through the implementation of fermented and pre-digested feeds produced from raw materials generated by aquaculture producers themselves or derived from local agro-industrial residues. This substitution has the potential to lower feeding costs by up to

50%, while simultaneously improving fish gut microbiota and metabolic performance (Gamble *et al.*, 2015; Samaddar, 2018; Yin-Mo *et al.*, 2020; Yao *et al.*, 2020; Omont *et al.*, 2021). In addition, these alternative feeds may enhance protein availability and reduce anti-nutritional factors, thereby improving the environmental and economic performance of aquaculture systems (Li *et al.*, 2019; Moniruzzaman *et al.*, 2020; Dawood & Koshio, 2020).

In addition, biofloc technology can address challenges such as water scarcity. This system is primarily composed of microorganisms that enhance intestinal enzyme activity in cultivated aquatic species, thereby significantly reducing the need for water exchange. The use of these microorganisms has been shown to improve feed conversion ratios in fish and shellfish. Moreover, biofloc has been reported to contribute to disease control due to its function as a natural probiotic, potentially reducing the use of antibiotics, antifungals, and other feed additives (Ahmad *et al.*, 2017; Khanjani & Sharifinia, 2020). This technology offers advantages that enhance both production and productivity in aquaculture systems by improving water quality through the regulation of carbon and inorganic nitrogen levels within culture ponds (Crab *et al.*, 2012; Bossier & Ekasari, 2017). The objective of the present study was to evaluate the effect of an alternative feed produced *in situ*, compared with commercial feed, during the production cycle of a sustainable aquaculture system using basa fish approximately three months of age.

MATERIALS AND METHODS

Study area

The design and implementation of the sustainable aquaculture module were conducted in the municipality of Paso de Ovejas, Veracruz (Figure 1). The municipality is located between 19° 08' and 19° 22' N and 96° 20' and 96° 38' W, at elevations ranging from 10 to 400 m (SEFIPLAN, 2021). Project implementation was carried out in collaboration with a local aquaculture production unit, which served as the cooperating farm throughout the operational phase of the study.

The municipality is located in a humid tropical climate zone, predominantly characterized by the AW1 climate type, which covers approximately 60% of the municipal



Figure 1. Geographic location of the municipality of Paso de Ovejas, Veracruz, and satellite view of the cooperating aquaculture farm.

territory, and the AW0 type, present in the remaining 40%. Both correspond to variants of a warm subhumid climate, characterized by a mean annual temperature of approximately 22 °C and annual precipitation ranging from 1,000 to 1,500 mm (CONABIO, 2022).

Treatments

The experimental design included one treatment (T_1) and one control (T_0). The treatment consisted of a sustainable alternative feed and included two replicates (R_1 and R_2). This feed was administered to basa fish approximately three months of age until completion of the production cycle during the Fall-Winter 2022 season. The T_1 diet consisted of a mixture of fermented and pre-digested corn, taro, and cassava, whereas T_0 received a commercial feed.

For the preparation of the sustainable alternative feed, guidelines developed by Bioaquafloc were used as a reference. The raw materials for T_1 were first ground and then boiled at 100 °C for 1 hour. Subsequently, the mixture was placed in containers located in a covered area exposed to sunlight. Whey was added as an additional protein source and probiotic agent to accelerate fermentation and predigestion. The mixture was allowed to ferment for three to five days under aeration until it developed a dark coloration and a sour odor.

During the fermentation process, fly larvae appeared and were incorporated as an additional protein source in the diet of the cultured organisms. Once the process was completed, the feed was supplied to three tanks. Additionally, biofloc technology was applied to reutilize residual organic matter within the tanks and to improve water quality.

Variables evaluated

A physicochemical analysis of the alternative feed was conducted in accordance with the standards of the Association of Official Analytical Chemists (AOAC) (Helrich, 1990). Monthly biometric measurements were performed on 32 organisms per tank from three circular tanks (25 m in diameter), each stocked with 3,000 organisms. Sampling procedures were designed to minimize stress and prevent negative effects on growth and development. Fish total length, body width, and weight were recorded (Figure 2).



Figure 2. Biometric measurements of basa fish.

Water quality parameters were also monitored weekly, including alkalinity, chloride, hardness, iron, pH, sulfite, and temperature.

Statistical analysis

Statistical analyses were performed using the open-access software RStudio. Descriptive statistics were calculated, and a one-way analysis of variance (ANOVA) was conducted to compare treatments evaluated on the same sampling date in order to determine differences or similarities among the studied variables.

RESULTS AND DISCUSSION

Physicochemical analysis

The physicochemical analysis of the alternative feed included moisture, ash, lipid, and protein content. Each sample was analyzed in triplicate. The results are presented in Table 1.

The results indicate that the protein content provided by the alternative feed was 21.6%, which falls within the range suggested by Zulfikar *et al.* (2005) and Sayeed *et al.* (2008) to support the growth and development of basa fish. These authors reported protein requirements ranging from 16% to 23.9%.

Biometric measurements

The evaluated variables (length, width, and weight) of basa fish during the Fall-Winter 2022 season did not show substantial differences between treatments. These results are presented in greater detail in Table 2, which displays the mean values and standard deviations of the data obtained from *Pangasius hypophthalmus* over a six-month period.

The final length values recorded for T₁R₁, T₀, and T₁R₂ were 30.2, 30.5, and 30.3 cm, respectively. Similarly, final weight values were 429.8, 433.8, and 442.0 g, respectively. For width, however, T₀ (8.9 cm) was greater than T₁R₁ (8.4 cm) but equal to T₁R₂.

The biometric results obtained during the Fall-Winter period differed from those reported by Khan *et al.* (2009), who recorded final length values of 29.86, 31.79, and 32.94 cm, with higher values in two of their treatments. However, their reported final weights (271.63, 340.12, and 386.55 g) were lower than those observed in the present study.

These findings indicate that the alternative feed adequately met the nutritional requirements of basa fish, comparable to the commercial feed, allowing continued growth and development. No significant differences were observed between replicates (R₁ and R₂) or between the treatment and control groups (T₁ and T₀), as illustrated in Figure 3.

Table 1. Physicochemical analysis of the alternative feed.

	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)
Corn	73.0	1.7	2.2	12.8
Taro	83.4	5.8	0.4	5.5
Cassava	91.3	7.0	2.8	3.3

Table 2. Biometric measurements conducted during the production cycle of basa fish.

Date	24/09/2022			22/10/2022		
Treatment	T ₁ R ₁	T ₀	T ₁ R ₂	T ₁ R ₁	T ₀	T ₁ R ₂
Length (cm)	25.3±2.3a	25.2±4.4a	26.7±2.1a	27.3±2.3a	27.0±2.0a	28.2±1.9a
Variance (cm ²)	5.4	19.8	4.5	5.5	4.0	3.9
Width (cm)	6.3±0.5b	6.5±0.5b	7.1±0.6a	7.3±0.5a	7.4±0.9a	7.7±0.7a
Variance (cm ²)	0.3	0.3	0.5	0.3	0.9	0.6
Weight (g)	219.6±45.8b	250.7±30.8a	248.5±37.6a	273.7±40.7b	283.9±60.8ab	311.5±51.6a
Variance (g ²)	2104.7	950.2	1419.7	1662.9	3691.5	2668.4
Date	19/11/2022			17/12/2022		
Treatment	T ₁ R ₁	T ₀	T ₁ R ₂	T ₁ R ₁	T ₀	T ₁ R ₂
Length (cm)	28.4±1.9a	27.8±1.7a	28.2±1.6a	27.6±2.2b	29.5±1.8a	28.4±1.6ab
Variance (cm ²)	3.7	3.0	2.8	5.2	3.4	2.6
Width (cm)	7.5±0.6a	7.5±0.7a	7.7±0.5a	7.5±0.7a	7.5±0.6a	7.7±0.5a
Variance (cm ²)	0.4	0.5	0.4	0.5	0.4	0.3
Weight (g)	318.7±36.4a	308.5±45.7a	315.9±41.6a	371.5±53.9a	374.2±56.7a	369.5±45.5a
Variance (g ²)	1325.8	2095.5	1734.6	2908.8	3209.9	2073.2
Date	14/01/2023			11/02/2023		
Treatment	T ₁ R ₁	T ₀	T ₁ R ₂	T ₁ R ₁	T ₀	T ₁ R ₂
Length (cm)	30.3±1.4a	30.1±1.7a	29.2±1.4b	30.2±2.2a	30.5±1.9a	30.3±1.7a
Variance (cm ²)	2.1	3.1	2.0	5.0	3.8	3.1
Width (cm)	8.6±0.5ab	8.7±0.7a	8.3±0.6b	8.4±0.7b	8.9±0.7a	8.8±0.9ab
Variance (cm ²)	0.3	0.5	0.4	0.6	0.6	0.9
Weight (g)	411.7±53.1a	423.8±66.5a	396.2±53.2a	429.8±83.2a	433.8±70.9a	442.0±75.8a
Variance (g ²)	2826.8	4419.4	2837.1	6916.9	5022.6	5743.3

*Mean value of 32 organisms ± standard deviation.

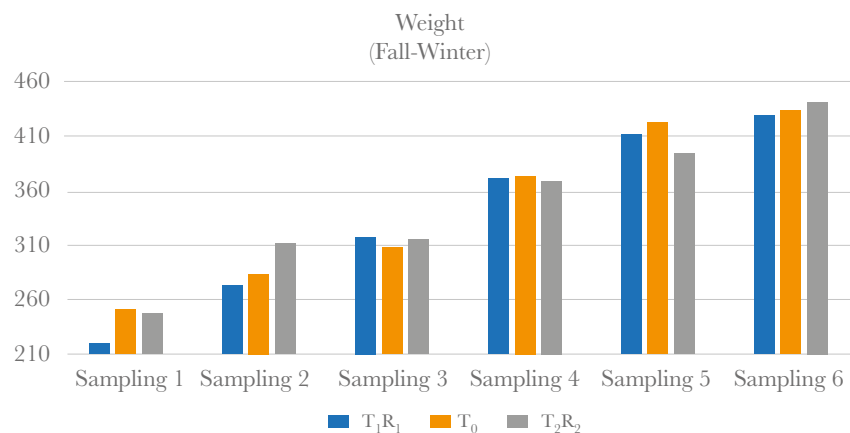


Figure 3. Monthly weight gain of basa fish. T₁R₁, T₀, and T₁R₂ correspond to Treatment 1, Replicate 1; Treatment 0 (control); and Treatment 1, Replicate 2, respectively.

Water quality analysis

Water quality analysis indicated that the measured parameters remained within normal ranges according to the classification criteria established by HANNA® Instruments. The detailed results are presented in Table 3.

The pH and temperature values were similar to those reported by Khan *et al.* (2009), who obtained pH values of 7.4, 7.3, and 7.2, and temperature values of 28.9 °C across their three treatments. These findings are consistent with the water quality parameters required for the optimal development of basa fish. In contrast, chloride and hardness levels differed between the treatment and control groups. These differences may be attributed to the greater incorporation of organic matter associated with the farm-produced feed.

Production costs

Table 4 presents the production costs of both systems. The cost of commercial feed was \$30,000 MXN, whereas the feed produced on the farm totaled \$11,500 MXN, plus \$3,000 MXN in labor costs, resulting in a total of \$14,500 MXN. This amount represents 48.3% of the cost of commercial feed.

Labor and energy (electricity and fuel) were also among the highest cost components in both systems. Commercial feed accounted for 60% of total production costs, while farm-produced feed represented 39.19%, reflecting an approximate 20% reduction in feed-related expenses.

Table 5 shows that the total reduction in production costs was 25.85%, making the farm-produced feed system more economically viable. This reduction is partly attributable to the lower cost of locally sourced ingredients and agricultural by-products used as raw materials. In contrast, commercial feeds often rely on imported ingredients, such as soybean meal, which increases costs for producers.

CONCLUSIONS

Fermented and pre-digested feeds produced on-farm using low-cost local and regional ingredients within sustainable aquaculture systems support growth and development in basa fish comparable to those achieved with commercial diets. This approach enables a reduction in production costs of up to 25%, thereby improving producers' income and overall economic performance.

Table 3. Water quality results.

mgL ⁻¹	T ₁ R ₁	T ₀	T ₁ R ₂
Alkalinity	27	18	24
Chloride	40	80	40
Hardness	180	165	183
Iron	0	0	0
pH	8	7.9	7.9
Sulfite	6	6	10
Temperature (°C)	28.6	28.1	28.2

Table 4. Production costs of both systems (MXN).

Item	Comercial	% of Total	Farm-Produced	% of Total
Fingerlings	3500	7.01	3500	9.46
Feed	30000	60.12	11500	31.08
Labor	5400	10.82	6000	16.22
Energy	4000	8.02	6000	16.22
Health Supplies	2000	4.01	2000	5.41
Infrastructure Depreciation	5000	10.02	5000	13.51
Feed Production Costs	0	0.00	3000	8.11
Total Cost	49900	100.00	37000	100.00

Table 5. Production costs of both systems.

Indicator	Comercial	Farm
Production (kg)	1,000.00	1,000.00
Cost per kg (MXN)	49,900.00	37,000.00
Total Savings (MXN)	-	12,900.00
Reduction (%)	-	25.85

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Anatomical Characterization of Ear and Kernel in Maize (*Zea mays* L.)

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ABSTRACT

Objective: To evaluate the physical quality of ears and kernels of hybrids and varieties cultivated in the municipality of San Luis Acatlán, Guerrero, Mexico. **Methodology:** Six white-kernel hybrids, two yellow-kernel hybrids, and two blue and white landraces were used. The maize was grown during the Spring-Summer 2020 growing cycle under rainfed conditions. Physical traits of the ear and kernel anatomy were evaluated.

Results: VS-Tuxpeño and Azul exhibited the greatest ear length among the evaluated varieties (18.70 and 18.56 cm, respectively), whereas H-565 showed the highest ear weight (204.40 g). H-515 and H-Tuxpeño presented a width-to-length index (WLI) of 0.81, corresponding to a round kernel type, whereas H-565 exhibited a more elongated kernel shape (0.65). All genotypes, except Ocotito, showed an endosperm proportion greater than 70%.

Limitations of the study: Given the edaphoclimatic conditions of the municipality of San Luis Acatlán and the increasingly pronounced effects of global climate change, adaptive strategies for this type of crop should be further investigated.

Conclusions: Hybrids H-565 and VS-Tuxpeño exhibited superior performance in most ear and kernel components, which makes them suitable for commercial production, whereas the Azul variety showed comparable performance with the additional advantage of natural pigmentation. H-562 and Ocotito presented higher proportions of endosperm and germ, respectively, underscoring their potential for the masa and tortilla industry. Overall, the results reveal significant differences among genotypes and support their selection according to the intended grain use in San Luis Acatlán, Guerrero.

Keywords: Grain, Hybrids, Maize (*Zea mays* L.), Morphology, Creole varieties.

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INTRODUCTION

Maize (*Zea mays* L.) is a grass species belonging to the family Poaceae, and Mexico is recognized as the center of origin and diversification of this cereal (Wellhausen *et al.*,



1951). It is considered the most important crop worldwide because of its high consumption and its relevance to industry, as the grain is used in the production of products such as oils, starches, proteins, and food sweeteners; furthermore, it serves as a supplement in animal feeding and as forage for livestock (Fernández-Suárez *et al.*, 2013). It is cultivated in two agricultural cycles, spring-summer and autumn-winter, adapting to diverse climatic conditions, soil types, and water availability (Luna Mena *et al.*, 2012). The selection of the best seed is a fundamental component of its improvement, and the principal traits to be considered include ear and kernel length and width, number of kernels per ear, number of rows, kernels per row, and kernel weight (Magdaleno-Hernández *et al.*, 2016; Delgado-Ruiz *et al.*, 2018). In addition, native or creole maize varieties present certain advantages in terms of adaptation to the diverse edaphoclimatic conditions found in Mexico (Magdaleno-Hernández *et al.*, 2016). Delgado-Ruiz *et al.* (2018) reported that, of 100% of rural producers, 37.2% cultivate hybrids, whereas 32.6% sow both creole varieties and hybrids, using the former for household consumption and the latter for commercialization.

Among the components of seed quality, factors such as the anatomical and nutritional characteristics of the kernel must be considered, since these are regarded as essential parameters required by the food industry at both small and large scales (Cortés Soriano *et al.*, 2010; Retes Mantilla *et al.*, 2014). The main structures are the pericarp, endosperm, germ, and pedicel. The endosperm accounts for the largest proportion of kernel weight; this structure is composed of starch, which may be vitreous (floury) or horny (hard), and the amylose-to-amylopectin ratio is responsible for these endosperm types. The pericarp protects the kernel from external environments and is composed of fiber. The germ (embryo) is the living structure of the kernel and is characterized by a high fat and protein content. The pedicel is the anchoring structure and consists of tubular cells that allow the transfer of nutrients from the cob to the kernel. The relationship among these structures confers characteristics that make the grain useful for the food industry (García-Lara *et al.*, 2019; Singh *et al.*, 2019). In the Costa Chica region of Guerrero, particularly in the municipality of San Luis Acatlán, maize is one of the crops of greatest importance, with an annual production of 50,546.72 t (SIAP, 2024), mainly cultivated under rainfed conditions. The objective of this study was to evaluate the physical quality of ears and kernels of hybrids and creole varieties cultivated in the municipality in order to select the best genotypes and achieve higher grain yield.

MATERIALS AND METHODS

Experimental establishment and biological material

The experiment was established during the Spring-Summer 2020 growing cycle at the experimental field of the Centro de Bachillerato Tecnológico y Agropecuario No. 178, located in the municipality of San Luis Acatlán, Guerrero. The predominant climate is warm sub-humid (Aw2), at an altitude of 250 m, with an average temperature of 25.9 °C and a mean annual precipitation of 1,309 mm (INEGI, 2024). Eight hybrids were used, six obtained from Universidad Autónoma Chapingo, one hybrid and one synthetic variety from Reycoll Seeds[®], and two creole varieties from the municipality of San Luis Acatlán (Table 1). The harvested ears were transported to the Laboratory of Agri-Food Technology

Table 1. Characteristics of the corn seed used in the experiment established in the town of San Luis Acatlán, Guerrero, Mexico.

Name	Genotype Grain Color	Genotype Grain Color
H-515	White Hybrid	White Hybrid
H-516	White Hybrid	White Hybrid
H-562	White Hybrid	White Hybrid
H-563	White Hybrid	White Hybrid
H-565	White Hybrid	White Hybrid
H-568	White Hybrid	White Hybrid
H-337	Yellow Hybrid	Yellow Hybrid
VS-Tuxpeño	Yellow Synthetic	Yellow Synthetic
Ocotito	White Creole	White Creole
Blue	Blue Creole	Blue Creole

and Innovation of the Regional Center for Higher Education of the Costa Chica of Universidad Autónoma de Guerrero.

Ear Quality Components

Ear weight

Fifteen ears were randomly selected, free of disease symptoms and physical damage. They were weighed using a VeLab-500 digital balance, and the results were expressed in g (Salinas-Moreno & Aguilar-Modesto, 2010).

Ear length and diameter

Ear length was measured with a ruler from the base to the last viable kernel (large, defect-free kernel). Diameter was measured at the middle section of the ear, and the results were expressed in cm (Salinas-Moreno & Aguilar-Modesto, 2010).

Number of kernels per ear

The number of rows and kernels per row in each ear was determined visually. Rows were counted in the middle section of the ear, whereas the number of kernels per row was counted from the base of the ear to the last kernel in four sections of the ear (Salinas-Moreno & Aguilar-Modesto, 2010).

Kernel weight per ear

The ears were shelled, keeping the kernels from each ear separately, and the obtained results were expressed in g (Salinas-Moreno & Aguilar-Modesto, 2010).

Kernel depth

Kernel depth was reported as the difference between ear diameter and cob diameter (Salinas-Moreno & Aguilar-Modesto, 2010), using the following equation:

$$KD = \text{ear diameter} - \text{cob diameter}$$

Row-to-ear diameter and ear diameter-to-length indices

The row-to-ear diameter index (REDI) and the ear diameter-to-length index (EDLI) were calculated according to Salinas-Moreno and Aguilar-Modesto (2010), using the following equations:

$$REDI = \frac{(\text{number of rows per ear})}{(\text{ear diameter})}$$

$$EDLI = \frac{(\text{ear diameter})}{(\text{ear length})}$$

Physical grain quality

Kernel size

Fifty viable seeds were selected in triplicate, and the length, width, and thickness of each seed were measured using a digital caliper (Truper[®]). The results were expressed in cm, and the kernel width-to-length index (WLI) was calculated according to Pérez Mendoza *et al.* (2006) and Rivera-Castro *et al.* (2020), using the following equation:

$$WLI = \frac{(\text{kernel width})}{(\text{kernel length})}$$

Thousand-kernel weight (TKW)

One thousand viable seeds were selected in triplicate and weighed using a digital balance (VeLab-500). The result was calculated and reported in g (Rivera-Castro *et al.*, 2020).

Kernel density

One hundred kernels were selected and placed in a 100 mL graduated cylinder; sample weight and displaced volume were recorded (Pérez Mendoza *et al.*, 2006). Kernel density was calculated using the following equation and expressed in g mL⁻¹:

Kernel anatomy

Fifty viable seeds were selected and soaked for 24 h; subsequently, the endosperm, pericarp, pedicel, and germ were separated using a scalpel. Thereafter, the structures were dried in a forced-air oven; once dried, they were weighed, and the results were calculated using the following equation (Figueroa Cárdenas *et al.*, 2013):

$$\text{Anatomical part (\%)} = \frac{\text{weigh of the anatomical part}}{\text{total seed weight}} \times 100$$

Experimental design

The experiment was conducted under a completely randomized design, in which each genotype was considered a treatment, with three replicates for each evaluated variable. The obtained results were subjected to analysis of variance (ANOVA), and a multiple comparison of means test was performed using Tukey's test ($\alpha=0.05$) with the statistical package MINITAB® 19.

RESULTS AND DISCUSSION

Ear quality components

Table 2 shows that all variables, except kernel weight ($p \geq 0.05$), exhibited statistically significant differences ($p < 0.05$), thereby confirming the existence of functional genetic variation among the evaluated materials. The greatest ear length observed in VS-Tuxpeño (18.70 cm) and the largest diameter recorded in H-562 (5.10 cm) suggest differences in reproductive architecture, possibly associated with photosynthetic efficiency and the availability of photoassimilates during kernel filling. These results fall within the ranges reported by Huerta *et al.* (2008), who indicated mean values of 14.90 and 4.78 cm for ear length and diameter, respectively, and by Ángeles-Gaspar *et al.* (2010), who reported intervals of 12.4 to 15.2 cm for length and 4.09 to 4.76 cm for diameter. The superiority of H-565 in the number of rows and kernels per ear reinforces the notion that this trait is strongly governed by genetic control, although it may also be modulated by environmental and management factors.

In contrast, kernel weight did not show statistically significant differences, suggesting that, despite the morphological variation among genotypes, there was physiological compensation in the distribution of assimilates to the kernel. This may be attributed to the adaptation of the landrace materials to local conditions, which favors a more stable response among genotypes, even in the presence of differences in ear architecture. This interpretation is consistent with Remache *et al.* (2017), who stated that light intensity, climate, and soil quality directly influence kernel filling. The values recorded for thousand-kernel weight and cob diameter were comparable to those reported by Jiménez-Juárez *et al.* (2012) and Ramírez Reynoso *et al.* (2020), whereas the similarity in kernel depth and morphological indices reflects adaptation and plasticity, even in comparison with hybrids, which partially agrees with the findings of Castellanos Reyes *et al.* (2017). Overall, the results reinforce the observations of Sánchez-Toledano *et al.* (1949), who proposed that differences among genotypes allow for specific uses and differentiated technological utilization.

The differences observed among the evaluated genotypes may also be explained by the level of phenotypic plasticity, particularly in landrace materials, which have coevolved with traditional agricultural practices and heterogeneous environments. This enables them to maintain stability in yield-associated variables, even when they differ in morphological ear traits. Several studies have indicated that, in native maize populations, coadaptation to local factors such as altitude, light intensity, soil type, and moisture availability promotes a physiological balance that is reflected in kernel filling and in the expression of quantitative traits, despite the existence of evident genetic contrasts among materials (Wellhausen *et al.*,

Table 2. Ear characteristics of maize genotypes cultivated in the municipality of San Luis Acatlán, Guerrero, Mexico.

RV	Genotypes									
	H-515	H-516	H-562	H-563	H-565	H-568	H-377	VS-Tux	Ocotito	Azul
EL (cm)	16.5±2.3ab*	14.6±1.1b	13.8±2.4b	16.2±2.3ab	16.0±0.8ab	15.1±1.4ab	17.4±1.9ab	18.7±1.4a	15.0±1.8ab	18.6±1.6a
ED (cm)	4.7±0.3abc	4.7±0.2ab	5.1±0.4a	4.4±0.2bcd	4.4±0.2bcd	4.6±0.2abcd	4.7±0.5ab	4.0±0.2d	4.7±0.2ab	4.1±0.1cd
RE	15.2±1.64ab	15.2±1.8ab	15.2±1.3ab	16.4±1.7a	16.8±1.a	14.4±2.2abc	14.8±1.1abc	12.8±1.1bc	15.6±0.9ab	12.0±0.7c
KR	33.7±4.24a	31.2±3.3a	32.2±5.1a	36.1±3.7a	38.2±3.1a	38.8±1.6a	37.9±2.7a	39.3±5.2a	35.2±6.7a	38.2±2.5a
EW (g)	177±45.74ab	163±28ab	187±25.2ab	142.6±24.4b	204.4±24.9a	170.6±23.5ab	208.2±27.7a	180.8±6.8ab	172.4±37.3ab	201.2±19.1ab
CD (cm)	2.9±0.4ab	2.9±0.2ab	3.2±0.2a	2.9±0.1ab	3.1±0.1a	2.7±0.2ab	2.9±0.3ab	2.4±0.2b	2.8±0.2ab	2.8±0.3ab
KW (g)	150.0±26.8a	138.4±25a	118.4±66.8a	131.2±43.8a	175.6±19.9a	146.3±19.8a	174.7±20.4a	150.2±15.4a	146.0±34.7a	186.7±25.9a
CW (g)	39.1±6.4a	24.8±3.9b	26.4±3.9b	27.2±10.5ab	29.2±5.5ab	23.5±4.4b	33.8±8.2ab	24.1±0.9b	26.6±5.2b	26.4±4.1b
KD (cm)	1.8±0.4ab	1.8±0.2ab	1.9±0.2a	1.6±0.2ab	1.3±0.2b	1.9±0.2ab	1.8±0.4ab	1.6±0.1ab	1.9±0.3a	1.3±0.3b
EDLI	0.28±0.02bcd	0.32±0.02ab	0.38±0.09a	0.28±0.04bcd	0.27±0.01bcd	0.30±0.03abc	0.3±0.05bcd	0.22±0.02d	0.3±0.03ab	0.2±0.03cd
REDI	3.3±0.3abc	3.2±0.3abc	2.9±0.3c	3.7±0.3ab	3.9±0.4a	3.1±0.4bc	3.1±0.3bc	3.2±0.3bc	3.3±0.29abc	2.9±0.1c
TKW (g)	295±4.8c	278±9.9c	319±8.2b	201.9±8.5d	280.7±19.2c	276.0±8.6c	329.5±8.2b	331.4±14.9b	283.7±2.6c	376.9±6.0a

(± standard deviation of the mean). *Different letters within rows indicate statistically significant differences according to Tukey's test (p≤0.05). RV: response variable; EL: ear length; ED: ear diameter; RE: rows per ear; KR: kernels per row; EW: ear weight; CD: cob diameter; KW: kernel weight; CW: cob weight; KD: kernel depth; EDLI: ear diameter-to-length index; REDI: row-to-ear diameter index; TKW: thousand-kernel weight; CV: coefficient of variation.

1951; Pressoir & Berthaud, 2004). This may explain why, in the present study, genotypes with greater ear length or diameter did not differ significantly in weight-related variables, thereby revealing a compensatory mechanism associated with the flow of photoassimilates toward the kernel.

Likewise, the results demonstrate that some landrace materials possess agronomic potential comparable to that of hybrids, not only because of their stability in key variables, but also because of the ear architecture observed. This behavior is consistent with recent reports highlighting the value of native maize as a strategic resource for genetic improvement schemes, particularly because of its adaptive capacity under biotic and abiotic stress, as well as its quality attributes and productive resilience (Ávila *et al.*, 2019; Arteaga *et al.*, 2021). In this regard, the similarity in kernel weight among genotypes in the present study, despite marked morphological differences, reinforces the relevance of landraces as a productive alternative in regions where hybrids do not always maximize their potential because of climatic or management conditions.

Physical grain quality

Table 3 presents the results obtained for kernel anatomy (Figure 1), in which statistically significant differences ($p < 0.05$) were observed in the pedicel, germ, and endosperm, but not in the pericarp. The latter constitutes the grain's first line of defense against the external environment, and its hardness is one of the principal criteria used to define its suitability for the food industry (Wolf *et al.*, 1952).

It was observed that H-568 (6.51%) and Ocotito (6.47%) exhibited the highest proportion of pedicel. This structure functions as the site of kernel attachment to the cob and is responsible for nutrient supply during kernel development; moreover, during nixtamalization, the pedicel, together with the pericarp, contributes to the formation of natural gums that confer an optimal texture to tortillas (Wolf *et al.*, 1952). Regarding the germ, differences among genotypes were minimal, with H-377 standing out at 22.5%. The germ represents the living part of the kernel and serves as the principal reserve

Table 3. Percentage of the main structures that make up the corn kernel of the evaluated genotypes.

Genotype	Pedicel (%)	Pericarp (%)	Germ (%)	Endosperm (%)
H-515	3.84±0.64 ^{c*}	5.34±1.22 ^a	13.49±1.70 ^{bc}	77.33±1.63 ^{ab}
H-516	5.12±1.07 ^b	5.27±0.25 ^a	11.54±0.80 ^c	78.07±1.15 ^a
H-562	3.89±0.32 ^c	4.61±0.31 ^a	13.70±2.74 ^{bc}	77.81±2.73 ^a
H-563	4.05±0.68 ^c	5.29±0.76 ^a	18.77±0.77 ^a	71.89±1.28 ^{bc}
H-565	3.36±0.26 ^d	5.43±0.20 ^a	19.50±0.68 ^a	71.72±0.95 ^c
H-568	6.51±1.45 ^a	4.94±0.96 ^a	18.10±2.71 ^{ab}	70.45±3.07 ^{cd}
H-377	2.64±0.56 ^d	4.40±0.55 ^a	22.50±1.67 ^a	70.46±1.88 ^{cd}
VS-Tuxpeño	4.99±0.48 ^b	5.16±0.34 ^a	19.79±1.17 ^a	70.06±1.35 ^{cd}
Ocotito	6.47±0.68 ^a	5.95±0.69 ^a	22.46±0.92 ^a	65.11±2.09 ^d
Azul	4.52±0.11 ^c	4.57±0.11 ^a	20.60±1.43 ^a	70.31±1.62 ^{cd}

±: standard deviation from the mean. *Different letters in the same column are statistically different (Tukey, $p \leq 0.05$). CV: Coefficient of variation.

of lipids, containing approximately 78% of the mineral content, with phosphorus as a key nutrient for initiating seedling emergence mechanisms (Agama-Acevedo *et al.*, 2013; Serna-Saldívar, 2019).

Regarding the endosperm, H-516 (78.07%) and H-562 (77.81%) exhibited the highest proportions (Table 3). The endosperm constitutes the principal source of carbohydrates and is subdivided into two types: 1) horny or vitreous, characterized by a higher amylopectin content and lower moisture, primarily destined for the flour industry (Luna Mena *et al.*, 2012); and 2) floury, characterized by a higher amylose content and greater moisture, and used in the production of masa and tortillas (Salinas-Moreno & Aguilar-Modesto, 2010).

Salinas-Moreno *et al.* (2013) reported, in hybrid maize produced in Oaxaca, values of 0.7–2.0, 0.63–5.4, 1.25–13.1, and 79.5–97.42% for pedicel, pericarp, germ, and endosperm, respectively. Corona-Terán *et al.* (2017) reported pedicel values ranging from 2.92 to 5.16%, germ values from 6.4 to 9.59%, pericarp values from 4.32 to 6.78%, and endosperm values from 78.47 to 86.36%. Rivera-Castro *et al.* (2020) reported, in kernels of native maize from the Costa Chica region of Guerrero, pericarp and endosperm values of 4.35–6.20 and 79.65–84.25%, respectively. The differences with the present study may be attributed to cultivation conditions, varietal background, and the type of irrigation used, as noted by Gaytán-Martínez *et al.* (2013).

Vázquez-Carrillo *et al.* (2010) indicated that a kernel of good nixtamalization quality should exhibit a pedicel proportion of less than 2% and a high pericarp proportion ($p \geq 5\%$), since the latter is responsible for generating the gel that confers texture to the tortilla. Rivera-Castro *et al.* (2020) demonstrated that there is a proportional relationship between germ size and fat content; that is, a higher germ percentage implies a greater lipid content. The results of the present study meet two desirable characteristics for the masa and tortilla industry: 1) a pericarp proportion greater than 3.2%, and 2) a germ proportion higher than 10.75%. These traits ensure that tortillas produced from these maize types will exhibit good texture and a high fatty acid content.

Table 4 shows significant differences ($p < 0.05$) in all evaluated variables. Azul and H-568 exhibited the highest values for length (12.84 and 12.66 mm, respectively) and width (9.29 and 9.54 mm, respectively), whereas H-563 showed the lowest values (length, 10.91

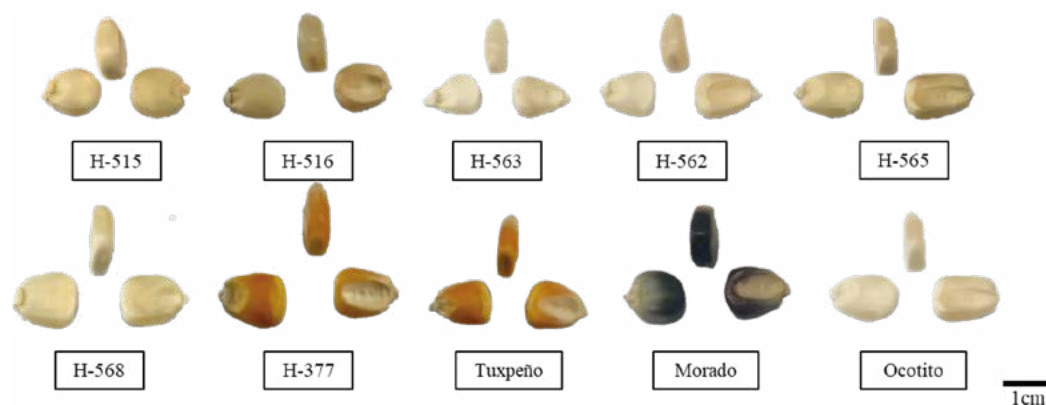


Figure 1. Morphology of the maize grain of the genotypes cultivated in the locality of San Luis Acatlán, Guerrero, Mexico.

Table 4. Physical characteristics of the grain of the evaluated maize genotypes that are grown in the municipality of San Luis Acatlán, Guerrero.

Genotype	Pedicle (%)	Pericarp (%)	Germ (%)	Endosperm (%)
H-515	3.84±0.64 ^{bc*}	5.34±1.22 ^a	13.49±1.70 ^{bc}	77.33±1.63 ^{ab}
H-516	5.12±1.07 ^{ab}	5.27±0.25 ^a	11.54±0.80 ^c	78.07±1.15 ^a
H-562	3.89±0.32 ^{bc}	4.61±0.31 ^a	13.70±2.74 ^{bc}	77.81±2.73 ^a
H-563	4.05±0.68 ^{bc}	5.29±0.76 ^a	18.77±0.77 ^a	71.89±1.28 ^{bc}
H-565	3.36±0.26 ^{bc}	5.43±0.20 ^a	19.50±0.68 ^a	71.72±0.95 ^c
H-568	6.51±1.45 ^a	4.94±0.96 ^a	18.10±2.71 ^{ab}	70.45±3.07 ^{cd}
H-377	2.64±0.56 ^{bc}	4.40±0.55 ^a	22.50±1.67 ^a	70.46±1.88 ^{cd}
VS-Tuxpeño	4.99±0.48 ^{ab}	5.16±0.34 ^a	19.79±1.17 ^a	70.06±1.35 ^{cd}
Ocotito	6.47±0.68 ^a	5.95±0.69 ^a	22.46±0.92 ^a	65.11±2.09 ^d
Azul	4.52±0.11 ^{abc}	4.57±0.11 ^a	20.60±1.43 ^a	70.31±1.62 ^{cd}

(± standard deviation from the mean). *Different letters in the same column are statistically different (Tukey, $p \leq 0.05$). C. V. coefficient of variation.

mm; width, 7.99 mm). Regarding thickness, VS-Tuxpeño presented the highest value (4.69 mm), whereas H-562 exhibited the lowest width-to-length index (WLI, 0.71); moreover, VS-Tuxpeño and H-515 showed the highest width-to-length relationship for this trait.

Figuroa Cárdenas *et al.* (2013) reported, in native maize, values ranging from 9.0 to 14.5 mm for length and from 6.2 to 10.9 mm for width, with an average thickness of 4.5 to 5.5 mm. Vázquez-Carrillo *et al.* (2010) reported densities of 29.3 to 35.5, whereas Ramírez Reynoso *et al.* (2020) reported a WLI close to 1, indicating rounder kernels. In the present study, the evaluated kernels were more elongated and flatter. The relationship between kernel depth and kernel length is consistent with that reported by Ramírez Reynoso *et al.* (2020) and Rivera-Castro *et al.* (2020). Wolf *et al.* (1952) emphasized that kernel dimensions are decisive in commercialization, with the sale by volume being more advantageous for larger kernels.

The results obtained reveal considerable variability in the anatomical composition of maize kernels, suggesting genetic adaptation to specific environmental conditions. This variability is consistent with the findings of Zepeda-Bautista *et al.* (2009), who reported significant differences in kernel structure between hybrids and landrace varieties, attributable to genotype-by-environment interaction. Landrace varieties, such as Ocotito, exhibited a higher proportion of pedicle and germ, which could indicate adaptation to local conditions that favor the preservation of essential nutrients during kernel development.

The anatomical composition of the kernel has direct implications for the food industry. According to Pérez de la Cerda (2007), the proportion of vitreous endosperm is related to the physiological quality of the seed, affecting germination and vigor. In the present study, hybrids with a higher proportion of endosperm, such as H-516 and H-562, may be preferred for flour production, whereas varieties with higher germ and pedicle content, such as Ocotito, may be more suitable for masa and tortilla production because of their textural characteristics and nutritional content.

When the present results are compared with previous studies, the proportions of pedicel, germ, and endosperm are similar to those reported by Salinas-Moreno *et al.* (2013) in hybrid maize from Oaxaca. However, the pericarp proportions observed in this study are lower than those reported by Rivera-Castro *et al.* (2020) in native maize from the Costa Chica region of Guerrero, which may indicate differences in agricultural practices and environmental conditions among regions.

Kernel quality also has implications for food security. Nixtamalization improves the bioavailability of essential nutrients such as phosphorus and calcium, while simultaneously reducing phytic acid, thereby enhancing mineral absorption. Varieties with a higher proportion of germ, such as Ocotito, may therefore be preferred for tortilla dough production, as they provide additional nutritional benefits that contribute to a more balanced diet.

Taken together, the results show that the evaluated genotypes possess anatomical characteristics that make them suitable both for the masa and tortilla industry and for specific food applications, thereby underscoring the importance of conserving the genetic diversity of native maize from Guerrero, which offers adaptive and productive advantages under diverse agroecological conditions.

CONCLUSIONS

Hybrids H-565 and VS-Tuxpeño stood out in most ear and kernel components, making them suitable for commercial production, whereas the Azul variety exhibited comparable performance, with the added value of its natural pigmentation. H-562 and Ocotito showed a higher proportion of endosperm and germ, respectively, indicating their potential for the masa and tortilla industry. Overall, the results reveal significant differences among genotypes and make it possible to select hybrids or landraces according to grain use, thereby fulfilling the objective of characterizing their physical quality in San Luis Acatlán, Guerrero.

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Effective adoption of the Pinto Saltillo common bean variety (*Phaseolus vulgaris* L.) in Durango and other states of México

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ABSTRACT

Objective: To analyze the area of effective adoption of the improved common bean variety (*Phaseolus vulgaris* var. Pinto Saltillo) in México.

Design/Methodology/Approach: The area planted with Pinto Saltillo was obtained from the Statistical Yearbook of Agricultural Production (SIAP) and other data sources. Variables such as planted area, seed yield, and total production under both rainfed and irrigated conditions were included. The data were analyzed using principal component analysis (PCA), descriptive statistics, graphs, trend lines, and maps showing the level of technological adoption.

Results: The results show a gradual reduction in the annual area planted with common bean in the state of Durango (39-97%). Extensive Pinto Saltillo plantings were observed in 11 states of México, mainly under rainfed conditions. The highest cumulative values were recorded in Durango. The state of Zacatecas ranked first due to its high Pinto Saltillo planting area under irrigation (47,838 ha year⁻¹), followed by Chihuahua (30,840 ha year⁻¹).

In México, the cumulative area planted with Pinto Saltillo between 2012 and 2024 reached 5.1 million hectares, with a total grain production of 2.8 million tons. Traits associated with the extensive adoption of Pinto Saltillo include disease resistance, high seed yield, slow seed coat darkening, and grain reduced cooking time.

Study Limitations/Implications: The study was conducted using information obtained from two databases; therefore, additional state, national, and international information sources should be considered in future analyses.

Findings/Conclusions: The productive and quality impact of Pinto Saltillo is evident on the common bean market, where the “Pinto Saltillo” commercial class has been recently introduced.

Keywords: *Phaseolus*, breeding, productivity, quality, sustainability.



INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the second most important staple crop in México (Félix *et al.*, 2019; Matul *et al.*, 2024). This staple product is used by farmers throughout the year for household consumption and for the generation of income through its gradual sale. For many years, common bean commercialization faced challenges associated with traditional pinto-type varieties, whose seed coat is subject to a rapid darkening (Hernández *et al.*, 2023). In response to this problem, common bean breeding programs focused on reducing seed coat darkening, which led to the development of the Pinto Saltillo variety. This common bean variety was developed from the improved line PT93004, selected in Ignacio Allende, Durango, based on its local adaptation, light seed color, and tolerance to seed coat darkening (Sánchez *et al.*, 2004; Junk *et al.*, 2008; Anaya *et al.*, 2021; Jiménez *et al.*, 2024).

The registration of the Pinto Saltillo common bean variety began in 1999 and was successfully completed in 2001 (Sánchez *et al.*, 2009). After its registration and release in Coahuila, seed multiplication and commercialization of the Pinto Saltillo variety began, mainly in the states of Chihuahua and Durango. In Chihuahua, the municipalities of Namiquípa, Cuauhtémoc, and Riva Palacio stood out for their adoption of Pinto Saltillo (Jiménez and Acosta, 2013). This variety was particularly adopted in northern Durango (municipality of Hidalgo) and the region known as “Los Llanos de Durango” (municipalities of Cuencamé and Guadalupe Victoria) (Ávila *et al.*, 2011). The rapid and widespread adoption of the Pinto Saltillo common bean variety by producers was favored by its competitiveness and consumption-related advantages (Hernández *et al.*, 2023).

The improved Pinto Saltillo variety offers several advantages, including tolerance to drought, high yield potential, and reduced cooking time, as well as an extended shelf life due to the slow darkening of the seed coat (Sánchez *et al.*, 2009; Hernández *et al.*, 2023). Consequently, the Pinto Saltillo common bean variety was widely adopted in the Semi-Arid Highlands of México (Ávila *et al.*, 2011). Subsequently, other common bean-producing states and regions of México accepted this variety. Farmers have continued cultivating Pinto Saltillo due to the high-income levels it generates, as a result of its high productivity, ease of commercialization, and the premium market price of the common bean (Hernández *et al.*, 2023).

Currently, the Pinto Saltillo common bean variety is cultivated in several states across the Semi-Arid Highlands, Subhumid Highlands, and even in the Dry Tropics of México. This variety has gained both commercial and dietary relevance, becoming a recognized commercial class in national and export markets. Consequently, several studies have characterized producers, determined the level of adoption, and evaluated the economic impact of the extensive use of the Pinto Saltillo common bean variety. In addition, González and Wood (2006), González *et al.* (2009), Ávila *et al.* (2011), and Hernández *et al.* (2023) recommended the identification of factors associated with the adoption process. The aim was the establishment of a strategic framework for the successful promotion of other improved common bean varieties.

Public and private sector agencies have generated different techniques that facilitate the study of the adoption process of technological developments. These techniques

include the use of large-scale databases and their analysis through different algorithms, which enable the development of highly accurate models. The objective of this study was to analyze the area of effective adoption reached by the improved Pinto Saltillo common bean variety in the Semi-Arid Highlands and other common bean-producing regions of México.

MATERIALS AND METHODS

A descriptive analysis focused on the interpretation of historical data to understand the context of common bean production in Durango from 1980 to 2024. Data on planted area, harvested area, disaster area, total production, and yield were obtained from the Statistical Yearbook of Agricultural Production–Statistical Yearbook of Agricultural and Livestock Production (SIAP, 2025).

Meteorological variables, including average monthly and annual minimum and maximum temperatures, as well as monthly accumulated precipitation, cropping cycle, and year, were obtained from the historical records of the National Meteorological Service (Sistema Meteorológico Nacional) (<https://smn.conagua.gob.mx/tools/DATA/Climatología/Pronóstico%20climático/Temperatura%20y%20Lluvia/PREC/1985-2024.pdf>). Additionally, the FAOSTAT historical database (FAOSTAT, 2025) was consulted to verify domestic yield information. A database was developed by using the collected data, sorting the information according to the water regime of common bean production —*i.e.*, under rainfed and irrigated conditions. Whenever possible, differentiated statistics were obtained for major varieties, such as Pinto Saltillo.

The data obtained were analyzed using descriptive statistics (Excel[®], Ver. 16.100.1) and Principal Component Analysis (PCA) performed with the R software (version 4.4.2). The results were presented using graphs and trend lines generated by Excel[®] v. 16.100.1 computing program. Additionally, maps were developed based on the technological adoption index at both the state level (Durango) and the national level. These maps were developed using the software packages Spyder Python v. 5.4.3 and ArcGIS Pro v. 2.5.0.

RESULTS AND DISCUSSION

The evaluated climatic parameters recorded high variability at the monthly, cropping cycle (July-October), and annual headings for common bean cultivation, as well as between years. These findings highlighted the importance of temperature and accumulated rainfall under rainfed (Figure 1a) and irrigated conditions (Figure 1b). Based on daily values and other evaluation periods, common bean have adapted to different temperature and precipitation patterns. Principal Component Analysis (PCA) required a high number of principal components to achieve a considerable accumulation in the explanation of variance (Table 1). Six principal components accounted for 70.1% of the variance under rainfed conditions and 68.0% under irrigated conditions. The variables had the highest influence on common bean response in Durango, under both irrigated and rainfed conditions, were the average minimum temperature and the average maximum temperature recorded during the year. Temperature, particularly minimum temperature, influences the growth and development rates of common bean

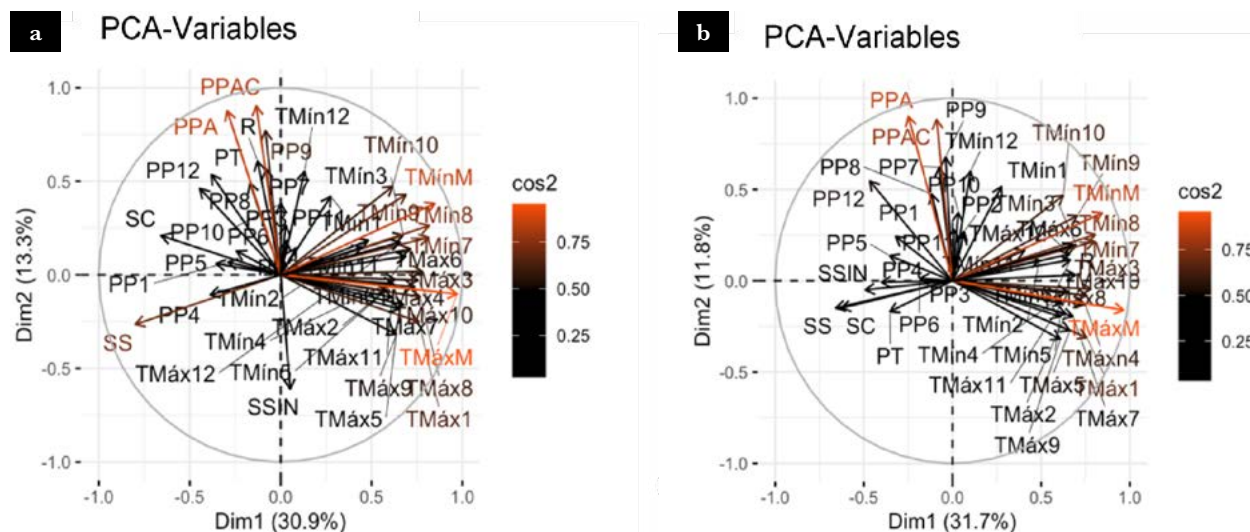


Figure 1. Cosine of the variables used in the Principal Component Analysis (PCA) for rainfed (a) and irrigated (b) common bean cultivation in Durango.

Table 1. Eigen values and levels of explanation of variance according to the Principal Component Analysis (PCA) for common bean cultivation variables in Durango (1985-2024).

Rainfed				Irrigated		
Principal Component	Eigen Value	Proportion (%)	Accumulated (%)	Eigen Value	Proportion (%)	Accumulated (%)
CP1	13.9	30.9	30.9	14.3	31.7	31.7
CP2	6.0	13.3	44.2	5.3	11.8	43.5
CP3	3.8	8.4	52.6	3.4	7.6	51.1
CP4	3.1	7.0	59.6	3.1	6.9	58.0
CP5	2.5	5.6	65.2	2.4	5.4	63.4
CP6	2.2	4.9	70.1	2.1	4.6	68.0

CP=Principal Component.

plants (Rosales *et al.*, 2001), which is associated with biomass accumulation, translocation of photoassimilates to the seeds, and common bean yield. In addition, temperature affects the survival, arrival time, and levels of damage caused by insect pests, such as the Mexican bean beetle (*Epilachna varivestis*) and the pod weevil (*Trichapion* spp.).

Other variables that influenced common bean cultivation were the accumulated precipitation during the cropping cycle and the total precipitation recorded throughout the year. The total precipitation also impacted common bean grown under irrigated conditions due to the influence of water on different biological factors, such as the presence of beneficial (*Rhizobium*) and pathogenic (*Fusarium*, *Rhizoctonia*, and *Pythium*) microorganisms. Additionally, precipitation hinders the survival and phases of arrival for insect pests, including white grubs (*Phyllophaga* spp.), the Mexican bean beetle (*Epilachna varivestis*), and the pod weevil (*Trichapion* spp.) and increases common bean productivity under both rainfed and irrigated conditions.

The study area should be expanded, and the accuracy of the observations should be improved to establish a direct relationship between agroclimatic variables and common bean productivity in Durango and other states of México. Currently, agrometeorological variables are often evaluated using damaged or obsolete equipment with limited maintenance. In addition, the personnel in charge of recording the meteorological data has little experience, multiple activities, and undergoes constant rotation. Likewise, there is a shortage of personnel and limited resources to conduct field sampling required to determine statistical data related to planted area, harvested area, and seed yield. Consequently, only incomplete and variable databases are available, reducing the accuracy and usefulness of the information for the study of the influence of meteorological factors on agricultural production.

The importance of common bean cultivation in the rural sector of México was confirmed: 613,345 production units were found throughout the country (INEGI, 2022). Nevertheless, a decreasing trend in planted area and fluctuations in yield under rainfed conditions were observed, particularly during the 2000s. This trend was associated with low productivity and the slow adoption of technologies aimed at the improvement of profitability and production sustainability (Sangerman *et al.*, 2010).

Drought (water stress) was another determining factor affecting common bean productivity, which was associated with the scarcity and irregular distribution of rainfall. Drought impacted the main common bean-producing regions in México and reduced competitiveness during several years within the evaluated period.

According to agricultural statistics, the low production recorded between 2011 and 2023 was the consequence of the great proportion (>90%) of the areas planted with common bean were grown under rainfed conditions (SADER, 2022). In addition, the lack of market regulation contributed to imbalances and inequalities among actors within the production chain. The area planted both under rainfed and irrigated conditions in Durango recorded a negative trend. Likewise, yield lacked a clear trend for rainfed common bean production (Figure 2a and 2b).

In Durango, the planted area and common bean production significantly declined starting in the 2000s (Figure 2; Table 2). Under rainfed conditions, the common bean planted area decreased by 57%, dropping from 347,000 ha in 1988 to 150,000 ha in 2023. This reduction was associated with the increasing preference for forage crops such as maize (*Zea mays*) and oats (*Avena sativa*), which are used for livestock feeding, particularly in La Laguna region. Under irrigated conditions, the common bean planted area decreased by 97%, giving priority to the production of forage species such as maize, alfalfa (*Medicago sativa*), sorghum (*Sorghum bicolor*), and oats. Additionally, perennial industrial species—such as pine (*Pinus greggii*) and cenizo agave (*Agave durangensis*) which are considered production alternatives for poor rainfed soils—were established. The compound annual growth rate (CAGR) decreased from 1.02% to −2.29% between 2010 and 2023. Common bean cultivation was gradually displaced toward marginal areas, where growing other plant species is almost impossible, negatively impacting the productivity and quality of grain produced in Durango.

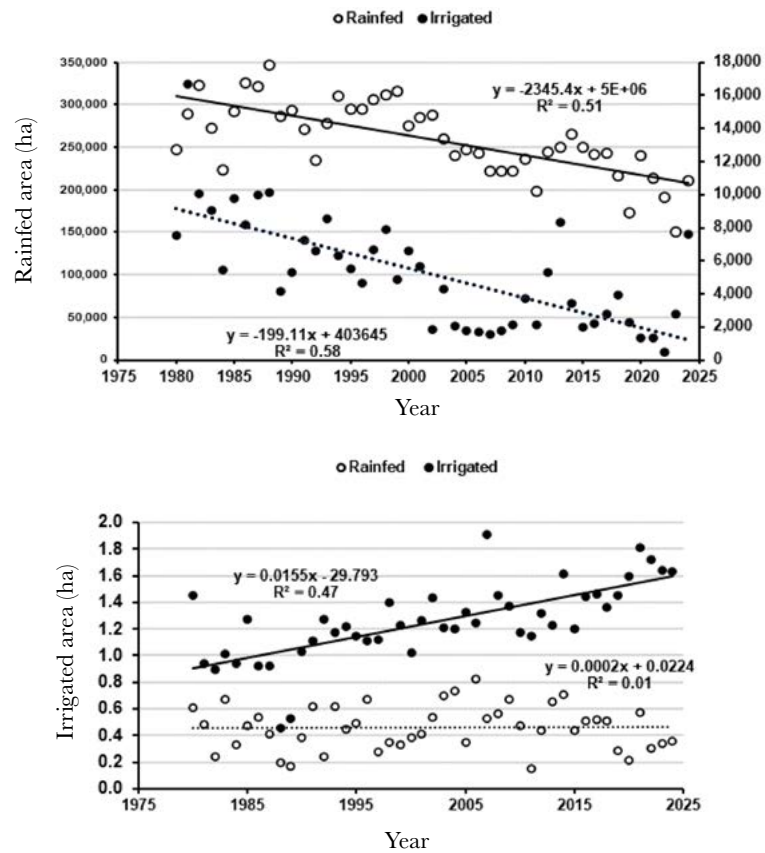


Figure 2. Planted area and common bean yield in Durango (1980-2023).

Table 2. Compound annual growth rate (CAGR) of common bean planted and production areas in Durango, México (1980-2023).

Period	Planted area (ha)	AAGR ¹ (%)	Total Production (t)	AAGR (%)
1980-1989	301,900	1.02	113,465	-4.01
1990-1999	297,644	1.70	121,542	-3.66
2000-2009	253,625	-2.74	135,574	0.29
2010-2023	225,426	-2.29	97,387	-4.42

¹AAGR=Average Annual Growth Rate. Source: Prepared by the author using data from SIAP, (2025).

The reduction in production volumes from 113,465 t to 97,387 t resulted in negative compound annual growth rates (from -4.01% to -4.42%) for total common bean production in Durango (Table 2). Consequently, since the 1980s, México became an importer of common bean and the main buyer of the United States (USA). Low yields, high production costs, and the deterioration of real prices were also recorded. These factors negatively impacted the competitiveness of common bean production, a situation that was further intensified by the cancellation of state marketing arrangements. These arrangements had been developed by the Compañía Nacional de Subsistencias Populares (CONASUPO), through price setting and market regulation (Ayala *et al.*, 2008).

Additionally, farmers faced water scarcity (drought), limited seed availability for improved varieties, lack of irrigation water, decapitalization, insufficient machinery, poor condition of agricultural implements, pests, diseases, and early frosts. Furthermore, farmers had limited access to technology transfer programs, training, technical assistance, and comprehensive support to restore degraded soils and move toward a sustainable common bean production (de los Santos *et al.*, 2017; Borja *et al.*, 2018).

An accelerated process of adoption of the Pinto Saltillo common bean variety was observed, particularly in Durango, Chihuahua (Ávila *et al.*, 2011), and Zacatecas (Table 3). Consequently, this variety was extensively grown, mainly under rainfed conditions. In Chihuahua, cumulative values reached 881,434 ha, while in Durango the cultivated area reached up to 1,832,307 ha from 2014 to 2024 (Table 3). In the case of Zacatecas, a shift in preference toward Pinto Saltillo was observed, aimed to replace the flor de mayo and flor de junio varieties (SIAP, 2025).

The state of San Luis Potosí also had a high level of Pinto Saltillo planted area, reaching 459,409 ha under rainfed conditions. The attributes of Pinto Saltillo favored its expansion across multiple states in México. Additionally, the levels of commercialization and consumption required the designation of a new commercial class: “Pinto Saltillo”. The slow darkening characteristic differentiated it from Pinto Nacional, a variety susceptible to seed coat darkening approximately one month after the harvest and threshing process.

The state of Zacatecas stood out with a large area sown with Pinto Saltillo, grown under irrigated conditions (56,306 ha), followed by Chihuahua (37,010 ha). This situation contributed to an increase in total production and seed yield average, favored by the genetic potential of the Pinto Saltillo improved variety. At domestic level, the cumulative values for the 2012-2024 period reached 5.1 million ha and 2.8 million metric tons of Pinto Saltillo common bean (Figure 3).

The results exceeded previous estimates, which had predicted a reduction in the total area planted with Pinto Saltillo beginning in 2012 (González *et al.*, 2009). This response was associated with the acceptance and significant expansion of the Pinto Saltillo planting area in the Semi-Arid Highlands and part of the Subhumid Highlands of México. High

Table 3. Cumulative values for variables related to the adoption of the Pinto Saltillo common bean variety (2012-2024).

State	Irrigated			Rainfed		
	*PA (ha)	TP (t)	SY (t ha ⁻¹)	PA (ha)	TP (t)	SY (t ha ⁻¹)
Chihuahua	37,010	66,349	1.92	881,434	567,523	0.70
Durango	25,614	40,595	1.62	1,832,307	760,392	0.44
Guanajuato	5,784	13,757	2.39	38,582	18,927	0.52
Nayarit	20,357	31,712	1.49	80,104	109,313	1.32
Querétaro	561	1,110	2.11	6,913	2,128	0.44
SLP	5,557	10,741	1.92	459,409	149,493	0.45
Sonora	10,064	20,416	1.99	--	--	--
Zacatecas	56,306	123,556	2.00	1,603,470	906,760	0.63

* PA=Planted area, TP=Total production, SY=Seed yield.

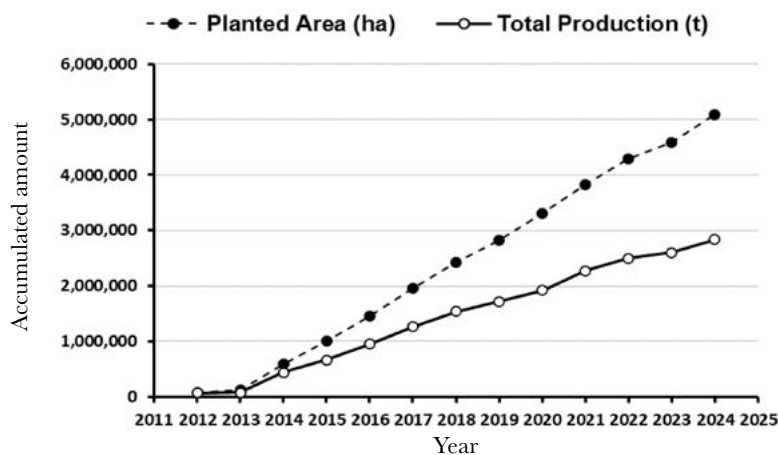


Figure 3. Cumulative values for planted area and production of the Pinto Saltillo common bean variety in México (2012-2024).

values were detected for the area planted with Pinto Saltillo under irrigated conditions in Zacatecas, Chihuahua, and Nayarit (Figure 4a). In the third state, sowing was related to the seed renewal (refreshment) process that takes place during the autumn-winter off-season (November-February); the seed is later used during the spring-summer cropping cycle (July-October) in Durango and other states of the Semi-Arid Highlands of México.

Under irrigated conditions, higher average for common bean yields were recorded for the Pinto Saltillo variety in Guanajuato, Querétaro, and Zacatecas ($>2.0 \text{ t ha}^{-1}$), as well as in Chihuahua, San Luis Potosí, and Sonora ($>1.9 \text{ t ha}^{-1}$). High yields were also reported in Nayarit under rainfed conditions (1.3 t ha^{-1}), associated with deep soils, high accumulated rainfall, and the use of supplementary irrigation. In contrast, the average yield values in the Semi-Arid Highlands ranged from 0.07 t ha^{-1} (2014) to 0.98 t ha^{-1} (2024). The Pinto Saltillo variety is mainly used in the Dry Tropics region for winter seed multiplication and this variety has also been adopted for grain production. In both Nayarit and Sinaloa, extensive areas were sown with Pinto Saltillo, to meet the demand from national and international grain and seed markets. Additionally, some areas in Nuevo León, Coahuila, and Tamaulipas were sown with Pinto Saltillo common bean variety.

In 2023, Pinto Saltillo was grown in 11 states of México, reaching an average yield of 1.8 t ha^{-1} in a sowing area of 18,341 ha established under irrigated conditions (Figure 4a). Under rainfed conditions, Pinto Saltillo was sown in 281,162 ha, accounting for an average yield of 0.64 t ha^{-1} . Compared to other varieties, the characteristics associated with the acceptance and widespread adoption of Pinto Saltillo include disease tolerance, high yield, and slow seed coat darkening. In addition, this variety has short grain cooking time, high consumer preference, and strong demand in the domestic market (Rodríguez *et al.*, 2010; SE, 2012).

Common bean productivity under irrigated conditions was more stable and showed an increasing trend, although its level of use was considerably lower than under rainfed conditions. Consequently, the area established with irrigated common bean production should be expanded in order to increase the certainty of production and seed yield. The

objective was to obtain at least the minimum volume required to meet the annual demand in the state of Durango (18,000 t) (Figure 4b). This objective could be achieved planting approximately 15,000 ha of Pinto Saltillo common bean under irrigated conditions, with an average yield of 1.2 t ha^{-1} .

Adoption of Pinto Saltillo in Durango

The Pinto Saltillo common bean variety has shown high growing potential across the common bean-producing regions of northern México (Figure 4a). This variety had a substantial impact during the 2021-2024 period, when it accounted for 79% of the growing area and 81% of the total common bean production in the state of Durango (Figure 4b). Within the state, the municipalities with the largest areas planted with Pinto Saltillo were Cuencamé and Guadalupe Victoria, followed by Durango, Pánuco de Coronado, Canatlán, and Nuevo Ideal. At the state level, the total sowing area ranged from 142,000 ha year⁻¹ (2014) to 278,000 ha year⁻¹ (2023). The area planted with Pinto Saltillo gradually expanded, reaching a total of 29 municipalities, where this improved variety is currently used for commercial common bean production. As a result, Durango has been the leading producer of Pinto Saltillo common bean since 2005.

The Pinto Saltillo variety has been widely adopted in Durango, largely due to the competitive advantages that this technological development has provided to farmers. The major benefits include the increase in the price paid to farmers for the grain, driven by a strong market demand and its tolerance to seed coat darkening, a trait associated with extended shelf life. This characteristic has allowed producers to extend the negotiation period with traders. In contrast, grains in traditional varieties such as Pinto Nacional and Pinto Villa typically darken approximately one month after harvest. The accelerated darkening of the seed coat caused the need of a rapid commercialization process of traditional pinto common bean varieties, due to the price reduction caused by the “old grain” appearance.

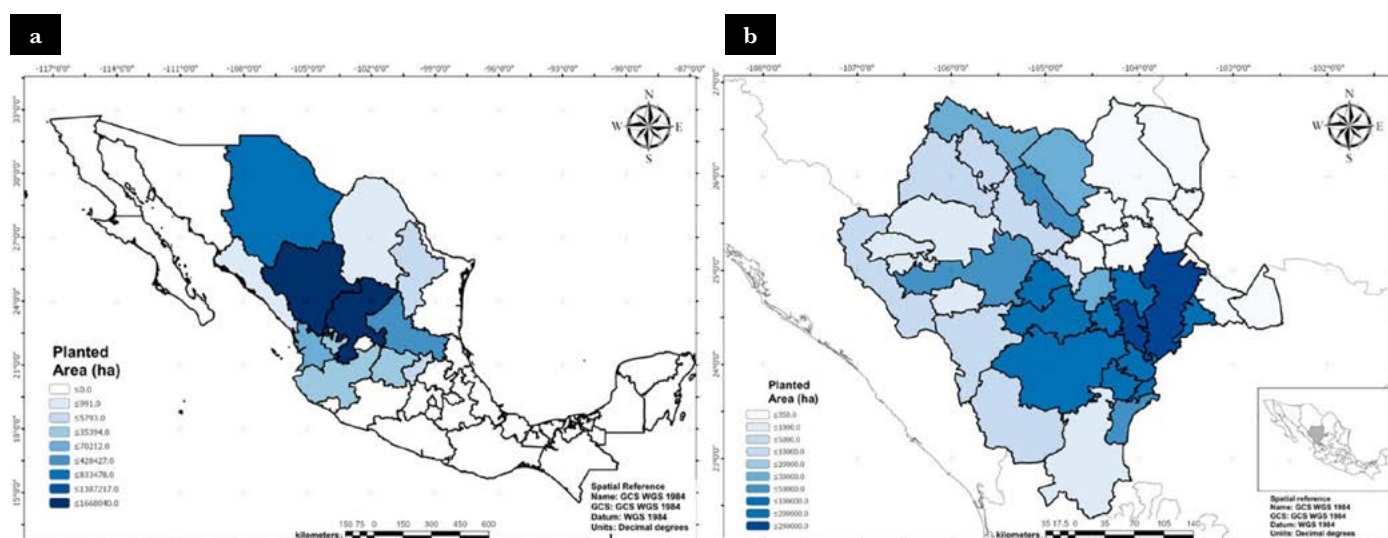


Figure 4. Level of adoption of the Pinto Saltillo common bean variety in México (a) and Durango (b).

Pinto Saltillo maintains its seed coat color and brightness for extended periods (>12 months), preserving its commercial value and consequently favoring greater economic returns for farmers. Short cooking time and easier household food preparation are other two important attributes of this variety. In addition, the reduced cooking time lowers the consumption of gas and other fuels, therefore decreasing the associated pollutant emissions. González *et al.* (2003) and Mutari *et al.* (2021) indicated that the main factors associated with the widespread adoption of improved varieties, such as Pinto Saltillo, include: (a) adaptability to particular field conditions, (b) disease resistance, and (c) high yield potential.

Additional government actions have complemented the productive and commercial attributes of the Pinto Saltillo variety, contributing to its widespread adoption. Various federal and state institutions have implemented initiatives to promote the use of Pinto Saltillo, including the Fundación Produce de Durango A. C. (FPD), the Secretaría de Agricultura, Ganadería y Desarrollo Rural of Durango (SAGDR), and the Secretaría de Agricultura y Desarrollo Rural (SADER) (SADER, 2020). The *Kilo por Kilo* program is one of the federal programs implemented to support common bean production. This program was developed by SADER in coordination with state agricultural agencies. Within this framework, suitable common bean seeds were provided to farmers as a support for sustainable agriculture. The program targeted farmers with growing areas of up to 30 ha (rainfed) and up to 5 ha (irrigation).

The common bean varieties distributed to producers through government programs included Negro San Luis, Pinto Saltillo, and Negro Michigan (SADER, 2020). Meanwhile, the *Frijol para México* government initiative has provided support to smallholder farmers through several strategies. These strategies include: (1) the Programa de Apoyo para Productores de Maíz y Frijol (PIMAF), which provides financial support for the acquisition of inputs (such as seed, fertilizers, and plant protection products) and technical support aimed at the improvement of production; and (2) the Programa Frijol del Bienestar, which offers a guaranteed price per kilogram of common bean, ensuring fair and direct income for farmers.

In 2021, the “Use of Certified Common bean Seed for Chihuahua, Durango, and Zacatecas, with expansion to San Luis Potosí, Nayarit, Quintana Roo, and Yucatán” strategic project was implemented, benefiting 5,945 producers across these states (SADER, 2022). As part of this initiative, the states of Zacatecas, Durango, and Chihuahua received support for the acquisition of Pinto Saltillo certified seed. This initiative contributed to the expansion of both the sowing area and the period in which this improved common bean variety can be used, while simultaneously it increased the economic returns on the investment made during its development and technological transfer.

In Durango, a gradual reduction in the area planted with Pinto Saltillo has been reported (SIAP, 2025). This trend has been associated with several factors, including delayed rainfall onset, reduced cumulative precipitation, and increased irregularity of rainfall distribution (FIRA, 2020). Additionally, a decline in the genetic purity of the variety has been observed, mainly due to mechanical mixtures and the authorization to use commercial common

bean grain as seed for sowing. These practices have limited the productivity and quality of common bean produced in México. Market dynamics have also influenced this trend, particularly the increasing demand for black common bean and the release of other pinto common bean varieties such as Pinto Centauro and PID 2, also considered in the Pinto Saltillo market class. Other important factors include market pressures and constant fluctuations in consumer prices, which have apparently contributed to the reduction in the area planted with Pinto Saltillo (Figure 5a). Despite these fluctuations, the area sown with Pinto Saltillo has varied throughout the years and, since 2009, has accounted for >70% of the total area planted with common bean every year in Durango (Figure 5b).

The results confirmed the widespread and persistent adoption of the Pinto Saltillo variety. In 2021, the adoption rate reached 87%, while farmers were 13% likely to abandon the technology within the following four years (Hernández *et al.*, 2023). These findings contradict earlier projections made during the evaluation of the adoption process. Previous studies predicted that since 2012, the use of this variety would gradually decline (rejection) in growing areas of Durango (González *et al.*, 2009).

Common bean genetic improvement in different regions of México increased yield by approximately 40% between 1961 and 2023. During this period, the national average common bean yield increased from 395.9 kg ha⁻¹ to 950.5 kg ha⁻¹ (Figure 6). This remarkable national achievement took place under highly diverse production

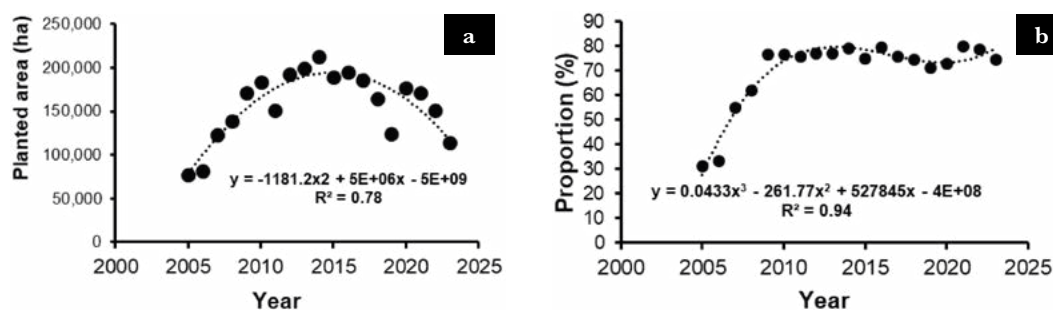


Figure 5. Planted area a) and proportion used by farmers b) for Pinto Saltillo common bean cultivar in Durango, 2005-2023.

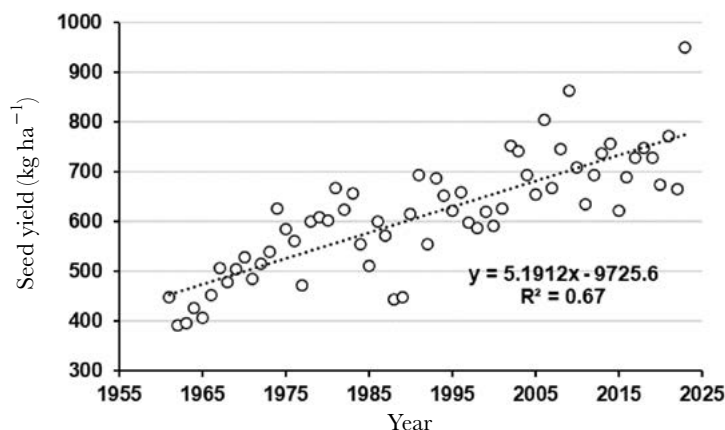


Figure 6. Common bean yield in México (FAOSTAT, 2025).

environments, including both rainfed and irrigated systems. The Pinto Saltillo variety has made a major contribution to domestic common bean production since 2005. The increase in productivity has maintained the national supply for domestic consumption despite the reduction in planted area. In some years, common bean imports were required (<200,000 t); however, this situation was most likely caused by industrial demand, import quotas established under international trade agreements, and credit facilities granted to Mexican traders and agro-industrial firms.

The effective adoption area achieved by the improved Pinto Saltillo variety exceeded every initial production expectations in the Semi-Arid Highlands and even extended to other common bean-producing regions of México. The area sown with Pinto Saltillo has increased steadily since 2005 and by 2023 the variety was grown in 11 states of the country. In 2023, an average of 281,162 ha year⁻¹ were cultivated using Pinto Saltillo under rainfed conditions, with a mean yield of 0.64 t ha⁻¹. Under irrigated conditions, 18,341 ha year⁻¹ were established, with an average yield of 1.8 t ha⁻¹. The highest cumulative values for the area sown with Pinto Saltillo were recorded in the states of Durango, Chihuahua, and Zacatecas, ranging from 833,000 to 1.7 million hectares. The cumulative area sown with Pinto Saltillo in México reached 5.1 million hectares, resulting in a total production of 2.8 million tons.

CONCLUSIONS

The high adoption levels of the Pinto Saltillo common bean variety were associated with several key attributes, including: disease tolerance, high yield potential, extended shelf life, and short cooking time. Adoption was also supported by the involvement of federal and state institutions that provided technical and financial assistance, as well as the implementation of certified seed distribution programs. Higher levels of common bean consumption in specific areas and favorable market prices also contributed to the widespread adoption of Pinto Saltillo, a variety developed by the common bean breeding program of the INIFAP in Durango.

The productive and commercial impact of Pinto Saltillo and other improved common bean varieties developed since 1960 has positively influenced domestic agricultural production in México. This impact is reflected in the increases of common bean yield and total production, which have been documented and validated by various national and international institutions. The use of the Pinto Saltillo variety contributed to the steady yield increase, particularly under irrigated conditions and favorable rainfed environments (>450 mm of rainfall). In addition, its adoption strengthened the food market through the introduction of a new commercial class known as “Pinto Saltillo.” Traders, agro-industrial processors, and consumers have also benefited from the adoption of this variety, which protects the value of the investment, reduces cooking time, and ultimately supports common bean consumption in México.

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Description of the Jipijapa palm (*Carludovica palmata* Ruiz & Pavón) agroecosystem in Santa Cruz Ex Hacienda, Calkiní, Campeche

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ABSTRACT

Objective: To describe the socioenvironmental and cultural components of the Jipijapa palm (*Carludovica palmata* Ruiz & Pavón) agroecosystem in the community of Santa Cruz Ex Hacienda, Calkiní, Campeche.

Design/methodology/approach: The sample included 10% of the farmers and artisans of Jipijapa palm (*Carludovica palmata*), selected after observing these plants in their backyards and through voluntary participation. A semi-structured questionnaire with 82 questions organized into five sections was designed and applied by means of interviews with 20 inhabitants of the community. The information obtained was analyzed through descriptive statistics using the Excel Microsoft Software.

Results: Of the interview respondents, 35% are men and 65% women, all originally from the locality and Maya speakers. The average age is 47 years, with the farmers being older than the artisans; 85% of the economic activities are developed locally, and the production of fine hats stands out. *Carludovica palmata* is the main species cultivated, but climate change, pests like moles, and the unsustainable use of shoots and leaves from the plant are affecting the crops and placing their production at risk. Results suggest that mothers are the ones that transmit knowledge about production and the skill. However, 63% of the community is not interested in continuing with these activities largely because of a tendency towards migration in the search for better opportunities.

Limitations on study/implications: The study presents limitations due to the reduced size of the sample (10% of the population), and the selection of participants based exclusively on current producers of Jipijapa plants.

Findings/conclusions: The community faces significant challenges, among them low schooling, youth migration, scarcity of raw materials due to climate change and pests, as well as dependency on intermediaries to sell their products. Although sustainable agricultural practices are implemented, it is fundamental to innovate in the productive management of the palm, to optimize agricultural techniques, and to strengthen commercial channels to guarantee the viability of production and the preservation of local productive traditions.

Keywords: Farmers, sustainability, productive systems.

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INTRODUCTION

In southeast Mexico, four species of the *Carludovica* genus have been identified, which belong to the family Cyclanthaceae: *C. chiapensis* Matuda, *C. tabascana* Matuda,



C. druidei and *C. palmata* (Haas *et al.*, 2016). From these, *C. palmata* Ruiz & Pav. is known as Jipijapa and is native to Center and South America (Bennett *et al.*, 1992; Muñoz and Tuberquia, 1999). Its morphology is composed of five main structures: petioles, adult leaves, shoots (young leaves), infructescence, and rhizomes, each with specific functions and different levels of exploitation (Haas *et al.*, 2016; Suárez-Yagual, 2018). Its capacity for adaptation to different edaphoclimatic conditions influences the quality of the fiber, making it a key source of raw materials and economic income for farmers and artisans (Cetzal-Ix *et al.*, 2018).

Historically, in Santa Cruz Ex Hacienda, Calkiní, Campeche, there was a plantation of approximately nine hectares where fruit trees were cultivated, as well as black-eyed peas (*Vigna unguiculata*), corn (*Zea mays*), squash (*Cucurbita*), henequen (*Agave fourcroydes*), and Jipijapa palm (*Carludovica palmata*), whose products were traded in nearby communities (Pinto de Estrada, 1975). Throughout the years, in addition to trading raw materials, the community began to transform Jipijapa into handicrafts, with the production of hats standing out (Medina-Vidal, 2018). However, faced with the lack of fiber, farmers opted for transplanting Jipijapa rhizomes to their backyards, establishing agroforestry agroecosystems (Muñoz-Sánchez *et al.*, 2021) of approximately two hectares (Perera-Hau *et al.*, 2025), which integrate agriculture with artisanal production (Gutiérrez-Cedillo *et al.*, 2008; Fernández and Bahena, 2012). However, asexual reproduction through rhizomes can lead to a homogeneous genetic structure, which could cause the loss of genetic variability, and consequently increases the vulnerability of this crop. In this context, it is essential to describe the Jipijapa agroecosystem in the community of Santa Cruz Ex Hacienda, to identify the problems associated with the sustainability of the species, the management of natural resources, and the preservation of artisanal practices.

MATERIALS AND METHODS

The study was conducted in the period of September to December 2024, in the community of Santa Cruz Ex Hacienda, Calkiní, Campeche, located on coordinates 90° 14' 25.869" W and 20° 23' 52.336" N and altitude of 3 masl, with average temperature of 28.1 °C and mean annual precipitation of 945 mm. The community has a total population of 1421 inhabitants, of which 703 are men and 718 women. The economically active population amounts to 510 people, of whom 322 are devoted to agricultural activities, including work with the Jipijapa palm (INEGI, 2020).

A methodology based on participatory action research was used for the study (Ander-Egg, 2003), actively integrating the members of the community (Zapata and Rondan, 2016), with the objective of fostering free participatory work. Of the total number of farmers and artisans in the locality, 10% were considered, selected systematically.

A semi-structured questionnaire made up of 82 open and closed questions was designed, organized into five sections: interview respondent's data, socioeconomic information, management of the crop and backyard, as well as cultural and environmental dimensions. The information collected was captured and organized in an Excel spreadsheet and analyzed using descriptive statistics.

RESULTS AND DISCUSSION

General information of farmers and artisans

In total, 20 people were interviewed, all originally from the community and Maya speakers; 35% were men and 65% women. The average age was 47.1 years (Figure 1), with a range between 26 and 77 years, which is comparable to the average age of Jipi artisans in Sucre and Bolívar, Colombia, which is 46 years (González-Vizcaya, 2021), and with that of agricultural corn producers in the state of Campeche, whose average age is 54 years (Uzcanga *et al.*, 2015).

The people who work with Jipijapa carry out two main activities: handcrafts and farming. In this sense, the artisans are in the range of 31 to 40 years old, while the farmers are between 61 and 80 years old. This age difference reflects the lack of generational replacement in farmers, attributed mostly to the indifference of young people, which places at risk both the continuity of the crop and the transmission of knowledge associated with its management. A similar situation is present in Barcelona and Ecuador, where only 9% of young people between the ages of 1 and 18 years participate in activities with the Jipi crop (Suárez-Yagual, 2018).

Regarding the time that they have been performing these activities, the farmers accumulate 33 years of experience, while artisans an average of 28.87 years. This result reveals that, initially, the inhabitants were devoted to the production of Jipi palm to obtain fiber, and then they made incursions into handcraft making. The community of Santa Cruz has been consolidated as the main producer of fiber in the northern region of the state of Campeche, and it is traded in the locality of Becal, Campeche (Medina-Vidal, 2018; Poot-Pool *et al.*, 2018).

When it comes to the time that they have been performing these activities, the study finds that both farmers and artisans accumulate broad experience, reflecting a sustained trajectory in the practice and transmission of knowledge related with the use of the Jipijapa palm. In this sense, one of the artisans described that the man who is his children's godfather was the first in the community to learn the art of weaving, thus becoming the first master in Santa Cruz Ex Hacienda. He taught all the men interested in learning, who

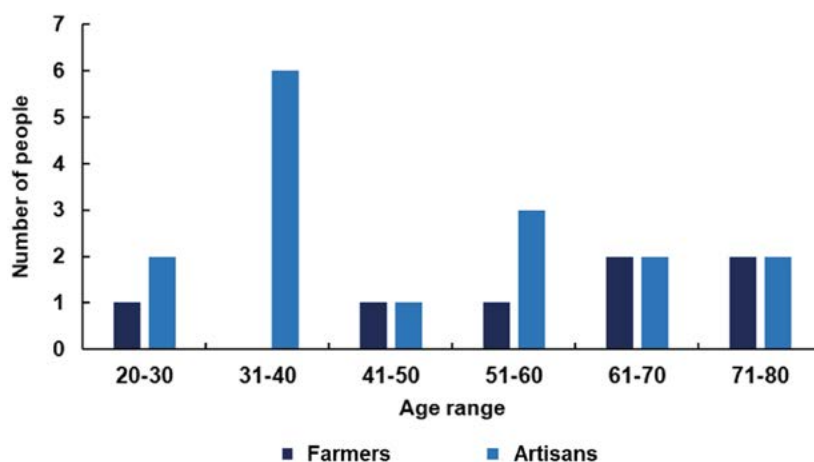


Figure 1. Ages of farmers and artisans from the community of Santa Cruz Ex Hacienda, Campeche, Mexico.

later transmitted the knowledge to their wives, and they in turn to their children, beginning the artisanal chain in the community. This type of transference of knowledge constitutes intangible heritage that strengthens collective identity and cultural continuity, similar to what happened in the community of Colosó and Huila, Colombia, where the networks of artisanal learning fulfill a central role in the preservation of ancestral knowledge (Pulecio and Cabrero, 2021).

The average level of schooling of interview respondents is 7.25 years; 25% do not have any studies, 20% finished primary school, 15% have not finished secondary education, 30% have studied high school, and 10% have finished higher education. This educational level is higher than what was reported for members of the cooperative “Loól Xa’án” with average schooling of 5.3 years (Fernández and Bahena, 2012).

Regarding the activities carried out by farmers and artisans, they were grouped into four main categories (P, A, AP and APR); 85% are carried out in the community and 15% are carried out in other municipalities in the state of Campeche and Yucatán; the latter include teachers, master bricklayers, and students. The category P includes those who are devoted solely to crop management, harvest and sale of green shoots to the artisans. Category A corresponds to people who elaborate handcrafts, acquiring raw material that is already processed or through a “handiwork” system, where the intermediary provides inputs and pays only for labor. The category AP groups those who are self-sufficient: they produce raw materials and elaborate their own handcrafts. Finally, category APR groups professionals who participate in artisanal activity as an additional source of income during vacation periods. The percentage of artisans and farmers with professional studies is reduced, and this is attributed to the fact that professionals tend to dissociate from family labor in favor of better paid jobs when they obtain a degree or migrate outside the community (Echeverría-Echeverría, 2018) (Figure 2). Likewise, when comparing with data reported by Fernández and Bahena (2012), a significant decrease in the number of farmers-artisans is observed, where 85% are classified as category AP, 8% as P, and 4% as producers-artisans-peasants.



Figure 2. Main activities in the Jipijapa agroecosystems. A) Farmers: cutting leaves and shoots, B) Farmer: reviewing the plantation, c) Artisan: selecting shoots, and D) Artisan: elaborating a hat.

Management of the Jipijapa agroecosystem

In relation to the selection of palm species within the agroecosystem, 45% of the producers indicated that they cultivate exclusively *C. palmata*, because of the preference of artisans for its quality of fiber and adaptability to the soil characteristics (Fadiman, 2001; Perera-Hau *et al.*, 2025). On the other hand, 10% mentioned that they plant both *C. palmata* and *C. drudei*, while another 10% mentioned that they cultivate three species, *C. palmata*, *C. drudei* and *Sabal mexicana*. The latter, although it increases plant diversity in the community, is not used commercially to obtain fiber. Finally, 35% of the interview respondents pointed out that they do not have any plant species that provide fiber to make handicrafts, a situation that could be attributed to diverse conditions, such as a lack of available lands for the crop, ignorance about the crop management, or disinterest for this activity.

The study identified that the agroecosystem is established by the interaction of *Carludovica* spp. with another 31 plant species distributed in 23 families (Table 1). Results show that the most abundant family in the agroecosystem is Rutaceae, followed by Moraceae and Zapotaceae. From the species recorded in this study, Pinto de Estrada (1975) mentions that the Jícara tree (*Crescentia cujete*) was the most abundant plant in the agroecosystems, followed by mango, avocado, Coyol palm, nance.

Table 1. Plant species with interaction in the Jipijapa agroecosystem.

Family	Species	Uses and contribution to the agroecosystem
Annonaceae	<i>Annona muricata</i>	Food and shade for the crop
Moraceae	<i>Artocarpus heterophyllus</i> , <i>Brosimum alicastrum</i>	Food-Shade, Fodder for livestock and shade
Malpighiaceae	<i>Byrsonima crassifolia</i>	Food-sale, shade
Meliaceae	<i>Cedrella odorata</i>	Timber-shade
Rutaceae	<i>Citrus sinensis</i> , <i>C. aurantium</i> , <i>C. latifolia</i> , <i>C. aurantifolia</i> , <i>C. paradisi</i> , <i>C. reticulata</i>	Food-sale, shade
Arecaceae	<i>Cocos nucifera</i> , <i>Acrocomia aculeata</i>	Food-sale, shade
Boraginaceae	<i>Cordia alliodora</i>	Food and timber, shade
Bignoniaceae	<i>Crescentia cujete</i>	Tools, moisture retention
Anacardiaceae	<i>Mangifera indica</i>	Food-sale, shade
Sapindaceae	<i>Melicoccus bijugatus</i>	Food-sale, shade
Musaceae	<i>Musa paradisiaca</i>	Food-sale of leaves, moisture retention
Lauraceae	<i>Persea americana</i>	Food-sale, shade
Euphorbiaceae	<i>Phyllanthus acidus</i>	Food-moisture retention
Piperaceae	<i>Piper auritum</i>	Medicinal-moisture retention
Sapotaceae	<i>Pouteria sapota</i> , <i>Chrysophyllum cainito</i>	Food-sale, shade
Rosaceae	<i>Prunus domestica</i>	Food-sale, shade
Lythraceae	<i>Punica granatum</i>	Food-moisture retention
Poaceae (Bambusoideae)	<i>Rhipidocladum pittieri</i>	Timber-sale, moisture retention
Cactaceae	<i>Selenicereus undatus</i>	Food, without contribution to the crop
Fabaceae	<i>Tamarindus indica</i>	Food, shade
Combretaceae	<i>Terminalia catappa</i>	Without added value-shade for the crop

Regarding the interaction with domestic fauna, the presence of cattle (*Bos taurus*), pigs (*Sus scrofa domesticus*), hens (*Gallus gallus domesticus*), turkeys (*Meleagris gallopavo domestica*), and ducks (*Anas platyrhynchos domesticus*) was identified. Drops from these animals are used as manure in the crops, which contributes to the recycling of nutrients inside the agroecosystem. In addition, it is recognized that backyard birds fulfill an important function in biological control, since they feed on insects considered pests (Figure 3).

Regarding the necessary soil conditions for the growth and development of the crop, 65% of the interview respondents pointed out that the species prefers black soil lands; 5% indicated that it can adapt to different types of soil, while 10% considered that its development is most favorable in red soil lands. The remaining 10% manifested not knowing which are the optimal soil conditions. This information agrees with what was reported by Jiménez *et al.* (2023), who highlight the importance of the black soil lands for productivity and vitality of the Jipijapa plantations. From an agroecological perspective, black soil lands (boox lu'um) are characterized by their high content of organic matter, good aeration, and excellent capacity for moisture retention, which provides favorable conditions for root development and nutrient absorption. These properties allow better stability in the availability of water during dry periods, and foster a more active soil microbiome, which can improve the resilience of the crop in face of environmental stressors. In this context, the preference for *C. palmata* in such soils responds not only to cultural and fiber quality factors, but also to a favorable interaction between the soil environment and the physiological demands of the plant.

Among the main problems identified, 52% of the interview respondents mentioned rotting of rhizomes and yellowing of leaves, while 13% reported the fall of petioles, and 35% manifested not knowing the causes that affect the crop. Leaf yellowing has been attributed to an unidentified fungus (Hass *et al.*, 2016), although its presence does not generate significant damage in the plantations. Another pest that negatively affects the crop is the mole (*Heterogeomys hispidus*), because they carve out tunnels in the soil and weaken the support for petioles, causing the plant to fall.

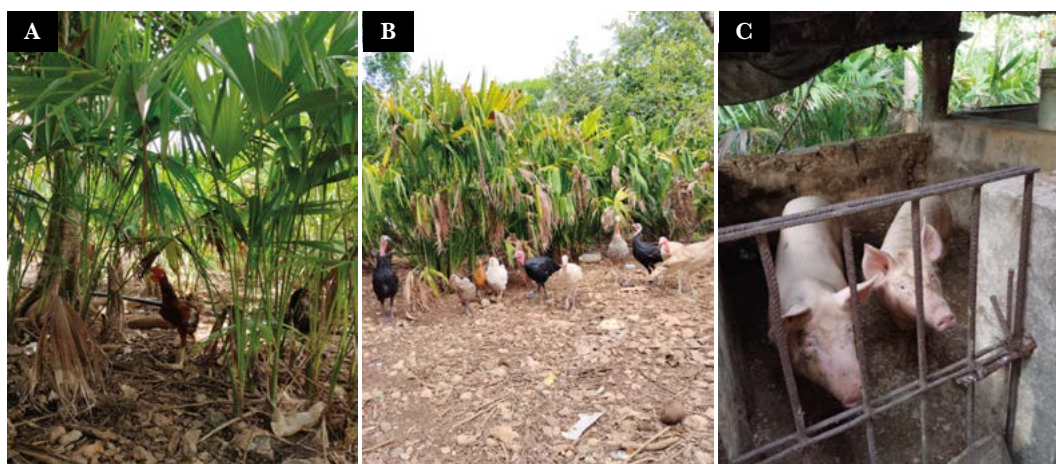


Figure 3. Animal species in the Jipijapa agroecosystem.

To minimize the loss of plants, interview respondents mentioned that it is fundamental to establish a daily irrigation regime of five hours, as well as performing periodical pruning. These two activities are considered essential for an optimal development of the crop (Suárez-Yagual, 2018). Regarding the pruning and management of plant waste, diverse variations were identified; however, most of the interview respondents agree in performing an evaluation of the state of the plants every six months. The residues from pruning are burnt and used as fertilizer, depositing the residues in high areas for them to be dragged towards the plantation through irrigation and to contribute to fertilizing the soil. Some producers decide to leave the plant residues directly around the plant, using them as mulch to conserve the soil moisture and reduce the evaporation.

Of the participants, 75% manifested that they do not use chemical products in their agroecosystems, favoring more sustainable agricultural practices, such as ecological agriculture. For their part, the remaining 25% indicated that they do use chemical products, highlighting that their use began less than two years ago, motivated by the participation in government programs from which they obtain these products.

Transference of knowledge

Regarding knowledge about the weaving techniques for handcrafts, 50% are from the mother, 30% from the father who fosters plant care, 5% is given by sisters, and in-laws (parents-in-law, brother- or sister-in law, etc.) represent 15% (Figure 4). This transmission dynamics of artisanal knowledge reflects not only a functional distribution of knowledge according to gender and family roles, but also the strength of affective links as catalysts of learning. The fact that 50% of the knowledge comes from the mother figure evidences her role as an educational and cultural nucleus in the heart of the household. At the same time, the participation of the father in 30% of the cases, centered on productive management, complements an integral model of teaching where the crop and the artisanal transformation are intertwined. The presence of sisters and in-laws as intermediaries or facilitators of generational transmission exposes an extended network of horizontal learning that is characteristic of communities which value cultural continuity beyond the immediate



Figure 4. Incorporation of young people in productive activities of the Jipijapa agroecosystem.

nucleus. In this context, the artisanal practice not only represents an economic activity, but also an expression of the economy of care, where work is carried out collectively, in spaces of coexistence and reciprocity. This interrelation between work, affection and memory allows for artisanal weaving to become, literally, a living metaphor of the community fabric.

Despite this, 63% of the interview respondents manifested not being interested in preserving the artisanal activity, 25% consider it to be unattractive, and 10% indicated that their priority is to continue studying or seeking new work opportunities outside the community. This trend reflects a symbolic and territorial process of migration, driven by the aspiration of a more individualized model of development and disconnected from local traditions (Figure 5).

In the presence of this scenario, it is essential to create conditions that encourage rootedness, articulating personal development with the strengthening of identity and cultural values. Artisanal activities, beyond their economic value, constitute expressions of collective memory, situated knowledge, and community cohesion. In this sense, working with children through workshops and courses promoted by local agencies (Campeche, 2024) represents a valuable strategy of cultural revitalization.

From a perspective of sociocultural sustainability, these efforts can be understood as practices of symbolic re-territorialization: actions that bring back meaning, belonging, and possibility to the community space in face of global homogenization. Recovering the value of tradition does not imply forcing its reproduction but rather offering tools and spaces where young people can redefine it from their own contexts and aspirations.

Commercialization of handicrafts and shoots

Traditionally, producers and artisans have taken economic advantage of shoots and rhizomes of Jipijapa as part of a system of integral exploitation (Poot-Pool *et al.*, 2018; Cetzal-Ix *et al.*, 2018). In the artisanal sector, the main income comes from the elaboration of hats; these articles have the highest demand and represent 70% of the preferences in sales. This trend contrasts with other artisanal regions from Colombia, like Colosó and

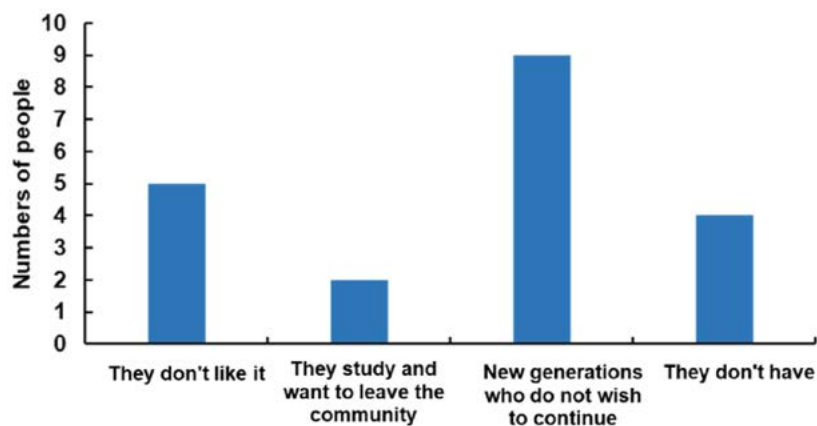


Figure 5. Reasons for loss of artisanal and agronomic work with the palm.

Sucre, where they elaborate mainly bomboneras, or localities from the Atlantic, Nariño and Caldas, which stand out from the commercialization of bags woven from Jipijapa fibers (Beltrán-Ayazo and Narváez-Santis, 2021).

In the community of Santa Cruz Ex Hacienda, commercialization of hats or shoots is carried out in 80% through local intermediaries, which reflects a strong dependency on the domestic market. These products are sold with an average income of 259.2 pesos. For their part, traders inside the community, who act as connections with external intermediaries, attain an average income of 280.5 pesos, which shows an economic gap between the artisans. The other 20% of the sales are channeled through external intermediaries that reach the community directly, acquiring the products at the same price as local re-sellers.

These commercialization models reflect the combination of local and external strategies to place the artisanal products in the market. Although economic differences between local traders are not markedly significant, important contrasts are identified in the value chain when the products reach external re-sellers, who obtain profit margins which double the incomes of original producers. This situation emphasizes the urgency of improving the distribution channels and strengthening the mechanisms of fair trade, given that one of the central problems mentioned by artisans is the deficiency in payment received for their products.

CONCLUSIONS

The study conducted in Santa Cruz Ex Hacienda confirms that agroecosystems of Jipijapa (*C. palmata*) continue to be a central activity in the community, both in the productive and in the cultural aspects. However, they face significant challenges, among them the scarcity of raw materials derived from the lack of adequate land, which affects the crop's productivity; the commercialization of handcrafts and shoots carried out through intermediaries causes a decrease in incomes, as well as a lack of motivation and social devaluation of handcrafts.

Although knowledge of palm cultivation and production has been kept in the family nucleus, ageing of producers and artisans, and migration of young people towards other economic opportunities threatens the continuity of these traditions. Therefore, it is crucial to implement strategies that foster interest in new generations for preserving this legacy and optimizing the conditions for a more sustainable production; also, investing in training, improving trade channels, and promoting the use of sustainable biotechnological and agricultural technologies that guarantee the future of artisanal and agricultural production in the region, as well as the certification or cultural denomination of origin.

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Thermal Variations and the Viability of Nile Tilapia (*Oreochromis niloticus*) Cultivation in Aquaculture Production Units in Zacatecas, Mexico

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ABSTRACT

Objective: To analyze variations in ambient and water temperature in order to evaluate their impact on the viability of tilapia farming in Zacatecas.

Methodology: Variations in maximum and minimum ambient temperatures recorded at 14 meteorological stations located near Aquaculture Production Units (APUs) in the state of Zacatecas were analyzed. Historical records were systematically processed, and simple linear regression analysis was applied. In addition, water temperature was evaluated at five APUs dedicated to tilapia production using HOBO Onset data loggers, which recorded hourly temperature values.

Results: Statistically significant increases ($p < 0.05$) in maximum temperature were identified at ten meteorological stations. In contrast, minimum temperature exhibited increases at three stations and decreases at two. With respect to water temperature, findings indicate that, overall, current thermal conditions are not optimal for sustainable production. This underscores the need to implement complementary technologies, such as greenhouse systems, to effectively mitigate the adverse effects of low temperatures.

Limitations: This study presents no major limitations.

Conclusions: The analysis of extreme ambient and water temperatures revealed a clear relationship between these variables. It was determined that Tabasco is the only Aquaculture Production Unit (APU) in which temperatures fall within the optimal range for productive development during the warm season (April -October), without requiring the implementation of additional technological support.

Keywords: Temperature, climate change, growth.

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INTRODUCTION

Tilapia (*Oreochromis niloticus*) has become one of the most important species in global aquaculture due to its remarkable adaptive capacity, rapid growth rate, and strong market demand (FAO, 2020). However, as an ectothermic organism, its optimal development



largely depends on environmental temperature, which directly regulates its internal body temperature (Boyd, 2018). This physical factor exerts a decisive influence on several physiological processes, affecting growth, locomotion, and reproduction (Volkoff & Rønnestad, 2020). Various authors have described, with some variation, the optimal temperature range for tilapia farming; this concept should be understood as the thermal interval within which organisms achieve their highest physiological and productive performance, reflected in superior growth rates, feed conversion efficiency, and survival. According to Swann (2000), this range lies between 23.9 °C and 32.2 °C. To optimize both weight gain and total biomass, conditions are particularly favorable when temperatures approach 28 °C (Kalule *et al.*, 2021). Nevertheless, other studies have proposed slightly different ranges. Importantly, the temperature to which fish have been previously acclimated influences both their preferred thermal range and the temperatures they tend to avoid when exposed to changes in water temperature (Acosta *et al.*, 2018). The capacity to tolerate extreme temperatures is determined by genetic factors and the organism's nutritional status (Oselame *et al.*, 2020). Likewise, the optimal temperature for aquatic organisms varies according to age and developmental stage (Volkoff & Rønnestad, 2020); when temperatures fall below 20 °C, metabolic activity decreases, negatively affecting feeding behavior and prolonging grow-out cycles (Hamed *et al.*, 2024). Temperatures outside the optimal range induce thermal stress in tilapia, disrupting hormonal regulation and compromising immune response, thereby increasing susceptibility to disease (Abram *et al.*, 2017; Zhou *et al.*, 2022). When temperature declines to approximately 13 °C, organisms may enter a state of paralysis, whereas values exceeding 32 °C trigger heat stress, impairing physiological homeostasis (Hassan *et al.*, 2017). These conditions adversely affect productive performance, reducing growth and yield, particularly when thermal fluctuations are intense or abrupt; conversely, gradual variations may allow partial acclimation processes (Borja *et al.*, 2006; Kovacevic *et al.*, 2019; Santillán-Espinoza, 2024).

In the state of Zacatecas, environmental conditions constitute a thermally restrictive system for tilapia farming. The high average altitude, exceeding 2,000 meters above sea level across much of the territory, promotes lower ambient temperatures than those observed in tropical regions, thereby increasing the frequency of thermal declines in water bodies. Moreover, climate change has intensified both the magnitude and frequency of extreme temperature events (Luo *et al.*, 2017); as a result, global temperature patterns have undergone significant alterations and are expected to continue fluctuating (Md Jakiul *et al.*, 2021). Within this framework, short- and long-term temperature variability underscores the necessity of understanding trends in maximum and minimum temperature fluctuations (Islam *et al.*, 2022; Nagayi *et al.*, 2022). Such analysis facilitates the identification of optimal thermal windows and critical production periods, thereby enhancing survival, growth, and overall production efficiency in tilapia farming systems. Based on this background, the research hypothesis was as follows: Ambient and water thermal variations recorded in tilapia Aquaculture Production Units (APUs) in the state of Zacatecas negatively affect the productive viability of the culture system, understood as the capacity of an aquaculture system to sustain adequate biological performance, expressed as yield under specific environmental and management conditions, thus enabling the functional continuity of the

production process. Accordingly, the aim was to analyze both ambient and water thermal variations in order to assess their effect on production viability. This approach may support informed decision-making regarding pond location, production-stage planning, and infrastructure investment for temperature management.

MATERIALS AND METHODS

Study site

The analysis of thermal profiles was conducted in Aquaculture Production Units (APUs) over the period from November 2022 to December 2024, located in the state of Zacatecas, Mexico, specifically within the municipalities of Morelos, Fresnillo, Jerez, Tabasco, and Villanueva (Figure 1). These units were selected based on their active operational status. Additionally, records from 14 official meteorological stations of the National Water Commission (Comisión Nacional del Agua, CONAGUA) (Table 1), situated in proximity to the APUs, were included in the analysis.

Acquisition and processing of meteorological data

For the analysis of ambient temperature, data were provided by the National Water Commission (Comisión Nacional del Agua, CONAGUA). The meteorological stations closest to the Aquaculture Production Units (APUs) were selected, prioritizing those with the most extensive datasets, covering historical periods ranging from 41 to 86 years. The number of observations per station varied due to differences in record length and data availability within the CONAGUA database.

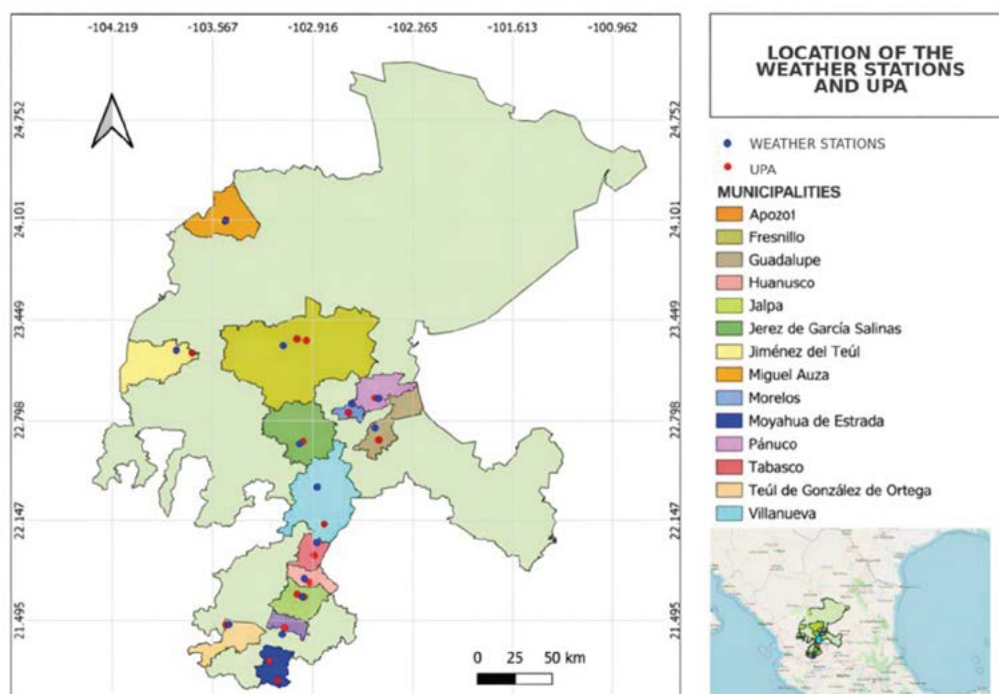


Figure 1. Location of the Aquaculture Production Units (APUs) and meteorological stations in the state of Zacatecas, Mexico.

Table 1. Spatial distribution of meteorological stations and Aquaculture Production Units (APUs) for thermal monitoring in Zacatecas, Mexico.

Station ID	Station	Latitude	Longitude	Elevation (masl)	UPA	Latitude	Longitude	Elevation (masl)
32191	Campo la Honda	24.0958	−103.4886	2191	Miguel Auza	24.1037	−103.4798	2193
32018	El Sauz	23.2817	−103.1089	2096	Fresnillo	23.3160	−102.9590	2042
32018	El Sauz	23.2817	−103.1089	2096	Fresnillo	23.3253	−103.0192	2062
32027	Jiménez del Teúl	23.2489	−103.8047	1924	Jiménez del Teúl	23.2334	−103.7003	2064
32047	San Antonio del Ciprés	22.9408	−102.4875	2173	Pánuco	22.9428	−102.5110	2259
32003	Calera	22.9086	−102.6597	2097	Morelos	22.8501	−102.6834	2203
32026	Jerez de García Salinas	22.6428	−103.0019	2000	Jerez	22.6610	−102.9780	2430
32121	Guadalupe	22.7469	−102.5067	2262	Guadalupe	22.6680	−102.4850	2292
32055	Tayahua	22.0978	−102.8719	1729	Villanueva	22.1214	−102.8414	1748
32013	El Chique	22.0003	−102.8892	1648	Tabasco	21.9170	−102.9050	1524
32025	Huanusco	21.7675	−102.9689	1490	Huanusco	21.7473	−102.9337	1439
32111	Jalpa	21.6522	−102.9814	1418	Jalpa	21.6501	−102.9668	1518
32111	Jalpa	21.6522	−102.9814	1418	Jalpa	21.6501	−102.9668	1532
32029	Juchipila	21.4047	−103.1142	1259	Apozol	21.4503	−103.1001	1302
32070	Teúl de González Ortega	21.4686	−103.4619	1909	Teúl de González Ortega	21.4667	−103.4833	1916
32125	Moyahua de Estrada	21.2589	−103.1569	1180	Moyahua de Estrada	21.6669	−103.0167	1560
32125	Moyahua de Estrada	21.2589	−103.1569	1180	Moyahua de Estrada	21.10722	−103.14295	1227

The APUs included in this study represent systems characteristic of the typical production management practices in the state of Zacatecas. No structural comparisons were conducted among units; therefore, variables such as pond type, operational volume, or depth were not considered analytical factors. The study focused exclusively on thermal variation. Some units operated under greenhouse infrastructure, whereas others functioned in open-air conditions; this distinction is described solely as part of the production context and was not incorporated as a comparative variable in the analysis. The duration of the record for each sensor was documented, along with any operational failures or data loss. The effect of water exchange on the thermal continuity of the time series was also considered. Instrument calibration checks were performed both before and after the monitoring period.

Subsequently, outliers and extreme values were removed, defined as those falling outside the range of the mean ± 1.96 times the standard deviation, approximating the 95% confidence interval. Once the dataset was refined, the intercept (β_0) and slope (β_1) were estimated using simple linear regression analysis in Excel (Microsoft). A significance level of $p < 0.05$ was established for the slope coefficient β_1 . The model applied was:

$$y = \beta_0 + \beta_1 X + \varepsilon$$

where: y corresponds to temperature (dependent variable); x to time in months (independent variable), β_0 to the intercept, β_1 to the slope reflecting the average change in temperature resulting from a unit variation in time and ε represents the error term.

The null hypothesis, evaluated at a significance level of $p < 0.05$, postulated a slope equal to zero ($H_0 : \beta_1 = 0$). Annual slopes, whether positive or negative, were transformed to a decadal scale ($\beta_1 \times 10$) to enable comparison among time series of differing lengths; this procedure was described by Santillán-Espinoza (2011).

Water temperature was recorded using HOBO Onset devices (Pendant Temp/Light UA-002-64). The equipment was synchronized prior to installation and configured to record at hourly intervals.

Sensors were positioned at an approximate depth of 1 m within the pond. The recording duration was not uniform across all devices, as installation and operation were conducted in collaboration with the aquaculture producers. This resulted in variability in monitoring periods and partial data loss associated with routine system management practices, including pond water exchange.

Development of thermal profiles

Scatter plots were generated for the Aquaculture Production Units (APUs) using Excel (Microsoft), in which mean monthly water temperature, as well as mean monthly minimum and maximum ambient temperatures, were represented. A reference line corresponding to the optimal temperature interval for tilapia farming (23.9-32.2 °C) was incorporated.

Given that the monitoring period differed among units and that each site presented specific technical failures (periods without records), the total number of valid observations was not consistent across cases. Mean monthly water temperature was calculated as the arithmetic average of valid hourly records obtained during each month, whereas environmental variables were aggregated to the same temporal scale to ensure comparability. The optimal thermal interval was uniformly applied to all APUs as a physiological reference based on the species' thermal requirements, according to Swann (2000), regardless of variations in management practices or local environmental conditions. No statistical tests were performed to evaluate differences among months, as the analysis was descriptive and oriented toward the visualization of thermal patterns. Likewise, thermal profiles for all APUs are not presented in order to avoid redundancy.

Ambient-water thermal range in relation to the optimal interval for tilapia farming

A comparative assessment of the thermal variation range in the APUs was conducted, considering the annual amplitude of both ambient and water temperatures in relation to the optimal interval required by the species.

RESULTS AND DISCUSSION

Trend analysis of minimum temperature

Of the fourteen stations evaluated, Jerez de García Salinas, El Chique, and Jalpa exhibited statistically significant positive slopes ($p < 0.05$) (Table 2). Conversely, San

Table 2. Results of the linear regression analysis of minimum temperature by station.

Minimum temperature trends					
ID	Station	β_0	β_1	P	Trend (°C decade ⁻¹)
32191	Campo la Honda	-203.7500	0.1018	0.0921	1.018
32018	El Sauz	-8.6349	0.0054	0.4477	0.054
32027	Jiménez del Teúl	24.7640	-0.0109	0.3663	-0.109
32047	San Antonio del Ciprés	232.3200	-0.1145	2.6747E-13	-1.145
32003	Calera	-19.4170	0.0110	0.3192	0.110
32121	Guadalupe	7.6521	-0.0012	0.9436	-0.012
32026	Jerez de García Salinas	-169.5100	0.0872	0.0002	0.872
32055	Tayahua	-37.8040	0.0220	0.0742	0.220
32013	El Chique	-48.8070	0.0290	0.0008	0.290
32025	Huanusco	102.9500	-0.0486	0.0017	-0.486
32111	Jalpa	-68.1560	0.0370	0.0349	0.370
32029	Juchipila	-0.5174	0.0040	0.7690	0.040
32070	Teúl de González Ortega	15.4820	-0.0051	0.6199	-0.051
32125	Moyahua de Estrada	79.9410	-0.0373	0.1513	-0.373

Antonio del Ciprés and Huanusco showed statistically significant negative slopes ($p < 0.05$).

Análisis de la tendencia de la temperatura máxima

Las estaciones Campo la Honda, Jiménez del Teúl, Guadalupe, Jerez de García Salinas, Tayahua, El Chique, Huanusco, Jalpa, Teúl de González Ortega y Moyahua de Estrada registraron pendientes positivas con significancia estadística ($p < 0.05$) (Cuadro 3). El Sauz presentó una pendiente negativa estadísticamente significativa ($p < 0.05$).

Analysis of the thermal profile of the APUs

The thermal profiles of the Aquaculture Production Units (APUs) revealed the variation patterns of maximum and minimum water temperatures in conjunction with the corresponding mean maximum and minimum ambient temperatures. The temperature interval considered optimal for tilapia farming (23.9-32.2 °C) was also delineated.

Morelos production unit

As shown in Figure 2, the mean maximum water temperature reached 26.6 °C, whereas the mean minimum was 16.7 °C, the latter falling below the optimal interval for cultivation. It is important to note that this unit operates under greenhouse infrastructure. Regarding ambient maximum and minimum temperatures, the trend remained stable, as no statistically significant changes were detected.

Table 3. Results of the linear regression analysis of maximum temperature by season.

Maximum temperature trends					
ID	Station	β_0	β_1	P	Trend (°C decade ⁻¹)
32191	Campo la Honda	-191.3800	0.1088	0.0011	1.088
32018	El Sauz	47.7640	-0.0092	0.0244	-0.092
32027	Jiménez del Teúl	-49.2180	0.0398	4.5039E-09	0.398
32047	San Antonio del Ciprés	46.1920	-0.0087	0.2900	-0.087
32003	Calera	41.6500	-0.0069	0.1932	-0.069
32121	Guadalupe	-52.7120	0.0405	0.0002	0.405
32026	Jerez de García Salinas	-84.2540	0.0573	1.5781E-05	0.573
32055	Tayahua	-41.7320	0.0375	1.557E-06	0.375
32013	El Chique	-48.6840	0.0412	4.8739E-11	0.412
32025	Huanusco	-137.0100	0.0856	1.5343E-15	0.856
32111	Jalpa	-152.2200	0.0933	2.3796E-16	0.933
32029	Juchipila	12.2390	0.0113	0.1666	0.113
32070	Teúl de González Ortega	-71.6210	0.0513	2.3733E-14	0.513
32125	Moyahua de Estrada	-17.8680	0.0264	0.0422	0.264

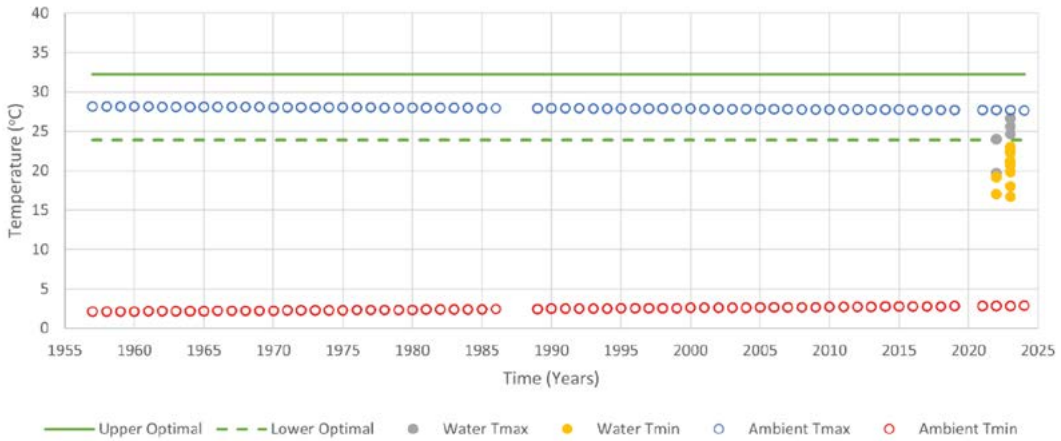


Figure 2. Historical ambient and water thermal profile in the Morelos Unit, with reference to the critical temperature range for tilapia farming.

Tabasco production unit

This unit exhibits relatively favorable conditions for cultivation, as mean maximum water temperatures reached 26.3 °C and mean minimum temperatures 19.9 °C. Furthermore, ambient temperatures showed an increasing trend, with a rise of 0.412 °C per decade in maximum temperature and 0.290 °C per decade in minimum temperature (Figure 3).

It is noteworthy that this unit operates under open-air conditions and benefits from access to a natural thermal water source characteristic of the region, which is strategically utilized to regulate pond temperature. This technological and environmental attribute

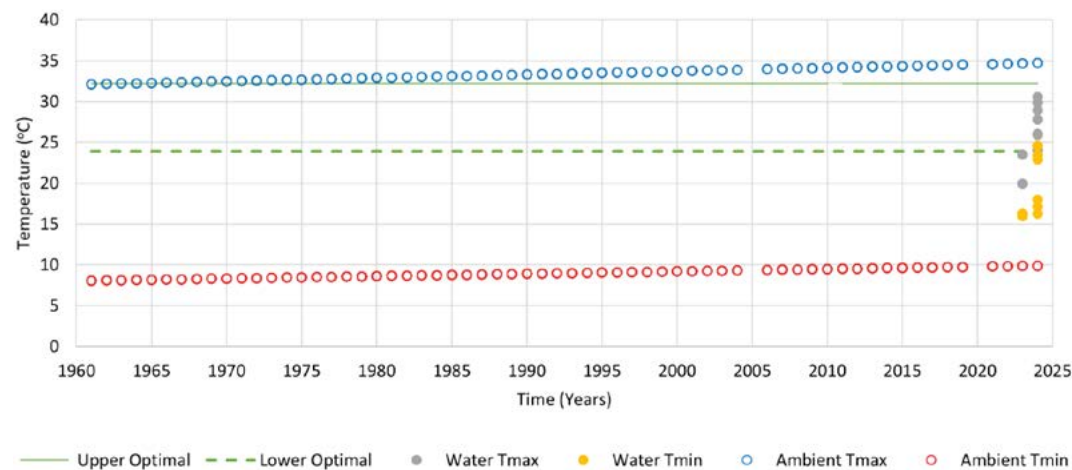


Figure 3. Historical ambient and water thermal profile in the Tabasco Unit, with reference to the critical temperature range for tilapia farming.

constitutes a competitive advantage over other production units located in areas with greater thermal risk.

Hourly temperature variation in the Morelos unit

As shown in Figure 4, water temperature remained within the optimal range for an average of 9.3 hours per day, with declines below this threshold occurring during the colder months.

Hourly temperature variation in the Tabasco Unit

The temperature record in the Tabasco Unit (Figure 5) indicated that, from November to March, water temperatures remained below the optimal range for cultivation. In

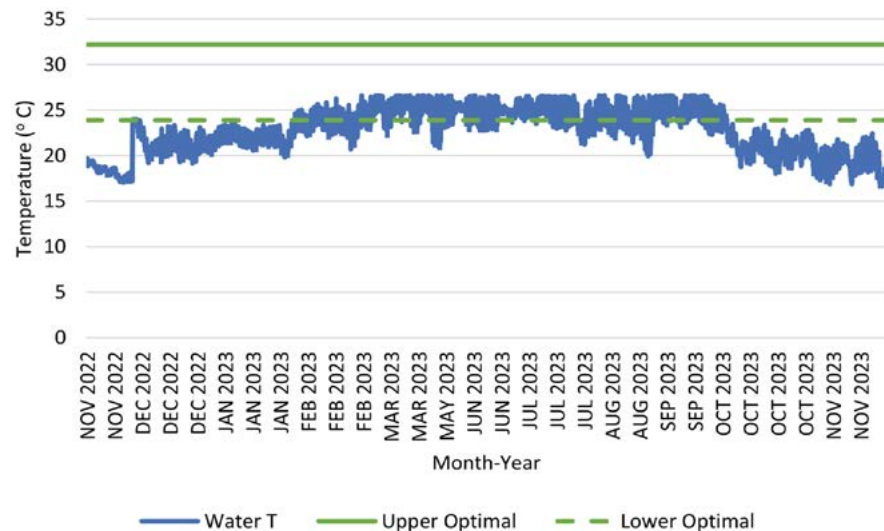


Figure 4. Water thermal profile in the Morelos Unit, with reference to the critical temperature range for tilapia farming.

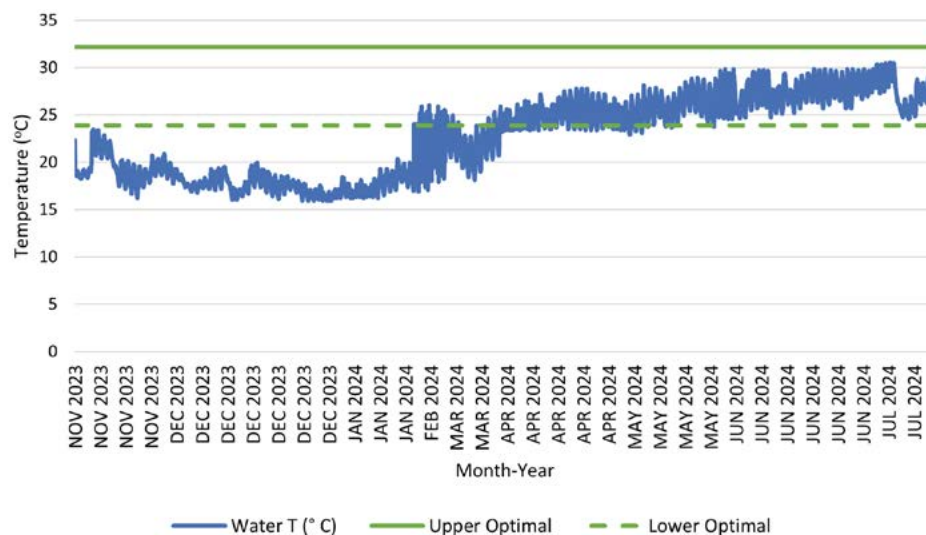


Figure 5. Water thermal profile in the Tabasco Unit, with reference to the critical temperature range for tilapia farming.

April, favorable thermal conditions were reached, with temperatures remaining within the optimal interval for an average of 22.3 hours per day, thereby enabling productive development without the need to implement supplementary technologies. Additionally, suitable conditions for cultivation were observed for approximately seven months of the year.

Ambient-water thermal range in relation to the optimal interval for tilapia

The assessment of thermal amplitude revealed marked variability among the APUs analyzed (Table 4). Ambient temperature amplitudes ranged from 23.2 °C to 29.5 °C, with the greatest amplitude recorded in Moyahua de Estrada. Regarding water temperature, amplitudes were comparatively lower, with values ranging from 4.2 °C to 9.9 °C. On average, water temperature across the APUs with available data was 21.8 °C.

The analysis of data obtained from meteorological stations located near the Aquaculture Production Units (APUs) enabled the identification of thermal variations within the study region. This information is essential for evaluating the viability of tilapia farming, as temperature constitutes a decisive factor in the sustainability and productivity of this activity. Of the 14 meteorological stations analyzed, 12 exhibited thermal trends, reflecting diverse scenarios. Maximum temperature increased at 10 stations and decreased at one, whereas minimum temperature increased at three stations and decreased at two.

This trend was observed in municipalities such as Miguel Auza, Jiménez del Teul, Guadalupe, Villanueva, Teul de González Ortega, and Moyahua de Estrada. With the exception of Moyahua de Estrada, this pattern resulted in an increase in thermal amplitude. According to Dos Santos *et al.* (2022), such conditions may constrain tilapia growth and weaken immune function. Despite the overall warming trend, water temperatures recorded in the Jerez Unit remain below the optimal values required for adequate tilapia growth,

Table 4. Variations in extreme temperatures.

APU	Ambient temperature (°C)			Water temperature (°C)				
	Tmax-Tmin	Mean	Amplitude	Tmin -Tmax (Optimal Range)	Optimal Mean	Tmax-Tmin	Mean	Amplitude
Miguel Auza	27.5-1.1	14.3	26.4	23.9-32.2	28.1			
Fresnillo	29.2-2.1	15.7	27.1	23.9-32.2	28.1	23.1-18.9	21.0	4.2
Jiménez del Teúl	30.1-3.1	16.6	27.0	23.9-32.2	28.1			
Pánuco	28.9-3.8	16.4	25.1	23.9-32.2	28.1			
Morelos	27.7-2.5	15.1	25.2	23.9-32.2	28.1	26.6-16.7	21.7	9.9
Guadalupe	28.4-5.2	16.8	23.2	23.9-32.2	28.1			
Jerez	30.6-5.4	18.0	25.2	23.9-32.2	28.1	24.7-18.7	21.7	6.0
Villanueva	33.2-6.2	19.7	23.7	23.9-32.2	28.1	23.7-19.2	21.5	4.5
Tabasco	33.2-9.1	21.2	24.2	23.9-32.2	28.1	26.3-19.9	23.1	6.4
Huanusco	33.8-6.0	19.9	27.8	23.9-32.2	28.1			
Jalpa	34.5-5.8	20.2	28.7	23.9-32.2	28.1			
Apozol	34.8-7.5	21.2	27.3	23.9-32.2	28.1			
Teúl de González Ortega	30.7-5.4	18.1	25.3	23.9-32.2	28.1			
Moyahua de Estrada	34.9-5.4	20.2	29.5	23.9-32.2	28.1			
Average	31.3-4.8	18.1	26.5	23.9-32.2	28.1	24.9-18.7	21.8	6.2

thereby limiting the potential benefits of this thermal increase. In contrast, the scenario in Tabasco is favorable during the warm season, as temperatures recorded during this period optimize productive development, as indicated by Abdou (2023). Stations with stable temperatures and no significant annual variation were also identified, as in the case of Calera.

Additionally, certain locations exhibited a decrease in minimum temperature while maximum temperature remained constant, such as San Antonio del Ciprés. In other cases, such as El Sauz, a decline in maximum temperature was recorded. According to Md Jakiul *et al.* (2021), Ahmed *et al.* (2023), and Khallaf *et al.* (2021), these scenarios pose a substantial risk to cultivation, as minimum temperatures below 0 °C are recorded during the coldest season of the year. After organizing temperature data according to geographic location from north to south, two distinct thermal trends were identified within the study area. The first group-Campo la Honda, El Sauz, Jiménez del Teul, San Antonio del Ciprés, Calera, Guadalupe, and Jerez de García Salinas-registered maximum temperatures below the upper optimal threshold.

This indicates that even during the warmest months, these regions present a high risk for tilapia production due to insufficient thermal conditions. It is noteworthy that the Calera station, although part of this group, did not exhibit a significant trend, indicating thermal stability; however, ambient conditions do not contribute to improving the situation in the Morelos Unit, as minimum temperatures remain below 0 °C.

In contrast, the second group Tayahua, El Chique, Huanusco, Jalpa, Juchipila, and Moyahua de Estrada showed average maximum temperatures of 33.7 °C and minimum

temperatures of 6.4 °C. Notably, the Tabasco station recorded maximum temperatures of 34.7 °C and minimum temperatures of 9.8 °C. Although Juchipila did not present a significant trend, its maximum temperatures exceed the upper optimal threshold, and minimum temperatures remain near 7.5 °C; under specific conditions, this scenario may be favorable for cultivation during the warm season. In the southern region of the state, municipalities such as Villanueva, Tabasco, Huanusco, Jalpa, Apozol, Moyahua de Estrada, and Teúl de González Ortega except for the latter exhibit a thermal pattern distinct from the rest of the state, characterized by warmer climates. According to the present analysis, mean annual ambient temperatures range approximately between 20 and 22 °C, consistent with reports by the National Institute of Statistics and Geography (INEGI, 2021). A notable case within the region is the municipality of Teúl de González Ortega, which contrasts with the general thermal pattern. Owing to its higher altitude, this area exhibits a lower mean temperature (approximately 18 °C), resulting in comparatively colder environmental conditions. This atypical thermal behavior implies that, although the municipality is located within a predominantly warm subhumid region adjacent to municipalities such as Juchipila and Moyahua de Estrada, its actual thermal conditions may limit tilapia development, as water temperatures seldom reach the optimal growth range. This local variability underscores the importance of conducting site-specific thermal assessments prior to establishing aquaculture production units. Following the analysis of thermal profiles, results from two representative APUs were presented to illustrate the characteristic patterns identified: Morelos, belonging to the first group where ambient maximum temperatures remain below the upper optimal threshold, and Tabasco, representing the second group where maximum temperatures exceed this threshold.

Observed trends indicate a direct relationship between ambient and water temperatures, suggesting that meteorological records may serve as a valuable tool for predicting thermal behavior in culture ponds. These findings are consistent with Tamara *et al.* (2024), who also reported a strong correlation between air and water temperature. Overall, the average maximum water temperature across the APUs was 24.9 °C, and the average minimum was 18.7 °C, resulting in a general mean of 21.8 °C. Tabasco recorded the highest mean water temperature (23.1 °C) compared to Fresnillo, Morelos, Jerez, and Villanueva, demonstrating more favorable environmental characteristics for cultivation during the warm season. In contrast, temperatures in the remainder of the state fall below the optimal range, thereby limiting growth performance, as suggested by Ahmed *et al.* (2023). Regarding thermal amplitude, the Tabasco Unit exhibited lower amplitude, reflecting greater thermal stability and consequently more stable conditions for cultivation. Conversely, the Morelos Unit displayed greater amplitude, indicating more pronounced fluctuations between minimum and maximum temperatures-factors that directly influence metabolism and growth.

CONCLUSIONS

Differences were identified between ambient and water temperatures, with the latter exhibiting less pronounced oscillations. Although water temperature partially buffers thermal fluctuations, this mitigation is insufficient to ensure optimal tilapia performance. The identification of zones with temperatures closer to the optimal cultivation range

facilitates the detection of areas requiring management adjustments under extreme thermal conditions. This approach supports the geographic prioritization of investments, the improvement of management practices, and the promotion of positive changes in production systems. The analysis of the production system's thermal profile revealed that, in general, temperatures are unsuitable for profitable tilapia development in most APUs. The exception is Tabasco, where optimal temperatures are reached during the warm season (April-October) without the need for technological intervention. These findings suggest substantial potential for the regionalization of Nile tilapia (*O. niloticus*) cultivation in the southern part of the state, particularly in the APUs located in Tabasco, Huanusco, Jalpa, Apozol, Juchipila, and Moyahua de Estrada.

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Estimation of the water table based on NDVI and NDWI Landsat, in the South Citrus Aquifer: a case study

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ABSTRACT

Objective: to assess the spatiotemporal changes of the vegetation of the Southern Citrus Aquifer and their possible association with the depth of the water table, through the analysis of the vegetation cover and the piezometric level with geomatics on Landsat images and the NDVI and NDWI spectral indices.

Design/Methodology/Approach: groundwater depth level data were collected from 63 monitoring wells distributed in the Southern Citrus Aquifer (DR-1914). And representative Landsat images corresponding to the years 2003, 2013 and 2022 (Landsat TM for 2003 and 2013, and Landsat OLI for 2022) were processed and analyzed with geomatics. The spatial distribution of the water table for each year was estimated by means of geostatistical interpolation techniques and the information was processed with QGIS[®] 3.28 and Google Earth Engine[®].

Results: our results showed a general trend of increase in NDVI values between 2003 and 2013; which changed towards signs of water stress in 2022. This means that there are changes in vegetation cover and vigor in relation to water availability and local anthropogenic pressure. The depth of the water table in 2022 differs significantly ($p \leq 0.001$) from the same value in 2003. This supports the existence of a progressive aquifer depletion process that spectral indices can detect, to assess the underground dynamics of the plant systems associated with the aquifer.

Limitations/Implications of the study: discontinuity was identified in the piezometric records, due to the absence of measurements in some years. This limitation was mitigated by interpolation estimation of average values from earlier and later records.

Findings/Conclusions: NDVI and NDWI indices proved to be effective tools for analyzing the relationship between vegetation dynamics, soil moisture availability, and water table depth. Remote sensing offers a methodological alternative for monitoring the hydrological and environmental dynamics of the aquifer. This methodology is efficient, spatially continuous, and temporally consistent.

Keywords: water table, Landsat, spatial distribution, vegetation health, geomatics.

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INTRODUCTION

The Southern Citrus Aquifer is a groundwater system that plays a vital role in supplying the agricultural, social, and ecological sectors, according to Mexico's Secretariat of Environment, and Natural Resources (SEMARNAT, 2020). This aquifer, identified as DR-1914, with an area of 4172 km², sustain agricultural cover (>20%), forest and

secondary vegetation (19%), shrubland (18%) and thorny vegetation [*Prosopis* as dominant] (1%), supplied by underground flows according to Mexico's National Commission for the Knowledge and Use of Biodiversity (CONABIO, 2021). It is recharged from the Sierra Madre Oriental with 75.1 hm³ per year; extractions for agriculture, urban and industrial use are the main discharges according to Mexico's National Water Commission (CONAGUA, 2024). Climate change has reduced the average annual recharge by 10-15% in two decades, which has caused an imbalance that has caused the water table to drop from 1.5 to 2 m per year (30 m accumulated since 1980). This has affected dependent ecosystems according to Mexico's National Institute of Ecology and Climate Change (CONABIO, 2018; INECC, 2019).

In Linares and Hualahuises (Nuevo Leon, Mexico) irrigated agriculture covers 14 650 ha and demands 96.8% of the water in the aquifer according to data from the Secretariat of Agriculture (SAGARPA, 2019). The study of vegetation by means of remote sensing allows the aquifer condition to be evaluated through spectral indices. The reflectance of healthy vegetation is characterized by low reflectance in the visible spectrum, and high in the near-infrared (NIR). Changes in this pattern indicate stress or damage (Chuvieco, 2002; Fiallos & Alexander, 2024). The NDVI (Normalized Difference Vegetation Index) is a key indicator that estimates plant vigor and water stress associated with the depth of the water table. Whereas the NDWI (Normalized Water Difference Index) estimates the moisture content in the vegetation cover from green and near-infrared (NIR) reflectance, which allows identifying variations in the humidity of the vegetation.

The objective of this study was to measure the spatiotemporal changes of the vegetation of the Southern Citrus Aquifer (DR-1914) and its possible association with the depth of the water table, by analyzing the vegetation cover and the piezometric level with geomatics based on Landsat images and the NDVI and NDWI spectral indices.

MATERIALS AND METHODS

The Southern Citrus Aquifer (DR-1914) belongs to the hydrological-administrative region of the Rio Grande Basin Organization. It is located in the southeastern portion of the state of Nuevo León (Mexico) and includes the municipality of Hualahuises, most area of the municipality of Linares and smaller proportions of the municipalities of Galeana, Montemorelos and Iturbide, plus a few areas of Rayones and General Terán (Figure 1). The area has a variability of BSoh(x') semi-warm arid climates, (A) C(w1) semi-warm sub-humid, (A)C(wo) semi-warm sub-humid, C(wo)x^f temperate sub-humid, and BS1(H') W warm semi-arid. Temperatures range from 18 °C to 22 °C, with rainfall distributed throughout the year and winter rainfall percentages of 5% to 10.2% according to the modified Köppen climate classification (García, 2004).

Depth of the water table

Information available on the depth of the water table of 63 measuring wells, distributed over the Southern Citrus Aquifer, with records in 2003, 2013 and 2022 was documented. In each of these years, an average of the total available records was obtained. Then, for

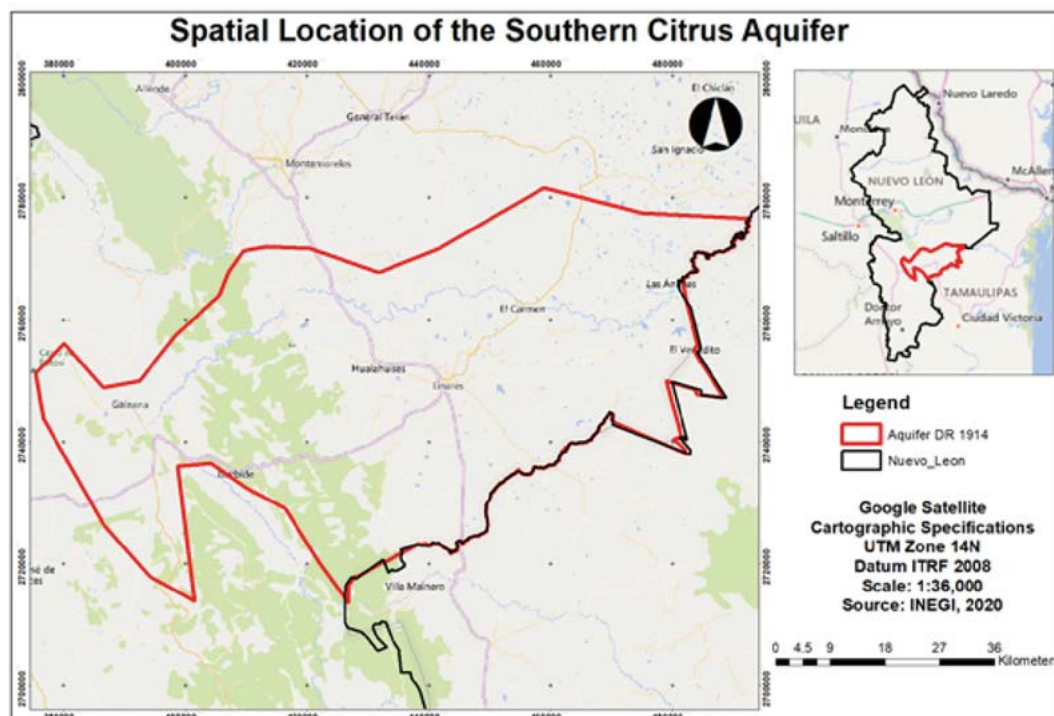


Figure 1. Location of the study area: the Southern Citrus Aquifer [DR-1914] in Nuevo León, Mexico.

each year, the spatial distribution of the depth of the wells was estimated by geostatistical interpolation in a 100×100 m mesh for a global variogram model, an exponential model, and kriging with the use of QGIS[®] 3.28 (QGIS[®] Development Team, 2023). Following this methodology, the values of the wells without data were estimated by spatial and temporal interpolation, and the trends were determined.

Afterward, statistical tests were performed to compare the periods with the Shapiro-Wilk normality test to verify compliance with the normality assumption. Then, the non-parametric Mann-Whitney test was performed to compare the distributions of water tables between different years, without assuming normality.

Remote sensing

Three Landsat Thematic Mapper satellite images dated November 22, 2003, May 11, 2013, and June 3, 2022, were analyzed, which were obtained from the Google Earth Engine[®] server. The information processing was done in QGIS[®] 3.28 and Google Earth Engine[®] to generate the scripts. The images were geometrically corrected with the methodology (GCPs) called the methodology of terrestrial control points and as a backup tool, we used the network of topographic map paths, scale 1:50000, of Mexico's National Institute of Statistics and Geography (INEGI, 2022).

In order to determine the water status of the vegetation, a spectral indicator was used throughout the area of interest. This indicator is widely used to assess plant health and vegetation density from spectral images. The Normalized Difference Vegetation Index (NDVI) is an index that identifies abnormal changes in plants and distinguishes vegetation

from other types of land cover (Rouse *et al.*, 1974). In the case of Landsat imagery, it is calculated according to the following equation:

$$NDVI = \frac{NIR - RED}{NIR + RED}$$

where, *NIR*: the reflectivities calculated from the near-infrared bands; *RED*: the spectral reflectance in the red.

To extract points in the satellite image, a systematic non-aligned sampling was done, to interpolate the data of depths of the piezometric level. The number of sampling points (*n*) was determined with the following equation:

$$n = \frac{z^2 \sigma^2}{L^2 + \frac{z^2 \sigma^2}{N}}$$

where, z^2 (95%): the abscissa of the normal curve for a given value of probability; σ^2 : the sample variance; *N*: is the size of the population; *L*: the margin of error allowed ($\alpha=5\%$).

The following equation was used to calculate the Normalized Water Difference Index:

$$NDWI = \frac{VIR - NIR}{VIR + NIR}$$

where, *NIR*: the spectral reflectance of the near-infrared; *VIR*: the spectral reflectance in the green.

RESULTS AND DISCUSSION

The statistical analysis of the depth of the water table of the Southern Citrus Aquifer allowed obtaining a record for each well in the years 2003, 2013 and 2022. The Shapiro-Wilk test showed that NDVI in 2003-2013 had a normal distribution ($W=0.9726$; $p=0.277 > \alpha=0.05$), while NDVI in 2003-2022 did not meet the normal assumption ($W=0.9357$; $p=0.00096 < \alpha=0.05$). Similarly, the NDWI in 2003-2013 maintained a normal distribution ($W=0.9823$; $p=0.3862 > \alpha=0.05$), in contrast to the NDWI in 2003-2022 which showed absence of normality ($W=0.9418$; $p=0.00196 < \alpha=0.05$). These values are evidence of greater variability in the periods of long-term analysis.

Considering the aforementioned results, the Mann-Whitney test was performed, which allowed comparing the distributions of the water tables between different years without assuming normality, to guarantee the robustness and statistical validity of the results obtained. The results showed that for the NDVI there were no statistical differences between 2003 and 2013 ($U=2324.0$; $p=0.0981$), while there were significant differences between

2003 and 2022 ($U=3285.0$; $p=2.25 \times 10^{-10}$); and between 2013 and 2022 ($U=2844.0$; $p=2.77 \times 10^{-5}$). In the case of the NDWI, the comparison between 2003 and 2013 did not show significant differences ($U=1606.0$; $p=0.0651$); while significant differences were identified between 2003 and 2022 ($U=849.0$; $p=3.06 \times 10^{-8}$), as well as between 2013 and 2022 ($U=1337.0$; $p=0.00159$).

The Mann-Whitney test provided evidence of significant changes in the distribution of water table depths (Figure 2). In 2003, the median was lower, and the distribution showed relatively shallow water tables, with less dispersion and few extreme values, indicating more homogeneous conditions. On the other hand, in 2013, a shift in the median towards greater depths and an increase in dispersion were observed, which suggests a general decrease in the water table and greater spatial heterogeneity. In 2022, this pattern intensified, the median reached the deepest values, variability increased, and more extreme outliers appeared, indicating areas with significant depletion of the water level.

In 2003, the NDVI showed high spatial variability, indicating patches of plant recovery, while the NDWI presented negative values associated with low surface-moisture. The NDVI-NDWI difference (Figure 3A) evidences the clear contrasts between areas with active vegetation and dry conditions. In 2013, NDVI remained stable or slightly lower than in 2003. The NDWI retained negative values with lower spatial dispersion, which suggests water homogenization. The NDVI-NDWI relation (Figure 3B) accentuates the differentiation between wells with different levels of plant vigor. On the other hand, in 2022 the NDVI recorded a generalized decrease and the NDWI reached more negative values, which reflects a more intense water deficit. The NDVI-NDWI (Figure 3C) amplifies the contrast and shows pronounced water stress in vegetation.

The comparisons (2003, 2013 and 2022) between the NDVI values per well showed temporal changes in plant vigor and its spatial variability. In 2003, NDVI values were concentrated in low to moderate ranges, with several wells close to zero or even negative.

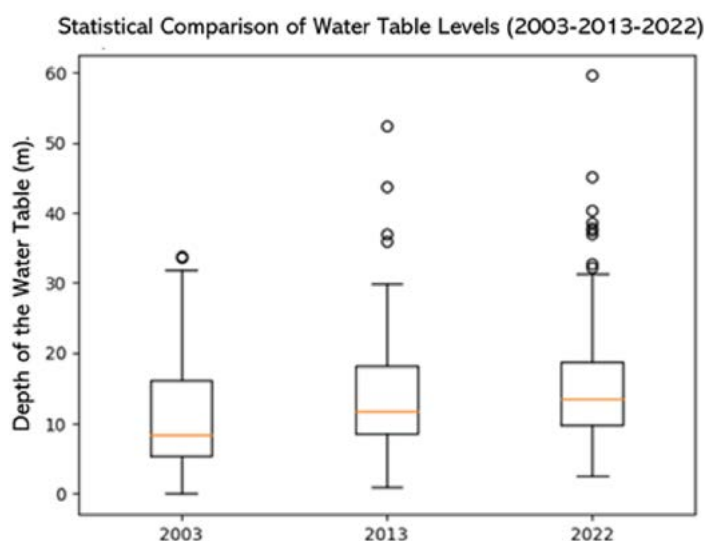


Figure 2. Distribution of the depth of the water table in the Southern Citrus Aquifer [DR-1914] in 2003, 2013 and 2022.

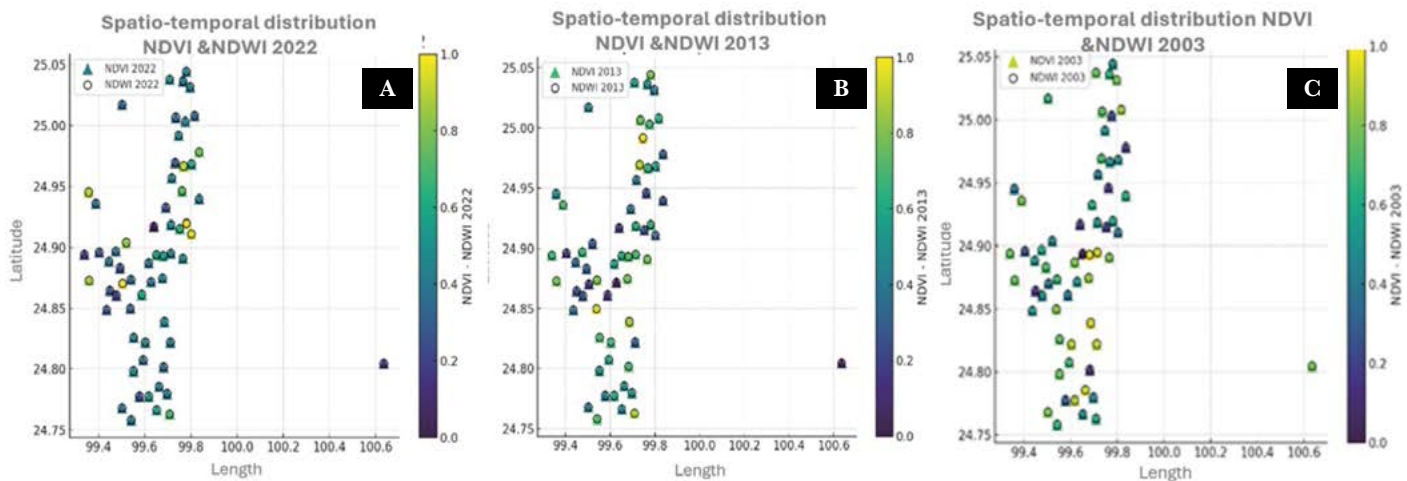


Figure 3. Spatio temporal evolution of vegetation and water table in the Southern Citrus Aquifer, through the NDVI-NDWI relation; 3A: 2003, 3B: 2013, 3C: 2022.

This indicates limited vegetation cover, consistent with water-stressed conditions. While in 2013 there was a general increase in NDVI in most wells, with predominantly positive values and less presence of negative extremes.

This suggests a better average vegetation condition and a more homogeneous system response, possibly associated with favorable climatic conditions. In 2022, although some wells reached higher maximum NDVI values than in previous years, spatial dispersion increased again, with coexistence of wells with high plant vigor and others with low or decreasing values. This pattern indicates a differential response of vegetation, compatible with localized water stress.

The correlation between the water table and the NDVI (Figure 4) shows a significant trend towards the margins of the Sierra Madre Oriental, where some wells showed an increase in their static levels. Vegetation, such as submontane scrub and the transition of pine and pine-oak forest areas, showed high NDVI values. This occurs because the Sierra acts as a hydrological regulator, and sustains high levels of plant vigor. To the north and south, NDVI values are medium to low, where vegetation cover is discontinuous and plant vigor is conditioned by water availability. Towards the northeast, the ranges are medium to high, due to agriculture and rural settlements, which generates less vigorous vegetation that is evidence of dry and anthropic conditions.

In an Eastern direction, low values are observed due to the fragmentation of the vegetation cover. In the center of the aquifer there are medium levels (0.2-0.05), where vegetation is moderate due to agricultural management practices and groundwater availability; in the Western portion, the highest values (>0.5) predominate, associated with mountainous topographic reliefs that reflect denser vegetation cover and continue to be favored by greater moisture uptake.

In the evaluation period (2003-2022), a decreasing relationship between NDVI and piezometric level variations was identified. In 2003, the trend showed a stable development between vegetation vigor and the availability of water tables (Figure 5). By 2013, an irregular

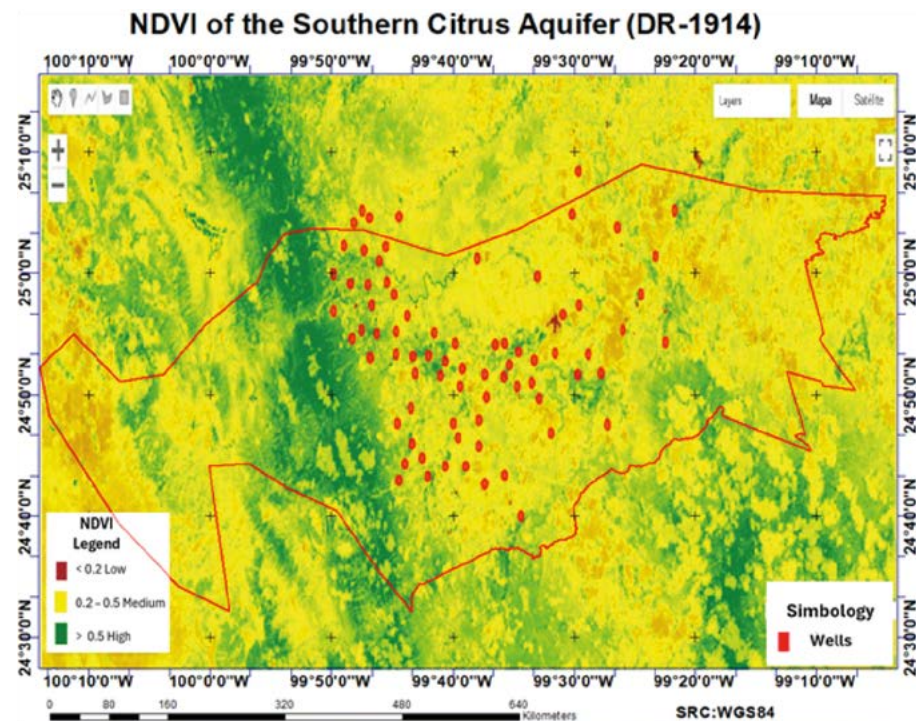


Figure 4. Correlation of NDVI with the water table in the Southern Citrus Aquifer [DR-1914].

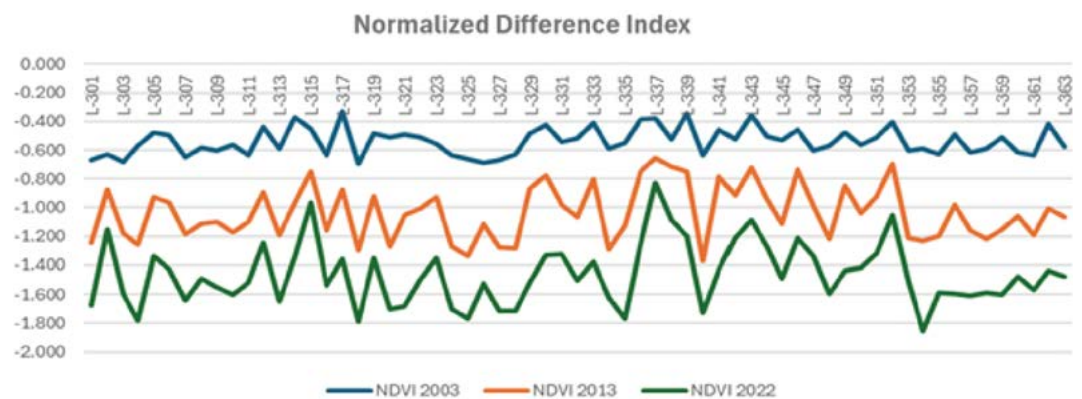


Figure 5. Relationship of depth records of the piezometric level in the South Citrus Aquifer [DR-1914] with the NDVI index.

behavior was observed, where the NDVI was maintained despite greater abatements. And in 2022, the most severe piezometric declines coexisted with high NDVI dispersion, indicating advanced groundwater stress and a clear loss of geological control over the water status of vegetation.

In the NDWI analysis, a consistent temporal evolution towards the reduction of surface water content in the sampled wells was observed. In 2003, the values reflected a relatively stable condition, with moderate variations and a balanced response between wells. By 2013, greater variability and a shift towards lower values were observed, indicating a perceptible decrease in humidity and a trend towards hydrological imbalance of ecosystems. And in

2022, the lowest and most dispersed values of NDWI predominated, which evidenced a generalized loss of surface moisture and confirmed the occurrence of advanced water stress, consistent with the progressive depletion of the Southern Citrus Aquifer.

The research presented an analysis of the average behavior and sustained trend of NDVI and NDWI for the 2003-2022 time series, associated with the piezometric levels of the Southern Citrus Aquifer. Overall, the statistical results and temporal trends showed that between 2003 and 2013 there were no statistically significant changes in either NDVI ($U=2324.0$; $p=0.0981$) or NDWI ($U=1606.0$; $p=0.0651$), indicating relative stability in both indices during this period.

In contrast, long-term analyses show statistically robust changes, with significant differences between 2003 and 2022 for both NDVI ($U=3285.0$; $p=2.25 \times 10^{-10}$) and NDWI ($U=849.0$; $p=3.06 \times 10^{-8}$), as well as significant differences between 2013 and 2022 in NDVI ($U=2844.0$; $p=2.77 \times 10^{-5}$) and NDWI ($U=1337.0$; $p=0.00159$). These comparative numerical trends confirmed that the variations of both indices intensified in the recent period and maintained temporal coherence. This is evidence that the response of vegetation, represented by the NDVI, is closely linked to the variations in surface water availability reflected by the NDWI, especially in the long-term multiple temporal analysis. The results identified that the 2003 period presented low NDVI values (<0.6) that coincide with Ceballos-Tavares & Ortega-Gaucin (2021), when determining that in that time interval the longest meteorological drought in the basin occurred. In 2013, a trend of NDVI continued with values similar to those of 2003, due to the fact that between 2011-2013 there was the greatest rainfall deficit in the basin (Palacios-Chapa, 2023). The NDVI and NDWI indices showed annual variations in their trends that did not exactly coincide with the climatic seasons, but decreased at the beginning of the year and tended to increase during the rainy season. These results are consistent with those reported by Ávila *et al.* (2020) and Carbajal & Paredes (2024), who in their research determined that in the dry season there were lower values in the spectral indices, while in the rainy season the health of the vegetation increased.

The values of the normalized water difference index ranged between -2 and -6 , which suggests that the vegetation maintained good vigor between 2013 and 2022. These data are similar to those determined by Longanezi *et al.* (2025), when using this index in the evaluation of land use in a basin in Brazil. The areas with the highest values of NDVI and NDWI are those belonging to mountains, slopes, and valleys where the dense and healthy natural vegetation cover was influenced by moisture capture due to the orography. These data contrast favorably with those reported by Xavier & Fortes (2023), who identified that native vegetation influences the results of high spectral indices and water availability.

Torrez (2024) used Sentinel 2 and Landsat-8 remote sensors for the evaluation of water tables and presented different correlations of each spectral band; the Landsat-8 sensor did not comply with the standards for the development of a methodology. Meanwhile, Sentinel-2 presented a moderate or high significance when evaluating pH and electrical conductivity. This allowed that author to generate mathematical models of water quality and predictive maps through a multivariate regression equation. On

the other hand, Castillo-Treminio (2022) mentioned that the methodology used to assess the state of the vegetation provides an effective tool to correlate the evolution of water levels in the aquifer; however, success depends on the quantity and quality of the data measured.

Likewise, Quiroz-Londoño *et al.* (2012) showed variations in frequency, applications and attenuations in the different parameters of ground movement. In that study, they determined with an analysis of piezometric levels and groundwater catchment patterns that the vegetation responds favorably to static surface levels. Finally, Uribe (2022) determined that, in the use of remote sensing and geographic information systems, 81.2% of the analyses are supported by the use of images from Landsat 7, 8, Sentinel 2A, unmanned aerial vehicles (UAVs) or drones, and new technological developments such as machine learning, neural networks and the use of smartphone cameras. Meanwhile, 18.8% use *in situ* sample collection to generate spatial distribution maps in geographic information systems with an inverse distance weighting (IDW).

This study demonstrates that the integrated use of remote sensing coupled with vegetation and humidity indices (NDVI and NDWI) is an effective and scientifically valid tool to analyze the relationship between vegetation and water tables of the Southern Citrus Aquifer (DR-1914). In contexts where traditional piezometric monitoring is limited by operational and spatial factors, remote sensing offers an efficient, spatially continuous, and temporally consistent alternative for monitoring hydrological and environmental dynamics.

The results confirm that NDVI is a reliable indicator of groundwater availability, particularly in arid and semi-arid regions where vegetation depends on the proximity of the water table. The temporal evolution of the index reveals a relative improvement in plant vigor in 2013, followed by greater heterogeneity and clear signs of water stress in 2022. This means vegetation that is increasingly conditioned by the decrease in water availability and by local anthropic pressure. Statistically, the Mann–Whitney test showed that the depth of the water table in 2022 differs significantly from the same value in 2003. This supports the existence of a process of progressive aquifer depletion, and this result reinforces the validity of the indirect approach based on spectral indices to detect substantial changes in the underground dynamics of the aquifer system.

CONCLUSIONS

The NDVI and NDWI vegetation and water indices proved to be adequate for the characterization of vegetation cover status; also, to differentiate vegetation status and recognize conditions associated with degradation, loss of cover and increased susceptibility to terrain instability processes, such as landslides.

The reduction of plant vigor emerges as a key factor in the identification of areas with greater environmental vulnerability. The use of Landsat imagery is confirmed as a reliable, accessible, and high-resolution source for the analysis of vegetation status and its relationship to groundwater.

The integration of these data with climate, land use, and water extraction information strengthens the comprehensive understanding of the factors that control the dynamics

of the aquifer. This methodology proved to be a strategic instrument for sustainable management, monitoring and planning of groundwater resources in regard to agriculture management.

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Carbon storage in total biomass and soil in a teak (*Tectona grandis* L.f.) plantation

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ABSTRACT

Objective: To estimate carbon storage in young teak plantations (*Tectona grandis* L.f.) in total biomass, dry litter, and soil due to the effects of chemical fertilizers and green manure (GM).

Methodology: A completely randomized block experimental design was used. At two years of age, chemical fertilizer (CF) (NPK) treatments were applied: control 0-0-0, 60-60-60, 90-60-60, and 120-60-60 kg ha⁻¹. At three and four years of age, treatments 0-0-0, 120-60-60, 150-60-60, and 180-60-60 kg ha⁻¹ were used. *Crotalaria juncea* was used as green manure (GM).

Results: Significant differences were observed in the interactions between CF and AV on total biomass at both ages, 3.2 and 4.7 years. The highest values were recorded in the control without AV and in the 180-60-60 kg ha⁻¹ treatment with AV. No significant differences were detected in litterfall. At 3.2 years of age, the plot without AV receiving the 90-60-60 kg ha⁻¹ dose exhibited the greatest increase in carbon storage, whereas at 4.7 years, the highest increase occurred in the control with AV. Significant differences were also observed in soil carbon storage (SOC), with an increase of 166.32 t C ha⁻¹ under the 150-60-60 kg ha⁻¹ treatment.

Conclusion: Total biomass at 3.2 years of age can store 35.05 t C ha⁻¹. Carbon in litter increases with the dose of chemical fertilization. Overall, a greater amount of carbon is stored in the soil than in above-ground and below-ground biomass and litter.

Keywords: allometric model, fertilization, green manure, litter, soil.

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INTRODUCTION

Carbon (C) stored in humid tropical forests results from the long-term balance between C assimilated during photosynthesis and its loss through respiration and tree mortality (Brando *et al.*, 2019).



Climate change is driven by increased CO₂ resulting from the disruption of the carbon cycle, highlighting the need for public policies aimed at mitigating these gases, implementing appropriate technologies, and adopting rational measures that both reduce emissions and maintain carbon balance in forests and commercial forest plantations (Correa-Guaicha & Romero-Hidalgo, 2016; Ávila *et al.*, 2001). The use of fossil fuels is a major source of greenhouse gases (GHGs), which contribute to global warming (Becerril-Piña *et al.*, 2007). Commercial forest plantations help mitigate GHG emissions caused by motor vehicles and industries, which act as the main pathways through which these pollutants are released (Erakhrumen & Inaede, 2018). Commercial forest plantations function as carbon sinks because they sequester large amounts of CO₂, primarily in their above-ground and below-ground biomass (Dhillon & Von Wuehlisch, 2013).

Importantly, forests and commercial forest plantations are significant carbon reservoirs, absorbing approximately 30% of annual CO₂ emissions (Pan *et al.*, 2011), making them strategic tools for atmospheric CO₂ sequestration. In the case of commercial forest plantations, wood serves as a short- to medium-term carbon sink when used to produce furniture and housing, while 30-40% of the total biomass remains stored in the soil due to litter incorporation and the below-ground biomass of trees (Gerardo-López *et al.*, 2018).

Teak (*Tectona grandis* L.f.) is a deciduous species during the dry season, which allows it to withstand severe droughts for up to five months of the year. It adapts well to humid sites and grows successfully in areas with year-round rainfall and no distinct dry season. Teak plantations have the potential to produce 26.7 t C ha⁻¹ year⁻¹ (González-Martínez *et al.*, 2014), although researchers such as Gerardo-López *et al.* (2018) report values of up to 151.37 t C ha⁻¹ year⁻¹. Due to the importance of carbon storage in commercial forest plantations and its relevance to climate change, carbon storage was estimated in total biomass, dry litter, and soil in young teak plantations (*Tectona grandis* L.f.) treated with chemical fertilizer and green manure (GM).

MATERIALS AND METHODS

Study area and experimental design

The study was conducted at a commercial site of the company Agropecuaria Santa Genoveva (ASGv) (17° 50' 47.64" N, 91° 12' 6.37" W), covering an area of 2.2 ha, located in the municipality of Balancán, Tabasco, Mexico. The climate of the site is warm subhumid with summer rainfall (Aw2(x)), an average annual precipitation of 1,500 mm, and a mean temperature of 26.5-27 °C (Aceves-Navarro & Rivera-Hernández, 2019). The site is situated at an altitude of 42 m above sea level.

At the start of the experiment, a preliminary soil fertility study was conducted. According to NOM-021-RECNAT-2000, the soil is sandy clay loam with a pH of 7.5 (moderately alkaline) and a medium organic matter content (SOM, 3.5%). The cation exchange capacity (CEC, 22.85 cmol_(c) kg⁻¹) is medium. Total nitrogen (Nt) content is medium (0.14%), and phosphorus (P, 7.65 mg kg⁻¹) is also at medium levels. Exchangeable bases show high calcium (Ca, 12.33 cmol_(c) kg⁻¹), low magnesium (Mg, 0.62 cmol_(c) kg⁻¹), and very low potassium (K, 0.15 cmol_(c) kg⁻¹) content.

The established teak plants were clones produced by Agropecuaria Santa Genoveva (ASGv). At the start of the experiment, they were two years old and planted in a square spacing of 3.5×3.5 m. Nitrogen (N), phosphorus (P), and potassium (K) fertilizer treatments were applied at doses of 0-0-0, 60-60-60, 90-60-60, and 120-60-60 kg ha⁻¹ when the plantations were two years old, increasing to 0-0-0, 120-60-60, 150-60-60, and 180-60-60 kg ha⁻¹ at ages three and four.

The fertilizer sources used were urea (46-00-00), diammonium phosphate (18-46-00), and potassium chloride (00-00-60). Fertilizers were applied using a handspike to a depth of 20 cm in two holes located to the north and south within the drip zone of each tree. The experimental design was a randomized block design with a split-plot arrangement. The factor tested was green manure (GM), *Crotalaria juncea*, sown at a density of 20 kg ha⁻¹ by broadcasting in the plantation alleys. After 60 days, it was incorporated into the soil to a depth of 15 cm using mechanized equipment.

Calculation of Carbon in Total Biomass and Dry Litter

Following the application of fertilizer and green manure treatments, the diameter at breast height (DBH, 1.30 m) was measured using a vernier caliper when the trees were two years old, and with a forest caliper once they were over three years old. Total height (H) was measured on five trees per plot using a Hagl f hypsometer (± 0.1 cm). To estimate the above-ground biomass of teak trees, a general allometric model developed by Kenso *et al.* (2020) was used:

$$AGB = 0.0649 \times DBH^{2.5702}$$

where: *AGB*: Above-ground biomass of the tree (kg dry weight); *DBH*: Diameter at breast height (DBH, 1.3 m).

The below-ground biomass of teak trees was calculated using the following allometric model (Kenso *et al.*, 2020):

$$BGB = 0.0460 \times DAP^{2.1763}$$

where: *BGB*: Below-ground biomass of the tree roots (kg dry weight); *DAP*: Diameter at breast height (DBH, 1.30 m).

To determine carbon in dry litter, biological traps made of fine plastic mesh, measuring 1.5×1.5 m, were randomly placed and secured to stakes to prevent contact with the soil surface and avoid contamination or mineralization effects (Pulido & D  az, 2005). Vegetative material was collected after treatment application and placed in perforated paper bags, labeled with site and treatment information. Litter samples were taken to the laboratory and dried at 65  C in a forced-air oven until constant weight was achieved (Murtinah *et al.*, 2016).

The values of dry litter, above-ground biomass, and below-ground biomass were multiplied by a factor of 0.52 to calculate carbon, as estimated by Kumar-Jha (2005) for teak. The results of the two biomasses were then summed to obtain total biomass. The carbon conversion factor corresponds to the percentage of carbon in the tree mass: carbon 50%, oxygen 41%, hydrogen 6%, nitrogen 1%, and ash 2%. In other words, the amount of carbon per ton of dry matter is approximately 500 kg (50%).

Calculation of Soil Organic Carbon

Soil sampling was conducted at the end of the experiment, when the teak trees were 4.7 years old, for each of the treatments. Samples were taken at a depth of 30 cm using a stainless-steel auger, placed in labeled plastic bags, and transported to the laboratory. The samples were air-dried, ground, and sieved (2 mm). Organic carbon was determined using the Walkley-Black method (1934), which is based on oxidation with a potassium dichromate solution and the heat generated by reaction with concentrated sulfuric acid.

From the laboratory results, soil organic carbon (SOC) content was calculated using the equation proposed by Rojas *et al.* (2009),

$$SOC = OC(Bd)Sd$$

where: SOC =Soil organic carbon ($t\ ha^{-1}$); OC =Organic carbon (%); Bd =Bulk density ($g\ cm^{-3}$); Sd =Soil depth (cm).

Statistical Analysis

Data on carbon storage in total biomass, litter, and soil were analyzed using the statistical software Infostat 2020, through analysis of variance and mean comparisons using Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

Carbon Storage in Total Biomass

At 3.2 years of age, statistical analysis of the interaction between chemical fertilization and green manure (GM) showed significant differences ($p<0.05$, Figure 1). The control treatment in the plot without GM and the 60-60-60 $kg\ ha^{-1}$ fertilization treatment * GM showed the highest carbon storage ($35.05\ t\ C\ ha^{-1}$), which gradually decreased as the fertilization dose increased. The opposite trend was observed in the plot with GM and 60-60-60 $kg\ ha^{-1}$ fertilization, where carbon in total biomass increased.

At 4.7 years of age (Figure 1), the interaction between chemical fertilization * GM again showed significant differences ($p<0.05$). Increases in carbon were observed when chemical fertilization was combined with GM, compared to fertilization without GM. In the latter, carbon storage was highest in the control treatment and decreased as nitrogen fertilization increased up to 150-60-60 $kg\ ha^{-1}$, before increasing again in the subsequent treatment.

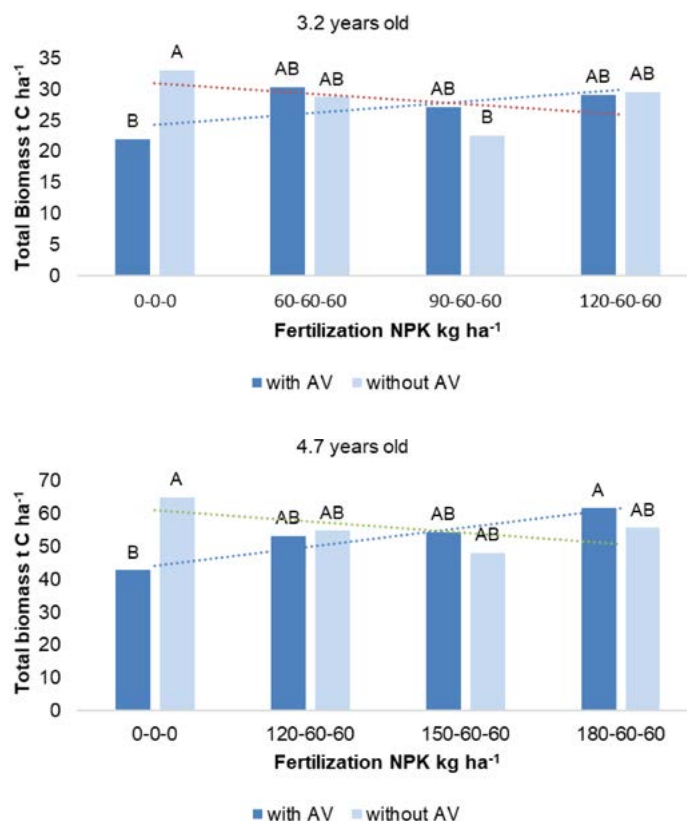


Figure 1. Interactions between fertilization * GM on carbon content in total biomass in teak.

Teak plantations, considering biomass quantity, have the potential to produce $26.7 \text{ t C ha}^{-1} \text{ year}^{-1}$ (González-Martínez *et al.*, 2014). In adult plantations, total above-ground biomass can range from 151.37 to $214.7 \text{ t C ha}^{-1}$, with 16.6% stored in roots (Gerardo-López *et al.*, 2018; N'Guessa-N'Gbala *et al.*, 2017). Differences in reported carbon storage between these studies and the present work are likely due to vegetation type and seed propagation. In this experiment, the higher carbon storage can be attributed to the use of highly productive clones (ASGv) at early ages.

Other species under five years old, such as *Gmelina arborea* Roxb. ex Sm., can store 15.8 t C ha^{-1} in above-ground biomass and 2.37 t C ha^{-1} in below-ground biomass (Tamang *et al.*, 2021), while *Hevea brasiliensis* Muell. Arg. can store $26.28 \text{ t C ha}^{-1}$ in above-ground biomass (López-Reyes *et al.*, 2016). In other vegetation systems, Pompa-García & Sigala-Rodríguez (2017) found that protected natural areas have higher above-ground carbon storage ($135.3 \text{ t C ha}^{-1}$) than primary forests and managed forests (105.1 and $115.4 \text{ t C ha}^{-1}$, respectively), with the lowest carbon storage rates observed in secondary forests (51.2 t C ha^{-1}), followed by reforested areas, commercial plantations, and agroforestry systems with 45.6 , 62.8 , and 35.8 t C ha^{-1} , respectively.

Carbon storage in total biomass (above-ground and roots) in teak plantations in this study was higher compared to other studies (Figure 1). Kumar-Jha (2015), in young teak plantations, reported 1.6 , 15.8 , 35.5 , 39.0 , 61.5 , and 73.2 t C ha^{-1} at ages of 1, 5, 11, 18, 24, and 30 years, respectively. Sreejesh *et al.* (2013) reported 21 t C ha^{-1} at five years of

age. In these studies, carbon values were obtained from plantations established from seeds, and differences in site index and soil fertility likely explain variations in above-ground and total biomass carbon among studies. Other experiments on carbon storage in teak total biomass in Nuevo Urecho, Michoacán, Mexico, found 77 t C ha⁻¹ at 11 years of age (Jiménez-Pérez *et al.*, 2020).

Carbon storage in litter

At 3.2 years of age, no significant differences were found among treatments ($p < 0.05$, Figure 2). The 90-60-60 kg ha⁻¹ dose without GM showed the highest increase, with 0.6 t C ha⁻¹. Although increases with GM were lower compared to treatments without GM, the highest dose (120-60-60 kg ha⁻¹) showed the largest increase with 0.5 t C ha⁻¹. When the teak plantations reached 4.7 years of age (Figure 2), no significant differences were observed ($p > 0.05$). The greatest increase was found in the control with GM, with 0.56 t C ha⁻¹. Among treatments without GM, the highest increases were observed in the 60-60-60 and 90-60-60 kg ha⁻¹ doses, with 0.53 and 0.52 t C ha⁻¹, respectively.

Carbon stored through dead litter is generally more labile and contributes significantly to the increase of soil fertility in forest systems. Kraencel *et al.* (2003) reported that 20-year-old teak plantations can store between 2.6 and 3.4 t C ha⁻¹ in litter. Average carbon contributions from litter in teak plantations and secondary forests are 4.7 and 2.3

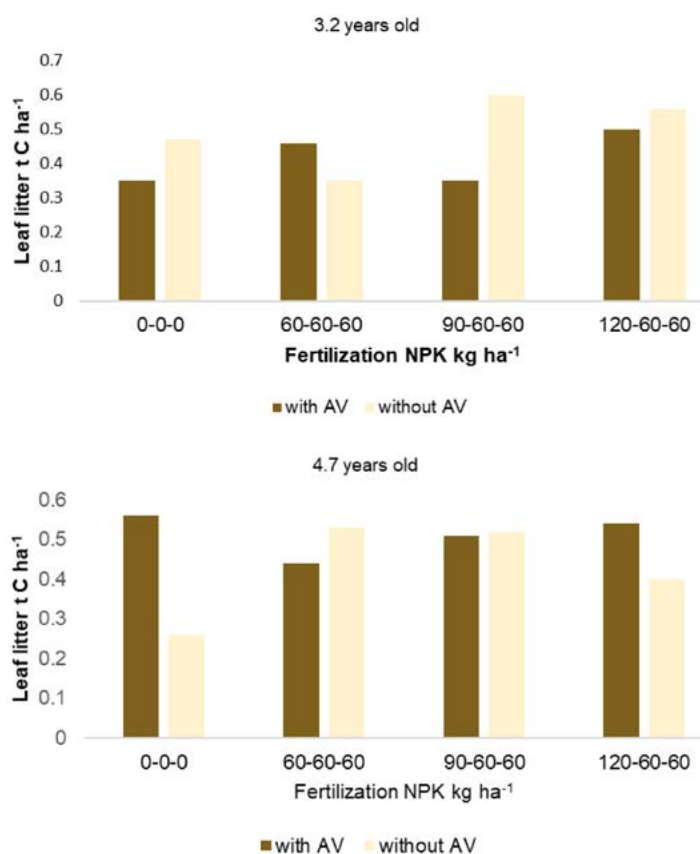


Figure 2. Interactions between fertilization * GM on carbon storage in teak litter.

t C ha⁻¹, respectively (N'Guessa-N'Gbala *et al.*, 2017). A large proportion of carbon in teak plantations comes primarily from litter and roots (Hansen *et al.*, 2013).

The amount of carbon stored in litter depends on the tree species. For example, in Sichuan Province, China, within the Wanglang National Nature Reserve, three forest types were compared: coniferous forests (*Picea purpurea* Mast.) stored the most carbon, followed by mixed forests (*Picea purpurea* Mast., *Abies faxoniana* Rehd. & E.H. Wilson, *Betula albosinensis* Burkill), and broadleaf forests (*Tilia tuan* Szysz., *Padus racemosa* L., *Salix paraplesi* C.K. Schneid), with 0.4, 0.36, and 0.14 t C ha⁻¹, respectively (Chen *et al.*, 2021). These values are lower than those found in the present study.

Carbon storage in soil

In soil carbon, interactions between chemical fertilization * green manure GM were observed (Figure 3), showing significant differences ($p < 0.05$). In the plot with GM, the 150-60-60 kg ha⁻¹ treatment recorded the highest carbon storage, with 166.32 t C ha⁻¹, while in the plot without GM, the same treatment resulted in lower carbon storage, with 150.52 t C ha⁻¹.

Under different management practices in five-year-old teak plantations, soil carbon storage can range from 39.55 to 45.84 t C ha⁻¹, which is lower than the values found in the present study. These differences may be related to soil fertility (Agyei-Kumi *et al.*, 2021).

The green manure *C. juncea* helps increase soil fertility by reducing degradation and water runoff, decreasing bulk density, and increasing soil organic carbon (SOC) and nutrient availability (Ma *et al.*, 2021). When green manure is established together with chemical fertilization, there is an increase in mineralizable and hydrolysable carbon, which improves microbial activity and promotes rapid decomposition of the green manure (Almagro & Martínez, 2014). In a study by Song-Juan *et al.* (2018) on rice cultivation in Hunan Province, China, the application of green manure resulted in dissolved organic carbon (DOC) storage of 711.10 mg kg⁻¹, which was significantly higher compared to the application of chemical fertilizers alone (428.50 mg kg⁻¹).

Similarly, agroforestry systems, such as teak plantations combined with *C. juncea*, absorb more CO₂ as soil organic carbon (SOC). De Stefano and Jacobson (2017) reported that

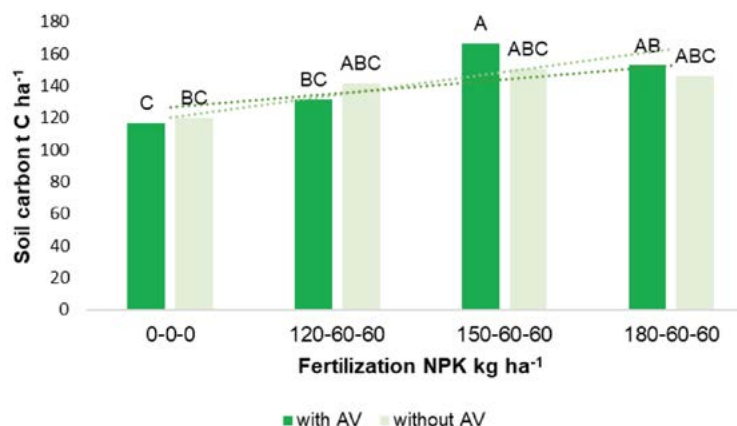


Figure 3. Interaction between chemical fertilization * GM on soil carbon storage in a teak plantation.

these systems can store between 12 and 228 t C ha⁻¹, a much higher amount than that stored in conventional agricultural crops.

Other studies, such as Tangsinmankong *et al.* (2007), recorded carbon storage in soils of deciduous mixed forests and teak plantations, finding that soil organic carbon at all sites generally decreased with increasing depth from the soil surface to the lower layer. In teak plantations of different ages, at 6, 24, and 15 years, and in the deciduous mixed forest (157.03, 105.67, 78.78, and 70.96 t C ha⁻¹, respectively), this difference in carbon storage may be due to forest fires, forest management, and topography. In teak plantations aged 12 to 20 years, Meunpong *et al.* (2010) reported soil carbon storage of 40 t C ha⁻¹ in Thailand. Soil organic carbon influences many physicochemical processes and biological properties (Smith *et al.*, 2013), playing a key role in soil productivity and quality (Lal, 2016). Forest type also affects soil carbon storage, along with characteristics such as tree species, litter quality, and primary productivity (Bai *et al.*, 2019).

CONCLUSIONS

Total biomass (root and above-ground biomass) at 3.2 years of age can store 35.05 t C ha⁻¹ in the control treatment without GM. At 4.7 years of age, carbon storage reaches 67.17 t C ha⁻¹ under chemical fertilization combined with GM. Carbon storage in litter increases with higher doses of chemical fertilization; however, the control plot with GM showed the highest litter carbon storage.

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Effect of thermal and water stress on growth, biomass and yield of native corn in the Yucatan Peninsula, Mexico

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ABSTRACT

Objective: The effect of thermal and water stress on growth, biomass production and yield in native corn (*Zea mays* L.) was evaluated.

Design/methodology/approach: The study grouped 17 native corn cultivars. Heat unit accumulation, leaf area growth, yield, total biomass dry weight, and other dry matter characteristics were evaluated. Individual analyses of variance and correlation were performed using SAS® statistical software. Means were compared using the Tukey test ($P \leq 0.05$).

Results: The effect of thermal and water stress allowed to observe a significant variation in the growth of the leaf area and production of dry matter and yield of native corn; on average, better expression was obtained through the genotypes Saak dzit baakal and Kàn Kàn xnuc naal that presented greater growth, biomass and yield based on thermal and water stress.

Limitations on study/implications: The study was limited to the application of water stress during the vegetative stage; it is suggested to cause stress during the reproductive stage.

Findings/conclusions: The native corns Saak dzit baakal and Kàn Kàn xnuc naal showed greater tolerance to thermal and water stress by presenting greater acclimatization that allowed them to present greater growth, biomass production and grain yield.

Keywords: temperature, genotype, physiology.

INTRODUCTION

Climate conditions affect the growth, development and yield of corn (*Zea mays* L.), including drought, heat stress, solar radiation, and the distribution of heat unit accumulation in the cultivation cycle (Ortez *et al.*, 2023). Thermal and water stress are abiotic stress



factors that affect the yield and quality of crops (Wang *et al.*, 2023). It has been reported that variation in climate had adverse effects on corn productivity in several agricultural regions throughout the world (Eck *et al.*, 2020). It has been shown that extreme climatic events, such as drought and thermal stress, are factors that affect the life cycle of corn (Sato *et al.*, 2024; Nawaz *et al.*, 2023), reducing light interception and increasing respiration (Zahra *et al.*, 2023). Therefore, thermal and water stress severely limit agricultural productivity, including corn yield (Kompas *et al.*, 2024).

Yang *et al.* (2023) indicate that both temperature and soil moisture are important covariables in corn yield; therefore, soil moisture could carry out a more important role than temperature, and the apparent adverse effect of temperature on yield variability could be the result of stress from concurrent drought instead of heat stress (Proctor *et al.*, 2022). Because of this, it is important to determine the range of temperatures and thus be able to measure growth through the daily integration of thermal energy or growing degree (D°) within the range, known as growing degree days (GDD) (Arista-Cortes *et al.*, 2018). Growing degree days (GDD) constitute the most extensive thermal indicator in use to predict plant development, obtained through daily maximum and minimum temperatures. This tool is essential to estimate the development rate and growth stage of plants, which are relevant for decision making (Arista-Paredes *et al.*, 2025). In corn, a deficient precipitation and heat can decrease the yield; likewise, it has been observed that for each additional growing degree day above 30 °C the yield decreases by 0.5% under irrigation and 2% in rainfed areas (Dhaliwal and Williams, 2022). The effects of temperature are increased by the water deficit, which demonstrates that understanding the interaction between temperature and water will be necessary to develop more effective adaptation strategies that compensate the impacts of extreme events of higher temperature associated with climate change (Hatfield and Prueger, 2015). Therefore, although this combination of natural variability and climate change is influencing corn productivity, it is important to direct studies about thermal and water stress to the physiological stages of corn. In addition to heat and drought, they are regularly linked to the corn production cycle in the Peninsula of Yucatan, where solar radiation and the distribution of heat unit accumulation during crop stages affect corn growth, biomass and yield. The objective of this study was to assess the effect of thermal and water stress on growth, biomass production and yield in native corns.

MATERIALS AND METHODS

Experimental site and genetic material

The experiment was carried out in plots in the locality of Uayamon, Campeche, Mexico, during the S-S 2024 cycle. Seeds from 17 native corn genotypes were used (Saak tux, Eh hu, Piix cristo, Chaac xmejen naal, Kàn Kán teel, Kàn Kàn xnuc naal, Saak Chan Teel, Eju, Chaak Choob, Tuxpeño, Chaak teel, Saak dzit baakal, San Pableño Blanco, Kàn Kàn dzit baakal, San Pableño Amarillo, Rosa San Juan and Morado MX).

Establishment and conditions of the experiment

A completely randomized block design with five repetitions was used. The experimental unit consisted of five rows, 6.0 m long, with 1.0 m separation between rows and 20 cm

between plants. The experiment was established manually in April 2024, when seed was deposited at 10 cm depth. For the agronomic management of the crop, the technological package from the National Institute of Forestry, Agriculture and Livestock Research (*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias*, INIFAP) for corn production was used, under rainfed conditions, in Campeche. During emergence at 24 days after sowing (das), the application of drip irrigation was done for a period of three hours. Likewise, water stress was applied based on suspending irrigation to all genotypes starting from 25 to 45 das (Figure 1B).

Variables statistical analysis

To calculate the growing degree days (GDD), the residual method was used, whose model is the following:

$$GDD = (T_{\max} + T_{\min}) / 2 - T_{\text{base}}$$

where: $T_{\max}$ =maximum daily temperature (°C); $T_{\min}$ =minimum daily temperature (°C); T_{base} =base temperature, 10 °C. The maximum and minimum temperature (°C) of the air were registered with an electronic thermometer, and the precipitation with a pluviometer in cm. To determine the variables, eight plants were used per repetition and reported based on the average.

The leaf growth area per plant (LAG) was determined by measuring the length (L) and width (W) of each leaf with a graduated ruler in cm and multiplied by 0.75 ($CAFP = L * A * 0.75$, cm²). The dry weight of the stem (SW, g), the leaf blade (LBW, g), the leaf sheath (LSW, g), the root (RW, g), the bracts (BW, g) and total biomass (TB, g), was obtained by separating each structure and drying it in a stove (Nieto REB 450) at 70 °C for 72 h, and then weighed on an analytical scale (METTLER®). The grain yield (GY, g) was determined by de-kernelling and weighing the corn cobs at physiological maturity. With the data obtained, individual analyses of variance and Pearson's correlation were conducted, as well as Tukey's means comparison ($P \leq 0.05$) using the statistical software SAS®, v. 9.1 for Windows.

RESULTS AND DISCUSSION

Heat unit accumulation

Significant differences ($P \leq 0.05$) were observed in GDD between genotypes for all the stages of corn growth and development (Table 1).

According to Beegum *et al.* (2023) corn EME needs between 120 and 125 GDD with maximum (32 °C) and minimum (28 °C) air temperature. However, the sowing depth (Villalobos *et al.*, 2023), soil-air temperature, and moisture can decrease or increase the number of GDD necessary for EME in corn such as Piix cristo which presented 242 GDD compared to Saak tux, Saak Chan Teel, Tuxpeño, Saak dzit baakal and Kàn Kàn dzit baakal, and San Pableño Amarillo which on average obtained 157 GDD at EME (Table 2).

Table 1. Analysis of variance based on the accumulation of GDD in the phenological stages of native corn, S-S 2024 cycle.

SV	DF	Phenological states				
		EME	HE	MF	FF	PM
G	16	6669.3**	81651.8**	79297.9**	60273.7**	32839.1**
R	4	1848.7**	3117.8*	795.2 ^{ns}	830.9 ^{ns}	19830.3**
Error	64	227.9	1134.6	353.0	418.4	2092.4
Total	84					
C.V. %		7.6	2.2	1.1	1.2	1.6

*= $P \leq 0.05$; **= $P \leq 0.01$; ^{ns}=non-significant; VF=variation factor; G=genotype; R=repetition; EME=emergence; SP=spiking; MF=masculine flowering; FF=feminine flowering, and PM=physiological maturity.

Table 2. Means comparison based on GDD accumulation for the different phenological stages of native corn, S-S 2024 cycle.

Genotype	GDD phenological states				
	EME	HE	MF	FF	PM
Saak tux	156 c	1373 f	1523 h	1562 g	2704 e
Eh hu	232 ab	1649 b	1795 b	1816 b	2910 a
Piix cristo	242 a	1880 a	2027 a	2027 a	2909 a
Chaac xmejen naal	213 ab	1373 f	1523 h	1605 fg	2717 e
Kàn Kàn teel	156 c	1393 ef	1543 gh	1605 fg	2737 de
Kàn Kàn xnuc naal	204 b	1397 ef	1543 gh	1627 ef	2749 b-e
Saak Chan Teel	156 c	1411 ef	1562 gh	1627 ef	2724 e
Eju	232 ab	1563 c	1708 c	1729 c	2906 a
Chaak Choob	237 ab	1541 c	1687 cd	1708 cd	2848 abc
Tuxpeño	156 c	1458 de	1605 ef	1669 de	2752 b-e
Chaak teel	227 ab	1441 def	1585 fg	1627 ef	2736 e
Saak dzit baakal	156 c	1501 cd	1647 de	1669 de	2841 a-d
San Pablén Blanco	161 c	1560 c	1647 de	1708 cd	2873 a
Kàn Kàn dzit baakal	218 ab	1458 de	1605 ef	1669 de	2749 b-e
San Pablén Amarillo	161 c	1458 de	1605 ef	1647 ef	2745 cde
Rosa San Juan	237 ab	1415 ef	1562 gh	1647 ef	2852 ab
Morado MX	232 ab	1397 ef	1543 gh	1562 g	2661 e

Mean values per column with different letters are statistically different ($P \leq 0.05$). GDD=growing degree days; EME=emergence; HE=heading; MF=male flowering; FF=female flowering; and PM=physiological maturity.

A different requirement of GDD between genotypes could be due to genotype-environment actions (Xu *et al.*, 2021).

Regarding the GDD at SP and MF, Saak tux and Chaac xmejen naal, were statistically more precocious, with 1373 and 1523 GDD. When FF happens, quite marked differences were observed in GDD, due to the lower precociousness of Piix cristo (2027 GDD). However, floral asynchronism in GDD of Piix cristo allows them to ensure the receptivity

of the stigmas at the time of anthesis and not with the differences of asynchronism in Chaac xmejen naal (82 GDD), Kàn Kàn xnuc naal (84 GDD), and Rosa San Juan (85 GDD) which risk fertilization, corncob development, and yield (Chen *et al.*, 2023). Eh hu, Piix cristo, Eju and San Pablèño Blanco presented higher GDD at MF with an average of 2899 GDD. The data reported here agree with what was reported by Arista-Cortes *et al.* (2018), when differences were reported in the GDD requirements of 10 corn accessions.

In corn, an important effect of water stress is the delay in feminine flowering, which results in an increase of the anthesis-feminine flowering interval, an important cause of losses in yield (Abdoul-Raouf *et al.*, 2016).

Growth of leaf area, biomass and yield

The analysis of variance detected differences ($P\leq0.05$) between genotypes for LAG, SW, LBW, LSW, RW, BW, TB and GY (Table 3). Heat exposure at 40 °C and water deficit during the vegetative stage (25 to 45 das), allowed observing a variation of the LAG (Figure 1), since Eh hu presents a difference of 42% (3757 cm²/plant) and 48% (4256 cm²/plant) compared to Chaak teel and Morado MX in LAG. However, a longer exposure

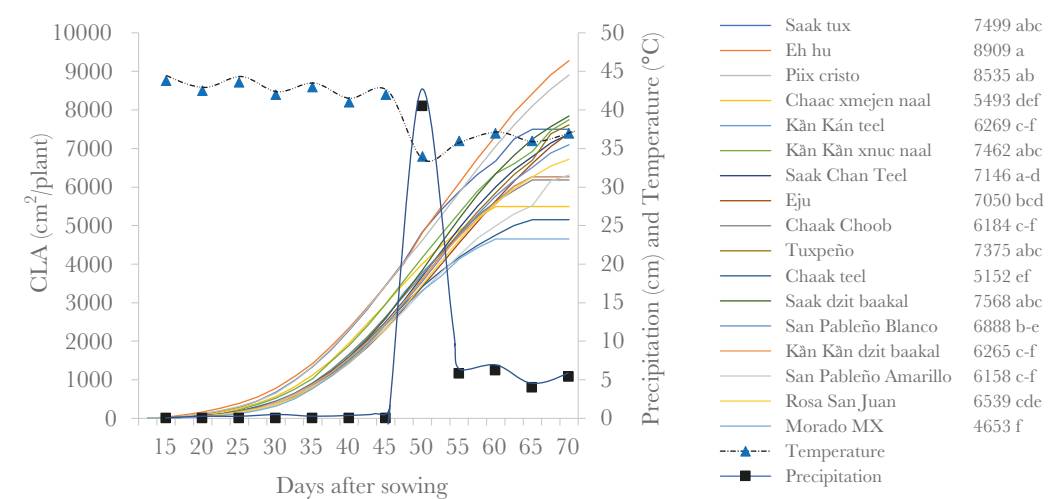


Figure 1. Leaf area growth accumulated by plant (LAG) based on tempera-ture (A) and precipitation (B), S-S 2024 cycle. *= $P\leq0.05$. Mean values per column with different letter are statistically different ($P\leq0.05$).

Table 3. Analysis of variance for leaf area growth per plant, dry weight of the blade and sheath of the leaf, dry weight of the root, bracts, total biomass and grain yield of the native corn, S-S 2024 cycle.

SV	DF	LA	DWS	DWBL	DWSH	DWR	DWBR	TB	YL
G	16	5794103*	4856*	359*	338*	5215*	355*	31104*	7943*
R	4	978357 ^{ns}	421 ^{ns}	29 ^{ns}	13 ^{ns}	640 ^{ns}	19 ^{ns}	4135 ^{ns}	584 ^{ns}
Error	64	549106	614.7	51.9	35.0	1467	26.1	4831	530
Total	84								
C.V.%		10.7	31.1	54.4	28.3	70.7	21.4	25.6	29.0

SV=source of variation; CV=coefficient of variation; DF=degrees of freedom; R=repetition; G=genotype; LA=leaf área growth per plant; DWBL=leaf blade dry weight; DWSH=sheath dry weight; DWS=stem dry weight; DWR=root dry weight; DWBR=bracts dry weight; TB=total biomass; YL=grain yield. *= $P\leq0.05$; ^{ns}=no significative.

(28 days) to heat (38 °C) causes even more serious effects on the LAG, with a reduction of 72% in net photosynthetic rate (Aziz *et al.*, 2023). Hatfield and Prueger (2015), found that the greater impact of high temperatures was produced during the reproductive stage of corn development and, in every case, the grain yield was significantly reduced, up to 90% compared to normal temperature.

The amounts estimated, recorded in Table 4, indicate that the highest proportions of dry matter were found in the SW with 30%, grain weight (GY) with 29%, and RW with 19%, and the lowest % in LBW with 5%, LSW with 8%, and BW with 9%. Likewise, significant changes were seen, since Piix cristo and Chaak Choob presented higher SW with 132 g; meanwhile, K̄n K̄n xnuc naal obtained the highest BW with 35 g. A high allotment to the plant stem would be the result of the plant maximizing photosynthesis, which produces more photosynthates than necessary to meet the growth demands of the roots and leaves (Walne and Reddy, 2022).

When comparing the LBW, LSW, RW and TB of the genotypes, it was observed that Piix cristo was superior ($P \leq 0.05$) with the exception of GY. However, the impact of the thermal and water stress was higher in Morado MX; since it had lower SW, LBW, LSW, RW, BW, TB and GY, because during the vegetative stage and floral initiation (Figure 1 and 2), the high temperatures and water deficit affected the growth (Zhu *et al.*, 2021), fertilization, development of the corn cob and yield (Chen *et al.*, 2023). Brief and prolonged episodes from high temperatures during the corn growth cycle (especially in the most

Table 4. Means comparison based on dry weight of native corn, S-S 2024 cycle.

Genotype	DWS	DWBL	DWSH	DWR	DWBR	TB
	g					
Saak tux	62 cd	6 c	16 def	39 bc	17 de	237 b
Eh hu	119 ab	27 ab	30 abc	100 ab	13 e	320 ab
Piix cristo	132 a	39 a	39 a	146 a	22 b-e	412 a
Chaac xmejen naal	68 bcd	13 bc	19 c-f	52 bc	24 a-e	234 b
K̄n K̄n teel	61 cd	7 c	15 ef	75 abc	31 abc	280 ab
K̄n K̄n xnuc naal	73 bcd	16 bc	29 a-d	66 abc	35 a	356 ab
Saak Chan Teel	72 bcd	9 c	19 c-f	66 abc	24 a-e	242 b
Eju	121 ab	14 bc	33 ab	60 abc	21 cde	293 ab
Chaak Choob	133 a	19 bc	25 b-e	65 abc	20 cde	319 ab
Tuxpeño	70 bcd	12 bc	20 b-e	34 bc	33 ab	294 ab
Chaak teel	60 cd	10 c	18 c-f	28 bc	27 a-d	243 b
Saak dzit baakal	106 abc	11 bc	24 b-e	44 bc	31 abc	347 ab
San Pableño Blanco	74 bcd	8 c	19 c-f	37 bc	28 a-d	285 ab
K̄n K̄n dzit baakal	69 bcd	7 c	15 ef	31 bc	24 a-e	221 b
San Pableño Amarillo	53 cd	11 bc	13 ef	26 bc	25 a-d	211 b
Rosa San Juan	56 cd	7 c	15 ef	44 bc	30 abc	262 ab
Morado MX	23 d	9 c	6f	8 c	0 f	46 c

Mean values per column with different letters are statistically different ($P \leq 0.05$). DWBL=leaf blade dry weight; DWSH=sheath dry weight; DWS=stem dry weight; DWR=root dry weight; DWBR=bracts dry weight; and TB=total biomass.

critical stage of flowering) cause metabolic and/or morphological alterations, which lead to irreversible reductions of the yield (Wagas *et al.*, 2021). Water stress inhibits corn growth, stem and root length, antioxidant activity, relative water content (RWA) and germination of the seeds. In addition, it causes the loss of turgidity and a decrease in the hormonal content (Rasheed *et al.*, 2023).

In this context, Saak dzit baakal and Kàn Kàn xnuc naal were the ones that presented highest GY with 131 and 137 g (Figure 2), and this could be because these genotypes presented higher tolerance to thermal and water stress during the physiological stages (MF, FF, PM), since they also presented higher LAG, BW and TB compared to Morado MX which presented more sensitivity to both stresses. Therefore, the effects of the thermal and water stress on growth, dry matter and GY (Figure 2) depend on the genotype (Shao *et al.*, 2023). In addition, the sensitivity to drought (Sah *et al.*, 2020) and high temperatures (Zhu *et al.*, 2021) could reduce the GY in different degrees based on the seriousness of stress and the development stage of the plant.

The results presented here agree with those reported by Rincón-Tuexi *et al.* (2006), since they found that temperatures higher than 35 °C caused a significant reduction of aerial biomass and grain yield in corn populations, and all the organs of the plant are affected except for the bracts; the reduction in grain yield caused by the high temperature was mainly a result of the reduction in number of grains per corn cob (73%). All the variables were correlated between one another in a positive way, except for the GY with SW, LBW, LSW, RW (Figure 3).

The highest correlations were between the LSW with SW (0.88) and TB (0.85); LBW-RW (0.85), GY-BW (0.84), LSW-RW (0.81), LBW-LSW (0.80), TB-SW (0.78) and LAG-TB (0.75) (Figure 3). That is, higher LAG contributed positively to a higher TB; for this study, this association was negative for Morado MX and positive for Saak dzit baakal and Kàn Kàn xnuc naal (Figure 1 and 2, Table 4). Other similar studies (Umer *et al.*, 2020) show that LAG is positively correlated with GY. The negative correlations observed between GY and SW, LBW, LSW, RW can happen because of limiting factors such as water stress and high

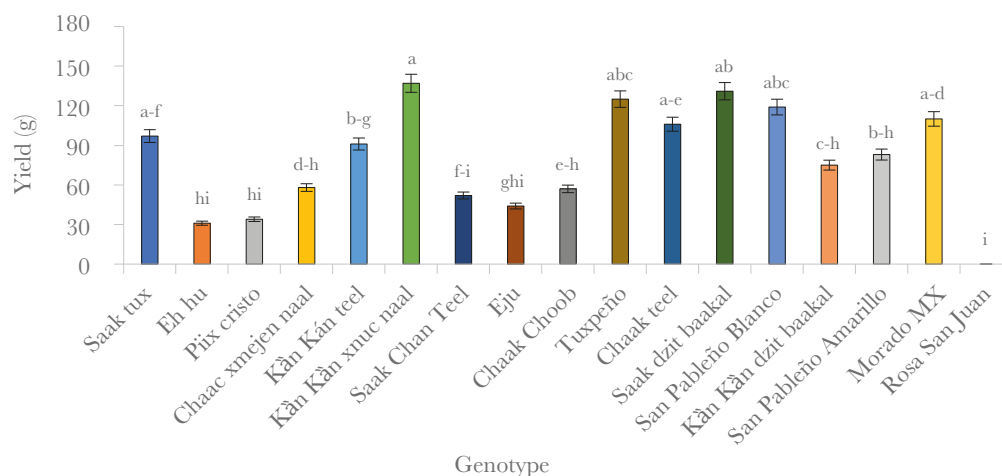


Figure 2. Means comparison of the grain yield, S-S 2024 cycle. Mean values per column with different letter are statistically different ($P \leq 0.05$).



Figure 3. Pearson's correlation coefficient from agronomic characteristics of native corn, S-S 2024 cycle. LAG=leaf area growth accumulated per plant; LBW and LSW=dry weight of the leaf blade and leaf sheath; SW, RW, BW=dry weight of stem, root, bracts, and TB=total biomass.

temperatures that affect yield significantly, that is, the distribution of plant assimilates is diverted from the main objective (grains) and directed to the growth of its vegetative parts (Quintero and Escalante *et al.*, 2025).

CONCLUSION

The 17 genotypes studied show different thresholds in the requirements of Tb and GDD, which expresses their versatility and broad climatic adaptation; likewise, a significant variation was found for the growth, biomass and yield between native corn genotypes. The best expression, on average, was obtained with the genotype Saak dzit baakal and K  n K  n xnuc naal, and this genetic variability based on thermal and water stress can be integrated into programs of genetic improvement that seek the adaptation of crops to climate change, thus contributing to food security.

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Proline influences the antioxidant capacity of Quelite (*Amaranthus hybridus* L.) and promotes its nutraceutical value

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ABSTRACT

Objective: To identify the proline content and antioxidant capacity in quelites of crop fields and marginal lands, for the delimitation of collection sites of plants with superior nutraceutical quality.

Design/Methodology/Approach: Plants of Mexican quelite (*Amaranthus hybridus* L.) in the fruiting stage were collected in two contrasting site conditions. The variables measured were plant height, number of lateral branches, and (visible) damage caused by insects. Aqueous extracts were obtained to measure the concentration of proline by the Bates free proline extraction method, to estimate antioxidant capacity as a percentage of inhibition of the DPPH radical.

Results: Results express average proline concentrations, 34.34 mg L⁻¹ in plants exposed to unfavorable conditions and 32.93 mg L⁻¹ in those associated with agricultural crops, this difference was significant ($p \leq 0.05$). Proline content was higher in plants subjected to stress. Regarding the percentage of inhibition of the DPPH radical with Ellagic Acid as a reference, in extracts of plants collected under marginal conditions, the average value was 22% (5.06 mg L⁻¹ EAeq), while in extracts of plants collected under favorable conditions it was 15.8% (0.98 mg L⁻¹ of EAeq). This is, plant extracts from sites with unfavorable conditions had a greater antioxidant capacity, which is related directly to proline.

Limitations/Implications of the study: The sample size of the quelite collections was small, even for non-parametric statistical tests. It is important to increment sample size to adequately measure the metabolites content of this plant with nutraceutical potential. High contents of proline, a bioactive compound with therapeutic properties place quelites, until now considered as weeds, as a functional food with the potential to improve human health.

Findings/Conclusions: The significant differences in proline concentration between sites, and the relationship of this aminoacid with the antioxidant capacity of quelite, highlight that site conditions increase the nutraceutical potential of these edible wild plants. This fact can lead to public health and food safety criteria for the sustainable use of marginal lands.

Keywords: proline, nutraceuticals, *Amaranthus hybridus*, antioxidant capacity, DPPH, functional foods.

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INTRODUCTION

The Mexican quelite (*Amaranthus hybridus* L.) is an herbaceous plant distributed in Mesoamerica that is consumed for its leaves, tender stems and inflorescences. It has high



biological value, with essential amino acids, iron and calcium, fiber, antioxidant vitamins (A and C), and phenolic compounds with anti-inflammatory, anti-anemic and liver-protective properties (Mateos *et al.*, 2020). Its tolerance to abiotic stress, especially drought, allows quelite to be used as an environmental bioindicator (González *et al.*, 2019). In addition, it reduces concentrations of Cu, Cr and Cd in contaminated soils; and it is outstanding as a useful species in phytoextraction and phytoremediation (Olawepo *et al.*, 2020).

Quelites are consumed fresh or cooked in various traditional dishes (López *et al.*, 2018). Cooking maintains bioactive compounds, such as polysaccharides with high antioxidant activity (Tang *et al.*, 2019). Hydroacetic, methanolic and aqueous extracts of quelite show antioxidant and antimicrobial capacity due to the presence of terpenoids, saponins and alkaloids (Okoye, 2018; Ndukwe *et al.*, 2020). Some compounds inhibit the xanthine oxidase linked to liver and kidney inflammation (Nana *et al.*, 2012). It also contains squalene, associated with anti-cancer and anti-tumor effects (Sharma & Vijayvergia, 2019). Regarding the amino acid content, the abundance of proline and leucine is highlighted (Aja *et al.*, 2021). Proline accumulates in response to stress, acts as an osmotic protectant, redox regulator, and participant in the synthesis of secondary metabolites. This amino acid is a physiological indicator of plant stress (Hosseinifard *et al.*, 2022). In humans, exogenous proline improves tolerance to oxidative stress in diseases such as cancer and fibrosis (Vettore *et al.*, 2021). In addition, it participates in collagen synthesis (Patriarca *et al.*, 2021) and is essential for the function of the retinal pigment epithelium (Du *et al.*, 2021).

Despite its benefits, quelite consumption has decreased due to dietary changes and perceptions of health risk in contaminated areas (De Loera *et al.*, 2019). In Guanajuato, particularly in Salvatierra, this trend is observed in a productive agricultural context with a persistent tradition of rural farmers (Cárdenas *et al.*, 2019). We proposed the hypothesis that stress increases the proline content and antioxidant potential in this wild plant, toward the sustainable use of this species. The objective of the study was to identify the proline content and antioxidant capacity in quelites of crop fields and marginal lands, for the delimitation of collection sites of plants with superior nutraceutical quality.

MATERIALS AND METHODS

To determine the variability in proline concentrations, a comparative sampling was implemented, from three sites located within the municipality of Salvatierra, Guanajuato, Mexico; at the geographical coordinates 20° 15' 18" N and 100° 56' 27" W. The criteria for the selection of the sites included two types of soil and climate conditions, an area in association with the crop, with favorable growing conditions due to indirect effects of agronomic management (irrigation, fertilization, pest control), and another in marginal (non-cultivated) lands, with unfavorable conditions for the development of Quelite caused by intense exposure to biotic and abiotic stress factors. The purpose of this strategy was to assess whether environmental stresses, particularly water and biotic stresses, significantly influence endogenous proline content.

The plants were collected during the fruiting stage, in which a large amount of bioactive substances can be found. Development variables, such as plant height and number of lateral branches, as well as the (visible) damage caused by insects were recorded. To measure plant

height, a tape measure was used; the number of lateral branches was counted manually, and insect damage was estimated visually. This is, a damaged plant was considered to be one that had leaves severed by the mouthparts of chewing insects. Plants that did not show these characteristics were considered healthy. These procedures were done at each collection site and for each growth condition.

Samples were stripped of leaves, dried in the sun and then ground in a domestic coffee mill. The resulting powder was sifted by a metal kitchen strainer, to ensure the homogeneity of the sample and eliminate coarse particles that interfere with extraction. For the quantification of proline, 24 g of each sample was weighed on an electronic scale (Escali Primo™ model P115M) with an accuracy of 0.1 g, and the compound was extracted with the technique described by Bates in 1973, adapted with the use of a domestic percolator (T-fal® Heliora). The solvent used was purified water in a proportion of 1.8 L per sample; an average volume of 1.75 L of extract per percolation session was obtained.

Before the determination of the proline content, it was necessary to obtain the pre-calibration curve with L-proline at 98.5% purity SAFCTM in progressive dilutions. A Bio-Rad® xMark™ spectrophotometer at a wavelength of 520 nm was used for this purpose. Then the quantification of proline in each of the preparations of the quelite collection was done. The procedure began with the use of a 2-mL Eppendorf-type tube, 600 µL of 3% sulfosalicylic acid was added, stirred to immediately add 400 µL of acid ninhydrin, continued with stirring, and then 400 µL of glacial acetic acid was incorporated. It was heated in a double boiler for an hour and the reaction was stopped with ice for 5 min; 600 µL of toluene were then added and stirred by means of a vortex to separate the phases. The upper or supernatant phase was transferred to a spectrophotometry reading plate with the spectrophotometer previously calibrated at 520 nm. Two measurements were made for both different extracts, each one with three replicates.

To determine the antioxidant capacity of a sample, as percentage of inhibition of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 150 µL of each extract were placed, then 150 µL of DPPH were added at 150 µM and mixed. Then, absorbance at the 490 nm wavelength was measured at 0, 30, and 60 minutes; three replicates were done per sample. In the end, the 60-minute data were not considered because they did not show changes in antioxidant capacity, compared to the samples measured at 30 minutes. To express the antioxidant capacity, we used the formula:

$$\%inhibition = \left(\frac{A_0 - A_s}{A_0} \right) \times 100$$

where, A_0 : Absorbance at 0 minutes; A_s : Absorbance at 30 minutes.

The pre-calibration curve with Ellagic acid was obtained to interpolate and express antioxidant activity as Ellagic acid equivalents (EAeq) per gram of sample. The data obtained from proline were analyzed with the statistical program Infostat® version 2020, analysis of variance ($p \leq 0.05$) and post hoc tests were performed with Tukey's multiple test,

for comparison of means when required. For the DPPH test, because of the number of data ($n=3$) of each treatment, a nonparametric Mann-Whitney U test was performed to explore significant differences; the error was set at a maximum $\alpha=0.05$.

RESULTS AND DISCUSSION

The morphological comparison between quelite plant collections showed differences in growth and endogenous proline accumulation. The plants obtained within the crop fields (favorable conditions) showed a more robust size, greater plant height, bigger number of leaves and lateral branches (Figure 1A). In contrast, specimens collected in marginal lands showed lower vegetative development, smaller size and number of leaves, as well as the presence of insect damage (Figure 1B).

With the analysis of variance, significant differences ($p\leq 0.05$) were found in plant height and percentage of damage by insects; there were non-significant differences in number of lateral branches (Table 1). Tukey means comparison test (Figure 3) confirmed that the greatest damage from partial or total defoliation by insects occurred in plants collected in marginal lands. While those collected in farmlands showed lower incidence of insects. This is probably due to the protective effect of agronomic management provided to the crop of interest in those farmlands.

The result of the analysis of proline content showed that the highest content of the amino acid was obtained from plants collected under unfavorable conditions (34.34 ± 0.82 mg L⁻¹), compared to those developed in association with a crop of interest (32.93 ± 0.87



Figure 1. Comparison between Quelite plants (*Amaranthus hybridus*) collected in the Ejido San Nicolás de los Agustinos, Salvatierra (Guanajuato) Mexico, in 2022. A: plant developed in farmlands. B: plant developed in marginal lands.

Table 1. Comparison of number of lateral branches in Quelite plants (*Amaranthus hybridus*) collected in sites with different conditions (favorable or unfavorable) in Salvatierra (Guanajuato) Mexico.

Treatment	Number of lateral branches
Plants collected in marginal lands	16.57±1.2 a
Plants collected in farmlands	18.37±1.7 a

Source: data analysis in Infostat® 2020 version. Data are means ± standard deviation.



Figure 2. Morphological characteristics and evidence of damage caused by insects in Quelite plants (*Amaranthus hybridus*) collected in Ejido San Nicolás de los Agustinos, Salvatierra (Guanajuato) Mexico, in 2022. Plant developed in marginal lands, with evidence of insect attack (left). Plant developed under favorable conditions (in farmlands, right).

mg L⁻¹) (Table 1). The difference was significant ($p\leq0.05$; Figure 4), which validates the initial hypothesis of the study, that stress increases the antioxidant response in Quelite plants. Also, it agrees with what was reported in other physiological models of response to oxidative stress in plants (Xie *et al.*, 2019).

When comparing the results obtained with values reported by Aja *et al.* (2021) in an investigation in Africa, where the Quelite plant reached proline contents of up to 0.0051 g per gram of dry matter (DM), the samples of our research showed lower values (0.00205 g of proline per g DM). However, these figures are relevant, because they were obtained through extraction techniques that can be repeated, by people in the community, without the need for specialized instruments.

The percentage of inhibition of DPPH and the interpolation of the equivalence with Ellagic acid concentrations, calculated with the pre-calibration curve, were notable for their high values (Table 2). As well as, because the ellagic acid used as a reference antioxidant has

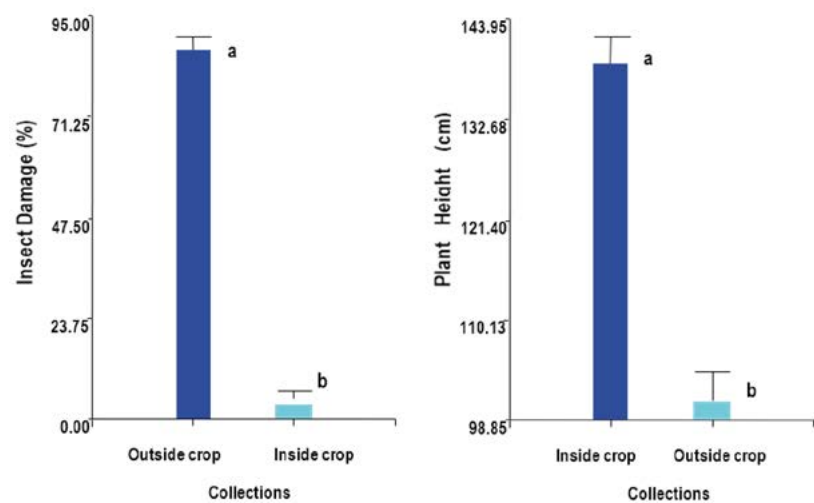


Figure 3. A: Insect damage (%) and B: plant height of Quelite plants (*Amaranthus hybridus*) obtained in favorable and marginal conditions, in Ejido San Nicolás de los Agustinos, Salvatierra (Guanajuato) Mexico, in 2022.

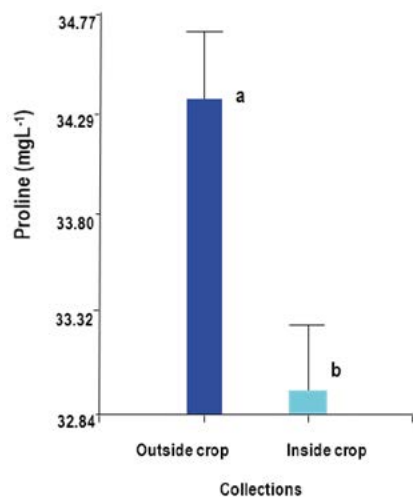


Figure 4. Proline content in Quelite plants (*Amaranthus hybridus*) collected under favorable and marginal conditions in Ejido San Nicolás de los Agustinos, Salvatierra (Guanajuato) Mexico, in 2022.

Table 2. Percentage of inhibition of the DPPH radical (antioxidant capacity) in plant extracts of Quelite (*Amaranthus hybridus*) in two contrasting collection sites in Salvatierra (Guanajuato) Mexico, in 2022.

Extract	Replicate	Inhibition of the DPPH radical (%)
Marginal lands	1	31.60
	2	18.10
	3	16.80
Average		22.00
Farmlands	1	20.90
	2	15.70
	3	10.90
Average		15.80

shown more promise than ascorbic acid, α -tocopherol, BTH (butyl-hydroxytoluene) and BHA (butyl-hydroxyanisole) in their antioxidant capacity, which coincides with Kilic *et al.* (2014). Arró *et al.* (2021) also proved this effect by testing extracts of the shiny oysterwood (or crabwood, *Gymnanthes lucida* Sw.) with the DPPH method; Those authors found that ellagic acid had an average inhibition capacity of $11.75 \pm 0.28 \mu\text{g mL}^{-1}$ and a reducing power of 107% compared to ascorbic acid.

Our data in this research showed a greater antioxidant capacity in the extract of plants collected in sites with greater abiotic deficiencies for the growth of quelite. In plants collected outside farmlands, the average inhibition value was 22%, equivalent to 5.06 mg L^{-1} of ellagic acid. On the other hand, the extract of the plants collected in the sites with better conditions for growth (in farmlands) showed 15.8% inhibition of DPPH, equivalent to 0.98 mg L^{-1} of ellagic acid. These indicate that plant extracts from sites with unfavorable conditions have a greater antioxidant capacity inhibiting the DPPH radical.

According to the results of the non-parametric Mann-Whitney U test (Table 3), although the percentage of inhibition of the DPPH radical was higher in extracts obtained from marginal lands, this test did not show significant differences ($U=2$, $p=0.40$). However, a slight trend towards greater antioxidant capacity was observed, suggesting a possible effect that should be confirmed with a larger sample size.

These results appear to confirm what was expressed at the beginning of this study, showing that the greater the stress condition, the greater the amount of proline and the greater the antioxidant capacity. This shows that the collection sites with the most unfavorable conditions are places with wide potential for the benefit of society. Although they do not have improved conditions from agronomic management, quelites collected there provide food, are innocuous, and cure ailments in the human population. These are valid foundations that can increase interest in the revaluation of many wild plants that are commonly considered weeds.

Our most important result is that proline content in quelite plant is significantly influenced by the ecological conditions of the growing site. Plants collected in marginal environments, exposed to higher levels of biotic and abiotic stress, accumulated higher levels of proline compared to those developed in association with crops, which agrees with Vargas *et al.* (2021). Statistical analyses supported the validity of using proline as a biomarker of nutraceutical quality in this edible species.

From a functional perspective, these findings reinforce the potential of quelites as a nutraceutical food, especially for sectors of the Mexican population that face economic and

Table 3. Results of the nonparametric Mann-Whitney *U* Test for percent of inhibition of the DPPH radical in Quelite (*Amaranthus hybridus*) plant extracts (Salvatierra, Guanajuato, México) in 2022.

U-Statistic	Value
<i>U</i> 1	2
<i>U</i> 2	7
The smallest <i>U</i> value	2
Critical <i>U</i> value at $\alpha=0.05$	2

nutritional constraints. The ability of proline to act as an antioxidant, cell stabilizer, and redox stress modulator places quelite plant (*Amaranthus hybridus*) as a valuable component in diets aimed at preventing chronic non-communicable diseases, including cardiovascular and inflammatory conditions, and certain types of cancer (due to squalene contents yet to be explored). This opens a window of opportunity to design evidence-based interventions that promote its consumption. As well as to articulate food education strategies with a view to sustainable agroecological management.

CONCLUSIONS

The Mexican quelite has an interesting nutritional profile for people to be consumed as a functional food. Such a profile highlights proline as an indicator and bioactive agent of nutraceutical interest. Adverse environmental growing conditions can favor the biosynthesis of beneficial compounds for human health in this plant. It appears as necessary to give a strategic value to systematic collection of quelite in wild environments.

Despite it is considered as weed, quelite represents a valuable and abundant resource, not only an accessible source of essential nutrients, but also a bio-cultural plant resource that can be integrated into public policies aimed at combating malnutrition, increasing food sovereignty and at the revaluation of local agricultural biodiversity. It is considered as a priority to establish sustainable collection protocols; to standardize processing methods that guarantee the nutraceutical quality of the plant, and to promote awareness campaigns that identify quelite as an accessible, culturally rooted functional food with scientific validation.

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One hundred weeks of solitude: did the pandemic impact the scientific output of female researchers in three Mexican public research centers?

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ABSTRACT

Objective: This study analyzes the evolution of scientific production by female researchers at three public research centers in Mexico (CIBNOR, CICESE, and CIAD) during the period 2014-2024, with an emphasis on the impact of the pandemic.

Design/methodology/approach: A bibliometric analysis was conducted based on the Scopus database, considering scientific articles, book chapters, and reviews.

Results: The overall results show that the pandemic did not cause a decline in output. An increase was observed at CIBNOR and CICESE, while CIAD experienced a decline in 2022 before recovering in 2024.

Limitations on study/implications: The study is restricted to the Scopus database, recognized for including the largest number of journals worldwide, although the omission of other databases could cause bias in the results. However, we consider that this limitation will not significantly affect the identified trends.

Findings/conclusions: This finding suggests that the impact of the pandemic on scientific productivity was not uniform and that institutional factors, adaptation strategies, and changes in funding and evaluation policies played a key role in the recovery. It is concluded that, although the overall trend was one of growth, the fluctuations observed reflect the complex interaction between external events, gender inequalities, and scientific policies.

Keywords: scientific articles, women, COVID-19, bibliometrics, gender equity.

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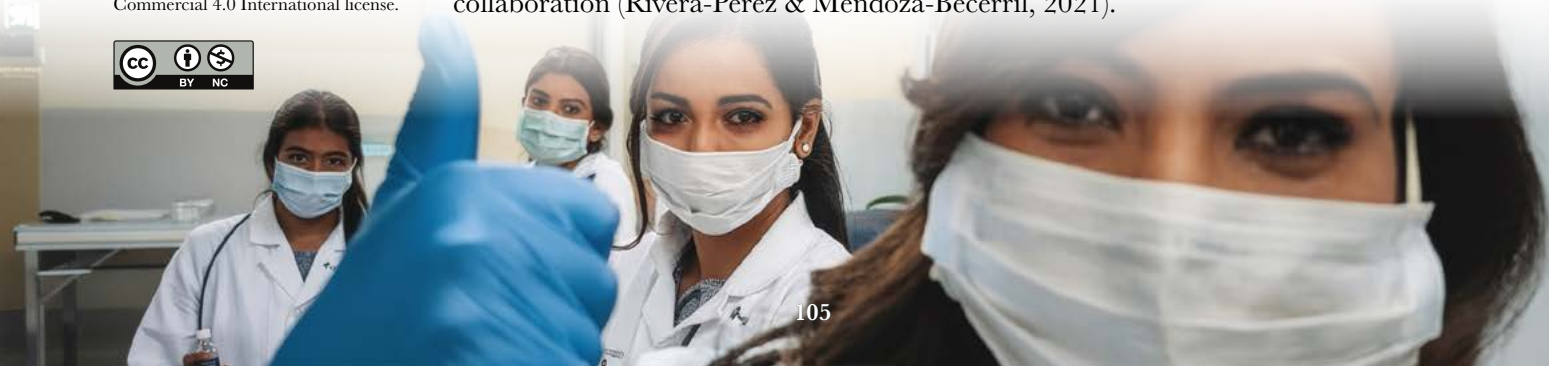
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INTRODUCTION

The COVID-19 pandemic brought with it a series of unprecedented challenges for the global scientific community, affecting both academic production and the work dynamics of researchers. In Mexico, the health contingency led to the closure of higher education institutions and public research centers, forcing researchers to adapt their activities to remote formats, facing limitations in access to laboratories, funding, and academic collaboration (Rivera-Pérez & Mendoza-Becerril, 2021).



According to various publications, one of the sectors most impacted by the pandemic was that of female researchers, who experienced a significant reduction in their scientific output compared to their male colleagues (Cui *et al.*, 2021; Lee *et al.*, 2023). Additionally, it has been documented that female academic faced a greater domestic and caregiving workload, which reduced their available time for research and affected their ability to generate new projects (Infante *et al.*, 2021; Cornejo Hernández, 2022). It has also been mentioned that administrative overload and the need to transform their teaching methodologies into digital environments have exacerbated the difficulties in maintaining their productivity (Cornejo Hernández *et al.*, 2023).

This study analyzes the scientific output of female researchers at three public research centers in Mexico over the last decade. Through the analysis of bibliometric indicators, it seeks to identify whether there was a significant variation in the number of articles published between 2020 and 2024, with an emphasis on the factors that may have influenced this trend, particularly the impact of the COVID-19 pandemic. The findings of this study will provide a better understanding of gender gaps in scientific output and contribute key information for the design of institutional strategies that promote equity in research. This study is based on a literature review that contextualizes the problem, analyzes relevant background information, and establishes the relationship with previous research.

MATERIALS AND METHODS

To assess the impact of the COVID-19 pandemic on the scientific output of female researchers in public research centers in Mexico, a bibliometric analysis was conducted based on data obtained from the Scopus database. A sample of 50 researchers was randomly selected from a total of 103 researchers involved in research in aquaculture, fisheries, aquatic biology, and related disciplines such as oceanography, aquatic ecology, marine biotechnology, or similar fields, registered at three Public Research Centers of the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SEHCITI):

- Centro de Investigaciones Biológicas del Noroeste (CIBNOR)
- Centro de Investigación en Alimentación y Desarrollo (CIAD)
- Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE)

The names of the researchers were provided directly by their respective institutions at the request of the postdoctoral researcher. The search for scientific output was conducted in the Scopus database, using the following criteria:

- Name of the researcher (as the main criterion).
- Name of the institution (as an affiliation filter).
- Analysis period: Publications between 2014 and 2024.

Scientific articles, book chapters, and reviews were included, regardless of the researcher's position on the list of authors. To avoid attribution errors, each publication was manually verified to ensure that it actually corresponded to the selected researcher.

The number of annual publications by each researcher was recorded in an Excel spreadsheet. Subsequently, timeline graphs were generated to analyze the evolution of scientific production at the following levels: i) By research center, at, where a line graph was created for each of the three centers, and ii) a consolidated analysis, in which the data from the three centers were integrated into a single general timeline graph.

This analysis made it possible to identify trends in the scientific output of female researchers, with a special emphasis on possible variations during the pandemic (2020-2024).

RESULTS AND DISCUSSION

The three public research centers show variable scientific output over the ten-year period.

Analysis of the scientific output of researchers at the three public research centers (CIBNOR, CICESE, and CIAD) between 2014 and 2024 shows a general upward trend, although with notable fluctuations throughout the period analyzed (Figure 4). Although the COVID-19 pandemic caused a drop in productivity in 2020, the recovery was not uniform across the different centers, suggesting that institutional factors and changes in funding policies may have influenced publication dynamics.

Production at CIBNOR follows a pattern of moderate growth, with fluctuations but an overall upward trend. In 2020, production registered 38 publications, while in 2022 there was a peak of 47 publications. In 2024, production declined slightly again to 34 publications.

At CIBNOR, scientific production showed significant variations in the period 2014-2018, with an increase in 2016 followed by a decrease in 2017 (Figure 1). From 2019 onwards, sustained growth is observed, reaching its peak in 2022. This increase could be related to the publication of works delayed by the pandemic or to an institutional reorganization that facilitated the productivity of female researchers. However, in 2023 there is a sharp drop, which could be due to changes in the criteria of the National System

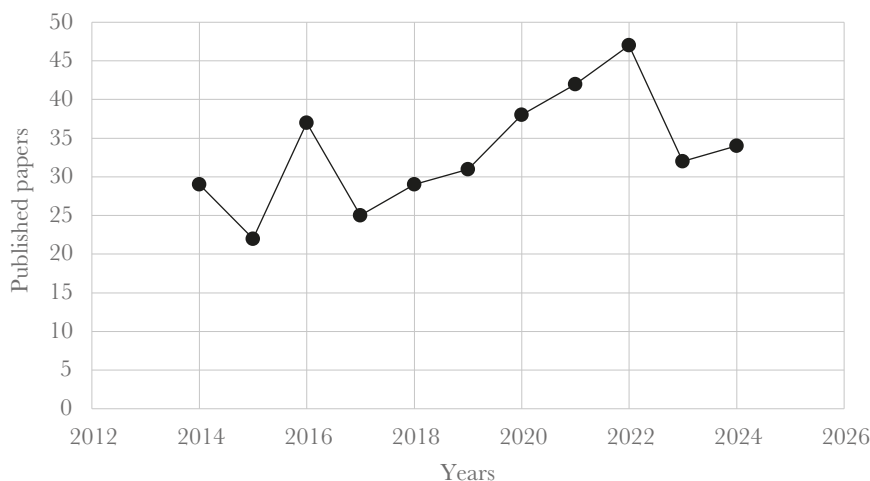


Figure 1. Timeline of scientific publications by female researchers at CIBNOR. Own elaboration based on data obtained from SCOPUS.

of Researchers (SNI) and the implementation of the National Postgraduate System (SNP), which may have affected the prioritization of academic activities. In 2024 there is a slight rebound, although without reaching the levels prior to the drop.

CICESE's productivity shows a sharp decline in 2020 (20 publications), making it the center with the greatest negative impact during the pandemic. The recovery is notable in the following years, reaching 41 publications in 2021 and 35 in 2022. In 2024, production stands at 29 publications, indicating that the recovery has not been sustained at this center. Production at CICESE shows a pattern of growth interrupted by fluctuations in different periods (Figure 2). Between 2014 and 2015, productivity was low, with fewer than 10 publications. From 2016 onwards, there was an increase to a peak of around 30 publications, followed by relative stability until 2018. However, in 2020, productivity declined significantly, coinciding with the global health crisis. Unlike CIBNOR, CICESE shows a significant rebound in 2021, exceeding 40 publications, suggesting that some research postponed during the pandemic may have been completed and published during this period. Nevertheless, from 2022 onwards, production begins to decline progressively until 2024, which could indicate a long-term impact of the health crisis or the reallocation of efforts to other academic activities.

At CIAD, the impact of the pandemic in 2020 does not appear to be as severe as at the other centers, as it maintains 40 publications, similar to 2019. However, in 2022, production falls to 33 publications, showing a later recovery. By 2024, CIAD has the highest number of publications among the three centers, with 53 publications.

In the case of CIAD, scientific production shows a more stable trend compared to CIBNOR and CICESE, although it also presents significant fluctuations (Figure 3). Between 2014 and 2016, productivity remained at around 30 publications per year, with no significant variations. In 2017, however, there was a sharp drop, reducing production to around 20 publications. Subsequently, between 2018 and 2020, production experienced sustained growth until stabilizing at around 40 publications. Unlike the other centers, in 2020 the pandemic does not seem to have caused an immediate decline in productivity, suggesting that researchers at this center may have found more effective strategies to continue

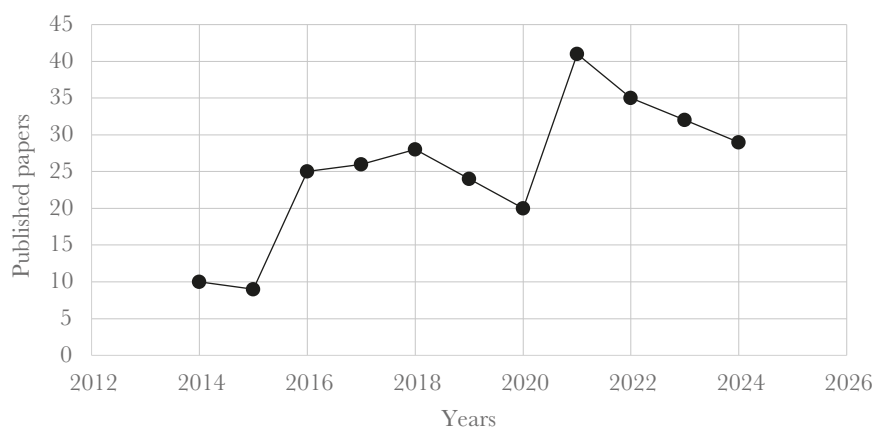


Figure 2. Timeline of scientific publications by female researchers at CICESE. Own elaboration based on data obtained from SCOPUS.

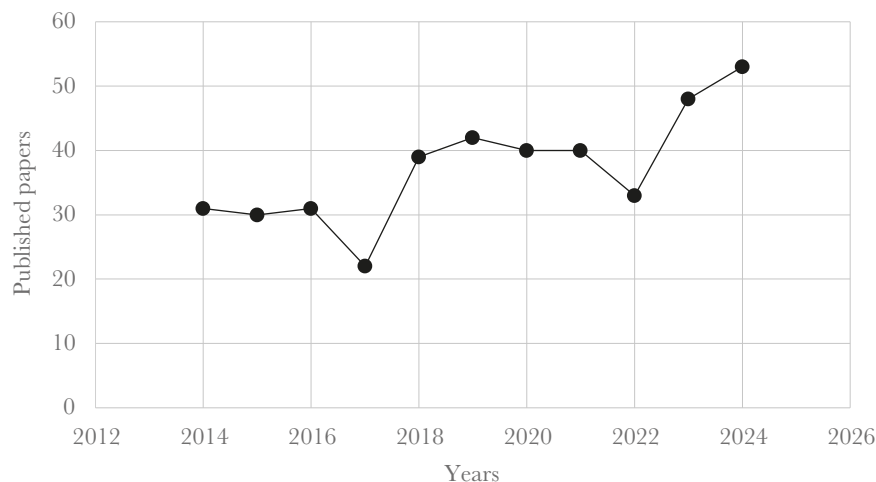


Figure 3. Timeline of scientific publications by female researchers at CIAD. Own elaboration based on data obtained from SCOPUS.

their projects under lockdown conditions. However, in 2022, there is a sudden new drop, which could be associated with the implementation of new scientific policies or difficulties in the allocation of funding. In 2023 and 2024, productivity recovers significantly, reaching the highest level of the period analyzed, with more than 50 publications in the last year.

In general terms, the graph of totals (Figure 4) shows a pattern of growth in the scientific output of female researchers in the three centers combined, although with moments of crisis. There was a decline between 2014 and 2016, followed by sustained growth until 2020. With the pandemic, production temporarily declined, but from 2021 onwards there has been a gradual recovery, with an upturn of 116 publications by 2024. However, the decline in 2023 suggests that the effects of the health crisis and changes in scientific policies are still impacting academic productivity.

These results show that, although female researcher's scientific output has shown a positive long-term trend, the fluctuations observed may be linked to factors such as the

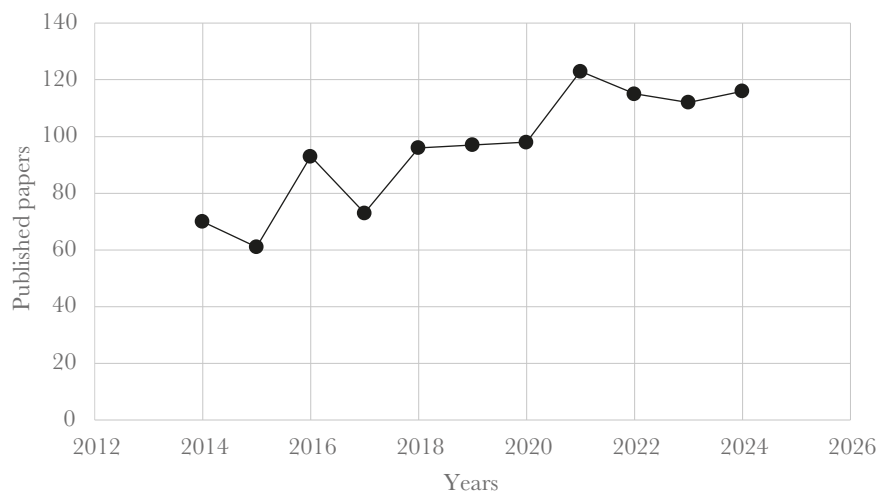


Figure 4. Consolidated timeline of scientific publications by female researchers from the three public research centers. Own elaboration based on data obtained from SCOPUS.

pandemic, the availability of funding, and changes in productivity assessment mechanisms in Mexico. The heterogeneity in post-pandemic recovery among the three centers suggests that institutional conditions played a key role in female researchers' ability to resume their scientific output.

The results show a general trend of growth in the scientific output of female researchers during the period 2014-2024. However, significant fluctuations are also observed, particularly in 2017, 2020, and 2023, which may be associated with structural, administrative, and social factors that have impacted the publication dynamics of female scientists in Mexico.

Overall, the COVID-19 pandemic (2020-2022) did not have an immediate negative impact on the total scientific output of female researchers at these centers. In fact, CIBNOR and CICESE saw an increase in productivity in the following years. This phenomenon can be explained by several hypotheses.

The drop in productivity in 2020 may have been a consequence of the interruption of ongoing research and the closure of laboratories. However, in subsequent years, many researchers managed to complete their projects and submit them for publication, generating a "rebound effect" in 2021-2022 (Squazzoni *et al.*, 2021). This phenomenon has already been documented in other studies, which indicate that the pandemic did not necessarily reduce scientific output permanently, but rather altered the dynamics of publication, with a temporary shift in articles (Myers *et al.*, 2020).

Digital infrastructure and access to collaborative networks may have been key factors in the recovery of scientific output. If CIBNOR and CICESE managed to implement effective remote working strategies and facilitated communication among researchers, their productivity may have remained steady or even increased compared to other centers. In contrast, CIAD experienced a decline in 2022, which could indicate that its researchers faced greater barriers to regaining their post-pandemic publication pace.

Although it has been reported that many researchers devoted more time to teaching and administrative tasks during the pandemic, it is possible that at CIBNOR and CICESE some scientists managed to reorganize their time and prioritize writing articles. It is also possible that certain research groups took advantage of this period to strengthen theoretical production and literature review, which may have facilitated the increase in publications at these centers.

One of the most relevant findings is the decline in scientific production in 2020, followed by a rebound in 2021-2022 and a further decline in 2023. This pattern has been reported in other studies on the impact of the pandemic on science, which highlight that female academics faced greater difficulties in maintaining their productivity due to domestic and care workloads, adaptation to online teaching, and lack of access to laboratories and scientific collaboration networks (Viglione, 2020; Squazzoni *et al.*, 2021). The gender gap in post-pandemic recovery has also been documented. Studies have shown that while male scientists were able to recover their publication levels more quickly, many women experienced a prolonged decline in their academic output due to extra-professional responsibilities that limited their research time (Cui *et al.*, 2021; King & Frederickson, 2021). This would partly explain the decline observed in 2023, when the

cumulative effects of work overload became more evident in the productivity of female researchers.

The decline in scientific output in 2017 and 2023 could be linked to changes in science funding and evaluation policies in Mexico. In 2017, the reform of the National System of Researchers (SNI) and adjustments to funding mechanisms may have generated uncertainty and a restructuring of research priorities, which affected the publication of scientific articles.

More recently, in 2022-2023, the implementation of the National Postgraduate System (SNP) to replace the PNPC, as well as changes in the SNI's evaluation criteria, may have influenced the prioritization of academic activities other than the publication of articles. Researchers may have focused on activities that were given greater weight in the new systems, such as outreach, dissemination, or teaching, pushing the production of scientific publications into the background.

The analysis by center reveals that CIAD showed the fastest recovery in 2023 and 2024, while CICESE and CIBNOR have experienced more pronounced declines in their recent publications. This suggests that institutional conditions and adaptation strategies have differed across centers.

Previous studies have pointed out that job flexibility and the availability of institutional support can make a difference in the ability of female scientists to resume their productivity after crises or structural changes (Myers *et al.*, 2020). CIAD may have implemented more effective strategies to support female researchers, facilitating their recovery, while at other centers, post-pandemic conditions may have made it difficult to return to previous publication levels.

The literature has pointed out that women scientists face additional barriers in academia, including heavier teaching and administrative loads, smaller international collaboration networks, and challenges in accessing funding and leadership in projects (Huang *et al.*, 2020; Malisch *et al.*, 2020). These inequalities may have influenced the variability observed in the scientific output of women researchers at the centers analyzed.

In addition, recent social movements, such as #MeTooAcadémicosMX (2019) and feminist strikes at Mexican universities (2020-2021), have highlighted structural problems of gender inequality in academia. It is possible that the increased attention to these issues has had an impact on the workload of female researchers, who have been actively involved in implementing gender equality policies at their institutions, taking time away from their research.

CONCLUSIONS

The results reflect the complex interaction between external events, scientific policies, and gender inequalities in the scientific output of female researchers in Mexico. Although the overall trend has been one of growth, the fluctuations observed in 2017, 2020, and 2023 suggest that female scientists have faced significant challenges at different points during the period analyzed.

The impact of the pandemic remains evident in the uneven recovery of productivity, with a late decline in 2023 reflecting the persistence of the cumulative effects of work

overload and changes in the science and technology system. Differences between research centers highlight the importance of institutional conditions and specific support for women researchers.

To achieve a sustained recovery and close the gender gap in science, it is essential to implement policies that facilitate the reconciliation of research with other academic and personal responsibilities, as well as to ensure equitable conditions in the distribution of funding and the evaluation of women's scientific output in academia.

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Economic valuation scenarios for native corn tortillas using Contingent Valuation and Discrete Choice Experiments

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ABSTRACT

Objective: To analyze respondents' willingness to pay (WTP) for tortillas with specific attributes handmade, produced from native corn, organic, and traditionally nixtamalized using models derived from the contingent valuation method (CVM) and discrete choice experiments (DCE), while varying the levels of socioeconomic variables and product attributes.

Design/Methodology/Approach: The study employed stated preference methods, specifically contingent valuation and discrete choice experiments, to estimate the economic value assigned to tortillas with differentiated attributes. Binomial, multinomial, and mixed logit models were estimated, and multiple scenarios were constructed for municipalities within the metropolitan area of Mexico City. The analysis examined changes in the probability of an affirmative response to the WTP question as a function of variations in respondents' income, educational level, number of dependents, and a 10% reduction in tortilla price.

Results: The findings showed that stated preference methods enabled the estimation of respondents' economic valuation of tortillas with differentiated attributes. The probability of answering "yes" to the WTP question varied according to income, education, number of dependents, and price reductions. Among the attributes evaluated, blue tortilla color was the most highly valued, followed by native corn, organic corn, and tortillas with added ingredients. The highest compensatory variation for organic, native-corn, blue tortillas with added ingredients reached 51.59 MXN kg⁻¹, whereas the lowest was 32.29 MXN kg⁻¹. At the municipal level, Coacalco de Berriozábal assigned the greatest value to tortillas with the analyzed attributes, followed by Texcoco, Tlalnepantla, and Cuautitlán Izcalli.

Limitations/Implications: Although stated preference methods are highly useful for valuing goods and services for which no alternative methodology is suitable, they continue to face criticism because they rely on hypothetical behavioral data. Nevertheless, their application provides valuable evidence for the valuation of differentiated products and for understanding potential market behavior toward new goods.

Findings/Conclusions: The combination of stated preference methods, contingent valuation, and discrete choice experiments constitutes a valid and reliable methodological framework for estimating the economic value of goods and services. The results were statistically comparable to those obtained through other economic valuation approaches, confirming the robustness of these methods for assessing consumer preferences and WTP for tortillas with value-added attributes.

Keywords: stated preference, willingness to pay, *Zea mays* L.

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INTRODUCTION

The economic valuation of a good or service can be conducted in different ways, either by using market price information or by eliciting consumer preferences through a wide range of non-market valuation methods, which are broadly classified into two major groups: revealed preference methods and stated preference methods. The latter have been frequently criticized because of their reliance on hypothetical behavioral data. According to Haab *et al.* (2020), stated preference methods should be regarded as approaches for providing estimates of the value of certain changes in the allocation of environmental and natural resources for which no alternative method can be applied. Among these, the Contingent Valuation Method (CVM) is the most widely used. One of its principal strengths lies in its flexibility; moreover, because of its hypothetical nature and its independence from existing markets, CVM can be applied to almost all non-market goods, as well as to past and future changes. It is also one of the few available methods capable of capturing all types of benefits associated with a non-market good or service, including those unrelated to current or future use, namely, the so-called non-use values (Hoyos and Mariel, 2010; OECD, 2018). Nevertheless, despite thousands of studies, substantial methodological advances, and extensive policy applications, contingent valuation remains a source of controversy. Critics argue that the values obtained are hypothetical, since payments are not actually made in cash, and that the method is also subject to numerous biases (Van den *et al.*, 2011). Even so, supporters of CVM have found that it produces value estimates comparable to those derived from revealed preference methods, such as the travel cost and hedonic pricing methods.

The popularity of CVM has declined as best practices have increasingly converged with Discrete Choice Experiments (DCE). The number of articles reporting DCE applications in food research evolved from a single publication in 2001 and 2002 to nearly 100 studies per year by 2019 and 2020. Likewise, the number of DCE studies addressing organic production or organic foods has grown steadily. Whereas organic production was previously regarded mainly as a healthier and safer alternative to conventional production, it is now also recognized as a provider of environmental benefits, extending beyond health and safety concerns. At present, DCEs have become a frequently employed research method in food studies due to their capacity to uncover the trade-offs involved in choosing among multiple alternatives, particularly when credence attributes are under consideration (Haab *et al.*, 2020).

The main difference between choice experiments and CVM is that the former involve trade-offs among alternatives, whereas in the latter respondents express their willingness to pay (WTP) on the basis of a proposed change. Currently, many economists tend to favor DCEs as an elicitation method on the grounds that the marginal values of goods and services are easier to estimate. In addition, DCEs provide richer information by offering respondents multiple alternatives, thereby reducing response problems and certain biases associated with CVM (Van den *et al.*, 2011).

As can be observed, stated preference methods are highly useful and encompass fields of economics that could not be investigated through any other methodological approach. One way to assess their validity is by constructing scenarios based on real data. Accordingly, the

objective of the present research is to analyze respondents' willingness to pay by generating scenarios and using one model obtained through CVM and another through DCE, while varying the levels of socioeconomic variables and product attributes. The hypothesis is that the combination of stated preference methods, contingent valuation, and discrete choice experiments constitutes a valid and reliable methodology for estimating the economic value of goods and services, yielding results that are statistically comparable to those obtained through other economic valuation methodologies.

MATERIALS AND METHODS

To obtain the data, face-to-face surveys were conducted from January to March 2024 in 15 municipalities of the State of Mexico, the most populated state in the country: Chalco, Chicoloapan, Chimalhuacán, Coacalco de Berriozábal, Ecatepec de Morelos, Ixtapaluca, Cuautitlán Izcalli, Naucalpan de Juárez, Nezahualcóyotl, La Paz, Tecámac, Texcoco, Tlalnepantla, Tultitlán, and Valle de Chalco Solidaridad.

The target population, defined as individuals older than 18 years, was identified from the total population of the State of Mexico (16,992,418 inhabitants, according to the Population and Housing Census) (INEGI, 2020). Following the recommendations of Ahmed (2024), the sample size was determined using an equation for large or infinite populations:

$$n = \frac{Z^2 pq}{d^2}$$

where: n is the sample size, Z is the z -value associated with the desired confidence level (1.96 for a 95% confidence level); p is the proportion of the population willing to pay for the improvement (equal to 0.5); q is the proportion of the population unwilling to pay (equal to 0.5); and d is the permitted estimation error or precision (7%).

For the survey design, focus group discussions were conducted with housewives, consumers, and subject-matter experts. Subsequently, preliminary studies were carried out with 100 consumers in order to detect possible misinterpretations of the questions. Using the sample size equation, the administration of 196 questionnaires was estimated; however, because four price levels were evaluated, a total of 216 surveys were conducted, distributed as 54 for each proposed price.

Contingent Valuation Method (CVM)

The referendum model consists of presenting the respondent with two response alternatives: yes or no. Since the dependent variable is discrete, the regression analysis is performed using either a logit or probit model. In the present research, the logit model will be used.

According to Tudela *et al.* (2018), the modeling process considers the random utility approach, as shown in Equation 1.

$$U_{ih} = v_{ih}(p_h, M_i, s_i) + \varepsilon_{ih} \quad (1)$$

where: the utility of alternative h for individual i is a function of s , which represents individual characteristics; p_h , which denotes the price of alternative h , and M , which corresponds to the individual's income. Utility is composed of a deterministic component (v_{ih}) and an unobservable random error component (ε_{ih}), which is independently and identically distributed (iid), with zero mean and constant variance. Individual i chooses the alternative that provides the greatest utility; therefore, the behavioral model is defined as follows: the individual chooses alternative h if and only if: $U_{ih} > U_{ij}, \forall h \neq j$. The probability that the individual chooses alternative h is given by equations 2 and 3:

$$\Pr(h) = \Pr\{U_{ih} > U_{ij}\} \quad (2)$$

$$\Pr(h) = \Pr\{\varepsilon_{ij} - \varepsilon_{ih} < v_{ih}(p_h, Z_{ih}, M_i, s_i) - v_{ij}(p_j, Z_{ij}, M_i, s_i)\} \quad (3)$$

To estimate welfare impacts, that is, willingness to pay (WTP) for a change from the status quo (alternative j) to the selected state (alternative h), Equation 4 is used:

$$\Delta v = v_{ih}(p_h, M_i - VC, s_i) - v_{ij}(p_j, M_i, s_i) \quad (4)$$

where VC denotes the compensating variation, which may be interpreted as the maximum amount of money an individual would be willing to pay in order to obtain a favorable change. In this case, for individual i , alternative h improves the level of well-being relative to alternative j .

Willingness to pay (WTP) for each respondent was estimated using Equation 5:

$$WTP_i = \frac{\alpha + \beta_2(INGR) + \beta_3(GEN) + \beta_4(EST) + \beta_5(DEP) + \beta_6(PRECIOKG)}{-\beta_1} \quad (5)$$

$$i = 1, 2, \dots, 216$$

where: *INGR* represents monthly monetary income; *GEN* denotes the respondent's gender; *EST* refers to the level of education; *DEP* indicates the number of economic dependents of the household head, and *PRECIOKG* corresponds to the price per kilogram of tortillas at the establishment where the respondent purchases them.

Binomial Logit Model

The willingness-to-pay (WTP) equation for the contingent valuation method (CVM) model was as follows:

$$\begin{aligned}
 WTP_i = & 5.45716259 - 0.13920993(BI) + 0.0000546293(INGR) \\
 & + 0.59357879(GEN) + 0.12629926(EST) - 0.09267390(DEP) \\
 & - 0.06552966(PRECIOKG)
 \end{aligned} \quad (6)$$

BI denotes the initial bid price, whereas the remaining variables were defined in Equation 5.

Scenarios to Be Analyzed

This analysis aims to determine the impact on predicted probabilities when a particular variable is changed over a range of values, while the remaining variables are held constant at their mean values. For this purpose, the following scenarios were considered:

- Scenario 1: A 10% reduction in the initial bid price (*BI*). One of the current policies of the federal government is to gradually reduce the price of tortillas by up to 10% over the six-year administration. To this end, the National Corn-Tortilla Agreement was signed with producers, traders, flour manufacturers, nixtamal producers, and tortilla makers (Rojas, 2025).
- Scenario 2: An increase of 1,000 pesos in the respondent's monthly monetary income (*INGR*). According to González-Juárez *et al.* (2024), lower income is associated with lower willingness to pay, whereas willingness to pay increases as income rises.
- Scenario 3: An increase of one additional level in the respondent's educational attainment (*EST*). Numerous studies have reported that consumers with a higher level of education exhibit a greater willingness to pay for differentiated goods or services (Lugo *et al.*, 2020; Melo *et al.*, 2022), including organic or blue tortillas (Jaramillo, 2016; Blare *et al.*, 2020).
- Scenario 4: A reduction of one economic dependent (*DEP*) in the respondent's household. This scenario is considered because, at the national level, the birth rate has been declining; in 2023, the reduction was 2.3% compared with the previous year (INEGI, 2024).
- Scenario 5: A 10% reduction in the price per kilogram of tortillas at the establishment where the respondent purchases them. This scenario was created for the same reason as Scenario 1, namely, the policy aimed at reducing the price of tortillas by 10% (Rojas, 2025).

Discrete Choice Experiments

Discrete choice experiments use surveys as a basis for inferring willingness to pay or willingness to accept compensation for a modification in a good or service taking place within a hypothetical market (Melo *et al.*, 2020). They have been applied in diverse fields, including environmental valuation, food studies (Lizin *et al.*, 2022), and health research (Smith *et al.*, 2022).

In a choice experiment, respondents are presented with a series of choice alternatives referring to different states of a good or service and are asked to select their preferred option. In general, these alternatives are organized into choice sets, in which one alternative is fixed and describes the current situation (*status quo*), while the remaining alternatives vary and represent changes relative to the current situation. The different alternatives contain attributes and levels. Attributes are used to explain to respondents both the current state and its possible modifications, whereas different attribute values, referred to as levels, are employed to describe changes from the current state. The importance of choice experiments in economic valuation lies in the estimation of welfare measures associated with the selection of different alternatives that affect individual well-being. For this purpose, multinomial discrete choice models are generally employed, such as the multinomial/conditional logit model and/or the mixed logit model (Tudela and Leos, 2017).

Multinomial Logit Model

$$V_{ij} = 0.53961(PAO) + 0.06621(HM) - 0.23527(MMN) + 0.55857(MCN) + 0.24736(AZUL) + 0.12875(CC) - 0.04251(PRECIO) \quad (7)$$

Mixed Logit Model with Interaction

$$V_{ij} = 0.52092100(PAO) - 0.91485637(HM) - 3.57294973(MMN) + 0.97411341(MCN) + 1.10813979(AZUL) + 0.07069074(CC) - 0.14942091(PRECIO) + 0.0000605394\text{ }ING1 + 0.41270665\text{ }EST1 + 0.0000307095\text{ }ING2 + 0.42460744\text{ }EST2 \quad (8)$$

where: V_{ij} stands for the probability of alternative j chosen by individual i ; PAO for Organic Agricultural Production; HM denotes Corn Flour; MMN refers to Improved Nixtamalized Corn; MCN represents Native Nixtamalized Corn; $AZUL$ corresponds to Blue Corn; CC indicates Tortilla with Added Ingredients; $PRECIO$ is the proposed price; EST refers to the level of education, and ING denotes monetary income.

Scenarios to Be Analyzed Using a Mixed Logit Model with Interaction

Scenario 1

Current situation: tortillas are made from corn and/or flour derived from conventional agricultural production.

Scenario: tortillas are made from corn and/or flour derived from organic agricultural production (PAO).

Based on the findings reported by different authors, consumers are willing to pay more for a product with the organic attribute than for one produced conventionally (Ching *et al.*, 2020; Rossi *et al.*, 2024). Similar results have also been reported for tortillas (Jaramillo, 2016).

Scenario 2

Current situation: tortillas are made from a mixture of improved nixtamalized corn and flour.

Scenario: tortillas are made from native nixtamalized corn (MCN).

This scenario is evaluated in light of the current federal government policy aimed at promoting the conservation, production, processing, and commercialization of native Mexican corn (Presidencia de la República, 2025).

Scenario 3

Current situation: tortillas are made from white corn.

Scenario: tortillas are made from blue corn (AZUL).

This scenario is evaluated on the basis of the same policy framework as Scenario 2.

Scenario 4

Current situation: tortillas are offered without any added ingredients.

Scenario: the dough or flour used to make tortillas is supplemented with seeds and/or vegetables such as nopal, epazote, chia, or others (CC).

In recent years, consumer preferences for healthier and more environmentally friendly products have increased, and consumers place greater value on the nutritional contribution of the foods they consume (Aprile *et al.*, 2016; Priya, 2023).

Scenario 5

Current situation: tortillas are made from a mixture of improved white nixtamalized corn and flour derived from conventional agricultural production.

Scenario: tortillas are made from native blue corn produced under organic agricultural practices and are supplemented with seeds and/or vegetables (ALL).

The multinomial logit model will be used to obtain the probabilities of each alternative.

RESULTS AND DISCUSSION

The models were estimated from a sample comprising 216 respondents, of whom 68% were women, with a mean age of 47 years. Most participants had completed high school education (27%), followed by a bachelor's degree (23%) and middle school education (22%). On average, households consisted of four members and two economic dependents. The respondents' average monthly income was 10,200 Mexican pesos. Regarding tortillas, the average price was 20 Mexican pesos per kilogram; 51% of respondents purchased tortillas every day; 65% knew what an organic product is; 40% were familiar with hybrid corn; 75% recognized creole or native corn; 80% knew what nixtamalization is; and 75% believed that the tortillas they consumed were made from a mixture of corn flour and hybrid corn dough.

The following graph (Figure 1) presents the attributes evaluated and their order of importance for respondents, considering 1 as the most important and 4 as the least important. It can be observed that, for most respondents, the attributes "agricultural

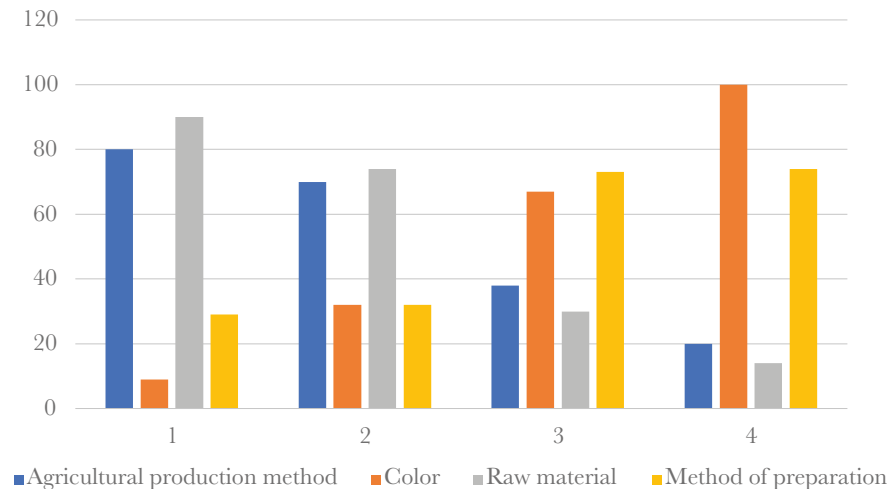


Figure 1. Importance of attributes in an artisanal tortilla. Source: Prepared by the authors based on survey data.

production method” and “raw material” were the most important, whereas color and method of preparation were regarded as less important.

The following graph (Figure 2) presents the average income and educational level across the municipalities evaluated. It can be observed that the municipality of Coacalco de Berriozábal exhibited the highest levels of both education and income, whereas the municipality of La Paz showed the lowest levels for these same variables. Overall, a clear pattern can be identified: as educational attainment increases, income level also rises, and *vice versa*. These findings are consistent with those reported by Stryzhak Olena (2020), who indicates that education contributes to higher income levels.

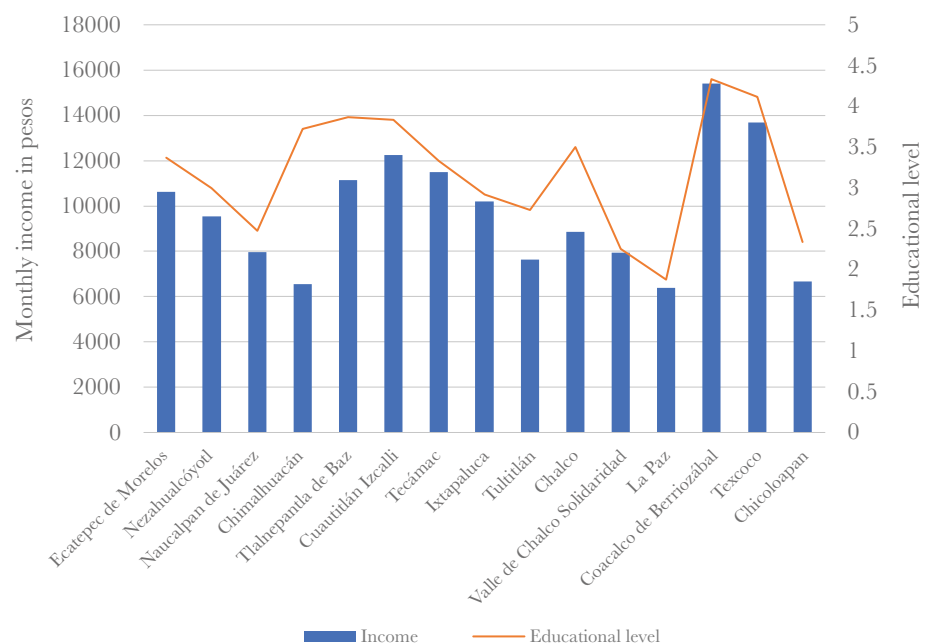


Figure 2. Educational level and income in the evaluated municipalities. Source: Prepared by the authors based on survey data.

Binomial Logit Model Scenarios

The following graphs (Figure 3) present the probabilities of willingness to pay in relation to the corresponding explanatory variables. The observed behavior is consistent with economic theory. The variables initial bid price (BI) (Figure 3-A), economic dependents of the household head (DEPEN) (Figure 3-D), and the price per kilogram of tortillas at the establishment where the respondent purchases them (PRECIOKG) (Figure 3-E) exhibit a negative relationship. In other words, as the initial bid price, the number of economic dependents, or the cost per kilogram of tortillas increases, the probability of willingness

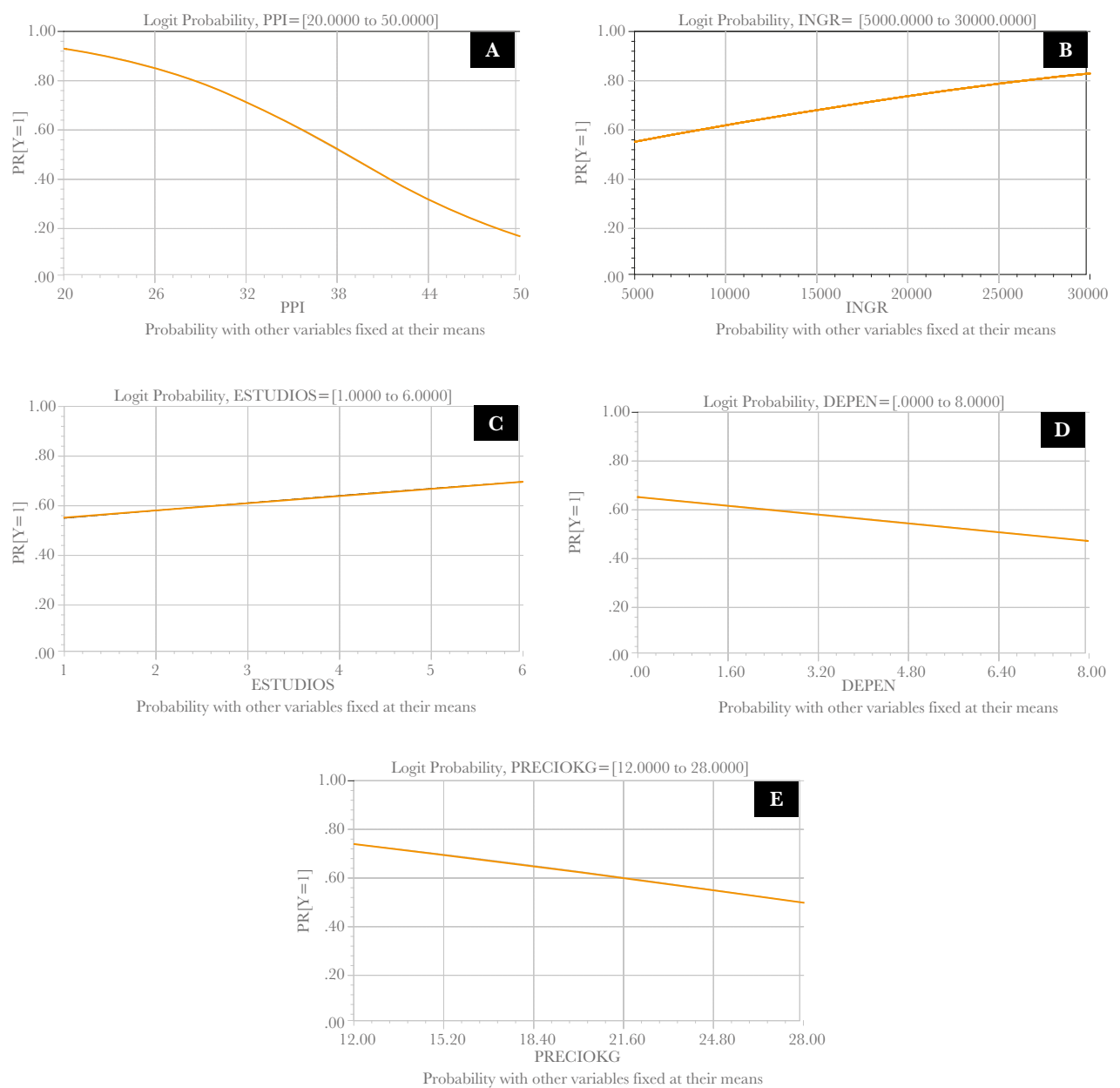


Figure 3. Predicted probability of willingness to pay (WTP) according to the initial bid price (A), monthly monetary income (B), educational level (C), economic dependents of the household head (D), and the price per kilogram of tortillas at the establishment where the respondent purchases them (E), using N-logit.

to pay decreases. By contrast, as monthly monetary income (INGR) (Figure 3-B) and educational level (ESTUDIOS) (Figure 3-C) increase, the probability of willingness to pay also rises, indicating a positive relationship.

The following table (Table 1) presents the results of the analyzed scenarios. In Scenario 1, a reduction in the initial bid price (BI) was proposed in response to the new federal government policies aimed at achieving a 10% reduction in tortilla prices (Rojas, 2025). If BI decreases by 10%, the probability of responding YES to the willingness-to-pay question

Table 1. Simulation results in the binomial logit model.

Scenario 1. Effect on aggregate proportions. The threshold T^* of the logit model to calculate the $\text{fit}=1[\text{Prob}>T^*]$ is .50000. Changing variable=BI, Operation=*, value=0.900.			
Result	Base	Scenario	Change
0	88=40.74%	58=26.85%	-30
1	128=59.26%	158=73.15%	30
Total	216=100.00%	216=100.00%	0
Scenario 2. Effect on aggregate proportions. The threshold T^* of the logit model to calculate the $\text{fit}=1[\text{Prob}>T^*]$ is .50000. Changing variable=INGR, Operation=+, value=1000.000.			
Result	Base	Scenario	Change
0	88=40.74%	84=38.89%	-4
1	128=59.26%	132=61.11%	4
Total	216=100.00%	216=100.00%	0
Scenario 3. Effect on aggregate proportions. The threshold T^* of the logit model to calculate the $\text{fit}=1[\text{Prob}>T^*]$ is .50000. Changing variable=STUDIES, Operation=+, value=1.000.			
Result	Base	Scenario	Change
0	88=40.74%	84=38.43%	-5
1	128=59.26%	132=61.57%	5
Total	216=100.00%	216=100.00%	0
Scenario 4. Effect on aggregate proportions. The threshold T^* of the logit model to calculate the $\text{fit}=1[\text{Prob}>T^*]$ is .50000. Changing variable=DEPENDENTS, Operation=-, value=1.000.			
Result	Base	Scenario	Change
0	88=40.74%	84=38.89%	-4
1	128=59.26%	132=61.11%	4
Total	216=100.00%	216=100.00%	0
Scenario 5. Effect on aggregate proportions. The threshold T^* of the logit model to calculate the $\text{fit}=1[\text{Prob}>T^*]$ is .50000. Changing variable=PRICEKG, Operation=*, value=0.900.			
Result	Base	Scenario	Change
0	88=40.74%	84=38.89%	-4
1	128=59.26%	132=61.11%	4
Total	216=100.00%	216=100.00%	0

Source: Results from the N-logit software.

increases by 13.89%, which is consistent with economic theory. Conversely, if monthly income increases by MXN \$1,000, the probability of responding YES rises by 1.39%, a result similar to that reported by Jaramillo (2016). If the level of education increases by one category, the probability of responding YES increases by 2.31%, in agreement with the findings of Hernández *et al.* (2019) and Lugo *et al.* (2020), who reported that a higher educational level is associated with a greater probability of obtaining an affirmative response. Similarly, if the number of economic dependents of the household head decreases by one, the probability of responding YES increases by 1.85%; this result is consistent with Valdivia *et al.* (2011) and Hernández *et al.* (2023). Finally, if the price of tortillas at the establishment where the respondent purchases them decreases by 10%, the probability of responding YES increases by 1.85%.

Using the scenarios analyzed with the binomial logit model, it can be concluded that consumers increase their willingness to pay when the price of tortillas decreases by 10%, when the number of economic dependents is reduced, or when their income or educational level increases.

Mixed Logit Model Scenarios

The following table (Table 2) presents the change in consumer welfare resulting from modifying or combining tortilla attributes. This change corresponds to the Compensating Variation (CV), which is a Hicksian monetary measure of welfare and serves as an approximation of willingness to pay (WTP). It is important to note that the attributes HM and MMN were not included because their negative signs indicate a loss of welfare for consumers.

Table 2. Changes in well-being due to different attributes of the tortilla (weights/kg⁻¹).

Municipality	PAO	MCN	AZUL	CC	ALL
Ecatepec de Morelos	28.8608036	31.8937955	32.7907676	25.8476359	43.269389
Nezahualcóyotl	26.1267014	29.1596933	30.0566653	23.1135337	40.5352868
Naucalpan de Juárez	22.198277	25.2312689	26.1282409	19.1851093	36.6068624
Chimalhuacán	28.3479558	31.3809477	32.2779197	25.3347881	42.7565411
Tlalnepantla de Baz	31.9529569	34.9859488	35.8829209	28.9397892	46.3615423
Cuautitlán Izcalli	32.448096	35.4810879	36.37806	29.4349283	46.8566814
Tecámac	29.1882195	32.2212114	33.1181834	26.1750518	43.5968049
Ixtapaluca	26.0645333	29.0975252	29.9944972	23.0513656	40.4731187
Tultitlán	23.4325615	26.4655534	27.3625255	20.4193938	37.8411469
Chalco	28.5038625	31.5368544	32.4338265	25.4906948	42.9124479
Valle de Chalco Solidaridad	20.9419541	23.9749461	24.8719181	17.9287864	35.3505395
La Paz	17.8863631	20.919355	21.8163271	14.8731954	32.2949485
Coacalco de Berriozábal	37.1837913	40.2167832	41.1137552	34.1706236	51.5923767
Texcoco	34.8992438	37.9322357	38.8292078	31.8860761	49.3078292
Chicoloapan	20.6328544	23.6658463	24.5628183	17.6196867	35.0414398

PAO: Organic Agricultural Production; MCN: Native Nixtamalized Corn; AZUL: Blue Corn; CC: Tortilla with Added Ingredients. ALL: All attributes combined.

The results show that the blue color attribute of tortillas (AZUL) is the most highly valued by consumers, a finding similar to that reported by Blare *et al.* (2020). However, in the direct question regarding the most valued attribute (Figure 1), color ranked fourth, that is, among the least valued attributes. Although some studies have reported that tortilla color is not a highly important characteristic (Escobedo and Jaramillo, 2019), in the present study this discrepancy may be due to respondents not having fully understood the direct question, although there is not sufficient evidence to confirm this assertion.

The attribute CC was the least valued, and these results are consistent with those reported by Espejel *et al.* (2016) and Escobedo and Jaramillo (2019), who indicate that consumers attach little importance to tortillas being supplemented with other products such as nopal, fiber, or vitamins. It was also observed that the municipality of Coacalco de Berriozábal assigned the highest value to the evaluated attributes, whereas the municipality of La Paz assigned the lowest value. These results may be explained by the fact that respondents from Coacalco de Berriozábal had higher income levels and higher educational attainment than those from La Paz (Figure 2). Moreover, several authors have reported a positive relationship between educational level, income level, and willingness to pay for a differentiated good or service (Jaramillo, 2016; Blare *et al.*, 2020; González *et al.*, 2024).

The highest compensating variation for organic, native-corn, blue tortillas with added ingredients was 51.59 MXN kg⁻¹, whereas the lowest was 32.29 MXN kg⁻¹.

Figure 4 graphically presents the mean changes in consumer welfare (MXN kg⁻¹) associated with tortilla attributes. The most highly valued attribute was blue corn (AZUL), followed by native nixtamalized corn (MCN), then organic agricultural production (PAO), and finally tortilla with added ingredients (CC). Nevertheless, tortillas combining all the aforementioned attributes (ALL) were valued more highly than any individual attribute considered separately. The figure also shows that Coacalco de Berriozábal and Texcoco

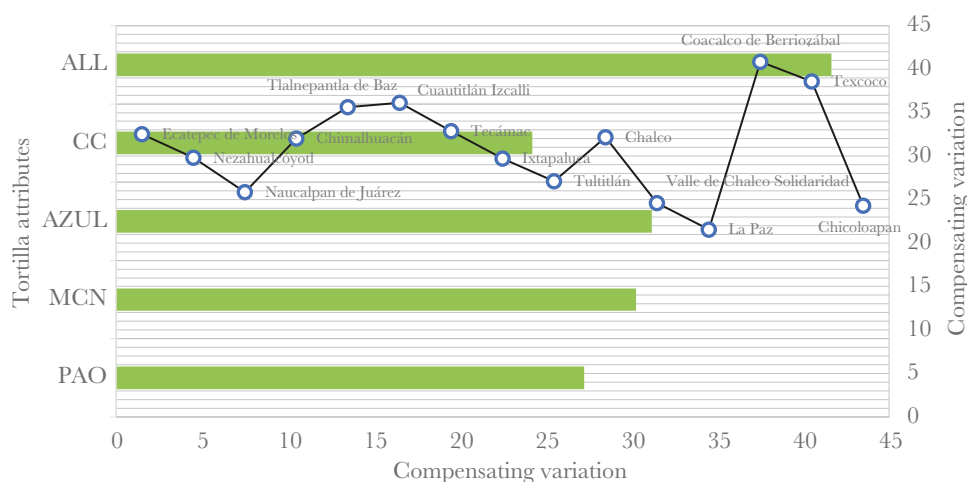


Figure 4. Changes in welfare associated with tortilla attributes (MXN kg⁻¹). PAO: Organic Agricultural Production; MCN: Native Nixtamalized Corn; AZUL: Blue Corn; CC: Tortilla with Added Ingredients; ALL: tortillas combining all the aforementioned attributes.

were the municipalities that assigned the greatest value to organic, native-corn, blue tortillas with added ingredients.

Based on the scenarios analyzed using the mixed logit model, it can be concluded that respondents placed greater value on the attributes of blue corn and native nixtamalized corn; however, they assigned even higher value to tortillas combining all the evaluated attributes. The municipalities of Coacalco de Berriozábal and Texcoco were those that valued this product most highly.

Multinomial Logit Model

Using the multinomial logit model, the probabilities of each alternative were obtained, as shown in the following table (Table 3). Alternative 1 and Alternative 2 consist of combinations of different attributes and levels, whereas Alternative 3 corresponds to the status quo. It can be observed that, when each alternative is analyzed individually, Alternative 3 has the highest probability of being selected. However, when the combined effect is considered, the probability of choosing Alternatives 1 and 2 reaches 0.54, which is 8% higher than that of the status quo alternative.

Table 3. Probability of each alternative.

ALT	EXP	PROBABILIDAD
3	1.5341868	0.4593990
2	0.5865062	0.1756242
1	1.2188585	0.3649767

ALT3: status quo; ALT1 and ALT2: combinations of different attributes and levels.

Social and Policy Implications of the Results

It was found that a 10% reduction in the price of tortillas, in line with the current federal government policy, increases the probability of responding YES to the willingness-to-pay question. A decrease in tortilla prices improves consumer welfare by enhancing purchasing power and increasing economic surplus. At the same time, a reduction in tortilla prices would increase demand for the product and for all the inputs required for its production, including corn.

Regarding the federal government policy aimed at promoting the conservation, production, processing, and commercialization of native Mexican corn (Presidencia de la República, 2025), these results confirm that artisanal tortillas constitute a viable means of adding value to native corn, since consumers value this attribute and are willing to pay a premium for it.

CONCLUSIONS

The use of the Contingent Valuation Method (CVM) with a binomial logit model, together with discrete choice experiments estimated through multinomial logit and mixed logit models, proved highly useful, as it made it possible to analyze different scenarios

involving changes in socioeconomic variables and tortilla attributes. Likewise, it allowed the evaluation of two federal government policies, leading to the conclusion that a reduction in tortilla prices improves consumer welfare and that artisanal tortillas represent a viable strategy for adding value to native corn, given that consumers value this attribute and are willing to pay a premium for it.

Furthermore, the probability of preference for the status quo was estimated, and the most highly valued attribute was identified as the blue color of the tortilla, followed by the native, organic, and added-ingredient attributes. The highest compensating variation (CV) for organic, native-corn, blue tortillas with added ingredients was 51.59 MXN kg⁻¹, whereas the lowest was 32.29 MXN kg⁻¹. The municipality of Coacalco de Berriozábal assigned the highest value to tortillas with the analyzed attributes, followed by Texcoco, Tlalnepantla, and Cuautitlán Izcalli. These findings support the validity of stated preference methods, since the results are consistent with those reported in other studies using different methodologies (Espejel *et al.*, 2016).

With regard to public policy, it is suggested that direct subsidies be granted to native corn producers with the following objectives: to make the cost of native-corn tortillas more affordable and to promote germplasm conservation. This is especially relevant because climate change is creating new agricultural challenges, corn is a crop of global importance, and Mexico possesses the genetic diversity that will be required to respond to such challenges. Simultaneously, the implementation of labels such as “Native Corn” or “Traditional Product” is recommended in order to add value to these tortillas, together with educational campaigns on native corn that provide clear information on its nutritional, cultural, and environmental benefits.

It is important to mention that, although the correct methodology was followed in the design and administration of the questionnaire, the study may still be subject to some degree of bias, particularly with respect to respondents’ understanding of the questions. Therefore, it is recommended that the study be replicated in other regions of the country. This would also make it possible to identify the preferences of a broader range of consumers, since such preferences are likely to vary according to the region under study.

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Yield of x'pelon bean *Vigna unguiculata* (L.) (Fabaceae) Walp. under inorganic fertilization

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ABSTRACT

Objective: The objective of this study was to evaluate the production of the X'pelón crop (*Vigna unguiculata* (L.) Walp) using two fertilization formulations.

Design/methodology/approach: In this research, two formulations based on nitrogen, phosphorus, and potassium were applied, along with a control, using a completely randomized block design. The data were analyzed using analysis of variance, Tukey's test for comparing means, and linear regression analysis.

Results: The results showed statistically significant differences in the number of pods (fruits) obtained per plant among the different fertilization treatments. However, no statistically significant differences were found between the fertilization formulas themselves, with the lowest production observed in the control group.

Limitations/implications of the study: No limitations were found in this research. It is worth noting that this type of research generates new knowledge and should therefore be conducted more frequently.

Findings/Conclusions: It is concluded that there are no statistically significant differences between the fertilization formulas, although both treatments resulted in higher pod production per plant than the control. Therefore, either fertilization formula can be recommended.

Keywords: Production, Fertilization, Fabaceae.

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INTRODUCTION

Legumes are characterized by their legume-shaped fruit. Legumes are seeds with a low-fat content. There are 150 species recorded as being used for human and animal consumption, the most important being beans (*Phaseolus* spp.); lentils (*Lens culinaris medicinalis* or *Lens esculenta* Moench.); peas (*Pisum sativum* L.); chickpeas (*Cicer arietinum* L.); broad beans (*Vicia faba* L.), and cowpeas (*Vigna unguiculata* (L.) Walp. or *Vigna sesquipedalis* Fruhw., *Vigna sinensis* (L.) Savi exd Hassk (Alonso *et al.*, 2010). In Mexico, legumes are economically important from a nutritional, cultural, social and primary sector perspective. Within this large group, there is the genus *Vigna savi*, which belongs to the Fabaceae family and among the species of this genus is *V. unguiculata* (Boukar *et*



al., 2019, Monge *et al.*, 2019). This plant is known by different names; worldwide, it is recognized as cowpea (Badiane *et al.*, 2014). In the country, due to the characteristics of the plant, it has been given different common names, for example, in the south it is known as x'pelón, espelón, black-eyed bean, sin tiempo, or simply pelón bean. (Lecuona-Villanueva *et al.*, 2006).

The cowpea (*Vigna unguiculata* [L.] Walp.) is a legume native to Africa (Morales-Morales *et al.*, 2019; Amelio *et al.*, 2023). It offers agronomic, environmental, and economic advantages in semi-arid tropical regions (Araméndiz-Tatis *et al.*, 2011). Estimates indicate that around 11 million hectares have been established worldwide (Cardeiro *et al.*, 2019). All parts of this species can be used for human and animal consumption, including its leaves, pods, grains, and flour (Gonçalves *et al.*, 2016). For human consumption, the main product used is the grain, which contains a high nutritional value, rich in proteins and essential amino acids. This makes it an important alternative to meet global food needs (Araméndiz-Tatis *et al.*, 2016; Gonçalves *et al.*, 2016; Carneiro *et al.*, 2019).

The x'pelón bean has been reported to have properties rich in protein, carbohydrates, dietary fiber, iron, zinc, vitamin C, thiamine, riboflavin, niacin, total folate, and saturated fatty acids. It is also used for medicinal purposes (Pandey, 2019). This legume, like others, is cultivated to improve soil fertility (Ríos-Castillo *et al.*, 2018). X'pelon is adaptable, and has therefore been cultivated in a wide variety of soils. Previous studies have reported that it obtains better results in well-drained soils with medium fertility and that it is resistant to heat compared to other legumes (Arnon, 1972). Likewise, x'pelon plays an important role in the agricultural system, as it compensates for the loss of nitrogen absorbed by other crops, thus causing a beneficial impact in the soil because it has the ability to fix atmospheric nitrogen (Mendieta López, 1999; Aguila *et al.*, 2014; Soriano Soriano *et al.*, 2019). X'pelon is currently considered an important economic source for local producers, together with the contribution of essential nutritional elements to the local population who consume it in different typical dishes of the region (Márquez-Quiroz *et al.*, 2015). Therefore, this research aimed to know the production of the *V. unguiculata* crop fertilized with two chemical formulas.

MATERIALS AND METHODS

Description of the study site

The study was carried out at the Technological Institute of Chiná, Campeche, Mexico (19° 46' 14.66" N and 90° 30' 6.95" O, with an elevation of 27 meters above sea level). The climate of the region is warm or hot climate with little precipitation, in the study period the minimum annual temperature was 19.6 °C and maximum 37.8 °C, and an annual precipitation of 1 132.5 mm, for the year 2025 (CONAGUA, 2023).

Experimental design

A 3×3 randomized block design was used in an area of 20 m². Treatments consisted of applying 100 g per strain (consisting of four plants) of two granulated chemical fertilizers composed of nitrogen (N), phosphorus (P), and potassium (K), for which two formulas N18-P46-K00, N17-P17-K17, and a control were applied. This is equivalent to 1.8 g of N, 4.6 g

of P, and 0.0 g of K in the case of the first formula, while in the case of the second formula, 1.7 g of N, P, and K of each of the elements would be applied to each plant strain. If this were applied to one hectare, 120 kg of N and 307 kg ha⁻¹ of P of the 18-46-00 formula would have to be applied. For the 17-17-17 formula, 113 kg ha⁻¹ of each element, N, P, and K, would have to be applied. Each treatment had 10 vines. Fertilizer application was carried out on each vine, burying the fertilizer 5 to 10 cm into the soil (10 cm from the stem of the vine) on two different dates. The first application was made 15 days after planting (50 g), once all the seedlings had emerged, and the second was applied 20 days after the first with the same amount of fertilizer (50 g).

V. anguiculata seeds were obtained from a cooperating farmer in the community of Pich, Campeche, Mexico, and showed 97% viability. Sowing was done by hand, using the club method, burying the seeds at a depth of 3 to 5 cm. Planting spacing was 30 cm between vines and 50 cm between rows, yielding approximately 66,667 vines ha⁻¹ in a ferric luvisol soil (Kankab in the Mayan classification). Planting took place on October and three harvests were performed at different times to evaluate pod yield. The first harvest was on December (60 days after planting), the second on February (112 days after planting), and the last on February (123 days after planting). The variables evaluated were pod number and pod weight. The data obtained were analyzed using ANOVA, means were compared using Tukey's Multiple Range test ($p < 0.05$), and a regression was performed between pod weight and grain weight to generate an equation for estimating grain quantity based on whole pod weight. These data were processed using INFOSTAT 2020 statistical software.

RESULTS AND DISCUSSION

Number of pods produced

First of all, it is important to mention that this research was not free from the presence of pests, the main one being the aphid (*Aphis fabae*), which was successfully controlled using garlic (*A. sativum*) and onion (*A. cepa*) extract as mentioned in the methodology. Regarding crop production, at the end of the evaluation period, the production in number of harvested pods did show statistically significant differences ($P < 0.0001$) between treatments. However, the opposite was true between treatments. Figure 1 shows that formula 17-17-17 is slightly higher (17.05 ± 11.13 pods/strain) than formula 18-46-00 (16.43 ± 10.16 pods/strain), while the lowest production was seen in the control (11.75 ± 7.08 pods/strain) (Figure 1).

Regarding the number of pods produced in the different harvest periods between treatments, a differentiated behavior was observed. In the first harvest (60 days after planting), the results show statistical differences ($p = 0.0026$), although the treatment with the 18-46-00 formula did not differ from the other treatments, of which the best was the 17-17-17 formula and the worst the control. In the second harvest (112 days after planting), statistical differences were also shown ($p = 0.0003$), where, as in the first harvest, there were no statistical differences between the fertilization treatments, and the control was again the one with the lowest pod production. In the last harvest (123 days after planting), no statistical differences were observed ($p = 0.2747$) between treatments,

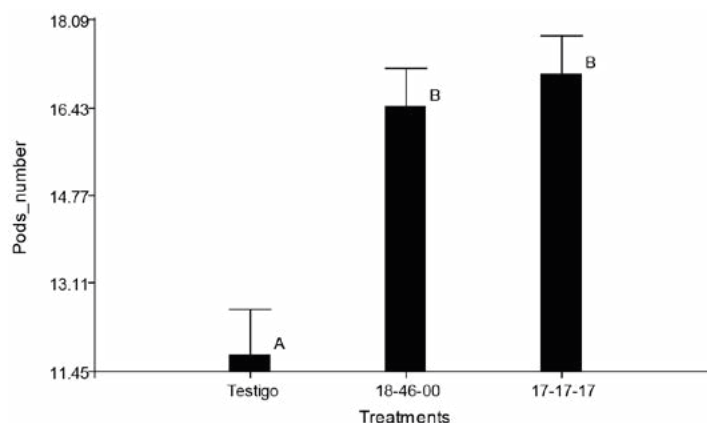


Figure 1. Production of x'pelón (*Vigna anguiculata* [L.] walp) pods with different inorganic fertilizers. Formula 17-17-17 (113 kg ha⁻¹ of N, P, and K) and 18-46-00 (120 kg of N, and 307 kg of P ha⁻¹) after 123 days of cultivation.

although, as observed on the different dates, the highest number of pods was obtained in treatments with chemical fertilizers (Figure 2).

Weight of pods with seeds

The weight of pods with seeds between treatments did not show statistically significant differences ($p=0.05$) at the end of the evaluated period (Figure 3). However, when comparing treatments between dates, only the first harvest (60 days after planting) showed statistically significant differences between treatments ($p<0.0001$), where the control (5.7 ± 1.57) and formula 18-46-00 (5.66 ± 1.61) were the ones that obtained the highest weights, while formula 17-17-17 obtained the lowest average value (3.06 ± 0.75) in pod weight with seeds. In the two subsequent harvests, there were no statistically significant differences between treatments (Figure 4).

This may possibly need to be reinforced with manure or other organic material mixed with inorganic fertilizers. Iwanthika and Thayamini (2023) report in their research work that they applied a mixture of 10 t/ha of poultry waste mixed with 75% triple

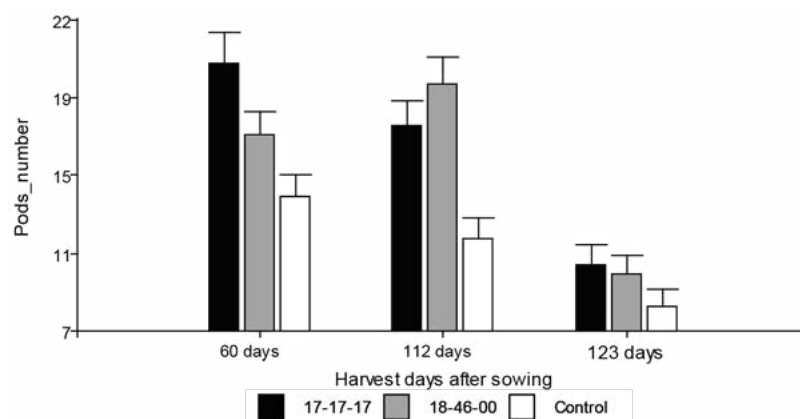


Figure 2. Number of x'pelón (*Vigna anguiculata* [L.] walp) pods produced under different inorganic fertilization treatments on different harvest dates. 60 days, 112 days, 123 days.

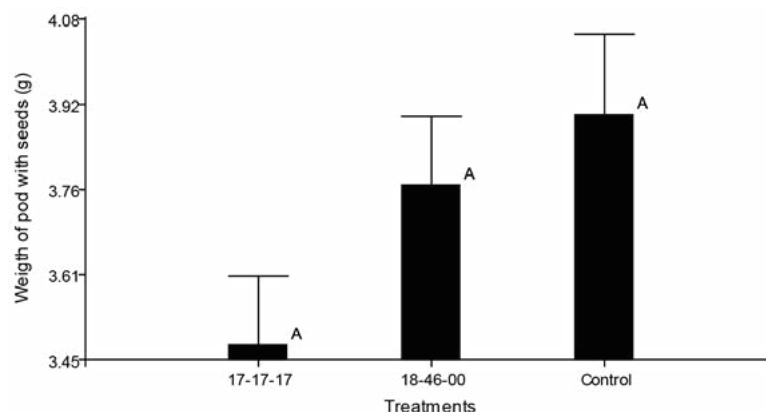


Figure 3. Weight of pods with x'pelón seeds (*Vigna anguiculata* [L.] walp) produced under different chemical fertilization treatments harvested after 123 days after sowing.

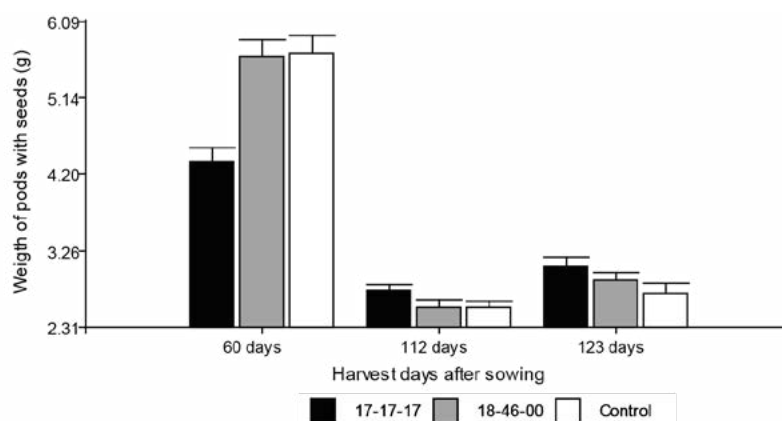


Figure 4. Weight of pods with seeds of x'pelón (*Vigna anguiculata* [L.] walp) produced under different chemical fertilization treatments harvested on different dates. 60 days, 112 days, 123 days.

superphosphate and 75% MOP (00-00-60), which showed a higher yield compared to other fertilization treatments in the cultivation of x'pelón in a sandy regosol soil. This was observed through a literature review, where different investigations report the positive relationship that exists between a combined fertilization of organic fertilizer + NPK + biofertilizers (Kaur and Rawat, 2020). Therefore, the use of inorganic fertilizers provides nutrients quickly, but they can affect, and affect the characteristics of the soil, which was observed in this research, being better in the number of pods, but not in their weight. For their part, da-Conceição *et al.* (2023), points out that the yield and productivity of x'pelón in the Yucatan Peninsula is better under irrigated conditions, since the humidity factor is decisive in the length of the fruits and the large size of the seed.

Linear regression analysis generated an equation to estimate the seed weight that can be obtained from whole pods. This will allow us to estimate the kilograms of *V. anguiculata* seeds that can be obtained by removing the hulls from the pods, with the advantage of not having to shell all the pods (Figure 5).

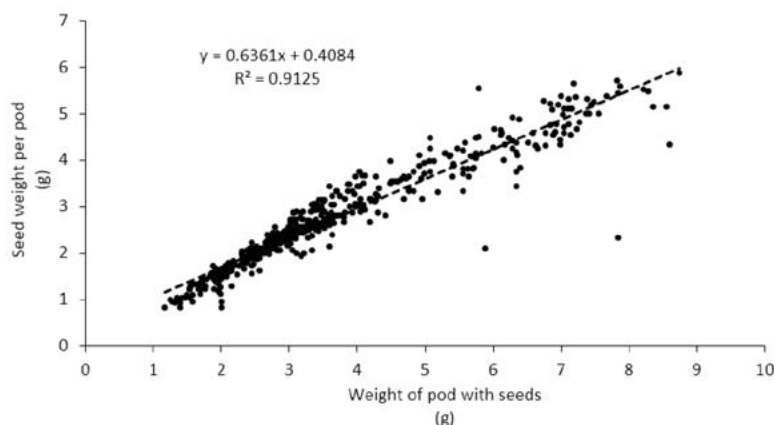


Figure 5. Linear regression analysis of the relationship between seed weight and whole pod weight of x'pelón (*Vigna unguiculata* [L.] walp) produced under different chemical fertilization treatments.

CONCLUSIONS

The objective of this research was to determine the yield of *V. unguiculata* crops fertilized with two chemical formulations. The results suggest that the use of chemical fertilizers does influence the yield of x'pelón crops, improving production in both fertilization formulas compared to the control (without fertilization), making it feasible to use either of the two fertilizers.

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Optimal growth in juvenile tilapia (*Oreochromis niloticus*), using different levels of fish meal replacement with cricket meal (*Acheta domesticus*)

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ABSTRACT

Objective: To evaluate juvenile tilapia (*O. niloticus*) growth, fed with different inclusion levels of house cricket meal (*A. domesticus*).

Design/methodology/approach: The juveniles (2.16 ± 0.46 g) were placed in 60 L semicircular tanks with 15 fish per tank, and fed at 5% of their biomass in 5 daily rations (08:00; 10:00; 12:00; 14:00, and 16:00 hrs.) for 45 days; temperature (28 ± 2 °C), oxygen concentration (7.2 ± 1 mg/L) and pH (7.02 ± 0.1) were constant. Five treatments were tested: T1-0%, T2-25%, T3-50%, T4-75%, and T5-100%, respectively, with biweekly biometrics. Growth parameters were determined using the biometric data.

Results: Results suggest that AWG, SGR, and PER were higher in T2 but without significant differences with T3; the highest FE was presented in T2 and T3, and the lowest FC, PC, and FCR were found in T3.

Limitations on study/implications: Using insects with sustainable and healthy production can be a viable response to the shortage of fishmeal in aquaculture. However, its use can cause a decrease in palatability and digestibility and a nutritional imbalance in the fish.

Findings/conclusions: Inclusions of 50% showed better growth levels with more vigorous and reactive fish, while higher inclusions affected the growth of *O. niloticus*.

Keywords: Tropical aquaculture, insect meal, alternative fed, food sovereignty, sustainable agriculture.

INTRODUCTION

In Mexico and around the world, the demand for feed for aquatic species has increased in recent years, where the most cultivated species are shrimp, trout, and tilapia; this has led to increased aquaculture production, while production costs are rising due to the shortage of fishmeal due to the decrease in natural fish populations from which it is extracted (FAO, 2020).

Given this situation, new sources of protein have been sought, proposing the use of insects as an alternative source of non-traditional, sustainable, and healthy protein to cover protein requirements in diets that help food security in both humans and animals (Hua, 2021; Maulu *et al.*, 2022; Bingqian *et al.*, 2024). Insect meal has been widely studied in fish diets in recent years. Among the most studied are the house cricket (*Acheta domesticus*), black soldier fly larvae (*Hermetia illucens*), and mealworms (*Tenebrio molitor*). However, it has been observed that the effects are diverse in the different species and are related to the levels of substitution by fishmeal, mainly affecting growth (Guerreiro *et al.*, 2022).

Acheta domesticus meal offers sources of vitamins and minerals with antioxidant properties and a high protein content that can be an alternative source to fish feed (Taufek *et al.*, 2016; Bingqian *et al.*, 2024). However, a nutrient deficiency could affect metabolic pathways and gene expression as a response to a nutritional imbalance, directly affecting growth. The present study aims to know the effects of cricket meal (CM) on the development of tilapia *Oreochromis niloticus*, using different levels of substitution of fish meal (FM) by *A. domesticus* meal.

MATERIALS AND METHODS

Obtaining and maintaining organisms

The Technological Institute of Centla Tabasco, Mexico, donated juvenile tilapia with an average weight of 2.16 ± 0.46 gr. The fish were transferred to the Multidisciplinary Academic Division of Jalpa de Méndez-UJAT (DAMJM), and placed in 80 L semicircular tanks with a density of 15 organisms per tank, a semi-closed recirculation system with two water changes per day, and a temperature maintained at 28 ± 2 °C, with a constant oxygen concentration (7.2 ± 1 mg/L) and pH (7.02 ± 0.1).

Diet formulation and preparation

The *A. domesticus* meal used in this experiment was purchased from the Insect Nutrition company in the Gamm industrial park, Apaseo el Grande, Guanajuato, Mexico. The diet formulation calculations, meticulously performed using the Mixitwin Plus 2.0 program, resulted in isoproteic and isocaloric diets with different levels of fish meal substitution by *A. domesticus* meal. The treatments were T1 control 0%, T2 25%, T3 50%, T4 75%, and T5 100% substitution, respectively (Table 1).

The treatments were prepared in the Academic Division of Biological Sciences, Aquaculture Nutrition Laboratory in the Universidad Juárez Autónoma de Tabasco. For their preparation, the macronutrients (meal used) and micronutrients were passed through a sieve with a 500-micron sieve to obtain the same particle size. The pellets were obtained

Table 1. Formulation of the experimental diets with different levels of inclusion of cricket meal (*A. domesticus*).

Ingredients	0% cricket meal	25% cricket meal	50% cricket meal	75% cricket meal	100% cricket meal
Soft wheat grain	43.44	42.56	42.74	41.86	41.6
Fish meal (herring)	26.01	20	12.83	5.72	0
Soybean meal 44%	20	18.9	18	18	17.03
Cricket meal	0	8	16	24	31
Fish oil	3.05	4	4	4	3.86
Soy lecithin	3	2.05	1.93	1.92	2
Gelatin	2	2	2	2	2
Vit-min premix	1	1	1	1	1
dehydrated fish solubles	1	1	1	1	1
Vitamin C	0.5	0.5	0.5	0.5	0.5
Nutrients	As fed	As fed	As fed	As fed	As fed
MEt Energy(m)	1355.36	1654.59	1987.15	2286.38	2564.29
Protein	35	35	35	35	35
Total fat	10	10	10	10	10
Fiber	2.89	3.54	4.1	4.75	5.27
Ashes	5.07	4.31	3.68	2.92	2.31

as a 4 mm diameter matrix and dried at a constant temperature of around 60 to 70 °C for 10 to 12 hours.

Feeding, parameters measurement and biometric parameters

The fish were fed five daily rations at 08:00, 10:00, 12:00, 14:00, and 16:00 hr for 45 days. Biometry of the weight and size of each organism was performed every 15 days to modify the food rations according to the body biomass of the fish (5%). The organisms were weighed on an analytical balance to calculate the biomass of each experimental unit; the size was measured with an ichthyometer from the head to the caudal fin. The pH reading was performed with a pH meter brand HI98108 pHep⁺[®]; temperature and oxygen were measured with a YSI 5 oximeter brand Hualix, and the readings were recorded twice a day (8:00 am and 4:00 pm), respectively, to keep the water in optimal conditions.

The biometric parameters were analyzed at the Universidad Popular de La Chontalpa, Absolute Weight Gained (AWG); Specific Growth Rate (SGR); Daily Growth Coefficient (DGC); Protein Consumption (PC); Feed Conversion Factor (FCR); Protein Efficiency Ratio (PER); Daily Weight Gain (DWG); Condition Factor (K) and Feed Efficiency (FE), were determined at the end of the experiment, using the following equations:

$$AWG(g / fish) = Wf(g) - Wi(g)$$

$$SGR(\%) = 100(\ln Wf - \ln Wi / T(days))$$

where: Wi and Wf are the initial and final weight of the fish respectively, and T is the number of days in the feeding period.

$$DGC(mg) = Wf^{1/3} - Wi^{1/3} / T(days)$$

$$PC = (Feed\ Consumption(g/day) - (Dietary\ Protein\ Level)) / 100$$

$$FCR = Dry\ Feed\ Provided(g) / Wet\ Weight\ Gain(g)$$

$$PER = Wet\ Weight\ Gain(g) / Protein\ Delivered(g)$$

$$DWG(g/day) = Weight\ Gain(mg) / Time(days)$$

$$K = Wf(g) / Standard\ Length(cm)^3 \times 100$$

$$FE = Wf(g) - Wi(g) / Feed\ Consumed(g)$$

Statistical Analysis

The data obtained at the end of the experiment were analyzed using the Kruskal-Wallis test. In addition, a Duncan test was used with a significance value of $P < 0.05$ for the treatment of means. These statistical analyses were carried out using STATISTICA™ v.7.0 software (Statsoft, Tulsa, USA). Graphics were elaborated using the Sigma Plot 12.0. (Grafiti LLC, Palo Alto, USA).

RESULTS AND DISCUSION

Table 2 shows the results of the nutritional profile of the treatments, which was analyzed in the Aquaculture Nutrition Laboratory of the National Autonomous University of Mexico in Merida, Yucatan. The nutritional profile was observed to be very similar between the treatments, but there is a difference in the percentage of lipids and ash. However, treatment T3 had the most significant similarity with the control, so the proximal content between the commercial feed and the feed made with cricket meal is very similar in nitrogen and carbon content.

At the end of the bioassay, T1 and T2 presented the highest values, showing no significant differences, but concerning T3, T4, and T5, the lowest values for AWG. In SGR, it was observed that T3 showed no significant differences concerning T1, and its difference concerning T2 was slightly significant, with T4 and T5 being where the lowest dietary use was observed (Figure 1a and 1b).

Table 2. Nutritional profile of the experimental diets.

Treatment	Meal (%)	Approximate sample weight sample	Nitrogen (%)	Carbon (%)	Moisture	Ash	Total lipids	Calories/g
T1	0 %	1.074	6.57	45.31	11.460	6.598	3.771	4573.192
T2	25 %	1.049	6.82	46.05	9.703	6.613	6.048	4678.849
T3	50 %	1.032	6.81	45.71	11.796	6.556	4.217	4868.103
T4	75 %	0.949	6.75	46.89	10.079	4.862	7.702	4419.706
T5	100 %	1.118	7.10	46.42	10.129	5.420	5.986	5125.193

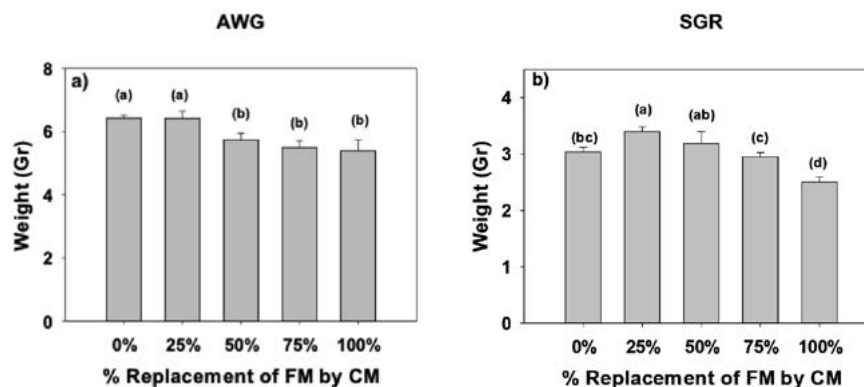


Figure 1. a) Weight gain and b) Specific growth rate of *O. niloticus* with different levels of substitution of fish meal by *A. domesticus* cricket meal.

On the other hand, the lowest FC was obtained in T3, being slightly significant with T2 and T4 and primarily significant with T1 and T5, respectively. Likewise, for the FCR values, T2 and T3 presented the best values concerning T1, T4, and T5, showing important significant differences (Figure 2a and 2b).

Regarding PC, T5 was not statistically different from T1, as T3 revealed the lowest values of crude protein consumed (Figure 3a). Regarding PER, treatments T2 and T3 present the highest values concerning T1, with a slightly significant difference between both treatments. In contrast, T5 presented the lowest protein efficiency, which is statistically different from the rest of the treatments (Figure 3b).

Table 3 shows the growth rate of the DWG in treatment T2 (25%), and it does not show significant differences compared to T1. However, it was significantly reduced in T4 and T5, while the DGC was also affected in treatment T5. Feed efficiency showed a downward effect in T5, and as regards survival, it had no effect in the treatments.

The continued growth of the aquaculture sector has led to an increase in the demand for fishmeal, the primary source of protein in the diet of farmed fish, raising its price and leading to a rise in production costs (FAO, 2020); Therefore, they require efficient and viable alternative protein sources, with studies carried out with plant and animal sources,

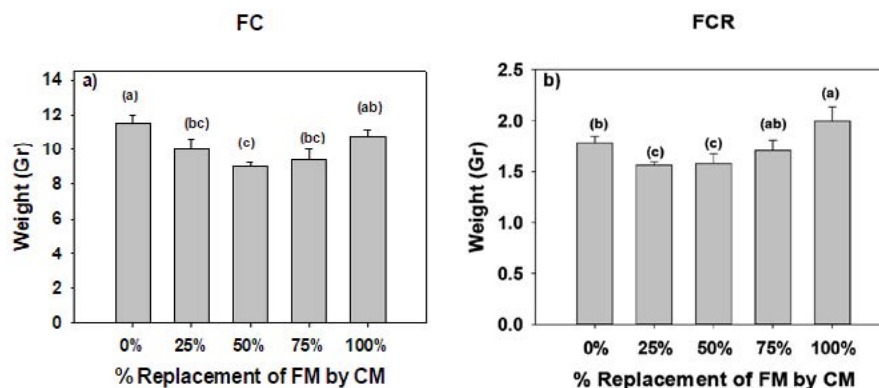


Figure 2. a) Feed consumed and b) Feed conversion rate of *O. niloticus* fed with different levels of replacement of fish meal with *A. domesticus* cricket meal.

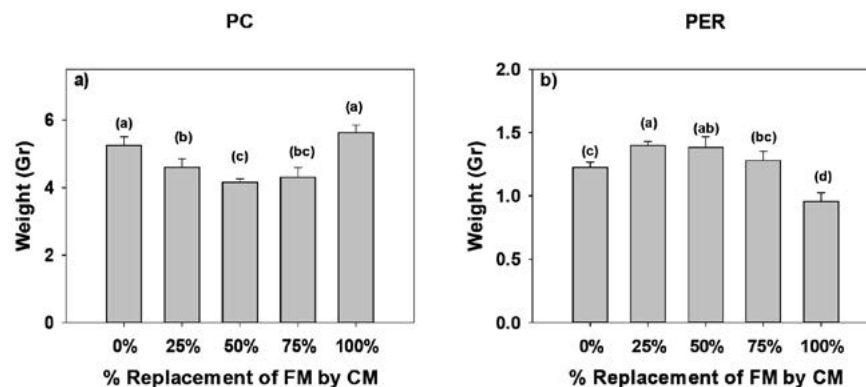


Figure 3. a) Crude protein consumed, and b) the protein efficiency rate of *O. niloticus* fed with different levels of replacement of fish meal with *A. domesticus* cricket meal.

Table 3. Growth of *O. niloticus*, fed with different dietary inclusion levels of cricket meal.

CT	Wi	Wf	LF	DWG	DGC	K	FE	SUP
0%	2.21±0.09 ^b	8.64±0.02 ^a	5.49±0.01 ^a	214.35±3.01 ^a	0.07±0.00 ^a	5.20±0.02 ^b	0.56±0.02 ^{ab}	100±0.00
25%	1.78±0.08 ^c	8.20±0.25 ^{ab}	5.40±0.20 ^a	214.12±7.24 ^a	0.07±0.00 ^a	5.22±0.42 ^b	0.64±0.01 ^a	100±0.00
50%	1.80±0.16 ^c	7.54±0.06 ^c	5.13±0.08 ^{ab}	191.38±7.10 ^b	0.06±0.00 ^{ab}	5.56±0.24 ^b	0.63±0.04 ^a	100±0.00
75%	1.98±0.09 ^{bc}	7.48±0.28 ^c	4.59±0.42 ^{bc}	183.27±7.21 ^b	0.06±0.00 ^b	7.94±2.05 ^{ab}	0.58±0.03 ^{ab}	100±0.00
100%	2.59±0.03 ^a	7.97±0.35 ^{bc}	4.30±0.11 ^c	179.54±11.81 ^b	0.05±0.00 ^b	10.01±1.04 ^a	0.50±0.04 ^b	100±0.00

however, within these sources, insects present a potential source unlike those mentioned above, since they can be produced massively, in less time and space, their production is environmentally friendly (Maulu *et al.*, 2022), it is highly rich in protein (42.1-63.3%), even higher than fish this amino acid profile is highly biodigestible by fish (Alfiko *et al.*, 2022), and its variation between species depends on their nutritional habits, being viable as a food ingredient for several species of omnivorous and/or carnivorous fish in their early stages of life, these dietary amino acids participate in the regulation of blood biochemical parameters and the gastrointestinal microbiota (Mikołajczak *et al.*, 2020), as well as modifying the gene expression of fish digestive enzymes, which in turn can affect digestibility and growth (López-Cerino, 2023).

In our study, juvenile *O. niloticus* showed a higher growth performance and SGR in the initial stage of the production cycle and lower in the later stages, like what was reported in Rohu (*Labeo rohita*) and *O. niloticus* by Rahman *et al.* (2021). Although there are studies that reveal a reduction in fish growth when more than 30% of FM is replaced by insect meals (Hua, 2021), the SGR in the present investigation showed that the inclusion of 50% cricket meal may be viable for the growth of tilapias, since it did not show significant differences compared to the control treatment. Likewise, a correlation was observed between a higher FCR value and a lower SGR value, previously reported in *O. niloticus* by Rahman *et al.* (2021); this is because the FCR is the relationship between the food consumed by the fish and the body weight gain obtained, while SGR expresses growth as the intuitively understandable percentage change in size per unit of time. In perch (*Perca*

fluviatilis), replacing 25% of the FM with a mixture of *A. domesticus* found a decrease in growth and an increase in FCR (Tilami *et al.*, 2020). It should be noted that not all fish have the same FCR, and this is because the utilization and use of nutrients vary among species. In our study, the lowest feed consumption and the highest FCR were found in T3, while the highest feed consumption and highest FCR were found in T5; the latter may be due to a nutritional deficiency that increased the intake of the fish as a mechanism to satisfy their needs (Concha-Frías *et al.*, 2018); this is because feed consumption is strongly linked to the amount of protein in the diet and modifies the satiety of the fish, something observed in our study. The same behaviors were reported by Nadaf *et al.* (2010), where they observed that the higher the FCR, the higher the SGR in juveniles of *L. rohita*. Likewise, weight gain, SGR, and FCR are significantly affected by the feeding rate, so sometimes a more significant amount of feed is required to increase growth as observed in the rabbitfish (*Siganus guttatus*), which will depend on the culture method according to Heriansah *et al.* (2022).

Regarding growth performance, such as weight gain and specific growth rate, these increased significantly in diets with a cricket meal level of up to 50% and then decreased with increasing cricket meal levels of up to 100%. Cadena-Cadena *et al.* (2023) report that the inclusion of 35% of *A. domesticus* in *O. niloticus* juveniles did not affect growth; likewise, in totoaba juveniles (*Totoaba macdonaldi*), Carvajal-Soriano (2022) reports similar data, recommending that substitution levels of up to 50% of fishmeal with *A. domesticus* meal are feasible without compromising survival or growth, while Lee *et al.* (2017) report that at optimal concentrations of cricket meal (60% *A. domesticus* and 40% rice bran) good growth occurs in *Oreochromis* sp, but at higher concentrations, they present liver damage.

In this study, PER was higher, and FCR was lower in fish-fed diets containing 50% inclusion than in the control. Hua (2021) has recommended these low values of insect meal replacement accepted by fish. They are due to the nutritional value of the meals since they present deficiencies in some amino acids and fatty acids essential for the physiology of fish, as well as high levels of chitin that decrease palatability and affect some metabolic and physiological pathways (Roncarati *et al.*, 2015).

However, it has been observed that 100% replacement of fishmeal with *A. domesticus* meal and field cricket (*Gryllus bimaculatus*) meal increases SGR, DWG, and AWG in *O. niloticus* fingerlings (Perera *et al.*, 2023). In Guppy fish (*Poecilia reticulata*), 100% replacement of fishmeal with *A. domesticus* and/or *G. bimaculatus* meal did not affect growth, noting that field cricket meal improved fish pigmentation (Perera & Bhujel, 2022).

Regarding the utilization of cricket meal in the study, T3 presented the lowest value and a higher PER, indicating a better utilization of the diet by the fish. Similar data are reported by Bhilave *et al.* (2012) for *C. idella* fed with diets formulated with soybean meal, highlighting that reducing the dietary protein level without affecting growth can reduce feed cost. Likewise, in mahseer (*Tor putitora*), an optimal protein requirement of 45% is reported, where the SGR and PER were higher with the lowest FCR (Ullah *et al.*, 2022), while Concha-Frías *et al.* (2018) report that a higher PER goes hand in hand with a lower CP, as well as a higher SGR and AWG in the common snook *Centropomus undecimalis*. It is that an excess of amino acids consumed by the fish must be eliminated from its system,

causing an extra energy expenditure in which amino acids are used for energy production (ATP) since the fish cannot accumulate these surpluses, thus reducing growth, due to an increase in the catabolism of lipids and carbohydrates in the same process (Concha-Frías *et al.*, 2018), this leads to an increase in feed consumption and little use of protein, similar to what was observed in our study where PER and FC are higher in T4 and T5 respectively.

Optimal replacement of insect meal in fish has shown a decrease in oxidative stress, which may be related to its chitin content and lipid fraction since the latter are more susceptible to oxidation (Guerreiro *et al.*, 2022; Bingqian *et al.*, 2024). However, in most studies, dietary inclusion of 50% and even 75% can present a significant decrease in the growth rate and a decrease in the feed efficiency of the fish, increasing oxidative stress (Bingqian *et al.*, 2024), which may be due to the deficiency of some essential amino acids in the tilapia diet, which could have an impact on the growth and health of the fish (Gasco *et al.*, 2016; Guzmán-García, 2023), as observed in our bioassay.

High inclusions of cricket meal (T4 and T5, respectively), showed a low growth of tilapia, which may be related to high protein digestion and, therefore a high energy cost for the elimination of excess amino acids (AAs) (Concha-Frías *et al.*, 2018), it may also be due to a deficiency in essential and/or limiting AAs in the diet, such as arginine, methionine, leucine, and lysine (Guerreiro *et al.*, 2020; Alfiko *et al.*, 2022), since the deficiency of these amino acids can cause a decrease in the production of various metabolites such as nitric oxide (NO), polyamines, urea, proline, and glutamate (Ahmad *et al.*, 2021), as well as a deterioration in defenses, protein synthesis, DNA methylations, decreased functions of antioxidants, metabolizers, immune system, disease resistance, cell signaling and endocrine system, cause DNA damage and therefore trigger cell apoptosis, cancer and even death (Yen *et al.*, 2002; Bingqian *et al.*, 2024), so high levels of inclusion of these meals could increase oxidative stress levels and decrease fish defenses (Taufek *et al.*, 2016; Maulu *et al.*, 2022). Guzman-García (2023) indicates that the increase in the concentration of cricket meal in the diet of the Mojarra Zacatera (*Cincelichthys pearsei*) caused an increase in the gene expression of BCL2 and BAX, P53, and Caspase involved in cellular DNA repair and apoptosis, which may be a reflection of the damage caused by the release of reactive oxygen species, due to oxidative stress caused by the diet, affecting the activity of antioxidant enzymes such as catalase and superoxide dismutase, etc., thus generating severe metabolic damage to proteins and lipids which is reflected in a decrease in fish growth (Song *et al.*, 2023).

Another nutritional component linked to poor growth in fish-fed insect meals is chitin, which is usually not digestible for many fish species and usually decreases the diet's palatability in large quantities. Some fish can digest chitin because they have enzymes such as chitinases, thus improving digestibility and facilitating the work of other digestive enzymes; they may also have the enzyme chitobiase in the intestine, which hydrolyzes chitin into β N-acetyl-glucosamine monomers to be absorbed by the enterocytes of the intestinal wall according to Gutowska *et al.* (2004). However, in fish that do not possess enzymes that allow the degradation of chitin, they increase the production of hepatocytes capable of managing metabolic needs; therefore, high concentrations of this carbohydrate decrease the palatability and digestibility of the diet, affecting the gene expression levels

of proteolytic enzymes (Carvajal-Soriano, 2022; López-Cerino, 2023), decreasing the activity of trypsin and causing low protein hydrolysis (López-Cerino, 2023). Likewise, organisms obtain most of their energy from lipids, which are part of the composition of the membrane, cell signaling, regulation of oxidative stress, and nutrients (Yoon *et al.*, 2021). Fish oil contains a higher concentration of omega-3 fatty acids than insect meals, which have a higher concentration of saturated fatty acids (linolenic acid and n-6 fatty acids), which is favorable for the health of fish consumers (Lee *et al.*, 2017; Tilami *et al.*, 2020) and low content of LC-PUFAs, while the concentrations of unsaturated fatty acids are higher in the domestic cricket (around 60-70%) (Gasco *et al.*, 2016; Alfiko *et al.*, 2022). An imbalance in the concentration of fatty acids required by the fish affects its health (Guerreiro *et al.*, 2020), activating oxidative stress through lipid peroxidation (PUFA oxidation), reducing antioxidant protection and fish growth.

CONCLUSIONS

The protein content of the T3 treatment based on 50% cricket meal did not show significant differences compared to the control treatment, which did not contain cricket meal. Regarding the deficiencies presented by cricket meal, these were supplemented with a premix of vitamins and minerals, such as soy lecithin, since these inputs are essential for the growth of tilapia. The productive parameters were not affected by the levels of substitution applied, with T3 (50%) being the most similar to the control treatment because the percentage of protein is very similar between these treatments. However, in higher concentrations, they affect growth in a negative way, which may be strongly related to an amino acid deficiency, lipid deficiency, and an excess of chitin. Using the house cricket to produce meals as feed for the cultivation of tilapia in the early stages of fry is feasible because it is rich in protein and has a simple production process with no ecological impact.

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Nutrition and Puberty in Tropical Heifers: A Systematic Review of Reproductive Performance and Endocrine Regulation

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ABSTRACT

This review article examines the effects of nutrition on puberty onset and reproductive performance in beef cattle raised under tropical and subtropical production systems. A systematic review of the scientific literature was conducted using PubMed, Scopus, Web of Science, and Google Scholar to identify studies focused on nutrition, puberty, and reproductive efficiency in cattle. The findings indicate that an appropriate nutritional strategy, particularly energy supplementation during critical stages of growth, can reduce the age at puberty by up to 22% and advance the age at first calving. This is especially relevant for addressing the reproductive delay characteristic of *Bos taurus* × *Bos indicus* crosses, in which puberty may occur as late as 30 months of age. In conclusion, adequate nutritional management enhances reproductive efficiency and fosters the sustainability of tropical livestock production systems.

Keywords: Bovine nutrition, supplementation, puberty, reproductive efficiency, tropical production systems.

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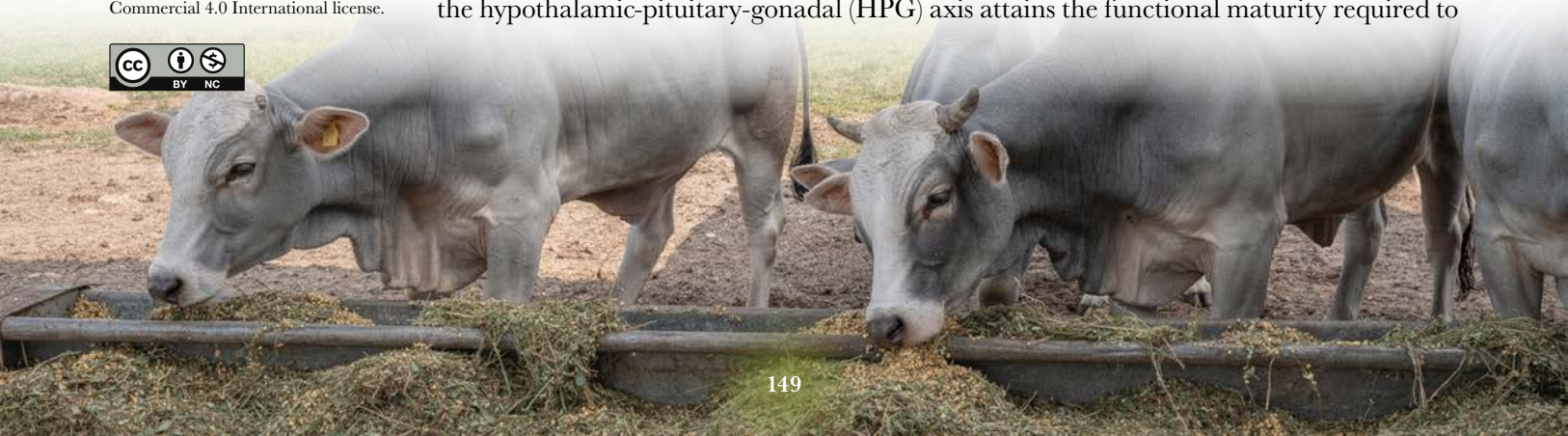
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INTRODUCTION

Beef production is essential to global food security and the livestock economy (Ricardo *et al.*, 2025), particularly in tropical regions, where a substantial proportion of the world's cattle inventory is concentrated (Cooke *et al.*, 2020). The sustainability of these production systems depends largely on the reproductive efficiency of females, as age at puberty and age at first calving directly influence replacement rate, productive lifespan, and herd profitability (Li *et al.*, 2019; Maquivar & Galina, 2010). In 2023, global production reached 60 million tons, with a projected growth of 9% over the next decade, underscoring the importance of optimizing production systems (FIRA, 2024).

From a physiological standpoint, puberty in heifers is defined as the process by which the hypothalamic-pituitary-gonadal (HPG) axis attains the functional maturity required to



permit ovulation, a phenomenon characterized by the pulsatile secretion of gonadotropin-releasing hormone (GnRH), a sustained increase in luteinizing hormone (LH), and the formation of the first functional corpus luteum (Barcellos *et al.*, 2014; Duittoz & Kenny, 2023). This event does not occur at a fixed chronological age; rather, it is influenced by factors such as genetics, environment, and, most decisively, nutrition (Amstalden M *et al.*, 2011). In tropical regions, *Bos indicus* heifers and their crosses may reach puberty as late as 30 months of age, with first calving occurring between 42 and 48 months, thereby prolonging the non-productive period and reducing the profitability of beef production systems (Maquivar & Galina, 2010).

Nutrition plays a pivotal role in regulating the onset of puberty, not only through its effects on somatic growth, but also through metabolic and endocrine signals that inform the central nervous system about the animal's energetic status (Fantuz *et al.*, 2024a). Hormones such as leptin, insulin, and insulin-like growth factor I (IGF-I) act as key mediators between metabolism and reproductive function, modulating the activity of the HPG axis and promoting sexual maturation when energy balance is positive (Allen *et al.*, 2017; Cardoso *et al.*, 2020). Adequate energy intake and early supplementation with concentrates accelerate sexual maturation; indeed, heifers that attain between 60% and 65% of their mature body weight exhibit earlier puberty and greater fertility (Duittoz & Kenny, 2023). Recent studies have demonstrated that nutritional strategies based on appropriate energy supplementation during early developmental stages can significantly reduce age at puberty and improve subsequent reproductive performance (Fantuz *et al.*, 2024a; Severino-Lendecky *et al.*, 2020).

Forage seasonality in extensive systems of the Mexican tropics represents a major challenge, as fluctuations in forage quality and availability limit the intake of nutrients required to achieve optimal body weight (Cooke *et al.*, 2020; National Academies of Sciences, Engineering, and Medicine, 2016; 2021). Consequently, reproductive efficiency is adversely affected due to reduced nutrient availability (Day & Nogueira, 2013).

Despite the extensive literature on bovine nutrition and reproduction, knowledge remains fragmented regarding the integration of nutrition, endocrine regulation, and the environmental conditions characteristic of tropical regions, particularly with respect to the role of metabolic signals in the neuroendocrine control of puberty. This situation hinders the development of specific nutritional recommendations tailored to tropical production systems.

Therefore, the aim of this systematic review with a narrative approach was to analyze the available scientific evidence on the influence of nutrition on the onset of puberty, reproductive performance, and endocrine regulation in heifers from tropical regions, in order to integrate physiological and productive foundations that contribute to improving reproductive efficiency and the sustainability of beef production systems.

SEARCH METHODOLOGY

The present study constitutes a systematic review of the scientific literature based on an adapted structured narrative synthesis protocol, designed to ensure the transparency and

reproducibility of the process. An exhaustive search for articles was conducted up to the most recent update, between October 15 and October 31, 2025.

The databases consulted included PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect, as well as governmental and international sources such as the Food and Agriculture Organization (FAO) and the National Institute of Statistics and Geography (INEGI). The search strategy combined key terms (“Nutrition,” “Puberty,” “Reproductive Efficiency,” “Cattle,” and “Tropics”) using Boolean operators (“AND” and “OR”). The inclusion criteria comprised studies addressing nutrition, puberty, and reproductive efficiency in tropical heifers, published in either Spanish or English. Studies without full-text access and those involving other species were excluded. The selection process included title and abstract screening, followed by full-text review. A total of 168 articles were initially identified, of which 57 were ultimately selected for qualitative analysis. The extracted data were classified according to nutritional factors and their physiological and endocrine effects, and a structured narrative synthesis was subsequently developed. Bibliographic organization was carried out using Zotero version 7.0.7.

This systematic methodology enabled the comprehensive compilation and organization of the relevant literature, thereby ensuring an integrated and up-to-date perspective on the nutritional effects influencing puberty and reproductive performance in heifers destined for beef production.

The synthesis of findings is based on 57 articles that fulfilled the established criteria. The results were grouped into subtopics addressing the nutritional and hormonal influences on reproductive performance. Table 1 summarizes the characteristics and quantitative findings of selected studies included in the review.

Productive Context of Cattle Farming in Tropical Production Systems

The increasing demand for beef has driven the need to improve the efficiency of production systems, with female reproductive performance representing a pivotal factor

Table 1. Characteristics and quantitative findings of studies included in the review.

Reference	Key quantified result
Luna-Pinto & Cronje (2000)	A diet formulated to support an average daily gain (ADG) of 0.3 kg/day increased age at puberty by 7% in Friesian heifers (P<0.01). Compensatory growth reached 0.74 kg/day ADG after restriction, exceeding that of the control diet (0.6 kg/day ADG).
Zieba <i>et al.</i> (2004)	Mean luteinizing hormone (LH) concentrations were significantly lower (P<0.001) in Brahman × Hereford heifers fed a diet designed for 0.3 kg/day ADG (0.66±0.01 ng/mL) compared with those receiving a diet designed for 0.68 kg/day ADG (0.76±0.01 ng/mL).
Gasser <i>et al.</i> (2006b)	Puberty was attained by 88% of heifers fed a diet designed for 1.5 kg/day ADG, compared with 0% of those fed a diet designed for 0.75 kg/day ADG. Age at puberty was 262±10 <i>vs.</i> 368±10 days, respectively.
Allen <i>et al.</i> (2012)	Heifers receiving diets designed for 1.0 kg/day ADG <i>vs.</i> 0.5 kg/day ADG showed the following values: insulin-like growth factor I (IGF-I), 103.75±9.9 <i>vs.</i> 52.01±4.39 ng/mL (P<0.005); leptin, 3.62±0.43 <i>vs.</i> 2.55±0.35 ng/mL (P<0.1); and insulin, 1.16±0.11 <i>vs.</i> 0.95±0.07 ng/mL (P<0.05).

ADG: Average daily gain; kg/day: kilograms per day; LH: luteinizing hormone; ng/mL: nanograms per milliliter; RPH: reproductive precursor hormones; IGF-I: insulin-like growth factor I; NS: not specified.

for enhancing productivity in both dual-purpose and beef systems (Cooke *et al.*, 2020). According to FIRA (2024), global production reached 60.0 million tons in 2023, with an annual growth rate of 0.4% and a projected output of 60.4 million tons for 2024. The Organisation for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization (FAO) forecast a global increase of 9% over the next decade, driven by genetic improvement and reduced forage-related costs. In Mexico, beef production reached 2.21 million tons in 2023 and is estimated at 2.26 million tons for 2024, with an average annual growth of 0.72%. Domestic consumption totaled 2.08 million tons in 2023, reflecting a 6.9% increase relative to 2022, with a modest projected annual growth rate of 0.11% over the coming decade.

As of 2022, Mexico reported a total cattle inventory of 36.6 million head, of which 92.62% were allocated to the beef industry, with Tabasco ranking as the second most productive state in the southeastern region (Servicio de Información Agroalimentaria y Pesquera [SIAP], 2024). Extensive cattle production systems in the Mexican tropics have substantially contributed to the social and economic development of the region (Nuncio Ochoa *et al.*, 2001).

The nutritional basis of these systems is grazing on natural or cultivated pastures; however, due to marked seasonality, both forage biomass production and nutritional quality are adversely affected, thereby limiting adequate nutrient intake and compromising the different physiological and productive stages of the animals (National Academies of Sciences, Engineering, and Medicine, 2016; 2021).

In general, the bovine biotype used for beef production in tropical climates is predominantly of *Bos indicus* origin or derived from *Bos indicus* crosses, owing to its parasite resistance and heat tolerance (Day & Nogueira, 2013; Rubio Lozano *et al.*, 2021).

What Is Puberty?

Puberty marks the onset of reproductive life and corresponds to the stage at which the hypothalamic-pituitary-gonadal axis acquires the functional capacity to sustain cyclic ovarian activity, as evidenced by the establishment of pulsatile gonadotropin-releasing hormone (GnRH) secretion, the progressive increase in luteinizing hormone (LH) release, and the appearance of the first functional corpus luteum (Barcellos *et al.*, 2014; Duittoz & Kenny, 2023; Estill, 2021). This process constitutes a key economic indicator in animal production (Ferraz *et al.*, 2018).

Nutrition and Prepubertal Growth

In tropical climates, the reproductive efficiency of heifers is markedly influenced by age at puberty; in *Bos taurus* × *Bos indicus* cattle, puberty occurs at approximately 2.5 years of age, delaying first calving until 3.5 to 4 years (Maquivar & Galina, 2010). In South America, *Bos indicus* heifers generally reach puberty between 2 and 3 years of age, and first calving occurs between 3 and 4 years (Day & Nogueira, 2013; Nogueira, 2004).

Nutrition plays a crucial role in the regulation of puberty in cattle. In particular, the rate of body weight increase, or average daily gain (ADG), during the growth period is considered the most relevant factor associated with age at puberty (Allen *et al.*, 2017).

Therefore, adequate nutritional management that ensures consistent weight gain rates of 700 to 900 g/day (van Niekerk *et al.*, 2021) may facilitate the earlier onset of puberty (Amstalden M *et al.*, 2011; Fantuz *et al.*, 2024b; Moriel *et al.*, 2017). More specifically, attaining 60% to 65% of mature body weight is essential to ensure that heifers reach puberty at a younger age (Duittoz & Kenny, 2023). Moreover, heifers exhibiting precocious puberty produce calves with greater weaning weights and higher growth rates than non-precocious heifers (Fernandes Júnior *et al.*, 2019).

In this context, Terakado *et al.* (2015) evaluated weaning weight in the offspring of precocious and non-precocious Nellore heifers and reported significantly higher weights ($P < 0.01$) in the offspring of precocious heifers when considering both the overall weaning weight of all calves and the weaning weight of calves at the third calving, with differences of 1.413 ± 0.118 kg and 0.768 ± 0.181 kg, respectively.

Effects of Nutrition: Deficiency Versus Excess

Both energy and protein supplementation play a significant role in the onset of puberty in heifers, as these nutrients are fundamental for body development and sexual maturation (Azari & Farrokhi Ardabili, 2019; Day & Nogueira, 2013; Severino-Lendechy *et al.*, 2020). Energy is essential for overall growth and the development of reproductive tissues, whereas proteins, as key components in hormone and tissue synthesis, directly influence reproductive function (Alves *et al.*, 2019; Azari & Farrokhi Ardabili, 2019; Praxitelous *et al.*, 2023). An adequate supply of both nutrients not only promotes weight gain and body condition but also contributes to the regulation of hormonal cycles, thereby accelerating sexual maturation and reducing age at puberty. In this regard, balanced nutrition that meets the energy and protein requirements of heifers during the growth phase is indispensable for optimizing reproductive development and ensuring an efficient transition into productive life (Fantuz *et al.*, 2024b).

Nutrition directly influences the reproductive efficiency of heifers: low energy availability induces negative energy balance (NEB), thereby impairing puberty, ovarian function, and fertility (Fantuz *et al.*, 2024b; Senosy *et al.*, 2017). However, excessive energy intake may lead to mammary fat deposition, reducing glandular development, milk production, and calf weaning weight (Cooke *et al.*, 2020). van Niekerk *et al.* (2021) reported that excessive consumption of low-quality forage or neutral detergent fiber (NDF) after weaning may reduce dry matter intake (DMI) and average daily gain (ADG), consequently increasing age at puberty. Nevertheless, precocious puberty may be induced through early weaning and the implementation of high-protein and high-energy diets during critical stages of growth, particularly between 4 and 9 months of age (Cardoso *et al.*, 2018).

Nutrition-Mediated Endocrine Regulation

The relationship between nutrition and puberty does not depend exclusively on body growth, but rather on the integration between energy metabolism and neuroendocrine regulation (Cardoso *et al.*, 2020; West *et al.*, 2024). Leptin, insulin, and insulin-like growth factor I (IGF-I) convey information to the hypothalamus regarding the animal's energetic status, thereby allowing the progressive activation of the hypothalamic-

pituitary-gonadal axis when energy balance is positive, which is essential for the initiation and maintenance of reproduction (Bruinje *et al.*, 2021; Childs *et al.*, 2021; Ferraz *et al.*, 2023; Godoy *et al.*, 2018).

Restricted feed intake results in low concentrations of leptin, insulin, and IGF-I, hormones that, for the purposes of this review, will be referred to as reproductive precursor hormones (RPH). This condition negatively affects growth rate and decreases ovarian steroidogenesis, thereby limiting estradiol production (Amstalden *et al.*, 2000, 2014; Estill, 2021). Owing to forage nutritional quality, negative effects on reproductive activity have been reported, including delayed puberty onset, altered ovulatory rate, compromised embryo quality, and impaired uterine environmental conditioning (West *et al.*, 2024). In a study conducted in Friesian heifers, energy intake restriction was shown to affect average daily gain (ADG), significantly increasing age at puberty ($P < 0.01$). In that study, the restricted-diet group (0.3 kg/day ADG) exhibited a 7% increase in age at puberty compared with the control group (0.6 kg/day ADG). Nevertheless, the restricted group displayed compensatory gain after the restriction period, reaching a mean ADG of 0.74 kg/day and surpassing the control group, which exhibited a gain rate of 0.58 kg/day. These results suggest that initial dietary restriction affected their capacity for accelerated growth once greater feed access was restored (Luna-Pinto & Cronje, 2000).

In addition to the hormones classically involved in the regulation of the hypothalamic-pituitary-gonadal axis, kisspeptin (KISS) has been identified as a key modulator of its function, acting as an integrator of signals that regulate both the somatotrophic and gonadal axes (Garza *et al.*, 2023; Navarro & Tena-Sempere, 2011). Metabolic conditions and nutritional status influence the function of KNDy neurons located in the arcuate nucleus, which express kisspeptin, neurokinin B, and dynorphin, and stimulate gonadotropin-releasing hormone (GnRH) secretion, thereby influencing the regulation of puberty and reproduction. Studies examining the effects of different metabolic stimuli have shown that kisspeptin acts as a pivotal mediator in the integration of nutritional and energetic signals controlling reproductive function (Sobrino *et al.*, 2022; West *et al.*, 2024).

KISS, or kisspeptins, constitutes a family of peptides classified according to length as KISS-54, KISS-14, KISS-13, and KISS-10 (Alamilla Rodríguez *et al.*, 2017).

Studies conducted in rats (Castellano *et al.*, 2006; Navarro & Tena-Sempere, 2011), sows (Lents *et al.*, 2008), goats (Hashizume *et al.*, 2010), and prepubertal heifers at five (Ezzat Ahmed *et al.*, 2009), seven (Kadokawa *et al.*, 2008), and seven to eleven months of age (Godoy *et al.*, 2018) have shown that administration of KISS-10 increased serum concentrations of LH and follicle-stimulating hormone (FSH), while in seven-month-old calves an increase in growth hormone was also observed ($P < 0.05$).

As illustrated in Figure 1, the connection between nutritional status and reproductive function is mediated by a complex cascade of endocrine signals acting on the hypothalamic-pituitary-gonadal axis. Adequate energy intake and increased body adiposity stimulate the release of key mediators such as leptin, insulin, and IGF-I. These molecules act as direct metabolic signals, integrating at the hypothalamic level to trigger pulsatile GnRH secretion, a process essential for the onset of puberty.

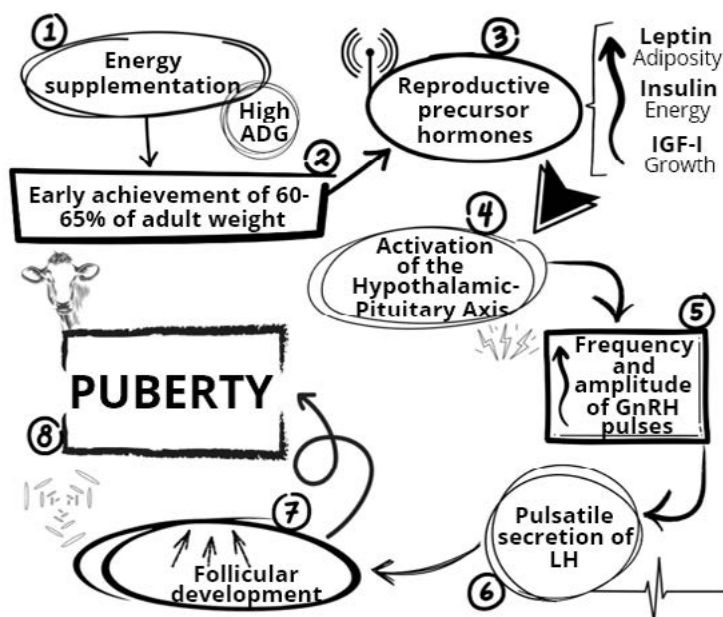


Figure 1. Metabolic and endocrine signaling pathways linking energy supplementation to puberty onset in heifers (figure prepared by the authors).

Energy

Several studies have evaluated the effect of energy supplementation on average daily gain (ADG) and age at puberty in *Bos indicus*, *Bos taurus*, and crossbred heifers. Gasser *et al.* (2006b) observed that heifers weaned at 2 to 4 months of age and fed high-energy diets (HD; 60% corn formulated to support 1.5 kg/day ADG) exhibited greater weight gain and a younger age at puberty than heifers fed the control diet (CD; 30% corn formulated to support 0.75 kg/day ADG), with gains of 1.27 ± 0.05 and 0.85 ± 0.05 kg/day for HD and CD, respectively. Moreover, 88% of the heifers receiving the HD exhibited precocious puberty (<300 days), whereas the CD group showed a rate of 0%, with ages at puberty of 262 ± 10 and 368 ± 10 days for HD and CD, respectively. Likewise, Severino-Lendecky *et al.* (2020) confirmed this premise using Girolando heifers (*Bos indicus* $\times$ *Bos taurus*) under tropical conditions, evaluating two ages and two supplementation levels: 8 and 10 months of age with supplementation (T1 and T3, respectively) and 8 and 10 months of age without supplementation (T2 and T4, respectively). The supplement contained 18% crude protein and 1.75 Mcal/kg of metabolizable energy. The authors found that heifers in groups T1 and T3 gained 50% and 58% more weight, respectively, than those in groups T2 and T4 (0.625 ± 0.05 kg/day and 0.650 ± 0.08 kg/day for T1 and T3 *versus* 0.315 ± 0.06 kg/day and 0.381 ± 0.04 kg/day for T2 and T4). In addition, heifers in group T1 reached puberty 155 days earlier than those in T2, while those in T3 did so 134 days earlier than those in T4.

In another study, the age at puberty in beef heifers was reduced by 22% through the use of two diets, a high-concentrate diet (60% corn; A) and a control diet (30% corn; C), applied across two feeding phases: 126 to 196 days of age (Phase 1) and 196 to 402 days of age (Phase 2). Four treatments were therefore implemented: AA (60% corn during both phases), AC (60% corn in Phase 1 and 30% corn in Phase 2), CA (30% corn in Phase 1 and 60% corn

in Phase 2), and CC (30% corn during both phases). A positive relationship was observed between continuous feeding with high-concentrate diets, increased ADG, and reduced age at puberty. Heifers fed the AA diet exhibited greater ADG (1.2 ± 0.04 kg/day; $P < 0.05$) than those in treatments AC and CA, between which no significant difference was detected, with mean ADG values of 1.0 ± 0.03 and 1.0 ± 0.02 kg/day, respectively. In contrast, age at puberty did not differ significantly between treatments AA and AC (271 ± 17 and 283 ± 17 days of age, respectively), although both differed from treatment CC (331 ± 11 days of age), whereas treatment CA showed a mean age at puberty of 304 ± 13 days and did not differ from the other treatments (Gasser, Grum, *et al.*, 2006a).

It has been demonstrated that feeding strategies with different energy levels can modify circulating concentrations of reproductive precursor hormones (RPH). However, despite the recognized relevance of dietary energy in the onset of puberty in heifers, studies using diets with the same protein content but different energy levels, specifically designed to isolate the effect of energy on sexual maturation, remain limited. Nevertheless, Schubach *et al.* (2019) implemented three feeding regimens with different energy levels and identical protein content in Angus $\times$ Hereford heifers. In that study, ADG during the growth phase was greater ($P < 0.001$) under the high-gain regimen (0.78 kg/day) than under the moderate-gain (0.60 kg/day) and restricted-gain (0.37 kg/day) regimens. Likewise, plasma IGF-I concentrations were higher ($P < 0.01$) in the high-gain regimen (200.2 ng/mL) than in the moderate-gain (177.2 ng/mL) and restricted-gain (131.8 ng/mL) regimens. Furthermore, high-gain heifers had greater body weight ($P < 0.01$) and a lower age at puberty ($P < 0.05$) than moderate- and restricted-gain heifers (340 kg and 345 days of age for the high-gain group versus 318 kg and 327 days of age for the moderate-gain group, and 295 kg and 358 days of age for the restricted-gain group).

Protein

Samadi *et al.* (2014) evaluated the impact of two nutritional regimens on growth, body condition, and puberty onset in Brahman (*Bos indicus*) heifers under subtropical conditions. Heifers in the enhanced nutrition group (ENG) received a diet with higher crude protein (CP) content and greater dry matter digestibility (DMD) than heifers in the moderate nutrition group (MNG). Crude protein content was 11.3% in ENG versus 8.9% in MNG, whereas DMD was 57% in ENG compared with 54% in MNG ($P < 0.01$). Heifers in the ENG exhibited a significant increase in final body weight (412 kg) relative to the MNG, which reached a final weight of 343 kg ($P < 0.01$). In parallel, ENG heifers showed higher concentrations of insulin (4.9 ± 0.19 μ U/mL versus 4.2 ± 0.13 μ U/mL), leptin (0.98 ± 0.03 ng/mL versus 0.75 ± 0.02 ng/mL), IGF-I (351 ± 9 ng/mL versus 300 ± 8 ng/mL), and glucose (4.4 ± 0.03 mmol/L versus 4.2 ± 0.03 mmol/L) than those in the MNG ($P < 0.01$), indicating a more favorable state of metabolic homeostasis.

Glucose

Glucose is a fundamental energy source for cellular metabolism and for the metabolic and physiological functions of the ovaries, which play a central role in reproduction by promoting follicular development, maturation, and ovulation, as well as the formation and

maintenance of a functional *corpus luteum* (CL) (Senosy *et al.*, 2017). The ovaries possess reproductive mechanisms that regulate glucose uptake; however, because glucose is a hydrophilic molecule, it cannot cross the plasma membrane directly and therefore requires sugar transporters (GLUT) to facilitate its absorption by tissues (Dupont & Scaramuzzi, 2016).

GLUT

GLUT4 is found in muscle and adipose tissue and functions as an insulin-dependent transporter. Consequently, glucose uptake by skeletal muscle and adipose tissue requires insulin-mediated signaling (Dupont & Scaramuzzi, 2016). It has been demonstrated that the ovaries can regulate glucose uptake and that the hormonal environment of the follicles may influence glucose uptake through the expression of GLUT1, GLUT3, and GLUT4, which together maintain glucose supply to the ovaries, thereby supporting follicular development, ovulation, and the formation and maintenance of the CL. According to the findings of Nishimoto *et al.* (2006), bovine corpora lutea and follicles expressed GLUT1, GLUT3, and GLUT4. Significant negative correlations ($P < 0.0001$) were observed between glucose concentrations in follicular fluid and the mRNA levels of GLUT1 ($r = -0.86$) and GLUT3 ($r = -0.87$), whereas significant positive correlations ($P < 0.0001$) were identified between glucose concentrations and GLUT4 mRNA levels in the theca interna cells of the follicles. In this sense, serum glucose concentration directly affects follicular glucose uptake; therefore, follicles are able to capture substantial amounts of glucose, provided that it is available in circulation.

IGF-I

In heifers, the consumption of high levels of energy and protein provides greater availability of utilizable nutrients for growth and simultaneously increases the concentration of IGF-I, a hormone produced in the theca cells of the ovaries that modulates the effects of growth hormone (GH), in addition to regulating gonadotropin-induced folliculogenesis and the activity of the hypothalamus and pituitary gland (Arboleda *et al.*, 2011; Armstrong *et al.*, 1992; Oyedipe *et al.*, 1982). This allows animals to express their growth potential, resulting in improved development at a younger age and greater body weight at puberty (Ferraz *et al.*, 2018). Thus, IGF-I serves as an indicator of adequate body condition, supporting an optimal metabolic environment for triggering the first ovulation. Indeed, increases in circulating IGF-I have been observed in heifers days or even hours before the onset of puberty (Alamilla Rodríguez *et al.*, 2017), a phenomenon driven by elevated LH concentrations (Arboleda *et al.*, 2011).

Leptin

Adequate body condition score (BCS) in heifers is indispensable for the onset of puberty; consequently, appropriate nutritional management promotes adipose tissue deposition, which in turn stimulates leptin secretion, a hormone closely associated with nutritional status and essential for the initiation of reproductive function (Cooke *et al.*, 2020). Leptin regulates the secretion of gonadotropins, such as luteinizing hormone (LH) and follicle-

stimulating hormone (FSH), which are responsible for modulating the hypothalamic-pituitary axis (Bruinje *et al.*, 2021). Thus, it acts as one of the key physiological signals involved in the onset of puberty (Childs *et al.*, 2021). Studies have shown that, in prepubertal heifers, increased leptin concentrations promote the initiation of puberty by enhancing LH secretion (Cardoso *et al.*, 2018). Furthermore, short-term fasting has been found to reduce leptin secretion by adipocytes and, consequently, to decrease LH secretion (Amstalden *et al.*, 2014).

Allen *et al.* (2012) conducted a study in newly weaned prepubertal heifers using two dietary treatments: a low-gain diet (LGD) with low energy content (1.48 Mcal/kg), formulated to promote an average daily gain (ADG) of 0.5 kg/day, and a high-gain diet (HGD) with higher energy content (1.53 Mcal/kg), designed to promote an ADG of 1.0 kg/day. The authors reported insulin concentrations of 1.16 ± 0.11 versus 0.95 ± 0.07 ng/mL ($P < 0.05$), insulin-like growth factor I (IGF-I) concentrations of 103.75 ± 9.9 versus 52.01 ± 4.39 ng/mL ($P < 0.005$), and leptin concentrations of 3.62 ± 0.43 versus 2.55 ± 0.35 ng/mL ($P < 0.1$), respectively, for HGD and LGD. Several years later, Allen *et al.* (2017) confirmed that, during the prepubertal development of crossbred beef heifers, animals with an ADG ranging from 0.8 to 1.5 kg/day exhibited higher concentrations of reproductive precursor hormones (RPH). Significantly higher leptin concentrations were observed at 24 ± 1 weeks of age ($P < 0.01$) and 26 ± 1 weeks of age ($P < 0.0001$) in the high-gain group (0.91 kg/day), which received a diet with a high concentrate content. Mean glucose concentrations increased by 13% ($P < 0.006$) in the high-gain groups compared with the low-gain groups (0.46 kg/day). Likewise, the high-gain groups exhibited significantly higher insulin concentrations at 20 ± 1 weeks of age ($P < 0.01$), 22 ± 1 weeks of age ($P < 0.1$), and 30 ± 1 weeks of age ($P < 0.04$) than the low-gain groups. However, no significant differences were detected in mean IGF-I concentrations between the study groups.

In this context, Cardoso *et al.* (2020) demonstrated that variation in RPH concentrations during post-weaning development in heifers promotes an increase in the pulsatile secretion of gonadotropin-releasing hormone (GnRH), LH, and FSH, thereby favoring an earlier onset of puberty. Conversely, Zieba *et al.* (2004) conducted a study in prepubertal Brahman $\times$ Hereford heifers using a restricted-growth diet (RG), designed to promote an ADG of approximately 0.3 kg/day, and a normal-growth diet (NG), designed to promote an ADG of approximately 0.68 kg/day. The authors observed that mean plasma LH concentrations were significantly lower ($P < 0.001$) in heifers fed the RG diet than in those fed the NG diet (0.66 ± 0.01 versus 0.76 ± 0.01 ng/mL for RG and NG, respectively).

To integrate the available evidence, Table 2 summarizes the principal effects of nutritional status on endocrine regulation and reproductive performance in heifers, taking into account the physiological stage as well as the associated metabolic and hormonal responses.

LIMITATIONS OF THE STUDY

Most of the available studies simultaneously combine variations in protein and energy levels, which makes it difficult to assess, in an isolated and precise manner, the specific impact of energy on puberty onset and the associated hormonal concentrations.

Table 2. Effects of nutritional status on endocrine regulation and reproductive performance in heifers.

Metabolic factor	Physiological stage	Metabolic effects	Endocrine response	Reproductive consequence	Reference
Energy deficiency	Prepubertal	Negative energy balance (NEB), low average daily gain (ADG)	Low levels of reproductive precursor hormones (RPH)	Delayed puberty	(Alves <i>et al.</i> , 2019; Ferraz <i>et al.</i> , 2023)
Adequate energy	Prepubertal	Optimal average daily gain (ADG)	Elevated levels of insulin-like growth factor I (IGF-I) and leptin, with activation of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH)	Precocious puberty	(van Niekerk <i>et al.</i> , 2021)
Excess energy	Prepubertal	Increased adiposity	Hormonal dysregulation	Anovulation and reduced milk production	(Cooke <i>et al.</i> , 2020; van Niekerk <i>et al.</i> , 2021)
Energy deficiency	Postpubertal	Negative energy balance (NEB), low average daily gain (ADG)	Low luteinizing hormone (LH) levels	Anestrus and reduced fertility	(Alamilla Rodríguez <i>et al.</i> , 2017; Ferraz <i>et al.</i> , 2023; Godoy <i>et al.</i> , 2018)
Protein deficiency	Prepubertal	Reduced tissue synthesis	Low insulin-like growth factor I (IGF-I) levels	Delayed puberty	(Alamilla Rodríguez <i>et al.</i> , 2017; Godoy <i>et al.</i> , 2018)
Adequate protein	Prepubertal	Greater structural growth	Elevated insulin-like growth factor I (IGF-I) levels	Improved ovarian development	

NEB: negative energy balance; ADG: average daily gain; RPH: reproductive precursor hormones; GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; IGF-I: insulin-like growth factor I.

In particular, further research is required to determine how energy levels, independently of other nutritional factors, may influence the concentrations of reproductive precursor hormones (RPH), which are involved in the regulation of growth and reproductive maturity. Such an approach could provide a more refined understanding of the underlying physiological mechanisms and, in turn, contribute to the optimization of nutritional management strategies aimed at improving reproductive efficiency in cattle production systems.

Despite the rigor of this review, several methodological limitations must be acknowledged, as they may affect the interpretation of the findings. The heterogeneity among studies in terms of breed, supplementation protocols, and climatic conditions precluded the performance of a meta-analysis with robust statistical validity. Publication bias may also be present, since negative or null results are less likely to be reported. Moreover, most studies have focused primarily on energy, with limited information available regarding microelements or the methodological quality necessary to support the reproducibility of the reported findings.

FUTURE RESEARCH DIRECTIONS

Based on the gaps identified, further investigation into reproductive management under tropical conditions is warranted. In particular, future studies should examine how early energy supplementation induces epigenetic changes in kisspeptin neurons that may affect puberty onset; analyze the synergistic influence of vitamins and minerals beyond the conventional focus on energy and protein; and develop predictive models for tropical systems that integrate weight gain, hormonal indices, and the temperature-humidity index in order to estimate the optimal breeding age with greater precision.

CONCLUSIONS

The evidence analyzed in this review confirms that nutrition plays a decisive role in pubertal development and reproductive performance in female cattle by directly and indirectly modulating the endocrine regulation of the hypothalamic-pituitary-gonadal axis. In particular, energy balance and an adequate supply of nutrients during the prepubertal stage influence the activation of metabolic signals, such as leptin, insulin, and insulin-like growth factor I (IGF-I), which enable the establishment of pulsatile gonadotropin-releasing hormone (GnRH) secretion and the functional maturation of the reproductive system.

Likewise, clear differences emerge between the nutritional effects observed during the prepubertal and postpubertal stages. Whereas, in the prepubertal phase, nutrition primarily determines age at puberty and initial ovarian development, in the postpubertal stage its impact is reflected in estrous cycle regularity, luteal function, and overall reproductive efficiency. Both nutritional deficiency and nutritional excess may compromise these processes, thereby highlighting the need for balanced feeding strategies tailored to the physiological status of the animal.

In tropical production systems, additional factors such as forage seasonality and heat stress intensify nutritional limitations, which may exacerbate metabolic and endocrine disturbances associated with delayed puberty, anestrus, or reduced fertility. Nevertheless, the available literature remains limited with regard to studies that simultaneously integrate nutrition, endocrine regulation, and the environmental conditions characteristic of tropical regions.

In this context, it is essential to strengthen research aimed at evaluating specific nutritional strategies for tropical production systems, considering not only productive indicators but also endocrine variables that may provide a more precise understanding of the physiological mechanisms involved. Such approaches will contribute to the design of more efficient nutritional management programs, with the potential to improve reproductive efficiency and the sustainability of beef cattle production systems in tropical regions.

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Composition and microbiological quality of edible insects subjected to two drying methods

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ABSTRACT

Objective: To compare the chemical composition and microbiological quality of *escamoles* (*Liometopum apiculatum* Mayr) and white maguery worm (*Aegiale hesperiaris* W.) subjected to freeze-drying and oven drying.

Design/Methodology/Approach: The presence of *Escherichia coli*, molds, and yeasts was quantified. In addition, titratable acidity, free fatty acids (FFA), carbohydrates (CHO), fat, moisture, protein, and ash contents were determined in both edible insect species.

Results: *Escamoles* showed the highest content of FFA ($0.65 \pm 0.05\%$), titratable acidity ($5.2 \pm 0.2\%$), and protein ($17.53 \pm 0.64\%$), while the white maguery worm recorded the highest ash content ($25.4 \pm 1.51\%$). Oven drying reduced the presence of *E. coli*, molds, and yeasts to levels that comply with the Official Mexican Standard NOM-210-SSA1-2014.

Study Limitations/Implications: Edible insects contain significant amounts of proteins, minerals, and vitamins that can contribute to improved human nutrition. The food industry has shown interest in processing these insects into flour, in order to include it into other products; however, information regarding their microbiological quality remains limited.

Findings/Conclusions: These results highlight the importance of applying appropriate heat treatments to guarantee the safety of edible insects, particularly if they are used as food ingredients by the industry. The combination of high nutritional value and adequate microbiological quality positions these insects as a promising and safe alternative for the development of innovative food products.

Keywords: protein, microbiological quality, *Liometopum apiculatum* Mayr, *Aegiale hesperiaris* W., drying.

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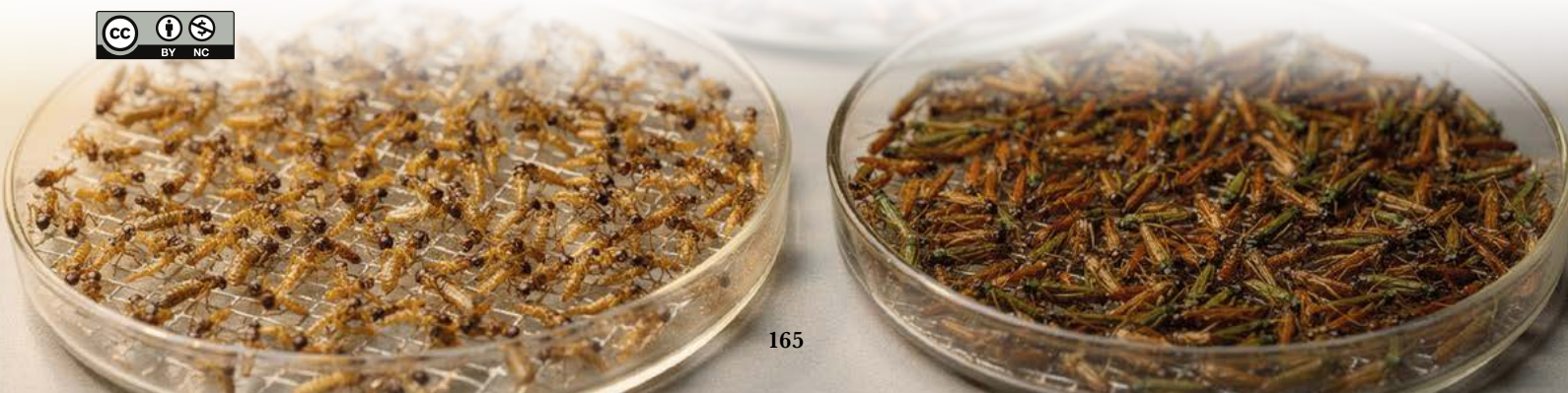
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INTRODUCTION

The UN (ONU, 2025) estimates that the world population will reach 10.3 billion people by 2080. This steady growth will increase the demand for resources and exacerbate malnutrition problems. Therefore, the search for sustainable protein sources has become critically important (Doğan and Çekal, 2022). Edible insects have been consumed in Mexico since ancient times (Melgar *et al.*, 2019). In the Potosino-Zacatecano Highlands, the consumption of *escamoles* (*Liometopum apiculatum* Mayr) and white maguery worm (*Aegiale*



hesperiaris W.) has social, economic, and environmental significance (Cruz-Labana *et al.*, 2018). However, studies addressing the microbiological quality of edible insects remain scarce (Arango *et al.*, 2004).

In this context, the use of edible insects provides an opportunity for the supplementation of balanced diets and for the whole gastronomy sector (Guiné *et al.*, 2021; Aigbedion-Atalor *et al.*, 2024).

Edible insects contain high levels of proteins, vitamins, minerals, amino acids, and essential amino acids (Doğan and Çekal, 2022; Papastavropoulou *et al.*, 2022). In addition, their production may be more sustainable than other protein sources, due to lower greenhouse gas emissions, lower land use, and potentially lower water footprint (Guiné *et al.*, 2021; Nowakowski *et al.*, 2022; Mishyna *et al.*, 2023).

The inclusion of edible insect flours into food products has increased iron and zinc contents (Ochieng *et al.*, 2023) and improved the texture of baked products such as cookies (Bottle *et al.*, 2024). However, prior drying of edible insects is essential, since molds, yeasts, and other pathogenic microorganisms have been detected in products made from these flours (Ibarra, 2017; Ochieng *et al.*, 2023).

Recently, interest in the processing of edible insects has increased in developing countries, as they can contribute to the improvement of human nutrition (Imathiu, 2020) and food quality. Nevertheless, due to harvesting processes and secondary contamination, pathogens such as *Salmonella* spp. and *Escherichia coli* have been detected in edible insects (Pal *et al.*, 2024). These agents are responsible for approximately 4% of all foodborne illnesses worldwide (Kapoor *et al.*, 2023). Therefore, the chemical composition and microbiological quality of *Liometopum apiculatum* Mayr and *Aegiale hesperiaris* W. were evaluated under two drying methods in order to determine their nutritional value and specific microbial load.

MATERIALS AND METHODS

The research was conducted in the Chemistry, Biochemistry, and Microbiology Laboratory of the Coordinación Académica Región Altiplano Oeste of the Autonomous University of San Luis Potosí, Mexico (22° 38' 28.5" N, -101° 42' 10.0" W).

Biological materials

Escamoles (*Liometopum apiculatum* Mayr) and white maguery worms (*Aegiale hesperiaris* W.) were used in this study. One kg of specimens of each species was provided by local collectors from Salinas, San Luis Potosí, Mexico, between March and June 2023 (Figuerola-Sandoval *et al.*, 2018). The insects were washed with purified water to remove impurities and stored frozen at -16 °C in polyethylene bags.

Variables and treatments

A proximate chemical analysis of the two edible insect species was performed. The presence of *Escherichia coli*, molds, and yeasts in insects subjected to freeze-drying and oven drying was compared with the microbial load found in fresh insects.

Moisture

Moisture content was determined in 5 g of fresh weight (FW) of each insect species using a Radwag[®] MA 50/1. R thermobalance, following the method described by Ileleji *et al.* (2010).

Free fatty acids (FFA)

Twenty-five mL of neutralized alcohol solution were added to 5 g (FW) of each insect species. Subsequently, 1 mL of phenolphthalein indicator was added and the solution was titrated with 0.01 N NaOH (AOCS, 2004). The FFA content was calculated using the following equation:

$$FFA = S \times N \times 28.2 \times W^{-1} \quad (1)$$

where: *FFA* is the free fatty acid content expressed as oleic acid (%); *S* is the volume of sodium hydroxide consumed (mL); *N* is the normality of sodium hydroxide (0.1 N); and *W* is the sample weight (g).

Titrateable acidity

The percentage of titrateable acidity was determined by titration. Ten grams of each insect sample were weighed and mixed with 90 mL of distilled water adjusted to a pH of 7 (10% sample). The mixture was homogenized in an Oster[®] BLST4655-013 blender and filtered through cheesecloth. Ten mL of the filtrate were titrated with 0.1 N NaOH using two drops of phenolphthalein as indicator (Melia *et al.*, 2022). The acidity percentage was calculated as follows:

$$Acidity(\%) = v \times N \times m_{eq} \times 100 \times m^{-1} \quad (2)$$

where: *v* is the volume of sodium hydroxide used (mL); *N* is the normality of NaOH (0.1 N); *m_{eq}* is the equivalent weight of lactic acid; and *m* is the mass (g) of the titrated sample.

Carbohydrates

To determine carbohydrate content, 5 g of FW of insects were mixed with 10 mL of distilled water, macerated in a mortar, and filtered through a cloth filter (cheesecloth). The samples were centrifuged at 4,000 rpm for 5 min (Hettich[®], Universal 320 R). Subsequently, 1 mL of the filtered sample was transferred to test tubes and 3 mL of sulfuric acid were added. The mixture was shaken for 30 s and then cooled in an ice bath for 2 min. Finally, absorbance was measured at 315 nm using a spectrophotometer (MAPADA, VISIBLE V-1600/1600 PC). Distilled water was used as blank. A standard calibration curve was prepared using D (+)-xylose (Millipore, 58-86-6) at concentrations of 1.25, 2.5, 5, and 10 mg/L. Results were expressed as xylose equivalents (%), according to Albalasmeh *et al.* (2013).

Fat

Fat content was determined using the AOAC method 920 (1990). Five g of previously dried and ground insect samples were placed in a porous extraction cartridge. Extraction was performed using a Soxhlet apparatus. The cartridge was placed in the extractor chamber. A total of 250 mL of hexane was heated at 130 °C. The extraction process was repeated for 8 h until complete extraction of the analytes from the sample. The solvent was evaporated at 100 °C, to obtain the dried hexane extract. Fat content was calculated using the following equation:

$$Fat = 100 \times (W_1 - W_2) (W^{-1}) \quad (3)$$

where: *Fat* is the fat content (%); *W* is the weight of the sample used; *W*₁ is the weight of the container with the dried hexane extract (fat residue); and *W*₂ is the weight of the empty container.

Protein

Protein content was determined using the Kjeldahl method 2.062 (AOAC, 1984). A 3 g insect sample and 1 g of protein catalyst were placed in a Büchi digestion unit. Subsequently, 2.5 mL of concentrated sulfuric acid were added until the sample changed from a dark to a clear color. Subsequently, 10 mL of distilled water were added to the sample, which was transferred to a tube in the distillation unit. Then, 10 mL of 60% NaOH were added. The distillate was collected in an Erlenmeyer flask, mixing 5 mL of saturated boric acid and two drops of methyl red indicator. Distillation stopped until the flask reached a final volume of 50 mL. Finally, the distillate was titrated with 0.125 N HCl. The crude protein percentage was calculated with the following equation, using the 6.25 factor:

$$Protein (\%) = 14.007 (v - b) \times (N) (factor) (100) (m)^{-1} \quad (4)$$

where: *v* is the volume of HCl used (mL); *b* is the blank volume (mL); *N* is the normality of HCl; and *m* is the mass of the sample (g).

Ash

Ash content was determined following the AOAC procedure 923.03 (AOAC, 1990). Five grams of fresh insect samples were weighed and placed in crucibles previously brought to constant weight. Samples were initially incinerated at low flame in a fume hood to avoid losses caused by smoke or liquid spillage. The crucibles were then placed in a Thermo Scientific® Thermolyne muffle furnace preheated at 550 °C for 30 min. Samples were maintained at this temperature for 2 h. Finally, the crucibles were transferred to a desiccator until constant weight was achieved. Ash content was calculated using the following equation:

$$Ash (\%) = 100 \times (W_2 - W_1) \times (W_0)^{-1} \quad (5)$$

where: W_0 is the sample mass (g), W_1 is the crucible mass (g), and W_2 is the mass of the crucible with ash (g).

Microbiological analysis

The presence of *E. coli*, molds, and yeasts was quantified in both fresh and dried edible insect species. The inocula were prepared following the Mexican Official Standard NOM-110-SSA1-1994 with four repetitions. One g of insect sample and 9 mL of diluent were placed in test tubes containing nine glass beads. The tubes were stirred in a Vortex[®] XH-D for 2 min and allowed to settle for 5 min. Monobasic sodium phosphate buffer (NaH_2PO_4 , 95%) was used as diluent for mesophilic molds and yeasts. The pH was adjusted at 7.2 with NaOH (0.01 N) and the solution was sterilized at 121 °C for 15 min.

Mesophilic molds and yeasts were quantified using potato dextrose agar (PDA, Difco[®], Detroit, MI, USA) according to NOM-110-SSA1-1994. One mL of each inoculum was placed at the center of 90-mm Petri dishes containing agar acidified with 10% tartaric acid. Subsequently, the inocula were spread using a Drigalski spatula with six movements from the center toward the edge of the plate. Inoculated plates were incubated at 25 ± 2 °C (Yamato Scientific[®], IN-804-115V). After 72 h of incubation, colonies were counted and microbial density was calculated as CFU g^{-1} using the following equation:

$$\text{CFU} \times \text{mL}^{-1} = A \times F \times B^{-1} \quad (6)$$

where: A is the number of colony-forming units; B is the inoculated volume (mL); and F is the dilution factor.

For the preparation of the *E. coli* inoculum, peptone water at pH 9 was used as diluent according to NOM-110-SSA1-1994. Detection was carried out using MacConkey selective agar (BD Bioxon[™], Mexico City, Mexico) following the procedure described by Cardona-López *et al.* (2020). Twenty-five mL of agar was poured into each sterile Petri dish and sealed with Parafilm[®] plastic film. Before inoculation, a 1:10 dilution was prepared by mixing 1 mL of inoculum with 9 mL of peptone water. One mL of the dilution was poured and spread over the agar surface using a Drigalski spatula as previously described. The plates were incubated at 35 °C for 24 h in a Yamato Scientific[®] IN-804-115V incubator. After incubation, Petri dishes showing intense pink colonies with precipitation halos were selected. Colony counts were performed using an UNICO PRO[®] 1001439 colony counter.

Data analysis

The chemical variables of both insect species were compared using a t-test ($\alpha=0.05$). Microbiological counts were subjected to an analysis of variance (ANOVA), followed by Tukey's multiple comparison test ($\alpha=0.05$). All analyses were conducted in R-project[®] v. 4.4.1 using the RStudio[®] interface v. 2024.04.2.

RESULTS AND DISCUSSION

Chemical composition

No significant differences were found in the moisture ($p=0.261$), carbohydrates ($p=0.480$), or fat content ($p=0.601$) of both edible insect species (Table 1). Their moisture content was similar to that reported by Rostro *et al.* (2012) for escamoles (71.90%) and white maguay worms (70.78%) collected in Teotihuacán, Mexico.

Edible insects showed a carbohydrate content close to 10% (Mayack and Naug, 2010). Melo-Ruiz *et al.* (2016) determined that escamoles contain relatively high carbohydrate levels (6.8-18.27%), which may be the result of the conversion of proteins into carbohydrates through the gluconeogenesis pathway. According to Rostro *et al.* (2012), the fat content of white maguay worms ranges from 8.64 to 9.80%, which matches the results obtained in this study ($9.5 \pm 2.86\%$). Rostro *et al.* (2012) also reported that the fat content of escamoles ranges from 10.39 to 12.40%. In contrast, the results of this study were lower ($7.9 \pm 3.92\%$).

Significant differences ($p \leq 0.05$) were found in the content of free fatty acids (FFA), titratable acidity, protein, and ash between insect species (Table 1). Escamoles showed higher FFA content than white maguay worms. Nevertheless, these values were lower than those reported by Atowa *et al.* (2021), who found levels ranging from 24.91 to 36.57%. According to Ewald *et al.* (2020), the content of vaccenic acid ranges from 0.8 to 1.5%; and values above 1% suggest the presence of beneficial fatty acids, for human growth and development. The percentage of titratable acidity in both edible insect species fell within the range reported for preserving sausages (5.07-6.2%) (Capita *et al.*, 2006).

Escamoles stood out for their protein content ($17.53 \pm 0.64\%$) compared with white maguay worms ($10.80 \pm 0.26\%$). Nevertheless, both values were higher than those reported by Rostro *et al.* (2012), who indicated that white maguay worms contain between 8.64 and 9.80% protein, while escamoles contain between 10.39 and 12.40%. White maguay worms showed the highest mineral content, reaching $25.40 \pm 1.51\%$, which exceeded the values reported by Rostro *et al.* (2012) (1.00-1.05%). The ash content of escamoles ($13.20 \pm 1.06\%$) was also higher than the percentages (6.53-7.85%) reported by Melo-Ruiz *et al.* (2016) (Table 1).

Table 1. Comparison of the composition of two species of edible insects.

Constituents	<i>L. apiculatum</i> Mayr.	<i>A. hesperiaris</i> W.
Moisture (%)	60.91 ± 11.04^a	70.78 ± 0.66^a
Free fatty acids (%)	0.65 ± 0.05^a	0.40 ± 0.13^b
Titratable acidity (%)	5.20 ± 0.20^a	4.20 ± 0.20^b
Carbohydrates (%)	8.64 ± 1.02^a	9.14 ± 0.21^a
Fat (%)	7.90 ± 3.92^a	9.50 ± 2.86^a
Protein (%)	17.53 ± 0.64^a	10.80 ± 0.26^b
Ashes (%)	13.20 ± 1.06^b	25.40 ± 1.51^a

¥ % on a wet basis. Means with the same superscript within each row are not significantly different ($\alpha=0.05$).

Microbiological quality

The drying method had a significant effect ($p<0.001$) on the growth of molds and yeasts after 72 h of incubation (Figure 1). Oven drying resulted in the lowest number of colony-forming units (CFU), but this pattern was not impacted by the edible insect species (Table 2). Both species in their fresh and freeze-dried states exceeded the limit for molds and yeasts (150 CFU g^{-1}) established by the Mexican Official Standard NOM-111-SSA1-1994. In contrast, oven-dried samples had 0.33 CFU g^{-1} , which made them microbiologically safe.

Edible insects are feasible option for human consumption; however, briefly heating crickets and cricket flour can reduce some bacterial populations, although *Bacillus cereus* may survive (Pal *et al.*, 2024), potentially explaining the reduction of the microbial load found in oven-dried insects (Table 2). Kolakowski *et al.* (2021) indicated that raw, unprocessed insects contain more bacteria than conventional meat samples; therefore, proper sterilization and handling are essential for both the food industry and consumers.

After 24 h of incubation (Figure 2), no significant differences ($p=0.409$) were found in the number of colony-forming units between species. The values recorded in this study for freeze-dried white maguey worms ($244.67\pm201.00\text{ CFU g}^{-1}$) and for *escamoles* ($335.00\pm135.60\text{ CFU g}^{-1}$) can potentially exceed the maximum limit (250 CFU g^{-1}) established by the Mexican Official Standard NOM-210-SSA1-2014 (Table 2). The presence of bacteria such as *E. coli* and *Salmonella* spp. poses a risk to human health, as they may cause foodborne diseases (Guragain *et al.*, 2024).

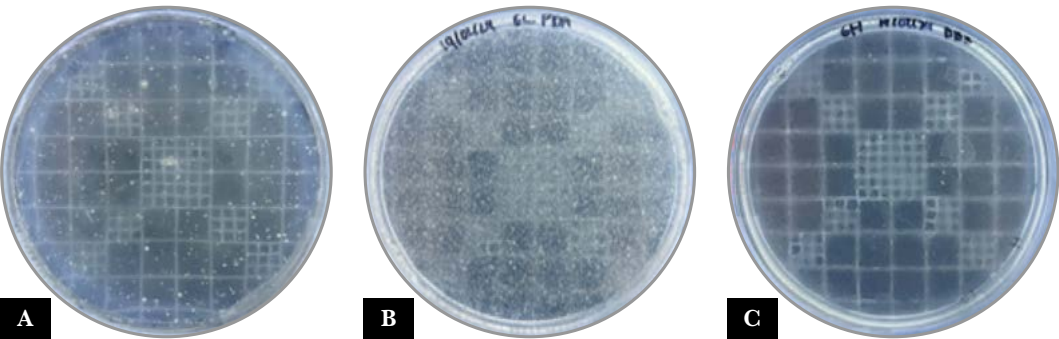


Figure 1. Growth of molds and yeasts in white maguey worms (*A. hesperiaris* W.): (A) fresh, (B) freeze-dried, and (C) oven-dried.

Table 2. Presence of molds, yeasts, and *E. coli* in two species of edible insects.

Species	Sample preparation	Molds and yeasts (CFU g ⁻¹)	<i>E. coli</i> (CFU g ⁻¹)
<i>L. apiculatum</i> Mayr.	Fresh	305±68.2 ^a	336.67±163.61 ^{ab}
	Freeze-dried	271±60.3 ^a	244.67±201.00 ^{ab}
	Oven-dried	0±0.0 ^b	0.67±1.16 ^b
<i>A. hesperiaris</i> W.	Fresh	371±136.0 ^a	405.00±134.81 ^a
	Freeze-dried	299±87.6 ^a	335.00±135.60 ^{ab}
	Oven-dried	0.7±1.2 ^b	1.00±1.00 ^b

*Values with the same superscript within each column are not significantly different ($\alpha=0.05$).

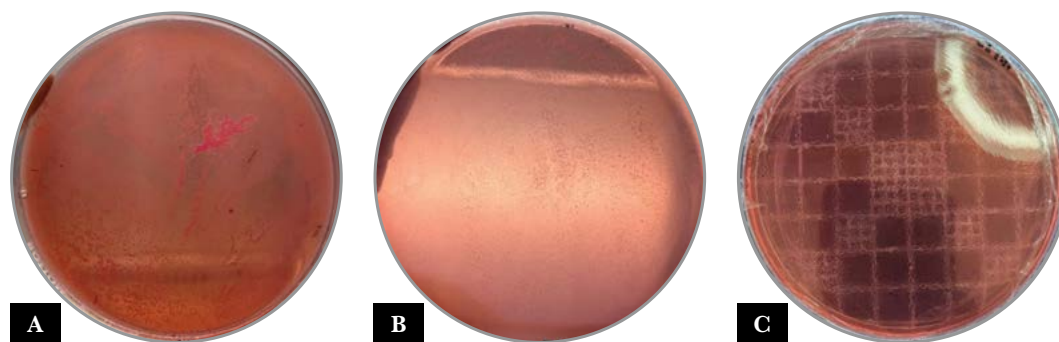


Figure 2. *E. coli* growth in escamoles (*L. apiculatum* Mayr): (A) fresh, (B) freeze-dried, and (C) oven-dried.

Significant differences ($p < 0.001$) were observed depending on the condition of the edible insects. Fresh samples showed the highest content of *E. coli* colony-forming units (370.83 ± 139.00 CFU g⁻¹), followed by freeze-dried insects (289.83 ± 161.00 CFU g⁻¹), and oven-dried insects (0.83 ± 0.98 CFU g⁻¹). These results suggest that the oven-drying method results in a microbiologically safe product, following the Mexican Official Standard NOM-210-SSA1-2014. According to Hernández-Álvarez *et al.* (2021), fresh edible insects pose a potential microbiological risk; however, this risk can be reduced through drying processes. These results are similar to the findings of this study. Fresh white maguery worms showed the highest *E. coli* colony-forming units (405.00 ± 134.81 CFU g⁻¹) compared with oven-dried insects, which recorded 1.00 ± 1.00 CFU g⁻¹ and 0.67 ± 1.15 CFU g⁻¹ for white maguery worms and *escamoles*, respectively (Table 2).

CONCLUSIONS

Escamoles (*L. apiculatum* Mayr) showed high free fatty acids, titratable acidity, and protein content. Meanwhile, white maguery worms (*A. hesperiaris* W.) were characterized by their higher ash content. The fresh samples of both insects indicated the presence of *E. coli*, molds, and yeasts at levels that may pose potential health risks. Oven drying significantly reduced the microbial load, proving its effectiveness as a hygienization method. Therefore, the implementation of this type of thermal processing is essential to guarantee the safety of these traditional foods.

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Antibacterial activity of polysaccharides from the fruiting bodies of *Boletus edulis* and *Lactarius indigo*

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ABSTRACT

Objective: This study aimed to evaluate the antibacterial activity of aqueous and alkaline polysaccharide fractions extracted from wild fruiting bodies of *Boletus edulis* and *Lactarius indigo* from central Mexico.

Design/methodology/approach: Polysaccharides were obtained and separated into aqueous (FAq) and alkaline (FAk) fractions, characterized by Fourier transform infrared spectroscopy (FTIR), and tested at concentrations of 500, 250, 125, and 62.5 $\mu\text{g mL}^{-1}$ using the broth dilution method against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Streptococcus mutans*.

Results: Four polysaccharide fractions were obtained (FAqBe, FAKBe, FAqLi, and FAKLi). All fractions exhibited antibacterial activity, with the highest inhibition (up to 97.6%) observed against *S. aureus* at 500 $\mu\text{g mL}^{-1}$ of FAKBe. A significant concentration-dependent inhibitory effect was detected.

Limitations on study/implications: However, further studies are required to elucidate the molecular mechanisms involved and to evaluate *in vivo* efficacy.

Findings/conclusions: These findings suggest that polysaccharides from wild edible mushrooms represent a promising source of bioactive compounds with antibacterial potential.

Keywords: Antibacterial activity, Aqueous and alkaline fractions, Bioactive polysaccharides, Functional foods, Wild edible mushrooms.

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INTRODUCTION

Edible mushrooms are valued as healthy foods with high nutritional content (Reis *et al.*, 2017). They contain a high concentration of carbohydrates and proteins, in addition to a considerable concentration of vitamins, minerals and fiber, with low levels of sodium and



unsaturated fats (Stamets, 2011). Edible mushrooms are also used in traditional medicine and are attributed with healing and therapeutic value (Guzmán, 2008), presenting immunomodulatory, anticancer and antibacterial properties (Cano-Estrada and Romero-Bautista, 2016).

Traditional knowledge has served as the basis for demonstrating the bioactivity of edible mushrooms against chronic degenerative diseases such as cancer, or cardiovascular, neurodegenerative and immunomodular diseases (Roncero-Ramos and Delgado-Andrade, 2017), as well as for the development of products for the food (Pizarro *et al.*, 2014; Reis *et al.*, 2017), pharmaceutical and cosmetic industries (Khan *et al.*, 2018).

Various studies have shown that the bioactivity of fungi is related to compounds present in their structure (Sari *et al.*, 2017; Muszyńska *et al.*, 2018). Polysaccharides are the most abundant structural compounds found in the cell walls of fungi (Arango and Nieto, 2013; Rathore *et al.*, 2017; Khan *et al.*, 2018). At structural level, polysaccharides can be found in the form of homopolysaccharides, heteropolysaccharides and glycoproteins (Donot *et al.*, 2012), and they have shown great potential as anticancer, antioxidant and antibacterial agents (Wasser, 2002; Chen *et al.*, 2012).

The polysaccharides present in basidiomycetes are composed of monosaccharides units linked by glycosidic bonds (Pizarro *et al.*, 2014). The bioactivity of polysaccharides is largely attributed to their structural variability (Synytsya and Novak, 2014), molecular mass, chain length (Wasser, 2002), solubility properties (Xiao *et al.*, 2004), branching frequency (Miyazaki *et al.*, 1979) and shaping in solution (Zhang *et al.*, 2004; Khan *et al.*, 2018).

It is important to note that, despite the great importance of edible mushrooms as functional foods (Wasser, 2002; Reis *et al.*, 2017), few studies have addressed wild edible mushrooms as a source of nutraceuticals (Arango and Nieto, 2013), particularly in traditionally valued yet understudied species such as *Boletus edulis* and *Lactarius indigo*. In addition to the nutritional, medicinal and economic value of *B. edulis* (Cano-Estrada and Romero-Bautista, 2016), studies have shown that some acetone and methanol extracts obtained from fruiting bodies of *B. edulis* exert a strong antimicrobial effect against bacteria such as *Escherichia coli* and *Staphylococcus aureus* (Kosanić *et al.*, 2012), as well as antioxidant activity (Tsai *et al.*, 2007) and antiproliferative and antitumor effects (Lemieszek *et al.*, 2013). *Lactarius indigo* is a species of both nutritional and pharmaceutical importance (Cano-Estrada and Romero-Bautista, 2016), since aqueous and organic extracts of its fruiting bodies have shown antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Salmonella enterica*, and antiproliferative activity against MCF7 cells (Ochoa-Zarzosa *et al.*, 2011), in addition to antitumor and anti-inflammatory effects (Cano-Estrada and Romero-Bautista, 2016). Moreover, various studies describe different antibacterial mechanisms, including the alteration and degradation of bacterial cell walls and cytoplasmic membranes (Cheng *et al.*, 2024; Liang *et al.*, 2024), as well as the penetration of polysaccharides into gram-positive bacteria due to the absence of an outer membrane composed of lipopolysaccharides or a phospholipid layer (Norhisham *et al.*, 2025). The aim of this study was to evaluate the antibacterial activity of an aqueous and alkaline fraction of polysaccharides from the fruiting bodies of wild *B. edulis* and *L. indigo* of central Mexico.

MATERIALS AND METHODS

Biological material

The study utilized the fruiting bodies of wild *B. edulis* and *L. indigo* collected in the Área de Protección de Flora y Fauna Nevado de Toluca (APFFNT), Mexico.

Obtaining biomass

The collected specimens (*Boletus edulis* 2000 g; *Lactarius indigo* 1600 g) were characterized and taxonomically identified according to Franco Maass *et al.* (2012). They were then oven-dried at 60 °C for 48 h in order to calculate the water content (Equation 1), then ground to obtain a powder, which was used for the extraction of polysaccharides.

$$\% \text{ of water} = \frac{(\text{fresh weight of biomass (g)} - \text{dry weight of biomass (g)})}{(\text{fresh weight of biomass (g)})} \times 100 \quad (1)$$

Extraction of crude polysaccharides

Biomass macerate: The dry biomass of the fruiting bodies of *B. edulis* (BFBe 103.87 g) and *L. indigo* (BFLi 142.37 g) was placed individually in 10 volumes of 70% ethanol for 24 h in order to eliminate apolar and low molecular weight compounds. This procedure was performed twice (Carbonero *et al.*, 2006). The biomass was subsequently recovered by filtering through filter paper of coarse porosity, discarding the ethanol.

Aqueous extraction: The biomass of the fruiting bodies recovered from the previous process was placed in 10 volumes of distilled water at 121 °C and 15 lbs for 60 min (Klaus *et al.*, 2011). The biomass was then filtered (cellulose filter of pore size 125 µm), obtaining an aqueous extract of crude polysaccharides in the filtrate and leaving the remaining biomass as a residue.

Alkaline extraction: The biomass from the aqueous extraction was recovered and placed in 10 volumes of an aqueous solution of NaOH at 2% (w/v) with 0.1% of NaBH₄, and heated at 80 °C for 4 h and subsequently neutralized with acetic acid (Klaus *et al.*, 2011). The resulting alkaline extract (Mizuno *et al.*, 1992) of crude polysaccharides was then filtered through a cellulose filter of pore size 125 µm and the biomass residue discarded.

Purification of polysaccharides

Ethanol precipitation: The aqueous and alkaline extracts of crude polysaccharides were placed individually in three volumes of cold ethanol (Smiderle *et al.*, 2013) in order to precipitate polysaccharides from the complexes of low molecular weight molecules (Carbonero *et al.*, 2006). Finally, the precipitates with polysaccharides corresponding to each extract were recovered.

Freezing-thawing of the precipitates: the precipitates with polysaccharides obtained were frozen and then slowly thawed at room temperature in order to eliminate linear compounds with a lower degree of branching. This process was carried out several times until there was no further formation of precipitate from the supernatant.

The precipitates from this process containing polysaccharides were recovered and centrifuged at 8000 rpm for 15 min at 15 °C (Smiderle *et al.*, 2013). The precipitates obtained from the centrifugation were then filtered through a cellulose acetate membrane of 0.2 μm in pore size. Two fractions of polysaccharides of each species were thus obtained: an aqueous fraction (FAq) and an alkaline fraction (FAk).

Identification of polysaccharides

A sample of each fraction (0.1 g) was oven-dried at 60 °C for 48 h until obtaining crystals, which were analyzed by FTIR under an absorbance range of 4000-400 cm^{-1} using the KBr disk technique (Mizuno *et al.*, 1992; Klaus *et al.*, 2011) to identify the vibrational mode of the characteristic bonds of polysaccharides (Alzorqi *et al.*, 2017). In addition, the regions associated with sugars, the anomeric region and the region associated with proteins were analyzed, corresponding to 3200, 2920, 1640, 1160, 1070, 1040, 970 and 890 cm^{-1} (Synytsya and Novak, 2014).

Antibacterial activity

The antibacterial activity test was carried out by dilution in broth according to the Clinical and Laboratory Standards Institute (CLSI, 2018). For this purpose, four strains of bacteria from the culture collection of the Laboratorio de Bioquímica de la Facultad de Odontología of the Universidad Nacional Autónoma de México (UNAM) were used: *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Streptococcus mutans*. These were reactivated through inoculation onto sterile Muller-Hinton agar (38 g L^{-1}) and incubated at 38 °C for 24 h. Bacterial cultures were diluted in saline solutions to make a bacterial suspension according to the McFarland 0.5 standard. In a 96-well plate, a volume of 200 μL of sterile Muller-Hinton broth was added per well and inoculated with 5 μL of the bacterial suspension (approximately $5 \times 10^5 \text{ CFU mL}^{-1}$). Moreover, a stock of each fraction was prepared and added to each well, with final concentrations of: 500, 250, 125 and 62.5 $\mu\text{g mL}^{-1}$ of polysaccharides. Sterile culture broth was used as a positive control, culture broth inoculated with bacteria as a negative control and sterile culture broth with different concentrations of polysaccharides was used as a blank. The plate was incubated at 38 °C for 24 h in an orbital shaker at 200 rpm. The two fractions of both species were applied in four concentrations to the four bacteria, with each treatment performed in quadruplicate in the wells (4 fractions $\times$ 4 concentrations $\times$ 4 bacteria $\times$ 4 repetitions in wells). Following incubation, the plate was analyzed in a plate reader in absorbance mode at 630 nm in order to determine the percentage viability of the bacterial inoculum compared to the untreated inoculum (Equation 2).

$$\%inhibition = \frac{(Treatment - Blank)}{(Negative\ control - Positive\ control)} \times 100 \quad (2)$$

where: the treatment was culture broth inoculated with bacteria and polysaccharides at different concentrations, the blank was culture broth and polysaccharides at different

concentrations, the negative control was medium inoculated with bacteria and the positive control was sterile culture broth.

Data collection and analysis

The data were collected and restructured into a long format, with each bacterial strain, treatment, and dosage combination treated as separate observations. The variable “dosage” was converted into a factor to perform categorical analysis. The dataset was structured to include the factors treatment, dose, and bacteria.

An analysis of variance (ANOVA) was performed to compare the inhibitory effects of the treatments, dosages, and bacterial strains. A simplified ANOVA model without interaction terms was used to identify the main effects. Tukey’s post-hoc test was applied to evaluate pairwise differences between the treatments, dosages, and bacterial strains, with significance set at $p < 0.05$. All analyses were conducted using R statistical software.

RESULTS AND DISCUSSION

Obtaining biomass

The fruiting bodies of *B. edulis* Pers Bull collected in *Abies* and *Abies-Pinus* forests at 3100-3500 m agreed with that reported by Franco Maass *et al.* (2012). The fruiting bodies presented a water content of 94.8 %. Macroscopically, they presented crowns of 8-15 cm in diameter, yellow-brown-reddish coloration, a midstipe of 7 to 20 cm in length and white to light brown in color. Microscopically, they presented basidia of $29.3 \times 11 \mu\text{m}$ and spindle-shaped and smooth basidiospores of 15.6- 4.6 μm . Likewise, the fruiting bodies of *L. indigo* (Schwein.) Fr. collected from *Pinus* forests at 3100-3500 m, agreed with that reported by Franco Maass *et al.* (2012).

They presented a water content of 91.1%. Macroscopically, the crowns were 7.5 cm in diameter, convex and umbilicus when ripe, indigo blue in color with grayish concentric areas and with slightly decurrent separated blades of indigo blue color. The stipe measured 4 cm and was blue and smooth. The basidia measured $5.1 \times 22 \mu\text{m}$, with a smooth-nailed shape, are hyaline and tetraesterygmatic, while the basidiospores were oval, measured $5.6 \times 7.3 \mu\text{m}$ and were hyaline and smooth.

Extraction and purification of polysaccharides

Total polysaccharides extracted from *B. edulis* were equivalent to 16.04 g of polysaccharides per 100 g of dry biomass, of which 12.36 g of polysaccharides per 100 g of dry biomass corresponded to the alkaline fraction (FAkBe) and 3.68 g of polysaccharides per 100 g of dry biomass to the aqueous fraction (FAqBe). Regarding *L. indigo*, 16.42 g of polysaccharides were obtained per 100 g of dry biomass, and 12.86 g of polysaccharides per 100 g of dry biomass corresponded to the alkaline fraction (FAkLi) and 3.56 g of polysaccharides per 100 g of dry biomass to the aqueous fraction (FAqLi). The alkaline fraction exhibited a higher concentration than the aqueous fraction.

Identification of polysaccharides

The FTIR spectra of the four fractions (Figure 1) show the characteristic vibrational mode of the bonds of polysaccharides (Movasaghi *et al.*, 2008). In the spectra of the fractions of *B. edulis* (FAqBe and FAkBe) (Figure 1A) and of *L. indigo* (FAqLi and FAkLi) (Figure 1B), there is a band at 3200 cm^{-1} corresponding to the vibrational mode of the hydroxyl bond OH, a band at 2920 cm^{-1} corresponding to the saturated CH bond (Šandula *et al.*, 1999) and a signal from 1160 to 1140 cm^{-1} related to the stretching of the C-O-C glycosidic bond, where the signal at 1040 cm^{-1} corresponds to the stretching C-O bond (Münzberg *et al.*, 1995). The characteristic bands of the carbon-glucoside bonds of a polysaccharide corresponding to 1160 , 1080 , 1040 , 970 and 890 cm^{-1} (Alzorqi *et al.*, 2017) were also presented, associated with the sugar region in the range of 1200 cm^{-1} to 950 cm^{-1} and the anomeric region in the range of 950 cm^{-1} to 750 cm^{-1} (Synytsya and Novak, 2014).

The four fractions (Figure 1) present an absorption signal at 1640 cm^{-1} , which is associated with the stretching vibrations of the carbonyl C=O bond of a polysaccharide-protein complex (Alzorqi *et al.*, 2017). The polysaccharides of these fractions of *B. edulis* and *L. indigo* could therefore be protein-coupled.

Antibacterial activity

The different concentrations (500 , 250 , 125 and $62.5\text{ }\mu\text{g mL}^{-1}$) of the aqueous and alkaline fractions of both species showed inhibition in the four bacterial strains (*S. mutans*, *S. aureus*, *E. coli* and *E. faecalis*). The analysis of variance (ANOVA) indicated significant main effects of treatment ($p=0.00501$), dose ($p=2.2\times 10^{-9}$), and bacteria ($p=1.43\times 10^{-7}$) on bacterial inhibition. These results suggest that both the type of treatment and dosage level significantly affect bacterial inhibition, and the response varies depending on the bacterial strain.

The concentrations with the highest percentage of inhibition (Figure 2) in *S. mutans* were of FAqBe at $500\text{ }\mu\text{g mL}^{-1}$ with 71.2% of inhibition and $250\text{ }\mu\text{g mL}^{-1}$ with 66.31% of

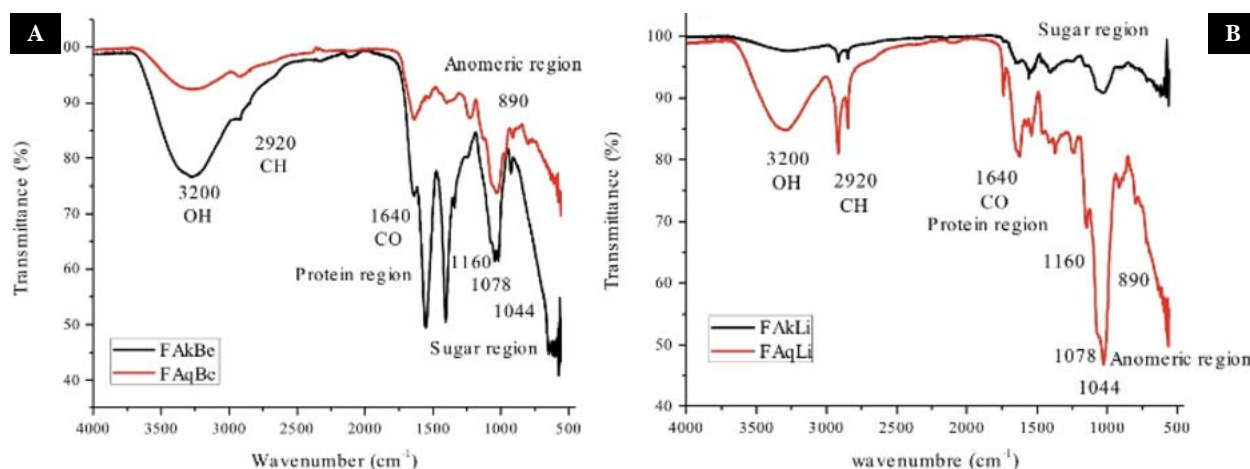


Figure 1. FTIR spectra of polysaccharide fractions obtained from *Boletus edulis* and *Lactarius indigo*: (A) aqueous (FAqBe) and alkaline (FAkBe) fractions of *B. edulis*; (B) aqueous (FAqLi) and alkaline (FAkLi) fractions of *L. indigo*.

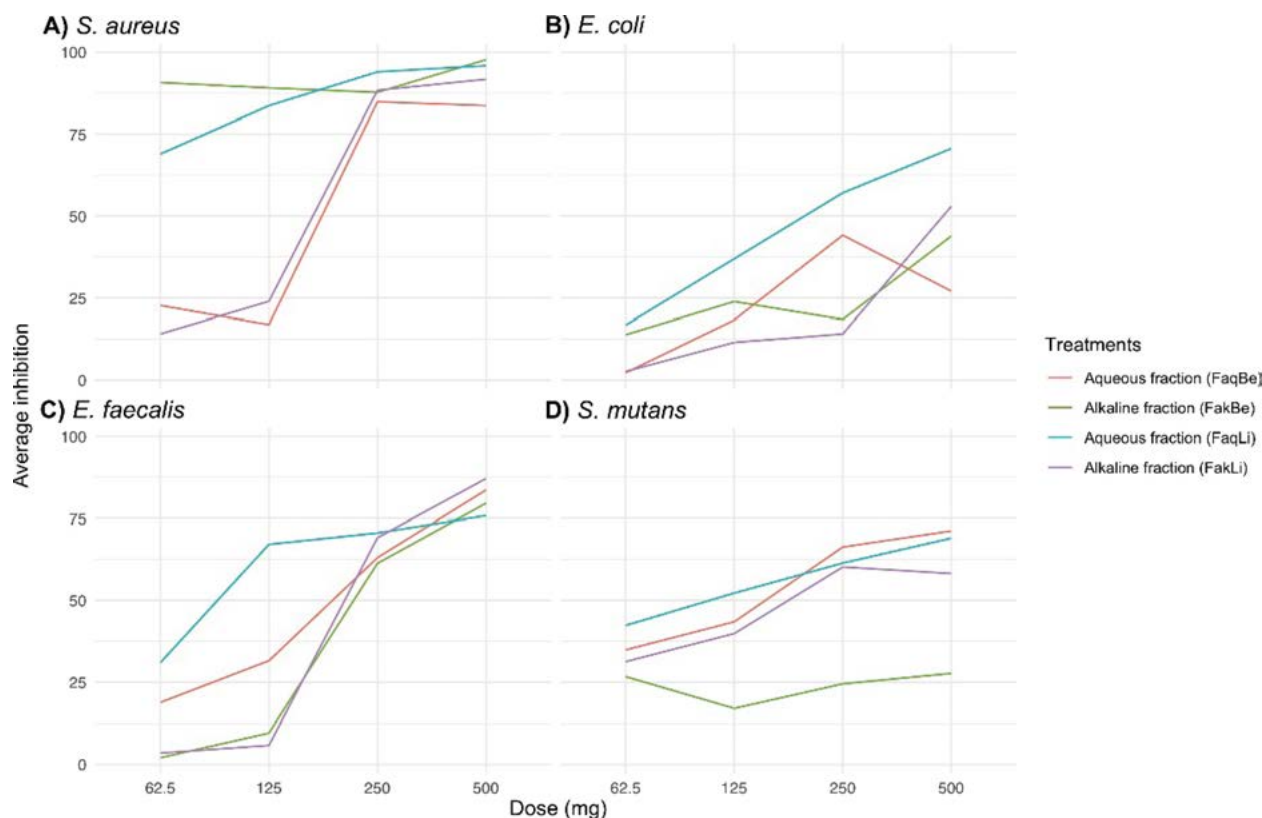


Figure 2. Antibacterial activity of polysaccharide fractions extracted from wild edible mushrooms. (A) Aqueous fraction of *Boletus edulis* (FAqBe), (B) alkaline fraction of *B. edulis* (FAkBe), (C) aqueous fraction of *Lactarius indigo* (FAqLi), and (D) alkaline fraction of *L. indigo* (FAkLi). Inhibition percentage (%) was determined using the broth dilution method at concentrations of 500, 250, 125, and 62.5 $\mu\text{g mL}^{-1}$ against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Streptococcus mutans*.

inhibition. For *E. coli*, FAkBe at 500 $\mu\text{g mL}^{-1}$ presented 43.91 % of inhibition, and FAqBe at 250 $\mu\text{g mL}^{-1}$ presented 44.15 % of inhibition. In the case of *E. faecalis*, both FAqBe and FAkBe at 500 $\mu\text{g mL}^{-1}$ showed the highest percentages of inhibition, with values of 83.60% and 79.55%, respectively. On the other hand, *S. aureus* reached values of up to 97.64% of inhibition at 500 $\mu\text{g mL}^{-1}$ of FAkBe.

Each fraction exhibited strong antibacterial activity on the four strains (Figure 2). In general, the highest percentage of inhibition of the four fractions was obtained at the highest concentration (500 $\mu\text{g mL}^{-1}$), approaching the positive control (sterile culture broth), while the lowest percentage of inhibition was at the lowest concentration (62.5 $\mu\text{g mL}^{-1}$), close to the negative control (bacterial inoculum with culture broth). However, FAqBe and FAkLi showed greater inhibition in *S. mutans* from the lowest concentration (62.5 $\mu\text{g mL}^{-1}$). Likewise, FAkBe and FAqLi presented greater inhibition on *S. aureus* at all concentrations.

Tukey's *post-hoc* test (Figure 3) revealed several significant pairwise comparisons: FAqLi was significantly more effective than both FAqBe ($p=0.0308$) and FAkBe ($p=0.0315$), particularly for *S. aureus* and *E. faecalis*. The FAkLi showed significantly less efficacy than FAqLi ($p=0.0061$), particularly for *S. mutans*. For dosage levels, 250 $\mu\text{g mL}^{-1}$ and 500 $\mu\text{g mL}^{-1}$

mL^{-1} were significantly more effective than $62.5 \mu\text{g mL}^{-1}$ ($p < 0.001$), and $500 \mu\text{g mL}^{-1}$ was more effective than $125 \mu\text{g mL}^{-1}$ ($p = 0.000006$). *Escherichia coli* exhibited significantly less inhibition compared to *S. aureus* ($p < 0.001$) and *S. mutans* ($p = 0.0389$), while *E. faecalis* showed intermediate inhibition levels. Besides, FAkBe exhibited the lowest inhibition for *S. mutans*, while FAqLi was the most consistent across all bacteria, with the highest median inhibition values.

Polysaccharides are major structural components that may constitute up to 50% of the dry weight of fungal fruiting bodies (Arango and Nieto, 2013). In this study, *B. edulis* was composed of 16.04 g of polysaccharides per 100 g of dry biomass, including the aqueous and alkaline fraction, while *L. indigo* comprised 16.42 g of polysaccharides per 100 g of dry biomass, including both fractions. Rathore *et al.* (2017) reported a direct relationship between biomass production and polysaccharide concentration, suggesting that biomass accumulation plays a key role in polysaccharide yield, particularly under natural growth conditions where substrates may induce the synthesis of enzymes involved in metabolite production (Ooi and Liu, 2000), which makes wild mushrooms a potential source of metabolites (Arango and Nieto, 2013).

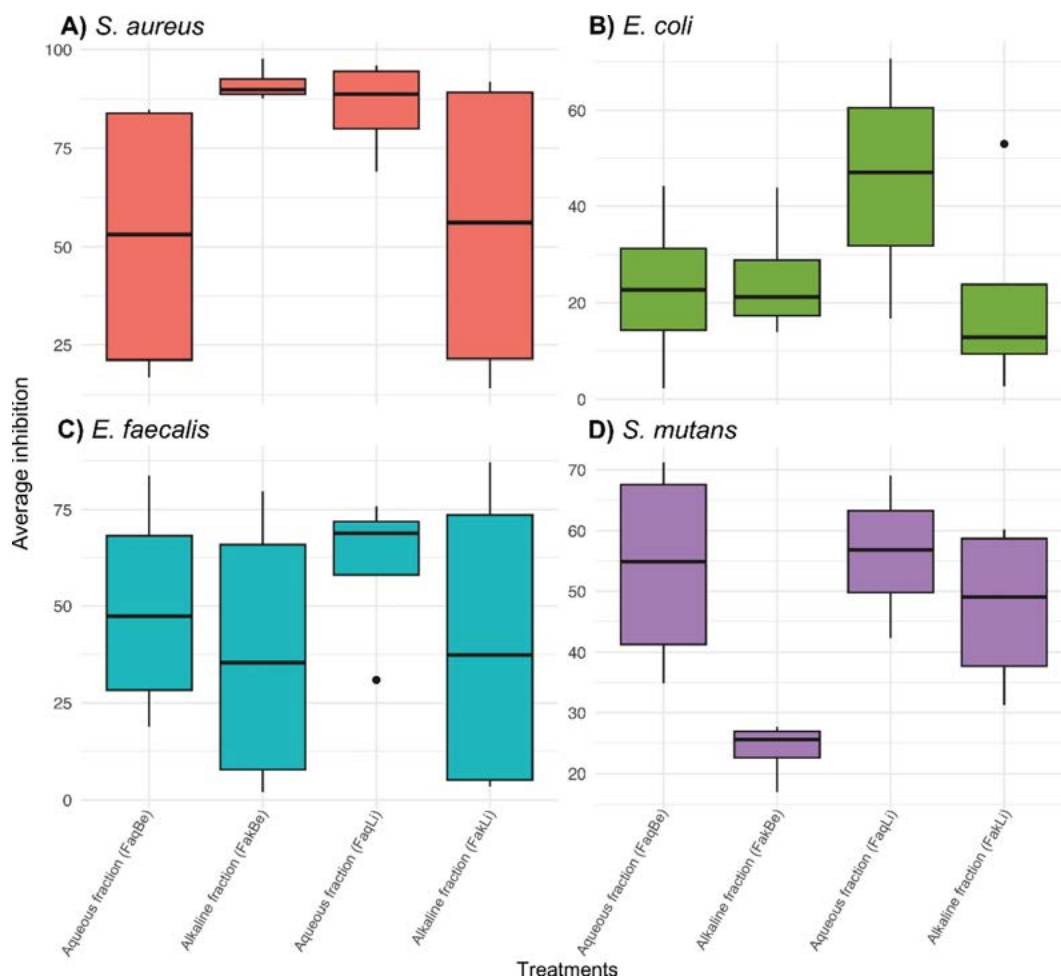


Figure 3. Tukey's post-hoc test of fractions from *Boletus edulis* and *Lactarius indigo*.

The concentration of polysaccharides can vary due to various factors such as substrate and extraction and purification methods. This was reported by Sari *et al.* (2017), who found that wild fruiting bodies of *B. edulis* contained 57.9 g of polysaccharides per 100 g of dry biomass in the pileus and 16.88 g per 100 g in the stipe. On the other hand, López-Vázquez *et al.* (2017) reported concentrations of polysaccharides present in fruiting bodies of *L. indigo* of 8.8 g of polysaccharides per 100 g of dry biomass. However, the presence of other compounds within the polysaccharide extracts cannot be ruled out, as the proposed method represents an efficient and low-cost alternative for extraction; nevertheless, it does not guarantee the complete removal of residual compounds during the extraction and purification processes.

Some *in vivo* studies have shown in which polysaccharides increase their resistance to bacteria by activating non-specific phagocytes of the immune system (Wang and Wang, 1997); however, the antibacterial activity of polysaccharides at the *in vitro* level remains to be described in detail. The bioactivity of polysaccharides could therefore be due to a direct effect on cells (Endo *et al.*, 2010). Several studies have demonstrated different mechanisms through which polysaccharides exert direct effects on bacteria, such as preventing bacterial adhesion, interfering with intracellular metabolic pathways, and altering or degrading plasma membranes and cell walls that are essential for bacterial growth. In addition, certain polysaccharides can inhibit bacterial enzymes and promote the production of antimicrobial compounds (Cheng *et al.*, 2024; Liang *et al.*, 2024).

Specific mechanisms have also been described for EPS and IPS extracts, which exhibit stronger antibacterial activity against Gram-positive bacteria (*e.g.*, *E. faecalis*), attributed to a greater affinity resulting from the absence of an outer membrane composed of lipopolysaccharides or a phospholipid layer, thereby facilitating polysaccharide penetration (Norhisham *et al.*, 2025). Therefore, the polysaccharides obtained from *B. edulis* and *L. indigo* may exert direct antibacterial effects on the evaluated strains. In this context, the antibacterial activity observed in this study may be associated with the physicochemical properties and structural characteristics of the extracted polysaccharides. According to Karácsonyi and Kuniak (1994), the bioactivity of the polysaccharides is related to the characteristics of the structure, its properties of solubility and the differences between molecular masses. It should be noted that polysaccharides can be coupled to a protein, so they have the ability to form supramolecular structures capable of increasing bioactivity through the formation of secondary structures (Chen and Seviour, 2007).

The concentration of polysaccharides presented in the fruiting bodies of both species was higher in the alkaline fraction. This may be attributed to the 2% NaOH extraction process, which disrupts the fungal cell wall and facilitates the release of alkali-soluble polysaccharides (Tseng *et al.*, 2008). This despite the fact that there are several studies that report the aqueous fraction to normally present higher bioactivity (Xiao *et al.*, 2004), probably due to the mechanisms mentioned above. Nevertheless, a concentration-dependent inhibitory effect was clearly observed for both aqueous and alkaline fractions.

CONCLUSIONS

The polysaccharides obtained from the wild fruiting bodies of *B. edulis* and *L. indigo* showed significant antibacterial activity against the evaluated bacterial strains; *S. mutans*, *S. aureus*, *E. coli* and *E. faecalis*. In general, higher dosage levels resulted in significantly greater inhibition across all treatments, with 500 $\mu\text{g mL}^{-1}$ showing the strongest effect. Likewise, the findings obtained in this study suggest that FAqLi is the most promising treatment and that higher doses are generally more effective in inhibiting bacterial growth, whereas FAkBe exhibited the lowest inhibition, particularly against *S. mutans*. On the other hand, *E. coli* was the least responsive to treatment, while *S. aureus* and *S. mutans* showed the highest inhibition rates.

Finally, it is important to recognize that wild species such as *B. edulis* and *L. indigo* are of high nutritional value as well as presenting an alternative for obtaining metabolites of medicinal, nutraceutical and biotechnological importance.

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The traditional coffee agroecosystem in Chiapas: a systemic analysis of its ecological, productive, and sociocultural relevance

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ABSTRACT

Objective: To analyze the traditional coffee agroecosystem in Chiapas from a systemic perspective to understand its ecological, productive, and sociocultural dimensions and to identify the relationships that explain its functioning and resilience.

Design/methodology/approach: A structured documentary review with a systemic and rational-deductive orientation was conducted using 42 scientific sources. The analysis was organized around ecological, productive, and sociocultural criteria and incorporated a SWOT matrix to synthesize the strengths, weaknesses, opportunities, and threats of shade-grown coffee systems in Chiapas and the Frailesca region.

Results: Three core attributes of the agroecosystem were identified: structural diversity, ecological and productive multifunctionality, and smallholder management supported by local knowledge. These characteristics contribute to productive stability and socioecological resilience. The review also revealed tensions associated with socio-environmental disturbances, phytosanitary vulnerability, and outmigration of young people. The SWOT matrix highlighted opportunities linked to differentiated coffees, the cultural value of the crop, and the role of shade in biodiversity conservation.

Research limitations/implications: The study relies on secondary sources and exposes gaps in systemic analyses of coffee production in the Frailesca region. Nevertheless, the review enabled the development of an integrative conceptualization of the agroecosystem and underscores the relevance of holistic approaches that articulate ecological, productive, social, and economic dimensions to guide future research and public policy.

Findings/conclusions: The traditional coffee agroecosystem is a complex system whose resilience depends on the interaction among diversity, multifunctionality, and smallholder management. Its long-term sustainability will require stronger ecological management, protection of local knowledge, and fairer market conditions for producers.

Keywords: Shade-grown coffee, agroforestry systems, biodiversity, climate resilience, family farming.

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INTRODUCTION

Coffee remains a significant commodity in international agricultural trade, with yearly production exceeding 10 million tons (FAO, 2020) and a consistent rise in global consumption (Delgado *et al.*, 2018). Cultivated primarily in tropical areas, coffee farming supports local economies and the livelihoods of countless smallholder producers.

Brazil, Vietnam, Indonesia, Colombia, and Ethiopia are the main producers, dominating the global supply chain (United States Department of Agriculture [USDA], 2023). Conversely, Mexico and Central America experienced a 7.7% decline in exports, attributable to adverse climatic conditions and escalating production expenses (International Coffee Organization [ICO], 2023).

Agroforestry systems, particularly those utilizing shade-grown coffee, furnish crucial ecosystem services. These services encompass biodiversity conservation, hydrological regulation, nutrient cycling, carbon sequestration, and erosion control (Muñoz-Villers *et al.*, 2020; Chazdon *et al.*, 2016; Villa *et al.*, 2020).

However, the availability of these services depends on how the system is structured and managed. This can be disrupted by poor practices or events that change the agroecosystem's structure.

According to the Agrifood and Fisheries Information Service (SIAP), Chiapas is Mexico's leading coffee-producing state, accounting for more than 37% of the country's total coffee production.

In this region, traditional coffee systems integrate native trees, polycultures, and subsistence crops, favoring soil conservation, production stability, and food security (Escamilla *et al.*, 2021).

The Frailesca region is a singular case. There, shade-grown coffee supports thousands of farming families, many of whom are indigenous, whose practices help preserve local knowledge, protect cultural and biological diversity, and mitigate the effects of climate change (Chazdon *et al.*, 2016).

Traditional coffee systems in Chiapas have undergone significant transformations driven by climatic variability, market fluctuations, changes in management practices, and pressures toward alternative land uses (Escamilla *et al.*, 2021). These changes have altered the ecological and socio-economic aspects of the agroecosystem, which has reduced its ability to recover. Therefore, a comprehensive approach is essential for understanding the complex interactions between biodiversity, management practices, ecological functions, and external pressures, as well as for assessing the Frailesca coffee system's complexity and interconnectedness.

Given the relevance of Chiapas to national coffee production, this article analyzes the current state of the traditional coffee agroecosystem in the Frailesca region in order to assess its ecological, productive, and sociocultural importance and to identify its main vulnerabilities in the face of climatic, economic, and socio-environmental pressures that threaten its continuity.

MATERIALS AND METHODS

This investigation employed a documentary review, leveraging a rational-deductive methodology (Hyde, 2000), with the objective of synthesizing and systematizing pertinent

data concerning the traditional coffee agroecosystem in Chiapas and the Frailesca region. The methodological approach used was a structured narrative review. This method is well-suited for organizing different types of evidence, identifying conceptual patterns, and analyzing inconsistencies within complex socio-agroecological systems (Grant & Booth, 2009; Snyder, 2019).

The literature search, which used Google Scholar, ResearchGate, Web of Science, SciELO, and institutional repositories, was conducted from January to October 2024.

The search used keyword combinations like “coffee in Chiapas,” “traditional agroecosystem,” “shade-grown coffee,” and “Frailesca region.”

The selection process followed predefined inclusion and exclusion criteria. The analysis included a total of 42 documents published between 2000 and 2024.

These documents provided information about the ecological structure of traditional systems, their sociocultural aspects, the limitations on production, and relevant statistics at local, national, and international levels.

Duplicate studies or those not directly related to the objectives of the study were excluded.

The information was organized using thematic matrices and a comparative analysis grounded in a systemic perspective, consistent with the agroecological conception of the agroecosystem as a complex unit in which ecological, productive, and sociocultural dimensions interact. In addition, a SWOT matrix was developed to synthesize the main strengths, weaknesses, opportunities, and threats facing the traditional coffee system in Chiapas and the Frailesca region.

RESULTS AND DISCUSSION

Productive context of coffee at the global, national, and state levels

Coffee is a strategically important crop worldwide. In the 2023-2024 growing season, global production was estimated at 171.4 million bags. Brazil and Vietnam were the leading producers. In contrast, Mexico and Central America have seen production declines due to climate-related issues and pests like coffee leaf rust (Avelino & Anzueto, 2020; USDA, 2023; SIAP, 2024).

Despite the observed decline, Mexico continues to hold a significant role in organic coffee production, specialty and quality coffees, and fair trade markets, demonstrating indications of a productive rebound (Rosales *et al.*, 2023; SIAP, 2024).

Nationally, Mexico's production reached one million tons, with Chiapas maintaining its status as the foremost producing state, contributing 390 thousand tons, succeeded by Veracruz and Puebla, which produced over 253 thousand and 223 thousand tons, respectively (Figure 1). This distribution reveals a high degree of territorial concentration in coffee production, as several states contribute only modest volumes, such as Querétaro (330 t) and Morelos (35 t).

In Chiapas, the state's coffee production shows a varied pattern. Municipalities like Motozintla, which produces around 94 thousand tons, Tapachula, at nearly 83 thousand tons, and San Cristóbal de las Casas, exceeding 54 thousand tons, dominate state production. These areas benefit from favorable agricultural and ecological conditions.

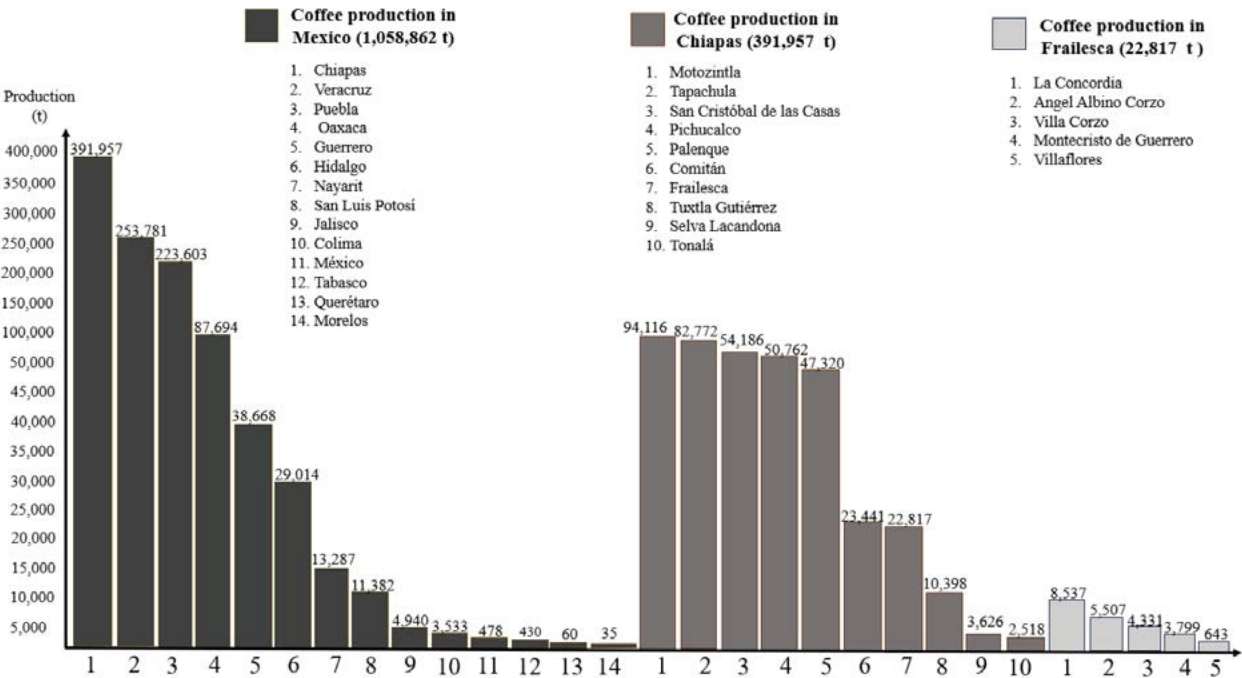


Figure 1. Coffee production in Mexico, Chiapas, and the Frailesca region. Source: elaborated based on the Statistical Yearbook of Agricultural Production (SIAP, 2024).

In contrast, municipalities like Tonalá and the Lacandon Rainforest region show low production levels.

Within this context, the Frailesca region contributes nearly 23 thousand tons, equivalent to approximately 2.2% of national production. Within the region, the municipalities of La Concordia (slightly more than 8,500 tons), Ángel Albino Corzo (around 5,400 tons), and Villa Corzo (just over 3,300 tons) concentrate most of the regional output, whereas Villaflores records considerably lower values, with slightly more than 600 tons. Although the Frailesca is not the main coffee-producing zone in the state, its relevance lies in the persistence of shade-grown agroforestry systems, characteristic of smallholder farms and diversified peasant management.

Agroecological overview of shade-grown coffee in Chiapas

Shade-grown coffee cultivation in Chiapas develops in mountainous environments characterized by diverse microclimates, volcanic-origin soils, and tree cover approaching 90%, conditions typical of the state’s traditional management systems (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad [CONABIO], 2015). This production, largely organized by smallholder farmers, has substantial social, economic, cultural, and ecological importance in the region (INCAFECH, 2022).

The activity is based on small-scale landholdings: most producers, including numerous households from indigenous peoples, cultivate plots smaller than five hectares, and approximately 180,000 families depend on coffee as their primary source of livelihood (Secretaría de Agricultura y Desarrollo Rural [SADER], 2020). Varieties of *Coffea arabica* L. predominate, such as Typica, Red Bourbon, and Yellow Bourbon,

adapted to altitudes between 1,000 and 1,300 m a.s.l. (Escamilla *et al.*, 2021). Family labor organization, in which women’s participation is fundamental, constitutes a central axis of productive dynamics (Merlín *et al.*, 2018).

From an ecological perspective, traditional coffee plantations form highly diverse agroforestry arrangements distributed across mountainous areas, humid tropical forests, and cloud forests. Their vertical vegetation stratification promotes soil conservation, hydrological regulation, and the persistence of a wide diversity of plants, vertebrates, insects, fungi, and microorganisms (CONABIO, 2015; Libert & Paz, 2018).

The canopy observed in the Frailesca region is characteristic of traditional shade-coffee management (Figure 2).

The types of coffee plantations present in the state vary according to shade density and tree species richness, ranging from highly diverse rustic arrangements to plantations established under full sun (Table 1). In the Frailesca region, traditional polyculture (TP) and the specialized system (SS) predominate. The former integrates native species as well as local or introduced fruit trees, whereas the latter features a canopy dominated by *Inga* spp., particularly *Inga vera* (chalum) (Escamilla *et al.*, 2021).

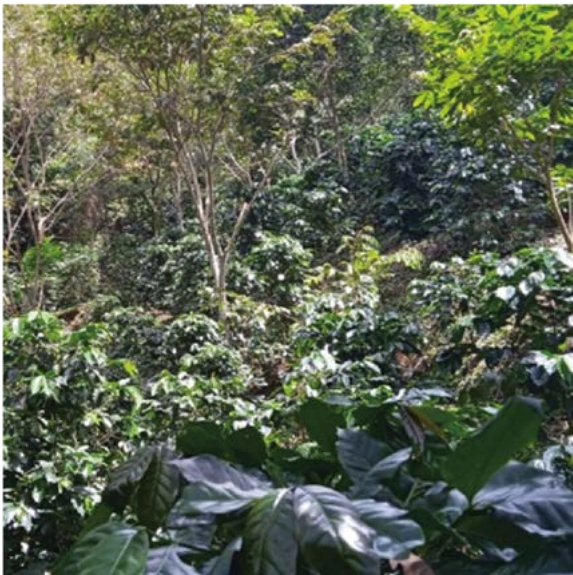


Figure 2. Multistratified traditional coffee plantation in Villa Corzo, Chiapas. Source: authors’ own elaboration, 2024.

Table 1. Canopy cover, shade tree richness, and coffee plantation types.

Coffee plantation type	Canopy cover (%)	Shade tree richness (number of species)
Rustic	71-100	>50
Traditional polyculture	41-70	21-50
Commercial polyculture	31-40	6-20
Shaded monoculture	10-30	1-5

Source: table elaborated based on Harvey *et al.* (2021).

This structural diversity confers both productive and ecological resilience, while also sustaining multiple environmental benefits. Breitler *et al.* (2022) describe traditional management as having several key features. These include: (1) polycultures that combine coffee with shade trees like *Inga* and *Erythrina*, along with crops for food and fruit trees; (2) farming methods that use few resources, such as pruning, composting, and biological pest control; and (3) the positive effects on soil health, climate regulation, and the conservation of wildlife habitats.

Conversely, shade-grown coffee systems in Chiapas are currently experiencing escalating pressures. The challenges facing coffee production stem from several factors: coffee leaf rust (*Hemileia vastatrix*), climate change, deforestation, the loss of shade cover, the use of less resilient coffee varieties, a lack of public policies that support conservation, and the aging population of coffee producers (Avelino & Anzueto, 2020; Venegas *et al.*, 2021; Harvey *et al.*, 2021).

These circumstances jeopardize the system's survival, thereby highlighting the necessity of evaluating its present condition through a comprehensive lens. Despite these challenges, shade-based management continues, supported by its cultural, environmental, and economic importance.

Within this framework, endeavors like the Payments for Environmental Services initiative, championed by the Mexican Carbon Program (PMC, 2016), have become increasingly pertinent as tools to bolster the model's sustainability and to safeguard carbon reserves and agrobiodiversity.

Considering the intricacies stemming from the interplay of productive, ecological, and sociocultural elements, a systemic approach is crucial. This approach facilitates a holistic comprehension of their interrelationships and fosters progress toward a conceptualization of the coffee agroecosystem within the Frailesca region.

Conceptualization of the coffee agroecosystem in the Frailesca region

The coffee-growing environment: territoriality and productive base

The territorial configuration of the Frailesca region, comprising the municipalities of Villaflores, Villa Corzo, La Concordia, El Parral, Ángel Albino Corzo, and Montecristo de Guerrero, located between the Sierra Madre de Chiapas and the Central Depression, establishes an altitudinal gradient ranging from 279 to 2,755 m and defines a marked environmental heterogeneity (Comité Estatal de Información Estadística y Geográfica de Chiapas [CEIEG], 2021) (Figure 3).

These agroecological particularities support diverse coffee production systems in which both organic and conventional management practices are implemented, while a high level of genetic diversity is conserved. In the region, *Coffea arabica* and *C. canephora* are cultivated, with varieties such as Bourbon, Typica, Costa Rica 95, Oro Azteca, Caturra, Colombia, Sarchimor, Marsellesa, Geisha, and Guacamayo. In addition, 89% of producers maintain several of these varieties in association with fruit trees, forming multistratified systems characteristic of shade-grown coffee (Escamilla *et al.*, 2021; Venegas *et al.*, 2021; Reyes *et al.*, 2024).

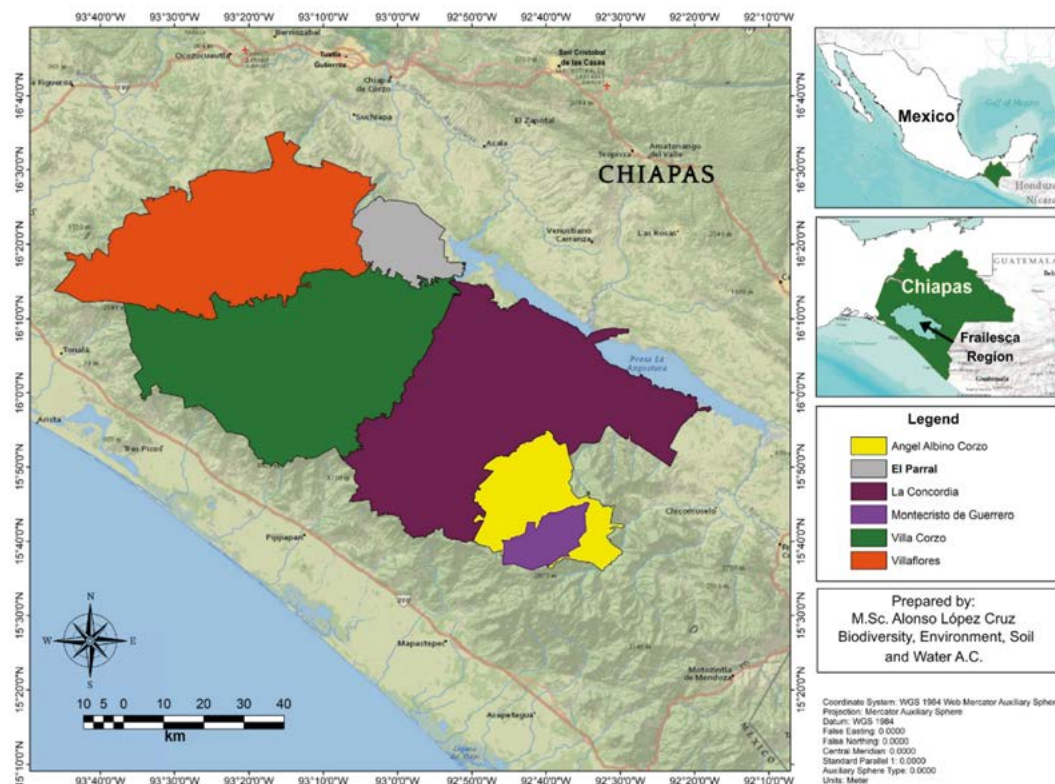


Figure 3. Frailesca region, Chiapas, Mexico.

Traditional coffee plantations incorporate a wide diversity of food and forest species that complement household diets and family incomes. These include pápalo (*Porophyllum ruderale*), purslane (*Portulaca oleracea*), black nightshade (*Solanum nigrum*), citrus species, mango, avocado, soursoap, cassava, and jackfruit (CONABIO, 2015).

The prevailing tree layer, encompassing *Inga* spp. (chalum), *Casimiroa edulis* (caspirola), cedar, avocado, orange, mango, and mandarin, offers shade and facilitates crucial ecological functions, concurrently yielding timber, firewood, food, and medicinal resources (Reyes *et al.*, 2022; FAO, 2016; Benayas, 2023; Delgado *et al.*, 2018). This canopy's existence supports associated wildlife, such as migratory birds, bats, and indigenous pollinators, which are indispensable for the ecological integrity and equilibrium of the agroecosystem (Meza & Rodríguez, 2022).

Consequently, these attributes collectively illustrate a multifaceted system whose productivity and resilience are contingent upon the interplay of biological diversity, smallholder agricultural methods, and varied environmental factors, thereby establishing the Frailesca region's strategic significance within Chiapas' coffee production.

Structure of the agroecosystem

From a systemic perspective, the coffee agroecosystem of the Frailesca region can be defined as a socioecological unit oriented toward the integrated reproduction of productive, ecological, and sociocultural functions. Its general structure is organized around the following components:

Agroecosystem objectives

- Productive-economic dimension: To ensure shade-grown coffee production as the main source of household income, complemented by fruit production, timber, firewood, and other secondary products that strengthen the rural economy.
- Ecological dimension: To conserve essential processes, biodiversity, microclimatic regulation, soil fertility, and water capture, which sustain system productivity and resilience.
- Sociocultural and family dimension: To ensure peasant social reproduction through the transmission of knowledge, family-based labor organization, and the persistence of the cultural landscape.

System boundaries

- Physical boundaries: These include the coffee plot, the shade canopy, native and fruit trees, and associated polycultures. They define the biophysical space in which productive processes take place.
- Functional boundaries: These involve ecological and sociocultural relationships, microclimatic regulation, nutrient cycling, farmers' management decisions, and the conservation of local knowledge, all of which help sustain the system beyond its merely physical boundaries.
- Temporal boundaries: These correspond to the annual coffee cycle and its alternation between harvest periods (characterized by high labor demand and economic flows) and maintenance phases (dominated by family labor and coffee plantation conservation). These boundaries vary according to climatic, economic, and labor conditions.

Inputs and outputs**Inputs:**

- Ecological energy: solar radiation, precipitation, volcanic-origin soils, and associated biodiversity.
- Cultural energy: local knowledge, management practices, and family labor organization.
- Labor energy: family labor and temporary labor support during the harvest season.
- Material inputs: seeds or seedlings, basic tools, agrochemical inputs, among others.

Outputs:

- Parchment coffee.
- Other products: fruits, timber, firewood, and medicinal plants.

Components or subsystems

The agroecosystem is organized into three interrelated subsystems:

- The ecological subsystem, encompassing volcanic soils, solar radiation, precipitation patterns, shade trees, and the biodiversity of both flora and fauna, functions to

regulate the microclimate, nutrient cycling, water infiltration, and biological control mechanisms.

- The productive subsystem, centered on coffee cultivation as its economic foundation, also includes native fruit trees, complementary crops, and agroforestry practices. These practices encompass pruning, shade regulation, replanting, weed management, and soil conservation techniques.
- The sociocultural and labor subsystem incorporates the peasant household, its internal organization, local knowledge, management decisions, and seasonal labor participation. This subsystem is essential for cultural reproduction and the human energy metabolism of the overall system.

The dynamics of the Frailesca coffee agroecosystem are sustained by three fundamental energy flows, ecological, cultural, and labor, whose interactions determine its functioning and resilience (Figure 4).

The biophysical processes underpinning the system's functionality are delineated by this flow, encompassing light absorption, the hydrological cycle, the breakdown of organic matter, natural biological regulation, and microclimatic control. The coffee plantation's agroforestry design, characterized by its shade canopy and the biodiversity it supports, augments these processes and contributes to the system's ecological equilibrium (Odum & Barrett, 2005; Gliessman, 2015).

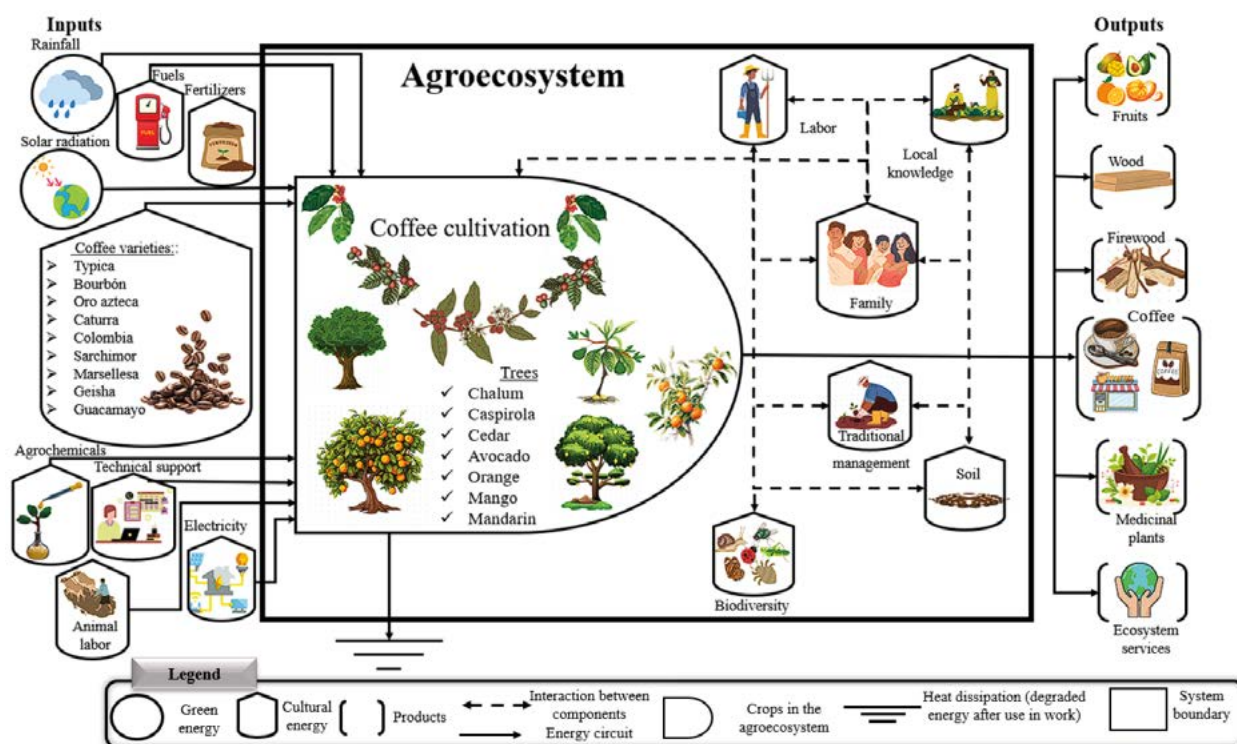


Figure 4. Functional dynamics of the traditional coffee agroecosystem in the Frailesca region, organized around ecological, cultural, and labor flows. Source: authors' own elaboration based on Odum and Barrett (2005).

Cultural flow

This flow pertains to the local knowledge, traditional practices, and social structures that inform management choices and influence the coffee plantation's configuration. It establishes the agroecological rationale of the system and its heritage aspect, thereby integrating technical, cultural, and familial knowledge (Toledo *et al.*, 2003; Altieri & Nicholls, 2017).

Labor flow

Labor flow encompasses the human effort that underpins management operations, coffee harvesting, and processing. The seasonal fluctuations inherent in this flow, characterized by labor surges during harvest periods and maintenance phases predominantly reliant on familial labor, govern the system's social metabolism and shape its productive temporality (Ruiz-Nájera *et al.*, 2016; Escamilla-Prado *et al.*, 2021).

Systemic integration

The interplay of these three flows gives rise to emergent properties that extend beyond mere productive results, including socioecological resilience, territorial stability, and adaptive capacity in response to climatic, economic, and environmental challenges.

Conceptual synthesis of the system

The coffee agroecosystem of the Frailesca region constitutes a complex socioecological unit grounded in mountain edaphoclimatic conditions and in local knowledge systems that organize coffee cultivation, the shade canopy, and associated goods. Its functioning integrates ecological, productive, and sociocultural components through flows of energy, matter, and labor that sustain community reproduction and territorial stability.

Beyond the plot scale, the system gains regional relevance by articulating productive, ecological, and cultural functions that are essential for rural well-being and territorial resilience. This configuration makes it particularly sensitive to a set of external and internal pressures that affect its adaptive capacity and continuity, while simultaneously offering opportunities derived from its biological diversity, agroforestry structure, and the strength of local knowledge.

Within this context, the factors conditioning agroecosystem stability are identified, including its weaknesses and threats, as well as its strengths and opportunities, which together define its potential for improvement and long-term sustainability.

Key aspects of the current coffee agroecosystem in the Frailesca region

The coffee agroecosystem in the Frailesca region faces structural challenges that compromise its ecological, economic, and social sustainability. Among the most relevant factors are the following:

1. Climatic and phytosanitary pressures.

Variability in climate, escalating temperatures, and inconsistent rainfall patterns negatively impact both the yield and the quality of beans. These environmental

factors create conditions conducive to the spread of pests, including coffee leaf rust and the coffee berry borer, thereby causing persistent economic setbacks for smallholder farmers (Quiroga *et al.*, 2020; Prado *et al.*, 2021).

2. Economic vulnerability.

Economic vulnerability stems from fluctuations in international market prices, dependence on intermediaries, and increased production costs, which collectively constrain the profitability of agricultural yields, particularly for small-scale family farms (Martínez & Manrique, 2014; Perfecto *et al.*, 2019).

3. Productive and technological constraints.

The advancement of coffee systems and improvements in coffee quality are hindered by insufficient rural infrastructure, limited financial resources, and the continued use of outdated technologies (Fernández *et al.*, 2020).

4. Environmental degradation.

The ecological resilience of the system is diminished, and soil quality is compromised, due to processes like deforestation linked to land-use changes and the heavy application of agrochemicals, which subsequently impacts long-term productivity (Sosa *et al.*, 2018; Sáenz *et al.*, 2023).

5. Social and demographic challenges.

A demographic aging of producers, outmigration of young people, and land fragmentation limit generational continuity and reduce local innovative capacity (Escamilla *et al.*, 2021; Henderson, 2019).

6. Insufficient public policies.

Government support programs do not always reach the most vulnerable producers, and the lack of productive diversification strategies increases dependence on a single crop (Martínez & Manrique, 2014).

These factors together define socio-environmental vulnerability, which threatens regional coffee production. Addressing this issue requires a combination of strategies, including climate change adaptation, community organization, fair market access, and improvements in production methods.

Consequently, the systemic characterization and contextual analysis are integrated via a SWOT matrix, which incorporates strengths, weaknesses, opportunities, and threats, thereby facilitating the identification of critical factors for bolstering the territorial resilience of the Frailesca coffee agroecosystem.

SWOT analysis of the coffee agroecosystem in the Frailesca region, Chiapas

The Frailesca coffee agroecosystem exhibits strengths associated with its agroecological diversity, sustained by shade-grown polycultures that promote biodiversity conservation,

soil protection, and community and family participation, particularly that of women. It provides essential ecosystem services, too, like capturing carbon and regulating water cycles. Beyond that, it plays a role in preserving high-quality coffee varieties, which rely on traditional knowledge to thrive.

Among the main opportunities are access to differentiated markets (organic and fair trade), Payments for Environmental Services programs, the growing global demand for sustainably produced coffee, and the availability of technological innovations aimed at improving productivity (Figure 5).

Weaknesses include an aging producer population, youth outmigration, dependence on intermediaries, high input costs, land fragmentation, and limitations in rural infrastructure.

Threats encompass increasing climatic variability, pests such as coffee leaf rust and the coffee berry borer, deforestation, volatility in international coffee prices, and the insufficiency of differentiated public policies. Taken together, these factors define a vulnerability framework that undermines the sustainability and profitability of the agroecosystem.

The SWOT analysis synthesizes the relationship between internal and external pressures. It indicates that the agroecosystem's future resilience depends on its ability to integrate ecological, productive, and sociocultural strategies. These strategies are essential for strengthening adaptation and ensuring long-term survival within the region.

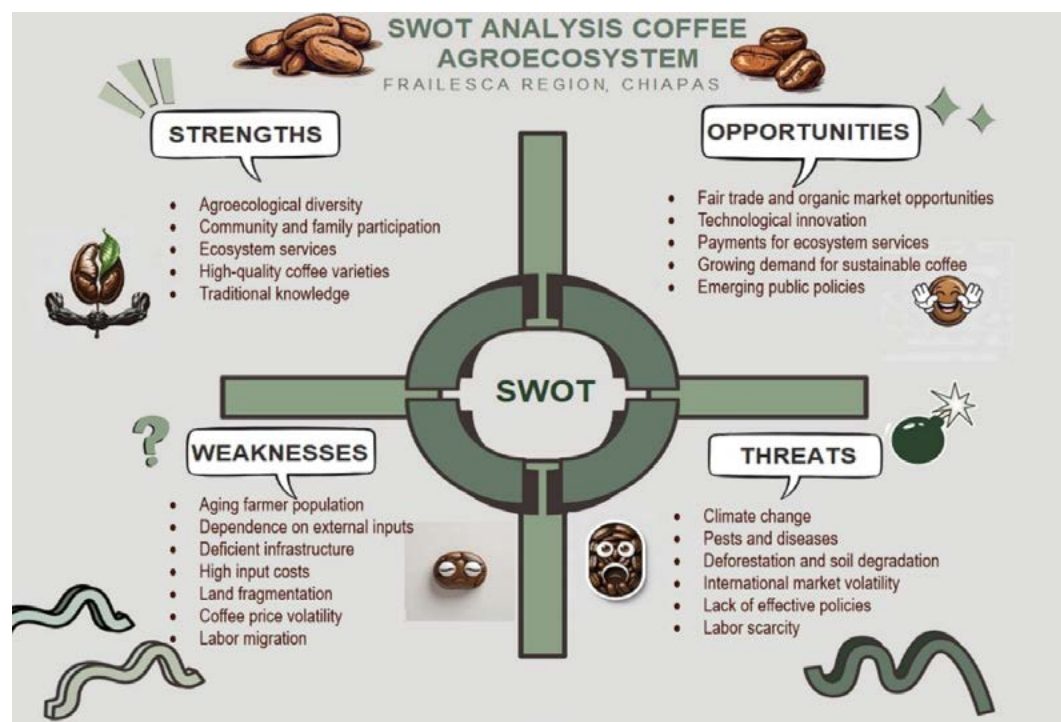


Figure 5. SWOT analysis of the coffee agroecosystem in the Frailesca region, Chiapas. Source: authors' own elaboration.

CONCLUSIONS

The systemic analysis shows that traditional coffee agroecosystems in Chiapas and the Frailesca region constitute socioecological units of high ecological, productive, and cultural relevance, sustained by biological diversity, agroforestry architecture, and local knowledge systems that confer territorial resilience.

It also identifies three critical pressures that threaten their continuity: (1) demographic vulnerability associated with aging producer populations and youth outmigration, (2) increasing climatic and phytosanitary stress, and (3) the absence of differentiated public policies tailored to small-scale coffee production.

The sustainability of these agroecosystems requires strengthening local capacities through strategies that integrate productive diversification, agroecological management, access to fair markets, and technical support. Integrating these elements is essential to ensure, in the long term, the ecological, economic, and sociocultural functions of shade-grown coffee systems in the region.

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Vermicompost doses on nutraceutical properties and yield of *Physalis ixocarpa* Brot. Ex. Hornem

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ABSTRACT

Objective: To evaluate different doses of vermicompost on the nutraceutical properties and yield of tomatillo (*Physalis ixocarpa* Brot. Ex. Hornem.) under field conditions

Design/methodology/approach: A randomized block experimental design was used with 4 treatments: T1 Synthetic fertilization (FS) (YaraMila Complex), T2 Vermicompost 50 g per plant (T2 50 g L), T3 Vermicompost 100 g L and T4 Vermicompost 200 g L, with 20 replicates and 3 blocks per treatment with a total of 240 experimental units.

Results: Statistical analysis showed significant differences between treatments ($P \leq 0.05$) for all evaluated variables, where a trend was observed that the best treatments were the applications of vermicompost of 100 and 200 gs per plant (T3 100 g L and T4 200 g L) for nutraceutical variables and a yield per plant of 8 kg.

Limitations on study/implications: include the contribution of vermicompost in the soil under study.

Findings/conclusions: The best treatments were doses of 100 and 200 gs of vermicompost per plant, which improves some nutraceutical characteristics of the tomatillo crop.

Keywords: tomatillo, acidity, °brix, polyphenols, DPPH.

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INTRODUCTION

In Mexico, one of the commercially important agricultural crops is the tomatillo (*Physalis ixocarpa*). In 2024, 694,327 tons were harvested from 37,504 hectares, resulting in a national average yield of 18.5 tons per hectare (SIAP, 2024). Sinaloa led the way with 136,593 tons produced, representing 19.7% of the national total. It was followed by Zacatecas with 79,953 tons (11.5%), Jalisco with 73,978 tons (10.7%), Puebla with 58,989 tons (8.5%), and Michoacán with 50,183 tons (7.2%) (SIEGSIN, 2025).



Due to their high demand in the food industry, tomatoes regulate and improve digestion. Furthermore, they are a source of bioactive substances that neutralize free radicals and slow down the aging process (Majkowska-Gadomska *et al.*, 2021). This crop is conventionally produced throughout much of Mexico, using high doses of synthetic chemical fertilizers (Cruz *et al.*, 2017), which represents a source of environmental pollution, leaching, contamination of groundwater and surface water, negative impacts on pH levels, deterioration of soil structure, and damage to microfauna, among other issues (González, 2019).

Therefore, alternatives are being sought to reduce the use of chemical fertilizers, highlighting organic fertilizers, leachates, and the use of vermicompost (Rivadeneyra-Manzanilla *et al.*, 2024). The addition of organic fertilizer with chemical fertilization at a 50% ratio equals yields and improves fruit quality in tomatillo (*P. ixocarpa*). It has been observed that the use of vermicompost improves the physicochemical characteristics of the soil, increases yields and fruit quality, and also improves the bioactive compounds in this Solanaceae. Aguiñaga-Bravo *et al.* (2020) report that the addition of organic fertilizer with chemical fertilization at a 50% ratio equals yields and improves fruit quality in tomatillo (*P. ixocarpa*) (Gamero-Vega *et al.*, 2022). However, research is needed to determine the appropriate doses to optimize yields for this crop. Therefore, the objective was to evaluate various doses of vermicompost on the nutraceutical properties and yield of tomatillo (*Physalis ixocarpa* Brot. Ex. Hornem.) under field conditions.

MATERIALS Y METHODS

Geographic location of the study area

The crop was established between February and May 2022 on a ranch located in the municipality of Coatepec, Veracruz, Mexico, at the geographic coordinates 19° 29' 36.12" N, 96° 56' 37.41" W, with a land area of 269 m² at an altitude of 1255 m above sea level. The soil has a silty loam texture, with a pH of 6.5, 6.6% organic matter, 33% total N, 42 ppm of P, 225 ppm of K, 3198 ppm of Ca, and 408 ppm of Mg. The average temperatures reported for the months of the experiment were 25-40 °C, and rainfall was 95-104.8 mm (CONAGUA, 2025).

Experimental design and description of treatments

A randomized block experimental design was established with 4 treatments: T1 Synthetic fertilization (FS) (YaraMila Complex), T2 Vermicompost 50 g per plant (T2 50 g V), T3 Vermicompost 100 g V) and T4 Vermicompost 200 g V, these last treatments also incorporated fertilizer at 50% with 20 replications and 3 blocks per treatment with a total of 240 experimental units.

Crop management

Green tomato (*Physalis ixocarpa*) seedlings of the Querétaro variety, from the Harris Moran company, were used, with an approximate height of 10 cm. These were transplanted into beds previously disinfected with the disinfectant FULL-GRO, applied at a dose of

9.75 mL·L⁻¹ of water. Simultaneously, 80-gauge plastic mulch was placed, and a drip irrigation system was installed.

Organic fertilization

During transplanting, 0, 50, 100 and 200 g of vermicompost based on horse pulp were applied per plant, which had the following characteristics: 84% organic matter, pH of 7.400, 0.250% phosphorus (P), 3.997% nitrogen (N), 2.140% potassium (K), 1.720% calcium (Ca), 0.800% magnesium (Mg), a C/N ratio of 12.219, 10.595% fulvic acids and 15.105% humic acids.

Fertilization and pest control

Fertilization of the fertilized treatment (T1Fs) was carried out eight days after transplanting with YaraMila Complex (12-11-18 EM), at a rate of 50 g per plant. The other treatments received only 25 g per plant of the same fertilizer. During the crop cycle, three applications of Bayfolan Forte were made to all treatments, using a dose of 1 mL·L⁻¹. Monochromatic blue and yellow traps were used for pest monitoring. Pest control was carried out using applications of neem extracts, Biopest Max, Micro Soil Control, and copper oxychloride, all at a dose of 1 g·L⁻¹.

Variables evaluated

56 days after transplanting, the following were evaluated: fruit weight per plant (g), polar (mm) and equatorial diameter of the fruit (mm), total soluble solids (TSS) expressed in °Brix with a digital refractometer, pH with a Redemon model potentiometer, titratable acidity, total polyphenols (mg GAE 100 g⁻¹ t.f.), with the method of Singleton and Rossi (1965), antioxidant activity by DPPH was carried out by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Brand-Williams *et al.* (1995) and total yield per plant (kg) amount of 5 harvests.

Statistical analysis

Normality tests were performed and, after verifying the statistical reliability of the data, they were analyzed using an ANOVA and Tukey's mean comparison with a significance level of 5% ($\alpha=0.05$) of the STATISTICA software (Version 8.0.360.0 StatSoft Inc., Tulsa, USA) for Windows (StatSoft Inc, 2007).

RESULTS AND DISCUSSION

Statistical analysis revealed significant differences between treatments ($P\leq 0.05$) for all variables evaluated, showing a trend towards the best treatments being the applications of vermicompost at 100 and 200 g per plant⁻¹ (T3 100 g V and T4 200 g V) (Table 1). A greater fruit weight (36.8 g) was observed in the treatments with high doses of vermicompost (T3 100 g L⁻¹ and T4 200 g V); similar values were reported by Singh *et al.* (2014) with 29.33-37.67 g.

Similarly, for the polar and equatorial diameter (43-36 mm), similar data were reported by Ramírez-Godina *et al.* (2013) in the same crop (4.65-3.99 cm). A higher amount of total

Table 1. Statistical analysis for growth variables.

Treatments	Weight of fruit (g)	Polar diameter of the fruit (mm)	Equatorial diameter of the fruit (mm)	TSS (°Brix)	pH
T1 SF	25.9±1.3c	40.0±1.1b	32.1±1.2b	4.55±1.1b	4.5±1.2b
T2 50 g V	26.8±1.2c	40.0±1.2b	32.3±1.4b	4.58±1.2b	4.5±1.3b
T3 100 g V	33.8±1.4b	43.0±1.1a	35.0±1.3a	5.67±1.1a	5.2±1.2a
T4 200 g V	36.8±1.1a	43.0±1.4a	36.0±1.5a	5.68±1.3a	5.5±1.1a

Values with the same letter, within columns, are statistically equal to each other (Tukey, $P \leq 0.05$). $\pm$ Standard deviation.

soluble solids was also observed in the treatments with the highest amount of vermicompost (5.67-5.68 °Brix). These results contradict those reported by Aguiñaga-Bravo *et al.* (2020) (6-7 °Brix), where synthetic fertilization influenced the finding of sweeter fruits. Regarding fruit pH, the treatments with SF and lower amounts of vermicompost were more acidic, unlike the higher doses of vermicompost, which were less acidic. The same trend was observed in the percentage of titratable acidity (5.2%) (Figure 1a). These results may be related to the concentration of salts present in synthetic fertilizer.

For the nutraceutical variables of the crop, significant differences were observed between the treatments evaluated ($P \leq 0.05$). For total polyphenols, the fruits with vermicompost at a dose of 300 g per plant were higher at 10.98 mg GAE in 100 g⁻¹ t.f. These results are

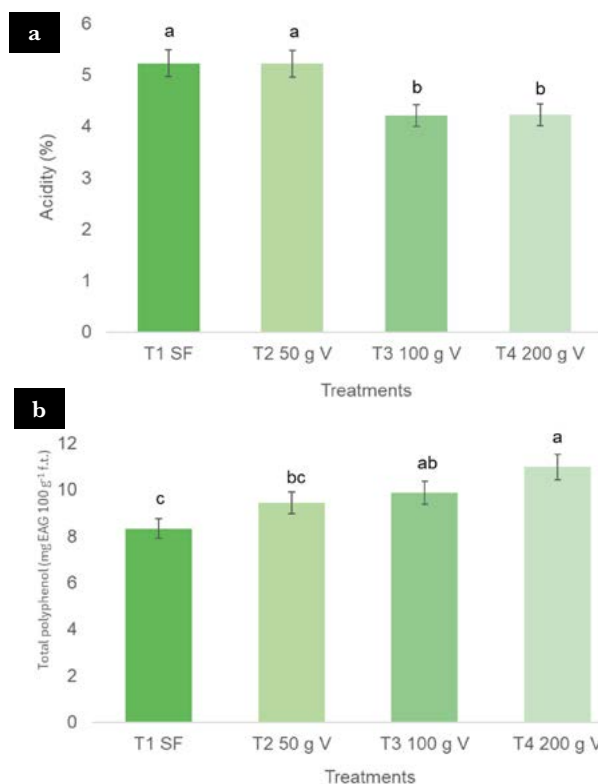


Figure 1. Nutraceutical properties of tomato fruits a) acidity, b) total polyphenols. Equal letters on the same bar are statistically equal to each other (Tukey, $P \leq 0.05$). $\pm$ Standard deviation.

similar to those reported by Majkowska-Gadomska *et al.* (2021), who reported 10.15 mg of total polyphenols in fruits from treatments with mulch.

For antioxidant activity, the same trend was observed as for total polyphenols, where the highest dose showed the highest content in tomato fruits: 1143 $\mu\text{mol ETrolox } 100 \text{ g}^{-1} \text{ t.f.}$ (Figure 2a). These results were higher than those reported by Aguiñaga-Bravo *et al.* (2020), who reported between 1119 and 1123 $\mu\text{mol ETrolox } 100 \text{ g}^{-1} \text{ t.f.}$ in treatments with synthetic fertilization and combined with organic fertilizers.

For yield per plant, it was observed that the treatments with vermicompost at high doses of 100 and 200 g per plant were similar to the fertilized treatment (Figure 2b), with an average of 8 kg per plant, which is higher than those reported by Aguiñaga-Bravo *et al.* (2020) for this same crop and that reported by Ramos-López *et al.* (2018) who report yields of 4.84 kg m^{-2} and 2.10 kg m^{-2} of the cv. Rendidora under greenhouse and field conditions. The results obtained in this research may be related to the physical, chemical, and microbiological properties present in vermicompost, as has already been mentioned in horticultural crops (Rivadeneyra-Manzanilla *et al.*, 2024).

They are also related to the rich organic matter content of the soil where the crop was established, since each year the crop is left fallow for a cycle, allowing legumes to be incorporated into the soil. These results are consistent with those reported by Quispe *et al.* (2021) in maize cultivation, where yields increased with the incorporation of organic fertilizers.

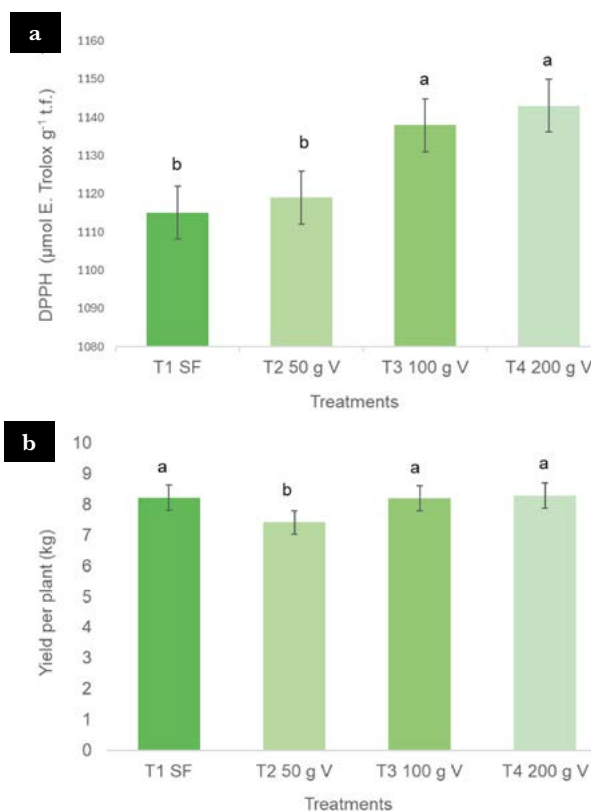


Figure 2. DPPH antioxidant activity a) and yield per plant (kg) b). Identical letters on the same bar are statistically equal to each other (Tukey, $P \leq 0.05$). $\pm$ Standard deviation.

CONCLUSIONS

According to the results, the statistical analysis showed that the best treatments were the doses of 100 and 200 g of vermicompost per plant. Therefore, the localized application of 100 g of horse manure vermicompost per plant could be recommended, as this improves some of the nutraceutical characteristics of tomatillo cultivation.

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Quantification of protein quality in a fermented mixture of orange bagasse, yeast and nitrogen additives to enhance nutritional quality

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ABSTRACT

Objective: To gauge the protein quality of a mixture of orange bagasse fermented with yeast and nitrogen compounds in order to determine its potential as an ingredient for animal diets.

Design/methodology/approach: The experimental design was a randomized complete block design with repeated measures. Four bagasse-based treatments were evaluated: Treatment 1: orange bagasse without additives; Trt 2: pulp + 200 g yeast + 60 g urea + 8 g (NH₄)₂SO₄; Trt 3: pulp + 100 g yeast + 30 g urea + 4 g (NH₄)₂SO₄; Trt 4: pulp + 50 g yeast + 15 g urea + 2 g (NH₄)₂SO₄. All treatments were mixed with 2 L of water per 10 kg of orange bagasse. Thus, 200 g samples were selected to measure physicochemical, nutritional, and microbiological variables at 0, 2, 4, 6, 24, 48, and 72 h of fermentation. Statistical analysis was performed using Proc Glimmix in SAS 9.4, and a Tukey mean comparison ($\alpha=0.05$).

Results: Trt 3 showed higher protein content, achieving a value of up to 18.55% at 48 h, suggesting a positive effect of the combined addition of yeast, urea, and ammonium sulfate.

Limitations of study/implications: The evaluation was only conducted at the ingredient level (bagasse); however, *in vitro* or *in situ* tests in animals are recommended.

Findings/conclusions: Bagasse fermented with nitrogen additives showed a greater tendency toward higher protein content, although no significant differences were observed, highlighting its potential as an ingredient in animal feed.

Keywords: fermentation, food waste, bagasse, ruminants, dietary constituent.

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INTRODUCTION

Globally, continuous population growth poses significant challenges to agri-food systems, which must meet a sustained and increasing demand for food. In this context, the 2030 Agenda for Sustainable Development highlights the need to achieve food security while promoting sustainable practices that help reduce environmental impact (UN, 2015).



In this regard, animal nutrition aims to provide animals with the appropriate quantity and quality of nutrients to ensure maximum productivity while maintaining good health status (Tripathi *et al.*, 2019).

In the case of ruminant animals, although pastures and forages constitute the basis of their diet, they are supplemented with concentrated feed (Flórez *et al.*, 2018). However, the cost of these feed inputs increases total production costs by 50% to 70% (Tripathi *et al.*, 2019), which has led to the exploration of alternative raw materials to reduce their dependence. Among these inputs, protein ingredients stand out, with soybean meal being one of the main representatives; however, its cost remains inaccessible. Therefore, crop residues and some agro-industrial by-products have been increasingly used as alternative protein sources to improve livestock productivity (Guerra *et al.*, 2021; Hernández *et al.*, 2019).

On the other hand, in Mexico there is still no Official Mexican Standard (NOM) that specifically regulates the use of orange bagasse in animal feed. This regulatory framework is essential to establish the scope for the use of this co-product, including conditions for its collection, transport, and storage, as well as process-related conditions such as prior analysis, washing, disinfection, among others, including the distribution cycle of the final product. The objective should be to avoid violating guidelines that could pose risks to animal health through the consumption of feed partially based on this new input. In its support, it can be mentioned that the General Law of Circular Economy was approved by the Senate in 2021, which promotes the use and valorization of waste, prioritizing the development of sustainable schemes (INECC, 2021).

Veracruz is the leading orange-producing state in Mexico, with a production of 2,271,524 tons, representing 50% of the national total (SIAP, 2023). As a result, the citrus sector in Veracruz generates approximately 630,000 tons of waste annually (Galindo-Segura *et al.*, 2023).

Although this by-product is abundant and relatively low-cost, its use in animal feed remains limited, mainly due to its low crude protein content (Panwar *et al.*, 2021). Therefore, it is advisable to subject it to biotechnological processes to increase its nutritive potential.

Orange bagasse can be directly transformed into a viable feed resource through bioconservation and biotransformation processes, among which aerobic fermentation and ensiling (anaerobic, with dry matter content control) stand out. Through these processes, undesirable flavors and odors from essential oils are eliminated and, by conferring a pasty consistency, the product becomes more acceptable to ruminants and stimulates their intake (Fernández, 2021), although both operate under different processing conditions.

Fermentation is a process in which fresh agricultural by-products can be transformed into improved feed through modifications in their nutritional composition, achieved by the consumption of carbohydrates by microorganisms, usually added to the substrate (Acosta *et al.*, 2015). Thus, the process can be carried out with minimal prior preparation and even accelerated with the integration of appropriate inputs, allowing the resulting ingredient to be incorporated into the animal diet. In addition, adjustments can be made during the process if necessary.

Therefore, the purpose of this study was to evaluate the degree of protein enrichment in orange bagasse fermented with yeast, urea, and ammonium sulfate, in order to obtain the best product under an appropriate dosage of inputs and the optimal processing time.

MATERIALS AND METHODS

Study location and experimental characterization

The orange waste fermentation process was carried out in the livestock area, and the microbiological analysis was conducted in the dairy laboratory of Colegio de Postgraduados, Córdoba Campus (COLPOS), located in the municipality of Amatlán de los Reyes. The average daily maximum and minimum temperatures range between 29-36 °C and 14-18 °C, respectively (SIEGVER, 2023).

The proximate chemical analysis was conducted in the Microalgae Biotechnology and Bioenergy Laboratory at the Instituto Tecnológico de Boca del Río, located at km 12, Veracruz-Córdoba Highway.

Biological material: Orange bagasse was obtained through donation from the company Jugos y Aceites del Golfo (JAG), located at Prolongación Josefa Ortiz de Domínguez No. 110, Peñuela, Veracruz.

Preparation of fermented orange bagasse: After collection and without prior storage, the bagasse was ground to obtain a particle size of approximately 10-40 mm in order to promote a stable fermentation process. Subsequently, the bagasse and materials were weighed, labeled, and identified for each treatment (Figure 1).

Treatments and experimental design

The experimental design was a randomized complete block design with repeated measures. The experimental unit consisted of 200 g samples in triplicate for each treatment, while physicochemical, proximate chemical, and microbiological variables were evaluated



Figure 1. Process flow for the fermentation of orange bagasse.

at time intervals of 0, 2, 4, 6, 24, 48, and 72 h. Prior to fermentation, the base inputs were mixed to obtain a homogeneous mass (Table 1).

Physicochemical analysis: Measurements were taken at the indicated time intervals (0, 2, 4, 6, 24, 48, and 72 h). pH was measured using a potentiometer (model PH10), previously calibrated with buffer solutions at pH 4 and 7 (HYCEL, Mexico) and equipped with a Conductronic electrode (Mexico). Soluble solids (°Brix) were measured using a refractometer (model HI96801; Hanna Instruments, USA). Both pH and °Brix were evaluated according to AOAC (1997) methods. Finally, sample temperature was recorded using an industrial digital infrared thermometer (BENETECH, model GM320), and ambient temperature was measured with a thermohygrometer (model HTC-2; HTC).

Proximate analysis: All samples were dried at 60 °C and, once a constant weight was reached, they were stored in Ziploc® bags for subsequent analysis. Dry matter (DM) and moisture content (M) were determined using AOAC method 925.09 (AOAC, 1997), while ash content (Ash) was determined using AOAC method 923.03 (AOAC, 1997).

The previously dried samples were sieved through a 680 µm mesh. Subsequently, 0.05 g of each sample was weighed and mixed with 50 mL of water in a beaker, followed by ultrasonication using a YUCHENGTECH ultrasonic processor set at a frequency of 24.20 Hz for 10 min at 60 °C in continuous mode with intermittent agitation. The volume was then adjusted to 100 mL in a volumetric flask to homogenize the sample. The resulting solution was used to determine total carbohydrates (TC) using the phenol-sulfuric acid method described by DuBois *et al.* (1956) and total protein (TP) using the method described by Lowry *et al.* (1951).

Yeast quantification: Serial dilutions (10^{-1} to 10^{-8}) were prepared and plated using the pour plate method according to the Official Mexican Standard NOM-111-SSA1-1994 (Secretaría de Salud, 1994). The plates were incubated in a culture incubator (Riossa, model ECF-82) at 30 °C for 168 h. After incubation, colony-forming units (CFU) were counted using a colony counter (model CVP-CM3) (Figure 2).

Statistical analysis

The results were analyzed using PROC GLIMMIX in SAS® 9.4. The variables were evaluated through analysis of variance (ANOVA), and multiple comparisons of adjusted means (LS-means) similar to Tukey's test were performed using conservative T-grouping ($\alpha=0.05$).

Table 1. Treatments and their corresponding dosages.

Input	Control (Trt 1)	Treatment high dose (Trt 2)	Treatment medium dose (Trt 3)	Treatment low dose (Trt 4)
Orange bagasse	10 kg	10 kg	10 kg	10 kg
Water	2 L	2 L	2 L	2 L
Urea		60 g	30 g	15 g
(NH ₄) ₂ SO ₄		8 g	4 g	2 g
Yeast of <i>S. cerevisiae</i>		200 g	100 g	50 g

Tr1, Tr2, Tr3, Tr4, correspond to treatment 1, treatment 2, treatment 3, treatment 4, respectively.

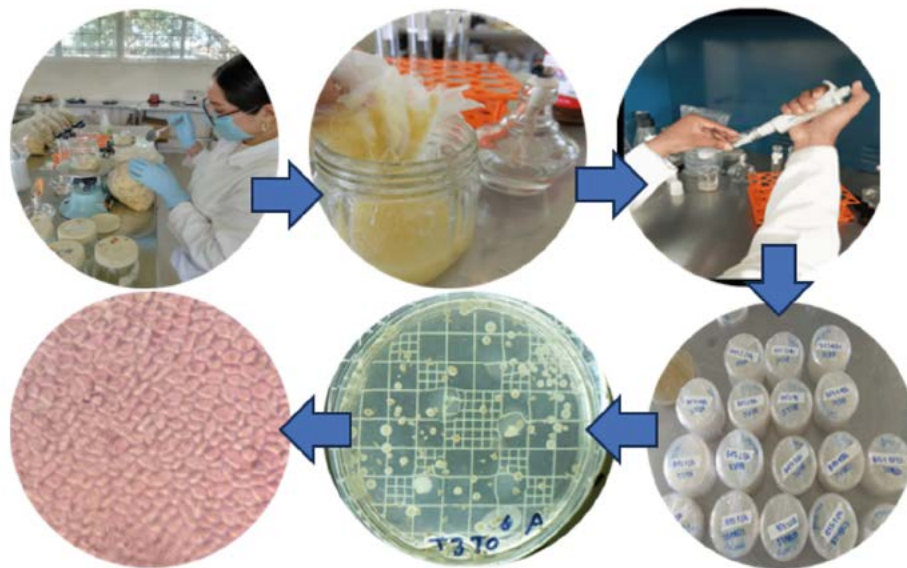


Figure 2. CFU quantification during the fermentation process.

RESULTS AND DISCUSSION

Physicochemical analysis: For all analyzed variables, a mixed model was applied using a TOEP(1) covariance structure and multiple comparisons of adjusted means (LS-means) similar to Tukey's test, using conservative T-grouping ($\alpha=0.05$).

Type III analysis of variance (ANOVA) for fixed effects on temperature showed a significant effect of time ($p<0.0001$), with no significant differences among treatments. Table 2 presents the adjusted mean comparisons of physicochemical parameters. Initial temperature values ranged from 22.36 to 22.70 °C, followed by a progressive increase until 48 h of fermentation, when the highest values were recorded: 28.05 °C (Trt 2), 27.84 °C (Trt 1), 27.67 °C (Trt 3), and 27.10 °C (Trt 4). At 72 h, temperature decreased to a minimum of 23.48 °C (Trt 4). The increase in temperature observed at the beginning of the experiment may be attributed to the metabolic activity of microorganisms, as described by Perwez and Al Asheh (2025) in a bioreactor study, where a low initial thermal incidence was recorded and temperature increased over time. Likewise, the temperature behavior observed in this study remained within the optimal range for metabolism and single-cell protein synthesis by *S. cerevisiae* (25-32 °C). This thermal increase can be attributed to metabolic heat release from yeast during the exponential growth phase, followed by a temperature decrease corresponding to the stationary phase (Walker and Stewart, 2016). The maximum temperatures recorded in this study were lower than those reported by Grizotto *et al.* (2020), who observed maximum temperatures of approximately 33 °C during the ensiling process of treatments containing 30% pelleted citrus pulp.

For the pH variable, the ANOVA showed a significant effect of time ($p<0.0001$), with numerically different but statistically non-significant differences among treatments. Initial pH values ranged from 4.00 to 4.03, followed by a progressive decrease until 48 h, reaching values of 3.76 (Trt 3), 3.74 (Trt 2), 3.68 (Trt 4), and 3.57 (Trt 1), and a slight

increase was observed at 72 h. The acidity trends observed in the bagasse are associated with the production of organic acids as a result of metabolic activity, a common process in fermentations (González-Guerra *et al.*, 2025). Therefore, a comparison among treatments indicates that those supplemented with yeast, ammonium sulfate, and urea showed the highest pH values. According to Yang *et al.* (2021), who compared urea and ammonium sulfate on the growth and fermentative metabolism of *S. cerevisiae*, urea promoted higher pH values. Likewise, Zhang *et al.* (2022) reported that urea hydrolysis contributes to pH increase in fermentation systems.

Regarding °Brix, significant differences were observed over time $p < 0.0001$ and in the interaction between time and treatments $p = 0.0230$. Initial values ranged from 5.78 to 6.23 at 0 h, and a progressive decrease was recorded as fermentation progressed. Trt 3 and Trt 4 showed more pronounced declines, reaching final values of 3.78 and 4.01, respectively. Trt 1 reached 4.19 at 72 h, while Trt 2 recorded the highest final value (4.91), which is associated with more efficient sugar utilization due to metabolic activity (Li *et al.*, 2021). The decrease in °Brix can be explained by yeast metabolism of simple sugars (sucrose, glucose, and fructose) during fermentation, as reported by Yafetto *et al.* (2023). Additionally, during orange juice extraction, soluble sugars are often inadvertently lost, reducing the sugar content of the bagasse (Rossit *et al.*, 2020). Therefore, soluble solids in citrus by-products typically range from 2 to 5 °Brix (Zhao and Yu, 2008), which may affect the reliability of recorded values.

Table 2. Least squares mean of physicochemical parameters during orange bagasse fermentation.

Temperature (°C)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	22.41 ^e	24.20 ^{edc}	25.15 ^{ebdac}	25.16 ^{ebdac}	25.04 ^{ebdac}	27.84 ^a	26.07 ^{bdac}
Trt 2	22.70 ^{ed}	25.52 ^{ebdac}	26.44 ^{bac}	25.75 ^{ebdac}	25.46 ^{ebdac}	28.05 ^a	26.83 ^{bac}
Trt 3	22.68 ^{ed}	25.18 ^{ebdac}	25.98 ^{ebdac}	25.62 ^{ebdac}	26.50 ^{bac}	27.67 ^{ba}	27.04 ^{bac}
Trt 4	22.36 ^e	24.89 ^{ebdc}	25.43 ^{ebdac}	25.16 ^{ebdac}	26.30 ^{bdac}	27.10 ^{ba}	23.48 ^{edc}
pH							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72h
Trt 1	4.00 ^{ba}	3.98 ^{bac}	3.94 ^{bac}	3.92 ^{bac}	3.72 ^{bac}	3.57 ^d	3.76 ^{bdac}
Trt 2	4.03 ^{ba}	4.05 ^a	3.97 ^{bac}	4.00 ^{ba}	3.90 ^{bac}	3.74 ^{bdc}	3.90 ^{bac}
Trt 3	4.03 ^{ba}	3.97 ^{bac}	4.00 ^{ba}	3.90 ^{bac}	3.76 ^{bdac}	3.76 ^{bdac}	3.97 ^{bac}
Trt 4	4.02 ^{ba}	3.97 ^{bac}	3.95 ^{bac}	3.92 ^{bac}	3.80 ^{bdac}	3.68 ^{dc}	3.85 ^{bdac}
°Brix							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72h
Trt 1	6.23 ^a	5.70 ^{ebdac}	5.63 ^{ebdac}	5.17 ^{ebdgcf}	4.95 ^{ebdhgcf}	5.40 ^{ebdacf}	4.19 ^{hgf}
Trt 2	5.93 ^{bac}	5.18 ^{ebdagcf}	5.18 ^{ebdgcf}	4.31 ^{hgf}	4.73 ^{ebdhgcf}	4.88 ^{ebdhgcf}	4.91 ^{ebdhgcf}
Trt 3	6.00 ^{ba}	5.63 ^{ebdac}	5.41 ^{ebdgcf}	4.86 ^{ebdhgcf}	4.73 ^{ebdhgcf}	4.58 ^{ehgf}	3.78 ^h
Trt 4	5.78 ^{bdac}	5.63 ^{ebdac}	5.63 ^{ebdac}	5.32 ^{ebdacf}	4.80 ^{ebdhgcf}	4.58 ^{ehgf}	4.01 ^h

Data were analyzed using a factorial ANOVA (treatment × time). Subsequently, Tukey's test ($p < 0.05$) was applied for mean comparison. Means with the same letter in the same column indicate no significant differences. Trat=group of treatments for each variable. Trt=treatments. Fermentation time: 0, 2, 4, 6, 24, 48, and 72 h.

Proximal analysis: For MS and H variables, the ANOVA showed a significant effect of time in both $p < 0.0001$ and a significant time $\times$ treatment interaction MS $p = 0.0408$; $H_p = 0.0026$, while the trend among treatments was not significant for either variable. Table 3 shows the behavior of MS and H, where initial values were close to 16-18% and final values were around 15-16% at 72 h, demonstrating that orange bagasse continues to exhibit a high moisture content. This agrees with López *et al.* (2023), who reported that at the end of the process orange bagasse registered a moisture content ranging between 75 and 80%. The reason for this is explained by Morovat *et al.*

Table 3. Least squares mean of chemical-proximal parameters during orange bagasse fermentation.

Dry matter (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	16.30 ^{ebdc}	16.40 ^{ebdac}	15.61 ^{edc}	14.94 ^e	15.40 ^{ed}	15.58 ^{ed}	15.87 ^{edc}
Trt 2	17.65 ^{bac}	16.41 ^{ebdac}	16.51 ^{ebdac}	16.45 ^{ebdac}	17.29 ^{bdac}	16.12 ^{ebdc}	16.00 ^{ebdc}
Trt 3	17.71 ^{ba}	17.10 ^{bdac}	16.52 ^{ebdac}	16.39 ^{ebdac}	16.58 ^{ebdac}	16.95 ^{ebdac}	16.58 ^{ebdac}
Trt 4	18.46 ^a	17.45 ^{bdac}	15.58 ^{edc}	16.66 ^{edac}	16.36 ^{ebdc}	16.19 ^{ebdc}	16.06 ^{ebdc}
Moisture content (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	83.72 ^{bac}	83.60 ^{bdac}	84.06 ^{bac}	84.59 ^{ba}	84.60 ^{ba}	84.42 ^{bac}	84.05 ^{bac}
Trt 2	82.35 ^{dc}	83.68 ^{bdac}	84.99 ^a	83.55 ^{bdac}	82.80 ^{bdac}	83.88 ^{bac}	83.61 ^{bdac}
Trt 3	82.29 ^d	82.90 ^{bdac}	83.48 ^{bdac}	83.61 ^{bdac}	83.42 ^{bdac}	83.06 ^{bdac}	83.42 ^{bdac}
Trt 4	81.53 ^d	82.56 ^{dc}	84.51 ^{ba}	83.26 ^{bdac}	83.64 ^{bdac}	83.81 ^{bac}	83.95 ^{bac}
Ashes (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	5.30 ^a	5.17 ^a	5.43 ^a	5.61 ^a	5.72 ^a	5.32 ^a	8.68 ^{ed}
Trt 2	5.38 ^a	5.48 ^a	5.53 ^a	5.30 ^a	5.09 ^a	5.26 ^a	11.33 ^{ebdc}
Trt 3	5.43 ^a	5.41 ^a	5.53 ^a	6.00 ^a	5.78 ^a	5.61 ^a	16.34 ^{bac}
Trt 4	5.42 ^a	5.78 ^a	5.20 ^a	6.07 ^a	6.07 ^a	5.71 ^a	15.80 ^{bac}
Total protein (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	8.21 ^e	10.17 ^{ebdc}	10.17 ^{ebdc}	10.51 ^{ebdc}	9.76 ^{ed}	8.68 ^{ed}	8.68 ^{ed}
Trt 2	10.80 ^{ebdc}	12.10 ^{ebdac}	12.10 ^{ebdac}	15.14 ^{ebdac}	16.01 ^{bac}	15.43 ^{bdac}	11.33 ^{ebdc}
Trt 3	15.24 ^{bdac}	15.76 ^{bac}	15.76 ^{bac}	16.03 ^{bac}	17.11 ^{ba}	18.55 ^a	16.34 ^{bac}
Trt 4	10.69 ^{ebdc}	11.90 ^{ebdc}	11.90 ^{ebdc}	12.31 ^{ebdac}	12.66 ^{ebdac}	13.07 ^{ebdac}	15.80 ^{bac}
Total carbohydrates (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	14.13 ^{hg^f}	18.47 ^{ebdac}	20.07 ^a	19.56 ^{ba}	19.33 ^{bac}	19.15 ^{bdac}	20.04 ^a
Trt 2	10.83 ^h	13.87 ^{hg}	14.76 ^{ehg^f}	15.44 ^{ebdg^c}	16.05 ^{ebdag^c}	16.75 ^{ebdag^c}	14.62 ^{ehg^f}
Trt 3	14.30 ^{hg^f}	13.45 ^h	16.04 ^{ebdg^c}	15.52 ^{ebdg^c}	14.45 ^{ehg^f}	17.43 ^{ebdag^c}	14.71 ^{ehg^f}
Trt 4	16.75 ^{ebdag^c}	15.03 ^{edhg^c}	14.55 ^{ehg^f}	15.22 ^{edhg^c}	17.00 ^{ebdag^c}	15.32 ^{ebdg^c}	14.68 ^{ehg^f}

Data were analyzed using a factorial ANOVA (treatment $\times$ time). Subsequently, Tukey's test ($p < 0.05$) was applied for mean comparison. Means with the same letter in the same column indicate no significant differences. Trat=group of treatments for each variable. Trt=treatments. Fermentation time: 0, 2, 4, 6, 24, 48, and 72 h.

(2014), who stated that during the fermentation of citrus by-products microorganisms metabolize fermentable sugars (glucose, fructose, sucrose, and maltose), which causes a partial reduction in dry matter.

Regarding the ash variable, ANOVA showed a significant effect of time ($p=0.0016$) and a significant treatment $\times$ time interaction ($p=0.0023$). Mean comparison indicated that the initial ash content was 5.30% (Trt 1), 5.38% (Trt 2), 5.43% (Trt 3), and 5.42% (Trt 4), and after 72 h of fermentation the values were 8.68% (Trt 1), 11.33% (Trt 2), 16.36% (Trt 3), and 15.30% (Trt 4). Thus, Trt 3 recorded the highest value at the end of fermentation. Ash content decreased predominantly in the control treatment compared to the others (Trt 2, Trt 3, and Trt 4), indicating lower metabolic activity, a result similar to that reported by Castillo *et al.* (2011) in a study on solid-state fermentation of agro-industrial by-products. Furthermore, Erismann *et al.* (2025) reported that ash content commonly fluctuates during fermentation due to the consumption and release of soluble mineral components and inorganic compounds.

Regarding protein, according to the ANOVA, the time factor significantly affected the variable $p=0.0021$ in the treatment $\times$ time interaction, while no significant differences were observed among treatments ($p>0.05$). Figure 3 shows the least squares means, where a relatively high standard error was observed, particularly at the beginning of the experiment, with higher values recorded in Trt 3. This dispersion may be due to environmental conditions during the process, the composition of the bagasse itself, and possibly the sensitivity of the Lowry method (Lowry *et al.*, 1951). This method was used because it quantifies true protein as soluble fractions and nitrogenous compounds, and the presence of yeast and nitrogen sources (urea and ammonium sulfate) increases the detectable fraction at the beginning of fermentation. Under this premise, protein content increased over time, with Trt 3 showing the highest values compared to Trt 1, which recorded the lowest values. The maximum protein value was observed at 48 h of fermentation, followed by a slight decrease at 72 h.

This finding corresponds to that reported by Morovat *et al.* (2014), who observed that lemon pulp supplemented with *Saccharomyces cerevisiae* and urea showed an increase in

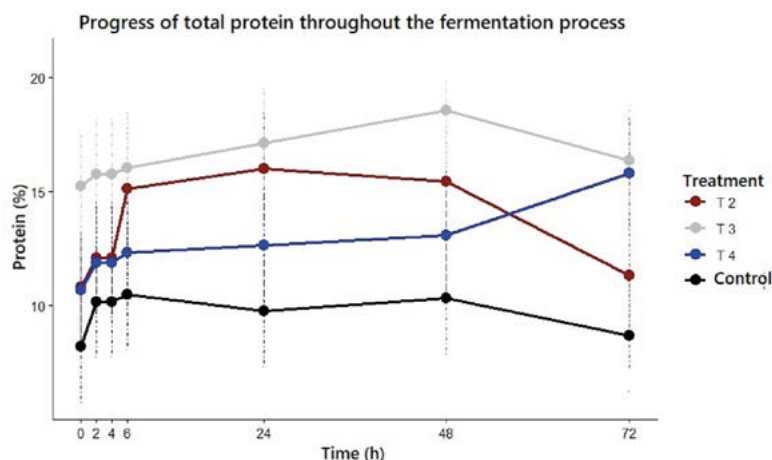


Figure 3. Evolution of the protein variable over the fermentation time in different treatments.

protein at the beginning of fermentation compared to the control. Likewise, the highest PT content throughout fermentation was observed in treatments that combined yeast with nitrogen sources. This can be explained by the premise that microorganisms utilize nitrogen sources (urea and ammonium sulfate) and transform non-protein nitrogen (NPN) into protein nitrogen (PN), which is partially incorporated into the cellular protoplasm (Rodríguez *et al.*, 2006).

Regarding total carbohydrate content, ANOVA showed a significant effect of time $p < 0.0001$ and a significant time $\times$ treatment interaction $p = 0.0024$, while no significant differences were observed among treatments ($p = 0.1801$). In Figure 4, total carbohydrate content exhibited a significant increase during the first hours of the process, reaching its highest value at 4 h (20.07 ± 1.59). Likewise, Trt 1 showed the greatest increase compared to the other treatments (Trt 2, Trt 3, and Trt 4). These latter treatments showed similar contents, although with irregular variations, and most of them reached their highest increase within the 24–48 h range.

Similarly, a contrasting effect was observed in carbohydrate content relative to protein. On the one hand, the increase in carbohydrates was lower in the treatments supplemented with nitrogen sources (Trt 2, Trt 3, and Trt 4); however, these registered a higher protein content. This behavior can be explained by the fact that these sugars are consumed by microorganisms, which use them as an energy source during fermentation (Peters, 2006). This assertion agrees with the findings of Rossit *et al.* (2020) and Zhao & Yu (2008), who report that added nitrogen sources, such as urea and ammonium sulfate, contribute to an efficient and rapid consumption of sugars.

Yeast count: Given the nature of yeast colonies —usually dispersed and forming large aggregates during colony development— the negative binomial model adequately fits cell count data (Lindén and Mäntyniemi, 2011). In addition, this model appropriately handles processes involving repeated analyses, as in this study, where the model fitting was performed using Microsoft Excel[®]. On the other hand, ANOVA results indicated significant differences among treatments ($p = 0.004$) and over time ($p < 0.0001$), with no significant time $\times$ treatment interaction ($p = 0.1092$). An exponential growth was observed

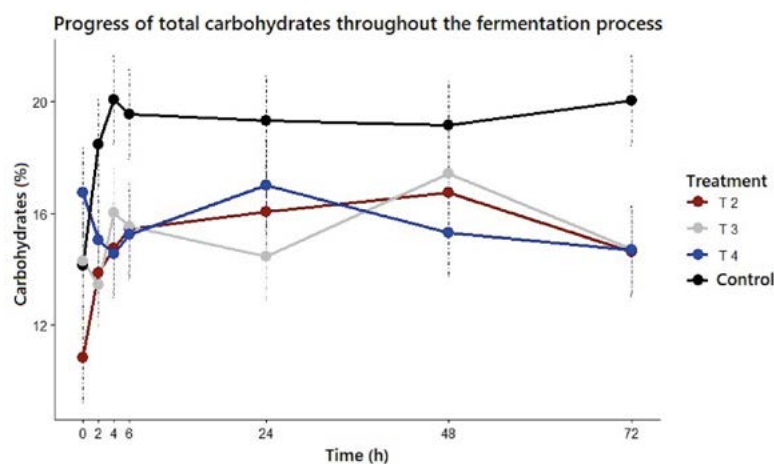


Figure 4. Evolution of the carbohydrate variable over fermentation time in different treatments.

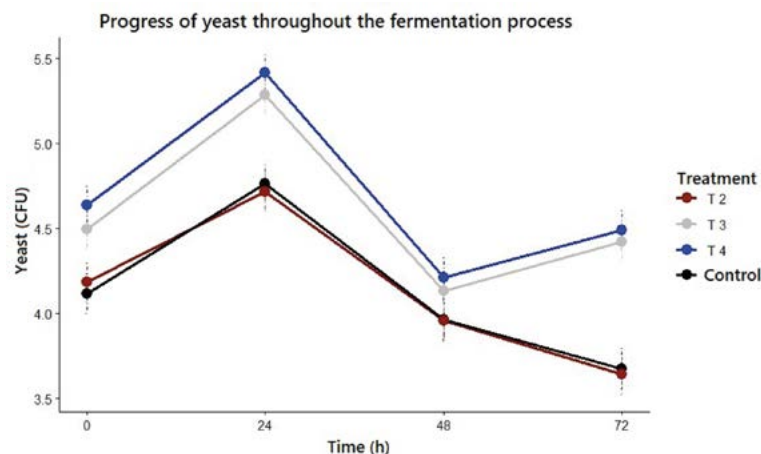


Figure 5. Evolution of yeast (CFU) over fermentation time across different treatments.

up to 24 h, followed by stabilization and a slight decrease at 72 h (Figure 5). Trt 4 showed the greatest increase (4.21×10^6 CFU/g), while Trt 1 and Trt 2 exhibited a similar and generally lower behavior compared to the other treatments. The highest yeast colony counts were recorded in treatments supplemented with nitrogen sources. This statement agrees with the findings reported by Schön *et al.* (2024), who indicated that the addition of nitrogen sources promotes the growth of *S. cerevisiae* yeast. Likewise, Li *et al.* (2024) reported that the use of nitrogen compounds contributes to the bioconversion of citrus residues, resulting in greater microbial biomass production, which in turn contributes to increasing the protein content of the substrate. In the same context, Grizotto *et al.* (2020) reported yeast counts close to 10^6 CFU/g in orange peel silage, confirming the reliability of the values reported in this study.

CONCLUSIONS

The fermentation of orange bagasse enriched with *S. cerevisiae*, urea, and ammonium sulfate did not show statistically significant increases in the physicochemical parameters (pH and temperature) or in the proximate chemical parameters (DM, M, Ash, CP, and TC) evaluated among treatments ($p > 0.05$). Fermentation time was the most determining factor in the numerical changes observed in the variables ($p < 0.05$). The mixture and dosage of inputs in Trt 3, in the physicochemical analyses of the studied parameters, recorded significant values, allowing the optimization and enhancement of fermented orange bagasse.

An 18.55% increase in protein was observed, higher than the 10.51% recorded in Trt 1 (control), requiring 48 h of processing. This value corresponds to a descriptive value across the different sampling times. In this regard, Trt 3 and Trt 4 exhibited the highest yeast counts. A reduction in TC, °Brix, and pH was also observed throughout fermentation, along with an increase in temperature as the process progressed.

These results provide preliminary information on the potential value of orange bagasse and support future research on its utilization.

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Evaluation of nutrition sources in cacao (*Theobroma cacao* L.) and their impact on yield

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ABSTRACT

Objective. The present study evaluated the effect of three fertilization sources —chemical fertilizer, organic compost, and a no-fertilizer treatment— on yield components of the cacao crop.

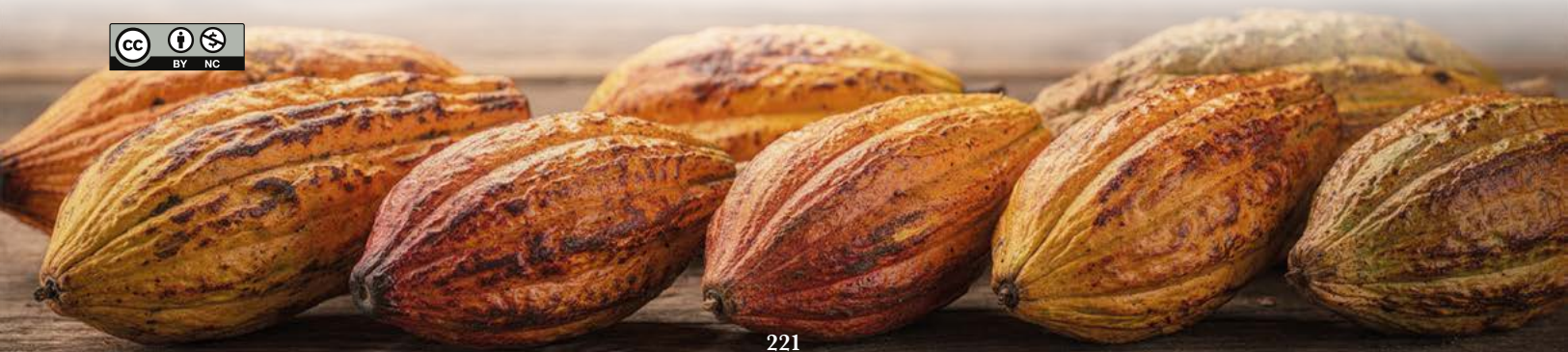
Materials and Methods. The experiment was conducted at the Rosario Izapa Experimental Field of INIFAP in Chiapas, Mexico, where the effect of three nutritional treatments (chemical fertilization, compost, and control) on yield components of six cacao clones (CATIE R4, Regalo de Dios, CAERI 1, CAERI 2, CAERI 4, and SCA12) was evaluated. The experiment was established under a randomized block design with three replications, each replication consisting of three trees per clone. Fruits were harvested from each clone, and variables were measured from 20 fruits per tree.

Results. Organic compost was the most effective nutrient source, showing significant improvements in the number of fruits and dry bean weight. Chemical fertilization showed a limited response, in some cases similar to the control treatment, indicating low efficiency under certain edaphic conditions. The control treatment showed values similar to organic fertilization in the total number of seeds variable.

Limitations/Implications. Cacao nutrition is a closed system in which many factors interact, particularly shade trees that either contribute nutrients or compete for them within the system, and whose effects are sometimes difficult to quantify.

Findings/Conclusions. These findings suggest that the use of compost not only improves cacao productivity but also represents a sustainable strategy that contributes to the agroecological resilience of the production system.

Keywords: chemical fertilization, compost, clones, fruits, dry bean



INTRODUCTION

Cacao (*Theobroma cacao* L.) is a crop of high economic, social, and ecological relevance in tropical regions, particularly in Latin America. Its production is closely linked to agroforestry systems and sustainable cultivation practices that contribute to food security and rural development (Muñoz *et al.*, 2019; Suarez-Venero *et al.*, 2019). In Mexico, cacao is cultivated on approximately 29,000 ha, with an average yield of 590 kg/ha (SIAP, 2025). Over the last three years, cacao prices have undergone a substantial increase in the price per kilogram of dry beans, rising from 60 to more than 200 Mexican pesos per kilogram (ICCO, 2025). This situation has boosted cacao cultivation in Mexico, particularly in tropical regions where agroclimatic conditions are favorable.

In recent decades, chemical fertilization has been widely used to increase agricultural productivity. However, several studies have reported its negative effects on soil health, edaphic biodiversity, and environmental balance (Castillo and Ramírez, 2018; Gómez and Cañizares, 2021). These practices may lead to natural resource degradation and increased dependence on external inputs, limiting the sustainability of production systems. As an alternative, organic fertilization, especially through compost application, has proven effective in improving the physical, chemical, and biological properties of soil, while also enhancing the vegetative development of crops such as cacao (Jiménez *et al.*, 2020). This type of nutrient management can promote more closed nutrient cycles, reduce the use of agrochemicals, and strengthen agroecosystem resilience. In this context, Paredes and Subia (2014) reported that cacao associated with leguminous species and forest trees can capture between 7.1 and 21.9 t ha⁻¹ of carbon.

Despite these advances, there is still limited comparative evidence on the impact of different fertilization sources on cacao yield components in agroforestry production systems. In this study, three nutritional treatments were evaluated: chemical fertilization, organic fertilization through compost, and an unfertilized control. The objective was to compare their effect on cacao yield and contribute to the design of more efficient and sustainable agricultural practices.

MATERIALS AND METHODS

The experimental site is located at the Rosario Izapa Experimental Station (CERI, for its acronym in Spanish) of the National Institute for Forestry, Agriculture and Livestock Research (INIFAP, for its acronym in Spanish) in the municipality of Tuxtla Chico, Chiapas, Mexico, at 14° 40' N latitude and 92° 10' W longitude, at an altitude of 435 m above sea level. The predominant climate in Tuxtla Chico is warm humid with abundant summer rainfall, with a minimum temperature of 24 °C and a maximum of 30 °C, and an annual precipitation ranging from 1,200 to 5,000 mm (INEGI, 2010).

Soils in the region are characterized by their formation from volcanic ash. The soils at CERI have a clay-sandy texture and belong to the Andosol group, with an approximate depth of 1.5-2 m. The soil has a pH of 6.5 and an organic matter content of 3%.

Plant Material

The cacao clones used in this study were selected based on their tolerance to moniliasis (*Moniliophthora roreri*) (Avendaño *et al.*, 2018; Avendaño *et al.*, 2019; Phillips-Mora *et al.*, 2012) Table 1, Figure 1).

Treatments

Prior to the establishment of the treatments, soil samples were collected from the experimental field where the study was conducted. The samples were analyzed in the laboratory to determine their nutrient composition, and the results are presented in Table 2.

Three treatments were considered in the study: chemical fertilization, organic fertilization, and a control treatment.

Table 1. Cacao clones used for evaluation under different nutritional treatments.

ID	Clon	Response to moniliasis
C1	CATIE R4	Tolerant
C2	Regalo de Dios	Tolerant
C3	CAERI 1	Tolerant
C4	CAERI 2	Tolerant
C5	CAERI 4	Tolerant
C6	SCA 12	Susceptible



Figure 1. Genotipos de *Theobroma cacao* L. CAERI-1 (a), CAERI-2 (b), CAERI-4 (c), CATIE-R4 (d), SCA-12 (e), Regalo de Dios (f).

Table 2. Soil characteristics of the experimental site.

M	pH	OM (%)	N_NO ₃ (ppm)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Ca (ppm)
1	6.37	8.73	15.37	305.5	4.9	192.2	1166
2	6.35	8.64	14.69	302.4	3.1	129.7	1163
3	6.27	8.90	14.41	311.4	3.6	139.8	1334
4	6.27	9.00	12.63	315.1	4.5	136.3	1373

Table 2. Continued...

M	pH	OM (%)	N_NO ₃ (ppm)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Ca (ppm)
1	114.35	10.30	28.48	2.68	3.10	1.044	0.102
2	108.88	5.89	29.41	2.71	2.91	1.027	0.103
3	127.07	7.36	26.66	2.63	4.68	1.096	0.098
4	124.08	1.47	25.86	2.70	4.65	1.04	0.103

M=Samples.

Chemical fertilization: Chemical fertilizer was applied starting six months after the establishment of cacao plants at a dose of 150 g per plant. The chemical composition of the fertilizer used is presented in Table 3. The fertilizer was applied manually in a circular band around the plant (50 cm from the stem) using a hoe and then covered with soil. Fertilization was carried out every 90 days until the productive stage of the plants.

Organic fertilization: The treatment was applied starting six months after the establishment of cacao plants at a dose of 1.5 kg of compost per plant. The chemical composition of the compost is presented in Table 4. The application was carried out manually, following the same procedure as chemical fertilization, with application intervals every 90 days until the productive stage of the plants.

Agronomic Management

For agronomic management, the plots were established with temporary shade (*Cajanus cajan*) and permanent shade (*Gliricidia sepium*). Cacao plants were established at a spacing of 3 m between plants and 3 m between rows. Weed control was carried out manually using a machete. Formative pruning was performed during the first six months

Table 3. Chemical composition of the fertilizer used in the experiment.

Component	Amount (%)
N-Total	21.0
N-Nitric	10.0
N-Ammoniacal	11.0
Phosphorus P ₂ O ₅	17.0
Potassium K ₂ O	3.0
Sulfur	4.0
Zinc	0.15
Magnesium MgO	0.4

Table 4. Nutrient composition of the compost.

Component	Amount
pH	7-8
C.E ds/m	4-10
Organic matter (%)	12-30
Total nitrogen (%)	1.2-2.5
Phosphorus P ₂ O ₅ (%)	0.5-2
Potassium K ₂ O (%)	0.53
Calcium CaO (%)	2-4
Magnesium MgO (%)	0.5-0.8
Sodium (%)	0.2
Sulfur (%)	0.2-0.45
Manganese (ppm)	550
Iron (ppm)	5000
Zinc (ppm)	150
Copper (ppm)	33
CEC Meq/100 g of soil	80-300

CE=Electrical conductivity, CEC=Cation exchange capacity. Control treatment. No type of soil fertilization or nutrient input was applied.

after plant establishment, followed by maintenance and fruiting pruning. For pest and disease management, diseased fruits were removed, pruning was conducted, and copper oxychloride was applied at a dose of 3 gL⁻¹ of water three times per year. Harvesting was carried out manually.

Experimental design and evaluated variables

A randomized block design with three replications was used, with each replication consisting of three trees per clone. Once the plants reached the productive stage, 20 fruits were harvested from each clone and taken to the laboratory for evaluation. Variables related to yield components were evaluated, including pod index (number of pods required to obtain 1 kg of dry beans), Pod length (PL, cm), Pod width (PW, cm), fruit exocarp thickness (FET, mm), total number of seeds (TNS), total fresh seed weight (TFSW, g), fruit weight (FWt, g), total dry seed weight (TDSW, g), and individual dry seed weight (IDSW, g). Dry seed weight was determined at 7% moisture content. Data collection was carried out twice per year (in each harvest cycle) for two consecutive years.

Statistical Analysis

An analysis of variance (ANOVA) was performed according to the randomized block experimental design model, followed by a mean comparison using Tukey's test at $\alpha=0.05$.

RESULTS AND DISCUSSION

Fruit Variables

According to the analysis of variance, no statistical differences were found for the source of variation “clones” in the variables TNS and FWt, while the remaining variables showed highly significant differences. For the source of variation “nutrition,” highly significant differences were found only in the pod index (PI). Finally, for the clone $\times$ nutrition interaction, a statistically significant difference was found only in fruit exocarp thickness (FET) (Table 5).

When comparing means among clones, regardless of the treatment applied, the CATIE R4 clone (C1) showed the lowest PI (25.9 pods), whereas the SCA12 clone (C6) showed the highest PI (40.1 pods). The CAERI 1 clone (C3) presented the highest PL (20.5 cm), while the lowest PL was observed in the SCA12 clone (C6, 15 cm). The CAERI 4 clone (C5) showed the highest PW (9.06 cm), whereas the lowest PW was recorded in the SCA12 clone (C6, 6.86 cm). CAERI 1 (C3) exhibited the highest FET (15.8 mm), while the lowest value was observed in SCA12 (C6, 10.3 mm) (Table 6).

In the variable total number of seeds, the clone CAERI 2 (C4) showed the highest number of seeds (37.6), while Regalo de Dios (C2) had the lowest number of seeds (27.9). However, in relation to total fresh seed weight, the clone CATIE R4 (C1) obtained the highest weight (142 g), whereas the lowest weight was recorded in the clone SCA12 (C6, 77.1 g). Finally, for the fruit weight variable, the clone CAERI 4 (C5) showed the highest average fruit weight (615.1 g), while the lowest fruit weight was obtained from the clone SCA12 (315.4 g) (Table 7).

Table 5. Sum of squares from the analysis of variance for fruit variables.

SV	DF	PI	PL	PW	FET	TNS	TFSW	FW
Clon	5	5069**	333.7 **	58.3 **	413.4 **	371.8 ns	59699**	139501ns
Nut	2	377.6*	10.3 ns	1.2 ns	10.3 ns	259.5 ns	50034 ns	55368 ns
Clon*Nut	10	48.2 ns	76.2 ns	11.1 ns	90.9 *	938.7 ns	8567 ns	246662ns

SV=source of variation; Nut=nutrition; DF=degrees of freedom; PI=pod index; PL=pod length; PW=pod width; FET=fruit exocarp thickness; TNS=total number of seeds; TFSW=total fresh seed weight; FW=fruit weight.

Table 6. Mean comparison of PI, PL, PW, and FET variables.

PI			PI			PL			FET		
Clon	Mean	Tuk	Clon	Mean	Tuk	Clon	Mean	Tuk	Clon	Mean	Tuk
6	40.1	a	3	20.5	a	5	9.06	a	3	15.8	a
2	31.9	b	2	19.8	a	3	8.53	b	2	15.3	a
4	29.1	b	1	18.3	b	1	8.55	b	1	15.0	a
5	26.7	b	5	17.5	b	4	8.25	b	5	14.6	a
3	26.4	b	4	17.3	b	2	8.16	b	4	14.5	a
1	25.9	b	6	15	c	6	6.86	c	6	10.3	b

Mean=Mean value, Group=Statistical group, PI=pod index; PL=pod length; PW=pod width; FET=fruit exocarp thickness; Tuk=Tukey's aggregation.

Table 7. Mean comparison of TNS, TFSW, and FW variables among cacao clones.

TNS			TFSW			FW		
Genotype (Clon)	Mean	Tuk	Genotype (Clon)	Mean	Tuk	Genotype (Clon)	Mean	Tuk
4	37.6	a	1	142.0	a	5	615.1	a
3	35.1	a	5	141.2	a	3	613.1	a
5	34.3	ab	3	125.6	ab	1	595.2	a
1	32.3	ab	4	113.6	bc	2	571.9	ab
6	31.8	ab	2	94.5	c	4	496.5	b
2	27.9	b	6	77.1	d	6	315.4	c

Mean=Mean value, Group=Group, TNS=Total number of seeds; TFSW=Total fresh seed weight; FW=Fruit weight; Tuk=Tukey's aggrupation.

When analyzing fruit variables according to the type of nutrition, it was observed that plants treated with compost and control plants had the lowest PI, while chemical fertilization required more pods to produce one kilogram of dry beans. The use of compost also had an effect on PL, PW, and FET, where the highest values were obtained. For TNS, no statistical differences were observed among fertilization types; however, the use of compost showed a higher number of total seeds (Figure 2). The effect of compost use was also reflected in TFSW and FW, presenting heavier fruits and a higher number of total seeds (Figure 3).

Seed variables

According to the analysis of variance for seed variables, highly significant statistical differences were observed among clones for all analyzed variables. For the source of variation type of nutrition, no significant statistical differences were observed only in ST (seed thickness), while the remaining variables showed highly significant statistical differences. For the clone × nutrition interaction, highly significant statistical differences were observed for all variables (Table 8).

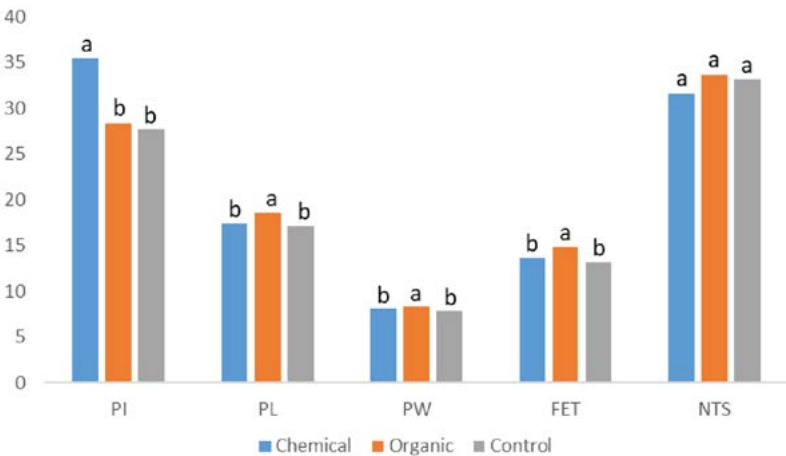


Figure 2. Mean comparison of variables. PI=pod index; PL=pod length; PW=pod width; FET=fruit exocarp thickness; TNS=total number of seeds.

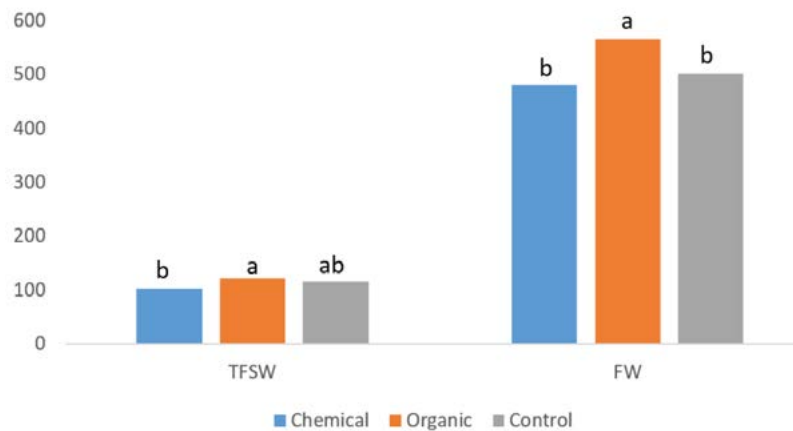


Figure 3. Mean comparison of TFSW and FW under three types of nutrition in cacao.

Table 8. Sum of squares of the analysis of variance for seed variables.

SV	DF	SL	SW	ST	FSWI	SI
Clon	5	2900.7 **	1272.8 **	850.6 **	338.6 **	46.1 **
Nut	2	146.3 **	17.6 **	1.2 ns	3.4 **	0.4 **
Clon*Nut	10	340.7 **	76.0 **	45.7 **	20.3 **	2.6 **

SV=source of variation; Nut=nutrition; DF=degrees of freedom; SL=seed length; SW=seed width; ST=seed thickness; FSWI=fresh seed weight per individual seed; SI=seed index.

When performing the mean comparison among clones, CATIE R4 (C1) showed the highest seed length (24.6 mm), while SCA12 (C6) showed the lowest seed length (19.7 mm); for ST, SW, and IS, CATIE R4 (C1) showed the highest values, with 12.6 mm, 9.4 mm, and 1.2 g, respectively, whereas SCA12 (C6) showed the lowest values of 9.5 mm, 6.5 mm, and 0.7 g, respectively (Table 9).

When analyzing the mean comparison among types of nutrition and their effect on seed variables, organic fertilization based on compost showed the highest values for SL, SW, ST, and SI, while chemical fertilization showed the lowest values for SL, SW, and SI (Figure 4).

Table 9. Mean comparison of seed variables SL, SW, GS, and SI among six cacao clones.

SL			SW			ST			SI		
Genotype (Clon)	Mean	Tuk	Genotype (Clon)	Mean	Tuk	Genotype (Clon)	Mean	Tuk	Genotype (Clon)	Mean	Tuk
1	24.6	a	1	12.6	a	1	9.4	a	1	1.2	a
5	23.4	b	5	12.1	b	2	9.3	a	5	1.1	b
4	22.9	c	3	11.9	c	3	8.9	b	2	1.1	bc
3	22.7	c	4	11.9	c	4	8.3	c	3	1.1	c
2	22.2	d	2	11.8	c	5	8.2	c	4	1.0	d
6	19.7	e	6	9.5	d	6	6.9	d	6	0.7	e

Mean=mean value; Group=statistical group; SL=seed length; SW=seed width; ST=seed thickness; SI=seed index; Tuk=Tukey’s aggrupation.

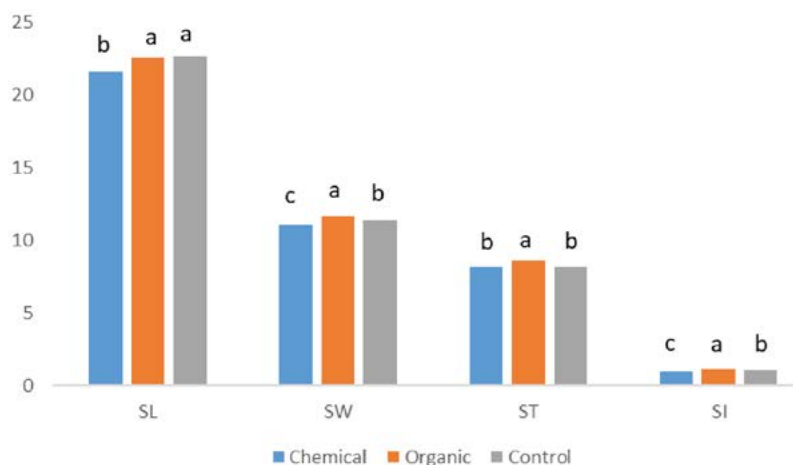


Figure 4. Mean comparison analysis of seed variables in cacao under three types of nutrition (SL=seed length, SW=seed width, ST=seed thickness, SI=seed index).

The data obtained in this study show that organic nutrition was the most effective source of nutrition in terms of cacao yield. This advantage can be attributed to its capacity to improve soil structure, promote microbiological activity, and release nutrients progressively. Studies such as Jiménez *et al.* (2020) indicate that the incorporation of organic residues significantly improves the vegetative and reproductive development of cacao, especially in soils with low initial fertility. Similarly, Mulia *et al.* (2017) demonstrate that under marginal and acidic soil conditions, organic amendments, particularly compost, have a significantly greater impact on cacao growth and productivity than mineral sources applied in isolation. Plants treated with compost showed greater height, higher flowering, and up to five times higher dry bean yield than treatments based exclusively on mineral fertilization.

Although chemical fertilizer provides rapidly available nutrients, in this study its performance was lower than that of organic nutrition. Previous research has found that synthetic inputs may have a transient effect on productivity and, in some cases, low efficiency depending on soil type (Castillo and Ramírez, 2018). This suggests that cacao may respond more favorably to organic sources when sustainability and resilience in cultivation are sought. The unfertilized treatment (control) showed the lowest yield, highlighting the need to implement plant nutrition practices to achieve economically viable production. According to Díaz *et al.* (2017), cacao cultivated without any type of fertilization produces a lower number of fruits per plant and lower dry seed weight, limiting its commercial potential.

Beyond yield, the use of compost as a source of organic nutrition represents a sustainable alternative that utilizes local organic residues and promotes a circular approach in agriculture. Authors such as Gómez and Cañizares (2021) state that agricultural systems integrating organic fertilizers improve soil biodiversity and contribute to climate change mitigation. In addition, several studies conducted in Mexico, Colombia, Ecuador, and other countries (Francisco-Santiago *et al.*, 2023; Vega *et al.*, 2021; Castillo-Garrido, 2024; Schmidt *et al.*, 2024) agree that the application of organic fertilizers, compost, and biofertilizers in cacao cultivation increases the number of fruits and yield per plant, improves

soil fertility and microbial activity, enhances productive stability and bean quality, and reduces dependence on chemical inputs, promoting more resilient agricultural systems.

Dogbatse *et al.* (2021), based on their results, conclude that inorganic fertilization is more effective in promoting rapid growth during the early stages of cacao; however, organic fertilization improves soil health and favors stronger root development. Therefore, they recommend an integrated strategy (organic + inorganic), as it combines vigorous growth, improved nutrient absorption, and soil sustainability. In this regard, Leiva-Rojas (2022) also states that the integration of organic and mineral sources is a recommended strategy, since cacao nutrition is a dynamic process dependent on the phenological stage, and nutrient uptake is closely linked to cacao physiology, while genetic and environmental variability conditions the response to fertilization. Similarly, López-Baez *et al.* (2015) highlight that cacao responds better to balanced and gradual nutrition than to intensive fertilizer applications. However, Marrocos *et al.* (2020) report that adequate mineral nutrition is a determining factor for productive sustainability, reflected in yield, physiological efficiency, and crop resilience. Sánchez *et al.* (2005) support the importance of implementing mineral fertilization programs in cacao systems where nutrition is limited, and Uribe *et al.* (2008) report that balanced mineral fertilization is a key tool to improve cacao productivity and can help overcome low yields.

CONCLUSIONS

Organic fertilization (compost) proved to be the most efficient source of nutrition, significantly improving cacao yield compared to chemical fertilizer and the unfertilized control. Chemical nutrition showed variable results, with a lower response compared to organic nutrition, suggesting that its use should be considered with caution depending on cacao clone, soil type, and agricultural management. On the other hand, the unfertilized treatment confirmed the need for effective nutrient management practices to achieve viable production.

Finally, organic fertilization (compost) offers additional benefits, such as improved soil structure, enhanced soil biological activity, and reduced environmental impact. This study provides valuable evidence to support the transition toward more sustainable agricultural production models in high-value tropical crops such as cacao under agroforestry systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Technical Assistance and Adoption of Technological Innovations: Analysis of GGAVATT Pig Producers in Guanajuato, Mexico

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ABSTRACT

Objective: To evaluate the level of adoption of technological innovations (TIs) and the effect of pig producers' participation in the GGAVATT model in the state of Guanajuato.

Design/methodology/approach: Monthly data from 5,162 records of 260 pig farmers who received technical assistance and training (TAT) through the GGAVATT model from 2014 to 2020 in Guanajuato were analyzed. The data included participation in meetings, training sessions, and technology transfer tours, as well as the implementation of 25 technical interventions (TIs). Five synthetic variables were constructed as adoption indices (AIs) of TIs: Technical Assistance and Training (TAT), Management (M), Nutrition (N), Reproduction and Genetics (RG), and Health and Biosecurity (SB). To evaluate the effect of technical assistance (TA) on the use of TIs, producers were classified into three levels according to their participation in GGAVATT: 1 year, 2-3 years, and 4-5 years. The five indices were calculated for each participation level.

Results: The estimated AI values by level of participation of pig farmers in the GGAVATT were 0.38, 0.40, and 0.46 for TAT; 0.51, 0.55, and 0.62 for M; 0.58, 0.61, and 0.69 for N; 0.53, 0.61, and 0.66 for RG; and 0.53, 0.58, and 0.63 for SB.

Limitations on study/implications: While all the AIs changed positively, the transition from a semi-technified to a technified production system was not achieved. Therefore, future studies should analyze other factors that limit IT adoption.

Findings/conclusions: The TAT activities provided to pig farmers through the GGAVATT model positively influence the adoption of technological innovations, with the strongest effect observed in the area of nutrition.

Keywords: Livestock technology, Livestock groups, Pig farming.

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INTRODUCTION

Livestock production focuses on generating animal protein to feed the population. In 2024, meat consumption in Mexico reached 10.8 million tons, of which 30.2% was pork, ranking second after poultry (COMECARNE, 2025). Meat production depends on the physiological development of sows and their offspring, which in turn is influenced by agroclimatic factors and the use of technology. In this context, technological innovations play a key role in ensuring the success of livestock production units (Montero *et al.*, 2015).



According to Quintero *et al.* (2021), technological innovation promotes changes in production processes through the adoption of technologies, procedures, new forms of organization and production management, which improve productivity and the efficient use of production factors. Although the concept of innovation has often been addressed from a business perspective, there is sufficient literature indicating its application to agricultural and livestock enterprises (Racewicz *et al.*, 2021; Sánchez *et al.*, 2022; Granada *et al.*, 2021).

Technological innovation materializes through adoption, which measures the outcome of producers' decisions to use or not a given innovation in the production process (Martínez *et al.*, 2018). In the case of agricultural and livestock innovation, this process is complex and is defined as a set of organizations with links and interactions among them, dedicated to: i) generation, ii) validation, iii) adaptation, iv) transfer, v) dissemination, and vi) adoption of technologies and knowledge for problem solving (Quintero *et al.*, 2021). For the first three stages, research institutions are primarily involved, although in some cases they also participate in technology transfer and dissemination. An example of this is the GGAVATT model (Grupos Ganaderos de Validación y Transferencia de Tecnología), which was developed by the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) in Veracruz. Its objectives are to validate and transfer livestock technology to organized groups of producers (Román *et al.*, 2021). One of the states where this model has been successfully applied is Guanajuato, where the state government has promoted the use of technological innovations under the GGAVATT model since 2002, supporting an average of 77 groups per year (Román *et al.*, 2021). One of the animal protein production systems that has benefited from this model is the pig production system.

In Mexico, pig production is highly important as it provides meat, generates employment, and adds value. It is based on three production systems: a) technified, b) semi-technified, and c) backyard or artisanal (Alonso and Rodríguez, 2024). The system that predominates in the state of Guanajuato is the semi-technified system, which is characterized by traditional facilities, medium productivity, and poor animal health.

In 2023, 140,169 tons of pork were produced, with 16,412 production units housing 1,746,492 pigs, with an average carcass weight of 80 kg (Alonso and Rodríguez, 2024). Pork production is one of the most dynamic livestock activities (SIAP, 2022); for this reason, GGAVATT groups of pig producers have been established in Guanajuato, receiving technical assistance and training from livestock technicians. Through this model, a technological package consisting of 25 technical assistance, training, management, and technological innovation activities is promoted, although the effect of this model on the adoption of these practices remains unknown. The objective of this study was to evaluate the level of adoption and the effect of participation in GGAVATT among pig producers in the state of Guanajuato, Mexico.

MATERIALS AND METHODS

Variables and Information Sources

The study was conducted in the state of Guanajuato using information from pig farmers organized in informal groups within the pork production system, who receive technical

assistance and training from livestock technicians (Román *et al.*, 2021). The methodological strategy applied with these producers is based on the GGAVATT model, which includes the establishment of technical, economic, and technology-use records (Román *et al.*, 2021). Therefore, monthly information on the application of a technological package is available.

For this research, monthly data from 5,162 records of 260 pig producers who received technical assistance through the GGAVATT model between 2014 and 2020 were compiled and analyzed. The study covered five municipalities: León, Moroleón, Pénjamo, Tarimoro, and Yuriria in Guanajuato (Figure 1).

The information was obtained from monthly monitoring forms in which each advisor recorded the participation of producers in GGAVATT monthly meetings, the training sessions provided, and the scheduled technology tours. Additionally, participation in management activities, practices, and technological innovations implemented by each producer during the month of record was documented, assigning a value of 1 if performed and 0 if not (Table 1). The data were compiled into a database using Excel®.



Figure 1. Location of GGAVATT pig producer groups in the state of Guanajuato.

Table 1. Synthetic variables used.

Synthetic variables	Activity, practice, and technological innovations
Technical assistance and training	1) Monthly meeting, 2) Training course or workshop, 3) Training in the use of artificial insemination, and 4) Technological tours.
Management	1) Permanent numerical identification, 2) Individual female record, 3) Recording of piglet birth date and weight, 4) Recording of piglet weaning date and weight, 5) Record of initial age and weight for fattening, 6) Record of age and weight at the end of fattening, and 7) Record of monthly income and expenses.
Nutrition	1) Use of concentrates/premixes and 2) Management of balanced diets by production stage.
Reproduction and Genetics	1) Use of farrowing crates, 2) Breeding stock selection, 3) Use of artificial insemination, 4) Raising of replacement gilts, and 5) Use of maternal lines.
Health and Biosecurity	1) Internal and external deworming, 2) Vaccination against viral diseases, 3) Washing and disinfection of facilities, 4) Biosecurity measures, 5) Ammonia control, 6) Rodent control, and 7) Temperature control.

Source: Author’s elaboration, based on data available from the Bajío Experimental Station, INIFAP.

Estimation of Technological Innovation Adoption Indices

Based on the previously mentioned database, five synthetic variables were constructed as technological indices by livestock area (Table 1). To estimate the technological level per production unit, the methodology of technological indices proposed by De Freitas and Pinheiro (2013) was applied, based on the use of the 25 technological activities, practices, or components, which are distributed across five areas (or synthetic variables). Within each area, the variables take a value of 1 or 0, indicating whether they were applied or not. To estimate the synthetic variables, the methodology proposed by Espinosa *et al.* (2018) was adapted. The formula is described below:

$$I_{ij} = \sum_{ij}^n \frac{\delta_{in}}{\delta_{i...n}}$$

Where: I_{ij} is the adoption index for livestock area i for producer j , δ_{in} is the actual sum obtained by the producer according to the number of activities, practices, or technological innovations implemented, and $\delta_{i...n}$ is the maximum sum of the n practices or technologies that a producer j can implement in livestock area i . The calculated index values range within the interval $0 > I_{ij} < 1$.

Effect of the GGAVATT Model on Innovation Adoption

To measure the effect of the GGAVATT model on innovation adoption, producers were classified into three levels according to their duration in the group receiving technical assistance: 1 year, 2-3 years, and 4-5 years. The five adoption indices were calculated for each level. Additionally, Pearson partial correlations between the estimated indices were calculated using the statistical software JMP® 17.

RESULTS AND DISCUSSION

GGAVATT for Pig Producers in Guanajuato

The GGAVATT model consists of an organized group for livestock production, in which all enthusiastic farmers receptive to technological changes can participate. Groups are formed with 10 to 20 “friendly” producers whose ranches or farms have similar characteristics and production purposes, such as milk and meat (Galindo, 2001). In Guanajuato, GGAVATT groups have been established for producers in the following production systems: beef cattle, dairy cattle, beekeeping, goats, sheep, and pigs. These producers receive technical assistance and training from livestock technicians to improve productivity, income, family self-employment, and the sustainability of their production units (Román *et al.*, 2021).

This model has been operating in the state since 2002, and in 2024 it served 1,100 farmers organized into 55 groups, who received technical assistance from 55 professionals, mainly veterinarians and livestock agronomists, as well as technical support from a researcher at the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), affiliated with the Bajío Experimental Station.

In the state of Guanajuato, an average of six GGAVATT pig producer groups were established per year during the 2014-2020 period, serving an average of 138 pig producers. This indicates that each GGAVATT consisted of 23 members, exceeding the number of producers suggested by the model designers, which was a maximum of 20 producers (INIFAP, 2005; Román *et al.*, 2021). The number of GGAVATT pig producer groups during 2014-2020 did not show major variations; however, the number of producers did. As shown in Figure 2, this is because the average number of producers per group increased from 16 in 2014 and 2015 to 30 in the following three years, and then decreased again in 2019 and 2020.

The distribution by year, municipality, gender, and years of participation in the GGAVATT model for the 5,162 records analyzed is presented in Table 2. It can be observed that most records correspond to men, from the municipalities of León, Pénjamo, and Yuriria, and to producers with four or more years in the model.

Adoption of Technological Innovations

The adoption indices of technological innovations by livestock area are presented in Figure 3. It can be observed that all values increased among pig producers who were more consistent in their participation in the GGAVATT.

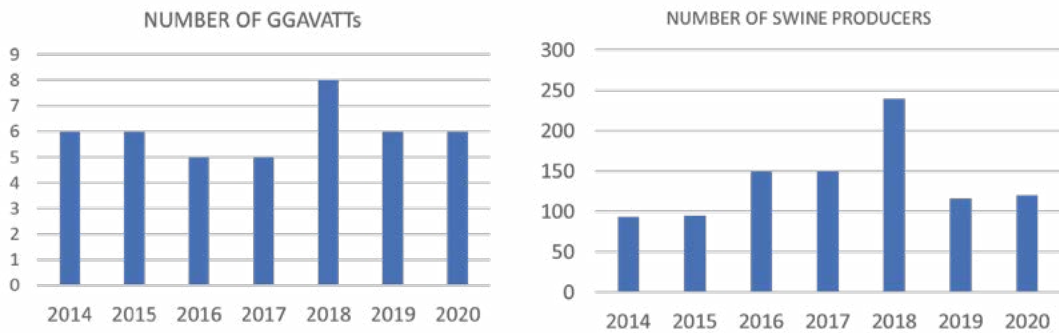


Figure 2. Trends in GGAVATT and the number of pork producers in the state of Guanajuato. Source: Author’s elaboration, based on data available from the Bajío Experimental Station, INIFAP.

Table 2. Distribution of pig producers by municipality, level, year, and gender.

Municipality	Frequency	%	Gender	Frequency	%
León	1,261	24.4	Male	4,324	83.8
Moroleón	630	12.2	Female	838	16.2
Pénjamo	1,279	24.8	Year	Frequency	%
Tarimoro	720	13.9	2014	245	4.7
Yuriria	1,272	24.6	2015	362	7.0
			2016	846	16.4
Level	Frequency	%	2017	1,110	21.5
One year	723	14.0	2018	1,110	21.5
2 to 3 years	2,193	42.5	2019	859	16.6
4 to 5 years	2,246	43.5	2020	630	12.2

Source: Author’s elaboration, based on data available from the Bajío Experimental Station, INIFAP.

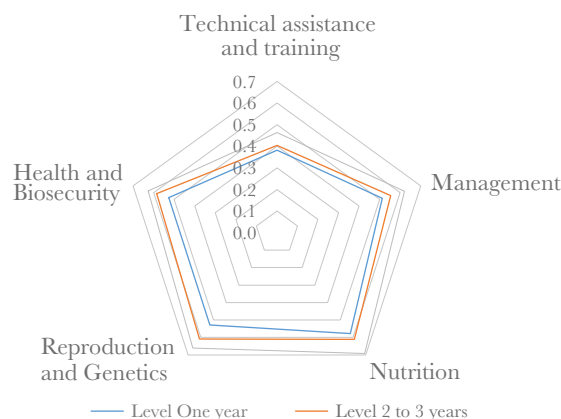


Figure 3. Adoption of health and biosecurity innovations in GGAVATT pig producer groups in Guanajuato, Mexico. Source: Author's elaboration, based on data available from the Bajío Experimental Station, INIFAP.

The index that reached the highest value, almost 0.7, was Nutrition, followed by the indices for Reproduction and Genetics (RG) and Health and Biosecurity (HB), each about three percentage points lower. This indicates that nearly 70% of pig producers incorporated technological innovations in these areas. This value is consistent with the study by Martínez *et al.* (2018) on beekeepers in Campeche, which reported adoption values of good production practices in the categories of artificial feeding and apicultural health of 0.90 and 0.68, respectively. It also aligns with the study by Espinosa *et al.* (2018) on livestock producers in the Mexican tropics, which reported adoption indices for innovations in the areas of nutrition and health of 0.70 and 0.71, respectively. Furthermore, these results show that technical assistance has a positive effect on the adoption of innovations.

By analyzing the change in adoption index values between pig producers with one year of participation in the GGAVATT and those with up to five years, it can be observed that the area with the greatest effect is Reproduction and Genetics, followed by Nutrition and Health and Biosecurity, with increases of 13%, 11%, and 10%, respectively. The positive effect of technical assistance and training in these three areas aligns with a study reported by Forero *et al.* (2013) for dual-purpose cattle producers in rural high-Andean areas of Colombia.

The behavior of the variables that make up each of the indices presented above is described below. Regarding technical assistance and training activities (Aguilar *et al.*, 2003), these are carried out monthly by the technicians with the pig producers. Participation in these activities is shown in Figure 4, where it can be observed that participation is highest in the monthly meetings, which follow a work program designed at the beginning of each year. About 70% of pig producers who join a GGAVATT attend the meetings, increasing their participation to 80% after five years in the program. Participation in training sessions is lower, although it increases among producers who remain in the GGAVATT, reflecting a positive change in their attitude toward training, as noted by González (2021). Finally, participation in technology tours is low, which is justified by biosecurity reasons that recommend restrictions when visiting or being visited by pig production farms (Montero *et al.*, 2015; Racewicz *et al.*, 2021).

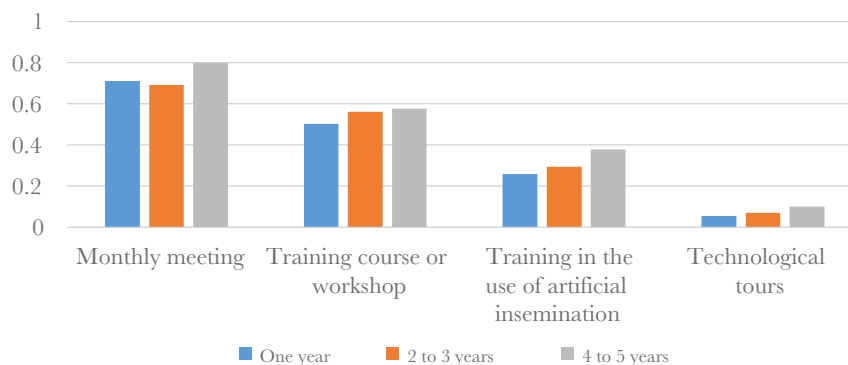


Figure 4. Participation of pig producers in Guanajuato in technical assistance and training activities of the GGAVATT. Source: Author's elaboration, based on data available from the Bajío Experimental Station, INIFAP.

Participation of pig producers in management activities is presented in Figure 5. Across the seven variables considered, a positive change is observed as producers spend more time in a GGAVATT. Recording the identification, dates, and weights of piglets and weaners showed the highest values, although progress in the use of economic records is also important, as it generates information for conducting technical and economic evaluations (Benítez, 2015).

Figures 6, 7, and 8 present the behavior of technological innovations in the Nutrition, Reproduction and Genetics, and Health and Biosecurity indices, respectively. The adoption of these innovations increased positively with longer participation in the GGAVATT, so that more than 50% of pig producers with five years of technical assistance applied most of the recommended innovations. The highest adoption values were observed for the administration of balanced diets by production stage, deworming, use of farrowing crates, use of maternal lines, and rearing of replacement animals.

The use of these innovations does not exceed 80% adoption in any case, which indicates that the technological level of pig farms participating in the GGAVATT in Guanajuato

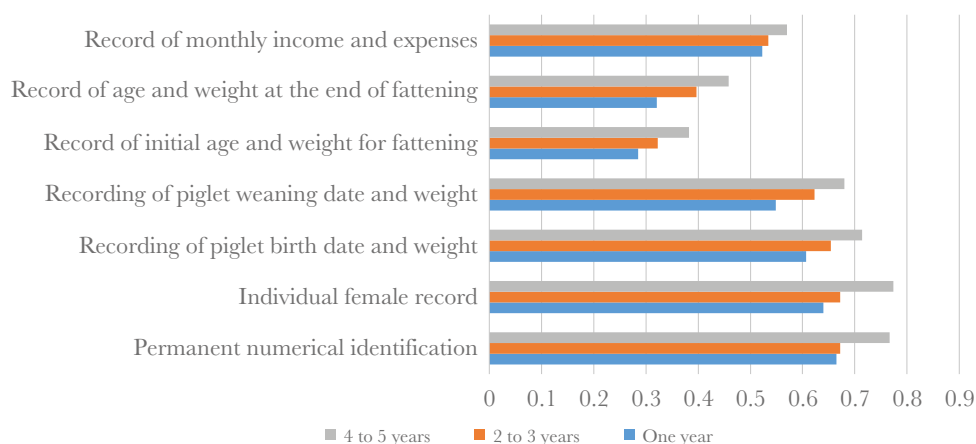


Figure 5. Adoption of management activities in GGAVATT pig producer groups in Guanajuato. Source: Author's elaboration, based on data available from the Bajío Experimental Station, INIFAP.

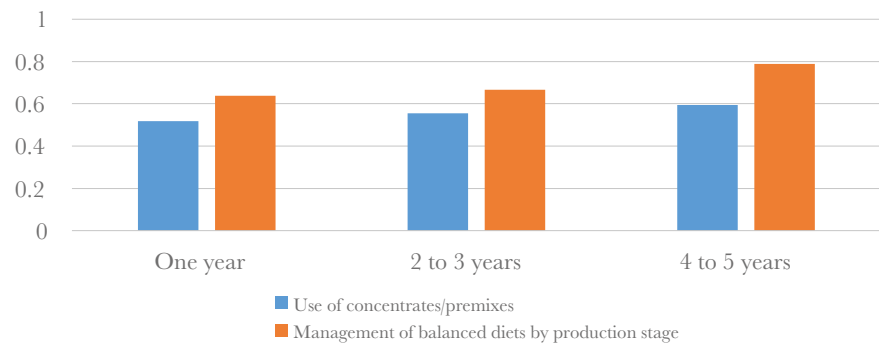


Figure 6. Adoption of animal nutrition innovations in GGAVATT pig producer groups in Guanajuato. Source: Author’s elaboration, based on data available from the Bajío Experimental Station, INIFAP.

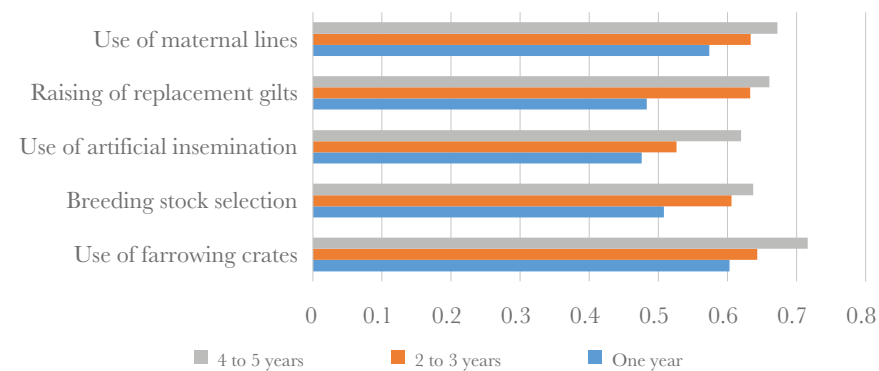


Figure 7. Adoption of reproduction and genetics innovations in GGAVATT pig producer groups in Guanajuato. Source: Author’s elaboration, based on data available from the Bajío Experimental Station, INIFAP.

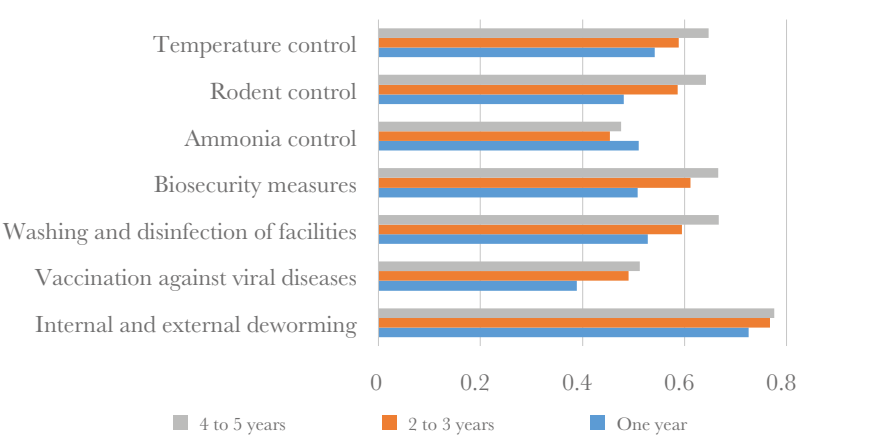


Figure 8. Adoption of health and biosecurity innovations in GGAVATT pig producer groups in Guanajuato, Mexico. Source: Author’s elaboration, based on data available from the Bajío Experimental Station, INIFAP.

is consistent with the semi-technified production system (Alonso and Rodríguez, 2024). This also shows a margin for improvement, which may not be achieved through technical assistance alone.

The correlation analysis showed positive and significant values between technical assistance and training and the indices associated with the adoption of technological

innovations. This confirms the positive effect that the technical assistance provided by the technicians implementing the GGAVATT model has on the adoption of technological innovations, as reported by Tejeda *et al.* (2019).

Table 3. Partial correlations.

Item	Technical assistance and training	Management	Nutrition	Reproduction and Genetics	Health and Biosecurity
Technical assistance and training	—	0.080***	0.045**	0.187***	0.165***
Management	0.080***	—	0.409***	0.585***	0.650***
Nutrition	0.045**	0.409***	—	0.447***	0.467***
Reproduction and Genetics	0.187***	0.585***	0.447***	—	0.709***
Health and Biosecurity	0.165***	0.650***	0.467***	0.709***	—

Note. * $p < .05$, ** $p < .01$, *** $p < .001$. Source: Author's elaboration, based on data available from the Bajío Experimental Station, INIFAP.

CONCLUSIONS

The technical assistance and training activities promoted among pig producers participating in the GGAVATT model in the state of Guanajuato have increased the adoption of technological innovations, with the greatest effect observed in the areas of Nutrition and Reproduction and Genetics. The adoption index values reached by pig producers with five years of technical assistance range between 0.46 and 0.69, with the lowest value corresponding to technical assistance and training and the highest to nutrition. This indicates that the level of technological innovation is consistent with a semi-technified production system.

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Application of nanotechnology in the production of nanoparticles of bioactive compounds: Case of rosemary (*Rosmarinus officinalis* L.) extracts

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ABSTRACT

Objective: To compile available information on techniques used in the production of nanoparticles involving functional compounds.

Design/Methodology/Approach: Published information and experiences from researchers working in the field of nanoparticles were compiled. Study protocols were created, and the advantages and disadvantages of nanoparticle fabrication were analyzed.

Results: The compiled information enabled the development of a flowchart for preparing nanoparticles from extracts containing bioactive compounds.

Limitations/Implications of the Study: Very little information is available. Specialized laboratories limit the use of these techniques.

Findings/Conclusions: A theoretical framework was developed to enable the use of nanoparticle fabrication techniques for research and to develop solutions that improve the shelf life of food intended for human consumption.

Keywords: bioactive compounds, nanoparticles, rosemary extracts, review.

INTRODUCTION

Nanotechnology has gained relevance in the food industry, particularly for enhancing antimicrobial and antioxidant properties and as an innovative alternative for food preservation. Chitosan-based nanoparticles have been used as vehicles for incorporating



various active substances due to their biocompatibility and non-toxicity. Their use in active packaging or in foods of animal origin directly impregnated with them has been employed to prevent lipid oxidation (Morales *et al.*, 2024).

Chitosan is a derivative of chitin, one of the most abundant polysaccharides, second only to cellulose. Chitosan is composed of three reactive functional groups: primary and secondary hydroxyl groups at C-3 and C-6, and an amino group at the C-2 position. It consists of N-acetyl-D-glucosamine and glucosamine units and is formed from the partial deacetylation of chitin, resulting in a copolymer. Chitosan can be obtained through three methods: physical, chemical, and microbial. Once obtained, the resulting product is non-toxic, colorless, and tasteless. In nature, chitosan is found in the form of chitin in the shells of marine organisms, such as crustaceans, in eggshells, and in the mucilage of silkworms (Wang *et al.*, 2024). Chitosan nanoparticles have a wide range of applications in various areas.

One line of research within our group has investigated the incorporation of rosemary extract into nanoparticle encapsulation and evaluated its functional activity. Rosemary extract (*Rosmarinus officinalis* L.) has been extensively studied because its properties make it a natural additive for food, as it can both improve and prevent lipid oxidation in meat. This is due to its content of bioactive compounds, such as flavonoids, terpenes, and the antioxidant rosmarinic acid (De Lima *et al.*, 2024).

The concept of nanoparticles

The history of nanoparticles dates back to China and Egypt, where they were used for healing and aesthetic purposes. Subsequently, numerous studies have evaluated nanoparticles, leading to the emergence of disciplines dedicated to their study, including nanomedicine, nanotechnology, and nanoscience. These fields generally encompass the 1-1000 nm range, and their characterization has been made possible through the use of electron microscopy and X-ray absorption spectroscopy (Reynaud *et al.*, 2020). The use and application of nanoparticles across areas such as medicine, cosmetics, and, more recently, food are advancing rapidly, and an increasing body of information is available to elucidate their mechanisms of action. The materials used in the production of nanoparticles must fulfill specific functions that provide them with particular properties and characteristics.

Application of nanoparticles

The study of nanoparticles has led to new applications in various fields, including food science and technology, which are now beginning to adopt their use. Nanotechnology allows for the modification of matter and the control of systems at the nanoscale (Ojeda *et al.*, 2019). In the food industry, the use of appropriate packaging and coatings has become a topic of considerable interest due to their potential to extend the shelf life of many food products, enhance food quality, and reduce packaging waste. In this regard, there is an incentive to explore new bio-packaging materials based on renewable resources. Therefore, the application of nanocomposites (obtained from natural extracts) in packaging promises to open new possibilities for improving not only the properties but also the efficiency,

contributing to the preservation of fresh food by extending its shelf life, as well as reducing packaging waste. Due to the nanometric size of the particles obtained by dispersion, these natural nanocomposites may represent an advance over conventional polymers (Marques *et al.*, 2012).

Extraction methods (bioactive compounds)

There are many alternatives when determining the method for extracting extracts of interest or essential oils. All techniques aim to preserve the components as much as possible; however, variables such as temperature, heat, energy, and time during extraction can lead to the loss of compounds of interest. The most commonly used techniques today are cold pressing and hydrodistillation (Bravo *et al.*, 2025). Other methods rely on mechanical forces for extended periods. For example, the solid-liquid method and the Soxhlet method are used to separate volatile compounds, but these have their disadvantages. They often pose health risks due to solvent use, and their components are heat-sensitive and may be lost during processing. On the other hand, ultrasound-assisted extraction demonstrates good performance in preserving phenolic compounds and, like microwaves, supercritical fluids, and solventless extraction, offers alternatives to traditional methods, yielding higher-quality extracts (Ramón *et al.*, 2021).

Physicochemical characterization of the extracts

The characterization of the extracts comprises a series of physicochemical tests whose primary purpose is to evaluate the compounds obtained during extraction. These tests include evaluation of antioxidant capacity using the DPPH and ABTS radical-scavenging methods and the iron-reducing antioxidant power (FRAP) assay. Additionally, the evaluation of phenols, flavonoids, and tannins enables the determination of these compounds and their quality (Manzanarez *et al.*, 2022). The nanoparticles are in the 1-1000 nm range; their size determines absorption, distribution, and accumulation. Functions associated with stability are also determined.

Principles of ionic gelation

The ionic gelation or ionotropic gelation method is used in the production of nanoparticles. It is based on electrostatic interactions between oppositely charged components, which form one or more ionic polymers. Chitosan and carboxymethylcellulose are the primary polymers used, along with stabilizing agents such as sodium tripolyphosphate (Hoang *et al.*, 2022). Other factors that stabilize the solution include pH and the physical forces of agitation. Furthermore, the rate and order of ingredient addition during encapsulation must be carefully controlled to ensure optimal nanoparticle performance (Vodyashkin *et al.*, 2022). Nanoparticles produced using the ionic gelation method have demonstrated stability and good encapsulation efficiency. The ratio for incorporating the components in the case of nanoparticles made from chitosan and tripolyphosphate is 3:1, and some authors use 2:1, with an optimal pH of 4.7-4.8, in adjusted acetic acid solutions, the results of size and morphology (Van Bavel *et al.*, 2023).

Particle size and polydispersity

Jeevanandam *et al.* (2018) identify factors that influence nanoparticle morphology and size, enabling classification of nanoparticles by origin. These can be type II, derived from synthetic nanomaterials, and distinguished by their size and morphology. In 2D and 3D, sizes greater than 100 nm with well-defined structures are observed. The dynamic light scattering technique enables the determination of particle size and the polydispersity index. Yuan *et al.* (2022) describe the challenges of preparing chitosan nanoparticles using sodium tripolyphosphate and explain how the preparation method, pH, stirring rate, and stirring time influence the process. All of these factors affect the degree of protonation of chitosan and its degree of interaction, thereby influencing its size and morphology, which makes it difficult to standardize its size, distribution, and morphology.

Z-potential

The most important properties for determining colloidal systems are those determined by the electrical charges produced by the particles that make up the system. This system forms when two phases with opposite charges come into contact. Once the system is formed, the electrokinetic degree of its components can be evaluated. The zeta potential determines this degree of interaction (Romero *et al.*, 2022). The zeta potential is the potential difference or electrical charge between the dispersion medium and the layer attached to the dispersed particle in the solution. This value is directly related to the stability of the colloidal medium (Mancini *et al.*, 2016).

Properties of rosemary extract

Rosemary is a plant that has been used throughout human history for a wide range of purposes, including in the production of cosmetics, anti-aging agents, and dyes, as well as in gastronomy due to its aromatic and flavor properties. This plant is rich in compounds such as antioxidants, enzymes, phenolic molecules, and glutathione, compounds that prevent the auto-oxidation of unsaturated triglycerides and oxidative stress in the cells of living organisms (Flores *et al.*, 2020).

Rosemary plant extract contains essential oils, which have been used for medicinal and other applications because they contain volatile molecules with biological, antioxidant, antimicrobial, antiseptic, anticancer, analgesic, antinephrotoxic, antitumor, antihepatotoxic, antifungal, antimutagenic, anti-inflammatory, and sedative properties. There is a wide variety of secondary metabolites in this plant, which are found mainly in the essential oil and extracts. These are grouped into three categories: flavonoids, hydroxycinnamic derivatives, and terpenoids (monoterpenes, sesquiterpenes, diterpenes, and triterpenes) (Flores *et al.*, 2020).

Scientific experience in the preparation of nanoparticles with rosemary extract

The preparation of chitosan-based nanoparticles loaded with rosemary extract is carried out according to the procedure described in Figure 1. Subsequently, these nanoparticles are analyzed for size, morphology, polydispersity index (PI), and zeta potential. The rosemary-extract-loaded nanoparticles exhibit a size of approximately 295 nm, with a PI

of 0.459 and a zeta potential (ζ) of +36.6 mV. The encapsulation efficiency should be greater than 60%, indicating a considerable fraction of the extract encapsulated within the nanoparticles (Figure 2). Table 1 shows the most common properties observed in the preparation of rosemary-extract nanoparticles.

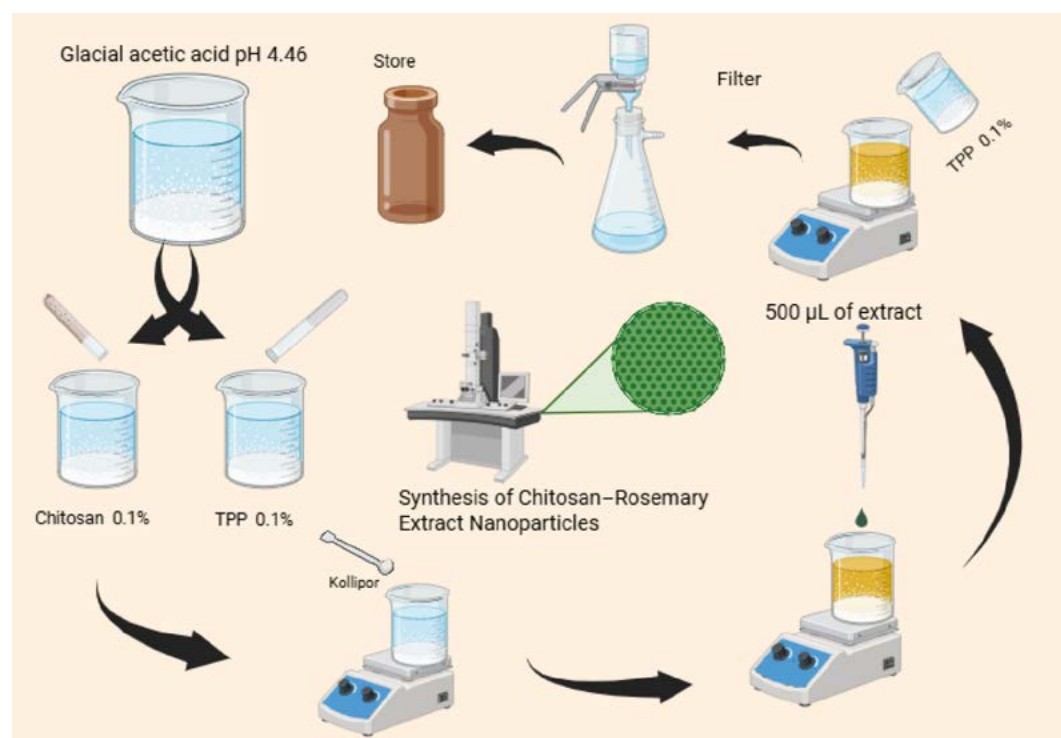


Figure 1. Procedure for obtaining nanoparticles.

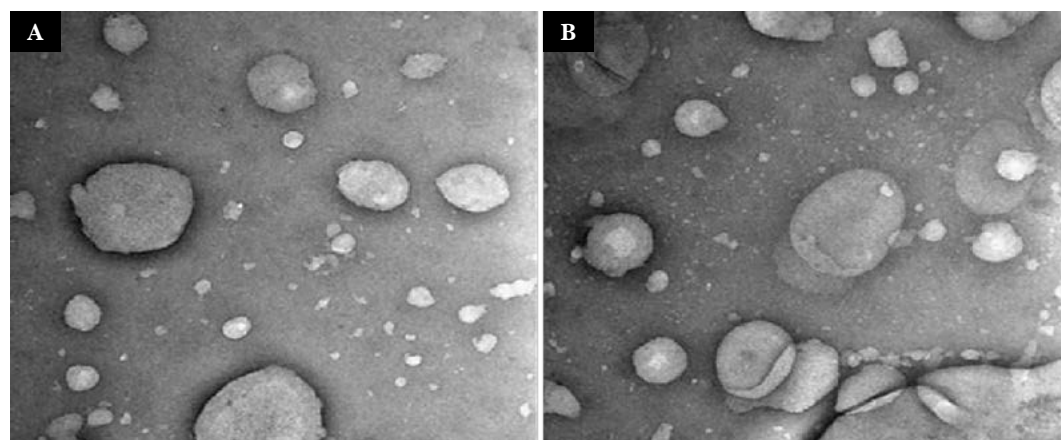


Figure 2. Transmission electron micrograph (TEM) of chitosan-based nanoparticles. (A) Nanoparticles loaded with rosemary extract (*Rosmarinus officinalis*) exhibit nano-oval morphology. With defined edges and a size distribution between 90 and 600 nm. (B) Empty chitosan nanoparticles with nano-spherical morphologies with less contrasting edges and a size distribution of 90 to 900 nm.

Table 1. Physicochemical characterization of the nanoparticles with rosemary extract.

Variable	Estimate	Unit	Brief interpretation
Size (peak intensity)	295	nm	Nano size is useful for coating
Polydispersity index	0.459	–	Moderate dispersion
Z Potential	36.6	mV	Positive charge; reasonable stability
Peak of the potential zeta	36.6	mV	Peak value in the distribution
z wall potential	30.5	mV	Auxiliary measurement of the equipment
Conductivity	0.664	mS/cm	Condition of the medium for ζ
Peak intensity of the area	95.7	%	Dominant mode (~96% of the area)
Quality factor	5.8	–	Acceptable signal-to-noise ratio

Note. ζ =zeta potential. Units according to equipment output (conductivity in mS/cm).

The use of nanoparticles facilitates the application of extracts and essential oils by preventing the degradation and volatilization of compounds of interest and enabling controlled release. Studies such as that by Kasem *et al.* (2022) have demonstrated that the encapsulation of extracts improves the stability of phenolic compounds and their antioxidant activity. On the other hand, Hoang *et al.* (2022) mention that chitosan nanoparticles produced by the ionic gelation method typically have sizes greater than 100 nm, a value that is also linked to the molecular weight of chitosan; the lower the molecular weight, the smaller the nanoparticles. Other factors, such as time and agitation force, also influence the size. Specifically, the ionization potential (IP) explains the relationship between low values close to zero and their uniform dispersion in the solution, and vice versa. For the Z (ζ) potential, the values range from –100 mV to 100 mV and represent the colloidal stability of the nanoparticles through electrical charges. Jeevanandam *et al.* (2018) classify nanoparticles into type II nanoparticles derived from synthetic nanomaterials, and, according to their dimensions (size and morphology), they present sizes greater than 100 nm with well-defined structures.

Meat is a highly perishable food; therefore, it is more susceptible to contamination and spoilage by pathogenic microorganisms. Improper handling of meat, along with factors such as temperature, humidity, and storage time, influences the quality and safety of meat products. Another significant factor is its high-water activity ($a_w>0.95$), which allows for the proliferation of microorganisms (Wornphan *et al.*, 2024). Currently, packaging is widely used to protect food from external factors that could alter its composition. It also provides safety by preventing damage during transport. Preservation extends shelf life, improves storage, and ensures proper handling (Bayona-Boneth *et al.*, 2023).

Meat is easily contaminated and is a perishable food because of numerous potential sources of contamination. Common examples include lipid oxidation, bacterial contamination, and enzymatic processes that reduce its shelf life, preventing its proper use. The industry relies on various tools to ensure adequate food preservation through temperature control during refrigeration. The use of polymers that serve as a physical barrier, preventing the entry of external contaminants, has also become more common (Kowalczyk *et al.*, 2024). Many materials are available on the market, the most commonly used for packaging being petroleum derivatives such as polypropylene (PP), high-density polyethylene (HDPE),

polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), expanded polystyrene (EPS), better known as Styrofoam, and flexible, soft polyethylene plastic film (PE). These materials are widely used for their desirable properties, including high strength, barrier properties, accessibility, and low cost (Momtaz *et al.*, 2024).

These act as barriers, enabling evaluation of changes in meat, such as moisture loss and color changes, thereby maintaining adequate control at each stage of the supply chain that affects the meat's shelf life (Brandelli *et al.*, 2024). The development of new packaging and preservation technologies is crucial for maintaining meat quality. In this case, the use of rosemary extract nanoparticles in vacuum-sealed packaging enhances meat quality and its physical and chemical properties, thereby helping to prevent lipid oxidation and providing antioxidant activity during storage.

CONCLUSION

Nanotechnology is the science that studies the design of functional and non-toxic materials for organisms. Its release systems enable the preservation and slow release of bioactive compounds, thereby maintaining their beneficial activity. This is the case with rosemary nanoparticles, which provide a means to preserve and release bioactive compounds, such as antioxidants, thereby extending the shelf life of meat.

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Identification of key meat quality parameters

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ABSTRACT

Objective: To analyze and synthesize the scientific literature related to the main physicochemical and technological parameters that determine meat quality, particularly pH, water-holding capacity (WHC), antioxidant activity, color, and texture.

Design/Methodology/Approach: A narrative review of scientific literature published in indexed journals, specialized books, and technical documents related to meat science and technology was conducted. Studies focusing on post mortem biochemical mechanisms and their influence on quality attributes were selected.

Results: The literature indicates that post mortem pH is a determining factor in protein structure and water-holding capacity, directly influencing texture and color. WHC is closely associated with myofibrillar spacing and the structural state of muscle proteins. Likewise, antioxidant activity plays a key role in oxidative stability, affecting color preservation and sensory profile. Together, these parameters interact dynamically and determine the technological and commercial quality of meat.

Limitations/Implications of the Study: Variability in production systems, management practices, genetics, and post mortem conditions makes direct comparison among studies difficult. Nevertheless, integrating these parameters provides a comprehensive understanding of the factors influencing meat quality and guides future research toward technological improvement strategies.

Findings/Conclusions: Meat quality results from complex biochemical and structural interactions occurring during the post mortem period. Understanding parameters such as pH, WHC, antioxidant activity, color, and texture is essential to optimize production and processing systems, as well as to ensure product stability and overall quality.

Keywords: Quality attributes, meat, physicochemical quality, meat science.

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INTRODUCTION

Meat is a fundamental source of essential nutrients due to its high digestibility and its content of high biological value proteins, heme iron, B-complex vitamins, and minerals such



as zinc and selenium. Its nutritional and functional quality depends on a complex chemical composition that may vary according to intrinsic factors, such as species, age, muscle fiber type, and metabolism, as well as extrinsic factors related to the production system, pre-slaughter handling, processing, and storage. These elements influence postmortem biochemical changes that determine key attributes such as pH, water-holding capacity, color, texture, and oxidative stability. Consequently, variations in these parameters affect not only the nutritional value and shelf life of the product, but also its technological quality and consumer acceptance (Lawrie, 1998).

Among the meats of greatest productive and commercial relevance, pork stands out due to its widespread global consumption, driven by its production efficiency, culinary versatility, and availability across different production systems. The growing market demand is focused not only on volume but also on the overall quality of the product. In this context, both consumers and the industry require meat that is not only safe from a sanitary standpoint and nutritionally adequate, but also exhibits optimal technological and sensory attributes, such as attractive appearance, uniform color, adequate water-holding capacity, tenderness, juiciness, and palatability (Eusse, 2009). These factors directly influence consumer acceptance and the competitiveness of the product within increasingly demanding agri-food supply chains.

pH in meat

The postmortem decline in pH is closely influenced by the predominant type of muscle fibers and by the level of physical activity of the animal prior to slaughter. Muscles with a higher proportion of fast-twitch fibers (type II or white fibers), characterized by predominantly glycolytic metabolism, generally have greater glycogen reserves and, consequently, produce more lactic acid after slaughter, reaching ultimate pH values close to 5.5. In contrast, muscles with a predominance of slow-twitch fibers (type I or red fibers), associated with oxidative metabolism and higher myoglobin content, tend to exhibit higher ultimate pH values, generally above 6.0 (Garrido, 2005).

Likewise, the degree of physical exertion before slaughter can modify muscle energy reserves. When the animal undergoes intense activity or prolonged stress, partial or complete depletion of glycogen occurs, limiting lactic acid production during postmortem glycolysis and leading to higher pH values (Dransfield, 1994). These variations in pH behavior directly influence key technological attributes such as water-holding capacity, color, and meat texture.

The decline in ultimate pH is closely associated with the accumulation of lactic acid in muscle tissue, resulting from anaerobic glycolysis that occurs after the interruption of oxygen supply following slaughter. During this process, muscle glycogen is degraded to generate energy in the form of ATP, leading to progressive lactic acid production and intracellular acidification. Both the rate of pH decline and the ultimate pH value are critical determinants of meat quality, as they directly influence protein denaturation, the structural integrity of myofibrils, and the stability of muscle pigments. Consequently, postmortem pH behavior affects essential properties such as color, texture, water-holding capacity, and technological yield. Alterations in this process may result in quality defects such as PSE

(pale, soft, exudative) or DFD meat, which negatively impact consumer acceptance and the industrial functionality of the product. When pH declines rapidly during the first hours postmortem, reaching values below 6.0 while muscle temperature remains high, or when the ultimate pH falls below 5.6, the meat may develop characteristics associated with the PSE condition (pale, soft, exudative). This phenomenon occurs as a result of accelerated glycolysis and excessive lactic acid accumulation, leading to denaturation of myofibrillar and sarcoplasmic proteins, reduced water-holding capacity, and color alterations due to changes in light scattering within the muscle tissue. The occurrence of PSE meat is closely linked to stress factors affecting the animal prior to slaughter, such as high environmental temperatures, improper handling, prolonged transport, overcrowding, or inadequate stunning methods. In addition, genetic components may predispose certain animals to a more intense metabolic response to stress. These conditions not only compromise the sensory and technological quality of the product but also generate significant economic losses in the meat industry (Bendall & Swatland, 1998; Ledward, 1992).

Water-holding capacity

According to Rosenvold & Andersen (2003), water-holding capacity (WHC) is a technological attribute highly sensitive to multiple pre- and post-slaughter factors. Among the most relevant is the stunning system used. Electrical stimulation can accelerate postmortem glycolysis, leading to a more rapid pH decline while muscle temperature remains elevated, thereby promoting protein denaturation and, consequently, lower WHC compared to methods such as CO₂ stunning, which tend to induce more gradual metabolic changes. Dietary supplementation with vitamin E has also shown positive effects on cellular membrane stability and reduction of oxidative damage, contributing to improved water retention in pork. Pre-slaughter fasting may influence muscle glycogen reserves; a moderate reduction in these reserves can increase ultimate pH at 24 hours postmortem, favoring WHC, although excessive depletion may lead to DFD-like conditions. Furthermore, carcass chilling conditions play a decisive role. Excessively rapid chilling, particularly when the muscle retains high energy levels, can induce cold shortening, altering myofibrillar structure and negatively affecting water-holding capacity. Overall, these factors demonstrate that WHC does not depend on a single mechanism, but rather on the complex interaction between postmortem metabolism, structural integrity of proteins, and technological conditions applied during processing.

All these factors, associated with the occurrence of PSE or DFD meat and with alterations in postmortem metabolism, decisively influence not only WHC, but also other fundamental quality attributes such as ultimate pH, color, and texture. Changes in the rate of glycolysis, glycogen availability, and muscle temperature affect the structure and functionality of myofibrillar proteins, directly impacting the stability of muscle tissue. In this context, PSE meat is characterized by a rapid decline in pH while muscle temperature remains elevated, leading to protein denaturation and a marked reduction in WHC, resulting in greater exudative losses. In contrast, DFD meat exhibits a high ultimate pH due to prior glycogen depletion, which promotes greater water retention and a darker, firmer appearance (Hoffman, 1987; Warner *et al.*, 2009). These differences affect not

only technological yield and shelf life, but also sensory perception and the commercial acceptance of the product.

Variations in WHC are primarily explained by changes in the spacing of myofibrillar filaments, which are closely related to modifications in the electrical charge and structural conformation of myofibrillar proteins, particularly myosin and actin. When the pH approaches the isoelectric point of these proteins, electrostatic repulsion between filaments decreases, reducing the intermyofibrillar space and limiting the muscle's ability to retain water. Additionally, contraction or expansion of muscle fibers alters the volume of intracellular and extracellular compartments, promoting the movement of water toward the extracellular space and eventually its loss through exudation. These structural changes explain why small variations in pH and in the physical state of proteins can generate significant differences in juiciness, yield, and the technological quality of meat (Offer & Knight, 1998).

Water activity

Water activity (a_w) influences the different chemical reactions occurring in the product, as well as the survival and resistance of microorganisms; therefore, a_w is a fundamental parameter for food preservation. Reducing water activity improves the shelf life and safety of meat products, as lower a_w levels limit the growth of microorganisms responsible for spoilage and foodborne illness. To prevent microbial proliferation, various preservation methods such as salting, freeze-drying, and others are applied. These techniques increase the osmotic pressure within the food matrix, which effectively reduces water activity (Zigerlig, 2008).

Antioxidant activity

In meat products, oxidation represents one of the primary mechanisms of deterioration during storage. Lipid oxidation leads to the formation of secondary compounds such as aldehydes, ketones, and alcohols, which are responsible for rancidity, off-odors, and flavor loss (Falowo *et al.*, 2014; Min & Ahn, 2005). Meanwhile, protein oxidation can cause structural modifications in myofibrillar and sarcoplasmic proteins, affecting attributes such as texture, color, and water-holding capacity, in addition to reducing nutritional value and shortening shelf life (Lund *et al.*, 2011).

In the case of wild boar meat, greater physical activity and a natural diet influence muscle composition. It generally exhibits higher concentrations of myoglobin and heme iron, as well as a greater proportion of polyunsaturated fatty acids (PUFAs). These factors may increase susceptibility to oxidation (Wood *et al.*, 2004; Min & Ahn, 2005), since iron acts as a catalyst in pro-oxidative reactions and PUFAs are more prone to free radical attack. Nevertheless, a diet based on natural resources may provide phenolic compounds and other endogenous antioxidants that could partially counteract these oxidative processes (Descalzo & Sancho, 2008; Falowo *et al.*, 2014).

Studies evaluating indicators such as thiobarbituric acid reactive substances (TBARS), total antioxidant capacity, or oxidative stability are still limited, making it difficult to establish definitive conclusions (Huang *et al.*, 2019). This knowledge gap highlights the

need for systematic research to better understand the oxidative behavior of both species and its impact on meat quality and shelf life (Huang *et al.*, 2019).

Texture

Texture is one of the most relevant sensory attributes in meat quality, as it directly influences the perception of tenderness and consumer acceptance. Several studies indicate that the production system and the animal's level of physical activity play a determining role in the mechanical properties of muscle. Graziotti *et al.* (2000) and Bote *et al.* (2000) report that in semi-extensive or outdoor production systems, increased physical activity promotes greater firmness or toughness of the meat, mainly associated with a higher proportion of type IIA muscle fibers, which exhibit intermediate characteristics between oxidative and glycolytic fibers.

On the other hand, Bosselmann *et al.* (1995) point out that collagen is the main component of intramuscular connective tissue and plays a fundamental role in determining meat texture. The total amount of collagen, its solubility, and its structural characteristics are closely related to the animal's age and physiological development (Fang *et al.*, 1999). As the animal ages, modifications occur in the predominant type of collagen along with a progressive increase in the number and stability of intermolecular cross-links. These cross-links, especially those of a non-reducible nature, increase thermal resistance and reduce collagen solubility during cooking, resulting in greater rigidity of muscle tissue and, consequently, tougher meat. Moreover, the more developed connective tissue in animals with high physical activity may intensify this effect.

Color

Color is one of the most important attributes in the evaluation of meat quality, as it constitutes the primary visual criterion influencing consumer purchasing decisions. This attribute is mainly due to the presence of myoglobin, a water-soluble sarcoplasmic protein whose physiological function is to store and facilitate oxygen transport in living muscle. The concentration of myoglobin in muscle tissue is determined by several factors, including species, animal age, level of physical activity, and the predominant muscle fiber type (Livingston & Brown, 1981).

From a chemical perspective, color also depends on the oxidation state of the iron present in the myoglobin molecule. The most relevant forms are deoxymyoglobin (purplish-red color), oxymyoglobin (bright red), and metmyoglobin (brown), whose proportions vary depending on oxygen availability, pH, temperature, and the degree of oxidation. In fresh pork, the characteristic color ranges from pink to light red tones, generally with a uniform appearance within each muscle (García, 2003).

A darker appearance may be associated with various factors, such as higher myoglobin concentration in older animals, greater physiological activity of the muscle (*e.g.*, flexor or extensor muscles with a predominance of oxidative fibers), reduced oxygen penetration at the surface, surface dehydration, microbial contamination, or the presence of the DFD (dark, firm, dry) condition. In contrast, excessively pale meat may be related to the PSE (pale, soft, exudative) condition.

In commercial terms, color is a key indicator of freshness and perceived quality. The bright red tone associated with oxymyoglobin is generally considered desirable by consumers, whereas brownish or excessively dark shades may lead to rejection. In the case of wild boar, the higher myoglobin content derived from greater physical activity may confer a more intense and darker coloration compared to domestic pork, representing both a distinctive characteristic and a potential challenge in terms of acceptance in conventional markets.

Table 1 provides a concise comparison of the main physicochemical, technological, and nutritional parameters of wild boar and domestic pork, based on representative values reported in the scientific literature. The reported ranges reflect variations attributable to differences in analytical methods, type of cut, and pre- and postmortem handling conditions.

Table 1. Comparison of physicochemical, technological, and nutritional parameters between wild boar and domestic pork.

Parameter	Wild Boar Meat (<i>S. scrofa scrofa</i>)	Domestic Pork (<i>S. scrofa domesticus</i>)	Impact/Significance
Ultimate postmortem pH	5.8-6.3 (higher and more variable values)	5.5-5.8 (typical in controlled systems)	Higher pH favors firmness and water-holding capacity; greater variability increases product heterogeneity
Water-holding capacity (WHC)	Moderate-high (depends on pH and handling)	High (more consistent)	Affects juiciness and industrial yield
Water activity (aw)	0.98-0.99 (fresh)	0.98-0.99 (fresh)	Similar values; refrigeration control is critical
Color - L* (lightness)	30-36 (darker)	38-45 (lighter)	Darker meat is associated with wild/game or gourmet products
Color - a* (red)	18-22 (more intense)	14-18	Higher intensity may indicate higher myoglobin levels.
Texture-Shear force (Newtons)	30-45 (higher)	25-35	Related to collagen content and prior physical activity
Intramuscular fat (%)	1.5-3.0 (lower)	2.5-5.5 (higher)	Influences flavor, juiciness, and lipid profile
Total protein (%)	21-24 (higher)	19-22	Leaner meats tend to have a higher proportion of protein
PUFA/SFA ratio	0.45-0.65 (more favorable)	0.30-0.45	Nutritional indicator; higher values are considered beneficial.
n-6/n-3 ratio	4:1-7:1 (more balanced)	8:1-12:1	Lower ratios are associated with nutritional benefits.
TBARS (mg MDA/kg)	0.3-0.6 (variable)	0.2-0.5	Higher values may indicate greater lipid oxidation.
Myoglobin content (mg/100 g)	2.5-3.5 (higher)	1.8-2.8	Influences color intensity and susceptibility to oxidation.
Total collagen (%)	1.8-2.3 (higher)	1.2-1.8	Influences firmness and tenderness.

CONCLUSION

Meat quality is determined by a complex interaction of biochemical and structural processes that develop primarily during the postmortem period. pH regulates the stability and organization of muscle proteins, directly influencing water-holding capacity, texture, and color. In turn, water-holding capacity is closely related to the structural state of myofibrils and serves as a key indicator of color, nutritional value, and product shelf life. Overall, these parameters do not act independently but rather form an interdependent system that defines the final properties of meat. Understanding these mechanisms is essential to optimize production and processing practices, as well as to ensure meat products with greater stability and quality.

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