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Contacto principal
Jorge Cadena Iñiguez
Guerrero 9, esquina avenida Hidalgo, C.P. 56220, San Luis Huexotla, Texcoco, Estado de México.
agroproductividadeditor@gmail.com

Contacto de soporte
Soporte
5959284703
agroproductividadsoporte@gmail.com

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Spondias dulcis propagation by seeds and stem cuttings

Roberta Castillo-Martínez[1*]; Héctor Cálix-de-Dios[2]

[1] Universidad Autónoma del Estado de Quintana Roo, Departamento de Recursos Naturales, División de Desarrollo Sustentable, campus Chetumal bahía. Blvd. Bahía s/n, Del Bosque, Chetumal, Quintana Roo. C.P. 77019.

[2] Universidad Intercultural Maya de Quintana Roo,Departamento de Desarrollo Sostenible, Carretera Muna Felipe Carrillo Puerto km 137 s/n, José María Morelos, Quintana Roo. C.P. 77870.

* Correspondence: robcasti@uqroo.edu.mx

ABSTRACT

Objective: To found simple and low-cost ways to increase the number of plants. In Chetumal, Quintana Roo, Mexico, a trial was established to evaluate two propagation forms from January to June 2023.

Design/Methodology/Approach: In soil enriched with bocashi, 22 seeds of seasoned fruits were sown. Also, four lots of stem cuttings were planted (five cuttings per lot) 30 cm in length, testing four average diameters (2.94, 2.34, 2.1 and 1.58, cm) that were also rooted in soil and bokashi, on the assumption that those with the largest diameter were also the oldest. The stem cuttings were obtained from 1.5- and 2.5-year-old trees. This latter experimental procedure was repeated, but stem cuttings were obtained from an 8-year-old tree, five lots of cuttings with the following average diameters (3.6, 1.92, 1.74, 1.7, and 1.16, cm) were evaluated; in addition, cuttings were treated with a stimulant to develop roots (Raizal® 400).

Results: The data analyzed in Excel™ showed that germination is slow and irregular, 77% germination was reached after 2.5 months. Only the thickest cuttings from the 1.5-year-old tree developed abundant roots and vegetative shoots.

Findings/Conclusions: The propagation of *S. dulcis* is better done by seeds. Propagation by cuttings is possible, but with low success, only 8.8%.

Keywords: germination, *Spondias dulcis*, vegetative propagation.

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INTRODUCTION

In Mexico, the fruit of the species *Spondias dulcis* Parkinson, Anacardiaceae (Mitchel and Daly, 2015), is practically unknown because its origin is not native American. *S. dulcis* is native to the Indo-Malayan region of Tahiti (de Souza *et al.*, 2021). The tree was introduced in Jamaica in 1782 and is currently cultivated in several Caribbean countries. Fruit exportation is an important source of profit from foreign exchange in those countries (Bauer *et al.*, 1993; Mohammed *et al.*, 2017). It goes by several common names as Kedondong, Yellow plum, Polynesian plum, Belize plum, or June plum, among others (Bauer *et al.*, 1993) and ciruela-mango in Mexico.

In this study we decided to use the latter name; there are two genetic lines of *S. dulcis*, large and miniature trees. On large trees the fruits reach in average, 5-6 cm in diameter and 9-10 cm in length, with 200 g average weight.

Whereas the fruits of miniature trees reach 4-5 cm in diameter and 5-6 cm in length, with 65 g average weight (Graham *et al.*, 2004; Mohammed *et al.*, 2017). The most widely cultivated genotype is that with the large fruit; the miniature type has received little attention (Graham *et al.*, 2024a), although it may have higher economical and nutritional value (Das *et al.*, 2019).

Both types of *S. dulcis* fruits can be consumed fresh or processed at home in various ways. In addition, they can be used harvested at different stages of development, either green, seasoned or ripe (Mohammed *et al.*, 2017). The fruits can even be processed into various added-value products and can be also valuable as raw material in the food and beverage industries. Likewise, the peel of ciruela-mango is a source of gelling agents for jam, candies, yogurt, and dairy drinks, among other products (Franquin *et al.*, 2005; Mohammed *et al.*, 2017). The fruit has nutraceutical properties; that is, it serves as food, and it helps to counteract symptoms of some diseases. For example, diabetes, indigestion, urinary tract infections, hypertension, and hemorrhoids (Islam *et al.*, 2013). Fruits are also used to treat heart and digestive problems; as well as for healing wounds, skin sores and burns (Das *et al.*, 2015).

The fruit is a drupe with a spiny-fibrous endocarp and is climacteric (Bauer *et al.*, 1993; Mohammed *et al.*, 2017). When the fruits are seasoned and ripe they are crunchy with pineapple scent and flavor; Graham (2004) and Bauer *et al.* (1993) described fruit flavor as sweet-and-sour. The miniature ciruela-mango has several ideal characteristics because fruits are available throughout the year, they are easily harvested due to the low height of the tree, which can be grown at high densities (Mohammed *et al.*, 2017). In addition, it is drought tolerant (Das *et al.*, 2015). Bauer *et al.* (1993) indicated that most *S. dulcis* plants are obtained by germination of seeds and few are obtained by cuttings. It is known that many seeds have certain difficulties in germinating. In fact, germination and dormancy are controlled by several genes that are affected by environmental and developmental conditions. In addition, the structures surrounding the seeds play a determining role in the inhibition and germination of the seed (Koornneef *et al.*, 2002).

For the large genotype of *S. dulcis*, 63% germination was reached at 60 days when combining mechanical scarification followed by imbibition in a solution of gibberellin plus cytokinin (350 mg L^{-1}) for 12 hours. In contrast to the control, which consisted of imbibition with water and without scarification; in this treatment germination was three times lower (de Souza *et al.*, 2020). Precisely because of the scarcity of the ciruela-mango trees in Mexico, the aim was to find a simple and cheap way to increase the number of plants, in such a way that it can be achieved by any producer, without depending on expensive or difficult to obtain products.

MATERIALS AND METHODS

This study was conducted from January to June 2023. The evaluated material was obtained from Spondias dulcis trees, an 8-year-old tree grown at the Autonomous University of the state of Quintana Roo, Campus "Bahía", located in the city of Chetumal. Also, from three other younger trees, two approximately 1.5 years-old and another 2.5 years old, these three trees are grown in a private home in Chetumal. The climate of Chetumal is warm sub-humid, the average annual temperature in the state is 26 °C, the average rainfall in the state is around 1300 mm per year (Comisión Nacional del Agua, 2017).

Propagation of *S. dulcis* plants was made in two ways, using seeds (seed plus endocarp) and stem cuttings. The seeds came from one of the 1.5-year-old trees and consisted of a batch of 22 seeds taken from seasoned fruits, *i.e.* at physiological maturity (Figure 1).

Once the pulp of the fruit was removed, the seeds were immediately washed in tap water to remove as much as possible the remains of the pulp. Subsequently, seeds were exposed to the sun for 12 hours in order to dry them and at the same time disinfect them. Afterwards, seeds were germinated in a substrate made with a mixture of equal parts of red soil and bocashi (Restrepo, 2007); the latter component made from sheep manure. The substrate was poured into 2 kg nursery bags and moistened to field capacity. Then, each seed was sown at 3 cm depth. For five months, irrigation was applied every Monday, Wednesday and Friday, along with daily records of any seedlings emergence, from January 15 to June 15, 2023. Records were supplemented with the description of the morphology of seedlings in different weeks of development.

On the other hand, for vegetative propagation, a first block of stem cuttings derived from two young trees (1.5 and 2.5 years-old) was formed, obtaining 20 stem cuttings 30 cm in length (four average diameters 2.94, 2.34, 2.1 and 1.58, cm), which were grouped into four lots (five cuttings per lot) according to thickness and color. Those cuttings that were thicker and grayish brown were considered as older, while those greener and thinner were estimated to be younger. Before planting the cuttings, they were left to airing for a day; they were then planted in a substrate made with a mixture of 70% humus soil and 30% bocashi. This substrate was contained in nursery bags with a capacity of 2 kg where the cuttings were planted and received three irrigations per week.

Data recording was conducted from April to June 2023. The propagation by cuttings was repeated (in the second block of stem cuttings) in the same way as it was done with Block 1. But this time the stems were obtained from the 8-year-old tree and were grouped into five lots (average diameters 3.6, 1.92, 1.74, 1.7, and 1.16, cm), following the criteria already described. This time, stem cuttings were introduced in a solution (200 g in 20 L of water) of a commercial stimulant for roots emission (Raizal® 400) for 10 minutes. In addition, according to the specifications of the product, each nursery bag was irrigated

Figure 1. *Spondias dulcis* fruits (green, seasoned and ripe), and seeds (in front of fruits).

with 80 mL of the same solution. The data obtained from germination and vegetative propagation were organized, analyzed and graphed in Excel™.

RESULTS AND DISCUSSION

Seed germination was very irregular (Figure 2); it began at 36 days and after 76 days (2.5 months) 77% germination was achieved. Whereas, at 148 days (almost five months), 81.87% germination was reached. This result was very similar to that reported by de Souza *et al*. (2020) for the large genotype of *Spondias dulcis*, for which 63% germination was recorded in 60 days.

Although it is true that, in this study, there was only about 40% germination at 60 days, just 15 days later 77% germination had already been reached. This is a high percentage if we consider that seeds were not scarified or treated with any accelerator for germination, in contrast with de Souza *et al*. (2020) where mechanical scarification was combined with hormones.

The irregularity and slowness of germination appear to be a characteristic of the genus. Martins *et al*. (2019) noted that germination of *Spondias mombin* L. occurs between 6 and 24 months. In addition, they found that germination was different according to size, color, and the seed scarification point. In fact, medium-sized, brown, laterally scarified seeds reached 50% germination at 336 days, whereas in other treatments that percentage was reached up to 489 days.

The endocarp that covers the seeds of *S. dulcis* may be responsible for the germination slowness; however, in this species it would not be easy to remove the endocarp without damaging the seed. In order to optimize costs and time, three months is the maximum suggested time to get a good number of *S. dulcis* germinated seeds. A longer waiting would not be worth it because the subsequent germination rate is negligible.

The number of seedlings that emerged per seed ranged from one to five (Figure 3). At the same percentage (27.7%), seeds produced one, two, and three seedlings; fewer seeds yielded four or five seedlings. The time of emergence of seedlings from a single seed was

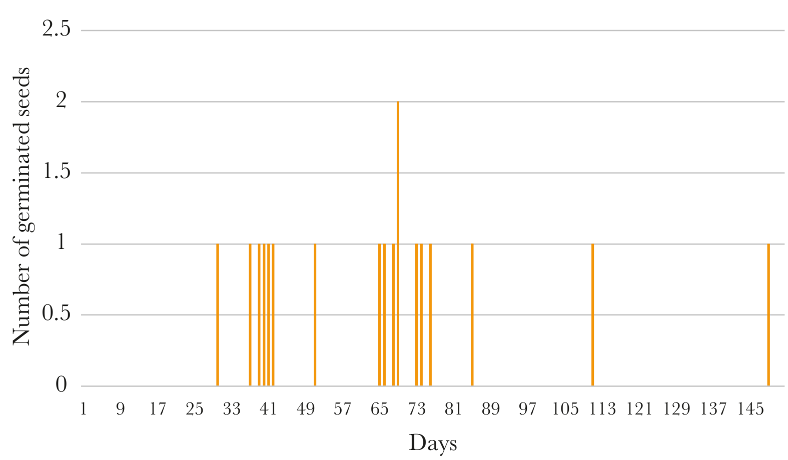

Figure 2. The irregular germination of *Spondias dulcis*.

Figure 3. Three *Spondias dulcis* seedlings which emerged from only one seed.

variable; some emerged up to 20 days after the first seedling emerged. The height and vigor of the plants originated from the same seed were also different.

According to the number of seedlings obtained from each seed, it was clear that within each one there may be between one and five embryos, perhaps more. Polyembryony is common in several genera of Anacardiaceae. Martínez-Ochoa (2010) reported 95 to 97.5% polyembryony rate in two cultivars of *Mangifera indica* L.; that author also found between 2 and 7 embryos per seed from zygotic and nucellar origin. Determination of the origin and number of embryos per seed will be one of the tasks to be solved for *S. dulcis*.

In *M. indica*, 2-5 seedlings were obtained when the seeds were germinated *in vitro* and only 2-3 when it was done *in vivo*. That same author stated that plants from nucellar embryos are believed to be more vigorous and are preferred for rootstocks. At five months, the survival of the plants was 100%, including those with up to five plants emerged from a single seed. Germination is hypogeal, the cotyledons are enclosed in the endocarp. The epicotyl lengthens and emerges curved just below the node of the two primordial leaves, both facing the ground.

Seedling growth is very fast; on the second day the seedling is fully erect, it has a length of about 4.5 cm, the single primordial leaves with smooth edges are about 2 cm long and are fully spread and opposite. While the first two nomophyl leaves (true typical leaves, pinnate) are compound and already exhibit the apical leaflet with serrated margin. The primordial leaves and the pinnate leaves are in a decussate arrangement, separated by very short internodes. On the third day the two pinnate leaves already show three leaflets, one at the tip and two laterals: the seedling measures approximately 5 cm (Figure 4).

Around the tenth day some primordial leaves turn yellowish and by the twentieth day they have already fallen off. As the development of the plant continues, the pinnate leaves increase in number of leaflets, all with serrated margins, increasing from three, to five, seven, nine, eleven, and thirteen leaflets. The latter was recorded in leaves of 35 cm

Figure 4. A three days-old *Spondias dulcis* seedling; two primordial smooth-edged leaves and two pinnate leaves, each containing three leaflets, are observed.

in length and 5 months-old plants, at this age the plants measured 89 cm in length, and they were still without lateral branches. However, it should be clarified that the plants continued in the same bags where germination occurred, that is, with some limitation for growth.

In relation to propagation by cuttings, those obtained from 1.5- and 2.5-year-old trees (Block 1) showed vegetative shoots after 10 days in four of the cuttings from lot 1. This lot consisted of the thickest stems obtained from a 1.5-year-old tree. Those same cuttings, after 22 days, all had emitted many shoots (between 5 and 7 per cutting); moreover, two cuttings already exhibited inflorescences in full development. Later, two months after planting, all four cuttings showed good vegetative growth (Table 1, Figure 5).

The well-developed leaves were about 35 cm long, each leaf with 11 or 13 leaflets. Likewise, in one cutting there were three clusters with fruits, nine fruits in total (Figure 6). Those fruits measured on average 0.7 cm × 0.8 cm in diameter and length, respectively. However, 10 days later the fruits were completely dry. In contrast, those cuttings with better-developed shoots also exhibited better roots, two months after planting (Figure 7).

Table 1. Average dimensions recorded in *Spondias dulcis* plants from Block 1, after 60 days.

	Lot 1					**Lot 2**					**Lot 3**					**Lot 4**				
Cutting Number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Cutting Length (cm)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Cutting Diameter (cm)	3.3	2.8	2.9	2.8	2.9	2.4	2.3	2.4	2.3	2.3	2.2	2	2.1	2.3	2.1	1.5	1.4	1.6	1.5	1.9
Average Shoot Length (cm)	58.6	0	11.75	3.94	2.5	4.4	0	0	0	0	1.4	4	0	0	0	0	0	0	0	0
Number of leaves (Cutting)	55	0	11.25	6.4	7.2	2	0	0	0	0	7	12	0	0	0	0	0	0	0	0

Figure 5. Four out of five stem cuttings of *Spondias dulcis* exhibited good vegetative growth.

Figure 6. *Spondias dulcis* fruits developed in one of the cuttings.

Figure 7. *Spondias dulcis* stem cuttings with good vegetative growth which also formed abundant roots; in contrast to those who showed poor vegetative development.

In regard to the cuttings from the other lots in Block 1, only a few cuttings from lots 2 and 3 emitted shoots. But at two months the leaves of the shoots were small and dark green; likewise, these cuttings had not formed roots (Figure 7).

The cuttings of lot 4, thinner and younger stem cuttings, exhibited wilted tips at 10 days and continued to dry from tip to base. On the other hand, the cuttings obtained from the 8-year-old tree (block 2), treated with Raizal® 400 (Table 2), began bud break between 15 and 20 days. At 15 days, three cuttings in lot 1, the thickest and supposedly oldest cuttings, had buds smaller than 1 cm. The same condition was observed in two cuttings of lot 3 and in one of lot 4.

Although these same cuttings emitted more shoots later, they grew little and dried out in a short time, in such a way that after 45 days only three cuttings exhibited reduced shoots, with leaves also very small. The longest leaves only measured 6 cm on average and were dark green. Although at 45 days there were two shoots with inflorescences, these dried up a week later. None of the cuttings in Block 2 had developed roots by 45 days.

Lot 5, consisting of the thinnest and youngest stem cuttings, was already dry after 15 days. The high survival of seedlings, as found in this study, lowers the costs of seed propagation in ciruela-mango; quite opposite to what would happen if it were decided to multiply *S. dulcis* by stem cuttings. In fact, it was only 8.8% successful if you consider the total of the cuttings of both treatments. Moreover, it occurred when the cuttings did not receive rooting and were obtained from a 1.5-year-old tree (thick and greenish cuttings). This suggests that success depends on the vigor and perhaps the age of the parent plant.

In this study, the youngest and thinner cuttings with or without root stimulant died quickly. While the other stems with both treatments lived longer and emitted vegetative shoots. However, these shoots reached little development because they used the cutting reserves because they did not develop roots. More research remains to be done in this regard. Vega-Escobedo (2010) did not find an interaction among three doses of IBA (0, 3000 and 5000 ppm), two ages of the parent tree (1-5 and 7-16 years) and the location

Table 2. Average measurements obtained in *Spondias dulcis* plants in Block 2 after 45 days.

	Lot 1					**Lot 2**					**Lot 3**					**Lot 4**					**Lot 5**				
Cutting Number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Cutting Length (cm)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Cutting Diameter (cm)	3.7	3.5	3.4	3.5	3.9	2.2	1.9	1.9	1.9	1.7	2.7	1.7	1.5	1.4	1.4	1.8	1.8	1.9	1.5	1.5	0.9	1	1.4	1.2	1.3
Average Shoot Length (cm)	2.3	0	0	0	0	0	0	0	0	0	1.08	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Number of Leaves (Cutting)	8	0	0	0	0	0	0	0	0	0	3.25	0	0	0	0	0	0	0	0	3	0	0	0	0	0

of the cutting site in the parent tree (basal, middle and apical). Tough, he did find 22% callus with 0% IBA in cuttings from the middle part of trees between 7 and 16 years old.

Jesus *et al.* (2004) also did not record root emission in cuttings of *S. dulcis* treated with different doses of IBA; while Rocha *et al.* (2020) concluded that vegetative propagation of *S. dulcis* was not feasible, but in *S. tuberosa* it achieved 11% success when cuttings were treated for 16 seconds in an IAA solution (10 g L^{-1}).

CONCLUSIONS

Germination of *Spondias dulcis* was relatively slow and irregular, with 77% germination in 2.5 months. Germination is hypogeal, the cotyledons remain within the endocarp. Seedlings develop two simple primordial leaves, while nomophiles (true leaves) are compound imparipinnates. Each seed germinated one and up to five seedlings, with a 100% survival rate at five months.

Vegetative multiplication of *S. dulcis* was successful without root stimulant, but it was only possible in the thickest and greenest cuttings, derived from a 1.5-year-old tree. In both treatments the younger, thinner cuttings died soon. Although all the other cuttings emitted vegetative shoots, most of these were completely dried by two months; the shoots that remained alive had very reduced growth because the cuttings had not emitted roots.

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Colegio de Postgraduados

Variation of the nutritional content of different genotypes of *Lotus corniculatus* L. under optimum and sub-optimum soil moisture conditions throughout the seasons of the year

Sahara Xoloctzi-Acoltzi[1]; Aurelio Pedroza-Sandoval[1*]; Ricardo Trejo-Calzada[1]; Gabino García-De los Santos[2]; Yáñez-Chávez Luis G.[1]

[1] Unidad Regional Universitaria de Zonas Áridas de la Universidad Autónoma Chapingo. Bermejillo, Durango. México. C. P. 35230.

[2] Colegio de Posgraduados, Montecillo, Texcoco Estado de México, México. Montecillo, Texcoco, Estado de México. C. P. 56264.

* Correspondence: apedroza@chapingo.uruza.edu.mx

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ABSTRACT

Objective: To evaluate the leaf nutrient content of four accessions and one variety of *Lotus corniculatus* L. trefoil throughout the seasons of the year and under optimum and sub-optimum soil moisture content, established under a shade mesh in northern Mexico.

Design/Methodology/Approach: During the 2021-2022 period, an experimental randomized block design, in a split-plot arrangement, with three replicates was established. The large plots had an optimum (26±1.5%) and sub-optimum (22±1.5%) soil moisture content. The small plots consisted of the trefoil accessions: 255301, 255305, 202700, 226792 origin code, and the Estanzuela Ganador variety.

Results: At optimum soil moisture content, the 202700 accessions recorded a K deficiency throughout the year. Meanwhile, regardless of the soil moisture content, the 255301 accessions recorded a Mg and a Mn deficiency only in summer. N, P, Fe, and Mn recorded deficient contents: N and P throughout the year and Fe and Mn only in winter and spring.

Study Limitations/Implications: These results could require a field validation, since the experiment was carried out in semi-controlled conditions, under shade mesh.

Findings/Conclusions: Developing an adequate annual nutrition program for *L. corniculatus* L. that improves its forage productivity requires several key factors, including the behavior of the nutritional status (dependent on the type of nutrient), the growth stage of the plant, the season of the year, and the soil moisture content.

Keywords: Plant nutrition, stress physiology, soil moisture, macro- and micro-nutrients, drought.

INTRODUCTION

Nutrition is fundamental for the growth of plants. Water plays a key role in the use of the different nutrients required by plants (Levinsh, 2023). Although plants require a minimum percentage of minerals, these are fundamental for their growth, development, and productivity (Pérez, 2017). Water deficit, marginal lands with low organic matter

Image by Petra at Pixabay

content, and extreme temperatures cause stress in plants (Bhattacharya, 2021). Some plant species have developed tolerance mechanisms to face biotic and abiotic limiting factors. This phenomenon promotes a better productive performance in marginal agricultural areas (Pedroza-Sandoval *et al.*, 2022).

The nutrition diagnosis of plants and soils —using critical, standard, or referential concentration indicators— enables the inference of the potential risks that may limit production, based on the deficit or the excess of nutrients (Kathpalia & Bhatla, 2018). Quintero-Azueta *et al.* (2021) reported that in several forage crops, the flowering stage is appropriate for the nutrition diagnosis of the plant.

Lotus corniculatus L. is a profitable pulse, with a nutritional quality similar to alfalfa (*Medicago sativa* L.) (18-22% raw protein); it has less cellulose and more non-structural carbohydrates (Álvarez-Vázquez *et al.*, 2018). Traditionally, the nutritional management of the common bird's-foot trefoil has been restricted to a P fertilization process, based on the idea that N can fix P in a soil in which pulses are grown (Barbazán *et al.*, 2008).

According to González-Espíndola (2024), the different seasons of the year impact the production of *L. corniculatus*. This situation is the consequence of extreme temperatures that modify several agronomic, such as the nutrients of the plants (Freire *et al.*, 2019; Márquez *et al.*, 2024). Therefore, the objective of this study was to determine the nutrient content of the leaves of different genetic plant materials of the common bird's-foot trefoil (*Lotus corniculatus* L.), under optimum and sub-optimal soil moisture content, throughout the seasons of the year. The hypothesis of the study was that the macro- and micro-nutrient requirements of the plant change, depending on the *L. corniculatus* L. genotype and the season of the year.

MATERIALS AND METHODS

The study was carried out in the experimental field of the Unidad Regional Universitaria de Zonas Áridas of the Universidad Autónoma Chapingo, in Bermejillo, Durango, Mexico (23° and 27° N and 106° and 102° W, at 1,130 m.a.s.l.). The area has a dry desert climate, with summer rains and cold winters. The region has a 258 mm annual mean precipitation and a 2,000 mm annual mean potential evaporation; the annual mean temperature is 21 °C, with 33.7 °C maximum and 7.5 °C minimum temperatures (Medina *et al.*, 2005).

Experimental design and management. An experimental randomized block design with divided plots and three replicates was used. The large plots had two moisture contents: optimum soil moisture content (OSMC: 26±1.5%) and sub-optimal soil moisture content (SSMC: 22±1.5%). Both were established based on the soil moisture drawdown curve determined by the pressure-membrane method proposed by Richards (1948), and through of this method the soil moisture constants were determined, corresponding to 26.5% field capacity (FC) and 17.5% permanent wilting point (PWP). The small plots used the *Lotus corniculatus* L. genetic plant materials of four accensions (255301, 255305, 202700, and 226792 identification codes) and the Estanzuela Ganador variety. The experimental unit consisted of a 20-kg plastic pot with a plant.

The pots were filled with 18 kg of a soil:compost:sand substrate (50:30:20 ratio). The substrate had a sandy-loam texture, with a 52:22:26 ratio of sand, clay, and slime, respectively. The experiment was carried out from March 2021 to May 2022, under shade-mesh conditions.

Irrigation was carried out every four days via gravimetry. The weight of the OSMC and SSMC pots was kept at 23.9 and 23.0 kg, respectively. An average of 0.6 L of water was added via irrigation to both moisture contents, reestablishing the upper limit of soil moisture of OSMC to 27.5% (slightly higher than FC) and of SSMC to 23.5%. Both percentages were allowed to decrease to 24.5% and 20.5%, respectively. A 3% margin (20.5-17.5) was considered as the usable moisture range, to prevent the plant from reaching PWP.

Measured variables. To determine the macro- and micro-elements, leaf samples from *Lotus corniculatus* L. were taken from the upper third of the plant. The samples were collected every 45 days (March 15, April 30, June 15, July 30, August 15, September 30, and November 15, 2021). From November 15, 2021 to March 15, 2022, the samples were collected every 90 days, because the low winter temperatures impacted plant growth. The Kjeldahl method (AOAC, 1984) and the Molybdenum blue method (Murphy and Riley, 1962) were used to determine N and P content, respectively.

The dry digestion method (Isaac & Kerber, 1971) was used to determine the Cu, Mn, Zn, Fe, Ca, Mg, K, and Na micro-nutrients, with a PerkinElmer AAnalyst 200 Atomic Absorption Spectrometer. On the one hand, the Ca, Mg, K, and Na macro-nutrients were established preparing a 1 mL + 9 mL triple distilled water dilution of the sample; on the other hand, 10 mL of the extract were used to determine the Fe, Cu, Zn, and Mn micro-nutrients.

The following formulas were used to determine the macro- and micro-nutrient percentages:

$$\text{Ca, Mg, K, Na}(\%) = \frac{ppm\ CC \times Dm \times Vd}{10\ 000} \quad (1)$$

$$\text{Fe, Cu, Zn, Mn}(\%) = ppm\ CC \times D_m \times V_d \quad (2)$$

Where: *ppm CC*=parts per million in the calibration curve; D_m=dilution of the mass (gauge volume/g of the sample); V_d=Volume dilution (gauge/aliquot, in case of dilutions).

Statistical Analysis. An analysis of variance (ANOVA) and Tukey's Multiple Comparison Test ($P \leq 0.05$) were used to process the database in the Statistical Analysis System version 9.0 software (SAS Institute, 2011). The statistical analysis was based on the corresponding model of the divided plot experimental design, where the treatments were the different genotypes within the moisture content. The Minitab v.18.0 statistical software was used to perform the Normality test (Johnson technique); this analysis was carried out before the ANOVA and the Tukey's Test.

RESULTS AND DISCUSSION

The Normality test recorded a positive value (P=0.956). Based on the results of the F-test, significant differences were found only between the optimum irrigation genotypes, throughout the seasons of the year (Table 1).

Based on the Normality test, the Ca, Mg, and Na values were P=0.325, 0.946, and 0.506, respectively. According to the analysis of variance, the F-probability identified a significant effect between the *L. corniculatus* genotypes, within each soil moisture content: Mg, Ca, and Na recorded no differences in sub-optimal soil moisture content, while Na recorded differences in the optimum soil moisture content (Table 2).

Behavior of the macro-elements between genotypes. The 202700 accession was the most sensitive and recorded the lowest K values in OSMC, throughout all the seasons of the year (Table 3); these results indicate that the plant consumes high amounts of K, which results in a low K content during the whole growth cycle (Sardans & Peñuelas, 2021).

Table 1. Analysis of variance of P content (%) of the different *Lotus corniculatus* L. genotypes, throughout the seasons of the year, with optimum and sub-optimal soil moisture content.

	Spring 2022		Summer 2021		Autumn 2021		Winter 2021-2022	
	OSMC	**SSMC**	**OSMC**	**SSMC**	**OSMC**	**SSMC**	**OSMC**	**SSMC**
Fc	62.9	1.57	8.39	2.49	6.85	0.82	5.58	1.78
Prob.	0.018*	0.295	0.008**	0.138	0.014*	0.55	0.024*	0.238

OSMC=optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

Table 2. Analysis of variance of the macro- and micro-nutrient content of the different *Lotus corniculatus* L. genotypes, with optimum and sub-optimal soil moisture content (summer 2021).

	Calcium (%)		Magnesium (mg kg^{-1})		Sodium (mg kg^{-1})	
	OSMC	**SSMC**	**OSMC**	**SSMC**	**OSMC**	**SSMC**
Fc	5.54	4.6	2.72	10.69	0.59	2.69
Prob.	0.023*	0.38*	0.01*	0.004**	0.681	0.000**

OSMC=optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

Table 3. Effect of the K content variation in *Lotus curniculatus* L. in different soil moisture contents and seasons.

Accessions	Spring (2022)		Summer (2021)		Autumn (2021)		Winter (2021-2022)	
	OSMC	**SSMC**	**OSMC**	**SSMC**	**OSMC**	**SSMC**	**OSMC**	**SSMC**
255301	5.0^{a}	3.9^{a}	5.8^{a}	4.0^{a}	5.7^{a}	3.6^{a}	5.3^{ab}	4.3^{a}
255305	4.3^{ab}	4.4^{a}	4.5^{ab}	4.4^{a}	3.9^{ab}	3.7^{a}	3.9^{ab}	3.7^{a}
202700	3.0^{b}	3.5^{a}	2.9^{b}	3.5^{a}	3.6^{b}	3.6^{a}	2.9^{b}	3.4^{a}
226792	4.7^{a}	4.11^{a}	4.7^{ab}	4.5^{a}	5.6^{a}	4.4^{a}	5.6^{a}	4.5^{a}
Estanzuela Ganador	4.3^{ab}	3.7^{a}	4.3^{ab}	3.8^{a}	4.9^{ab}	3.7^{a}	4.1^{ab}	3.5^{a}

Tukey's Test (P≤0.05). Figures with the same letters within each column are statistically equal. OSMC=optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

The Ca, Mg, and Na macro-elements only recorded effects during summer. The 255301 accession recorded de lowest Ca and Mg values, regardless of the soil moisture content. These results indicate that these elements are the most required during summer season (Catzistathis *et al.*, 2010). The high temperatures recorded during this season resulted in a higher photosynthetic activity. Regarding SSMC, the Estanzuela Ganador variety recorded high Na values. The plant did not record negative effects, which suggests a potential salinity tolerance (Orosco *et al.*, 2018) (Table 4).

Temporal behavior of *L. corniculatus* L. regarding the macro- and micro-nutrient average

Nitrogen content. The average N concentration of the plants recorded was 1% in OSMC and 1.1% in SSMC, with a 0.79-1.19% seasonal range and a significant difference ($P \leq 0.05$) between seasons. The highest (1.4%) and lower (0.79%) N content for both soil moistures were recorded in winter and summer, respectively (Figure 1A). Based on the 2% critical level for alfalfa recommended by Barbazán *et al.* (2008), the *L. corniculatus* L. recorded a N deficiency throughout the year.

Phosphorous content. The average P concentration reached 0.1%, regardless of the soil moisture content. No statistical difference ($P \leq 0.05$) was found, and the range recorded was 0.08-0.11%. Figure 1B shows that the P content is lower than the critical values (0.24%) proposed by Pinkerton *et al.* (1997). The P deficiency symptoms are similar to those of N: a growth delay of all the plant organs (mainly leaves and stems). These phenomenon increases the impact on foraging crops, because these organs are the main source of fresh and dry biomass productivity (Marín, 2011).

Potassium content. The plants recorded an average K concentration of 4.4% (OSMC) and 4.0% (SSMC), with a 3.86-4.72% range between seasons (Figure 1C); however, there were no differences ($P \leq 0.05$) between seasons. In addition, these values are higher than the 1.4% critical value reported by Barbazán *et al.* (2008). Just like in the case of N and P, K travels from the old to the new organs of the plant. When a plant has a K deficiency, the leaves turn slightly yellow and then develop necrotic spots (Marín, 2011).

Based on these results, the N, P, and K concentrations are expected to change according to the season, showing deficiency levels as the metabolic activity of the plant increases

Table 4. Effect of the Ca, Mg, and Na content variation in *Lotus curniculatus* L., with different soil moisture contents (summer 2021).

Accession	Calcium		Magnesium		Sodium	
	OSMC	SSMC	OSMC	SSMC	OSMC	SSMC
255301	3.4^{ab}	2.5^{a}	1.04^{abc}	0.77^{c}	0.34^{a}	0.25^{bc}
255305	3.5^{a}	2.8^{a}	1.14^{ab}	0.89^{abc}	0.32^{a}	0.22^{c}
202700	3.1^{ab}	3.6^{a}	1.16^{a}	1.12^{a}	0.39^{a}	0.27^{bc}
226792	2.5^{ab}	2.6^{a}	0.78^{c}	0.84^{bc}	0.37^{a}	0.40^{b}
Estanzuela Ganador	2.4^{b}	3.1^{a}	0.85^{bc}	1.07^{ab}	0.44^{a}	0.64^{a}

Tukey's Test ($P \leq 0.05$). Figures with the same letters within each column are statistically equal. OSMC= optimum soil moisture content (26±1.5%); SSMC=sub-optimal soil moisture content (22±1.5%).

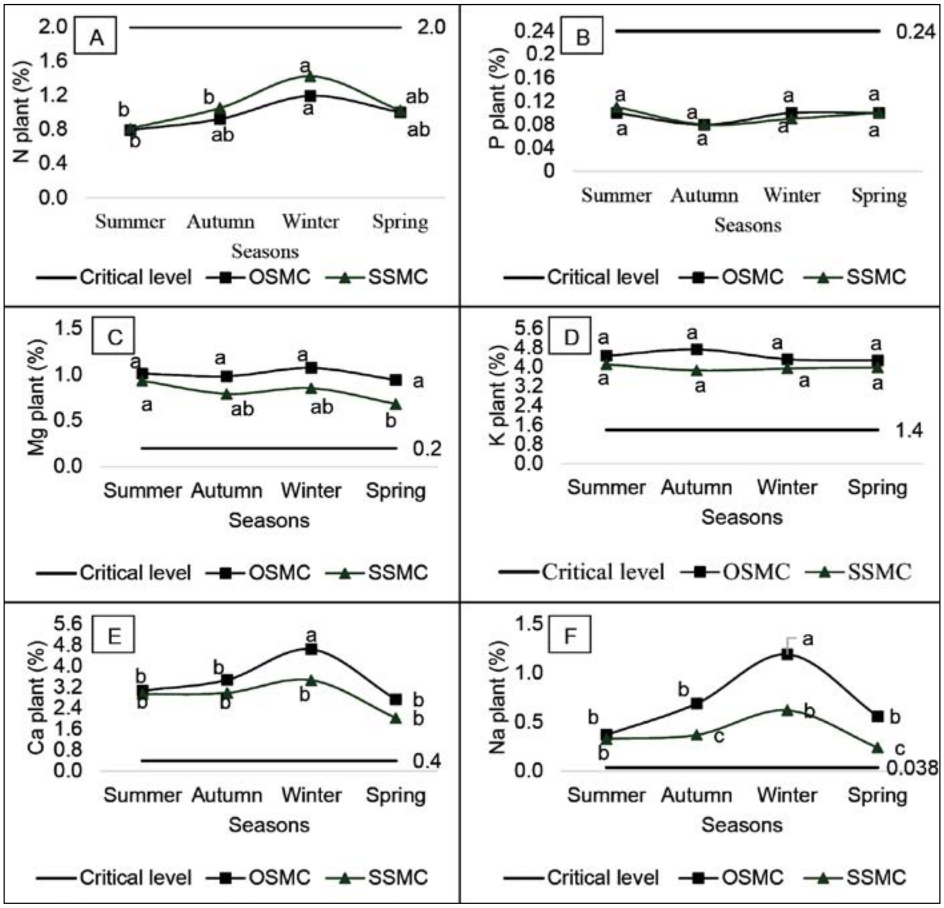

Figure 1. Seasonal behavior in the macro-nutrient content of the leaves of *L. corniculatus* L., with optimum soil moisture content (OSMC) and sub-optimal soil moisture content (SSMC). A=N concentration (%); B=P concentration (%); C=Mg concentration (%); D=K concentration (%); E=Ca concentration (%), and F=Na concentration (%).Tukey's Test (P≤0.05). Figures with the same letters on the same line of each graph are statistically equal. OSMC: optimum soil moisture content (26±1.5%). SSMC: sub-optimal soil moisture content (22±1.5%).

during spring and summer. The nutrients of a plant change with the seasons; this change is an indicator of how the plant and its organs absorb, use, redistribute, and extract nutrients (Galindo-Reyes *et al.*, 2011).

Calcium content. Ca concentration showed significant differences (P≤0.05) between seasons. Winter recorded the highest concentration (4.64%). The Ca average reached 3.5% (OSMC) and 2.9% (SSMC) and the range between seasons was 2.9-3.5% (Figure 1D). These results surpassed the critical values (0.4%) reported by Thor (2019) for different plant species.

Magnesium content. The average Mg concentration recorded was 1% (OSMC) and 0.8% (SSMC), with a seasonal range of 0.68-1.07% (Figure 1E). Winter recorded the highest Mg concentrations (1%); however, the values decreased as the plant grew old. Nevertheless, the Mg values were always higher than the critical values (0.2%) of most plant species (Ishfaq *et al.*, 2022).

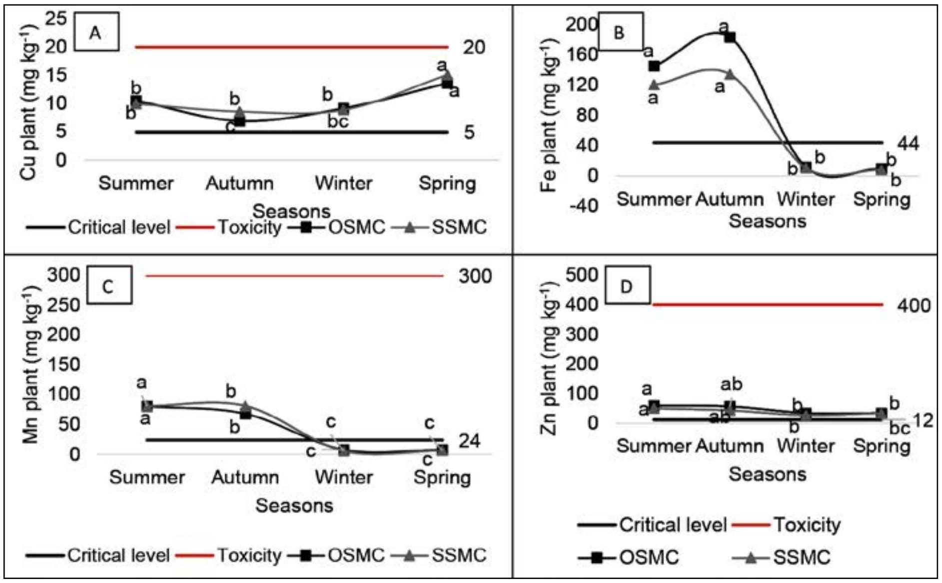

Figure 2. Seasonal behavior of the micro-nutrient content of *L. corniculatus* L., with optimum soil moisture content (OSMC) and sub-optimal soil moisture content (SSMC). A=Cu concentration (mg Kg^{-1}); B=Fe content (mg Kg^{-1}); C=Mn concentration (mg Kg^{-1}); D=Zn concentration (mg Kg^{-1}). Tukey's Test ($P \leq 0.05$). Figures with the same letters of each graph are statistically equal. OSMC: optimum soil moisture content (26±1.5%). SSMC: sub-optimal soil moisture content (22±1.5%).

Sodium content. The average Na concentration was 0.7% (OSMC) and 0.39% (SSMC), with a 0.24-1.19% range. The highest Na concentrations were recorded during winter, but only in the case of OSMC. The values decreased in spring (Figure 1F); however, they were always higher than the critical levels (0.038) established by Miloševi and Miloševi (2012).

Micro-nutrient content. A Cu deficiency was recorded throughout the year; however, Fe and Mn deficiency was recorded only in winter and spring. A similar response trend was reported by Ishfaq *et al.* (2022) in their study about alfalfa: 5, 44, 24, and 12 mg kg^{-1} for Cu, Fe, Mn, and Zn, respectively. At physiological level, the micro-nutrients impact the redox processes, which are important for the appropriate development of photosynthesis and the detoxification of free radicals in the oxygen (Marín, 2011).

Based on the results of the seasonal behavior of the micro-nutrients of the common bird's-foot trefoil (*L. corniculatus* L.), a higher seasonal influence was observed, depending on the type of nutrient, the moisture condition, and chemical characteristics of the soil. The behavior is modified by the relationships established between the genotypes and the plant species, strongly influenced by environmental conditions (particularly extreme temperatures and precipitation periods) (Prause and Fernández, 2012).

The nutritional state of a plant is partly related to the soil moisture content. However, its relation to the season of the year is even more important. The N and P macro-nutrient content is related to spring and summer, while the micro-nutrient content is related to winter. This information must be taken into account to improve the nutritional state of a plant using fertilization.

CONCLUSIONS

The nutritional state of *Lotus corniculatus* L. changed depending on the genotype, the type of nutrient, season, and, in a lower degree, the soil moisture content. The 202700 accession recorded K deficiency all year long in an optimum soil moisture content; meanwhile, the 255301 accession recorded Mg deficiency only during summer, regardless of the soil moisture content. N, P, Fe, and Mn recorded the highest deficiencies. N and P recorded deficiencies throughout the year, while Fe and Mn only in spring and summer. A plant and soil nutrition diagnosis is fundamental to develop an appropriate plant nutrition program.

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Colegio de Postgraduados

Kinetics of ammonium volatilization in the form of ammonia in soils through a textural gradient

Perez-Rosales, Alejandro[1*]

[1] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Zacatepec. Carr. Zacatepec-Galeana, km 0.5. Col. Centro. Zacatepec, Morelos. C.P. 62780.

* Correspondence: fitotecni@hotmail.com

ABSTRACT

Objective: To estimate the ammonia volatilization rate, using a texture gradient with increasing doses of ammonium in alkaline soils.

Design/Methodology/Approach: The experiment took place in the Análisis Químico de Suelos lab of the Soil Department of the Universidad Autónoma Chapingo. The incubations consisted of 25 g of soil from the former Texcoco lake, with 12.5% clay. The soil was air-dried and sieved with a no. 10 mesh. The soil was mixed with three different concentrations (17.5%, 22.5%, and 32.5%) of bentonite, in order to increase clay content. The mixtures were placed in plastic containers with airtight seals. Twelve-point five mL of an ammonium sulfate solution (with 150, 300, 450, 600, and 750 mg of nitrogen kg soil^{-1}) was added. The ammonia was recovered in a container with a boric acid solution. Volumetry was used to quantify ammonium. A completely randomized design with two factors (clay content and ammonium dose) was used. Data were analyzed with a regression analysis, analysis of variance, and Tukey Multiple Comparison Test, using the SAS OnDemand for Academics software.

Results: The ammonia volatilization has a linear trend, with the concentration of the applied nitrogen. The volatilization rate ranged from 0.02 to 0.03 mg of ammonium per milligram of the nitrogen applied per kg soil^{-1}. Significant statistical differences were recorded between the effect of the N dose and the clay content on the ammonia volatilization rate.

Study Limitations/Implications: Clay content in the soil and ammonia volatilization rate can be used as an indicator to estimate ammonia losses and the ammonia adsorption capacity of the soil.

Findings/Conclusions: Ammonia volatilization is independent from clay content and takes place immediately after its application. It has a linear trend regarding the ammonia dose applied. Ammonia volatilization rate decreases (asymptotic trend) as the clay content increases in the soil.

Keywords: clay, ammonium, alkaline, cationic exchange capacity.

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INTRODUCTION

The nitrogen (N) available for plants in the soil is the highest limiting factor (Martínez-Dalmau *et al.*, 2021). Consequently, during the last decade, the annual use of nitrogen fertilizers has increased by ≈1.4% worldwide; however, their recovery efficiency is $<$50% (Panday *et al.*, 2020; Mahmud *et al.*, 2021). Approximately 80% of the N used in agriculture is lost as a result of leaching, runoff, and gaseous emissions. The predominant gases are nitrous oxide (N_2O-N) and ammonia (NH_3-N), with 4 Tg N year^{-1} and 12 Tg N year^{-1}, respectively (Krol *et al.*, 2020). The NH_3-N volatilization accounts for 10-60% of the total N applied as ammonium fertilizer. This process takes place a few days after the application (Chu *et al.*, 2020).

The NH_3-N volatilization is the result of the ammonium (NH_4-N) adsorbed by the colloids and its interaction with the physical and chemical properties of the soil (Wang *et al.*, 2023). Volatilization is highly sensitive to pH and NH_4-N concentration. The increases of pH promote the formation of NH_3-N (Shi *et al.*, 2024) —*i.e.*, a >7 pH increases the formation of NH_3-N, as a consequence of the high concentration of the hydroxyl radical (HR) that reacts with the NH_4-N (Skorupka and Nosalewicz, 2021). This dependency between pH and the NH_4-N/NH_3-N balance (Pelster *et al.*, 2019) increases exponentially the formation of NH_3-N (Ohnemus *et al.*, 2021). In calcareous soils, the losses account for 50% (Powlson and Dawson, 2021).

Clays, cation exchange capacity (CEC), texture, organic matter (Cassity-Duffey *et al.*, 2020), and damping capacity of soils determine the NH_3-N volatilization rate, because they correlate with higher NH_4-N adsorption rates. Clay soils have a lower volatilization than sandy soils (Pelster *et al.*, 2019; Mazloomi and Jalali, 2019): the amount of N lost in the volatilization is significantly influenced by soil texture and it is higher in coarse-textured soils than in fine-textured soils (Al-Badrani *et al.*, 2023). The NH_4-N adsorption and desorption in the soil is highly dependent of the type and quantity of clays; these minerals have chemical reactivity and are grouped according to their silicon tetrahedra (1:1) and aluminum octahedra (2:1). The 2:1 type clays have a high CEC and a higher specific surface than the 1:1 type clays, increasing their NH_4-N adsorption (350-3,800 kg ha^{-1} at ≤30 cm of depth); vermiculites, illites, and smectites predominate in these soils (Kome *et al.*, 2019). Consequently, the objective of this research was to estimate the ammonia volatilization rate, using a textural gradient and increasing doses of ammonium in alkaline soils.

MATERIALS AND METHODS

The experiment was carried out in the Análisis Químico lab of the Soil Department of the Universidad Autónoma Chapingo. To determine the volatilization rate, soils that had been previously air-dried and sieved with a no. 10 mesh were incubated.

Increasing concentrations of bentonite were added to the soil samples (texture gradient) to increase clay content. Bentonite is an expandible clay of the montmorillonite group (2:1) that has strong affinity and cation adsorption capacity in its internal and external layers (specific surface) (Alexander *et al.*, 2019). Bentonite has the following characteristics: $pH_{10:1}$=9.42, $EC_{10:1}$=1.36 dSm^{-1}, CEC=63.23 meq 100 g^{-1} S, carbonates=1.5 $mmol_c$ L^{-1}, bicarbonates=82.49 $mmol_c$ L^{-1}, and clay (particles>0.002 mm)=95.84%. Adding this mineral allowed the simulation of soils with higher contrasts regarding clay content (17.5%, 22.5%, and 32.5%). Soil without bentonite was used in the case of the 12.5% concentration. The soil samples collected from the former Texcoco lake had the following characteristics: 12.5% clay obtained with the pipette method (Van Reeuwijk, 2003); 9.6 pH, 389 μScm^{-1} EC, and 32.0 mg kg^{-1} phosphorous (SEMARNAT, 2002); 8.78 Cmol kg S^{-1} CEC (Pleyser *et al.*, 1986); and 1.60 g kg^{-1} soluble organic carbon (Harvey *et al.*, 2009). Once the sample and the bentonite were mixed, 25 g were weighted and placed in 15 cm × 20 cm transparent polypropylene bags with airtight seals. An ammonium sulfate solution (12.5 mL) was added to the bags. This solution was determined based on the effective porosity saturation (Wallach, 2019); the percentage used in this experiment was 50% (W/V).

The N doses applied were based on the proposals of Liu *et al.*, 2007 and Fan *et al.*, 2011. Ammonium sulfate was used as source of N (150, 300, 450, 600, and 750 mg N kg soil^{-1}). A modified version of the quantification methodology proposed by Ahmed *et al.* (2008), Chen *et al.* (2014) and Palanivell *et al.* (2015) for the recovery of ammonium was used: a 100 mL container with a 50 mL boric acid solution (20 g L^{-1}) was mixed with the bromocresol green and methyl red indicators and placed in the airtight sealed bag with the soil sample (previously treated with ammonium sulfate). Finally, the bag was airtight sealed to prevent water loss and gas and it was incubated at room temperature for three days. Volumetry was used to determine the amount of ammonium recovered from the boric acid solution (Shen *et al.*, 2020); in addition, the sulfuric acid concentration was adjusted to 0.005 N. The experiment was carried out using a completely randomized design with two factors (NH_4-N concentration and clay content of the soil) and three replicates. The analysis of the volatilization rate was carried out with a simple linear regression analysis. The NH_4-N doses were analyzed per clay level. The linear regression analysis determined the NH_3-N volatilization rate. Subsequently, a graph of these volatilization was developed per clay level, in order to evaluate the influence of clay content on the NH_3-N volatilization. The recovered NH_3 (mg) was subjected to an analysis of variance and to a Tukey Multiple Comparison Test, with the SAS OnDemand for Academics software.

RESULTS AND DISCUSSION

Effect of the clay content on the soil and the NH_4-N dose on the NH_3-N volatilization
According to the analysis of variance and the regression analysis for the five NH_4-N levels in each clay layer, when α=0.01 (Table 1), the behavior of the NH_3 volatilization has a proportional linear trend as the NH_4-N levels applied increase, given the positive correlation between NH_3-N emissions and NH_4-N concentration (Li *et al.*, 2020).

After the samples had been incubated for three days, a linear trend was identified between the concentration of NH_4-N applied and the amount of NH_3-N recovered, regardless of the clay content; this linear behavior is found in each clay layer evaluated, because high NH_4-N doses increase NH_3-N emissions (Wan *et al.*, 2021). The NH_3-N volatilization rate ranged from 0.02 to 0.03 mg per mg of N applied per kg of soil, with

Table 1. Analysis of variance of linear regression for four soil clay contents and five NH_4-N doses on NH_3-N volatilization.

Source	DF	Sum of squares			
		12.5	17.5	22.5	32.5
		Clay (%)			
Reg	1	500.89**	430.56**	331.95**	282.87**
Error	13	0.48	0.57	0.61	0.69
Total	14				

DF: Degrees of Freedom; *α=0.05; **α=0.01; NS: Not Significant.

>97% correlation coefficients (Figure 1). Minato *et al.* (2020) mention that the accumulated NH_3-N volatilization has a sigmoid pattern with gradual increases. Its first stage had a high NH_3-N emission rate, reaching its maximum point 72 h after the application (0.4 kg ha^{-1} day^{-1} NH_3-N). It remained constant during the first 5 days.

The NH_3-N losses recorded during the incubation with the various N doses (150, 300, 450, 600, and 750 mg·kg $soil^{-1}$) suggest that, under experimental conditions, this situation takes place immediately after application and that, during the first stages, the soil lacks the capacity to absorb NH_4-N from the clay. Viero *et al.* (2014) report a similar trend: a maximum loss of 0.40 and 0.53 kg ha^{-1} day^{-1} was registered 5-10 days after the application. Dari and Rogers (2022) determined that maximum volatilization takes place from 4 to 8 days after the application (8 to 11% of the total N applied). These emissions are generated by the reactivity of NH_4-N to the OH^- content in the soil —*i.e.*, the post-application volatilization is mostly a chemical process, since the increase from 6 to 10 in pH increases the anionic association sites that react to NH_4-N, reducing the NH_4-N concentration and, in turn, the adsorption capacity of soil clays (Fan *et al.*, 2021).

The analysis of variance found significant differences on the effect on the NH_3-N volatilization of clay content, NH_4-N doses, and the interaction between clay content and NH_4-N doses (Table 2). Da Cruz Corrêa *et al.* (2021) established that an increase in the N doses results in a linear increase of volatilization, irrespective of the ammonium source. The NH_4-N adsorption increases as clay content in the soil increases, given the negative correlation between the NH_3-N volatilization, the clay content in the soil, and the CEC (Chuong *et al.*, 2020).

NH_3 volatilization is a chemical reaction determined by the pH of the soil; however, the clay content in the soil tends to diminish volatilization, since the ammonium applied in alkaline soils and soils with many clay layers or high CEC levels is absorbed by the sites where these clays are exchanged (Wester-Larsen *et al.*, 2022).

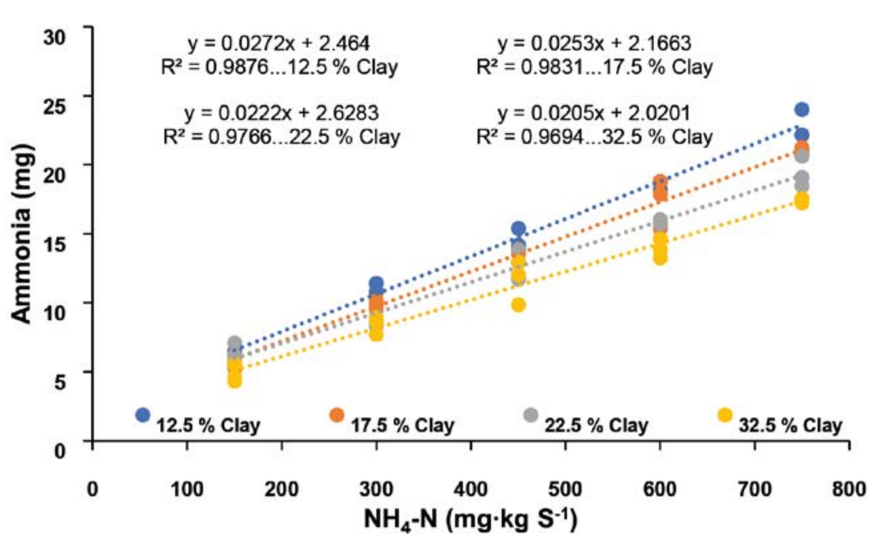

Figure 1. Linear regression model for the effect on ammonia volatilization of five levels of ammonium concentration and four levels of clay concentration found in the soil.

Table 2. Analysis of variance of the effect of four soil clay contents and five NH_4-N doses on NH_3-N volatilization.

Source	DF	SS (NH_3 mg)	F_c
Soil clay content (A)	3	98.9925	53.73**
Doses of ammonium sulphate (B)	4	1528.1210	622.03**
AB	12	23.0417	3.13**
Error	40	24.5667	
Total		1674.7218	

DF: Degrees of Freedom; SS: Sum of Squares; *α=0.05; **α=0.01; NS: Not Significant.

The multiple comparison of means determined that a greater clay content in the soil diminished NH_3-N volatilization. Likewise, statistical differences were found for each level evaluated (Table 3). An increase in the clay content of the soil increases cation exchange sites, enhancing the capacity of the soil to adsorb NH_4-N. Foereid *et al.* (2019) point out that the application of 10 t ha^{-1} zeolite increases NH_4 adsorption, without limiting the amount available for the crop.

Each level of the NH_4-N doses applied had statistical differences regarding NH_3-N volatilization. The 750 mg NH_4-N kg $soil^{-1}$ dose generates the largest volatilized NH_3-N; likewise, there is a proportional relation between the NH_4-N dose applied and the NH_3-N generated (Table 4). These results match the findings of Wang *et al.* (2021) who mentioned that NH_3-N volatilization (4.3 mg, 5.27 mg, and 6.32 mg) gradually increases as the N dose applied increases (26.6 mg, 48.3 mg, and 69.0 mg).

The clay content of the soil and the application of ammonium sulfate showed statistical differences regarding NH_3-N volatilization: 750 and 600 mg kg $soil^{-1}$ doses recovered

Table 3. Effect of soil clay content on NH_3-N volatilization.

Soil clay content (g kg S^{-1})	NH_3 (mg)
125.0	17.7333 a*
175.0	13.5467 b
225.0	12.6067 c
325.0	11.2267 d

* Means with the same letter are statistically equal (Tukey, α=0.05). MSD=0.767.

Table 4. Effect of ammonium sulfate application on ammonia volatilization.

Doses of ammonium sulphate (mg kg S^{-1})	NH_3 (mg)
750	20.1417 a*
600	16.4167 b
450	13.3083 c
300	9.5167 d
150	5.7583 e

* Means with the same letter are statistically equal (Tukey, α=0.05). MSD=0.9138.

more NH_3-N, an amount that gradually decreases as the N dose decreases (Table 5). Xie *et al.* (2019) reported a similar phenomenon: NH_3-N volatilization increases as a result of the fertilization dose applied.

Effect of the clay content in the soil on the NH_3-N volatilization rate

Figure 2 shows a slight decrease in volatilization speed as the clay content in the soil increases, based on the volatilization rates (27.2, 25.3, 22.2, and 20.5 μg NH_3 mg NH_4-N kg soil^{-1}) with the clay content (125, 175, 225, and 325 g kg soil^{-1}), which seemingly indicates an asymptotic trend for the study interval. This volatilization has a negative relation with the CEC and the clay content and its trend is adjusted to an exponential regression model (Hearn *et al.*, 2023), since bentonite is a 2:1 clay with a strong capacity to adsorb NH_4-N (19.01 mg NH_4-N g^{-1} of clay) and high CEC (40-130 Cmol kg^{-1}). NH_4-N adsorption in a soil to which bentonite has been added is a result of its electrostatic interaction with the negative charges of the surface, resulting from isomorphous replacement (Han *et al.*, 2020). Mazloomi and Jalali (2019) studied the relation between adsorbed NH_4-N and the concentration of the NH_4-N applied in soils treated with vermiculite and determined that the NH_4-N adsorption capacity of soils with 0% vermiculite is 285 mg kg^{-1}. It increases by 10.5%, 14%, and 24.6%, with

Table 5. Effect of soil clay content and ammonium sulfate application on ammonia volatilization.

Soil clay content (g kg S^{-1})	Doses of ammonium sulphate (mg kg S^{-1})	NH_3 (mg)
125	750	22.80 a
175	750	20.93 ab
225	750	19.40 bc
125	600	18.60 bc
325	750	17.43 cd
175	600	17.37 cde
225	600	15.80 def
125	450	15.00 efg
325	600	13.90 fgh
175	450	13.80 fgh
225	450	12.83 ghi
325	450	11.60 hij
125	300	11.20 ij
175	300	9.87 jk
225	300	8.60 kl
325	300	8.40 klm
225	150	6.40 lmn
125	150	6.07 mn
175	150	5.77 n
325	150	4.80 n

* Means with the same letter are statistically equal (Tukey, α=0.05). MSD=2.4241.

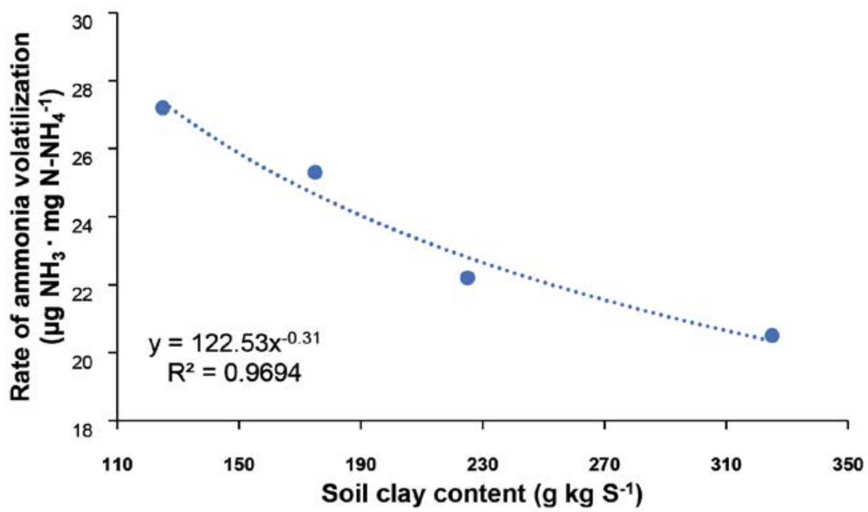

Figure 2. Effect of the clay content on the ammonia volatilization rate.

the addition of 2%, 4%, and 8%, respectively. These results indicate that the adsorption capacity of the soil is directly dependent on clay, which can be interpreted through the CEC.

Significant differences were found as a result of the analysis of variance of the effect of clay content on NH_3-N volatilization. These results suggest that ammonia volatilization speed tends to decrease with a higher clay content; however, these losses continue (Table 6), since the adsorption capacity of bentonite behaves according to the Langmuir adsorption isotherm (Zaini *et al.*, 2021). Likewise, Linggi *et al.* (2020) mention that the capacity of various soils and clays (*e.g.*, bentonite) to adsorb ammonium can be explained with a non-linear model, using the Langmuir isotherm.

Significant differences were recorded in the effect of the clay content of the soil on the NH_3-N volatilization rate: the lowest clay content in the soil (125.0 g kg soil^{-1}) is more volatile (27.2409 μg NH_3 mg NH_4-N kg soil^{-1}) (Table 7). The decrease in the NH_3-N volatilization rate is the result of the NH_4-N adsorption in the exchange sites. This phenomenon prevents their reaction with the OH^- found in the soil solution, because bentonite has a wide specific surface, high CEC, and a high cation adsorption capacity (Barakan and Aghazadeh., 2020). In addition, it has negative charges generated through isomorphous replacement, which are not affected by pH (Jolin *et al.*, 2020).

Table 6. Analysis of variance of the effect of soil clay content on ammonium volatilization rates with three days of incubation with ammonium sulfate α=0.01.

Source	DF	SS (μg NH_3 mg NH_4-N kg S^{-1})	F_c
Tratamientos	3	83.03	12.26**
Error	8	18.07	
Total	11	101.10	

DF: Degrees of Freedom; SS: Sum of Squares; *α=0.05; **α=0.01; NS: Not Significant.

Table 7. Effect of soil clay content and on the NH_3-N volatilization rate.

Soil clay content ($g\ kg\ S^{-1}$)	**Rate of ammonia volatilization ($\mu g\ NH_3\ mg\ NH_4\text{-}N\ kg\ S^{-1}$)**
125.0	27.2409 a*
175.0	25.2560 ab
225.0	22.1760 bc
325.0	20.4711 c

* Means with the same letter are statistically equal (Tukey, α=0.05). MSD=3.9292.

CONCLUSIONS

Ammonium losses are recorded immediately after the application of ammonium sulfate, regardless of the clay content in the soil and they are proportional to the amount of ammonium that was applied. Significant differences have been recorded regarding the amount of ammonia produced as a result of the increase in the clay content in the soil and the ammonium doses. Clay content in the soil reduces the speed of ammonia volatilization —*i.e.*, a greater clay content in the soil reduces the ammonia volatilization rate, as a consequence of the increase in the ion exchange sites in which the ammonium found in the solution is adsorbed. Ammonia volatilization takes place in alkaline soils and is more accentuated in sandy soils. Therefore, under these conditions, the use of ammonium sources must be limited and complemented with the application of soil improvers (organic carbon, fragments of recalcitrant carbon, and soil acidifiers) that increase exchange sites, in order to increase ammonium adsorption in the soil.

The ammonia volatilization rate helps to evaluate ammoniacal nitrogen sources, doses, and corrections, in order to increase the time that ammonium remains in the soil, as well as the efficiency of its recovery.

Clay content in the soil (measured as CEC) is an indicator of the ammonium adsorption capacity of alkaline soils. Although the NH_3-N volatilization is ruled by the pH, it is minimized by the type and amount of clay.

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Yield and nutritional value of *Cenchrus purpureus* VC Maralfalfa grass

Maldonado-Méndez, José de J.[1]; Gálvez-Marroquín, Luis A.[2*]; Guerra-Medina, Cándido E.[1]; Basurto-Gutiérrez, Ricardo[3]; Escobar-España, José C.[4]; Monterrosa del Toro, A.[4]

[1] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental Rosario Izapa, Tuxtla Chico, Chiapas, México, C.P. 30870.
[2] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental Valles Centrales de Oaxaca, Santo Domingo Barrio Bajo, Villa de Etla, Oaxaca, México. C.P. 68200.
[3] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal, Ajuchitlán, Colon, Querétaro, México. CP. 76280.
[4] Universidad Autónoma de Chiapas, Facultad de Ciencias Agrícolas, Campus IV. Huehuetán Chiapas, México. C.P. 30660.
* Correspondence: galveluis2010@gmail.com

ABSTRACT
Objective: Characterize the yield traits and nutritional values of Maralfalfa grass (*Pennisetum glaucum* L. × *P. purpureum* Schumacher), under humid tropical conditions.
Design/methodology/approach: The study was carried out in the Germplasm Bank from Rosario Izapa Experimental Unit, plant height, number of tillers, and fresh herbage and dry matter production were evaluated. Crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF) and *in vitro* dry matter digestibility (IVDMD) of grass samples were determined at 28, 42, 56, 70, 84 and 97 days.
Results: Plant height, number of trillers, and fresh herbage and dry matter production increased as cutting age increased. In relation to the nutritional value, the crude protein content decreased as the cutting age increased, but the NDF and ADF content increased, consequently, the DVDMS decreased.
Limitations of the study/implications: The study was carried out in the humid tropics; the evaluation is required to be carried out under dry tropic conditions.
Findings/conclusions: The appropriate harvest time of Maralfalfa grass in humid tropical conditions is between 42 and 56 days of regrowth, whereas nutritional values decrease over time.

Keywords: forage, feeding, cattle, humid tropics.

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INTRODUCTION

High solar radiation and year-round high temperatures characterize tropical climates; however, rainfall regimes are variable, and water excess or deficiency is the primary limitation for forage production in the tropics (Cardoso *et al.*, 2015). This limitation is reflected in forage production (kg of dry matter day^{-1}) available for grazing livestock (León *et al.*, 2018; Boval and Dixon, 2012). Additionally, grass age influences nutritional quality, with older grasses exhibiting increased proportions of structural carbohydrates in the cell wall and decreased cellular protein content (Verdecia *et al.*, 2011).

The Maralfalfa grass is originally from Colombia and was obtained through hybridization between *Pennisetum glaucum* (L.) R. Br. (syn. *Americanum* (L.) Leeke) and *P. purpureum* Schumacher. It thrives in altitudes ranging from 10 to 2,400 meters on soils with high organic matter content and good drainage. These soils are predominantly loamy-clay and loamy-sandy types, found in relatively dry climates with soil pH ranging from 4.5 to 5.0 (Castrejón *et al.*, 2017).

Maralfalfa has been utilized by livestock farmers in various regions of Chiapas; however, inadequate management has hindered its full production potential. Due to this lack of knowledge, it has not been effectively utilized in feeding systems to enhance meat and milk yields. In a study conducted by Citalán *et al.* (2012) in the Chiapa de Corzo region, production ranged from 9 t ha^{-1} of green matter at 30 days to 65.33 t ha^{-1} at 90 days. In Villaflores, Cárdenas *et al.* (2012) observed increases in total dry matter yield from 4.78 to 20.20 t ha^{-1} defining 60 days as the optimal age for cutting.

Research in other states demonstrates Maralfalfa's yield potential and quality. In Veracruz, Calzada *et al.* (2014) reported yields of 37,297 kg DM ha^{-1}. Ventura *et al.* (2017) achieved yields ranging from 8.2 to 20.2 t DM ha^{-1}. López *et al.* (2020) observed maximum growth rates at 56 days in fertilized plots and a maximum production of 10.4 t ha^{-1}. In Nayarit, Villanueva *et al.* (2022) observed yields of 50.2 t DM ha^{-1}. Gómez *et al.* (2020) concluded that Maralfalfa has superior nutritional value compared to most tropical grasses, with dry matter production of 17.0 t ha^{-1} at 60 days of regrowth. However, Hermitaño *et al.* (2022) mention that the optimal nutritional value based on *in vitro* dry matter digestibility and crude protein content occurs at 45 days of regrowth. In the eastern region of Yucatán, applying nitrogen and phosphorus resulted in the highest dry matter production for Maralfalfa during the rainy season, surpassing varieties such as Taiwan, OM-22, and CT-22 (Ramos *et al.*, 2015).

Maralfalfa is suitable for forage production during the dry season in the tropics, and due to its quality, it yields less than 200 mL g DM^{-1} and less than 50 mL of CH_4 g DM^{-1} (Camacho, 2020). In a study by Vargas and Cruz (2023), the amount of available carbon for Maralfalfa was quantified at 8.28 t of CO_2. Therefore, it is a forage grass with high yield and nutritional quality that, when properly managed, can be used by livestock farmers to transition from traditional livestock farming to alternative production systems with greater economic benefits and high environmental efficiency.

Accordingly, in humid tropical conditions, the cutting age of Maralfalfa grass is crucial for dry matter production and nutritional quality. Therefore, it is considered necessary to estimate the optimal cutting point to harvest the highest nutrient yield per hectare.

MATERIALS AND METHODS

The study was conducted at the Rosario Izapa Experimental Field of INIFAP, located in Tuxtla Chico Municipality, Chiapas, at coordinates 14.961864 latitude, −92.153272 longitude, and an elevation of 398.41 meters above sea level. The climate is warm and humid (INEGI, 2024), with monthly temperatures recorded as follows: average, maximum, and minimum of 26.2, 27.0, and 25.6 °C, respectively. The average annual precipitation is 3,894.6 mm, with the rainy season occurring from June to September. The driest month still receives over 40 mm of rainfall, and the winter rainfall percentage ranges from 5 to 10.2% of the total annual precipitation. The annual total evaporation is 1,384.2 mm, with an average of 115.35 mm, and maximum and minimum values of 155.0 and 111.5 mm, respectively. Solar radiation intensity averages 7 ± 1 hours of sunshine (CONAGUA, 2024). The soil type is classified as Eutric Sideralic Cambisol (Clayic, Humic) (INEGI, 2024), with varying textures among horizons: clayey, clay loam, and clay loam crumbs. The pH ranges from 5.7 to 5.9, organic matter content varies from 9.2 to 1.3%, and total nitrogen content ranges from 0.05 to 0.35% (Gómez *et al.*, 2018).

The grass was established under a randomized complete block design with a split-plot arrangement, comprising three replications. The main plot consisted of Maralfalfa grass, while the subplot treatments were cutting frequencies (Rao, 1998). Cutting frequencies were applied at 28, 42, 56, 70, 84, and 97 days after uniformization cutting. Yield component variables such as plant height (cm), number of tillers per m^2, and green matter yield (GM) in t ha^{-1} were measured. GM yield was determined by weighing the fresh weight of plants harvested from a square meter area cut at 15 cm height. To estimate dry matter yield (DM) in t ha^{-1}, a homogeneous 200 g wet sample was dried to constant weight at 60 °C. The initial weight was subtracted from the final weight, divided by the initial weight, and multiplied by 100. This percentage was then multiplied by the green matter yield to obtain the dry matter yield.

The dried samples were ground using a Wiley mill with a 2 mm sieve and scanned using a Near-Infrared Reflectance Spectrophotometer (NIR) to determine the percentages of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Bonilla *et al.*, 2015; Basurto *et al.*, 2009), percentage of *in vitro* dry matter digestibility (DIVMS), and of rumen degradable protein (PDR) (Hoffman *et al.*, 1999). These analyses were conducted at the National Center for Research in Animal Physiology and Improvement of INIFAP, located in Ajuchitlán, Querétaro.

The data for plant height, number of tillers, green matter yield (GM) in t ha^{-1}, dry matter yield (DM) in t ha^{-1}, percentages of crude protein (CP), rumen degradable protein (%RDP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and *in vitro* dry matter digestibility (%DIVMS) were analyzed using the General Linear Model (Proc GLM) procedure of SAS (2011).

RESULTS AND DISCUSSION

Yield Components of Maralfalfa Grass

The results observed in Figure 1 (A) and (B) demonstrate that Maralfalfa grass has the capability of producing 18 to 42 tillers from 28 to 97 days. Simultaneously, plant height increases from 0.54 to 2.74 m. The tillering capacity of Maralfalfa is influenced by the activation of growth meristems; during the vegetative state, tillering occurs from meristematic centers that produce new plants. Tillers are structural units where new leaves, stems, and roots originate (Buguet and Bavera, 2001).

The green matter yield was 4.55, 9.93, 15.40, 26.25, 29.65, and 40.56 t ha^{-1} at 28, 42, 56, 70, 84, and 97 days of maturity, respectively. Maralfalfa's high capacity to accumulate green matter under tropical climate conditions provides ample availability of green forage to support feeding a larger number of animal units per day. This capability has been observed in various environments by other authors such as Prudencio *et al.* (2020), who reported green matter productions of 98.06 t ha^{-1} at 226 days post-planting. Similarly, Peña *et al.* (2020) achieved green matter yields ranging from 23.89 to 25.23 t ha^{-1} at 90 days of regrowth, using different fertilizer sources and doses. Cuzco *et al.* (2021) observed a green biomass accumulation of 17.4 t ha^{-1} at 45 days after cutting.

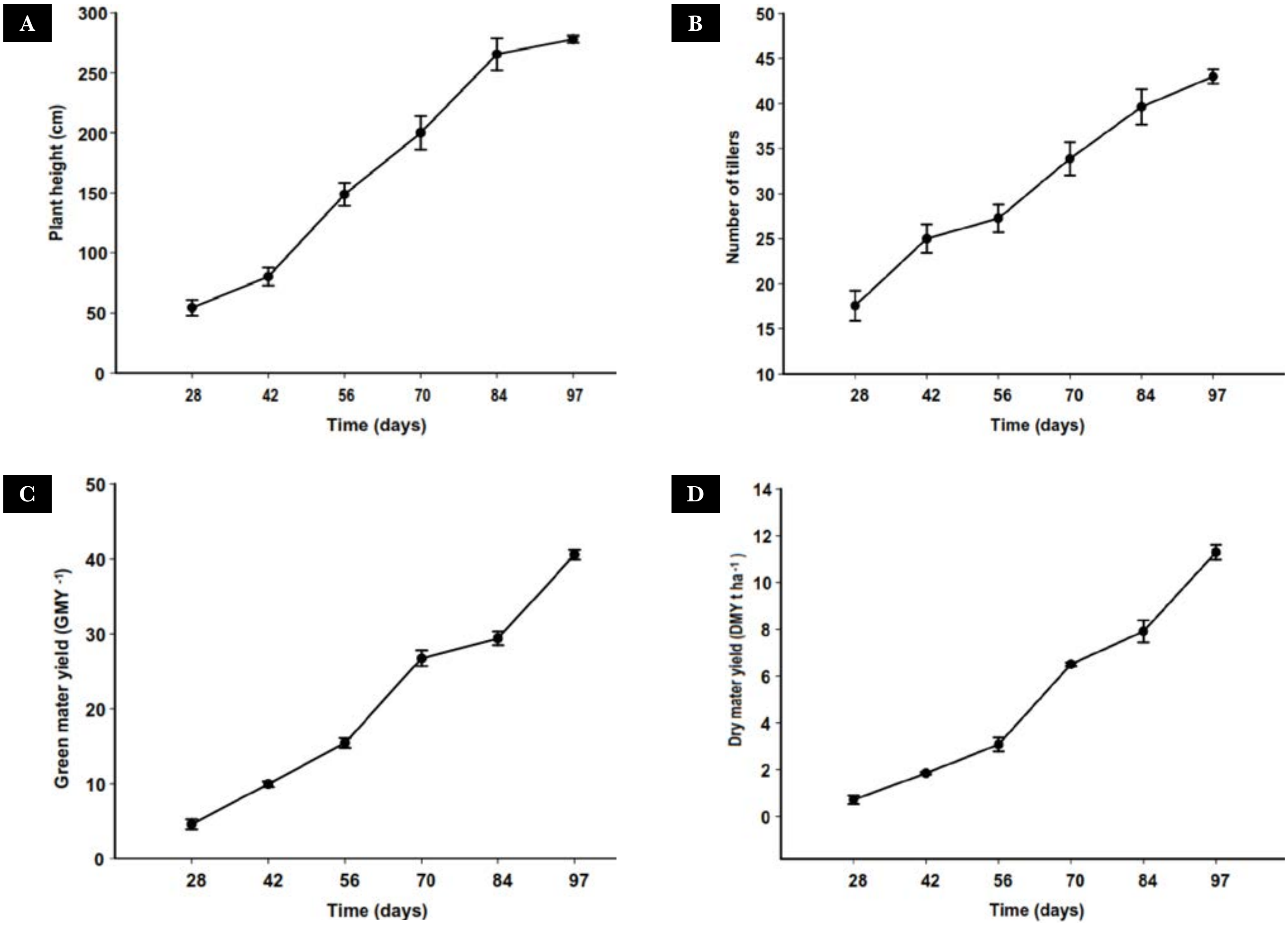

Figure 1. (A) Average number of tillers + SD; (B) Influence of maturity on average height (cm) + SD; (C) Average green matter yield t ha^{-1} + SD; and (D) Average dry matter yield t ha^{-1} + SD of Maralfalfa grass under the climate and soil conditions of Tuxtla Chico, Chiapas.

The dry matter yield of Maralfalfa grass was 0.71, 1.84, 3.18, 7.94, 8.00, and 11.38 t ha^{-1} at 28, 42, 56, 70, 84, and 97 days, respectively. Other studies highlight the yield potential in various environments: Del Aguila *et al.* (2023) achieved 557.8 kg DM ha^{-1} per cutting^{-1} at 49 days of regrowth in the humid tropics of Pucallpa, Peru. Reyes *et al.* (2021) reported 20.9 t DM ha^{-1} at 60 days of regrowth with the application of a complete formula, 30 g per plant of NPK-Mg-S (21-0-28-2-3). Arrieta *et al.* (2024) observed dry matter yields of 67.03 t ha^{-1} in the rainy season, 67.37 t ha^{-1} in the dry season, and an annual yield of 134.41 t ha^{-1}, demonstrating Maralfalfa's genetic capacity to maintain stable production under different environmental conditions. Maldonado *et al.* (2021) assert that the regrowth age of the plant significantly affects dry matter yield.

The chemical and nutritional composition of Maralfalfa grass was affected by maturity. Table 1 shows that the percentage of crude protein (CP) decreased from 16.26% to 7.16% from 28 to 97 days. These findings differ from those observed by Ramos *et al.* (2014), where an increase in plant age at cutting from 40 to 70 days significantly reduced protein content from 19.1% to 10.3%. Conversely, Márquez *et al.* (2007) recorded a protein content of 7.28% at 49 days after cutting. In a separate study, Correa (2006) reported protein values of 21.8% at 56 days and 11.9% at 100 days.

When the CP content of grass decreases, the availability of ruminal ammonia nitrogen also decreases, limiting microbial activity, reducing digestion rate, and slowing digesta transit (Hanigan *et al.*, 2021; Castillo *et al.*, 2014). Additionally, dry matter intake is affected (Mora *et al.*, 2015). In the present study, it was observed that rumen degradable protein (PDR) decreased from 9.95% on day 28 to 3.35% on day 96 after cutting. At the beginning of the growth stage, PDR constitutes 60% of the forage protein, decreasing to less than 50% after day 70.

As the vegetative cycle of the grass progresses, the NDF content increases due to cell wall lignification, which correlates negatively with forage digestibility (Estrada, 2002). To achieve optimal production results, it is recommended to provide forages containing 32 to 60% NDF (Shimada, 2005). Accordingly, Maralfalfa grass would ideally be harvested at a maximum of 28 days of age. However, at this age, its growth and dry matter accumulation are low. Therefore, there needs to be a balance between nutritional value and dry matter production, aiming for maximum production of digestible nutrients per hectare. Nava *et al.* (2021) detected differences in NDF content among regrowth ages, increasing from 67.7% at 72 days to 76.3% at 114 days. Álvarez *et al.* (2021) observed that as the age of the Maralfalfa plant progresses, the NDF content increases. In full plant analysis, they observed 67.06% at 33 days of regrowth, up to 79.06% at day 167, and they suggest that the optimal cutting time may be at 61 days.

The ADF (acid detergent fiber) is the fraction of dry matter that is not digestible, and its content showed a similar trend, increasing from 33.31% to 45.75% as the cutting age increased from 28 to 96 days. Lignification negatively impacts the digestion of cell walls, thereby reducing *in vitro* dry matter digestibility (IVDMD) with increasing ADF content.

In the present study, the IVDMD of Maralfalfa grass decreased as the cutting age increased (Table 1); specifically, it was 78.50% at day 28 and decreased to 64.91% at day 96. Ortíz *et al.* (2018) observed a reduction in true in vitro dry matter digestibility as the sampling period progressed, with values of 79.4% at 39 days of regrowth and 60.7% at 90 days. In another study, Knowles *et al.* (2008) reported an IVDMD of 62.3% at 45 days of regrowth. Sosa *et al.* (2006) obtained an IVDMD of 68.11% at 70 days, while Clavero and Razz (2009) achieved 62.45% after three weeks of growth.

Table 1. Influence of maturity on nutritional quality variables in Maralfalfa grass under the climate and soil conditions of Tuxtla Chico, Chiapas.

Quality variables (%)	**Days of maturity at cutting**					
	28	**42**	**56**	**70**	**84**	**96**
Crude protein	16.26	14.91	9.44	7.54	7.33	7.16
Rumen degradable protein	9.95	8.99	5.70	4.29	3.6	3.35
Neutral detergent fiber	60.47	62.9	71.19	72.2	72.59	73.50
Acid detergent fiber	33.31	36.31	42.42	42.48	43.96	45.75
in vitro dry matter digestibility	78.5	76.56	70.17	66.59	64.69	64.91

The results suggest the importance of considering the optimal cutting age between 42 and 56 days to maximize dry matter yield and maintain a balanced relationship between nutritional quality and forage productivity. The implications of this study highlight the relevance of adjusting Maralfalfa grass management practices according to its maturity to optimize its value as a feed resource for livestock in sustainable livestock production systems in humid tropical climate regions.

CONCLUSIONS

As the cutting age of Maralfalfa grass increases, there is an increment in tiller number, plant height, and both fresh and dry matter yield. However, protein content (PC) and rumen degradable protein (PDR) decrease, while neutral detergent fiber (FDN) and acid detergent fiber (FDA) contents increase, resulting in a reduction in *in vitro* dry matter digestibility (IVDMD). Therefore, the optimal cutting age for Maralfalfa grass ranges between 42 and 56 days of regrowth to maintain a balance between forage productivity and nutritional quality in livestock production systems in humid tropical climate regions.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Analysis of the natural regeneration of the white mangrove (*Laguncularia racemosa* (L.) C.F. Gaertn. with dasometric variables during the drought season

Sánchez-Díaz, Baltazar[1,2]; Sol-Sánchez, Angel[1*]; Hernández-Melchor, Gloria I.[3]; Gerón-Cano, Hugo E.[1]; Zaldívar-Cruz Juan M.[1]

[1] Colegio de Postgraduados - Campus Tabasco, Periférico Carlos A. Molina S/N Km. 3, Periférico Carlos A. Molina S/N, Ranchería Río Seco y Montaña, Heroica Cárdenas, Tabasco, México, C. P. 86500.
[2] Tecnologico Nacional de México - Campus Comalcalco, carretera vecinal, Paraíso - Comalcalco Km. 2, RA Occidente 3ra sección, Comalcalco, Tabasco, México, C. P. 86650.
[3] Universidad Autónoma de Chiapas, Facultad Maya de Estudios Agropecuarios, Carretera Catazajá-Palenque, Km. 4, . Catazajá, Chiapas México, C. P. 29980.
* Correspondence: sol@colpos.mx

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ABSTRACT

Objective: To analyze the natural regeneration of the white mangrove (*Laguncularia racemosa* L.) with dasometric variables, during the drought season, in the ejido La Solución Somos Todos in Paraíso, Tabasco, Mexico.
Design/Methodology/Approach: The structural variables of white mangrove —diameter at breast height (DBH), height, and canopy diameter— were measured in three sampling plots. A statistical analysis was performed with the resulting data and the mean data were determined for the three sampling plots.
Results: The following data were recorded for each sampling plot: DBH (0.34, 0.37, and 0.42 cm), height (134, 128, and 158 cm), and canopy (44.18, 38.56, and 39.83 cm).
Study Limitations/Implications: In the dry season, erosion can expose rocky and sandy substrates that are not suitable for mangrove growth.
Findings/Conclusions: The white mangrove —which undergoes its brinzal (seedling) stage during the dry season— recorded statistically significant differences in growth between sampling plots using the dasometric variables (DBH, height, and canopy diameter).

Keywords: mangroves, conservation, sustainability, water, salinity.

INTRODUCTION

The natural regeneration of mangroves is a vital process for the health of these ecosystems, but it can be severely hindered by various factors, especially alteration of environmental conditions (Vargas-Fonseca, 2014).

Laguncularia racemosa L. is a very attractive species for restoration programs, because of its better chances for survival. Furthermore, its rapid growth rate and its ability to colonize degraded areas makes it a promising species for natural regeneration (Guerra-Santos *et al.*, 2015).

Image by WikimediaImages at Pixabay

The relationship between allometric measurements and the evaluation of natural regeneration of *Laguncularia racemosa* during the drought season are crucial to understand and address its adaptation and growth difficulties (Flores-de-Santiago *et al*., 2012). In this context, dasometric measurements (*e.g*., height, canopy diameter, and breast height diameter) can help to evaluate the response of plants to drought conditions and the adaptation strategies that might be effective (Sobrado and Ewe, 2006). These strategies could provide information on which traits are of the utmost importance for the survival and growth of *Laguncularia racemose* under drought conditions, which in turn could provide management and conservation guidelines (Sánchez-Toruño *et al*., 2022).

Given the crucial role of mangroves for ecosystem services and their importance in climate change mitigation, ongoing research and conservation efforts are essential. Measuring the dasometric variables in these ecosystems is fundamental to establish effective conservation and restoration policies and actions. Therefore, this research aims to analyze the natural regeneration of the white mangrove (*Laguncularia racemosa* L.) with dasometric variables, during the drought season in the ejido La Solución Somos Todos in Paraíso, Tabasco, Mexico.

During the drought season, conditions such as water level, salinity, and light availability are expected to significantly impact the growth, structure, and survival of the white mangrove (*Laguncularia racemosa* L.), which will be reflected in remarkably different the structural and dasometric variables of *Laguncularia racemosa* in different sample plots.

MATERIALS AND METHODS

Study site

The study was carried out at the UMA (Unit for the Conservation, Management and Sustainable Use of Wildlife) La Solución Somos Todos, located in the municipality of Paraíso, Tabasco, north of the state capital, in southeastern Mexico. The UMA is located in 18° 19’ 00” and 18° 23’ 00” N and 93° 04’ 00” and 93° 07’ 00” W (Figure 1).

Data collection

Field data collection was carried out during the drought season (February to June 2023), when no precipitation was recorded. During the drought season the solar radiation reaches its maximum point in the study area and, consequently, the mangrove canopy is in a dormant state and photosynthetic activity requires a careful use of water. Under these conditions, the temperature increase can affect or limit the growth of the white mangrove (Berlanga-Robles *et al*., 2019). Three measurements were taken in February, April, and June. For this purposes, three 10×10 m plots were established (Fonseca *et al*., 2007).

Measurements

Each mangrove plant was labeled at the beginning of the study. A 6” Truper® stainless steel analog vernier caliper (std and mm) was used to measure the diameter at chest height (30 cm above the soil level). The height of the mangroves was measured with a 3 m long graduated ruler, while the diameter of the canopy was recorded with a measuring tape. In total, 586 plants were monitored, out of which 207, 197, and 182 were located in plots

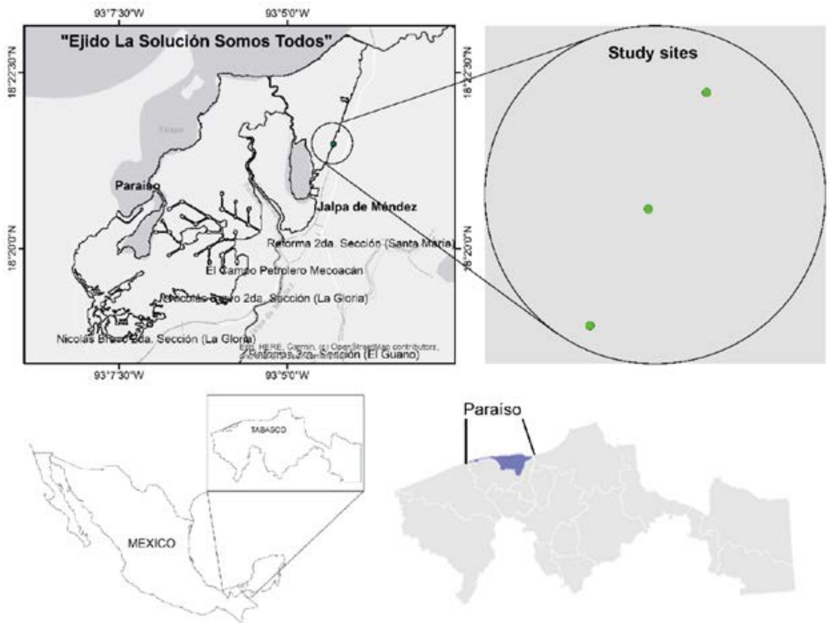

Figure 1. Geographical location of the UMA La Solución Somos Todos, Paraíso, Tabasco.

1, 2, and 3, respectively. The monitoring period was 6 months during the drought season (February to July); no precipitation was recorded during this period.

Dasometric variables

Allometric functions are used to estimate growth in relation to certain physical parameters of the tree, such as diameter at breast height, total height, and canopy diameter (Aye *et al*., 2022). The dasometric analysis includes variables such as DBH, height and canopy diameter that are crucial to understand the structure and growth of individual trees.

Statistical analysis

The recorded data were analyzed using the non-parametric Kruskal-Wallis test to estimate the growth of the species during this period.

RESULTS AND DISCUSSION

White mangrove height

Statistically significant differences ($p<0.0001$) were recorded among the variables for the three plots. The height means for each of the plots were 134 cm, 128 cm, and 158 cm, respectively. The resulting data can be compared with the findings of other researchers. For example, Elster (2000) reported that the height of *L. racemosa* in the mangroves of Cénaga Grande de Santa Marta in Colombia during the drought season ranged from 109 cm (minimum) and 140 cm (maximum). Figure 2 shows the mean height of white mangrove brinzal in each of the three monitored plots: no statistically significant differences were reported in plots 1 and 2, but plot 3 had statistically

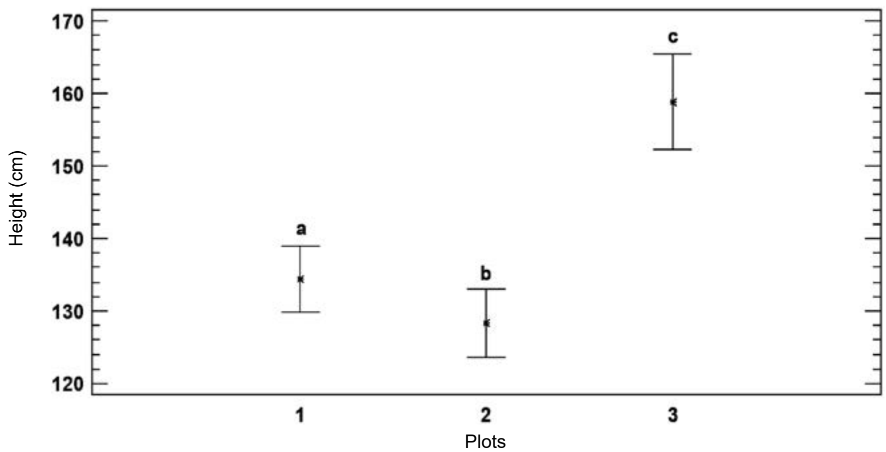

Figure 2. Statistical differences in mean *brinzal* height between white mangrove monitoring plots. Different letters indicate the average and significant statistical differences.

significant differences regarding the other two plots. According to the physiological requirements, light incidence and water availability may have influenced the growth of the plants during the evaluation period. Meanwhile, the database shows that the water depth on the same site reached 26 cm and 49 cm on May 5 and on May 22, respectively; comparatively speaking, the development of the roots of the plants was much slower and therefore the smallest were the least favored. Soil salinity and inter- and intra-specific competition are other factors that influence the growth of these species The site salinity data recorded 49 and 51 parts per thousand (ppt) on May 5 and 22, respectively; nevertheless, this is an acceptable range. The lack of water in the plant generates stress and, at a higher concentration of salt, the basal increase of white mangroves is negatively affected. Consequently, adult plants could have few expectations (Monroy-Torres *et al.*, 2014; Rodríguez-Rodríguez *et al.*, 2016).-

Diameter of white mangrove at breast height

Statistically significant differences were recorded among the three plots ($p<0.0001$) regarding this variable. The values obtained per plot were 0.34, 0.37, and 0.42 m (Figure 3). The data obtained are comparable with other researches. For example, the *L. racemosa* mangroves from Samaná Bay, Los Haitises National Park, Dominican Republic, recorded a 0.46 cm DBH during the drought season (Sherman *et al.*, 2000). Meanwhile, the DBH of *L. racemosa* mangroves in Puerto Rico, during the drought season, was 0.56 cm (Branoff, 2020).

Canopy diameter of white mangrove

Statistically significant differences ($p=0.0009$) were recorded for this variable among the three plots. The canopy diameter values obtained per plot were 44.18, 38.56 and 39.83 cm (Figure 4). Increased soil temperature and decreased humidity during the drought season can lead to a reduction in the leaf area index (LAI) of white mangrove. This decrease in LAI may have implications for the health and productivity of the

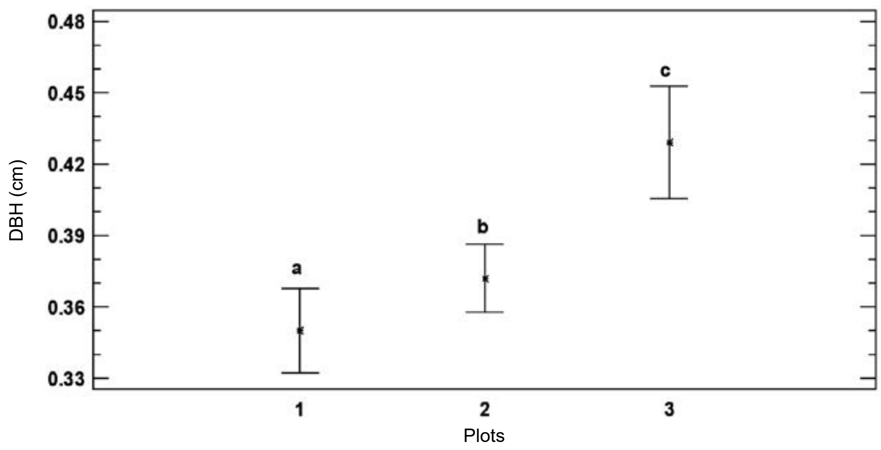

Figure 3. Mean diameter at breast height of white mangrove *brinzal* in the different plots. Different letters indicate the average and significant statistical differences.

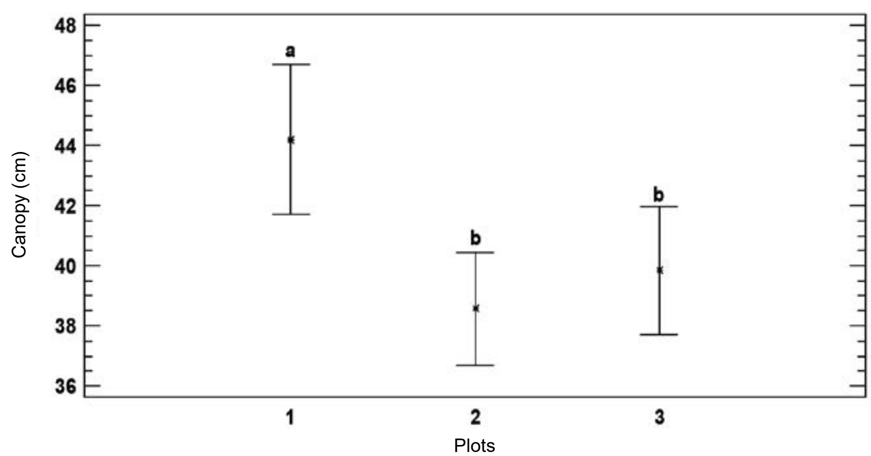

Figure 4. Mean canopy diameter of white mangrove *brinzal* in the different plots. Different letters indicate the average and significant statistical differences.

mangrove ecosystem, since the white mangrove is a key species in these coastal habitats (Flores-de-Santiago *et al.*, 2012).

CONCLUSIONS

The study recorded significant differences in the height, diameter at breast height (DBH), and canopy diameter among three monitoring plots of white mangroves. These differences indicate variations in environmental conditions such as light incidence, water availability, and soil salinity. These dasometric measurements are crucial for understanding the growth and adaptation of white mangroves, as they reveal the species' response to environmental stressors like drought and salinity. Understanding the complex nature of the mangrove ecosystems requires multiple variables, such as water level and salinity, to assess their health and survival. The study suggests that the death of seedlings might be a consequence of the increased salinity caused by a reduction in water intake, although this conclusion is not backed by any corroboration measurements. Further research into the structural variables of white mangroves during different growth stages and seasons is recommended. Additionally, incorporating factors like water level and salinity into growth studies can help to make informed decisions regarding sustainable mangrove use, ensuring viable harvesting practices and the long-term health of the species.

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Evaluation of three forages as a source of fiber in diets of fattening rabbits in Aguascalientes, Mexico

Mejía-Haro, Ignacio[1,*]; Tirado-Estrada, Gustavo[1]; Ramos-Dávila, Mauricio[1]; Pinales-Jiménez, Francisco J.[2]; Tirado-González, Deli N.[1]; Aréchiga-Flores, Carlos F.[3]

[1] Instituto Tecnológico El Llano, Aguascalientes, Tecnológico Nacional de México, Km. 18 Carretera Aguascalientes-S.L.P., El Llano, Aguascalientes, México, C. P 20330.
[2] MSc in Agricultural Biotechnology by ITEL (thesis pending).
[3] Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Zacatecas, Zacatecas, México.
* Correspondence: ignacio.mh@llano.tecnm.mx

ABSTRACT
Objective: To evaluate three forages as a source of fiber in the diets of fattening rabbits.
Design/Methodology/Approach: Whole grain diets with forage oat, mesquite pod, and alfalfa were used. Thirty-six weaned male rabbits were randomly distributed into three treatments (T1, forage oat diet; T2, mesquite pod diet; T3, alfalfa diet). Feed consumption, daily weight gain, total weight gain, and feed conversion were recorded. The animals were slaughtered to evaluate carcass yield. The data were statistically evaluated by analysis of variance and Tukey's test.
Results: T1 recorded greater fattening than both T2 and T3 ($P<0.05$) and the last treatment surpassed T2 in daily weight gain, total weight gain, and feed digestibility. Regarding feed conversion, T1 and T3 had lower results than T2. In carcass yield, T1 was higher than T2 and T3 —which, on its turn, surpassed T2. Finally, no differences were observed in feed consumption between treatments ($P>0.05$). There were also no significant differences in growth.
Study Limitations/Implications: Mexicans have a low consumption of rabbit meat. The mesquite pod could be a viable alternative due to its low cost and availability in semi-arid areas.
Findings/Conclusions: Forage oat recorded the best productive parameters, followed by alfalfa and mesquite pod; however, the latter had a greater economic advantage.

Keywords: Rabbit production, productive parameters, forage oat, mesquite.

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INTRODUCTION

Nowadays, large companies sell rabbit food in the market at very high prices, which limits the profitability of production (Maertens, 1999; Nieves and Calderón, 2001). Likewise, producers are not allowed to change the nutritional composition according to the registered progress and requirements of the rabbits; therefore, producers would benefit from the capacity to formulate and prepare their feeds, using ingredients and forages that are more accessible and inexpensive than alfalfa, which is commonly used in rabbit diets

(Machado *et al.*, 2007; Asar *et al.*, 2010). Forages have different fiber and component contents, which provide them with a particular quality that is reflected in digestive health, mortality rate, feed digestibility, and response in productive parameters (Maertens, 1999; Gidenne, 2015).

In the past, diets were formulated based on the content of crude protein, crude fiber, and some amino acids (NRC, 1977; Nieves *et al.*, 2002; Akande, 2015). Subsequently, European research teams have studied the importance of the quality of the fiber contained in forages, determining the minimum level of acid detergent fiber required to reduce the mortality of weaned rabbits (Gidenne, 2015). On the contrary, alfalfa is the standard forage used as a source of fiber in feed formulation; although it has an acceptable quality, its cultivation requires an enormous volume of water and, in some seasons of the year, it is very expensive. Therefore, using regional, less expensive (Machado *et al.*, 2007; García-Sánchez *et al.* 2023), and more readily available forages which require less water than alfalfa would be advantageous. In this sense, other forages should be analyzed for their inclusion in the diets of rabbits and their productive and digestibility response must be evaluated.

Dairy producers in Aguascalientes have employed several alternative forages that could also be used in rabbit diets due to their good quality. These forages include corn (*Zea mays*) and sorghum (*Sorghum bicolor* L. Moench) silage, as well as winter crops, such as triticale (× *Triticosecale* Wittmack. Ex.), oats (*Avena sativa*), and wheat (*Triticum aestivum* L.) (Llamas and Núñez, 2002). Green forages could be an alternative for rabbit diets, including partially-dehydrated hydroponic corn (Sánchez-Laino *et al.*, 2010).

Feeding costs account for 60 to 70% of the total expenses of a rabbit farm (García-Sánchez *et al.*, 2023). Meanwhile, forages included in more than 30% of the mixed diet provide the minimum fiber required by rabbits. Usually, commercially available feeds use hayed alfalfa as a source of fiber. Alfalfa cultivation has a high-water consumption and its price in the market is consequently high and variable throughout the year, doubling in winter or periods of drought. In contrast, forage oat is a winter crop with low water consumption, high availability, and an adequate and constant price, while the mesquite pod is collected when the fruit ripens and falls and only entails milling costs (Mejía-Haro *et al.*, 2023).

The objective of this research was to evaluate three forages as additional sources of fiber in fattening rabbit diets, considering their availability, cost, and response in terms of productive parameters.

The hypothesis was that using forage oat and/or mesquite pods in a mixed diet for fattening rabbits can yield similar productive parameters to hayed alfalfa.

MATERIALS AND METHODS

The study was carried out from May to December 2022 at the CUNICARNE rabbit farm, located in the community of Las Palomas, municipality of Aguascalientes, in km 12 of highway 42, at 21° 44' 48" N and 102° 21' 04" W, and at 1,885 meters above sea level. It has a semi-dry climate, a mean annual temperature of 17.4 °C, and a mean rainfall of 526 mm (Instituto Nacional de Estadística y Geografía [INEGI], 2017).

Experimental design

Thirty-six one-month old weaned male rabbits, with an average weight of 400 grams, of the California breed, were distributed with a completely randomized design into three treatments: T1, diet with forage oat (*Avena sativa* L.); T2, diet with mesquite pod (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst.); and T3, diet with alfalfa hay (*Medicago sativa* L.). The experimental period lasted 50 days. The first 30 days were considered the growth stage and the following 20 days, the fattening or finishing stage (García-Sánchez *et al.*, 2023).

Ingredients and nutritional composition of forage

The forages were milled with a Rayken® RKP 3000 mill (Raiker), with a particle size of 2.54 cm. They were incorporated into the formulation of a diet with 16% crude protein and 2.8 Mcal/kg of metabolizable energy. The diets were isoproteic and isocaloric (Table 1) and were processed in a mixer (Garmak, Michoacán, Mexico) and finally shaped into pellets (Rommel, model Wkl 120c, China). The diets were subjected to a proximal analysis to determine crude fiber (CF), crude protein (CP), fat, and ash by wet chemistry (AOAC, 2012), as well as to analyze their neutral detergent fiber (NDF) and acid detergent fiber (ADF) content (Van Soest *et al.*, 1991).

In addition, the Ca and P contents were analyzed by atomic absorption spectrophotometry (Dorta and Ciarfella, 2014). The metabolizable energy was calculated considering the equations of Nolan and Savage (2009).

The rabbits underwent a 10-day pre-experimental adaptation in a stall, divided into individual metal cages with a feeder and a nipple drinker. The animals were weighed with a YL TRD® gram scale, on day 1 of the experimental period and every week until day 50. They were fed *ad libitum*, with food served every day at 9 AM throughout the experimental period. Rejection and consumption per day were also recorded.

Evaluation variables

Feed consumption (g dry matter (DM) and g DM/kg live weight (LW)), daily weight gains (DWG) and total weight gains (TWG) were measured. The feed conversion (FC=WG/dry matter (DM) intake) was calculated with those data. At the end of the fattening period, the rabbits were slaughtered to evaluate the yield and characteristics of the hot carcass. The offal (head, hide, tail, and legs), viscera (liver, heart, kidneys, and lungs), digestive organs (stomach, intestines, and cecum), and perirenal and scapular fat were weighted.

Subsequently, an *in vivo* dry matter digestibility (DMD) test was carried out, using six adult rabbits per treatment. The rabbits were fed 70% of their daily consumption for two weeks to guarantee that they would fully eat the same amount each day. Feces were collected in trays daily and data were recorded for the last 6 days. The feces were dried in a Felisa® 293 D air circulation oven for two days at 60 °C to determine the DM. The indigestible part was then calculated and, the percentage of DMD was calculated by difference (DMD=Dry feces/DM Intake*100).

Table 1. Ingredients and nutritional composition of the rabbit diet (g/100 g on a wet basis).

Ingredients*	T1	T2	T3
Alfalfa hay	0	0	35
Ground *Prosopis laevigata* pods	0	31	0
Oat hay	30.9	0	0
Soy bean meal	9.5	12.2	5.9
Canola meal	4	4	4
Ground corn	32.8	39.5	32
Ground sorghum	14.2	8.7	13.5
Vegetable oil	4.4	0.5	5.6
Fish meal	3	3	3
Monocalcium phosphate	0.7	0.5	0.4
Ca CO_3	0	0	0
Salt	0.3	0.3	0.3
Vitamins**	0.1	0.1	0.1
Trace minerals	0.1	0.1	0.1
TOTAL	100	100	100
Nutritional Composition %			
Dry matter (DM)	88.4	86.6	86.6
Ash	5.2	3.9	6
Crude Protein (CP)	16.0	16.0	16.0
Fat	7.7	4.6	7.1
Crude Fiber (CF)	17.9	17.1	16
Acid Detergent Fiber (ADF)	15.3	13	14.1
Neutral Detergent Fiber (NDF)	23.6	18.2	20.3
Non-Fiber Carbohydrates (NFC)	46.7	47.0	44.7
Metabolizable Energy (Mcal/Kg)	2.824	2.854	2.835
Calcium (Ca)	0.50	0.52	0.67
Phosphorous (P)	0.35	0.32	0.32
Lysine	1.27	1.13	1.19
Methionine + Cisteine	0.91	0.83	0.90

Non-Fiber Carbohydrates *=100−(% NDF+%CP+%Fat+%Ash), Metabolizable Energy=TDN* 4.4* 0.82. *Ingredients expressed in kg as fed basis. **Vitamins Vitafort.

Statistical analysis

The data were evaluated with the Statistical Analysis System (SAS; V. 9.2; 2013) package, using general linear procedures (PROC GLM). The analysis of variance (ANOVA) considered the effect of forage type, according to Model 1. Treatment means were compared using Tukey's test (P=0.05).

$$\text{Model (1)} \quad Y_{ij}=\mu+T_i+\varepsilon_{ij}$$

Where Y_{ij}=Feed consumption, DWG, TWG, DMD, μ=general mean, T_i=effect of the *i*-th type of forage, and ε_{ij}=experimental error.

RESULTS AND DISCUSSION

Productive behavior variables

Feed consumption. No significant differences ($p>0.05$) were recorded between treatments in the growth and fattening phases (Tables 2 and 3). This could be partially due to the pellet shape of the feed in all treatments, avoiding the selection of particles (Loor-Mendoza, 2016). Additional causes can include the similarity in the CP, ME, NDF, and ADF content of the diets and in the initial weight of the rabbits since consumption is related to LW. Another factor that could have influenced the lack of differences in consumption between treatments was the low daily food rejection, which suggests a lower food selectivity between the cylindrical and powdered or divided particles. In addition, consumption/kg LW also showed no differences between fiber sources ($p>0.05$). Contrary to the results of this experiment, García *et al.* (2023) reported lower consumption in diets with 15-30% mesquite pod flour than in the control (alfalfa as a forage source). The nutritional composition of forages has a wide range, mainly regarding the quantity and composition of detergent fibers, which affects digestibility and production parameters (García-López *et al.*, 2019).

Weight gain. The DWG, TGW, and final gain did not show differences between treatments ($p>0.05$) in the growth stage. Meanwhile, the rabbits fed on forage oat (T1) in the fattening or finishing stage had better indicators of productive behavior than rabbits fed on the T2 and T3 forage sources ($p<0.05$) (Table 2). Despite the lack of differences among treatments in feed consumption, other factors were involved with TGW, such as differences in the DMD coefficient. This phenomenon was reflected in differences in the feeding efficiency of the diets and a difference in weight of 3-5 g/d and 63-100 g/d in the period among treatments. The DMD coefficient was higher in the treatments with alfalfa and forage oat (T3 and T1) than those in which rabbits were fed on mesquite pod (T2), potentially influencing the efficiency of feed use and improving total weight gains. Similar results were reported by García *et al.* (2023) in diets with 0, 15, and 30% mesquite pod flour, where rabbits fed on 30% mesquite pod flour gained less weight and recorded lower *in vivo* digestibility than rabbits that received other treatments. In contrast, Igwebuike *et al.* (2013) did not find statistical differences in weight gain and digestibility of dry matter and other nutrients in rabbit diets with 10, 20, 30, and 40% of *Prosopis africana* pulp (Guill. and Perr.) and reported lower values in the three forages evaluated than those reported in this work. The fiber composition of forage oat and alfalfa hay is more advantageous than mesquite pod since the seed of the latter is covered by a very hard cuticle that is difficult to degrade and is not always completely milled, causing differences in its composition and digestibility (Mejía-Haro *et al.*, 2023). The daily weight gains recorded in this research are higher than the 37 g reported by Herrera-Soto *et al.* (2018) for the productive behavior test of different breeds of New Zealand rabbits in fattening.

Feed conversion (FC). The FC of the rabbits in the growth stage did not show differences between the treatments ($p>0.05$) (Table 2). However, in the finishing or fattening stage, the FC of the rabbits fed on forage oat (T1) was lower than the FC of rabbits fed on mesquite pod and alfalfa hay ($p<0.05$) (Table 3). Feed conversion efficiency is highly influenced by fiber digestibility, which is associated with the lignin content of food (Gidenne, 2015). Although forages sometimes contain similar concentrations of acid detergent fiber —which is composed of cellulose and lignin— their use has behaved in different ways since they vary both in the lignin content and in the form in which it is embedded in the cellulose molecule (Moore and Jung, 2001).

Mora-Valverde (2010) mentions that more lignified fibers reduce the size of intestinal villi and can atrophy epithelial tissues, altering the normal functioning of intestinal epithelial cells. According to the results, the quality of forage oat cut at its optimal point as feed for fattening rabbits can surpass the quality of mesquite pod and even alfalfa —largely due to its fiber content and composition. Although forage oat and alfalfa have a similar ADF value, the lignin content of alfalfa is almost three times higher than in oats (Gidenne, 2015),

Table 2. Productive parameters of growing rabbits fed on diets with different fiber sources.

Variable	T1	T2	T3	SE	P Value
Initial weight (g)	403[a]	400[a]	391 [a]	31.4	0.09
Feed intake (g)	2078[a]	2054[a]	2085[a]	66.8	0.09
Final weight (g)	1482[a]	1463 [a]	1474[a]	32.51	0.10
TG (g)	1079[a]	1062 [a]	1083[a]	41.5	0.08
ADG (g)	36[a]	35.3 [a]	36[a]	1.28	0.09
FC (kg/Kg)	1.93[a]	1.94[a]	1.94[a]	0.096	0.10

T1, Oat hay; T2, *Prosopis laevigata* pods; T3, Alfalfa hay; ADG=Average daily gain; FC=Feed Conversion (Kg of feed per Kg of live weight gain); TG=Total gain in the period; SE, Standard error; P value, probability value; *Feed intake per rabbit during the period; **Same letters among rows indicates no significant differences among treatments ($P<0.05$).

Table 3. Productive parameters of fattening rabbits fed on diets with different fiber sources.

Variable	T1	T2	T3	SE
Feed intake (g)*	2265[a]	2276[a]	2267[a]	133.6
Feed intake, g DM/kg LW d^{-1}	57.5 [a]	60.3 [a]	57.1 [a]	3.259
Initial weight, (g)	1482[a]	1463[b]	1474[a]	32.5
Final weight (g)	2433[a]	2315[c]	2389[b]	82.3
TG (g)	952[a]	852[c]	915[b]	94.0
ADG (g)	47.6[a]	42.7[c]	45.7[b]	4.7
FC (Kg/Kg)	2.37[c]	2.67[a]	2.49[b]	0.26
In vivo Digestibility (% DM)	70.7[a]	67.7[b]	69.8[a]	0.785

T1, Oat hay; T2, *Prosopis laevigata* pods; T3, Alfalfa hay; ADG=Average daily gain; FC=Feed Conversion (Kg of feed por Kg of live weight gain); TG=Total gain in the period. *Feed intake per rabbit during the period. Feed intake, g/kg LW d^{-1}=Feed intake per kg LW per day; SE, Standard error; P Value, probability value. **Different letters in the same row indicate significant differences among treatments ($p<0.05$).

which partially affects its quality. Meanwhile, the mesquite pod has a higher ADF content than the two other forages and a high level of lignin, which limits its quality (Angeles-Hernandez *et al.*, 2022; Mejía-Haro *et al.*, 2023). For its part, the digestibility of dry matter in oats and alfalfa was higher than in mesquite pods, reflecting a better feed conversion. However, the feed conversion difference between the three forages is only 10 to 12%.

García *et al.* (2023) reported 2.4 and 2.5 feed conversions in diets with 15 and 30% mesquite pod, respectively; these results are similar to the findings of this study. Meanwhile, Adamu *et al.* (2013) reported feed conversions of 6.45, 6.83, 6.30, and 6.31 in rabbits, replacing corn with 10, 20, 30, and 40% *Prosopis africana*, respectively. The genus *Prosopis* contains anti-nutritional compounds that can negatively impact the digestibility of the diet and productive parameters of rabbits, depending on the concentration and the species. This characteristic must be taken into account during the formulation of diets (Akande and Alabi, 2021).

Carcass yield and characteristics

Table 4 shows that, regarding carcass weight and yield, the forage oat (T1) diet had better results than T2 and T3 ($p<0.05$). These carcass yields are closely related to the weight at slaughter and the weight of the viscera. T1 rabbits had a higher weight at slaughter than T2 and T3 rabbits, while the weight of the viscera was lower in T1 than in T2, perhaps due to the type of fiber. The lower digestibility of mesquite pod could cause greater growth of the viscera, mainly in the fattening period, because the development of the rabbit's digestive system increases according to age, weight, and type of diet at the moment of weaning (Carabaño *et al.*, 2020). García *et al.* (2023) also reported lower carcass yields in rabbits fed on diets with 15 and 30% mesquite pod than rabbits fed on diets that included 30% alfalfa. For their part, Igwebuike *et al.* (2013) reported no differences in the carcass yield of rabbits fed on diets with 0, 10, 20, 30, and 40% of *Prosopis africana* and lower values than in this study. Overall, if the digestive organs (mainly, stomach and intestines) are heavier, the carcass yield will be lower (Hernández et al., 2015). In addition, the carcass yield will be influenced by the weight of the rabbits at the time of slaughter, as well as their diet, sex, and the climatic conditions in which they develop (Pilco et al., 2018).

Table 4. Yield and characteristics of rabbit carcasses.

Variable	T1	T2	T3	SE	P Value
Weight of offal (g HB)	411^{a}	319^{c}	386^{b}	2.957	<0.0001
Viceras weight (g HB)	530^{b}	619^{a}	520^{c}	4.75	<0.0001
Weight of peri-renal and scapular fat (g HB)	107^{c}	118^{b}	146^{a}	1.92	<0.0001
Slaugther Live weight (g HB)	2387^{a}	2261^{c}	2342^{b}	17.1	<0.0001
Carcass weight (g HB)	1337^{a}	1203^{c}	1290^{b}	9.796	<0.0001
Carcass (%)	56^{a}	53^{c}	55^{b}	0.08	<0.0001

T1, Oat hay; T2, *Prosopis laevigata* pods; T3, Alfalfa hay; Offal=skin, head, feet; Viceras=Kidnies, liver, lungs, heart, intestines, cecum and urunary bladder. SE, Standard error; P value, probability value. *Different letters in the same row indicate significant differences among treatments ($p<0.05$). HB=Humid basis.

Economic analysis of the diets

Feed costs (Table 5) show that kg of feed formulated with mesquite pod meal is the less expensive (T2), followed by forage oat (T1) and alfalfa hay. Overall, alfalfa maintains a high price throughout the year, and in certain months, it is even more expensive since it is a forage preferably used for other species, such as horses and dairy cows (Estrada-Prieto, 2018). On the contrary, rural communities collect mesquite pods from the ground and subsequently dry and grind them (Mejía-Haro *et al.*, 2023), resulting in lower expenses. They can also be obtained from other farmers at affordable prices. As for forage oat, it is available at affordable prices throughout the year, without major fluctuations. Taking into account the production cost of one kg of rabbit (*i.e.*, feed) and considering alfalfa as the standard forage, using mesquite pod and forage oat as a source of forage instead of alfalfa reduces costs to 80% and 89%, respectively. Additionally, this replacement contributes to lower or even zero water consumption.

Table 5. Costs of diets that included different forage sources.

Variable	T1	T2	T3
Cost per kg of feed ($)	8.9	7.85	9.8
Feed intake in the growing period (g DM)	2078	2054	2085
Feed intake in the fattening period (g DM)	2265	2276	2267
Total feed intake (g DM)	4343	4330	4352
Weight gain in the growing period (kg)	1.079	1.062	1.083
Weight gain in the fattening period (kg)	0.951	0.852	0.915
Total weight gain (kg)	2.03	1.914	1.998
Cost of feeding ($)	38.65	33.99	42.65
Cost of feeding/kg of live weight gain ($)	19.04	17.76	21.35
Cost of feeding/kg of live weight gain (%)	89	80	100

T1, Oat hay; T2, *Prosopis laevigata* pods; T3, Alfalfa hay. $, mexican pesos (MXN).

Implications

According to the results of this study, forage alternatives to alfalfa achieve similar productive parameters and improve the economic yield of the meat of fattening rabbits in Aguascalientes. Rabbit producers should include locally-sourced forage in their diets and reduce their costs and the use of water; they would also no longer need to purchase high-cost balanced feed to sustain their rabbit meat production business.

CONCLUSIONS

The three forages evaluated have good productive parameters; however, the diet with forage oat recorded the best parameters in weight gain, feed digestibility, and feed conversion, followed by alfalfa and, in third place, the mesquite pod. Although there were no differences in consumption between treatments, rabbits whose diet included forage oat had the best feed conversion. In conclusion, using mesquite pods as a source of fiber in the diet is more profitable than forage oat and alfalfa.

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Colegio de Postgraduados

Characterization and description of beekeeping agroecosystems in Hopelchén, Campeche, Mexico

Chiquini-Medina, Ramón A.[1]; **Candelaria-Martínez, Bernardino**[1]; **Ramírez-Bautista, Marco A.**[1]; **Chiquini-Medina, Ricardo A.**[1]; **Flota-Bañuelos, Carolina**[2*]

[1] Tecnológico Nacional de México campus Instituto Tecnológico de Chiná, Posgrado en Agroecosistemas Sostenibles. Calle 11 s/n entre 22 y 28 CP. 24520, Chiná, Campeche, México.
[2] CONAHCyT-Colegio de Postgraduados Campus Campeche. Carretera Haltunchén-Edzná km 17.5, Sihochac, Champotón, Campeche, México, C.P. 24450.
* Correspondence: cflota@colpos.mx

ABSTRACT

Objective: to characterize honey bee (*Apis mellifera*) production systems in the municipality of Hopelchén, Campeche, Mexico.

Design/methodology/approach: the sample was made up by 10% of the beekeepers in Holpechén, identified through the snowball method. A semi-structured questionnaire was designed with 94 questions grouped into five sections (general information, structure of the systems, product trade, technologies used, and decision making). The questionnaire was applied through the technique of interviewing those responsible for the 118 beekeeping production systems. The information obtained with the questionnaires was analyzed with descriptive statistics.

Results: beekeeping is an important productive activity in the region of Los Chenes and La Montaña in Hopelchén, which are undergoing the process of generational replacement and are currently maintained by young adult producers who are younger than 45 years old. They own 55 hives on average per producer, which are located at a distance of 7.7 km from their households. The main technology is the use of sugar for feed, by 50% of producers, followed by the diagnosis of Varroa infestation and determination of moisture with 35 and 21.5% of beekeepers.

Limitations on study/implications: the limited access to regions with beekeeping potential, as well as the lack of monitoring by outside staff, does not ease open-mindedness of beekeepers toward researchers.

Findings/conclusions: the characterization of beekeeping production systems with high potential allows proposing improvement strategies to promote the development of small-scale producers.

Keywords: Beekeeping, characterization, sustainability.

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INTRODUCTION

In Mexico, an activity of great economic and social relevance is beekeeping, because it generates employment, income and currency (Güemes *et al.*, 2003; Tiatrini, 2002). It is considered to be an important activity of the livestock subsector, and its production volume and level of productivity place Mexico in the sixth place globally; in America, it is third in both categories, as well as in exports (Magaña *et al.*, 2007). This livestock production

Image by PollyDot at Pixabay

activity, in addition to its social and economic importance, is relevant from the ecological point of view due to its positive impact on cross-pollination of wild and cultivated species (SADER, 2016; Martinez-Gonzalez *et al.*, 2018). In addition to honey, beekeeping is a source of other products of excellent quality and uses in agroindustry, such as pollen, royal jelly, wax, propolis and bee venom.

The Yucatan Peninsula is an important region in honey production at the national level, approximately 95% of its production is destined to the international market, and due to its quality conferred by the botanical origin, it is a product accepted in the European and United States market (Güemes-Ricalde *et al.*, 2003). The state of Campeche is positioned in the second place nationally in bee honey production (SIAP, 2023).

It is estimated that approximately 6,226 families from the rural sector depend directly on the beekeeping activity in this state (SADEr, 2018), and the highest honey production is concentrated in the municipalities of Campeche and Hopelchén, which contribute more than 75% of the annual state production (SIACON, 2017). Hopelchén has a higher proportion of rural areas and therefore more agriculture and livestock activity. This municipality is divided into two large regions called La Montaña and Los Chenes.

La Montaña represents a higher proportion, with approximately 200,000 ha, and it is located in the southern part of the municipality of Hopelchén. It is considered a high priority conservation area, part of the muffling area of the Calakmul Biosphere Reserve and Mesoamerican-Mexican Biological Corridor (Porter-Bolland *et al.*, 2008). The climate of the municipality of Hopelchén corresponds to warm tropical sub-humid type (García, 1973), the mean temperature and annual precipitation are 26 °C and 1,050 mm, although between both regions there is a moisture gradient that is higher in the La Montaña region, where vegetation is represented mainly by sub-evergreen and deciduous medium forest, while in the region of Los Chenes it is low deciduous forest (Porter-Bolland *et al.*, 2008).

Beekeepers in Campeche have been grouped into 31 organizations and eight honey stockpiling companies operate in the state (Cruz-Zamudio, 2017). However, the organization has had a lower impact as a driving element of beekeeping, probably because the production systems differ and their particular characteristics have not been considered in the elaboration of strategies to potentiate the activity. Organizing among beekeepers is important for the knowledge exchange process and the creation of technical and financial cooperation networks, as well as to represent themselves as a union in the presence of authorities, to gain access to government backing, and to confront threats to their activities (Contreras-Uc *et al.*, 2018).

From this perspective, it is important to understand the structure and management of beekeeping production systems to identify areas of opportunity that allow their development in the short and long term. Therefore, the objective of this study was to characterize the honey bee (*Apis mellifera* L.) production systems in the municipality of Hopelchén, Campeche.

MATERIALS AND METHODS

The study was carried out from March to July in 2022. Of the honey-producing localities, 10% were considered, with a total of 13, from the two geographic regions of the municipality of Hopelchén, Campeche (Figure 1). In the valley region (Los Chenes), the localities were: Ich ek, El Poste, Katab, Xcalot Akal, Huechil, Bolonchén and Xculoc; and in the region of La Montaña, the localities were: Dzibalchén, Iturbide, Chunchintoc, Ukum, Xmejia and Xmaben.

To determine the sample size, 10% of the beekeepers reported by the local authorities were considered. The selection of producers to be interviewed was according to the criterion of free decision to participate, and the snowball method was used. A sample population of n=118 Beekeeping Production Systems was obtained (Table 1).

A semi-structured questionnaire was designed with 94 questions grouped into five sections: general information, structure of the systems, product trade, technologies used, and decision making. The questionnaire was applied with the technique of interviewing those responsible for each of the beekeeping production systems considered in the study.

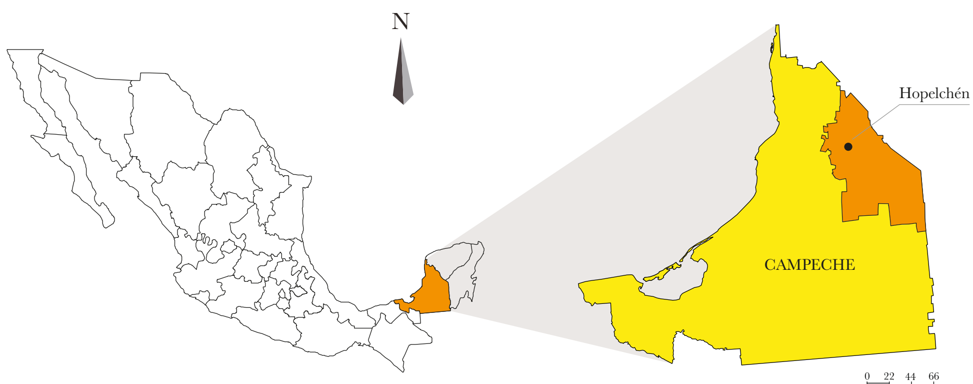

Figure 1. Location of the municipality of Hopelchén, Campeche, Mexico.

Table 1. Localities and number of beekeepers interviewed in the municipality of Hopelchén, Campeche, Mexico.

Region	Location	N	%
La Montaña	Ukum	14	11.86
	Chunchintoc	11	9.32
	Dzibalchen	9	7.62
	Iturbide	11	9.32
	Xmaben	10	8.47
	Xmejia	10	8.47
Los Chenes	Xculoc	9	7.62
	Katab	10	8.47
	Ich ek	7	5.93
	El poste	10	8.47
	Xcalot	10	8.47
	Bolonchen	5	4.23
	Huechil	2	1.69
Total		118	100

The information obtained with the application of questionnaires was arranged in an Excel spreadsheet and analyzed through descriptive statistics.

RESULTS AND DISCUSSION

Age of the beekeepers

The average age of those responsible for beekeeping systems is 44.1 years; 43.8 and 44.3 in the systems of La Montaña and Los Chenes, respectively (Figure 2). At the time when the interview was applied, the youngest age recorded was 18 years and the oldest 85 years. In La Montaña, 29.2% of those responsible are older than 50 years while in Los Chenes 41.5% of those responsible are in that age range.

In this regard, Rogers (2003) pointed out that 114 studies with producers showed that age has an impact on the attitude of older adults toward acquiring new knowledge, implementing new practices, and assuming risks in the production. In this sense, Escamilla *et al.* (2019) mention that age is a defining factor for the adoption of technology and innovation in agriculture and livestock systems, because older producers present resistance to change, while young ones are more innovative.

For both regions, it was seen that beekeeping is an activity that is undergoing a process of generational replacement since a higher proportion of beekeeping systems are kept by young adult producers less than 45 years old, this being the case in 56.9 and 53.7% in La Montaña and Los Chenes, respectively.

Number of apiaries and beehives

In all, the 118 producers interviewed in the two zones own 270 apiaries with a total of 4,825 hives (Table 2). In La Montaña, 156 apiaries were counted and 3,146 hives, corresponding to 20 hives apiary^{-1}. In the same region, on average 2.4 apiaries and 49.1 hives were recorded by producer. In the region of Los Chenes, 114 apiaries were counted and 1,679 hives, corresponding to 14.7 hives apiary^{-1}, and on average 2.2 apiaries and 33.8 hives were recorded per producer.

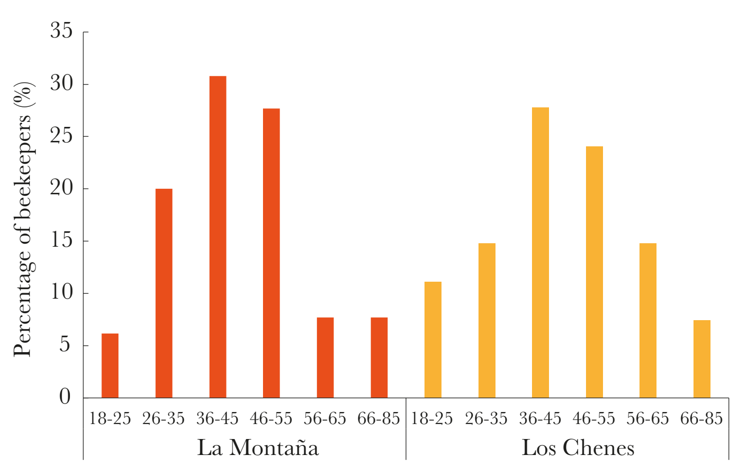

Figure 2. Age of beekeepers in the regions of La Montaña and Los Chenes, Hopelchén, Campeche, Mexico.

Table 2. Number of apiaries and hives belonging to beekeepers in the regions of La Montaña and Los Chenes in the municipality of Hopelchén, Campeche, Mexico.

Region	Location	Apiariess	Hives
La Montaña	Ukum	33	495
	Chunchintoc	26	640
	Dzibalchen	14	244
	Iturbide	17	280
	Xmaben	35	703
	Xmejia	31	784
Los Chenes	Xculoc	14	296
	Katab	25	285
	Ichek	14	302
	El poste	21	273
	Xcalot	16	177
	Bolonchen	20	285
	Huechil	4	61
Total		270	4,825

Years devoted to beekeeping

The family has been devoted to beekeeping 16.8 years on average in the region of La Montaña and 19.7 in Los Chenes. In the region of La Montaña, 41.5% of the families are located within the range of 11 to 20 years, while 35.9% of the families are within the same range in the region of Los Chenes (Figure 3). Likewise, the longest-lived beekeeping systems in La Montaña (1.5%) were found to be within the range of 41 to 50 years of existence, while in Los Chenes 5.5% of the systems have been operating for 60 years. It is important to highlight that 35.3% of the systems considered in La Montaña have existed for less than 10 years, while in Los Chenes 29.6% are under this condition.

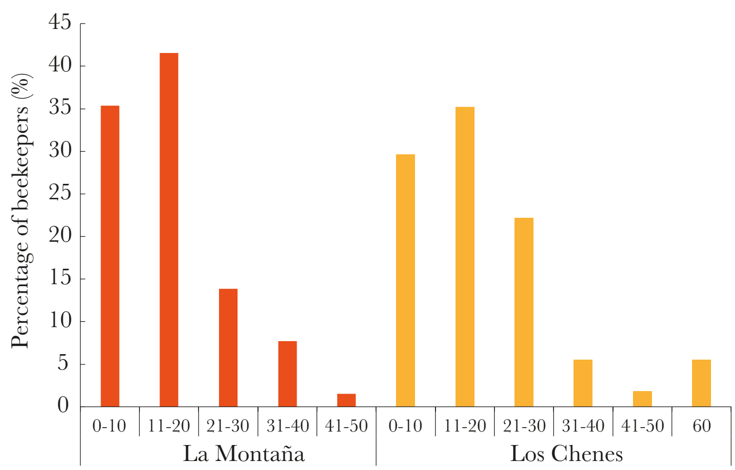

Figure 3. Years of having been devoted to beekeeping in the regions of La Montaña and Los Chenes in the municipality of Hopelchén, Campeche, Mexico.

Main economic activities

In La Montaña, 56.92% of interview respondents have three economic activities, while for the region of Los Chenes 61.11% of the families reported two economic activities (Figure 4). This phenomenon may be because producers from the two regions consider beekeeping as an alternative source of income due to its inconsistency and risk, but it is also seen as an alternative for the future. Another explanation could be risk management by producers, who make sure to diversify their sources of income throughout the year. The risk is defined as the uncertainty regarding the results that can involve damages or losses.

When the consequences of performing a specific activity are uncertain, especially unfavorable or negative, it is said that such an activity carries a related risk (Elton *et al.*, 2014). The decision of taking that risk is influenced by the characteristics of the person, the situation and often who with and when they interact (Finger and Weber, 2011).

Economic importance of beekeeping

When the importance of beekeeping in the family's income was analyzed, it was seen that it is the main source of income for the region of La Montaña (72.3% of producers), while in the region of Los Chenes it is 66.7% (Figure 5). This could be because producers from both regions consider beekeeping as an alternative source of income with some inconsistency and risk, so producers devote only part of the workday to beekeeping tasks. Producers also mentioned that the growing establishment of farming areas in the municipality results in more deforestation and segmentation of the ecosystem, which increases the distance between the flowering zone and the apiary.

Average education in years

It was found that 88.05% of the family members in the beekeeping systems of La Montaña had access to education, while in the region of Los Chenes it was 95.17%. Average schooling of beekeepers responsible for the systems is 8.4 years and 9.2 years in La Montaña and Los Chenes, respectively. It was also seen that the maximum average

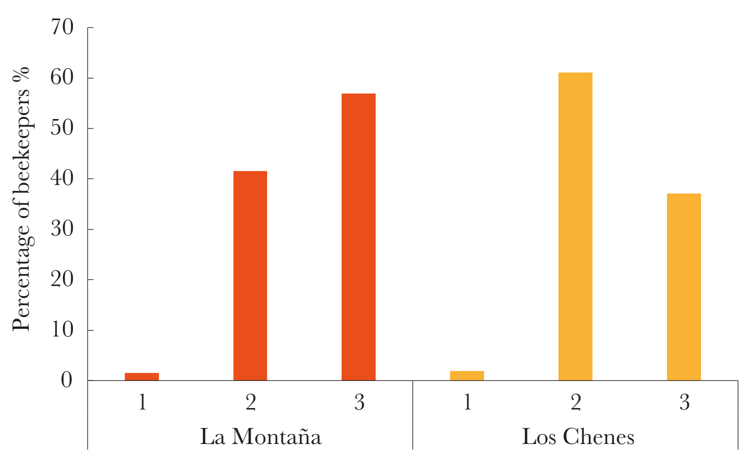

Figure 4. Economic activities of beekeepers from the regions of La Montaña and Los Chenes, municipality of Hopelchén, Campeche, Mexico.

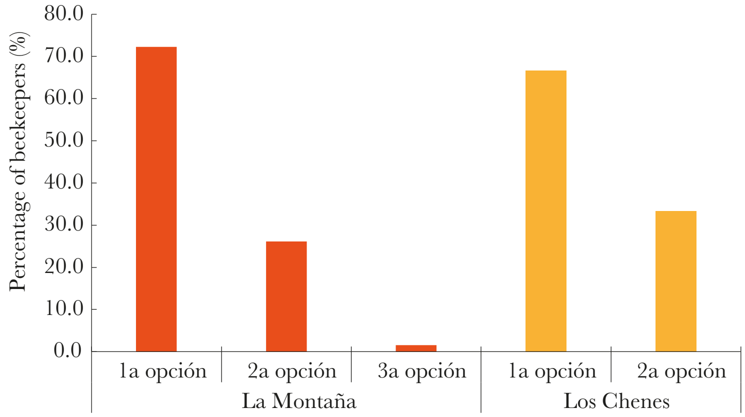

Figure 5. Economic importance of beekeeping for families from the regions of La Montaña and Los Chenes, Hopelchén, Campeche, Mexico.

education reached by any member of the beekeeping family is 11.5 years in La Montaña and 12.5 years in Los Chenes. The range with highest proportion of education by region observed in the families considered in this study was 30.77% for the region of La Montaña within a range of 7 to 9 years of schooling (Secondary), while it was 35.19% between 13 and 17 years (Higher Education) in the region of Los Chenes (Figure 6). This difference could be because the municipal township is located in the region of Los Chenes, so the educational offer is greater.

Distance from the apiary to the household of the beekeeping family

When it comes to the distance at which beekeeping systems are found from the household of those responsible, averages of 8.7 and 6.7 km were recorded in La Montaña and Los Chenes, respectively. Most apiaries are located at less than 10 km from the household of the person responsible, 64.6 and 81.5% in La Montaña and Los Chenes, respectively (Figure 7). Therefore, more proximity to the apiaries results in better and more constant control and care of the systems, since the visits are more frequent;

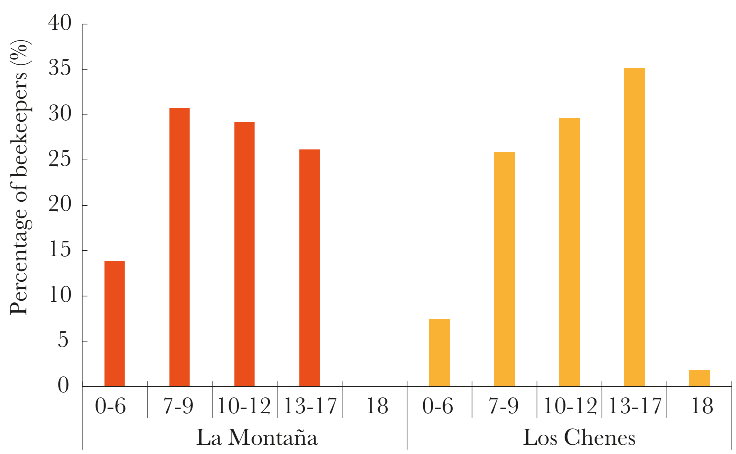

Figure 6. Schooling average of beekeeping families in the regions of La Montaña and Los Chenes in the municipality of Hopelchén, Campeche, Mexico.

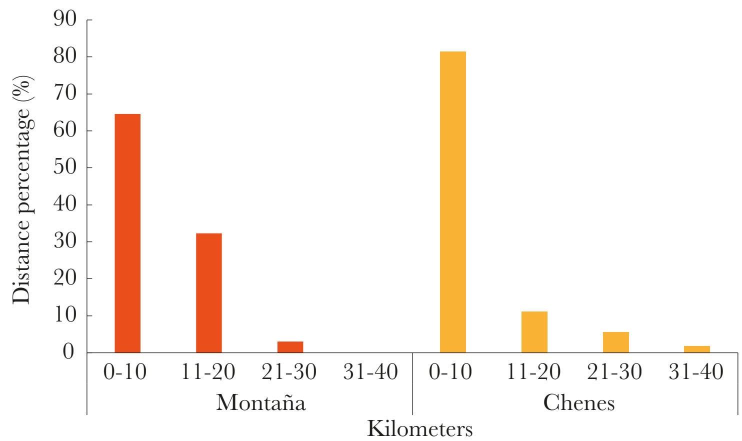

Figure 7. Distance (km) from the apiary to the beekeeper's household in the regions of La Montaña and Los Chenes, Hopelchén, Campeche, Mexico.

inputs, equipment and products can be transported with greater ease and there is more availability and integration of the family nucleus into the system's activities, generating empathy and enjoyment of family members toward beekeeping in the future.

Technologies applied in the apiary

The use of six technologies for preventive and corrective control was observed in all the systems analyzed in the two regions of study. It could be seen that feeding with sugar is the most widely used practice inside the apiaries, with a record of 48.7 (La Montaña) and 49.5% (Los Chenes). It is a useful strategy in the drought season to maintain the hive colonies strong and healthy.

The source from which beekeepers learn technologies varies between regions; in La Montaña the greatest proportion of beekeepers, approximately 37.6%, learn from another producer, while in Los Chenes the greatest proportion (39.4%) learn new technologies on their own. It stands out that in La Montaña the highest proportion (41.4%) of learning about sugar feeding is obtained from the family's father.

Decision making for apiary management

It could be seen that in the two regions the head of the family and person responsible for the beekeeping system makes 90% of the decisions related to apiary management, because he/she contributes resources, carries out management, and has more knowledge about beekeeping. For the remaining 10%, decision making was different between regions. In La Montaña, 6.15% was shared between the man and woman heads of household, 1.53% by another member of the family, and 1.53% by several members of the family. In Los Chenes, 5.55% of the decisions are made by several members of the family, 1.85% by another member of the family, and 1.85% by all the members of the family. With this, a marked tendency toward the head of household can be seen in the two regions, generally the owner or person responsible for the system, just as he/she contributes the resources and has more knowledge about their management.

Table 3. Technologies used by beekeepers from the regions of La Montaña and Los Chenes, Hopelchén, Campeche, Mexico.

Region	Technologies	Producers (%)	Years applying the technology	Where learned *
La Montaña	Sugar feeding	48.7	14.4	a=13, b=7, c=13, d=24, e=3
	Protein feeding	19.3	9.6	a=12, b=1, c=5, d=5, e=0
	Diagnosis of varroa (*V. destructor*)	38.6	12.0	a=25, b=3, c=1, d=7, e=10
	Varroa traps (*V. destructor*)	7.6	4.6	a=2, b=1, c=0, d=4, e=2
	Determination of moinsture in honey	29.4	19.1	a=7, b=21, c=3, d=2, e=2
	Beetle traps	6.7	4.5	a=2, b=0, c=0, d=4, e=2
Los Chenes	Sugar feeding	49.5	13.2	a=6, b=17, c=14, d=10, e=3
	Protein feeding	30.3	7.9	a=9, b=8, c=2, d=9, e=2
	Diagnosis of varroa (*V. destructor*)	32.3	13.0	a=10, b=10, c=6, d=4, e=2
	Varroa traps (*V. destructor*)	15.1	11.6	a=3, b=5, c=3, d=2, e=2
	Determination of moinsture in honey	22.2	16.2	a=1, b=17, c=0, d=3, e=1
	Beetle traps	15.1	4.3	a=5, b=2, c=1, d=5, e=2

* Where: a=from another producer, b=on own account, c=another producer, d=from the father, e=did not respond

After performing a general analysis of the data gathered, it could be seen that there is a marked trend in decision making about the apiary management, since the head of the family is the one responsible for this activity with an average of 90.7% of the decisions made, and this is notable taking into account that the average number of members per family is 3.6 (La Montaña) and 3.2 (Los Chenes), with a range of 1 to 10 members. Likewise, the educational level of those members represents 11.5 (La Montaña) and 12.5 (Los Chenes) years on average, which shows that the academic degree has been increasing among the young population, although this does not mean that they are taken into account to participate and make decisions in the system.

This results in a very marked decreasing trend in the application of resources and technologies within the production process, since the number of practices where there was shared decision was 0.32 (La Montaña) and 0.09 (Los Chenes), from within a range of 0 to 9 available practices to be performed in the apiary. Therefore, the application of technologies that could be used in the apiary also decreases when the head of household does not have interest or knowledge of the technologies, so that he/she does not intervene in the process; there was an average of 2.8 (La Montaña) and 3 (Los Chenes) technologies applied from a range of 0 to 7 available ones.

Based on the data gathered, it could be observed that although beekeeping has been growing in development, and since it already represents the main source of income for the great majority of the producers, decision making continues to be markedly by the head of the family; and although there are more members of the families involved in the apiary activities from an early age, this has not allowed for their participation in decisions and application of technologies to have an influence on the system.

CONCLUSIONS

Knowledge of the characteristics of different types of beekeeping systems present in Hopelchén, Campeche, allows explaining their structure and management, as well as the cultural, technological and economic factors that define them, making it possible to detect important components that could allow promoting their development. The organization among beekeepers is of utmost importance to ease the access to government backing and qualified technical assistance, to promote knowledge exchange, and to approach sustainability. With this, the shortcomings that beekeepers face in production within their apiaries could be minimized, and the economic benefits and sustainability they seek to find in beekeeping could increase.

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Colegio de Postgraduados

Applications of nanoparticles in veterinary science: A promising technology for animal health

Rodríguez-Marín, Erik[1]; Ortega-Cerrilla, María Esther[1*]; Castillo-Juárez, Israel[2]; Crosby-Galván, Elsa Margarita[1]; Herrera-Haro, José Guadalupe[1]; Miranda-Jiménez, Leonor[1]

[1] Colegio de Postgraduados Campus Montecillo, Programa de Recursos Genéticos y Productividad-Ganadería. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de México, C.P. 56264.

[2] Postgraduados Campus Montecillo, Programa de Botánica. Colegio de Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de México, C.P. 56264.

* Correspondence: meoc@colpos.mx

ABSTRACT

Objective: To revise and describe the importance of nanoparticles in veterinary sciences to improve the health and welfare of animals.

Design/methodology/approach: The use of nanoparticles in veterinary sciences has the aim of using the unique properties of these structures at the nanometer level in order to solve medical and therapeutic problems in the field of veterinary medicine.

Limitations on study/implications: Although the nanoparticles have great potential in veterinary medicine, they also have certain limitations that must be considered in their application, among which the following stand out: bioavailability, distribution, toxicity, immunologic interactions, and costs.

Conclusions: The field of veterinary medicine is in constant evolution, which is why nanoparticles have become a cutting-age tool with transforming potential. Their application in the diagnosis, treatment and prevention of diseases offers ways to improve animal health and welfare. The continuous search for solid research and solutions to overcome the limitations will allow taking advantage of all the potential of these technologies.

Keywords: nanoparticles, veterinary sciences, animal health.

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INTRODUCTION

In a world in constant evolution, where science and technology come together to improve life, nanotechnology is the manipulation and use of materials at a nanoscale, which has opened new horizons in several fields of science, including veterinary medicine (Ali *et al.*, 2021). Nanoparticles have become a fascinating innovation frontier. These small structures vary in size between 1 and 100 nanometers, and they have unique physical and chemical properties that have allowed them to supply minerals, vitamins, medications and other compounds, providing innovative approaches for the management of animal health (Cervera *et al.*, 2023).

Veterinary medicine, responsible for the care and welfare of animals, constantly seeks to solve medical problems, improve the diagnosis, optimize the treatment, and prevent diseases. Therefore, nanoparticles have become a promising tool to change the way in which animal health is approached (Scott, 2005). Nanoparticles have a series of unique characteristics due to their size at nanometer scale, such as a special surface-volume relationship and optical, magnetic and chemical properties (Urrejola *et al.*, 2018). These characteristics allow them to interact with biological systems in a specific and accurate way, which opens up a series of possibilities in veterinary medicine and zootechnics. From an early detection of diseases to specific treatment, nanoparticles have become a multifaceted tool useful to improve animal health (Reséndiz and Arvízu, 2020). This review has the objective of understanding some applications of nanotechnology in the field of veterinary medicine, as well as considering ethical safety and the balance necessary to take advantage of the entire potential of this emerging technology.

Applications of nanoparticles in veterinary medicine

Diagnostic application

Nanoparticles were used to improve diagnostic procedures in animals. Their capacity to interact with specific biomarkers allows a detection of highly sensitive and specific diseases (Tyler and Kang, 2015). This makes the early detection of diseases easier, which at the same time leads to more effective interventions and more positive results. The tests based on nanoparticles can detect certain biomarkers in a faster and more sensitive way (Kyle and Saha, 2014). For example, gold nanoparticles used with antibodies can bond with specific antigens allowing the early detection of infectious diseases or cancer in animals (Guo *et al.*, 2017). This test also has the potential to be used in diagnostic tests of zoonotic diseases, so it is important both for animal health and for public health.

Administration of medications

The accurate administration of medications is a challenge in veterinary medicine. Nanoparticles can be loaded with drugs and they can be liberated in a controlled way, which allows a prolonged and specific liberation in the animal's body (Irache, 2008). This is particularly useful in prolonged treatments or to minimize the secondary effects of the medication. Nanoparticles can also go through biological barriers such as the blood-brain barrier, which allows the treatment of disorders of the central nervous system in animals. Nanoparticles offer a series of advantages in comparison with the formulation of traditional drugs (Murthy, 2007). Their nanometric size allows a better penetration in specific tissues and cells, thus improving the bioavailability of the drug. In addition, the superficial properties of nanoparticles allow the conjugation with target cells, permitting a controlled and specific liberation of the medication in the site of action. This reduces the necessary dose and minimizes side effects (Masri *et al.*, 2018).

Prevention and control of diseases

Nanoparticles were also used in the prevention and control of diseases in animals, such as increasing the immunogenicity of cows, which results in a stronger and more lasting immunity response (Durán *et al.*, 2020). In addition, antimicrobial nanoparticles are being researched as an alternative to combat infections that are resistant to antibiotics, of great importance in the livestock industry. Nanoparticles offer a new way of preventing infectious diseases in animals. It has been proven that antimicrobial nanoparticles, such as those from silver and copper, have strong bactericide and antiviral properties (Alvarracin *et al.*, 2021). These nanoparticles could be incorporated to equipment and lining of surfaces in livestock facilities, reducing the propagation of pathogens and the need for treatment with antibiotics.

In parasitology, nanoparticles can be loaded with antiparasite drugs and administered through feed or topical formulations to control endo and ectoparasites in the livestock.

Applications in food safety

In addition, there are many applications for nanoparticles to improve food safety. They can be used as antimicrobial lining on surfaces and food containers, limiting the growth of bacteria and other pathogens (Villamizar and Monroy, 2015). The nanoparticles from silver, zinc and copper have shown efficient antibacterial properties against various pathogens transmitted in food. Biosensors based on nanoparticles can quickly detect pathogens and contaminants in foods, allowing early detection and a timely response to the problems of food safety. Similarly, they can be used to control the liberation of antimicrobial and antioxidants to help extend the useful life of perishable foods (Khaliefa *et al.*, 2017).

Application in animal nutrition

Studies have shown that nanoparticles are valuable to improve the absorption and bioavailability of essential nutrients in animal feed (Cuca, 2018). Minerals such as zing and iron tend to have low bioavailability in the diet of animals. Encapsulating these nutrients in nanoparticles could increase their solubility and absorption in the digestive tract, allowing a better availability (Kociova *et al.*, 2020). The digestive tract plays an important role in animal health and the absorption of nutrients; nanoparticles can have beneficial effects on intestinal health by modulating the microbiome and the integrity of the mucosa (Cano, 2022). Some nanoparticles act like prebiotics, promoting the growth of beneficial bacteria in the intestine, improving the digestion and absorption of nutrients. Oxidative stress is a factor that can affect negatively the yield and the health of animals, and some nanoparticles have antioxidant properties that can neutralize the free radicals and reduce the oxidative stress in animal tissues (Flores *et al.*, 2022). This is important, particularly in the case of animals subjected to stressful conditions, such as intensive breeding.

Importance and advantages of nanoparticles

The introduction of nanoparticles in veterinary medicine has changed the definition of animal health. The benefit is clear, since an early and accurate detection of diseases can be achieved thanks to their capacity to interact with specific biomarkers (Rodríguez *et al.*, 2021). In addition, customized therapy has become a reality, helping to control medications and to reduce the side effects. Disease prevention has also been improved by making vaccines more effective and combating infections that are resistant to antibiotics (Gómez *et al.*, 2016).

The use of nanoparticles in veterinary medicine has proven to be an innovative and promising strategy to address various animal health and welfare challenges, from the accurate diagnosis to customized therapy and the improvement of livestock (Aguilera *et al.*, 2021). As research in the field of nanotechnology advances, it is important to face the challenges and maximize the potential of this technology in benefit of the animals.

Challenges and disadvantages

However, these opportunities entail challenges. The bioavailability and distribution of nanoparticles in animals can be unpredictable, which can affect their efficacy. The safety and toxicity of these particles must be evaluated carefully to avoid adverse effects (Chavez, 2018). Although there can be beneficial effects for the animal, they can also cause inflammatory reactions and even toxic ones at cellular level. The biodistribution of nanoparticles in animals is a reason for great worry because they can be accumulated in certain organs and have long-term effects that are not completely understood yet. Immune interactions and differences between species are also problems, since the administration and approval of therapies based on nanoparticles could be a complex and costly process. In addition, the issues of ethics and sustainability require continuous evaluation (Lozano, 2022).

The future of nanoparticles in veterinary medicine

Despite these problems, the balance between advantages and disadvantages suggests that the use of nanoparticles in veterinary medicine has great potential. Studies indicate that it is possible to attain reliable solutions to overcome these limitations, with which greater benefit will be obtained from these technologies (Grande, 2007). We must remember that all scientific achievements are associated with ethical responsibilities and the need for a careful application (Wing, 2006). On the other hand, it is important to consider that nanoparticles offer great potential to improve the diagnosis, treatment and prevention of diseases which allows a more customized and efficient care and treatment for the animals (Betancur, 2016).

CONCLUSIONS

Nanoparticles have proven to be versatile and promising tools in veterinary medicine. Nanoparticles are an innovative tool to enhance animal nutrition, improving the bioavailability of nutrients, promoting intestinal health, and reducing oxidative stress. They also enhance the capacity to improve early diagnosis, therapeutic efficacy and disease prevention in animals, which has the potential of changing the veterinary practice. However, more research is needed to understand the problems of safety and efficacy, and to maintain high ethical standards when these technologies are used in animal care. This is why it is necessary to continue the research in this area, as well as to analyze their advantages and disadvantages to ensure that their use in the practice of veterinary medicine can be a supporting tool in animal science.

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Colegio de Postgraduados

Economic evaluation of the “Healthy Water for La Laguna” project

Guerrero-Borrás, Luis A.[1]**; Mora-Flores, José S.**[1*]**; García-Salazar, José A.**[1]**; Ramírez-Valverde, Gustavo**[1]

[1] Colegio de Postgraduados Campus Montecillo, Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de México, México, C.P. 56264.

* Correspondence: saturmf@colpos.mx

ABSTRACT

Objective: To identify the costs and benefits attributable to the “Healthy Water for La Laguna” project, quantifying and evaluating them throughout their useful life, with the aim of judging the convenience of conducting this project.

Design/methodology/approach: An Economic-Financial Evaluation was conducted determining the main economic indicators: Internal Rate of Return (IRR), Net Present Value (NPV), and Cost-Benefit Rate (C/B R).

Results: The project “Healthy Water for La Laguna” was profitable given the following indicators: Social Net Present Value of $26’819,451,175 in a horizon of evaluation of 34 years considering a discount rate of 10%, an Internal Rate of Return (IRR) of 35.29%, and a Benefit-Cost Rate of 2.8. In the sensitivity analysis, it was determined that the project shows a low sensitivity to the increase in investment and operation costs. However, it was seen that the variable that could have the most significant impact is that of benefits, since a reduction of more than 64.33% would make the Net Present Value (NPV) lower than zero.

Limitations on study/implications: The one limitation found in the study was the lack of information, since some missing data had to be estimated.

Findings/conclusions: It can be concluded that the execution of the project is essential and necessary to achieve an immediate increase in the consumption of drinking water in the locations covered by the project, in addition to serving as a departure point to improve the quality of the water used and to reduce the impacts of overexploitation.

Keywords: Evaluation, water, sustainability, price.

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INTRODUCTION

The Comarca Lagunera or Lagoon Region, known as La Laguna, is in the central-northern zone of Mexico, on the border of the states of Coahuila and Durango. This region is made up by 15 municipalities, 10 of which belong to Durango (Gómez Palacio, Lerdo, Tlahualilo, Mapimí, Rodeo, Nazas, Simón Bolívar, San Juan de Guadalupe, San Luis del Cordero, and San Pedro del Gallo) and the other five belong to Coahuila (Torreón, San Pedro, Matamoros, Francisco I. Madero and Viesca) (Mazcorro, cited by López López and Sánchez Crispín, 2010).

According to Maeda (2006), the problem of water arsenic poisoning in the Comarca Lagunera region was evident through an epidemiological study carried out in 1963, although its history remounts to at least 1953. This is because of a prolonged drought that affected the region between 1952 and 1958, which led to the need to extract underground water to satisfy the demands of supply for the community. During the period of 1953 to 1955, 160 cases of people who had epidermoid-basocellular cancer were detected, whose cause was unknown; these patients came from ejidos belonging to the municipalities of Tlahualilo, Mapimí, Francisco I. Madero, and San Pedro. After performing a series of medical studies and field research, the conclusion was reached that an environmental factor was at the origin of this problem.

The municipalities most affected by water arsenic poisoning are Tlahualilo, Francisco I. Madero, San Pedro, Matamoros, and Viesca (Maeda, 2006). This problem extends from these municipalities toward lower areas, including Matamoros, Coahuila, and the main aquifer, as the levels of water decrease. Maeda also suggests that the best way to approach this problem is for the population to stop consuming water with high levels of arsenic (As), that is, to relocate them in areas free of pollution. In addition, he points out that the measures implemented, both physical and chemical, such as solar filters, inverse osmosis plants, and reducing agents such as iron sulfate and flocculants, have not had positive results.

The quandary in the zone related with the low consumption of water from sustainable sources has become a persistent problem because of the absence of alternatives for healthy supply. The lack of alternative supply sources forces the continued extraction of underground water, which increases overexploitation, to keep urban centers functioning despite the risks and costs involved. Furthermore, as water continues to be extracted from the aquifer, it is highly likely for the concentration of arsenic and other salts to exceed the limits allowed for human consumption.

Therefore, it is imperative to carry out projects that increase water production from sustainable and healthy sources, with the aim of improving the quality of life of the population and to avoid placing public health at risk.

According to the project and for this study, the main objective consisted in establishing the infrastructure required to provide enough high-quality and renewable water. The hypothesis set out is that if the non-sustainable sources are partly substituted, that is, incorporating renewable water of good quality (viability of the project "Healthy Water for La Laguna") and water ceases to be extracted from deep wells, the sustainable production will increase.

MATERIALS AND METHODS

An economic-financial evaluation was conducted using an approach based on the model described by Pavón (2012). This analysis evaluated the key economic indicators, which include profitability, the internal rate of return (IRR), the net present value (NPV), and the benefit-cost rate (B/C R), with the purpose of evaluating the viability of this project.

According to what was established in the guidelines for the elaboration and presentation of the cost and benefit analysis of the investment programs and projects (SHCP, 2013), the project is qualified as a project for economic infrastructure, because it is for the construction of fixed assets to supply services in the water sector. Table 1 shows the investment costs in the project.

Table 1. Investment costs of the project.

Concept	Unit	Amount	UP	Annual cost ($ at 2020 prices)	
				without tax	with tax
Aqueduct	m	46,371	57,190	2,651,936,690	3,076,246,560
Storage tanks	m^3	6,000	16,984	101,901,126	118,205,307
Water treatment plant	Lot	1	2,017,036,929	2,017,036,929	2,339,762,838
Diversion plant	Lot	4	7,627,288	30,661,698	35,567,570
Trunk network	Derivations	10	275,627,764	2,756,277,636	3,197,282,057
Land management	Lot	5	562,193	2,810,963	2,810,963
Pumping plant	Lot	1	547,530,460	547,530,460	635,135,334
Supervision	Lot	1	302,438,229	405,267,227	470,109,983
Management of water rights through technical development	Hm3	150	11,773,760	1,766,064,000	1,766,064,000
Total				10,279,486,729	11,641,184,612

Source: Comisión Nacional del Agua, Organismo de Cuenca Cuencas Centrales del Norte.

Table 2 presents the execution programming of the main components of the project. The execution calendar is expressed in years, programming to execute the tasks in a period of four years, from 2021 to 2024.

The project is executed through financing of a federal program, whose origin of the resources can be a contribution of 50% by the state government and the other 50% federal resources, as presented in Table 3.

The useful life period of the project is restricted by the duration of the materials of the infrastructure that will be built. For example, the specifications of the manufacturer indicate that steel piping has a useful life of 50 years. Other components of the infrastructure can have a similar useful life, or at least 50 years in the case of the civil works. Therefore, it is estimated that the system will function adequately during a minimum of 50 years, as long as adequate conditions of operation, maintenance, and replacement of electromechanical

Table 2. Calendar of project investments. Percentages.

Component	2021	2022	2023	2024
Aqueduct	25.0	35.0	25.0	15.0
Storage tanks	20.00	35.00	25.0	20.0
Water treatment plant	10.0	35.0	30.0	25.0
Diversion plant	0	100.0	0	0
Trunk network	10.0	35.0	40.0	15.0
Land management	100.0	0	0	0
Pumping plant	10.0	35.0	25.0	30.0
Supervision	15.0	35.2	31.3	18.5
Management of water rights through technical development	15.9	32.9	51.20	0

Source: Comisión Nacional del Agua, Organismo de Cuenca Cuencas Centrales del Norte.

Table 3. Calendar of project investments.

Program	Origin of investment resources	Input %	2021	Input %	2022	Input %	2023	Input %	2024	Input %	Total
Federal	State	0%	0	59%	2,392,854,527	59%	2,351,083,044	59%	1,076,654,735	50%	5,820,592,306
	Federal	100%	1,763,227,724	41%	1,667,988,874	41%	1,638,871,196	41%	750,504,512	50%	5,820,592,306
	Sum		1,763,227,724		4,060,843,401		3,989,954,241		1,827,159,247		11,641,184,612
Total	State	0%	0	59%	2,392,854,527	59%	2,351,083,044	59%	1,076,654,735	50%	5,820,592,308
	Federal	100%	1,763,227,724	41%	1,667,988,874	41%	1,638,871,196	41%	750,504,512	50%	5,820,592,306
	Sum		1,763,227,724		4,060,843,401		3,989,954,241		1,827,159,247		11,641,184,612

Source: Comisión Nacional del Agua, Organismo de Cuenca Cuencas Centrales del Norte.

equipment are kept. Nevertheless, with the purpose of evaluating the benefits of the project, a useful life of 30 years has been established since the project finalization. Year "0" of the project corresponds to 2021, and the construction period is programmed for the 2021-2024 period, covering a total of four years. Therefore, the horizon of evaluation is extended to 34 years.

RESULTS AND DISCUSSION

The essence of the project analyzed consisted in providing a sustainable supply of high-quality water in sufficient amount for the municipalities of Francisco I. Madero, Matamoros, San Pedro, Torreón, and Viesca in the state of Coahuila, as well as Gómez Palacio, Lerdo, and Tlahualilo in the state of Durango. The project will allow increasing the availability and the consumption of sustainable drinking water in the zone of influence of the project. The social benefits have been determined through the increase in consumption by the population, which has been quantified and valued following the methodologies published by the Center for Preparation and Evaluation of the Projects (CEPEP, 2018) and the National Water Commission (CONAGUA, 2019).

According to Table 4, which details the flow of funds of the project and includes the valuation of the social costs and benefits identified, the project "Healthy Water for La Laguna" proves to be profitable from a social perspective. This is reflected in a Social Net Present Value (SNPV) of 26,819,451,175 pesos during an evaluation period of 34 years, considering a social rate of 10% discount. This rate was established for an Investment Unit through the Oficio Circular number 400.1.410.14.009, with date January 13, 2014.

The effects that the modification of the relevant variables would cause on the main profitability indicators of the project: NPV, IRR and B/C ratio, were evaluated. The effect for different percentage variables was considered, also determining the percentage variation with which the NPV is equal to zero. The results are presented next, with the PV expressed in pesos at social prices of 2020, the IRR in percentages, and the B/C rate is adimensional. For the case of variations in the investment amounts, the project is not very sensitive in this sphere, since when applying increments of 50, 100, and 250%,

Table 4. Summary of the evaluation.

Concept	Unidad	Valor
Evaluation horizon	Years	34
Social discount rate	Percentage	10.0%
PV social investment costs	Pesos	8,943,619,260
PV social operating costs	Pesos	5,930,449,677
PV total costs	Pesos	14,874,068,937
PV gross profit increased consumption	Pesos	41,693,520,112
PV total benefits	Pesos	41,693,520,112
Social net present value (SNPV)	Pesos	26,819,451,175
Social internal rate of return (SIRR)	Percentage	35.29%
PVB/PVC ratio	Without	2.8

Where: Present Value (PV), Social Net Present Value (SNPV), Social Internal Rate of Return (SIRR), and Benefit Cost Ratio (Total Benefits/Total Costs). Source: Prepared by the authors with data from Comisión Nacional del Agua, Organismo de Cuenca Cuencas Centrales del Norte.

the indicators of profitability continued being favorable. When it comes to the dead point analysis, it was determined that an increase higher than 299.9% in the investment amounts would be required for the NPV to be less than zero. When it comes to the analysis with the operation and maintenance costs, it was determined that it is the least relevant variable on the project's profitability.Increases of 50, 100, and 250% were also considered, which remained favorable in every case. When the analysis of indifference was conducted with this variable, it was determined that an increase higher than 452.2% would be required for the NPV of the project to be negative, which shows lower impact of this variable on the profitability of the project. Finally, Table 5 expresses the analysis that considers possible reductions in the generation of benefits, which is mainly conditioned by variations in the demand. That is, if the demand increases (for example, if physical losses increase or if the population grows at a faster rhythm than what was foreseen), the *per capita* consumption decreases and, as consequence, the benefits are reduced. Initially, variations of 10%, 25%, and 50% were considered in the quantification of these scenarios, and the project remains profitable in these cases. However, a negative Net Present Value

Table 5. Effects under assumptions of benefit reduction.

Indicador	−10.00%	−25.00%	−50.00%	−64.33%
VACS	14,874,068,937	14,874,068,937	14,874,068,937	14,874,068,937
VABS	37,524,168,101	31,270,140,084	20,846,760,056	14,874,068,937
SNPV	22,650,099,164	16,396,071,147	5,972,691,119	0
SIRR	31.74%	26.23%	16.36%	10.00%
B/C	2.52	2.1	1.4	1
IRR	30.37%	24.25%	14.04%	8.20%

VACS: VABS: SNPV: Social Net Present Value; SIRR: Social Internal Rate of Return; B/C: Benefit/Cost; IRR: Internal Rate of Return. Source: Prepared by the authors with data from the Comisión Nacional del Agua, Organismo de Cuenca Cuencas Centrales del Norte.

(NPV) was observed when a reduction of more than 64.25% in the generation of benefits was assumed.

CONCLUSIONS

The conclusion can be reached that the execution of the project "Healthy Water for La Laguna" is important for the Región Lagunera, because it supplies healthy water sustainably for the nine most populated municipalities of the Comarca Lagunera, because all the indicators (NPV, IRR, B/C Rate) are positive. The sensitivity analysis shows that if the costs of infrastructure substantially increase, the project will continue to be profitable. Similarly, if the benefits are reduced to slightly over half, the project will continue to be profitable. With the execution of the project, health problems generated by the consumption of water contaminated with arsenic (As) and other metals would be reduced. Likewise, the overexploitation of water tables would be avoided as a result of the substitution with water from other sources, such as superficial water (dams).

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Colegio de Postgraduados

Characterization of production and commercialization systems of camedor palm (*Chamaedorea elegans* Mart.)

Briones-Ruiz, Gregorio[1]; Díaz-José, Julio[1*]; Leyva-Ovalle, Otto R.[1]; Ávila-Castro, Jesús O.[1]; Murguía-González, Joaquín[1]; Andrés-Meza, Pablo[1]

[1] Universidad Veracruzana, Facultad de Ciencias Biológicas y Agropecuarias, Región Orizaba-Córdoba. Peñuela, Amatlán de los Reyes, Veracruz, México. CP 94945.

* Correspondence: juliodiaz@uv.mx

ABSTRACT

Objective: To describe the cultivation systems of *C. elegans* through the analysis of production and commercialization in three municipalities in the high mountain region of Veracruz, Mexico.

Design/Methodology/Approach: The study was conducted in Tepatlaxco, Zongolica, and Omealca, Veracruz, Mexico. From January to July 2022, a survey was administered to 84 producers of Camedor palm using convenience sampling. In addition, interviews with key informants and participant observation were conducted.

Results: Camedor palm production occurs in small production units ranging from 1±0.05 to 7.0 hectares, primarily managed by male producers (93%) with an average age of 48 years and 6 years of schooling. Planting densities range from 35,000 to 100,000 plants per hectare, and leaf cutting is conducted on a quarterly basis, yielding between 1000 to 3700 rolls per hectare at an average price of $14.00 Mexican pesos per roll paid to the producer. Prior to cultivating Camedor palm, 56% of producers were growing coffee. The main driving factor for cultivation is the steady generation of income.

Limitations/Implications: This is a specific case study; therefore, the results are limited to descriptive statements about the study area.

Findings/Conclusions: Producers utilize non-timber forest products as alternatives to crises in other crops and investment constraints within production units. The production of *C. elegans* is accessible, requires low investment, and is compatible with family farming. However, yields in some cases are low, and the marketing network shows high intermediation.

Keywords: Foliages, livelihoods, Chamaedorea.

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INTRODUCTION

Non-timber forest products (NTFPs) are biological goods derived from forests that are tangible objects distinct from wood (FAO, 2020). They are widely used by rural communities as a means of livelihood (Awono *et al.*, 2016), and their cultivation or collection has low ecological impact (Téllez-Velazco, 2017), making them promoted as an option for rural development (Belcher *et al.*, 2005) and conservation (Huynh *et al.*, 2016). The growing use of NTFPs has globally driven research (Timko *et al.*, 2010), the development of public policies (Bárcena, 2018), and standards for their commercialization (UNEP-CITES, 2021). In

Mexico, in 2017, 256 permits were issued for the use of NTFPs (covering 380,000 hectares), mainly in the states of San Luis Potosí, Zacatecas, and Veracruz, which concentrated 86% of the production totaling 562,000 tons. Of these, 477,700 tons consisted of leaves, with the remainder being stems, inflorescences, roots, and fibers, primarily cultivated in *ejidos* (communal land) and communities (SEMARNAT, 2019).

In 2022, the export value of Mexican NTFPs reached nearly 25 million US dollars, marking a 48% increase compared to 2020. The primary destinations included the United States of America, Netherlands, Canada, and Japan (Banxico, 2022), where foliage has shown growing demand (AIPH, 2019). *C. elegans* is particularly valued for its attractive foliage (Castillejos-Musálem, 2014) and its role in generating employment and economic resources in the communities where it is harvested (Blancas Vázquez *et al.*, 2017).

In the state of Veracruz, some reports highlight the importance of the species *C. elegans*, leading to studies focused on diversifying agroforestry systems with palms (Meneses *et al.*, 2012) and canopy cover (Lascurain-Rangel *et al.*, 2019). However, there is limited information regarding the dynamics of change in cultivation patterns, production, and commercialization processes in these plantations. Therefore, this study aimed to characterize the production of *C. elegans* in three municipalities of Veracruz, Mexico, through analyzing the dynamics of production and commercialization systems.

MATERIALS AND METHODS

The study was conducted in the high mountain region of Veracruz, Mexico, specifically in the municipalities of Tepatlaxco, Zongolica, and Omealca. These municipalities were selected due to their significance in the production of *C. elegans* and their shared natural, social, and economic characteristics.

From January to July 2022, a survey was administered to n=84 producers of camedor palm. Convenience sampling was employed, and the survey covered: characteristics of the producers, production unit details, production aspects, and commercialization practices. Data collection was conducted using the KoboToolbox platform (https://www.kobotoolbox.org/). In addition to the survey, techniques such as interviews with key informants (Hay, 2016) and participant observation (Flowerdew & Martin, 2005) were utilized. Interviews and participant observation data were analyzed using ATLAS.ti software to construct the marketing network. Land use change trends were analyzed using conditional probability methods (Borovcnik, 2012).

RESULTS AND DISCUSSION

Production unit characterization

Camedor palm production takes place in remote areas with limited access to services. Producers have an average age of 48 years, and the majority are men (93%). The average educational level is 6 years, with 14% having not completed basic education (Table 1). Similar characteristics are reported by the Agricultural Census of Mexico (INEGI, 2022), which indicates that the agricultural sector is predominantly male and has an average educational attainment at the primary level.

Table 1. Characteristics of the producers and production unit.

Socio-economic factor	Median value	Low level	High level	Std. Deviation	Confidence interval (0.05)	%
Age	48	17	79	16.6	3.56	
Level of formal education	6	0	16	3.7	0.89	
Plot size (ha)	1	0.05	7	0.9	0.19	
Altitude (masl)	1272	801	1530	249	78.14	
Land property (communal)						13
Land property (private)						87

* Original work based on 2022 survey data.

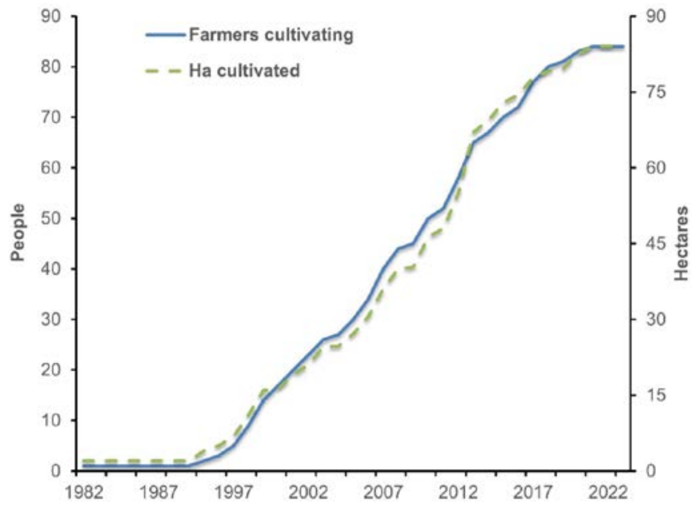

Figure 1. Evolution of producers and cultivated area of *C. elegans*. Survey 2022.

The cultivation is recent, with an average experience of ~12 years, a maximum of 40 years, and a minimum of three years (Figure 1).

The average plantation size is one hectare, which aligns with the findings of Sánchez (2007), who found that in the municipality of Pajápan, Veracruz, 90% of *C. elegans* producers have less than one hectare. The *C. elegans* crops in the study region are distributed between 800 and 1600 meters above sea level, under the canopy of lowland evergreen forests and cloud forests, with slopes exceeding 30%. These conditions match those described by Perez & Geissert (2008). Planting density in the study area varies depending on the slope and the history of land use. In Tepatlaxco, densities of more than 100,000 plants per hectare were found, while in Zongolica and Omealca, the average is ~30,000 plants per hectare (Figure 2). These differences impact crop management, income, and ecosystem effects. The densities in Tepatlaxco exceed INIFAP's recommendations by 40% (Cervantes, 1999).

Land Use Change Dynamics to NTFPs

Foliage cultivation began as an alternative to generate income but has gradually become the primary crop. According to survey data, 56% of producers indicated that

Figure 2. Plantations of *C. elegans* A) Without planting framework and low density in Zongolica; B) With planting framework and high density in Tepatlaxco. J. Orlando Ávila Castro (2022).

the space currently used for foliage cultivation was previously occupied by coffee crops, 26% by forest, 5% by secondary vegetation, and the remaining 13% by maize and banana. This land use change process was also reported by Granados (2004) in producing communities in the municipality of Cuichapa, Veracruz. The land use change process indicates that *C. elegans* cultivation will continue to expand at the expense of coffee cultivation (Figure 3).

In the region, foliage crops are associated with unfavorable soil conditions, steep slopes (>30%), and a high percentage of rocky areas, which hinder the development of other crops. Additionally, respondents mentioned that the cultivation of *C. elegans* is driven by various factors, including continuous economic income, low labor demand, and low investment requirements (Figure 4). Authors such as Sánchez (2007) mention that cultivation is encouraged by the lack of productive options, low coffee prices, consistent income, public incentives, and ease of trade. These factors make it a viable option for the livelihoods of communities, consistent with the findings of Aguirre-Cadena *et al*. (2016).

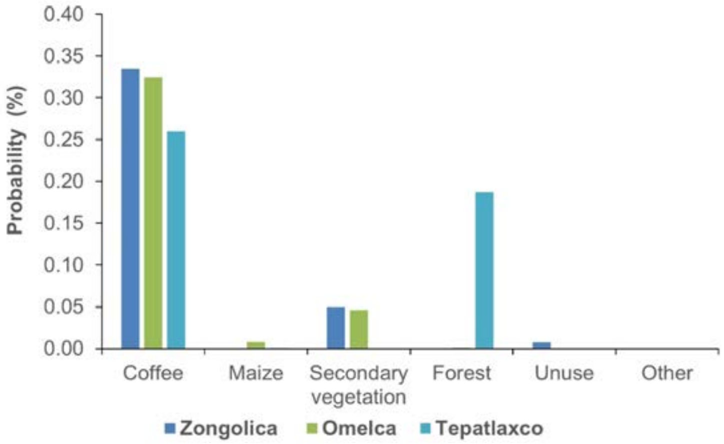

Figure 3. Conditional probability, land use change to *C. elegans*. Survey 2022.

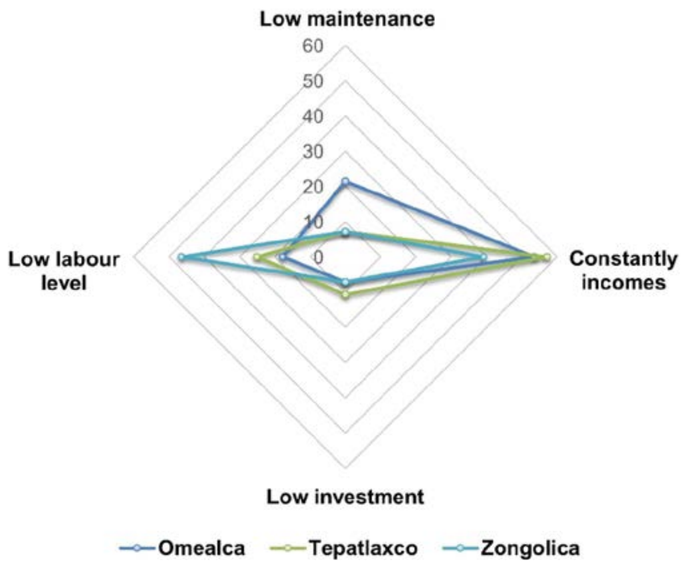

Figure 4. Factors favoring the cultivation of *C. elegans*, in percentages. Survey 2022.

In remote rural areas, NTFPs are cultivated or collected on small plots of marginal land with steep slopes (>30%), where other activities are not profitable.

Productivity of *C. elegans* systems

Leaf cutting is conducted quarterly in 75% of cases. Plantations smaller than one hectare yield an average of 1000 rolls (each rolls contains ~40 leaves) per hectare, whereas plantations larger than two hectares exceed 3700 rolls per hectare. Leaves are harvested using various techniques and tools, such as natural fiber threads and knives (Figure 5-A). For sales, producers use different units of measurement agreed upon with intermediaries.

The palm is regularly sold in rolls containing ~40 leaves each, at an average price of MX $14.00 per rolls (Figure 6-B), which are organized into bunches of 60 rolls (Figure 5-D).

Commercialization

The commercialization network is characterized by a high degree of intermediation (Figure 6). The distribution channel consists of producers, intermediaries, retailers, and consumers, operating within an informal system where each actor operates independently. The latter complicates communication and increases the complexity of commercial relationships. Similar systems are observed across the country among palm producers (CONABIO, 2003). These production systems, like other agricultural products, feature long distribution channels under an informal buying and selling system, where producers receive a minimal proportion of the product's value. This situation is exacerbated by the unregulated status of these productive activities (D. Sánchez & Valtierra, 2003; Scudder *et al.*, 2019).

Figure 5. A: Cutting process, B: Bunch preparation, C: Bundles, D: Packaging (right side). By J. Orlando Ávila Castro (2022).

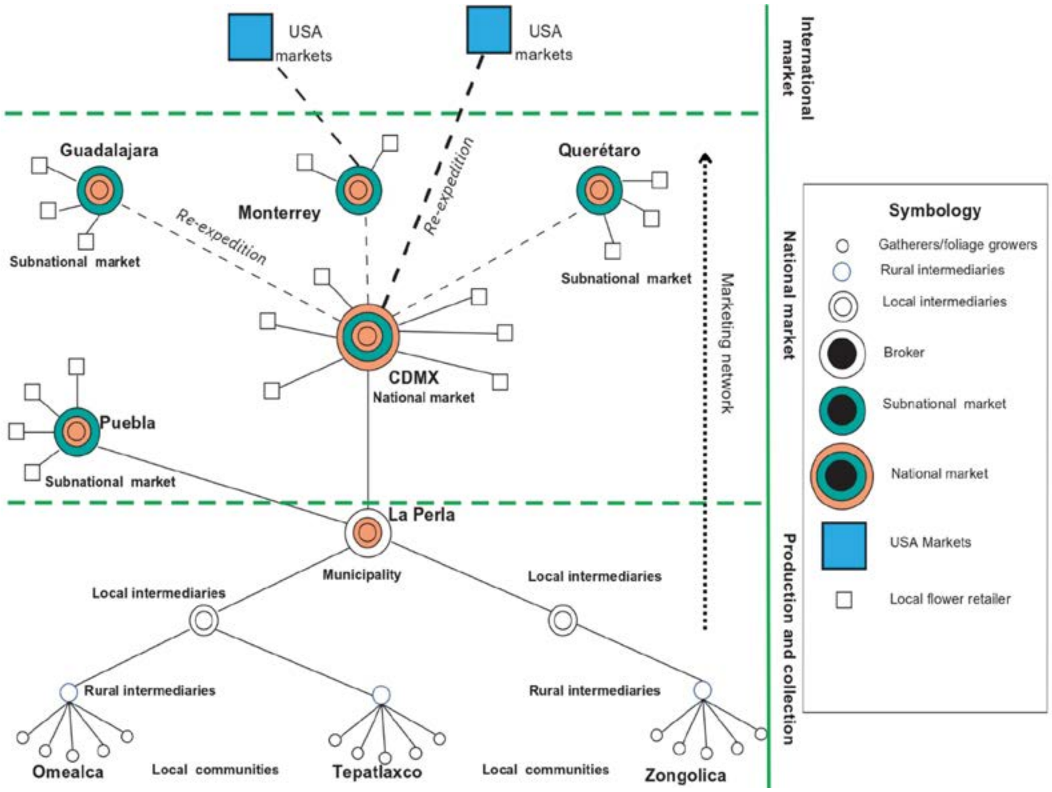

Figure 6. Commercialization network. Data from key informants (2022).

CONCLUSIONS

Small-scale producers in the high mountain regions of Veracruz cultivate NTFPs because they represent one of the few sources of income in these areas. Activities related to *C. elegans* are accessible and require low investment. They are compatible with small-scale agriculture and traditional gender roles, where men perform production activities and women participate in harvesting. Although yields are low in some locations in Zongolica, their importance lies in income generation and reducing income risks from other crops. NTFPs are also crucial for food security, climate change adaptation, and replacing declining crops such as coffee. The socioeconomic contributions of NTFPs in the study communities are affected by factors like intermediation in commercialization.

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Early prediction of lactation milk yield of Holstein-Friesian cows in Querétaro, Mexico

Guevara-Escobar, A.[1]; **Cervantes-Jiménez, M.**[1]; **García-Muñiz, J.G.**[2*]; **Lemus-Ramírez, V.**[3]; **Yabuta-Osorio, A. K.**[3]

[1] Universidad Autónoma de Querétaro, Facultad de Ciencias Naturales. Juriquilla, Querétaro, Querétaro, México. C.P. 76210.
[2] Universidad Autónoma Chapingo, Departamento de Zootecnia, Posgrado en Producción Animal. Chapingo, Texcoco, Estado de México, México. C.P. 56230.
[3] Universidad Nacional Autónoma de México, Centro de Enseñanza, Investigación y Extensión en Producción Animal en Altiplano. Carretera Federal Tequisquiapan a Ezequiel Montes, km. 8.5. Tequisquiapan, Querétaro, México. CP 76790.
* Correspondence: jgarciamppa@hotmail.com

ABSTRACT

Objective: To obtain a machine learning (ML) model to predict the milk yield adjusted to 305 d (MY305) from the same lactation period.

Design/methodology/approach: A database of test days (TD) was used, made up by 11,892 records of daily milk production from cows with more than 150 days in milk (DIM), from 19 farms in Querétaro, Mexico. The milk production was standardized to specific DIMs (5, 10, 20, 30 and 40) and estimations of MY305 were obtained with these, using ML models. The following were also incorporated as explicative variables of the herd: month of birth of the cow, month of start of lactation, number of lactation, number of days for three daily milking events, and the two first linear scores of somatic cells.

Results: The best goodness of fit was achieved with ensemble models, obtaining a deviance of 1503584 in the training with 80% of data chosen randomly, while with 20% of the data reserved to evaluate the deviance model it was 1576776. The relationship between data observed and predictions of MY305 of the ensemble models had a coefficient of determination of $r^2=0.79$ and RMSE of 1256. In the best individual model (deviance of 2281420) of 'deep learning' type, the most important variables were daily milk production at 30, 10, 5 and 20 DIM (19.9, 16.6, 16.2 and 12.8%, respectively).

Limitations on study/implications: The value of RMSE was high. Although TD databases are generated regularly and following systematic measurement procedures but not many farms are represented.

Findings/conclusions: For the database examined, milk production in the early phase of lactation together with a set of automatic learning models resulted in an adequate prediction of MY305.

Keywords: machine learning, somatic cells, lactation curve, test day.

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INTRODUCTION

The predictions of daily cow milk production (MY), including the current lactation, are important to identify critical situations of productive interest and with that to make decisions about nutrition, feed management, reproduction, preventive health, genetic

improvement, among others [1], [2]. The prediction of MY is possible with data-based models of: prior and/or contemporary lactation events of the cow or herd [3]-[5]. Traditionally, MY data in test days (TD) are used to predict the future individual production of cows based on the lactation curve; in these cases, the incomplete gamma function proposed by Wood in 1967 [6], is one of the most frequently used to describe the lactation curve [7]. When the MY is modelled in cow groups, the data of genotype, weight and age of the cow, body condition, frequency of milking and somatic cell count (SCC) in the milk, among others, are also useful.

Presently, there are machine milking systems that record the MY in every milking, in addition to other variables. Currently, the integration of this type of database is one of the most important challenges in the dairy industry due to the diversity of technologies available and the overflowing of data that happens in dairy operations [8]. However, the databases of MY in TD continue being essential for the study of prediction models of MY [4]. Modelling of individual lactation curves or groups of cows with empirical models, such as the Wood function [6] or mechanistic ones [9], allow performing punctual or accumulated MY estimations in time. To perform comparisons between cows, there is a well-established metric, where the MY is adjusted to 305 days in milk (DIM) and to a mature equivalent (MY305) [10], [11]. The MY305 can be adjusted according to the milk solids content and by the number of lactations, allowing to establish comparisons between cows.

Currently, the predictive models of MY based on machine learning (ML) algorithms are increasingly more frequent in the literature and their performance has shown greater advantages than the regression models adjusted by least squares [2], [12]. The applications of the ML models in the milking systems are broad [12], [13]. Ensemble models with strategies of classification and/or regression can be obtained with the ML approach, using incomplete data records, crossed-validation routines, selection of variables, exploration of hyper-parameters, selection of models and automatic ensemble models, with the intention of avoiding the model's overfitting [14]. With the ML models, there have been advancements in the interpretability of the model, allowing to identify the importance of the variables included in the model and the contribution to the solution of specific data records or in the set of the database [15].

For this study, the data available from the early lactation phase of Holstein-Friesian cows were used to predict the MY305. With this approach, Pereira *et al.* [16] identified the Bayesian empirical method and only 5 TD, as the most effective in the prediction of MY305 in Holstein livestock. The neural networks have performed better to predict the MY with early lactation data or from the transition period [4], [17]. Although the data from the transition period of the milking cow are important for the health and future milk yield [18], it is a challenge to predict the future MY, since the TD during early lactation tend to be few and also the health state of the cow can be compromised [4]. The use of daily milk production records based on TD data are proposed, together with the data that allow classifying cows according to their month of birth and their calving, number of lactation, herd and somatic cell count. The objective was to obtain a ML model to predict MY305 based on data from early lactation of Holstein-Friesian cows in Querétaro, Mexico.

MATERIALS AND METHODS

Data from lactation periods that started in 2006 were used, generated in Mexico by Mexico's Holstein Association (*Asociación Holstein de México*, AHM), from 19 herds in the state of Querétaro corresponding to 13,935 lactation records of Holstein-Friesian cows. The cows recorded were from lactation one to ten. Each lactation record had up to ten milk weighing moments. Most of the herds were kept in the milk production confinement system.

The Wood function (Equation 1) was used to model the individual lactation curve for the records with more than five TD and DIM higher than 150 (n=11,892). The milk production adjusted to mature equivalent obtained by the AHM (MY305, kg) was predicted through explicative variables: herd, month of birth of the cow, month of start of lactation, number of lactation, number of days in three times daily milking (3x), first two scores of the linear scoring (LS) system based on the recount of somatic cells (LS 1 and LS 2, Equation 2) (adopted by the Dairy Herd Improvement Association, DHIA), and the estimated milk production ($M\widehat{Y}$, kg) with the Wood function for days: 5, 10, 20, 30 and 40 ($M\widehat{Y}5$, $M\widehat{Y}10$, $M\widehat{Y}20$, $M\widehat{Y}30$ and $M\widehat{Y}40$). The $M\widehat{Y}$s were selected due to the irregularity in the intervals of TD to different DIM. The discreet variables that classify the cows were used based on the report by Grzesiak *et al.* [7] to model the daily production of milk using neural networks.

$$Y_t = at^b \; e^{-ct} \tag{1}$$

where: Y_t is the daily milk production (kg) on day t of lactation, e is the base of the natural logarithms, a, b, c, are adjustment parameters [6].

Calculation of linear scoring based on the somatic cell count (SCC):

$$LS = Log_2\left(SCC / 100\right) + 3 \tag{2}$$

where: LS is the linear scale of the somatic cell count, Log_2 is the logarithm base 2, SCC is the somatic cell count (cells ml^{-1} of milk). The conversion of LS to SCC is achieved with the following expression: $SCC = 100 \times 2^{(LS-3)}$ [19].

Machine learning (ML) algorithms were used to obtain a predictive model of MY305 through the H2O package version 3.40.0.1 [14] of the R language [20]. The AutoML function results in a leaderboard of models and ensemble models generated from models of same leaderboard. The deviance was used as a metric of goodness of fit in the training of models and also to order the ensembles and individual models. The model with best adjustment was the one that produced lowest deviance. For the best individual model, the importance of the variables with their SHAP values was determined (SHapley Additive exPlanations). The code was executed without a time limit, with 50 processing threads on a dual Xeon E5-2680 v4 cluster and with a maximum of 360 Gb of RAM to execute the

AutoML function. Memory was reserved with another 152 Gb of RAM for external code routines of XGBoost (Optimized distributed gradient boosting machine) and within the Ubuntu 22.04.2 LTS operating system.

The AutoML function implies the pre-processing of data, normalization, model selection, optimization of hyper-parameters, analysis of predictions, and control of the over-parametrization of the model [21]. The ML algorithms used in this exercise were: DL (Deep learning), GBM (Gradient boosting machine), XGBoost and GLM (Generalized linear model). However, AutoML explores up to 15 types of algorithms to solve problems of classification and regression [14]. Each ML algorithm is executed many times to explore the effects of regularization and crossed validation on the accuracy of the model [22].

The database was divided randomly in data for the training (80%) and 20% for the model testing [13]. During the training phase, the crossed validation was used to verify the stability of the solution; that is, an internal validation of the model defined by the parameter nfolds=5. Thus, the training data were divided randomly into five groups; four groups were used to train the model, and the fifth to test the performance of the previously trained model. In each training run, the training database was again divided into five groups.

The SHAP values [23] are based on the concept of the Shapely values [24] and they quantify the influence that each variable included in the model has for each individual prediction to deviate from the average prediction. However, each variable contributes in a different way in function of the k variables incorporated into the model; that is, 2k combinations. The SHAP values can be evaluated locally (local explanations) where the contribution of each variable on the result from each prediction is examined. Globally, the local explanations were aggregated to understand the impact of specific variables on the entire model. For individual models it is possible to obtain local and global explanations, but it is not possible for the case of ensemble models.

With the best ML model, estimations of values observed for the MY305 from the database reserved for testing were obtained. The relationship between values observed and estimated was explored with a simple linear regression model and the coefficient of determination (r^2), the root of the mean square error (RMSE), and the bias were obtained; the latter through the blandr package according to Bland and Altman [25].

RESULTS AND DISCUSSION

In the management of dairy cows from the herds studied, extended lactation periods and three daily milking moments were identified as important practices. Lactation with less than 150 DIM were not analyzed (Table 1). In the database, the records from the first lactation were the most numerous (40%), and from these lactation events, 31% were with 150 to 305 DIM and with three times daily milking. No first lactation longer than 305 DIM had days to three times daily milking. The average MY305 of the data used was 11,282 kg, although there was variation according to the duration of lactation and the number of lactation (Table 2). The herds with less than 100 lactation records had a lower MY305 than the herds with more than 100 records (9,233 and 11,310 kg, p=0.012).

Table 1. Number of production records used according to their number of lactation, days in milk (DIM), and the practice of three milking moments per day (3X) for Holstein-Friesian cows in Querétaro, Mexico.

DIM	Lactation number				Subtotal	3x
	1	2	3	4+		
>150 to <305	1803	1357	735	741	4636	2969
>=305 to 365	1343	880	466	449	3138	1654
>=365 to 730	1559	1074	613	740	3986	2057
>730	70	27	18	17	132	91
Total	4775	3338	1832	1947	11892	6771

Table 2. Means ($\bar{x}$) and standard error of the mean (se) for the milk production to mature equivalent obtained by the AHM (MY305, kg) of Holstein-Friesian cows in Querétaro, Mexico.

DIM	Lactation number							
	1		2		3		4+	
	$\bar{x}$	se	$\bar{x}$	se	$\bar{x}$	se	$\bar{x}$	se
>150 a <305	11638	65	11287	73	10875	103	10066	95
>=305 a 365	11697	74	11569	89	11197	130	10789	132

The best result to predict the MY305 was a model ensemble of ML algorithms used. In this case, the deviance was 1503584 and a RMSE of 1226 for the training data, while with the data reserved for the evaluation, the deviance was 1576776 and a RMSE of 1256. These measurements of goodness of fit suggested that the ensemble was a good representation of the database and that there was no over-adjustment. This indicated that the AutoML function solved the possible multicollinearity that could exist between the $M\widehat{Y}$ values. The second best result was an ensemble of the best family of algorithms used, with deviance of 1717832 and RMSE of 1311 for the training data. Four other ensembles followed in the leaderbord (data not shown). Verification of the best ensemble resulted in r^2 of 79.9 between observed and predicted values of MY305 (Figure 1). The bias was −10.42 with a confidence interval at 95% of −63.8 to 43.0; these values were interpreted as the magnitude of underestimation of the model in relation to the MY305 observed. In this case, the confidence interval did not include zero, which indicated that the bias was significant.

The best individual model was the DL type, which remained in sixth place in the leaderboard and their deviance was 2281420 and RMSE of 1510, which resulted in a lower goodness of fit than those of the ensembles. Out of 797 models and ensembles generated, the worst model was GLM type, with deviance 7621002 and RMSE of 2760. This result was explained because the GLM algorithm was highly penalized in the regularization process carried out by the AutoML function to reduce the over-adjustment caused by the multicollinearity in variables. In contrast, with ML algorithms such as neural networks and ensemble methods, this problem can be handled [26], [27]. For the prediction of MY305, the most important variables were the $M\widehat{Y}$s at 5, 10, 20 and 30 DIM, followed by the somatic cell count and lactation number (Table 3). The variables of month of birth

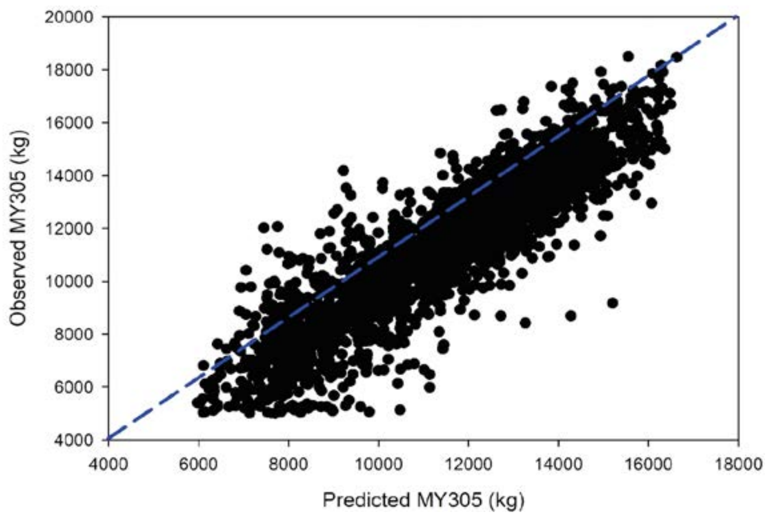

Figure 1. Relationship between values observed of milk production at mature equivalent adjusted to 305 d (MY305) and the corresponding values estimated by ensemble models of machine learning, $y = -951 + 1.074x$, $r^2 = 0.79$.

Table 3. Percentage importance of the variables adjusted in individual models of machine learning to predict the production of milk to mature equivalent (MY305) of Holstein-Friesian cows in Querétaro, Mexico.

	Model			
	DL	GBM	GBM	XGboost
$M\hat{Y}30$	19.9	55.1	56.1	34.7
$M\hat{Y}10$	16.6	6.5	6.3	9.6
$M\hat{Y}5$	16.2	7.5	7.5	13.4
$M\hat{Y}20$	12.8	4.4	4.0	10.2
CS 2	6.4	2.3	2.2	3.2
Lactation number	6.2	8.9	9.2	9.1
CS 1	5.2	2.5	2.4	3.3
Herd	4.9	6.8	7.1	7.3
Month of calving	4.3	2.8	2.6	3.4
3X	3.9	1.2	1.0	2.8
Cow's month of birth	3.8	2.0	1.7	2.9
Ranking in the table of models	6°	8°	9°	11°
Deviance	2281420	2672503	2703187	2762434
RMSE	1510	1634	1644	1662

of the cow and calving month were variables of lower importance, just as 3X. Although calving month had importance of 4.2% in the DL model (and lower in other models), seasonal differences were observed for MY305 with milk productions from lactation started in winter, summer and spring being similar between them (11,111, 11,112 and 11,181 kg, $p > 0.05$), winter and summer were different from fall (11,349 kg, $p < 0.05$), yet at the same time fall and spring were similar. This result suggests that the variables with low importance in the ML models also must be considered in modelling.

Figure 2 shows the SHAP contribution of each variable and each observation (lactation records) in the prediction of MY305 by an individual model of XGBoost type (eleventh on the scoring table). Using the H2O package, it is only possible to obtain the SHAP values for the models XGBoost, GBM and Random Forest. When the SHAP values were graphed, the aggregated trend of the contributions of each observation was appreciated where some of them contributed more than others. In the XGBoost values, the larger magnitude of the SHAP values of $M\hat{Y}30$ and $M\hat{Y}5$ indicated a higher predicted value of MY305 (reddish colors with positive SHAP value). In this model, the $M\hat{Y}30$ variable was more important than the $M\hat{Y}5$ variable (Table 3), because the SHAP values of $M\hat{Y}30$ were more useful to build a classification tree. For the $M\hat{Y}10$ and $M\hat{Y}20$ variables, the SHAP values of highest magnitude contributed to predicting lower milk yield (reddish colors with negative SHAP values). Likewise, as the SHAP value of number of lactation increased, the contribution was in the sense of lower magnitude of the MY305. For the linear scale (LS) of the somatic cell count, a negative contribution was observed in the model, but with comparatively lower SHAP values than the variables already mentioned.

The distribution of the SHAP values for the $M\hat{Y}$ variables suggested that there are thresholds for these variables associated with a higher value of MY305. The graphs of partial dependency indicate that these thresholds are different between the ML models generated and in addition that the dependency was not linear between the MY305 and each variable included in the model, for example, for $M\hat{Y}30$ (Figure 3). The general linear model (GLM) did not show dependency of the MY305 in function of the range of $M\hat{Y}$ values. For the ensemble models, the partial dependency graph suggested that values of $M\hat{Y}30$ lower than 20 kg are not important to predict MY305; the same happened with values of $M\hat{Y}30$ higher than 40 kg when the dependency decreased. The grey bars in Figure 3 represent the frequency distribution of the data of MY305.

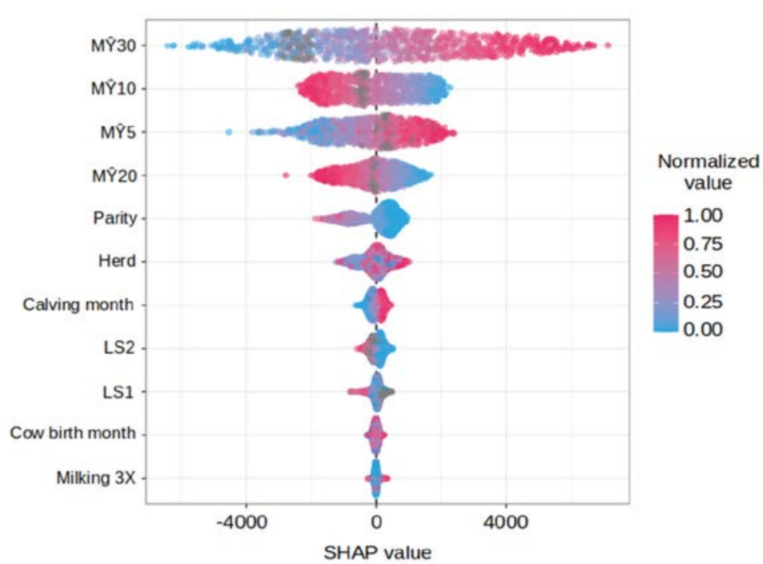

Figure 2. SHAP values for the XGBoost model presented in Table 3.

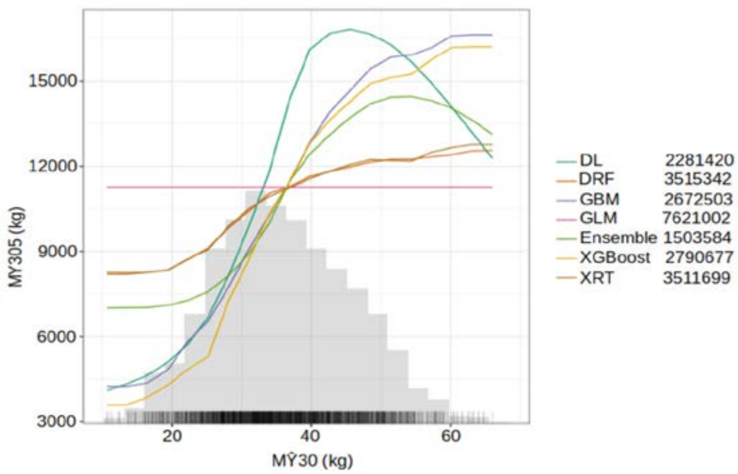

Figure 3. Partial dependency of MY305 on $M\hat{Y}30$ for different ML models. The deviance for each model is presented. The frequency histogram of $M\hat{Y}30$ is shown in grey.

The average values and standard deviations of MY305 of the first and second lactation events in the database examined (11,586±2,760 kg and 11,473±2,701 kg) were found within the range reported by Toledo-Alvarado *et al.* [28] in Holstein cows in Mexico, where the MY305 for the first lactation was 11,473±2,443 kg and for the second one 11,612±2,652 kg; other lactation events showed slightly lower total yield than those reported in this study. Since the heifers are genetically superior, they generally have higher MY305 than the mature cows of lower genetic quality [29]. Likewise, the values of MY305 reported here were numerically lower than the average of 12,662 kg for Holstein in the United States [30].

We used estimated values of daily milk production ($M\hat{Y}$) during the early lactation based on modelling with the Wood function. These data fulfilled the function of representing measurements of early lactation; in many farms these data could come directly from the registry of the automatized milking system. Based on our result, the daily registry of milk production can be explored during early lactation to obtain estimates of the MY305 or daily MY. This would be similar to the approach of [2], who used the machine learning algorithm XGBoost. In that study, with climate data of the 60 previous days, the identification, age and weight of buffalo, their feed consumption and their days in milk, the daily frequency of milking, milk production, and fat and protein composition in the next 28 days (r^2=0.90) were predicted.

The variance and auto-correlation of deviations of the daily milk production during early lactation could be related to clinical mastitis [1].

The variables of somatic cell count were included in the models, but they were not the most important. Of the 13,935 records, the LS did not have a value in any of the two dates examined (4,661 and 4,361 records) and 4,206 records did not have a value in any of these dates. It is possible that the low importance of LS 1 and LS 2 was a reflection of a disperse database.

It is also possible that the management of some herds was satisfactory and the incidence of mastitis low. The herds with less than 100 records of lactation had higher counts than the herds with more than 100 records (LS 1 of 29.56 and 22.52 and LS 2 of 27.40 and 18.31, $p<0.001$). However, this result can be an effect from the dilution of the LS, since the herds with more than 100 cows had higher MY305. Correcting the LS in function of the MY would eliminate the effect of dilution [31].

The proposal by Singh *et al.* [17] to predict the MY305 ($r^2=0.82$) of buffalo in the first lactation is the one most similar in the literature to this study's approach. They considered the milk production in test days at 6, 36, 66 and 96 DIM, the daily milk production in the peak of lactation, and the age at first calving. Cook *et al.* [29] considers the first test day (around 20 DIM) as an important predictor of yield at 305 d, because many of the health problems during the transition period would explain a low daily milk production at first DIM.

In this study, the decision to use $M\hat{Y}$ at specific lactation days was made, with the aim of avoiding a disperse database. Although the ML algorithms can deal with disperse databases, their efficiency in training the model is low because the processing time increases and the complexity of the models also increases. In this sense, the use of the daily milk production records during early lactation would still have to be explored, even when the records of the number of test days and the days in milk of the measurement do not agree, as is the case with disperse databases. One possibility would be to group the measurements of test day by week of lactation, and thus to reduce the number of lactation records without data for a given week.

CONCLUSION

This study showed that ensemble models of machine learning allow estimating the milk production adjusted to mature equivalent and that the deep learning algorithm is capable of generating the best individual model. Among the ten best models, six ensembles had the lowest deviance, followed by a deep learning model and the last three were gradient boosting machine. The daily milk production variables in the early phase of lactation, from 5 to 30 days, were important in the models; however, this was not the case for the variables that classify the livestock, such as month birth, month of calving, the herd and others. No model included the daily milk production at 40 days of lactation.

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Colegio de Postgraduados

Heliconias (Heliconiaceae) in rural landscapes

Baltazar-Bernal, Obdulia[1*]

[1] Colegio de Postgraduados - Campus Córdoba, Carretera Federal Córdoba-Veracruz Km 348, Congregación Manuel León, Municipio de Amatlán de los Reyes, Veracruz, México, C. P. 94946.
* Correspondence: obduliabb@colpos.mx

ABSTRACT
Objective: To research native, endemic, and introduced heliconia species and their size for use in rural landscaping and to describe the phenology of two species.
Design/Methodology/Approach: A bibliographical review was carried out in search for native and endemic heliconia species; a list of the introduced heliconias was developed with field data. The phenology of *H. psittacorum* and *H. wagneriana* was evaluated from June 2021 to September 2023. The *H. psittacorum* and *H. wagneriana* rhizomes used for this research measured 40 and 60 cm, respectively. They were washed with tap water and then immersed in 1 g L^{-1} of Captan® 500 for 10 min. Subsequently, they were established in a propagation bed for three weeks and then they were planted in the field, to record the vegetative and flowering stages.
Results: According to the bibliography, Mexico has 21 heliconia species and one hybrid; five of which are endemic species, while the rest are native. Eight of them are used in landscaping. *H. psittacorum* and *H. wagneriana* had a vegetative period of 10 and 33 weeks, respectively; meanwhile, *H. psittacorum* flowered at five weeks, while *H. wagneriana* flowered at eight weeks.
Study Limitations/Implications: The phenology of two species was just evaluated in the field..
Findings/Conclusions: Only eight out of the 21 species of Mexican heliconias are used in landscaping; however, 20 are introduced cultivars are used in Mexico landscaping. The production of flowers began after 10 (*H. psittacorum*) and 33 (*H. wagneriana*) weeks; both species are used in floral designs once they bloom. Heliconias are used in the gardens of rural tourism enterprises and educational institutions.

Keywords: native heliconia, endemic heliconia, introduced heliconia, heliconia phenology.

INTRODUCTION

The family Heliconiaceae is native to the American tropics (Kress *et al.*, 2001). It consists of over 250 species, 90 of which can be found in Colombia (Berry and Kress, 1991), while Mexico has 16 native species, including *H. veracruzensis* C. Gut. Báez (Gutiérrez-Báez *et al.*, 2016) and three species which are endemic to southeastern Mexico (Villaseñor, 2016). Heliconias are herbaceous perennials with very attractive and exotic inflorescences (Kress *et al.*, 2001; Loges *et al.*, 2016). According to their size, they are classified as: small (1-1.5 m), medium (1.5-3.0 m), and big (>3 m) (Baltazar-Bernal *et al.*, 2011).

Heliconias achieve a better development in the valleys and hillsides than in the top of tropical and subtropical

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forests (Tokarz *et al.*, 2019), where they engange in multitrophic interactions with hervibores, pathogens, pollinators, and seed spreaders (Benítez-Malvido *et al.*, 2014). Their roots play a major role in the conservation of the undergrowth soil; when they flourish, they are food for hummingbirds (Torres-Vanegas *et al.*, 2019) and bats (Benítez-Malvido *et al.*, 2014).

These plants grow from seeds, in their natural environment, and from rhizomes, in *ex situ* enviroments (Berry and Kress, 1991). They have vegetative, reproductive, and senescence phases. Small heliconias flower all year long; however, medium-sized and big heliconias have reproductive peaks throughout the year (Baltazar-Bernal *et al.*, 2011). The aim of this research was to determine the starting point and the duration of the flowering. *Heliconia wagneriana* Peterson, *H. orthotricha* L. Anderson, *H. bihai* L., and *H. stricta* Huber flower sooner than *H. rostrata* Ruiz & Pavon. Meanwhile, *H. wagneriana* starts blooming 30 weeks after planting (Huaranga-Herrera, 2019).

As a resource, landscape provides information about the overall state of the environment (Picuno *et al.*, 2019). Rural landscape is the result of the interaction between natural elements and human activities, in time and space. According to Xie *et al.* (2022), it has various characteristics resulting from strictly cultural aspects. Therefore, preserving the forests that host the greatest heliconia diversity is a very important task (Benítez-Malvido *et al.*, 2014; Bruna *et al.*, 2023). Since heliconias are a shared property in rural landscapes, studying the biotic and abiotic factors that influence their establishment is fundamental, along with the preservation of their rural landscapes.

Consequently, the aim of this research was to study native, endemic, and introduced heliconia species, as well as their size and use in the rural landscape, describing the phenology of two species. Therefore, this study sought to determine which heliconias can be considered Mexican and which heliconia size is used in landscaping. They hypothesis of this work was that the heliconias found in rural landscapes are mostly introduced medium-sized species.

MATERIALS AND METHODS

Based on the bibliographical information available, heliconias found in Mexico were classified according to their origin (native, endemic, and introduced), their size, and their use in landscaping. In the case of introduced heliconias, the information was taken from the field databases that this team has collected. For more than ten years, this team has established and advised clients regarding the handling of heliconias in anthropized landscapes. Two species were established in the field for phenological purposes.

Study area

The study was carried out in the experimental area and the gardens of the Colegio de Postgraduados-Campus Córdoba (CPCC), located in Amatlán de los Reyes, Veracruz (18° 51' 21" N, 96° 51' 35" W, 627 m.a.s.l.). The climate is humid subtropical, with abundant summer rains, an annual precipitation of 2,150 mm, and a mean annual temperature of 22 °C (INAFED, 2023). The local vegetation is composed of plants that have been introduced from the mesophytic forests and low rainforests (Baltazar-Bernal *et al.*, 2020).

Plant material

The phenology of heliconias was studied from June 2021 to September 2023. Forty *Helicona psittacorum* L. f. (Figure 1A) and 20 *H. wagneriana* Peterson (Figure 1B) rhizomes were used. Both species are native to Mexico. The former is small in size and flowers all year long, while the latter is medium-sized and has flowering peaks throughout the year (Figure 1). The rhizomes of *H. psittacorum* and *H. wagneriana* (Figure 2) measure 40 and 60 cm, respectively; they have two shoots, with an average length of 8 cm. They weight 60 (*H. psittacorum*) and 400 g (*H. wagneriana*). The rhizomes were rinsed with tap water and then immersed in a water solution with a 1 g L^{-1} of Captan® 500 solution for 10 min.

Establishment of the experiment

The rhizomes were established in a propagation bed for three weeks (Baltazar-Bernal *et al.*, 2011). Afterwards, they were planted in a previoulsy prepared plot (Figure 3). The

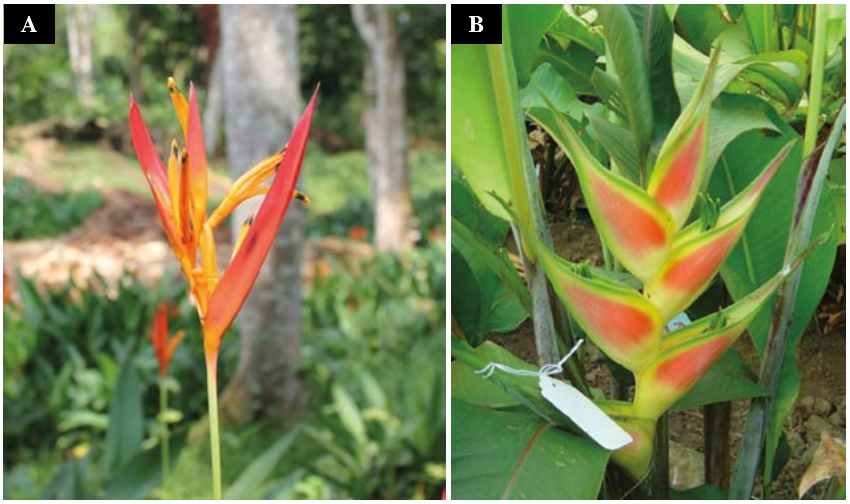

Figure 1. Inflorescences of *Heliconia psittacorum* L. f. (A) and *H. wagneriana* Peterson (B).

Figure 2. Rhizome of *Heliconia wagneriana* Peterson.

Figure 3. Establishment of *Heliconia wagneriana* Peterson.

soil was irrigated to a saturation point. Subsequently, humidity was kept at field capacity irrigating by hand with a water stream, twice per week. The number of weeks required to reach the flowering stage was recorded.

RESULTS AND DISCUSSION

Native Mexican and introduced heliconias used in landscaping

Based on the bibliographic review, 21 native heliconia species were found in Mexico (Table 1). Villaseñor (2016) classified *Heliconia mooreana* R. R. Sm., *H. uxpanapensis* Gutiérrez-Báez, and *H. wagneriana* Peterson (Figure 1B) as endemic species, while Gutiérrez-Báez *et al*. (2016; 2022) included *H. veracruzensis* C. Gut. Báez and *H. chiapensis* Gutiérrez-Báez in this category. For their part, Curiel *et al*. (2022) generated a hybrid of heliconia Karely. *H. psittacorum* L. f. —a native species commonly known as avecilla ("little bird")— is not only used in landscaping (Figure 1A), but also as an indoor plant, as a result of its small size (<1.5 m) (Baltazar-Bernal *et al*., 2011). *H. adflexa* (Griggs) Standl. is seldom used in lansdcaping, in slightly more temperate climates, with temperatures of ≤5 °C. Little value has been attached to *H. latispatha* Benth, likely as a result of its wide distribution as a wild species; however, it s a versatile species with high commercial potential. *H. librata* Griggs requires a lot of care and is used in Teapa, Tabasco, for lanscaping purposes. *H. rostrata* is one of the most popular landscaping species, in Mexico and the whole world (Pinheiro *et al*., 2012; Naik *et al*., 2019), as a result of its high adaptability, its attractive colors, and its pendulum-like bracts (Berry and Kress, 1991). The use of *H. collinsiana* has increased in landscaping; nevertheles, its great size (>3 m) (Table 1) limits its use (Baltazar-Bernal *et al*., 2011). Heliconias are part of the tropical landscapes of the world (Baltazar-Bernal *et al*., 2011; Benítez-Malvido *et al*., 2014). In Mexico, the most beautiful and most widely distributed heliconias are *H. collinsiana* Griggs, *H. latispatha* Benth., and *H. schiedeana* Klotzsch (Villaseñor, 2016).

The states that shows more than 12 native species are Veracruz, Oaxaca, Chiapas, and Tabasco; meanwhile, Mexico City, the State of Mexico, and the states of Chiapas

Table 1. Size of the endemic and native heliconias in Mexico.

Cientific name	Origin	Size	Used in landscape
Heliconia aurantiaca Ghiesbr. ex Lem.	N	S	No
H. psittacorum L. f.	N	S	Yes
H. adflexa (Griggs) Standl.	N	M	Yes
H. latispatha Benth.	N	M	Yes
H. librata Griggs	N	M	Yes
H. lophocarpa G. S. Daniels & F. G. Stiles	N	M	No
H. chiapensis Gutiérrez-Báez	E	M	No
H. rostrata Ruiz & Pavon	N	M	Yes
H. schiedeana Klotzsch	N	M	No
H. spissa Griggs	N	M	No
H. subulata Ruiz & Pav.	N	M	No
H. tortuosa Griggs	N	M	No
H. vaginalis Benth.	N	M	No
H. wagneriana Peterson	E	M	Yes
H. uxpanapensis Gutiérrez-Báez × *H. latispatha* Benth. Var. Karely	---	M	---
H. bihai L.	N	B	Yes
H. bourgeana Petersen	N	B	No
H. collinsiana Griggs	N	B	Yes
H. dielsiana Loes.	N	B	No
H. mooreana R.R. Sm.	E	B	No
H. uxpanapensis Gutiérrez-Báez	E	B	No
H. veracruzensis C. Gut. Báez	E	B	No

Origin: Endemic (E), Native (N)
Size: Small (S), Medium (M), Big (B)
Source: Elaborated with data from Villaseñor, 2016; Gutiérrez-Báez *et al.*, 2016; Gutiérrez-Báez *et al.*, 2022; Curiel *et al.*, 2022 and field data.

and Veracruz stand out for the development of *H. adflexa* in their more temperate climate (Villaseñor, 2016) (Figure 4).

Heliconias have been introduced to Mexico by flower farms and institutions such as the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The aim of INIFAP was to train producers, mainly in the production and commercialization of heliconias as cut flowers. However, over the course of the last decade, the price of cut flowers has fallen more than 50%; consequently, producers are forced to sell rhizomes of the introduced cultivars at $15 to $100 Mexican pesos, to be used in landscape gardening. The flowers of introduced cultivars of *H. bihai* are very popular, because they are smaller and more colorfoul than wild species (Table 2). Nevertheless, before producers use a new cultivar, they should study their physiological characteristics (Naik *et al.*, 2019). It is important to carry out research with native heliconias so that they are used in the landscape, instead of using introduced heliconias.

Figure 4. Number of Mexican heliconia species per state. Source: Figure developed by the authors based on data recorded by Villaseñor (2016), Gutiérrez-Báez *et al.* (2016), Gutiérrez-Báez *et al.* (2022), Curiel *et al.* (2022), and field data.

Table 2. Size of heliconia cultivars introduced in Mexico.

Heliconia cultivars	Origin	Size	Used in landscape
Heliconia psittacorum L. f. cv. Andromeda	I	S	Yes
H. psittacorum L. f. cv. Lady di	I	S	Yes
H. psittacorum L. f. cv. Sassy	I	S	Yes
H. psittacorum L. f. cv. St. Vincent red	I	S	---
H. psittacorum × *H. spathocircinat*a cv. Golden Torch	I	S	Yes
H. psittacorum × *H. Spathocircinata* cv. Golden Torch Adrian	I	S	Yes
H. psittacorum × *H. spathocircinata* cv. Alan Carle	I	S	Yes
H. stricta Huber	I	M	Yes
H. stricta Huber cv. Fire Bird	I	M	Yes
H. stricta Huber cv. Fire Opal	I	M	Yes
H. stricta Huber cv. Tagami	I	M	Yes
H. orthotricha L. Anderss. cv. She lisa	I	M	---
H. bihai Huber cv. Lobster claw two	I	B	Yes
H. bihai Huber cv. Aurea	I	B	Yes
H. bihai Huber cv. Nappi	I	B	Yes
H. caribaea Lamarck cv. Chartreuse	I	B	Yes
H. chartacea Lane ex Barreiros cv. Sexy Pink	I	B	Yes
H. chartacea Lane ex Barreiros cv. Sexy Scarlet	I	B	---
H. caribaea × *H. bihai* cv. Jacquinii	I	B	Yes
H. champneiana Griggs	I	B	Yes
H. latispatha Benth. cv. Butter	I	B	---
H. stricta Huber cv. Iris Red	I	B	Yes

Origin=Introduced (I);
Size: Small (S), Medium (M), Big (B)
Source: Elaborated with field data.

Heliconia phenology

The vegetative stage of *H. psittacorum* lasted 10 weeks, far shorter than the period reported by Da Silva *et al*. (2017) for *Heliconia psittacorum* × *H. spathocircinata* 'Golden Torch' (18 weeks). On the one hand, *H. psittacorum* is smaller and, on the other hand, rhizomes with two shoots were planted; nevertheless, the vegetative phase falls within the normal parameters reported by Berry and Kress (1991) for small heliconias (16 to 20 weeks). The vegetative phase of *H. wagneriana* ended 33 weeks after its establishment, a longer period than the one reported by Huaranga-Herrera (2019). This difference may be attributed to the slighty lower altitude and higher temperatures of the experiment site, which reduced the vegetative phase of *H. wagneriana* to 30 weeks.

The flowering phase of *H. psittacorum* (Figure 5A-C) and *H. wagneriana* takes place at five and eight weeks, respectively. Once this phase starts, *H. psittacorum* continues flowering throughout the year; nevertheless, the plantation should be replaced every five years, because the quality of the plant decreases and empty spaces appear between them. Consequently, a new *H. wagneriana* plantation should be established every seven years.

Heliconias in landscaping

Heliconia are not only used as cut flowers; they are also used in gardening and landscaping (Pinheiro *et al*., 2012; Malcar and Biswas 2022). Several *H. psittacorum* cultivars have been introduced to Mexico, because they are readily available and their small size facilitates the renovation of a plantation, although some medium-sized cultivars also exist (Table 2). Only introduced cultivars are sold in selling points. Meanwhile, native species can be found in some institutions and botanical gardens in Mexico.

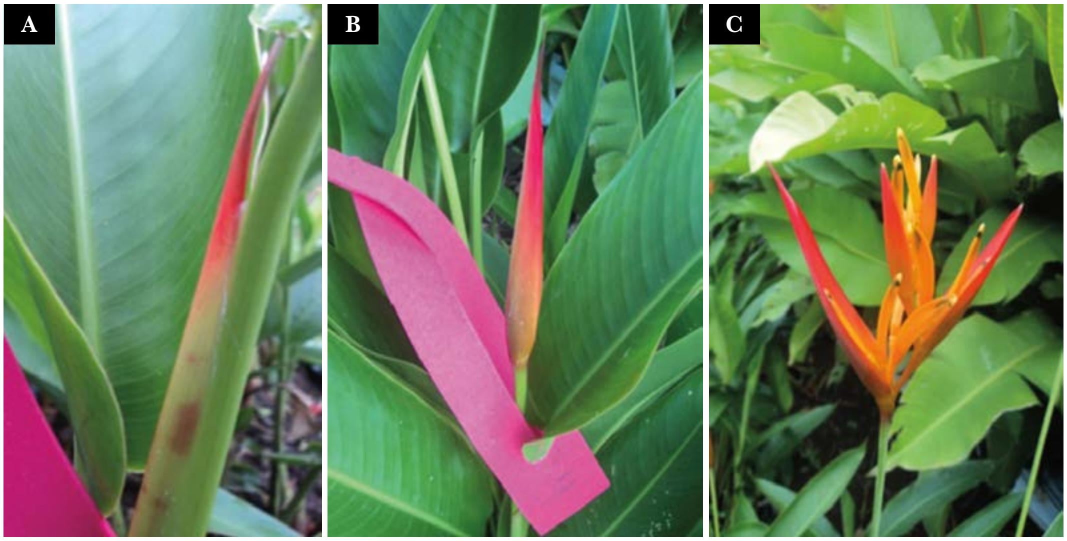

Figure 5. Phenology of the flowers of *Heliconia psittacorum* L. f. at the first (A), third (B), and fifth (C) week of development.

The exotic colors of their bracts (commonly called "flowers") make heliconias a tropical genetic resource and an alternative for rural landscaping. Therefore, to sucessfuly recreate a heliconia landscape that resembles natural landscapes, heliconias must be reintroduced to wide spaces. Additionally, as a result of their evolution, the most important environmental interaction of heliconias is with hummingbirds (Janeček *et al.*, 2020). Finally, the rhizome growth of heliconias protects the soil and consequently has a positive impact in the landscape (Woods *et al.*, 2022; Krishna *et al.*, 2023).

The flowers and leaves of heliconias grown by entrepreneurship tourism rural are also used for the floral designs (Figure 6A and 6B) that decorate bedrooms and halls of ecotourism centers and hotels.

Using native heliconias for landscaping is a feasible effort: however, their propagation and establishment should be researched to guarantee the continuation of the species, taking into consideration the microclimatic conditions required for their development. Additionally, heliconias should be subject to appropriate management practices to transform *ex situ* landscapes into shelters for hummingbirds (Torres-Vanegas *et al.*, 2019) and bats (Benítez-Malvido *et al.*, 2014), both of which feed on the nectar of heliconias.

Figure 6. Floral designs made with heliconia flowers (A) and leaves (B) as focal points.

CONCLUSIONS

Only eight out the 21 Mexican heliconias, along with 20 introduced species of small and medium-sized plants are used for landscaping. *H. psittacorum* and *H. wagneriana* bloomed between 10 and 33 weeks after they were planted. *H. psittacorum* blooms all year long and *H. wagneriana* has two flowering periods. The use of native heliconias in landscaping is crucial to guarantee their conservation.

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Colegio de Postgraduados

Socioeconomic impacts on Valencia orange (*Citrus sinensis* [L.] Osbeck) farming in Martínez de la Torre, Veracruz, Mexico

Carreño-López, Magda V.[1]; Almanza-Sánchez, M.[1]; Arvizu Barón, E.[2]; Franco-Valderrama, A.[1*]

[1] Universidad Autónoma Chapingo. Carretera México-Texcoco Km. 38.5, Chapingo, Texcoco, C. P. 56230, Estado de México.

[2] Colegio de Posgraduados, campus Montecillo. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, C. P. 56264, Estado de México.

* Correspondence: mareducacion2020@gmail.com

ABSTRACT

Objective: To analyze the socioeconomic impacts on Valencia orange farming in Martínez de la Torre, Veracruz, Mexico.

Methodology: A study was carried out using semi-structured surveys applied to a sample of 40 Valencia orange producers during the 2023 agricultural cycle. Convenience sampling was carried out, considering demographic, agronomic, socioeconomic and environmental variables. The methodology included the analysis of production costs, profits and profitability. A multiple regression analysis was conducted to evaluate the relationships between the variables, using the statistical SPSS software version 29.0. The variables considered were profitability of the crop (dependent variable), and the independent variables: sowing density, age of the crop, yield, and production cost.

Results: The coefficients of regression show a direct relationship with the dependent variable, which is why the model as a whole is useful to explain the variability in the profitability of the Valencia orange crop.

Conclusions: The current production model highlights key challenges and opportunities for the producers. It is considered that the socioeconomic, agronomic, commercial and environmental variables drive the sustainability and competitiveness of the crop in the region.

Keywords: production, profitability, yield, Valencia orange, costs.

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INTRODUCTION

Globally, orange [*Citrus sinensis* (L.) Osbeck] leads production and trade in the citrus production sector. As a country, Mexico has given high priority to citrus farming because of its competitiveness in the production and export of lemons, limes, grapefruits and oranges, and it is the fourth global producer of grapefruit and fifth of orange (SADER, 2022).

According to the last report from the statistical yearbook of agricultural production in the year 2022, published by the Agrifood and Fishing Information Service (*Servicio de Información Agroalimentaria y Pesquera*) (SIAP, 2023), citrus production in Mexico is concentrated mainly in 10 states, with the following standing out: Tamaulipas, Veracruz, Tabasco, Yucatán, Nuevo León, Puebla, Sonora, Colima, Michoacán and Oaxaca. Among them, Veracruz is the undisputed leader in the production and export of citruses, performing a crucial role in the supply of oranges of varieties as relevant as Valencia, marrs and bitter orange.

In particular, the state of Veracruz has achieved a spillover of more than 7 million pesos through orange farming. With an average annual yield of 14.64 tons per hectare and a surface sown that exceeds 171,719 hectares, the region has proven to be a fundamental pillar in the domestic citrus production industry. The five municipalities that stand out in this region due to their contribution to orange production are: Álamo Temapache with 722,238 tons, followed by Tihuatlán with 200,328 tons, Papantla with 199,030 tons, Castillo de Teayo with 172,176 tons, and Martínez de la Torre with 134,168 tons.

There is special emphasis on Martínez de la Torre, located in the northern zone of the state of Veracruz, Mexico; this municipality has become a key stakeholder in the history of citrus production in the country throughout time. This prominence is due, on the one hand, to its outstanding production and export of Persian lime, and on the other, to the diversification that it presents by hosting the three varieties of orange previously mentioned, in contrast with the other municipalities that are limited to the cultivation of the Valencia variety.

Farmers from Martínez de la Torre, Veracruz, have generally faced a complex environment in constant change that includes fluctuations in the national and international markets that affect the profitability of orange production, as well as challenges related to pests, diseases, and climate change. There are also structural factors such as investment in technology, access to credit, and dynamics in the supply chain that have influenced the capacity of producers to adapt and to respond. In this context, socioeconomic consequences are evidenced in Valencia orange farming in Martínez de la Torre. From the year 2003 until 2022, the surface sown with orange crop has reduced by 11,221 hectares (SIAP, 2023).

Based on this, the socioeconomic impacts on Valencia orange farming were analyzed, taking into account the combination of socioeconomic variables adapted to the specific conditions in the region of study. Semi-structured surveys were conducted during the agricultural cycle corresponding to the year 2023, directed at a sample of 40 Valencia orange producers selected through convenience sampling.

MATERIALS AND METHODS

Characteristics of the study zone

Throughout its rich trajectory in the citrus industry, the municipality of Martínez de la Torre, Veracruz, has stood out as an important producer and exporter of citruses.

The results from the agricultural cycle of the year 2022 by SIAP (2023) reported the cultivation of Persian lime with a harvested surface of 15,579 hectares. Likewise, other citruses such as orange reported a harvested surface of 9,741 hectares, followed by grapefruit with 2,280 hectares, and varieties such as tangerine with 155 hectares and mandarin with 23 hectares harvested, which all contribute to the productive landscape. The value of the joint production of these citrus crops resulted in a total production value that exceeded 2,600 million MX pesos.

These impressive data reflect the continuous importance and success of Martínez de la Torre in the citrus production sphere, consolidating it as a key protagonist in the production and export of citrus at the national and international level.

Location of the study zone

According to the National Institute of Statistics and Geography (*Instituto Nacional de Estadística y Geografía*) (INEGI, 2021) together with the Ministry of Finance and Planning of the government in Veracruz de Ignacio de la Llave (SEFIPLAN, 2021), the geographic coordinates locate the municipality of Martínez de la Torre in the northern area of Veracruz, between parallels 19° 58' and 20° 17' latitude North and 96° 56' and 97° 10' longitude West, with a surface of 402.1 km^2 and an altitude that ranges from 10 to 400 masl. This municipality destines 79.07% of its areas to agriculture, while it only occupies 5.36% for the urban zone, leaving 15.57% to grasslands and rainforest.

Research techniques

A total of 40 semi-structured exploratory surveys were conducted, through convenience non-probabilistic sampling with different Valencia orange producers. The characterization of the crop in study was carried out in the first section, in agreement with the application of the survey with the aim of establishing the relationship between production costs and profitability of the Valencia orange crop; the second section represents the multiple linear regression econometric model. For the data analysis obtained, the IBM Statistical Package for Social Science (SPSS) version 29.0 was used for statistical tests and the multiple linear regression model.

MATERIALS AND METHODS

In the specific context of evaluating the profitability in Valencia orange production, a multiple regression analysis was conducted, where the multiple linear regression model is considered as a model to predict the value of a dependent variable based on the value of two or more independent variables; that is, Y is the dependent variable that it seeks to predict; (β_0), is the constant that represents the expected value of Y when all the predicting variables (X_1, X_2, ..., X_n) are zero; (β_1, β_2, ..., β_n) are the regression coefficients that indicate the relationship between each predictor variable and the dependent variable. For the study case, the dependent variable was profitability, and the following were independent variables: density of sowing, age of the crop, yield, and production cost.

The general equation of the multiple linear regression model is:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_n X_n + \ldots + \varepsilon$$

Production costs of Valencia orange

The key method to evaluate the production costs of Valencia orange implied the conventional approach of economic theory.

This method is based on the relationship between the total production cost and the amount of oranges generated, expressed through the general formula:

$$Unit\ Cost = (Total\ Production\ Cost) / (Orange\ Yield)$$

Obtaining an accurate estimation of the total cost requires the meticulous collection of detailed data on fixed and direct costs, classifying them as labor, inputs, agricultural machinery, land, leasing, distribution and financing, among others. The sum of these elements generates the total production cost. The orange yield is measured in tons per hectare.

It is imperative to consider that the costs associated with the production activities and the agricultural inputs were obtained through two different methods: surveys and data about prices provided by commercial houses near the study area in the year 2023. The comparison between these two sources allows a more complete and accurate evaluation of the costs involved in Valencia orange farming.

Production profitability of Valencia orange

Measuring the profitability of the Valencia orange crop for the agricultural cycle 2023 was obtained through the formula:

$$Profitability = \left(Profit - Investment \,/\, Investment\right) * 100$$

The profit was calculated using the "Margin of Contribution" formula, which represents the difference between total income and the variable production costs:

$$Profit = Total\ Income - Total\ Production\ Cost$$

The "Total Income" refers to what is generated by the sale of Valencia oranges, and the "Total Production Cost" includes the variables of labor, inputs, agricultural machinery, land, leasing, distribution and financing, among others.

For the determination of costs and yields, data from interviews with producers and statistics reported by the SIAP portal for the year 2023 were compared. In order to avoid bias in the information, however, the cost (price) taken into account for the analysis was the one manifested by the producers of Valencia orange through the interview.

RESULTS AND DISCUSSION

Characterization of Valencia orange farming

During the observations, various activities have been identified in the farmland, classifying them into two categories: farming tasks to maintain the orange plantation and work associated to the process of fruit cutting.

The farming tasks for maintenance are carried out by day laborers and they are divided into two sub-categories: those carried out with their own tools, such as machetes and scissors, and those carried out with tools provided by the owner, such as clearers and tractors, which are handed out at the beginning of the workday and returned at the end. Payment for these tasks is weekly. In contrast, the work of orange cutting is paid daily, with workdays that begin between 6:00 am and 7:00 am, and conclude around noon or 1:00 pm.

An approach to maintaining the plantation has been observed, with practices such as periodic clearing and pruning of Valencia orange trees. This implies the elimination of unproductive branches and parasite vines that affect the tree development.

Soil fertilization is also conducted to improve the quality and presentation of the fruit. When it comes to the harvesting process, day laborers use cloth *ayates*, baskets and ladders to cut the oranges. While some cut and deposit the oranges in baskets, others transport these baskets to fill the transport truck with the fruit that has already been cut.

Finally, the transport process of the Valencia orange for its trade is divided into three markets, each subdivided into juice and fruit. The sale of oranges for juice is done through the sale on the scale or at the *juguera* (juice-maker). The sale of oranges as fruit is carried out in central markets destined to domestic trade.

Qualitative analysis: demographic, agronomic, economic, commercial and socio-environmental aspects

Evaluation of the field data about the Valencia orange producers has made it possible to offer a meticulous vision of many variables related to production. The interpretation is presented next.

Age: the average age of Valencia orange producers is 58 years old, suggesting an older population. This characteristic can be beneficial due to the accumulated experience and deep knowledge in crop management. In addition, age could influence the planning of succession in the operations of orange farming.

Sex: the participation of men predominates among producers, with 72.5% compared to 27.5% of women.

Marital status: 70% of the survey respondents declared being in domestic partnership, 20% are widows, 7% married and only 3% single.

Education: the educational disparity is evident, with 57.5% of the producers who only completed primary school, 30% secondary school, and only 12.5% with high school education.

In Martínez de la Torre, the management system for Valencia orange farming continues to follow a conventional approach, although the income generated does not satisfy the basic needs of producers.

Although it is not imperative to obtain credits, the annual production costs on average were the following:

Leasing of a hectare was $9,200; farming tasks around $1,400; soil fertilization $5,585.63; and weed control, $890.75. Thus, this represents an economic challenge.

The total annual production cost per hectare is $17,076.38 MX pesos, with an average unit cost per ton of $965.

Meanwhile, the average annual profit reaches $28,305.73, highlighting the absence of leaf fertilization and the use of heavy machinery. The average profitability is 1.41, indicating that a yield of 1.41 is obtained for each investment unit.

The sale price of conventional plants is $17.00 pesos, in contrast with $35.00 pesos for certified plants. The producers devote on average two hectares to Valencia orange cultivation, with a density of 267 trees per hectare and a mean age of 32 years. A single cut is carried out per year, with an average yield of 14.9 t ha^{-1}. In terms of trade, 45% is sold to intermediaries, another 45% through auctions, and only 3% is destined to the sale to juice-makers, with the collection center known as "sale at the hole". Environmental changes include 55% soil erosion and 45% contamination of water sources due to ill management of agricultural residues. The climate challenges are notable, with 95% facing drought and only 5% affected by torrential rainfall. The main threats are *Diaphorina citri*, mites, woodlouse, aphids, leaf miner, and diseases such as Huanglongbing, citrus tristeza, alternaria, antracnosis and canker.

Results from the econometric multiple linear regression model

Results were obtained based on the multiple linear regression model, which considers profitability as dependent variable and density of sowing, age of crop, yield and production cost as explicative independent variables. They are declared in the following way:

$$Profitability = \beta_0 + \beta_1 * Density\_Sowing + \beta_2 * Age\_Crop + \beta_3 * Yield * \beta_4 * Production\_Cost$$

Coefficients of correlation (R) and determination (R^2)

Table 1 shows the summary of the model in the context of the R and R^2 test where both values are significant, of R: 0.935 and R^2: 0.875. These values indicate that 87.5% of the variability in the profitability can be attributed to the independent variables incorporated in the model. A high R^2 points to a solid capacity of the model to explain the variation observed.

Analysis of variance test (ANOVA)

Another result obtained by the multiple linear regression (Table 2) is the ANOVA test, whose results reveal that the F statistic is high, reaching a value of 61.058. This substantial

Table 1. Summary of the model[b].

Model	R	R square	R square corrected	Typical error of the estimate
1	.935[a]	.875	.860	.11968

[a] Predictor variables: (Constant), Production_Cost, Crop_Age, Sowing_Density, Yield

[b] Dependent variable: Profitability

Source: Prepared by authors using Software SPSS v. 29.0, field research 2023.

Table 2. Analysis of variance (ANOVA).

Model		Sum of squares	gl	mean square	F	Sig.
1	Regression	3.498	4	.874	61.058	.000[b]
	Residual	.501	35	.014		
	Total	3.999	39			

[a] Dependent variable: Profitability

[b] Predictor variables: (Constant), Production_Cost, Crop_Age, Sowing_Density, Yield

Source: Prepared by authors using Software SPSS v. 29.0, field research 2023.

value indicates that the model has statistical significance, confirming that the predicting variables exert a significant impact in the Profitability of the Valencia orange crop. The degree of Signification (Sig.) associated to the F statistic is equal to 0.000, evidencing that the model is highly significant. When Sig. is lower than the conventional significance of 0.05, it suggests the rejection of the null hypothesis.

Interpretation of coefficients

Table 3 presents the coefficients corresponding to the independent variables, revealing the following relationships:

Density_Sowing: An increase of one unit in the density of sowing results in a reduction of 0.001 units in the profitability, keeping the other variables constant; Age_Crop: An increase of one unit in the age of crop translates into an increase of 0.001 units in profitability, keeping the other variables unchanging; Yield: An increase of one unit in the yield is associated with an increase of 0.162 units in profitability, keeping the other variables constant; Production_Cost: An increase of one unit in the production cost is related to a decrease of 9.21E-005 units in profitability, keeping the other variables constant.

Ultimately, the model is revealed as significant, offering a substantial explanation of the variability in profitability. The individual coefficients provide information about the relationship of each independent variable with the dependent variable. In other words, the model as a whole is a valuable tool to explain the variability in profitability. Next, the expression of the multiple linear regression model:

$$\begin{aligned} Profitability = 1.005 - 9.21 \times 10 - 5 \times Production\_Cost + 0.001 \times Age\_Crop \\ - 0.001 \times Density\_Sowing + 0.162 \times Yield \end{aligned}$$

Table 3. Coefficients[a].

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.
		B	Typical error	Beta		
1	(Constant)	1.005	.309		3.247	.003
	Seeding_Density	−.001	.001	−.055	−.906	.371
	Age_Crop	.001	.004	.016	.261	.796
	Performance	.162	.016	.674	9.850	.000
	Production_Cost	−9.212E-005	.000	−1.032	−15.248	.000

[a] Dependent variable: Profitability

Source: Prepared by authors using Software SPSS v. 29.0, field research 2023.

CONCLUSIONS

A demographic profile mostly composed by experienced producers is revealed, with an average age of 58 years, which indicates deep knowledge in crop management. However, educational disparity stands out.

From an agronomic and economic perspective, a conventional orientation is seen in the crop management, with significant annual costs and a lack of implementation of sustainable practices, which contravenes the growing global demand for agricultural sustainability, especially if the significant relationship between socioeconomic variables and profitability is taken into account. In terms of opportunities and challenges, the importance of training programs stands out, as well as access to financial resources, exploration of new markets, and diversification of products to increase competitiveness. Investment in research and development, together with innovative agronomic practices, becomes essential to approach challenges and improve perspectives in the long term. Environmental sustainability is perceived as a central worry, with soil erosion and water pollution identified as critical aspects. The multiple regression model applied to evaluate the profitability shows a robust explicative capacity, with R^2 of 87.5%, which suggests that the variables selected are key elements to understand the variability in profitability. In sum, the study provides an integral and balanced view of the landscape of Valencia orange farming in the region, highlighting both the positive aspects and the challenges, and offering orientations to improve the sustainability and competitiveness of the citrus industry in Martínez de la Torre.

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Colegio de Postgraduados

Determining co-movements of tomato prices in the United States and macroeconomic variables in Mexico for 2023

Borbón-Morales Carlos G.[1*]; Arvizu-Armenta, Marisol[1]; Rueda-Puente Edgar O.[2*]

[1] Centro de Investigación en Alimentación y Desarrollo, Hermosillo, Sonora, México, C.P. 83304.
[2] Universidad de Sonora Departamento de Agricultura y Ganadería, Hermosillo, Sonora, México, C.P. 83000.
* Correspondence: cborbon@ciad.mx; edgar.rueda@unison.mx

ABSTRACT
Objective: To analyze the co-movements of macroeconomic variables in Mexico and prices of Mexican tomato exports and to estimate the prices of Mexican tomatoes in American and Canadian supply markets based on Mexican macroeconomic variables.
Design/Methodology/Approach: The research was conducted using Pearson's coefficient —calculating the standard scores for X and Y. We determined the co-movements of Mexican tomato market prices and Mexico's GDP, the Interbank Equilibrium Interest Rate (IEIR), natural gas prices, and consumer inflation. Econometric techniques were thus combined with agricultural sector variables as a reliable precedent of the relation intensity between said variables.
Results: The coefficient of determination showed an acceptable degree of linear relationship between the market prices of Mexican tomatoes in different cities and the selected macroeconomic variables, with an average correlation of 20%. We concluded that the variables are not entirely independent since they show a weak linear relationship between them.
Study limitations/implications: It is crucial to conduct studies to determine whether the coefficients of determination support linearity or independence between the evaluated macroeconomic variables.
Findings/Conclusions: Econometric techniques were combined with agricultural sector variables as a reliable precedent of the relation intensity between said variables. The coefficient of determination showed an acceptable degree of linear relationship between the market prices of tomatoes in different cities and the selected macroeconomic variables. We recommend the creation of a price forecasting model.

Keywords: Agricultural producer regions, Vegetables, Growth, Market, Fluctuation.

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INTRODUCTION

According to the Agri-food and Fishing Information Service (Servicio de Información Agroalimentaria y Pesquera, SIAP, 2023), the Mexican regions devoted to agricultural production generate food and raw materials for the agri-food industry. In 2022, these regions produced 297.6 million tons of food, increasing to 301.3 million tons by 2023. This increase occurred for fruits, vegetables, and forages. In the first group, oranges, bananas, apples, and lemons stand out, with 11,340,000 tons. In the second group, red tomato stands out with 3,392,000 tons. In the last group, alfalfa registered the most significant increase —35,119,000 tons (SIAP, 2023).

The Economic Information System (Sistema de Información Económica, SIE, 2023) points out that the fluctuations in supply and demand for these and other commodities in the markets have a complex explanation from the rich theoretical viewpoint of micro and macroeconomics. While demand is explained by the price of goods, the income of consumers, the demand for substitutes and complements, and consumers' tastes and preferences, supply can be explained by the price of goods, costs, competitive supply, joint supply, and unexpected events (Young, 1987).

Hence, price plays an important role when observing these fluctuations. From Oxenfeldt (1973) onwards, we have strived to know which methods are best to understand price behavior. To this day, there are still difficulties in determining the sensible reasons underlying said behavior. In this context, Chunrong, Arjun, and Song (2006) show how fluctuations observed in macroeconomic variables can signal the price trend of daily consumption goods. According to the same authors —who cite Pindyck and Rotemberg (1990); Scott (1999); and Pagan (1999)— there is evidence that the prices of seemingly unrelated products move together, even after controlling macroeconomic indicators such as inflation, industrial production, and interest rates, among other variables.

Thus, the "excess co-movement hypothesis" calls into question the rationality of commodity markets and opens the debate to the relevance of the competitive model of price formation.

Therefore, the analysis of price co-movement has proven to be an appropriate tool to delve into price fluctuations by establishing a correlation between variables. Still, applying this or other similar techniques to determine price behavior continues to pose empirical challenges to explain the phenomenon of fluctuation. Based on the above, the objective of our research was to estimate the correlation between Mexican tomato prices in American and Canadian supply markets and some Mexican macroeconomic variables such as GDP, natural gas prices, IEIR, and inflation.

MATERIALS AND METHODS

This study was conducted following Chunrong, Arjun, and Song (2006), who maintain that a) markets enable transactions between buyers and sellers; b) specific quantities of goods are sold at specific prices; c) in a perfectly competitive market, there is only one price: the market price. The authors argue that, in markets that are not perfectly competitive, each company can charge a different price for the same product attempting to attract customers from its competitors or knowing that customers are loyal to the brand, which allows some companies to charge higher prices.

In this study, market price refers to the average price of all brands and/or supermarkets. Market prices for most goods fluctuate over time, and for many products, fluctuations can be fast, especially for those sold in competitive markets (Pindyck and Rubinfeld, 2009).

Regarding competitive market prices for agricultural products, it must be noted that all producers know the conditions under which their production will be placed on the market. Prices vary from city to city. The data used in the current work are tomato market prices in 17 cities of the United States and Canada where Mexican tomato is sold. The data was collected from the US Department of Agriculture website and modified to reflect quarterly periods from 2010 to 2022.

The Instituto Nacional de Geografía y Estadística (INEGI) measures and publishes the Gross Domestic Product quarterly. Such information was considered to relate the values of the TIIE (Interbank Equilibrium Interest Rate), which is a representative rate of credit operations between banks. The IEIR is calculated daily (for terms of 28, 91, and 182 days) by the Banco de México based on quotes presented by banking institutions and through a mechanism designed to reflect the conditions of the money market in national currency.

To learn the behavior that natural gas had during the years analyzed in this study, we considered the INEGI (2023) database. INEGI presents the main indicators of the recorded price evolution for various goods and services marketed by different sectors of the economic activity.

To calculate the National Consumer Price Index (NCPI) starting from the first half of January 2011, the second half of December 2010=100 was taken as a reference, along with the Monthly National Consumer Price Index and the Fortnightly National Consumer Price Index.

Inflation is a continuous increase in the general price level, the magnitude of which is measured from one period to another using a percentage rate. The inflation rates refer to a) the previous month, interannual monthly inflation, or annual accumulated inflation, and b) the biweekly price index, *i.e.*, the variation in prices compared to the previous fortnight (INEGI, 2023).

RESULTS AND DISCUSSION

The data collected from the US Department of Agriculture website and modified to reflect quarterly periods from 2010 to 2022 shows that Mexican tomato was not sold in 2012 and 2023, nor in the city of Chicago in 2016 (Figure 1). Prices vary from USD $2.25 to USD $37.00, depending on the season and destination. It should be noted that

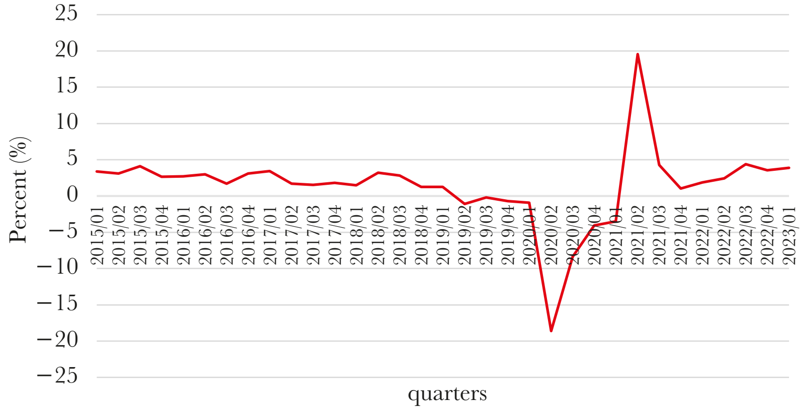

Figure 1. Tomato market prices from 2010 to 2022 in cities of the United States and Canada. Source: Own elaboration based on data from USDA (2023).

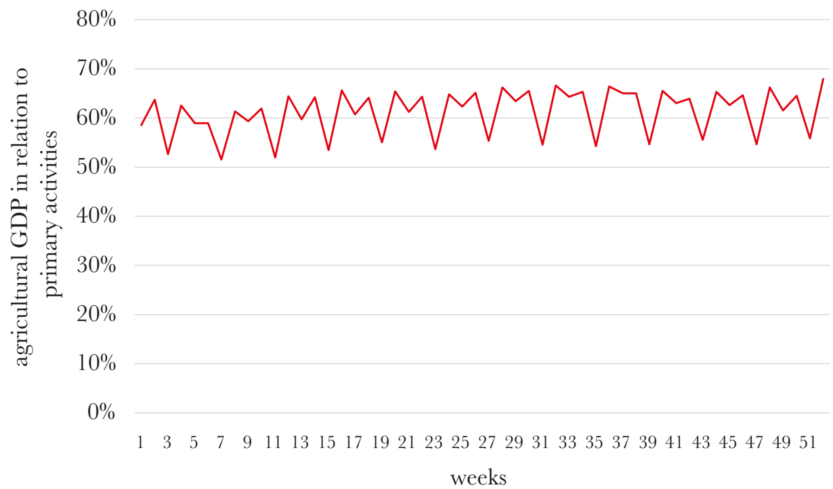

Figure 2. Quarterly GDP, base year 2013: Behavior of primary activities as regards total activity in 2010-2022. Source: Own elaboration based on INEGI, Sistema de Cuentas Nacionales de México, 2023.

the prices pertain only to products of Mexican origin and greenhouse production, which means they are comparable. Figures 1 and 2 show that primary activities diminished as regards global economic activity during 2020, in times of the COVID-19 pandemic. However, starting from the first quarter of 2021, there is a recovery and a stabilization, reaching pre-pandemic percentages with an upward trend.

Economic activity in Mexico during the COVID-19 pandemic dropped almost 20% in the first quarter of 2020. After the pandemic, participation increased conservatively for the same period of 2021, showing moderate growth and reaching a total value of 18,925,112.06 pesos on average for 2023.

Figure 3 shows that agriculture is a crucial part of Mexican primary activities. On average, between 2010 and 2022, agriculture accounted for 62.95% of that economic

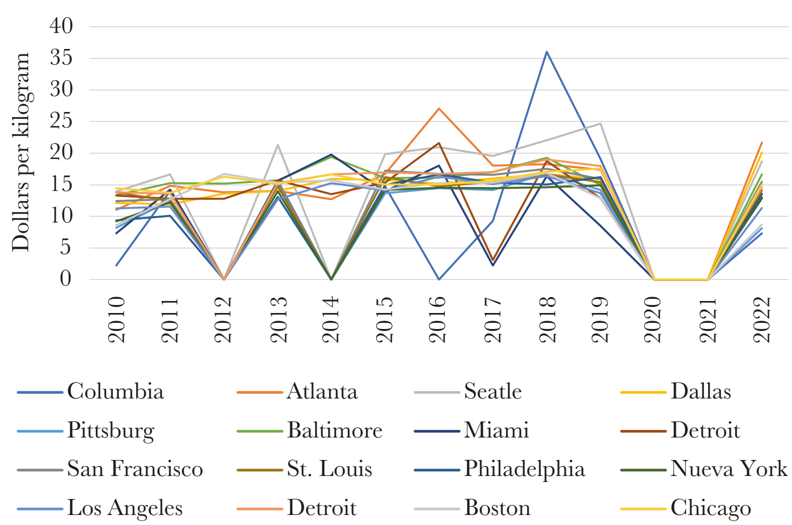

Figure 3. Quarterly GDP, base year 2013: Behavior of agriculture as regards primary activities, 2010-2022. Source: Own elaboration based on data from USDA (2023).

sector, which leads us to assert that it directs the behavior of primary activities in Mexico.

The long-term trends established by global economic activity are an important tool to determine the future behavior of specific activities characterized by uncertainty. According to Canova (1996), studying economic cycles presents difficulties because there are multiple parametrizations for their definition and classification. However, the study of some economic variables with stable behavior and available time series offers the opportunity to determine whether there is any point of comparison with other variables in the sector. Figure 2 shows the decrease of primary activities as regards global activity. The export market offers Mexican rural producers the chance to improve the quality and price of their merchandise. To access this market, producers must comply with certain non-tariff barriers imposed by the market, such as quality, safety, transportation, logistics, and company administration, which will allow them to take advantage of the marketing opportunity. These barriers can be addressed using controlled systems, as happens in protected agriculture —a form of agricultural production that cultivates and protects plants using metal structures and a translucent plastic cover that prevents atmospheric phenomena from damaging the harvest and whose objective is to reproduce or simulate the most appropriate climatic conditions for the growth and development of plants, providing some degree of independence from the outside environment and spacious enough for people to work inside (NMX-E-255-CNCP-2008). Most of the merchandise produced in protected agriculture is sent to the export market. Therefore, in this study, the market price of the goods produced under such system constitutes the guideline to determine whether there are co-movements between the macroeconomic variables mentioned above and the market price as a proxy for the relationship between said variables and protected agriculture.

Concerning the GDP —measured and published quarterly by INEGI— we confirmed that the information matched the series prepared considering the base year 2003. These series use the same conceptual and methodological framework as the estimation of the Goods and Services Accounts within the System of National Accounts of Mexico, which, in turn, uses the same criteria as the North American Industrial Classification System (NAICS). The figures pertain to the total economy, as well as to each of the 20 sectors into which it is divided according to the NAICS classification. The information is supplemented with the Implicit Price Indices, which result from relating the current values with the GDP constants for each quarter. To select the appropriate indicators, we considered the conceptual and methodological scope of the activities and the type of goods and services produced (INEGI, 2023). Figure 4 shows how the product maintains an increasing trend during the analyzed period. A similar behavior occurred with the IEIR obtained from the Banxico database (2023), displayed in Figure 5.

Throughout the Mexican territory, different regions have contrasting climates, altitudes, and meteorological conditions. Many of them could use protected agriculture. Figure 6 shows the behavior of natural gas prices during the years studied in this analysis. We can see how it reaches a maximum level during the third quarter of 2015 and a minimum during the first quarter of 2020. Fluctuations respond to various infrastructure

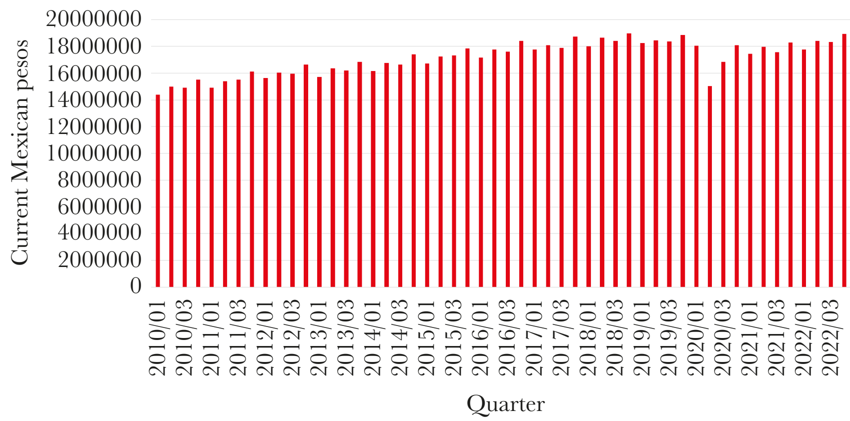

Figure 4. Quarterly GDP in Mexico, 2010-2022 (second quarter).
Source: Own elaboration based on data from INEGI (2023).

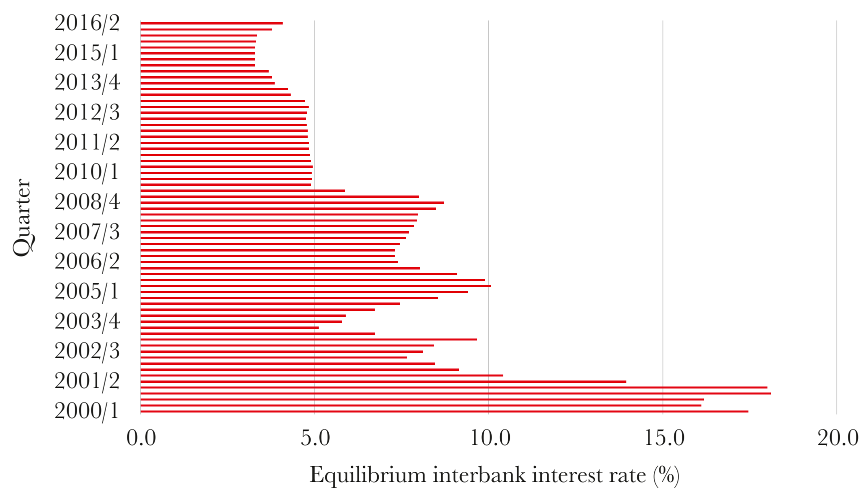

Figure 5. Quarterly IEIR (28-day term), 2010-2022.
Source: Own elaboration based on data from Banxico (2023).

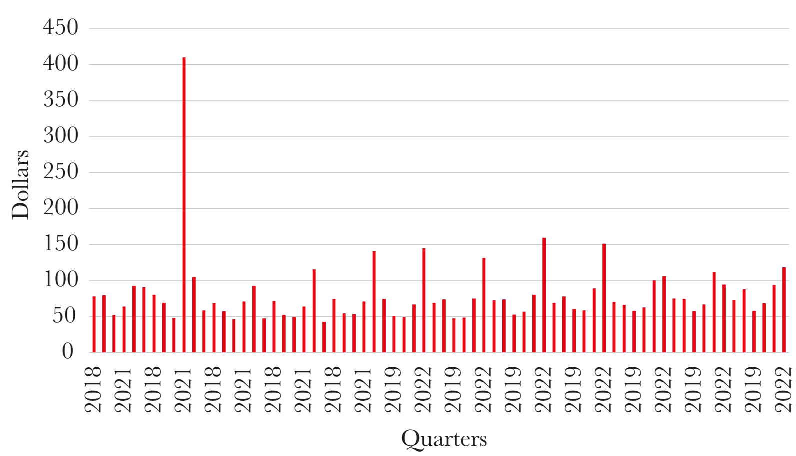

Figure 6. Price of natural gas in dollars, 2010-2022.
Source: Own elaboration based on data from CRE (2023).

developments, distance from the border, labor facilities, government support, and natural gas availability (we must note that this type of gas is cheaper than propane). Mexico can achieve considerable progress in protected agriculture by leveraging its geographical conditions (CRE, 2023). Figure 7 shows how inflation levels from 2010 to 2023 have a sustained increase.

The Pearson coefficient was used to determine the possible cyclical co-movements of the variables that make up the market prices of Mexican greenhouse tomato exports, considering the total GDP of the economy, the IEIR, the prices of natural gas, and inflation. Table 1 presents the price correlation coefficients of the proposed macroeconomic variables by city. We include 15 cities from the United States and two from Canada, each fulfilling the abovementioned considerations.

We can observe how the GDP and inflation maintain a direct, although not perfect, correlation with the market prices in each studied city. These coefficients range between 0 and +1, with the highest being 0.799 for the inflation variable in the city of Dallas and the lowest being 0.113 for the GDP variable in Pittsburgh (Table 1). As for the IEIR variable, it maintains an inverse correlation, although not perfect either, in all studied cities. Its extreme values are observed in Dallas, with −0.601, and Toronto, with 0.066.

In the case of natural gas (Table 1), the Pearson correlation coefficient shows levels of direct correlation in almost every city except for Dallas, where the coefficient shows an inverse correlation with a value of −0.004.

Overall, the coefficient of determination shows an acceptable degree of linear relationship between market prices in the different cities and the selected macroeconomic variables, with an average correlation of 20%. We can conclude that the variables are not entirely independent since they show a weak linear relationship between one another.

However, we must stress the high linear relationship between the proposed variables shown by Dallas and Atlanta, with more than 63% and 45%, respectively (Table 2).

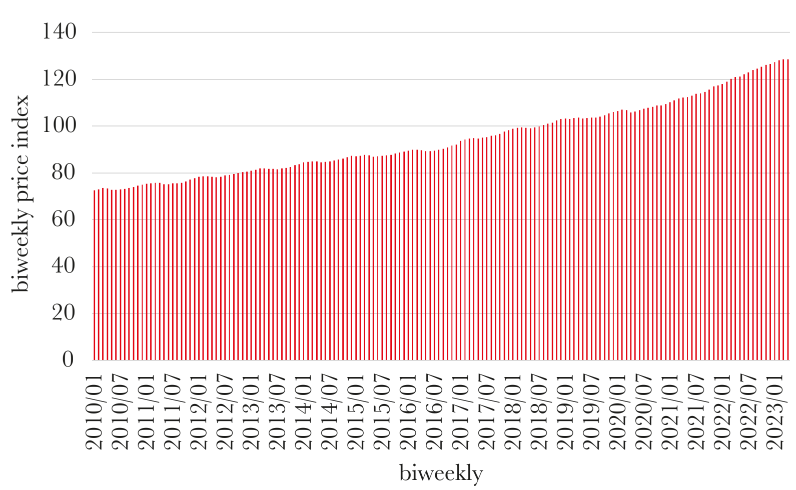

Figure 7. Inflation in Mexico, 2010-2023.
Source: Own elaboration based on data from INEGI (2023).

Table 1. Pearson correlation coefficient between GDP and market prices by city, 2023.

City	Gross Domestic Product (GDP)	Equilibrium interbank interest rate (EIIR)	Natural gas price	Inflation
Atlanta	0.672	−0.489	0.103	0.643
Baltimore	0.489	−0.434	0.151	0.529
Boston	0.186	−0.335	0.259	0.221
Chicago	0.381	−0.351	0.094	0.439
Columbia	0.196	−0.189	0.129	0.247
Dallas	0.777	−0.601	−0.004	0.799
Detroit	0.520	−0.229	0.383	0.496
Los Ángeles	0.469	−0.198	0.458	0.397
Miami	0.161	−0.170	0.215	0.207
Montreal	0.379	−0.173	0.303	0.380
Nueva York	0.549	−0.252	0.190	0.539
Philadelphia	0.571	−0.278	0.245	0.562
Pittsburgh	0.113	−0.056	0.360	0.120
San Francisco	0.578	−0.197	0.233	0.531
Seattle	0.381	−0.151	0.248	0.390
St. Louis	0.547	−0.158	0.171	0.510
Toronto	0.466	−0.066	0.439	0.383

Source: Own elaboration based on (Banxico, 2023) (CRE, 2023) (INEGI, 2023) (USDA, 2023).

Table 2. Coefficient of determination for GDP *vs.* market prices by city.

City	Gross Domestic Product (GDP %)	Equilibrium interbank interest rate (EIIR %)	Natural gas price (%)	Inflation (%)
Atlanta	45.16	23.96	1.07	41.37
Baltimore	23.95	18.82	2.27	28.00
Boston	3.46	11.25	6.72	4.90
Chicago	14.55	12.31	0.87	19.29
Columbia	3.83	3.56	1.66	6.12
Dallas	60.31	36.10	0.00	63.87
Detroit	27.01	5.25	14.69	24.56
Los Ángeles	22.03	3.92	20.95	15.78
Miami	2.60	2.88	4.61	4.27
Montreal	14.39	3.00	9.15	14.43
Nueva York	30.10	6.34	3.60	29.10
Philadelphia	32.66	7.71	6.00	31.59
Pittsburgh	1.27	0.31	12.96	1.45
San Francisco	33.46	3.87	5.45	28.24
Seatle	14.55	2.29	6.13	15.24
St. Louis	29.88	2.51	2.93	26.01
Toronto	21.68	0.43	19.29	14.67

Source: Own elaboration based on (Banxico, 2023) (CRE, 2023) (INEGI, 2023) (USDA, 2023).

CONCLUSIONS

The variables considered in this study were the quarterly GDP from 2010 to 2022, the 28-day IEIR considered by quarterly average, the price of natural gas reported by the Comisión Reguladora de Energía in Mexico, and inflation for the same period. We observed that GDP and inflation maintain a direct, although not perfect, correlation with the market prices of each studied city. The IEIR variable showed an inverse correlation. In the case of natural gas, the Pearson correlation coefficient shows direct correlation in almost all cities, except for Dallas. The coefficient of determination shows an acceptable degree of linear relationship between market prices in the different cities and the selected macroeconomic variables. Finally, we conclude that the variables are not entirely independent since they show a weak linear relationship.

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Colegio de Postgraduados

Evaluation of aromatic plants hydrosols on the growth of *Trichoderma harzianum*

Ruíz-González, Miguel A.[1]**; Colmena-Chino, Diego R.**[1]**; Jerónimo-Ponce, Fernando**[1]**; Solano-Vidal, Roney**[1]**; Serrato-Cruz, Miguel A.**[1*]

[1] Universidad Autónoma Chapingo. km 38.5. Carretera México-Texcoco, Chapingo, Estado de México. C. P. 56230.

* Correspondence: serratocruz@gmail.com

ABSTRACT

Objective: To evaluate the compatibility of hydrosols from aromatic plants on the growth of *T. harzianum*.

Design/Methodology/Approach: All hydrosols of *Foeniculum vulgare*, *Plectranthus coleoides*, *Tagetes arenicola*, *T. coronopifolia*, *T. erecta*, and *T. lucida* were evaluated *in vitro* on the mycelium *Trichoderma harzianum*. Their growth rates and inhibition percentages were recorded. *T. harzianum* was inoculated in 50 g of sterilized corn cob enriched with *F. vulgare* hydrosol (3, 5 and 7%), this mixture was incubated at 28±2 °C; spore counting, and viability tests were performed on PDA medium. The Tukey test ($p \leq 0.05$) was used for mean comparisons.

Results: All hydrosols inhibited *in vitro* the mycelial growth of *T. harzianum* at 100%, but at 5% concentration, some hydrosols promoted the growth of the fungus more than the control. The concentration of *F. vulgare* hydrosol influenced the production and viability of *T. harzianum* spores in the cob substrate.

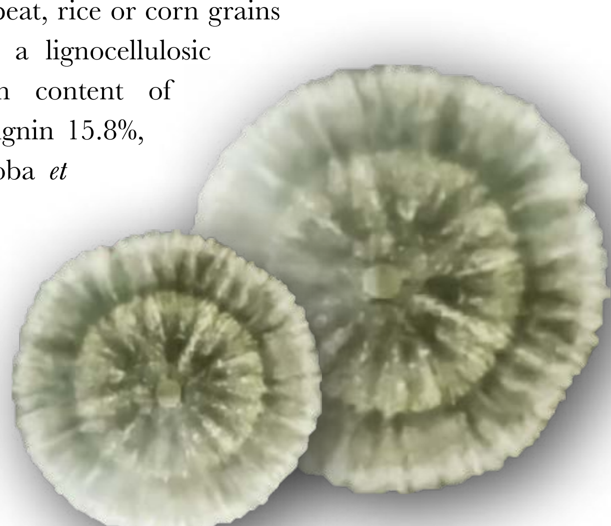
Study Limitations/Implications: This study provides information on the use of hydrosol, which are typically considered waste products.

Findings/Conclusions: At low concentration, *F. vulgare* hydrosol can be used to enrich corn cob and promote the growth of *T. harzianum*.

Keywords: *Trichoderma harzianum*, hydrosol, growth, corn cob.

INTRODUCTION

Due to the harmful effects of synthetic fungicides, there is noticeable interest in the biological activity of organisms such as *Trichoderma harzianum* to control phytopathogenic fungi. *T. harzianum* is characterized by its rapid growth and colonization of substrates (Saravanakumar *et al.*, 2017). Several species of this organism are used as an organic input, marketed in forms such as wettable powder, granules, liquids, and solids added to sorghum or corn straw, cob, cane bagasse, coco peat, rice or corn grains (Bellino & Marroquín, 2015). Cob is a lignocellulosic agricultural by-product with a high content of hemicelluloses (33.6%), cellulose 45%, lignin 15.8%, and particularly xylanases 94% (Córdoba *et al.*, 2013). This represents an advantage, since *Trichoderma* spp. can utilize and degrade lignocellulosic residues composed of cellulose (40-55%), hemicellulose (25-50%), and lignin (10-40%) (la Grange *et al.*, 2010). Depending on the substrate used, the growth and production of spores



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of *T. harzianum* can take 10 to 15 days (Kumar *et al.*, 2014) or 20 to 21 days (Simon, 2011). The evaluation of adjuvants in substrates where *Trichoderma* multiplies could help accelerate the production of fungal biomass and reduce the time needed to obtain it, making the exploration of diverse sources pertinent.

Until now, limited attention has been paid to distillates from aromatic plants as auxiliaries in biotechnological procedures for the reproduction of *Trichoderma*. Although essential oils and hydrosols obtained from hydrodistillation of aromatic species have fungal inhibitory properties (Taglienti *et al.*, 2022), some essential oils do not inhibit the growth of some *Trichoderma* species (Ali *et al.*, 2011; Infante *et al.*, 2013). In the case of hydrosols from aromatic plants, these contain traces of essential oil and are expected to have a biological effect that stimulates mycelial growth (Fiori *et al.*, 2000). Additionally, hydrosols from non-aromatic plant species, such as *Chenopodium*, have been found to favor the growth of fungi such as *Fusarium oxysporum* (Covarrubias *et al.*, 2013).

In general, not enough is known about the effect of hydrosols on fungal growth. Furthermore, in the hydrodistillation processes of aromatic plants, the production of hydrosols, compared to the essential oil, is much higher and this by-product is not always utilized. In Mexico, the families Amaranthaceae, Anacardiaceae, Apiaceae, Asteraceae, Burseraceae, Euphorbiaceae, Fabaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Rubiaceae, and Verbenaceae include aromatic species as resource potentially useful in agriculture (Calvo-Irabién, 2018). In the immediate future, experimental evaluation of these species as complementary material for the growth of antagonistic fungi, such as *T. harzianum* (an organism very useful in the control of phytopathogenic fungi), could anticipate a new source of natural adjuvant to stimulate and shorten the production time of *Trichoderma* inoculum. For this reason, in the present study evaluates the compatibility of hydrosols from some aromatic plants in the growth of *T. harzianum*.

MATERIALS AND METHODS

The biological material used included *Plectranthus coleoides* Benth. voucher 22336 (Lamiaceae), *Foeniculum vulgare* Mill. 19561 (Apiaceae), and four species of *Tagetes* (*T. arenicola* Panero & Villaseñor: 35882, *T. coronopifolia* Willd.: 36282, *T. erecta* L.: 35869, and *T. lucida* Cav.: 35872- Asteraceae), these plants have taxonomic support in the Jorge Espinosa Salas Herbarium of the Departamento de Preparatoria Agrícola of the Universidad Autónoma Chapingo, State of Mexico. In October 2022, stems, leaves, and flowers of these plants, grown without fertilization in a greenhouse in Chapingo, Mexico, were subjected to a hydrodistillation process in an Italian-type glass still to obtain hydrosols. The distillation time was 45 min from the beginning of precipitation, the hydrosols were preserved in plastic bottles until use.

In vitro bioassay

Using the poisoned food technique (Balouiri *et al.*, 2016), hydrosol treatments were evaluated, in five replicates per treatment and a control. The experimental unit was a Petri dish with Potato Dextrose Agar (PDA) culture medium and a completely randomized design was used. Each hydrosol was prepared in three concentrations, 5, 50, and 100%.

The flasks containing the treatment substances (hydrosol, PDA, and double-distilled water) were sterilized for 17 minutes in an autoclave at 120 °C and poured into sterile 90 mm glass Petri-dishes.

For this experiment, a strain of *Trichoderma harzianum* kept in the Laboratorio de Resistencia Genética at the Universidad Autónoma de Chapingo, previously identified at the molecular level (accession number MK752565.1 in GeneBank), was used. After 24 hours, a PDA disc with *T. harzianum* inoculum, obtained from a five-day-old colony using a 3.5 mm diameter sterile punch, was placed inverted in the center of the Petri dishes. These dishes were incubated in dark conditions at 28±2 °C in a culture oven. The radial growth of the fungus was measured every 24 hours using a digital vernier, Mycelial inhibition (%I), growth rate (GR), and growth percentages (%G) were calculated with the following formulae:

$$\%I = \frac{D1 - D2}{D1}(100) \qquad GR = \frac{Fd - Id}{Ft - It} \qquad \%C = \frac{C1(100)}{CG}$$

Where: %*I*: percentage of inhibition; *D*1: diameter of mycelial growth of the control (mm); *D*2: diameter of mycelial growth of the influenced (mm); *GR*: growth rate (mm day^{-1}); *Fd*: final growth diameter (mm); *Id*: initial growth diameter (mm); *It*: initial growth time (days); *Ft*: final growth time (days). *C*1: treatment growth (mm), *CG*: control growth (mm).

After carrying out the *in vitro* bioassay, the treatment that promoted the greatest growth of *T. harzianum* was chosen and the second experimental phase, which involved its inoculation in corn cob substrate.

Cob growth

Corn cobs were ground into 1 cm pieces using a forage mill. This substrate was soaked in different concentrations (3, 5 and 7%) of the best hydrosol until it reached a humidity of 55%. The moisture content was estimated on a thermobalance (MA37, Sartorius, United States) and adjusted to pH 6 with 0.2 M citric acid. Then, 50 g of substrate were placed in Erlenmeyer flasks, covered with cotton, and sterilized for 20 min in an autoclave at 120 °C. After cooling to room temperature, inoculation was performed. A suspension of spores (1×10^5) was prepared from a ten-day-old colony of *T. harzianum* and 1×10^5 spores were added to each gram of dry substrate. The flasks were incubated in a culture oven under dark conditions for seven days at 28±2 °C until colonization and sporulation were observed in the control substrate.

Spore count and viability

After eight days, 3 g of sporulated substrate (cob) were weighed and placed in a flask with 10 mL double-distilled water and Tween 20 (0.1 mL L^{-1}). It was then shaken, and the spore count was determined using a Neubauer chamber. This process was repeated three times. To determine viability, one hundred spores were sown in Petri dishes with PDA medium and incubated at 28±2 °C. The number of germinated colony-forming units (cfu) was counted 48 hours after sowing. This process was repeated four times.

Analysis of data

The recorded data were subjected to Tukey's mean comparison test ($p \leq 0.05$) using SAS Academic Software.

RESULTS AND DISCUSSION

In vitro bioassay

Pure hydrosols inhibited mycelial growth, decreasing the percentage and growth rate of *T. harzianum*. However, at concentrations of 50 and 5%, the percentage and growth rate increased significantly, while the percentage of inhibition decreased (Table 1). The concentration of hydrosols at 5% stimulated mycelial growth similar to the control treatment. Hydrosols contain very small amounts of essential oil, so a high concentration could inhibit mycelial growth (Revilla-Medina *et al.*, 2020). The rapid mycelial growth of *T. harzianum* with some hydrosols at low concentration is likely due to the presence of some non-volatile compounds, such as carbohydrates, which are carried

Table 1. Mean values of growth rate, growth percentage, and inhibition percentage after four days of growth.

Treatments	Growth velocity (mm day^{-1})	Growth (%)	Inhibition (%)
Experimental control (witness)	10.7 ab	100 ab	0 e
P. coleoides 100 %	0.59 e	1 h	99 a
O. coleoides 50 %	5.8 e	38.4 fg	61 b
O. coleoides 5 %	11.8 a	92.6 ab	7 e
T. erecta 100 %	6.08 d	41.4 efg	58 bc
T. erecta 50 %	8.4 bcd	63.5 cd	36 d
T. erecta 5 %	11.0 ab	100.4 ab	0 e
T. coronopifolia 100 %	10.2 abc	62.2 cde	38 d
T. coronopifolia 50 %	10.5 abc	82.1 bc	18 e
T. coronopifolia 5%	11.8 a	98.6 ab	1 e
F. vulgare 100 %	7.7 cd	31.1 g	69 b
F. vulgare 50 %	8.4 bcd	58.2 def	42 cd
F. vulgare 5 %	11.9 a	105.4 a	0 e
T. lucida 100 %	10.4 abc	82.7 bc	17 e
T. lucida 50 %	9.4 abc	93.7 ab	6 e
T. lucida 5 %	11.5 a	104.9 a	0 e
T. arenicola 100 %	8.3 bcd	82.7 bc	17 e
T. arenicola 50 %	10.6 abc	96.7 ab	3 e
T. arenicola 5 %	11.1 ab	100 ab	0 e
CV	13	12	30
MSD	2.91	20	18

CV: Coefficient of variation; MSD: Minimum significant difference.

over during distillation and could serve as nutrients for the organisms (Labadie *et al.*, 2015). The observation that a high concentration inhibits the mycelial growth, and a low concentration stimulates it (Table 1) is consistent with the hormesis phenomenon (Jakobsen *et al.*, 2021).

The 5% *F. vulgare* hydrosol completely covered the Petri-dish by the third day (Figure 1), while the control did so on the fourth day. Due to the rapid growth observed with this hydrosol, concentrations of 3% and 7% were also evaluated. The 7% concentration showed similar growth to the 5% concentration, while the 3% concentration resulted in lower growth (Figure 2).

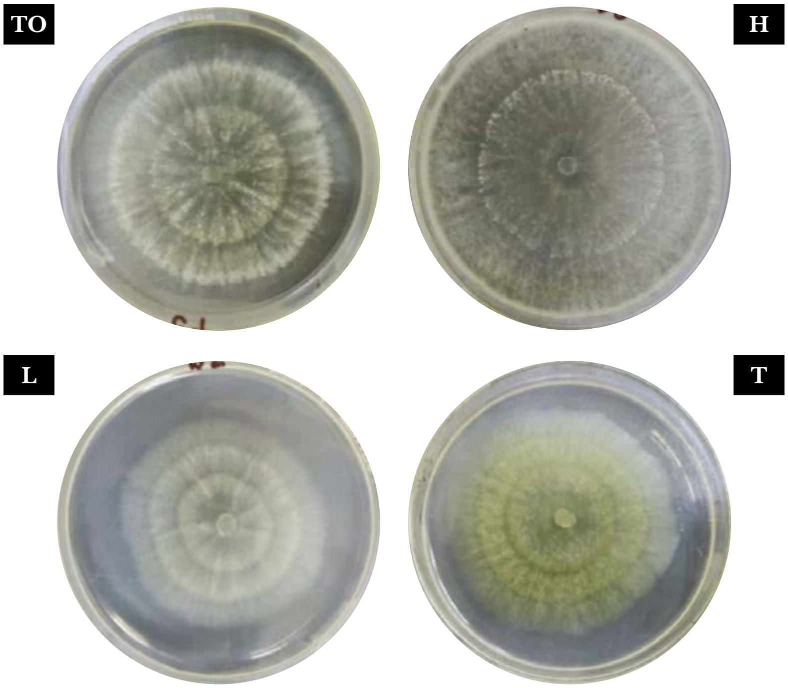

Figure 2. Growth kinetics of *T. harzianum* grown in culture medium (PDA) enriched with *F. vulgare* hydrosol at three concentrations. T0: Witness; H7: *F. vulgare* 7%; H5: *F. vulgare* 5%; H3: *F. vulgare* 3%.

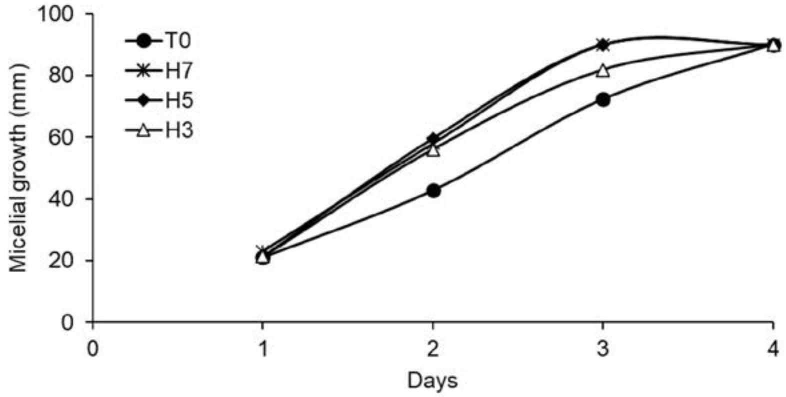

Figure 1. Growth of *T. harzianum* on the third day with 5% hydrosol. TO: Witness, H: *F. vulgare* 5%, L: *Tagetes lucida* 5%, T: *Tagetes erecta* 5%.

Thus far, hydrosols have been used as antifungal (Boyraz & Özcan, 2005) and antiviral agents (Taglienti *et al.*, 2022). However, this study demonstrates their potential to promote the growth of *T. harzianum* is shown. Additionally, due to the inhibition observed at the 100% concentration in the same fungus, further evaluation against phytopathogenic fungi would be promising.

Growth in substrate

The concentration of fennel (*F. vulgare*) hydrosol at 5% was notable for the *in vitro* growth of *T. harzianum* (Table 1). However, when evaluated in corn cob substrate conditions at concentrations of 3, 5, and 7%, only the 3% hydrosol concentration led to greater spore production ($7{,}205\times10^7$), compared to the control and the other fennel hydrosol concentrations, over a period of seven days (Table 2). Michel-Aceves *et al.* (2008) obtained 4.43×10^8 spores mL^{-1} in a period of 21 days using chopped corn cobs. Corn cob contains glucose, xylose, arabinose, galactose, mannose, acetyl groups, lignin, ash, and uronic acid (Da Silva *et al.*, 2015). *T. harzianum*, through its enzymes, converts these hemicelluloses into assimilable sugars (Filho *et al.*, 2017). The hydrosol also influenced the viability of the spores: the lowest concentration (3%) resulted in 70% viability, while the 5% concentration resulted in 56% viability (Table 2). This could be due to the presence of monoterpene alcohols, aldehydes, ketones, and traces of essential oil (Garneau *et al.*, 2014) that can affect spore germination.

Table 2. Production and viability of spores of *T. harzianum* grown on corn cobs enriched with *F. vulgare* hydrosol.

Treatments	Number of spores mL^{-1}	Viability of spores (%)
Witness	3.285×10^7 b	36 b
F. vulgare 3 %	7.205×10^7 a	70 a
F. vulgare 5 %	3.3125×10^7 b	56 ab
F. vulgare 7 %	4.53×10^7 b	53 ab
CV	12.13	20
MSD	1.45	29

CV: Coefficient of variation; MSD: Minimum significant difference.

This study demonstrates that crushed corn cob enriched with 3% *F. vulgare* hydrosol is suitable for the growth and sporulation of *T. harzianum*. This finding represents an advantage, as it is feasible to use low-cost substrates to achieve higher spore density and reduce cultivation time (Singh and Nautiyal, 2012). Additionally, this enriched substrate can be applied directly to crops or soil (Woo *et al.*, 2014).

CONCLUSIONS

Hydrosols in low concentrations can stimulate the growth of *T. harzianum*. Specifically, *F. vulgare* hydrosol can be used to enrich organic substrate such as corn cob, thereby promote the productivity of *T. harzianum* and reducing the time required for its cultivation.

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Assessment of dry matter yield, nutritional value, and calf weight gain in Mombaza-Kudzu pasture associations

Fragoso-Islas, Abraham1; Bolaños-Aguilar, Eduardo Daniel2*; Enríquez-Quiroz, Javier Francisco3; Montero-Lagunes, Maribel3

[1] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. Sitio Experimental "Las Margaritas", Hueytamalco, Puebla, México. CP 73583

[2] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. Campo Experimental Huimanguillo. Huimanguillo, Tabasco, México. CP. 86600.

[3] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. Campo Experimental "La Posta". Paso del Toro, Veracruz, México. CP. 94277.

* Correspondence: bolanos.eduardo@inifap.gob.mx

ABSTRACT

Objective: To evaluate the biomass production, nutritional value and animal production of an association with fabaceae species and grasses with grazing calves.

Design/Methodology/Approach: The study compared Mombaza (*Megathyrsus maximus*) – Kudzu (*Pueraria phaseoloides*) association pasture against a Mombaza in monoculture control. Ten calves, five in each pasture type, were grazed in rotation for seven months. Dry matter yield, proportion of Kudzu in the association, protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, and daily weight gain were evaluated every 36 days.

Results: Dry matter yield did not differ between pasture types, but varied by sampling dates, being higher at the onset of the rainy season (May 20th). The proportion of Kudzu in the association was low, decreasing from 8.42% to 1.71% over the study period. Lignin content increased in the association during the driest month (April). Daily weight gain was 978 g in the monoculture pasture and 678 g/day in the association, with no significant difference.

Study Limitations/Implications: The low persistence of the fabaceae in the association limits the nutritional value of the pasture, consequently, the improvement of grazing animal production.

Findings/Conclusions: During the drought season, both pastures exhibited the highest protein concentration and the greatest weight gain at the beginning of this period. However, the association did not result in greater weight gains due to the low persistence of the fabaceae.

Keywords: *Pueraria phaseoloides*, dry matter yield, protein, grazing, animal production.

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INTRODUCTION

Natural pastures are the main source of feed in tropical areas. The humid tropics support grass growth most of the year due to frequent rains, however, there are drought periods, lasting 3 to 4 months, during which grass production can decrease by up to four times (Pardo *et al.*, 2020). This reduction in forage leads to changes in its nutritional value and a reduced stocking rate (Juárez *et al.*, 2011), necessitating adaptive pasture management (occupation/rest) to control usage and nutritional value. Seasonal forage distribution, nutritional value changes, and stocking rate cause fluctuations in weight gain or milk production. An alternative solution is pasture association of fabaceae species and grasses (Fabaceae-Poaceae). Pardo *et al.* (2020), reported that the *Urochloa humidicola* with *Arachis pintoi* association produced 0.8 t ha^{-1} more dry matter (DM) compared to

Humidicola in monoculture, and the association recorded 19.21 g kg^{-1} more protein than the monoculture. Diverse pastures achieve greater production stability, since forage species reach their phenological stages at different times (Prieto *et al.*, 2015). Fabaceae fix atmospheric nitrogen in the soil, allowing grasses greater access to nutrients, enhancing nutrition and tolerance in drought periods (Schmitz *et al.*, 2023). Fabaceae-grass associations also improve dietary nutritional value due to the high protein content of fabaceae, resulting in increased animal production (Brink *et al.*, 2008).

Few experimental studies with livestock exist in the humid tropics of Mexico, so information on the benefits and persistence of fabaceae within these associations in tropical climates is limited.

Therefore, this study aimed to determine the effect of grazing on biomass production and the nutritional value of fabaceae-grass association by assessing the daily weight gain of grazing calves at different times of the year.

MATERIALS AND METHODS

The study was established in 2019 and was conducted in 2020 at the "Las Margaritas" Experimental Site of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP), located east in the state of Puebla (19° 45' N, 97° 27' W) at 450 meters above sea level. The soil was loam texture, high in OM (6.5%), in Fe (17.1 mg kg^{-1}), in Zn (0.10 mg kg^{-1}), Mn (7.13mg kg^{-1}) in Cu (0.79 mg kg^{-1}), and in Al (28.4 mg kg^{-1}), low in Mg (53.2 mg kg^{-1}), with apparent density of 1.1 g cm^{-3}, and acidic pH (5.9). The surface of the study was 3.0 ha. Secondary vegetation was removed through slash and burn. The main weed, *Paspalum virgatum*, was eliminated with the herbicide glyphosate (N-(phosphonomethyl) glycine) applying 1kg ha^{-1}. In June 2019, two types of pasture were established: monoculture and associated fabaceae-grass. Each type covered 1.5 ha separated by barbed wire. Grass and fabaceae seeds were sowed on lines drawn 1 m apart using minimum tillage, with 5.0 cm deep blows every 50 cm. The monoculture pasture consisted of *Megathyrsus maximus* var. Mombaza sowed at 7 kg ha^{-1}, while the associated pasture consisted of a mix of Mombaza (6 kg h^{-1}) and Kudzu (*Pueraria phaseoloides*) (3 kg h^{-1}). Seeds were commercially purchased, and no fertilization was applied. Pastures were established in december 2019 and divided into four (0.370 m^2) subplots per type, with electric wire for rotational grazing (12/36 days occupation/rest). The climate data is shown in Figure 1. Ten Zebu × Simmental calves (eight months old) were divided into two groups of five, each assigned to a different pasture. Group 1 averaged 225 kg in weight (182 to 288 kg) and was assigned to the Mombaza-Kudzu association. Group 2 averaged 229 kg (212 to 266 kg) and was assigned to Mombaza monoculture. During January 2020, adaptation grazing was carried out. The study ran from February 14^{th} to September 3^{rd}, 2020, with data collection until October 9^{th}.

The animals were weighed every 36 days at the end of each grazing cycle, covering both the drought and rainy season. For forage sampling, one of the four subplots of Mombaza in monoculture and one of the Mombaza-Kudzu association were chosen. Sample collection of the available forage in both subplots occurred every 36 days before the entry of the

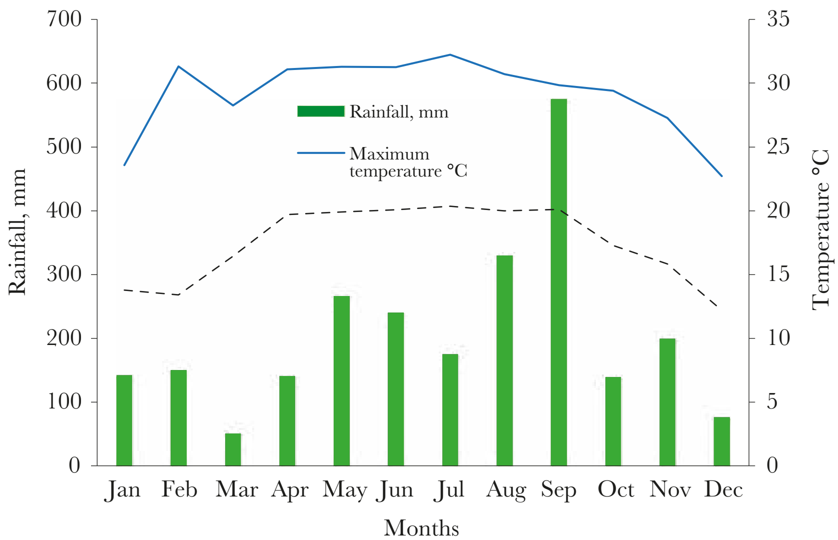

Figure 1. Average climate data during the study period, year 2020.

animals. Samples were collected using a randomly assigned 1 m^2 plot in both pastures, following the method proposed by Toledo & Schultze-Kraft (1982). Four repetitions were conducted from March 9th to October 9th, 2020. Sampling involved harvesting the biomass or fresh matter (FM) within each 1 m^2 plot with cuts made 15 cm above ground level. When the 1 m^2 plot contained individual Kudzu plants, they were harvested 7 cm above ground level. Additionally, in the associated pasture extra samples of Kudzu were taken for individual evaluation in dry matter yield, nutritional value, and its proportion in the pasture. The harvested material was weighed on a portable electronic scale with a capacity of 10 kg ± 1 g. To determine the dry matter yield (DMY, kg ha^{-1}), subsample of 300 g of FM were separated and dried in forced air ovens at 65 °C for 48 h. The DMY was calculated based on the DMY of the 300 g of FM and the total FM of the 1 m^2 plot, extrapolated to one hectare. For the determination of the DMY (kg ha^{-1}) and proportion of the fabaceae in the total biomass, a second subsample of 200 g was used, which was separated into its grass and fabaceae components. The proportion of fabaceae (%) in the total biomass was obtained by dividing its dry weight by the dry weight of the total biomass (grass + fabaceae). The concentrations (in g kg^{-1} of DM) of protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin from Mombaza and Mombaza-Kudzu, were obtained from representative dates of the drought (April 14th) and rainy season (July 29th). These determinations were from the dry samples of 300 g of FM, ground to a particle size of 1 mm in a Wiley brand mill, according to the AOAC (1990).

Statistical analysis

The analysis of variance for available forage, nutritional value, and daily weight gain of the calves was performed using the SAS GLM program (SAS Institute, 2010). The effect of harvest date, pasture type, and the interaction date by pasture type, was analyzed in completely randomized designs in a divided plot arrangement with four repetitions, considering harvest date as the main plot, and pasture type as the subplot. Means were compared using Tukey test ($p \leq 0.05$). Daily weight gain was analyzed separately in a completely randomized design with five repetitions, considering each animal a repetition, the effect of each type of pasture was analyzed considering the average daily gain.

RESULTS AND DISCUSSION

Dry matter yield (DMY)

There was no interaction ($p > 0.05$) between harvest date and pasture type (Table 1). DMY followed a similar chronological pattern in both pastures throughout the study period, with no significant differences ($p > 0.05$). These results differ from those of Carrillo-Hernández *et al.*, (2022) who reported higher yields in Mombaza-*Canavalia brasiliensis* than in Mombaza alone.

The average DMY of both pastures was 2428.23 kg DM ha^{-1} varying from 1040.96 to 3930.93 kg ha^{-1} for the monoculture, and from 775.69 to 3398.5 kg ha^{-1} for the associated pasture. However, harvest date significantly affected ($p \leq 0.001$) the DMY in both pastures (Table 1). On March 9th and April 14th, the lowest DMY was recorded, with average values of 908.3 kg ha^{-1} and 1069 kg ha^{-1}, respectively for both pastures (Table 2). Between May and October, the DMY increased 200%, with no significant differences ($p > 0.05$) between dates, with average values of 2259.5, 2495.1, 3340.8, and 3664.8 kg ha^{-1} compared to May 20th, June 23rd, July 29th, September 3rd, and October 9th. This meant a production of 196 kg of DM ha^{-1} more, in the May-October period, compared to March-April. This increase is associated with higher rainfall, from January-April (121mm) to May-October (317 mm) (Figure 1). These results are consistent with records of peak growth for grasses and forage fabaceae in the summer (Pardo-Aguilar *et al.*, 2020).

The DMY of Kudzu in the association was low, and significantly affected ($p \leq 0.05$) by the sampling date (Table 1). The yield was lower on April 14, with 21.0 kg of DM ha^{-1} less compared to the average of 67.0 kg of DM ha^{-1} for the rest of the evaluated dates (Table 2). The DMY of the fabaceae remained low but persisted in the association. This

Table 1. Mean squares of dry matter yield (DMY) and nutritional value of *Megathyrsus maximus* var. Mombaza in monoculture, and the Mombaza-Kudzu (*Pueraria phaseoloides*) association, at 36 days post-grazing.

Variables measured	Average	Date (D)	Meadow (M)	D × M
DMY, kg ha^{-1}	2428.2	9.656 ***	0.4816 NS	0.1154 NS
Legume, kg ha^{-1}	64.3	0.0007 *	----------	---------
D.F.		6	1	6
Protein, g kg^{-1} DM	80.19	1142.70 **	13.44 NS	102.66 NS
NDF, g kg^{-1} DM	697.42	4442.90 ***	40.70 NS	13.02 NS
ADF, g kg^{-1} DM	458.28	4704.48 **	376.32 NS	406.0 NS
Lignin, g kg^{-1} DM	50.17	51.66 NS	108.60 *	122.24 NS
Ashes, g kg^{-1} DM	109.8	375.20 *	14.30 NS	55.04 NS
D.F.		1	1	1

DF: Degrees of freedom; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber. Statistical differences *** ≤0.001, ** ≤0.01, and * ≤0.05; NS, not significant.

Table 2. Dry matter yield (DMY, kg ha^{-1}) of the different components of the pasture in monoculture and associated on different dates.

Harvest dates	Mombaza in monoculture	Mombaza + Kudzú association	Mombaza of the association	Kudzú of the association	Kudzú ratio (%)
March 09	1041.0 c	775.7 b	710.3 c	65.31 ab	8.42 a
April 14	1181.4 c	957.7 b	957.8 bc	46.83 b	4.75 b
May 20	2201.0 bc	2318.1 ab	2234.0 abc	84.06 a	3.73 bc
June 23	2466.3 abc	2523.8 ab	2449.8 ab	74.19 ab	3.13 bcd
July 29	3543.5 ab	3138.1 a	2240.8 abc	72.64 ab	2.36 cd
Sept 3	3930.9 a	3398.6 a	3341.8 a	56.92 ab	1.72 d
October 9	3282.8 ab	3236.5 a	3186.3 a	50.17 ab	1.71 d
LSD	1588.4	1755.0	1715.9	35.3	1.97

LSD: Least significant difference; Means within the column followed by different letters are statistically different ($p \leq 0.05$).

persistence can be attributed to the irregular defoliation of the animals, which induces the formation of small bare areas that allow light penetration and stimulates the development of branches of their axillary buds (Zegler *et al.*, 2018). Therefore, the proportion of Kudzu in the pasture decreased ($p \leq 0.05$) with the advancement of sampling dates, from 8.42% on March 9th to less than 2% on October 9th (Table 2). This decrease was also due to the increase ($p \leq 0.05$) in DMY of Mombaza grass, recorded from May 20th onward. This indicated that compared to Mombaza, Kudzu showed a lower efficiency in converting soil nutrients and water into dry matter.

Nutritional value

The nutritional value did not show an interaction between the sampling date and the type of pasture ($p > 0.05$) in the evaluated variables. However, it was affected by the time of year (sampling date), except for lignin ($p > 0.05$; Table 1) which did not vary between sampling dates, averaging 50.17 g/kg of DM (Table 3).

The protein concentration was higher on April 14th (drought season), with 15.52 g kg^{-1} of DM more than on July 29th (rainy season).

Table 3. Nutritive value (g kg^{-1} of DM) of *Megathyrsus maximus* var. Mombaza in monoculture and the Mombaza-Kudzu (*Pueraria phaseoloides*) association on representative climate dates in 2020.

Variable	Averages of two seasons of the year			Dry Season (April 14)			Rainy Season (July 29)		
	April / 04	July / 09	LSD	Mombaza	Mombaza + Kudzú	LSD	Mombaza	Mombaza + Kudzú	LSD
Protein	9.95 a	0.43 b	1.6	5.96 a	93.0 a	2.5	2.30 a	8.56 a	4.3
NDF	78.18 b	16.66 a	5.9	75.30 a	81.07 a	3.2	15.86 a	17.46 a	1.4
ADF	38.48 b	78.08 a	5.1	27.07 a	49.90 a	3.5	78.30 a	77.86 a	2.5
Lignin	8.10 a	2.25 a	1.3	1.90 b	4.30 a	1.3	2.43 a	2.06 a	1.2
Ashes	15.43 a	04.25 b	3.0	18.66 a	12.20 a	6.4	03.20 a	05.30 a	1.1

LSD: Least significant difference; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; Means in the same row with different letters are different ($p \leq 0.05$).

The higher protein concentration in the drought season is explained by the low DMY, causing less dilution within the plant (Reyes *et al.*, 2015). Conversely, the highest concentration ($p \leq 0.05$) of NDF and ADF was recorded in the rainy season. This indicates that the DMY was made up mainly of stems, which are the support organs of the plant and have a higher concentration of fiber. The increase in DMY during the rainy season in forage species has been attributed to the decrease leaf:stem ratio and leaf senescence (Calzada-Marín *et al.*, 2019), which may also explain the reduction in nutritional value during this time of year. Higher nutritional values have also been recorded in the drought season in other tropical species (Portillo-López *et al.*, 2021).

The higher concentration of total ashes (115.43 *vs.* 104.25 g kg^{-1} of DM in rainy season), representing the plant's minerals (calcium, potassium, phosphorus, magnesium, and copper), in the drought season could be due to soil minerals (which have no nutritional value for animals), since the dusty conditions of the drought season increase this contamination. This ash concentration (124.7 g kg^{-1} DM) is similar to that reported by Schmitz *et al.* (2023) in Mombaza associated with *Arachis pintoi*, although it is lower (134.01 g kg^{-1} DM) than that reported by Enwete *et al.* (2023). On the contrary, the lower concentration of ash in the rainy season can be attributed to the greater growth of the plant, which results in a higher dilution, similar to what occurs with the concentration of protein.

The protein concentration in the Mombaza-Kudzu association was not higher ($p > 0.05$) than Mombaza in monoculture (Table 1), even though Kudzu recorded an average value of the two seasons, of 206.72 g kg^{-1} of DM (Table 4). This was because the proportion of Kudzu in association was not sufficient to improve the protein concentration of the pasture.

Daily Weight Gain (DWG)

There was no interaction ($p \leq 0.05$) between weighing date and pasture type of the DWG. A trend towards greater gains was observed on the second weighing date, from March 9th to April 14th, where DWG in the Mombaza monoculture pasture was 300 g day^{-1} higher than that recorded in Mombaza-Kudzu association (Figure 2). However, the difference was not significant ($p > 0.05$) due to the high variation of the data (c.v.=28.0%). Therefore, DWG between pasture types did not differ ($p > 0.05$), with an average gain of 485.6 g day^{-1} across both pastures during the study period.

Similar results were reported by Schmitz *et al.* (2023) who did not observe differences in DWG between a Mombaza pasture and a Mombaza-Arachis pasture, attributing it to

Table 4. Nutritive value, in g kg^{-1} of Kudzu (*Pueraria phaseoloides*) dry matter on two dates of the year with contrasting climates.

Season	Protein	NDF	ADF	Lignin	Ashes
Dry (april 14)	208.99 a	536.58 a	399.41 a	105. 85 a	63.30 a
Rainy (july 29)	204.46 a	554.13 a	421.74 a	102.81 a	66.58 a
LSD	22.8	26.5	28.7	16.8	4.4

LSD: Least significant difference; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; Means in the same row with different letters are different ($p \leq 0.05$).

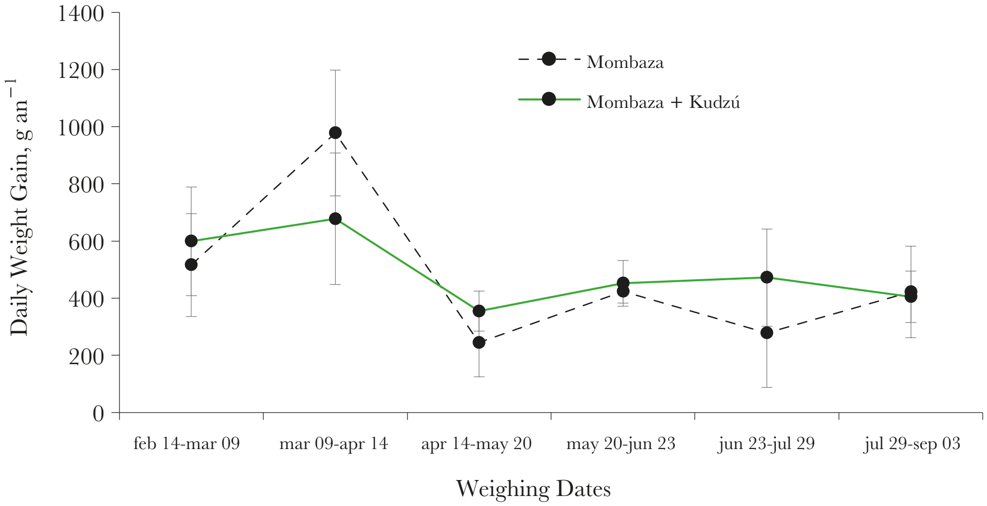

Figure 2. Daily weight gain (DWG) of calves in pastures with monoculture Mombaza and Kudzu-Mombaza association on four grazing dates.

the animal's low preference for the fabaceae species. The DWG was affected by the date of the year ($p \leq 0.05$), particularly from March 9th to April 14th, when there was greater DWG in both pastures (Table 5). During this period the animals recorded 600 g more per day with moniculture Mombaza, compared to the average gain (377 g d^{-1}) for the rest of the evaluation dates (March 9th to April 14th and September 3rd), while in the Mombaza-Kudzu association was 221 g more per day, compared to the average (457 g d^{-1}) for the rest of the dates studied.

This higher DWG from March to April (drought season) was associated with the highest protein concentration and the lowest fiber concentration of the available forage (Table 3). The lowest DWG in rains (July 29th) corresponds with Rueda *et al.* (2020), who observed that in the rainy months, grasses increase their NDF, a protein insoluble in neutral detergent, and decrease metabolizable energy, which limits the weight gain of calves.

Table 5. Daily weight gain of calves in pastures with *Megathyrsus maximus* var. Mombaza in monoculture and in Mombaza-Kudzu (*Pueraria phaseoloides*) pastures in 2020.

Evaluation period	Mombaza (g an^{-1} d^{-1})	Mombaza + Kudzú (g an^{-1} d^{-1})
February 14 – March 09	517 b	600 ab
March 09 – April 14	978 a	678 a
April 14 – May 20	245 b	356 b
May 20 – June 23	424 b	453 ab
June 23 – July 29	278 b	472 ab
July 29 – September 03	422 b	405 ab
LSD	322.1	307.1

LSD: Least Significant Differences; Means within the column with different letters are statistically different ($p \leq 0.05$).

CONCLUSIONS

Kudzu demonstrates less association capacity than Mombaza as its proportion in the pasture decreased over time. Therefore, there were no differences in nutritional value or daily weight gain of the animal between the Mombaza and Mombaza-Kudzu pastures, due to the low availability of the fabaceae. However, the higher protein concentration at the beginning of the drought period induced higher DWG in both pastures, additionally, both pastures responded similarly to the distribution of rains, registering 2.9 times more DMY during the rainy period than in the first months of evaluation with less precipitation.

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Colegio de Postgraduados

Design of an orchid conservation trail in Ixtaczoquitlán, Veracruz, Mexico

Hernández-García, A.[1]; **Baltazar-Bernal, O.**[1*]; **Zavala-Ruiz, J.**[2]

[1] Colegio de Postgraduados - Campus Córdoba, Amatlán de los Reyes, Veracruz, México. C. P. 94953.
[2] Universidad Autónoma Metropolitana, Prolongación Canal de Miramontes 3855, Col. Ex-Hacienda San Juan de Dios, Alcaldía de Tlalpan, Ciudad de México, C. P. 14387.
* Correspondence: obduliabb@colpos.mx

ABSTRACT

Objective: To raise awareness about the ecological importance of orchids and to promote the species commercialized by a company focused on their production through the design of a trail.

Design/Methodology/Approach: A diagnosis of the plot was carried out to identify its characteristics and points of interest. A trail was designed to connect the most outstanding points. Native trees, which are the habitat of orchids, were subsequently planted to establish the outdoor collections.

Results: The appeal of the place and the established phorophytes were taken into consideration to design the trail. Likewise, recommendations were made on what species of orchids should be planted in each station along the trail.

Study Limitations/Implications: Evaluating visitor experience is pending. The trail lacks infrastructure for people with disabilities. The trail is located in an area subjected to anthropization and it will take several years before orchids can be established on the trees planted in July 2023.

Findings/Conclusions: Trails are a useful tool to promote the conservation of native orchids; however, under anthropization conditions, it should be subjected to a systematized implementation to provide the climate and phorophyte conditions necessary for the survival of orchids.

Keywords: Trail design, orchid trail, phorophytes, native orchids, introduced orchids, alternative tourism, hiking.

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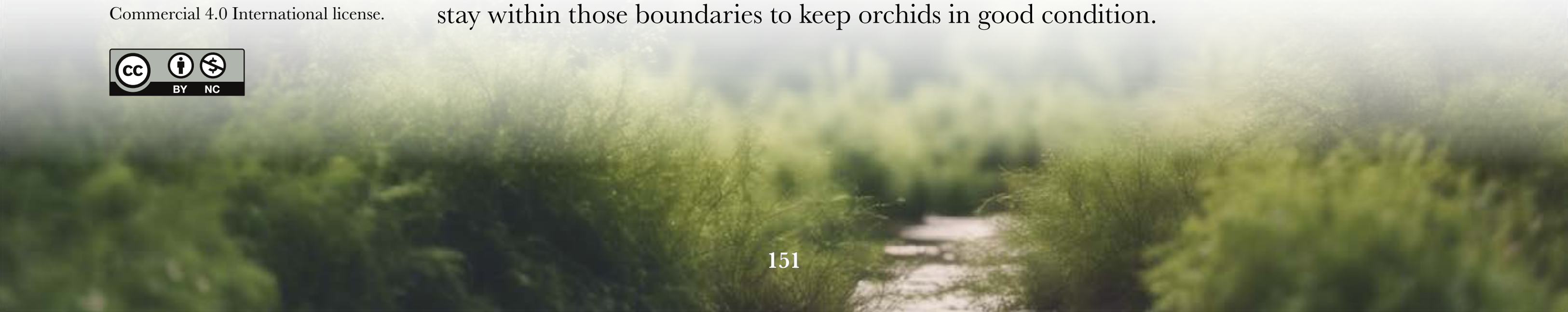
INTRODUCTION

Trails, as part of the natural environment, facilitate recreational activities and contact with nature (Phillips *et al.*, 2014). Outdoor activities (*e.g.*, hiking and picnics) are considered part of alternative tourism or rural tourism (Phillips *et al.*, 2014; Baltazar-Bernal, 2024). Therefore, each trail must be designed according to its target audience (Hernández-Ulate *et al.*, 2015). Consequently, designing a trail requires surveys and assessments to identify the biotic and abiotic elements of the area, therefore avoiding soil erosion, soil compaction, and disturbance of local species, among other factors (Phillips *et al.*, 2014). In this sense, trails must be designed comprehensively to avoid damaging natural resources and to encourage the study about the diversity of flora, including native orchids and their environmental functions (Rahadi *et al.*, 2018; Baltazar-Bernal, 2024). Therefore, Light y Macconaill (2008) suggests establishing clear limits on the trails and instructing visitors to stay within those boundaries to keep orchids in good condition.



There are two types of trails: guided and self-guided. As their name indicates, the former require a guide and the latter are designed for areas in which a high number of visitors walk the trail on their own, which requires sufficient signage and/or information material to enhance the enjoyment of the visit (Lucero *et al.*, 2021). Forest orchids are potentially threatened by deforestation and illegal felling (Solano-Gómez *et al.*, 2019); therefore, conservation awareness should be increased, recognizing threats to reduce their impact and having adequate management (Wraith and Pickering, 2017). The diversity of wild orchids and bromeliads found in forest trails could become tourist attractions (Baltazar-Bernal *et al.*, 2014). Outdoor orchid collections have different species displayed in a natural space, enhancing the experience of the visitors (Semiarti *et al.*, 2020).

Orchid trails promote the integration of conservation activities, based on the observation of the different elements that compose the environment (Rodríguez-Gutiérrez *et al.*, 2019; Baltazar-Bernal, 2023). Similar studies have been carried out in the state of Veracruz aimed at designing an orchid trail in anthropized environments, such as a rural community (Baltazar-Bernal *et al.*, 2014) and an educational institution (Baltazar-Bernal, 2023). However, further research about trails is fundamental to promote orchid conservation in other types of anthropized environments, such as private companies. Therefore, the objective of this study was to design an orchid trail for a company in Ixtaczoquitlán, Veracruz, Mexico. The ultimate purpose was to raise awareness about the ecological importance of orchids and to promote the species that they commercialize. The hypothesis was that the study site will have the climate, phorophytes, vegetation cover, and natural appeal required to design an orchid trail.

MATERIALS AND METHODS

The methodology proposed by SECTUR (2005) and modified by Baltazar-Bernal *et al.* (2014) was used to design this trail. The design phases were: (1) diagnosis and planning and (2) design and construction.

First phase: Diagnosis and planning

The first phase consisted of a bibliographic review, searching for information on abiotic and biotic elements, including: soil characteristics, climatic conditions, altitude, and vegetation (Table 1). Subsequently, a site analysis was carried out to record the conditions of the land, the different natural and artificial elements that make up the landscape (including an inventory and identification of the trees and orchids), and the various points

Table 1. Study site characteristics, Ixtaczoquitlán, Veracruz, México.

Vegetation	Soil	Clima	Temperatura media anual	Precipitación anual	Altura
Cloud Forest	Vertisol crómico (CONABIO, 1995)	Semicálido húmedo, con lluvias en verano (García, 2004)	17 °C (García, 2004)	1,977 mm (García, 2004)	1,160 msnm

Table 2. Tree inventory, orchid species and study site attractions, Ixtaczoquitlán, Veracruz, México.

Species/Atractive	**Common name**
Trees	
Ficus sp.	Higuera
Eriobotrya japonica (Thunb.) Lindl.	Níspero
Heliocarpus appendiculatus Turcz	Jonote
Bursera simaruba (L.) Sarg.	Mulato tree
Spondias sp.	Mexican ciruela
Orchids	
Isochilus linearis (Jacq.) R.Br.	
Prosthechea ochracea (Lindl.) W.E. Higgins	
Maxillaria densa (Lindl.)	
Site atractives	
Water spring "Cascada de la Calavera"	
Matzinga river tributary stream	
Water pond	
Lookout	
Greenhouses	
In vitro culture laboratoty	

of interest (Table 2). The company's characteristics were also described, based on three surveys with a Garmin® Etrex10 GPS device, obtaining the coordinates of the site, the altitude, and the paths. Finally, the area of the land was calculated.

The polygon was measured and the tree cover was calculated. The coordinates determined during the visits were used to generate a polygon with the Google Earth Pro software, in order to obtain the total area of the land. To calculate tree cover, the polygon tool was applied to a satellite image of the plot. Finally, the difference between both areas was calculated using the rule of three (Cecato *et al.*, 2020) and the information collected and the photographic record were integrated.

Study site

The study site is located in the municipality of Ixtaczoquitlán, in the state of Veracruz, Mexico, between 18° 50' 21" and 18° 50' 26" N and between 97° 04' 34" and 97° 04' 39" W. Its south border is a tributary stream of the Matzinga River, part of the Papaloapan River basin; to the North, its limit is the Privada de la Calavera Street and several chayote crops; to the East, it borders with chayote crops; and finally an uncultivated land lies to the West. The total area is 12,380 m^2 and has a perimeter of 563 m (Figure 1).

The participating company is also located in the municipality of Ixtaczoquitlán, in Veracruz, Mexico. It produces and commercializes *in vitro* and potted orchids; additionally, it sells pots and cultivation supplies. The company sells hybrids of the genera *Phalaenopsis*,

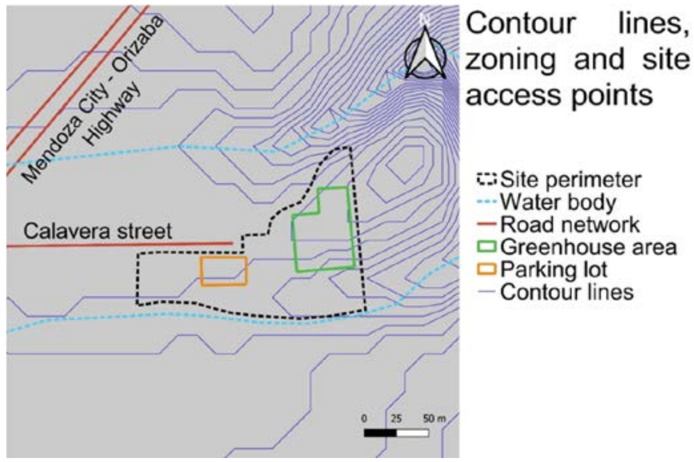


Figure 1. Study area, Ixtaczoquitlán, Veracruz, Mexico.

Cattleya, and *Dendrobium*. Its facilities include greenhouses, an *in vitro* culture laboratory, a recreational area, a tributary stream of the Matzinga River, a spring, and a pond. In addition, it has a Wildlife Conservation Management Unit (UMA), which provides the legal framework for the commercialization of native orchid species included in NOM-059-SEMARNAT-2010.

Second phase: Design and construction of the trail

Based on the methodology proposed by Baltazar-Bernal *et al.* (2014) —which takes into account site information, like inventory of trees and orchids, and the attractive features of the site—, different points of interest identified during the visits were chosen, including: active constructions, trees, paths, river, spring, and pond, as well as the physiography of the plot. The opinions and suggestions of the company's technicians were also considered. During the verification visit, the tree cover in most of the plot was calculated at 16-30%. Consequently, a zoning-based planting was the chosen option, since most orchids require shade to thrive (Morales-Linares and Menchaca-García, 2021).

Native tree planting

After carrying out the diagnosis of the place, a planting scheme was designed to increase the tree cover, vegetation cover, and green areas. Different species of native trees and those produced in regional nurseries were chosen for reforestation. Since most orchids are epiphytes, phorophytes were planted to increase plant coverage. Reforestations efforts were carried out at different points of the trail without or with scarce tree vegetation (Figure 2).

Trees were planted in the first days of July to coincide with the rainy season (Figure 3). Sixty-two trees were planted 8 to 10 m apart from each other (Table 3). It will take several years before the transplanted trees can be used as phorophytes; in the meantime, some other fast-growing plants could be used.

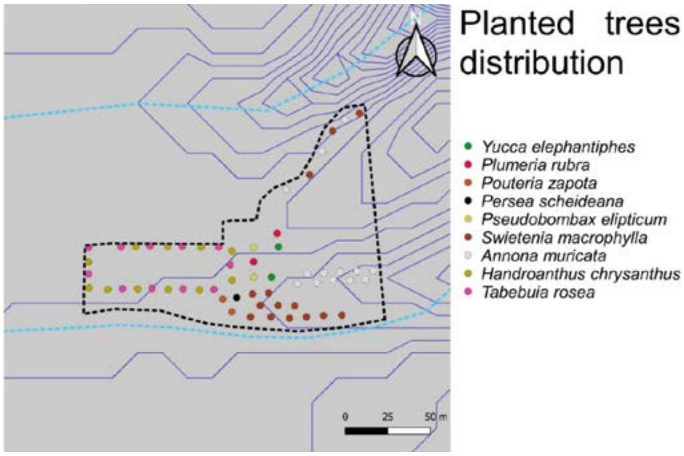


Figure 2. Trees planted in the study area, Ixtaczoquitlán, Veracruz, Mexico.

Figure 3. Tree planting in the study area, Ixtaczoquitlán, Veracruz, Mexico, A) *Tabebuia chrysantha* (Jacq.) G. Nicholson, B) *Tabebuia rosea* (Bertol.) DC., C) *Persea schiedeana* Nees, and D) *Annona muricata* L.

Plot zoning

Parking area

At the main entrance to the property, 20 trees of the species *Tabebuia mexicana* (Bertol.) DC and *Tabebuia chrysantha* (Jacq.) G. Nicholson were planted, 6 to 8 m apart from each other. These colorful trees will provide shade for vehicles.

Table 3. Tree species planted on the study site, Ixtaczoquitlán, Veracruz, México.

Scientific name	Common name	No.	Zones
Tabebuia rosea (Bertol.) DC.	Five leaves	12	1
Swietenia macrophylla King in Hook.	Caoba	15	3
Annona muricata L.	Guanábana	16	2
Tabebuia crhysantha (Jacq.) G.Nicholson	Primavera	12	1
Plumeria rubra L.	Plumería	2	2
Yucca elephantipes Lem.	Izote	2	1
Persea schiedeana Nees	Chinene	1	2
Pseudobombax elipticum (Kunth) Dugand	Lele	2	2
Total		62	

Hillside area

In this area, 10 *Annona muricata* L. trees and two *Persea* spp. were planted 8 m apart from each other. These tree species have strong roots that will maintain soil stability and atmospheric moisture. These conditions will allow the establishment of orchid species. While those trees grow, bamboo, a fast-growing species, could be planted.

River bank area

The flow of water that runs through the plot is important, since it provides a constant source of atmospheric moisture and is used to irrigate greenhouses. In this area, trees that commonly grow on river banks will prevent water erosion and maintain the humidity and temperature required by the orchids. Twenty *Swietenia macrophylla* King in Hook were planted 10 m apart from each other (Figure 4).

RESULTS AND DISCUSSION

Trail design

After the planting, the trail was designed at the company facilities, with the aim of raising awareness about the ecological importance of orchids and promoting the different species

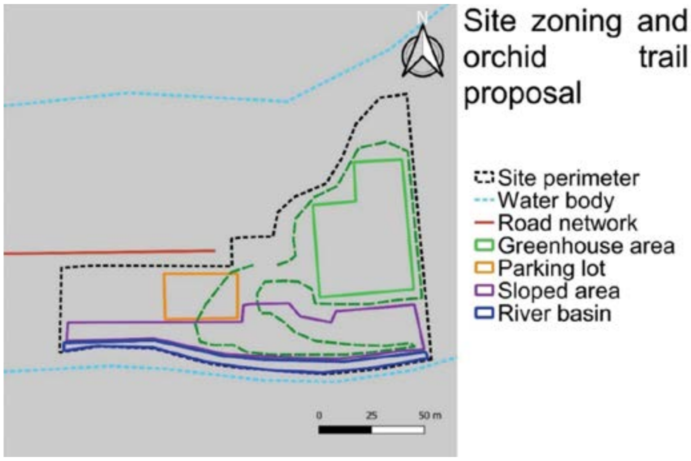


Figure 4. Lot zoning in Ixtaczoquitlán, Veracruz, Mexico.

that the company sells. With the information collected during the visits, the trail proposal was developed, using the pre-existing paths. The trail design included seven stations, where visitors will be able to observe some native orchid species and their phorophytes (Figure 5).

Stations

***Inicio / bienvenida* (*Start / Welcome*)**

Visitors will be welcomed at this point. They will be given an introduction about the diversity of orchids in the region (Krömer *et al.*, 2021) and their ecological importance, as one of the largest families in the world, which includes more than 30,000 species (Hágsater *et al.*, 2015; Solano-Gómez 2019). Species of orchids that tolerate sunlight exposure, such as *Myrmecophyla grandiflora* (Lindl.) Carnevali, J.L.Tapia and I.Ramírez, *Cyrtopodium macrobulbon* (Lex.) G.A., Romero-González and Carnevali, *Oncidium sphacelatum* Lindl., and *Catasetum intergerrimum* Hook may be used in this part of the path. Orchids can be grown in adult trees of *Ficus* sp., and *Eriobotrya japonica* (Thunb.) Lindl. (loquat). In few years in *Tabebuia rosea* (Bertol.) DC. and *Tabebuia chrysantha* (Jacq.) G. Nicholson recently planted. The importance of having a similar vegetation cover than in the forest (humidity, shade, and temperature) must be emphasized to establish and conserve the orchids that will be suggested for each station of the trail.

***Higuera* (*Ficus tree*)**

At this point, the importance of tall adult trees for the natural reproduction of orchids will be explained (Hernández-García *et al.*, 2021). The *Ficus* sp. (fig tree) tree will be pointed out as an example of a phorophyte, since several epiphytes are housed in its mature bark and it also provides shade (Figure 6A) (Hernández-Pérez *et al.*, 2018; Izuddin *et al.*, 2018). These characteristics of the *Ficus* sp. allow native species such as *Isochilus* sp. and *Prosthechea ochracea* (Lindl.) W.E. Higgins to inhabit it. This station can feature species that are resistant to dry conditions or that require less shade, such as: *Laelia anceps* Lindl., *Oncidium sphacelatum* Lindl., and *Epidendrum melistagum* Hágsater.

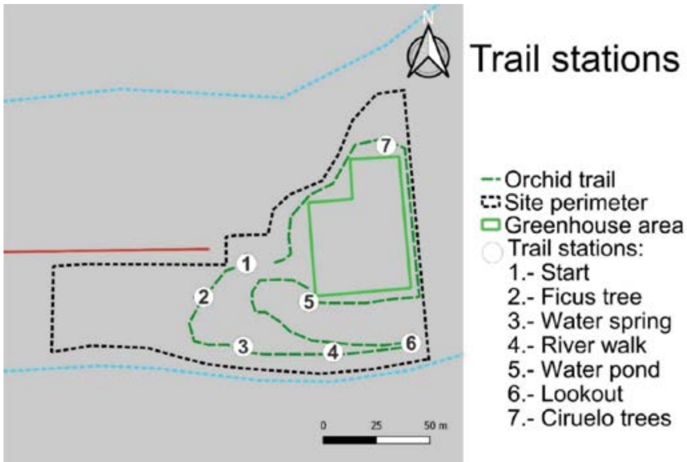


Figure 5. Design of the trail and its stations in a company in Ixtaczoquitlán, Veracruz, Mexico.

Figure 6. Stations of the trail: A) La Higuera, B) El Manantial, C) El Río, D) El Estanque, E) El Mirador, and F) Los Ciruelos.

***Manantial* (*Water spring*)**

This station offers a view of the beautiful, crystal-clear water spring "Cascada de la Calavera;" the overall importance of water bodies and their positive influence on orchids and flora will be explained at this point in the path. The role that environmental moisture plays in the development and maintenance of orchid populations will also be explained. Emphasis will be made on the risk that orchid populations face as a result of the reduction of moisture and decrease of the shade provided by the trees (Anastacio-Martínez *et al.*, 2016; 2019; Solano-Gómez *et al.*, 2019). Although this station will not include any phorophytes suitable for the adaptation of epiphytes, the walls of the spring can house species that require a lot of environmental moisture, such as *Epidendrum parkinsonianum* Hook and *Erycina pusilla* (L.) N.H.Williams & M.W.Chase. The area features adult *Bursera simaruba* (L.) Sarg. (gumbo limbo) trees; however, orchids are not able to anchor on their slippery and constantly renewing bark. Therefore, species such as *Pouteria sapota* (Jacq.) H.E.Moore & Stearn (Figure 6B) and *Persea scheideana* were established.

***Camino del río* (*River walk*)**

In this part of the trail, visitors will be told that most orchids prefer to live on trees located in river banks, because the greater abundance of vegetation cover protects them better (Hernández-Pérez *et al.*, 2018; Morales-Linares and Menchaca-García, 2021). Consequently, more than 60 trees were planted on the trail. In adult trees of

Heliocarpus appendiculatus Turcz (Figure 6C), orchids such as *Myrmecophyla grandiflora* Lindl. Carnevali, J.L.Tapia & I. Ramírez, *Laelia anceps* Lindl., and *Oncidium sphacelatum* Lindl will be stablished.

Estanque (*Water pond*)

This station is halfway along the path and has an area with a palm-roof palapa, where visitors can rest and relax. The great hydrological wealth of the state of Veracruz and the importance of Pico de Orizaba as a water source for the entire region will be explained (Rivera-Hernández *et al.*, 2019). Furthermore, the importance of water bodies (Figure 6D) —which help to maintain environmental moisture— for the development of native orchids will be highlighted: moisture explains the great diversity of orchids in the cloud forest (Hágsater *et al.*, 2015). The native species suggested for this area include *Oncidium sphacelatum* Lindl, *Laelia anceps* Lindl., *Heliocarpus appendiculatus* Turcz., and *Chamaedorea tepejilote* Liebm.

Mirador (*The Viewpoint*)

At this station, visitors will be invited to the commercial orchid exhibition area. The viewpoint should be rearranged to allow visitors to enjoy the view. The *in vitro* cultivation processes, acclimatization, hardening of orchid plants for commercialization, and the care they require will be explained (Chen *et al.*, 2020), especially those of native species (Krömer *et al.*, 2021). Once the *Plumeria rubra* L., *Pseudobombax elipticum* (Kunth) Dugand, and Yucca elephantipes Lem species (Figure 6E) reach their adult stage and provide vegetation cover, *Oncidium sphacelatum* Lindl. can be established.

***Ciruelos mexicanos* (*The Mexican plums*)**

Several adult Mexican plum trees (*Spondias* sp.) can be found in this station. They host such orchids as *Isochilus linearis* (Jacq.) R.Br., *Prosthechea ochracea* (Lindl.) W.E.Higgins, and *Maxillaria dense* (Lindl.). The importance of native trees as hosts of orchids will be emphasized at this point (Hernández-García *et al.*, 2021); orchids will be admired along the trail (Lucero *et al.*, 2021; Hernández-Pérez *et al.*, 2019; Hernández-Pérez *et al.*, 2018). In addition, visitors will be invited to establish epiphytic orchids on some trees with the following procedure: orchid plants with four pseudobulbs will be attached to the trunk of the phorophyte with a cotton thread, at a height of 1.2 to 2.0 m. (Hernández-García *et al.*, 2021) (Figure 6F). At this point, the conclusions will also be given.

The walk through the 420-m long orchid conservation trail in Ixtaczoquitlán, Veracruz, lasts 90 minutes. It has been designed for the public interested in learning about the orchids that grow in their phorophytes. Although it does not have access and infrastructure for people with disabilities, it is relatively accessible for seniors and children, which makes it a family-friendly site. Its operation is a work in progress and it will take from 6 to 10 years, before the trees that were established can house orchids and provide greater vegetation cover. However, given the appeal of the place and, if the plot is properly prepared, visits can be made to validate the trail, before it is opened to the public.

CONCLUSIONS

The orchid trail designed for the company requires greater plant coverage to establish and conserve the suggested orchids at each station of the trail, which is why 62 native trees that develop in the region as orchid phorophytes were planted.

The trail is in an anthropized area with low tree cover, therefore, it will take between six and ten years to be consolidated. However, it is recommended to validate the design of the trail, so that in the medium term it can be used to raise awareness about the ecological importance of orchids, and promote the species they produce.

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Colegio de Postgraduados

Economic cointegration of the North American agricultural sector

Cruz-Lázaro, Luis M.[1]; **Banda-Ortiz, Humberto**[1*]; **Vivanco-Palacios Paulina I.**[1]

[1] Universidad Autónoma de Querétaro, Querétaro, México, C. P. 76010.
* Correspondence: Humberto.banda@gmail.com

ABSTRACT

Objective: To determine if the Mexican, Canadian, and American agricultural industries are cointegrated.

Methodology: Six cointegration tests were carried out between the Mexican, Canadian, and American agricultural, forestry, fishing, and hunting industries, as well as the Mexican animal husbandry and exploitation sector. The USA was the independent variable in all cases.

Results: The Mexican sector, with an α of 5% (with and without trend) is not cointegrated with the USA and Canada, while Canada and the USA, with an α of 5% (with and without trend) are cointegrated.

Study Limitations/Implications: The agricultural sector of the three countries were not analyzed separately and the Engle-Granger causality test was not used. Although some products from Mexico's agricultural sector have managed to make inroads in the USA and Canada, further advances are still possible. Therefore, there are areas of improvement for Mexican products. Likewise, NAFTA and the USMCA/CUSMA have failed to achieve their objective of cointegrating the agricultural sectors of the three nations.

Conclusions: The Mexican sector was not cointegrated with the American and Canadian sectors during the analysis period —*i.e.*, the Mexican sector is not influenced by and does not have the same long-term behavior (with delays) than the USA and Canadian sectors. However, the Canadian sector is cointegrated with the USA sector —*i.e.*, the Canadian sector is influenced and has the same long-term behavior than the USA sector.

Keywords: North America; agricultural sector; cointegration; NAFTA; USMCA; CUSMA.

INTRODUCTION

Globalization included integration processes carried out through trade agreements. One of the most important agreements was the North American Free Trade Agreement (NAFTA) which came into force in January 1, 1994 and sought to integrate the economies of México, the United States, and Canada. It consisted of several measures, such as lifting tariffs. Nevertheless, Mexico has signed other important trade agreements with several countries, including Colombia, Nicaragua, and Israel. The most recent agreement is the Comprehensive and Progressive Agreement for Trans Pacific Partnership (CPTPP) (Puyana, 2020; Nava, 2021; Infante *et al.*, 2021). In addition, NAFTA was replaced in 2020 by a new treaty between Mexico, the United States, and Canada (USMCA/CUSMA). Both NAFTA and USMCA/CUSMA have impacted the economies of the three countries and they have led to an economic cointegration, as evidenced by the percentage of exports (85%) and imports (40%) of Mexico to and from the United States in 2019 (Santa, 2019; Morales *et al.*, 2016; Garza-Rodríguez, 2016; Valencia *et al.*, 2021; Leos and García, 2018).

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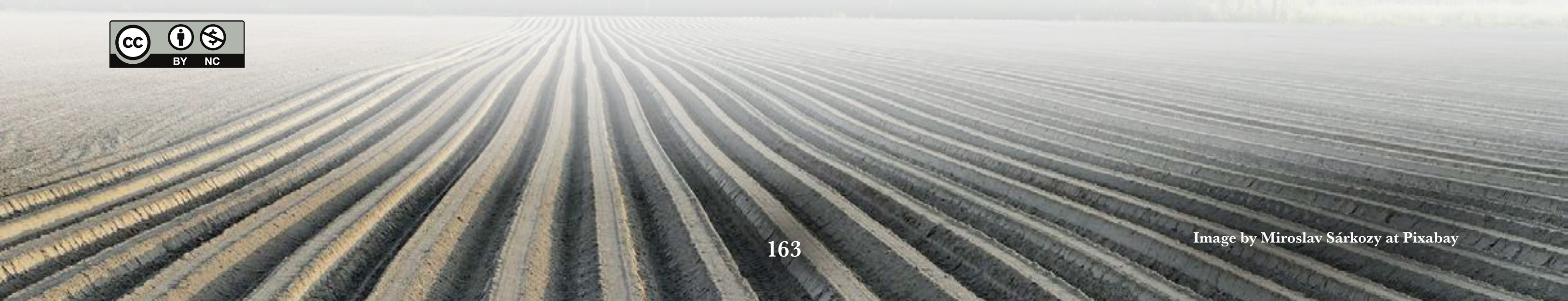

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The aim of both treaties was to promote trade and to achieve the long-term integration (*i.e.*, the cointegration) of the Mexican, American, and Canadian economic sectors (such as agriculture). This cointegration would allow the economic sectors of the three countries to react to changes in demand in the other members of the group. For example, if consumer demand in the United States increases (independent variable), due an income increase, the Mexican agricultural sector would increase its production (dependent variable), although this reaction on the part of Mexico would be delayed. In this regard, some of the countries' agri-food products are co-integrated, as a result of several factors, including: long-term relationships between producers, traders, and consumers in the three countries; and the large percentage of products that some sectors of the Mexican agricultural sector export to the United States (*e.g.*, fruits and vegetables). The cointegration of Mexico with Canada and the USA is evidenced by the increase and diversity of Mexican exports. Likewise, some products from Mexico's agri-food sector, such as vegetables and fruits, have taken advantage of trade agreements to position themselves in the American market. Furthermore, Mexican producers have focused on the production of export goods, contributing to the cointegration of Mexico with Canada and the USA (Jaime *et al.*, 2015; Chávez *et al.*, 2019; González, 2017).

Meanwhile, not all sectors and markets in the three countries have achieved cointegration, particularly the labor market. Additionally, the commercial and financial integration within the region is heterogeneous. Finally, the evidence provided by theoretical and empirical models has not been conclusive with regard to the synchronization of economic cycles and the cointegration of the abovementioned sectors. Specifically, the cointegration of the agricultural sectors of the three countries will depend on the type of crop, the type of relationships formed between the countries, and the type of producer. Producers who have greater organization, education, and economic resources will be able to export their products and establish strategic alliances with groups from other countries (González, 2017; Pérez, 2019; Anguiano and Ruiz, 2022).

Mexican farmers are at a disadvantage compared with American producers, whose goods are subsidized, while in Mexico such support has decreased (Jaime *et al.*, 2015; Chávez *et al.*, 2019; Infante *et al.*, 2021). In this regard, if Mexico's agricultural sector is to take advantage of commercial openness and achieve co-integration, it must overcome the sector's structural lags, increase its productivity with technology, and design long-term strategies. In this sense, Pérez *et al.* (2019) indicate that the growth of the agricultural sector responds to: the production area that is irrigated; the economically active population of the agricultural sector; and the volume of fertilizers applied.

Meanwhile, the unbalanced rules and conditions established in the treaties that negatively affected Mexico have prevented the cointegration of the productive sectors; these factors were not taken into account during the negotiation of the trade agreements. For instance, the USA imposed its rules, such as the periods of tax relief on imports and the establishment of non-tariff barriers. The USA applied these rules to the entry of crops that could have a negative effect on its competitiveness. There is also an inequality between the economies of the three countries: for example, in 2017, the *per capita* income of Mexico, the USA, and Canada amounted to $8,688, $61,247, and $44,487 USD, respectively. Likewise, there are differences in the competitiveness of the sectors in the three countries. For example, some agricultural products from the USA and Canada are more productive than their Mexican equivalents. Finally, the USA has had an enormous influence on Mexico's economy, even before the entry into force of the trade agreements (Puyana, 2020; Chávez *et al.*, 2019; Jaime *et al.*, 2015; Infante *et al.*, 2021). Therefore, the cointegration of the three countries must analyzed to determine the impact of trade agreements in Mexico's agricultural sector, detect potential areas of improvement, and develop policies to take advantage of those opportunities. In conclusion, the objective of the research was to determine whether the agricultural industries of Mexico, Canada, and the United States are cointegrated.

METHODOLOGY

The methodology of this research aims to determine if the agricultural industries in Mexico, Canada, and the United States are co-integrated. For this purpose, six cointegration tests of the three countries were carried out. In the case of Mexico, the *agricultura*, *cría y explotación de animales*, *aprovechamiento forestal*, *pesca y caza* (agriculture, animal husbandry and exploitation, forestry, fishing, and hunting) heading was extracted from the National Institute of Statistics and Geography website (INEGI, 2023); in the case of Canada, the agriculture, forestry, fishing, and hunting heading was obtained from the Statistics Canada website (StatCan, 2023); finally, the agriculture, forestry, fishing, and hunting sector was extracted from the Bureau of Economic Analysis of the U.S. Department of Commerce website (BEA, 2023). The analysis covered the quarterly databases from the January, 2012-December, 2022 period.

These sectors were chosen in view of their comparativeness, although not all countries have divided these sectors in the same way. In addition, the values of the Mexican and Canadian databases were changed to U.S. dollars. The value of the agriculture, animal husbandry and exploitation, forestry, fishing, and hunting sector in Mexico was changed from Mexican pesos to US dollars. A quarterly average of the FIX exchange rate extracted from the Banco de México website (BANXICO, 2023) was used; in the case of Canada, a quarterly average of the official exchange rate of the Bank of Canada (Statcan website, 2023) was used to calculate the exchange rate from Canadian dollars to US dollars. Averages were applied, because the reports for the three sectors are developed on a quarterly basis; likewise, the three sectors were already deflated. Cointegration tests were performed on the three sectors (Table 1).

Table 1. Cointegration tests between the North American countries.

Cointegration tests	Expected results
México-Estados Unidos without a trend	That they are cointegrated
México-Estados Unidos with a trend	
México-Canadá without a trend	
México-Canadá with a trend	
Canadá-Estados Unidos without a trend	
Canadá-Estados Unidos with a trend	

Source: Table developed by the authors.

The six cointegration tests (three with a trend and three without a trend) shown in Table 1 were carried out to determine the existence of a long-term relationship and to establish whether or not the relationship between the Mexican agriculture, animal husbandry and exploitation, forestry, fishing and hunting sector with the agriculture, forestry, fishing, and hunting sector in Canada and the United States —along with the relationship between the same sectors in Canada and USA. The expected result is that the three sectors are co-integrated. These tests are based on the authors mentioned in the theoretical framework, who state that NAFTA and the USMCA/CUSMA have co-integrated the economic sectors of Mexico, Canada, and the United States. For instance, some Mexican produces (such as vegetables) have managed to position themselves in the American market.

Hence, in two of the cointegration tests, the independent variable is the agriculture, forestry, fishing, and hunting sector of the USA and the dependent variable is the agriculture, animal husbandry and exploitation, forestry, fishing, and hunting sector of Mexico. In the other two, the independent variable is the agriculture, forestry, fishing, and hunting sector of Canada and the dependent variable is the agriculture, animal husbandry and exploitation, forestry, fishing, and hunting sector of Mexico. In the two last ones, the independent variable was the agriculture, forestry, fishing, and hunting sector of the United States and the dependent variable was the agriculture, forestry, fishing, and hunting sector of Canada. A bibliographic review supports the following conclusions: that the USA, as the largest of the North American countries, influences the Mexican and Canadian economic sectors; that NAFTA and USMCA/CUSMA have successfully co-integrated the economic sectors of the three countries; and that there is an asymmetry in the size of the economies of the NAFTA and USMCA/CUSMA countries. Therefore, the cointegration tests are free of endogeneity, since the variables are not correlated with the non-observed variables.

According to Gujarati and Porter (2010) and Wooldridge (2010), the initial step of cointegration tests is to determine if the variables meet the non-stationary condition and if they are all in the order of integration one. Therefore, to determine if the variables are non-stationary, a unit root test of original order must first be done for each one. According with the abovementioned authors, there are several unit root tests, including the augmented Dickey-Fuller test (DFA), which has greater statistical properties (*e.g.*, it does not assume that the error term is not correlated) than other tests, including the Dickey-Fuller test. The DFA tests, in original order, were executed using the Eviews software and the methodology described by Gujarati and Porter (2010) and Wooldridge (2010). In total, six DFA tests were performed in their original order, two for each of the three variables (three with a trend and three without a trend). The DFA test in its original order is shown in Equation 1.

$$\Delta Y_t = \beta_1 + \beta_2 t + \delta Y_{t-1} + \sum_{i-1}^{m} \alpha_i \Delta Y_{t-1} + \varepsilon_t \quad (1)$$

Where: ε_t is a pure white noise error term. ΔY_{t-1} =the number of lagged difference terms that are frequently included.

The Durbin-Watson statistic will be examined in its original order, using the first six DFA tests to establish that they have no autocorrelation problems. Therefore, the Durbin-Watson statistic value must be above the critical value (α=5%), with its respective k and n values. The p values of the original order tests were then analyzed. If they were greater than 0.05, the series had a unitary root and it would not be stationary. Meanwhile, if they were lower than 0.05, the series had no unit root and it was therefore stationary. Determining that the three analyzed variables are non-stationary in their original order (with and without trend) meant that the variables were of order of integration one.

For the variables to be in order of integration one, they should be stationary in the first difference. According to the abovementioned authors, a second DFA test must be carried out, including diverse variables (three with a trend and three without a trend). In other words, six DFA tests were performed. The Durbin-Watson statistic is examined once more to establish that there are no autocorrelation problems. The p value of the DFA tests with differences is analyzed next to establish that the variables analyzed are of integration order one. In the first difference, the series must not have a unit root and will therefore be stationary, with a p value lower than 0.05.

If the variables are non-stationary and of integration order one (Table 1), the six integration tests (three with trend and three without trend) would then be performed. The Eviews software will be used for these tests and, following Gujarati and Porter (2010) and Wooldridge (2010), the Augmented Engle-Grenger (EGA) method will be applied. Therefore, six co-integral regressions were performed as shown in Equation 2.

$$Y_i = \beta_1 + \beta_2 X_{1i} + u_i \tag{2}$$

Where: Y_i = one of the sectors of the countries for a given quarter i. β_1= intercept. β_2 = cointegrating parameter. X_{1i} = one of the sectors of the countries for a given quarter i. u_i = estimated residuals from the cointegrating regression. i = year within the study period.

Based on Gujarati and Porter (2010) and Wooldridge (2010), the cointegrated residuals are determined with the six cointegrated regressions. The EGA unit root test was applied to the six cointegranted residues, in order to obtain the Engle-Granger tau statistic and, subsequently, to establish if the residues are stationary and to determine if the variables are cointegrated. If the p value of the Engle-Granger tau statistic is less than 5%, the cointegrated residues have no unit root and, therefore, are stationary. This result would imply that the series are cointegrated in the long term, whereas, if the p value of the Engle-Granger tau statistic is greater than 5%, the cointegraded residues have roots and are not stationary, showing that the series are not cointegrated in the long term.

RESULTS AND DISCUSSION

As indicated in the methodology, the six DFA unit root tests (three without trend and three with trend, in original order) were estimated first for the analyzed variables. The DFA unit root test results are shown in their original order in Table 2.

Table 2. Results of the six DFA unit root tests (with and without trend) of the variables analyzed from the original order.

Variable	P value (DFA test at original order)	P value of the last lag	P value of the trend	Value of the Durbin-Watson statistic with an alpha of 5% and n=42.	Significance point of the Durbin-Watson statistic	Is there a positive serial correlation?
Agriculture, animal husbandry and exploitation, forestry, fishing and hunting sector of Mexico without trend	0.152	0	-	1.904	1.72	No, because 1.90 is greater than 1.72
Agriculture, forestry, fishing, and hunting sector of the USA without tred	0.117	0.094	-	1.933	1.666	No, because 1.933 is greater than 1.666
Agriculture, forestry, fishing, and hunting sector of Canada without tred	0.137	0.966	-	1.999	1.958	No, because 1.999 is greater than 1.958
Agriculture, animal husbandry and exploitation, forestry, fishing and hunting sector of Mexico with trend	0.377	0	0.496	1.924	1.776	No, because 1.924 is greater than 1.776
Agriculture, forestry, fishing, and hunting sector of the USA with tred	0.095	0.591	0.038	1.99	1.776	No, because 1.99 is greater than 1.776
Agriculture, forestry, fishing, and hunting sector of Canada with tred	0.204	0.602	0.183	2.024	2.022	No, because 1.024 is greater than 1.022

Source: Table developed by the authors.

Table 2 shows no evidence of a positive serial correlation, because the value of the Durbin-Watson statistic in all cases is above the significance point (with its respective k and n values). In the six DFA unit root tests (with and without trend, in original order), the p values were always >0.05, indicating that the series had a unit root, with and without trend. In conclusion, variables with and without trend are non-stationary in their original order ($\alpha=5\%$). Table 3 shows the DFA unit root tests, although their initial differences are included to determine the order of integration.

Table 3 shows the DFA unit root tests, with and without trend and with their initial differences, proving the lack of a positive serial correlation. In all cases, the value of the Durbin-Watson statistic is above the significance point (with its respective k and n values). Additionally, all the p values of the DFA unit root tests, with and without trend, with initial differences, are lower than 0.05, proving that the series have no unit root and are stationary. Therefore, all variables are in order of integration one. Table 4 shows the results of the six EGA unit root tests applied to the six residues of the cointegrated regressions (three without trend and three with trend).

In the case of the relationship of Mexico with the US and Canada, the p values of the Engle-Granger tau statistic from the EGA test, applied to the residues of cointegrant regressions, are greater than 0.05 (Table 4). According to Gujarati and Porter (2010) and Wooldridge (2010), variables with an α of 5% have unit roots and, therefore, are not stationary, since they are not cointegrated, with and without a trend. Consequently, they do not have a long-term relationship. Meanwhile, the p values of the Engle-Granger tau statistic from the EGA test for the relationship between Canada and the US, applied to the residues of cointegrant regressions, are lower than 0.05. According to Gujarati and Porter

Table 3. Results of the six DFA unit root tests (with and without trend) of the analyzed variables with first differences.

Variable	P value (DFA test with first differences)	P value of the last lag	P value of the trend	Value of the Durbin-Watson statistic with an alpha of 5% and n=42.	Significance point of the Durbin-Watson statistic	Is there a positive serial correlation?
Agriculture, animal husbandry and exploitation, forestry, fishing and hunting sector of Mexico without trend	0	0	-	2.122	1.666	No, because 2.12 is greater than 1.66
Agriculture, forestry, fishing, and hunting sector of the USA without tred	0	0	-	2.064	1.615	No, because 2.064 is greater than 1.615
Agriculture, forestry, fishing, and hunting sector of Canada without tred	0	0.77	-	2.06	1.835	No, because 1.06 is greater than 1.835
Agriculture, animal husbandry and exploitation, forestry, fishing and hunting sector of Mexico with trend	0	0	0.581	2.139	1.72	No, because 2.139 is greater than 1.72
Agriculture, forestry, fishing, and hunting sector of the USA with tred	0	0	0.815	2.067	1.666	No, because 2.067 is greater than 1.666
Agriculture, forestry, fishing, and hunting sector of Canada with tred	0	0.639	0.409	2.064	1.895	No, because 2.064 is greater than 1.895

Source: Table developed by the authors.

Table 4. Results of the six cointegration tests.

Cointegration test	Independent variable	Constant	Trend	P-value of the Engle-Granger tau statistic	Are they cointegrated?
México-Estados Unidos without trend	0.013	−0.002	-	0.431	No
México-Estados Unidos with trend	−0.102	−0.024	0.001	0.629	No
México-Canadá without trend	0.01	−0.001	-	0.384	No
México-Canadá with trend	0.01	−0.022	0	0.619	No
Canadá-Estados Unidos without trend	3.694	−0.03	-	0	Yes
Canadá-Estados Unidos with trend	3.169	0.068	0.004	0	Yes

Source: Table developed by the authors.

(2010) and Wooldridge (2010), variables with an alpha of 5% do not have unit roots and, therefore, are stationary —*i.e.*, they are cointegrated, with and without trend.

The agriculture, animal husbandry and exploitation, forestry, fishing, and hunting sectors of Mexico were not co-integrated with the agriculture, forestry, fishing, and hunting sectors of the United States and Canada during the analysis period (January, 2012-December, 2022); therefore, the Mexican sector is not influenced and does not have the same long-term behavior (with delays) than the American and Canadian sectors. However, Canadas' agriculture, forestry, fishing, and hunting sectors are co-integrated with their American equivalents —in other words, the Canadian sector is influenced and has the same long-term behavior (with delays) than the US sector.

Consequently, NAFTA and USMCA/CUSMA had not successfully integrated the Mexican agriculture, animal husbandry and exploitation, forestry, fishing, and hunting sectors with the agriculture, forestry, fishing, and hunting sectors of the United States and Canada during the analysis period (January, 2012-December, 2022). Therefore, trade agreements have not achieved their objective for these sectors (Puyana, 2020; Puchet *et al.*, 2014; Anguiano and Ruiz, 2022; Nava, 2021; Infante and López, 2019).

The results match the conclusions of Pérez (2019), who points out that not all economic sectors are co-integrated, since co-integration can vary from sector to sector. Meanwhile, Anguiano and Ruiz (2022) established that the cointegration of economic sectors is not always achieved. For their part, González (2017), Jaime *et al.*, (2015) and Chávez *et al.*, (2019) agreed that some products from the Mexican agricultural sector (such as fruits and vegetables) are co-integrated with the USA, because Mexico exports a large percentage of its production to that country. However, these results have not been enough for the co-integration of this Mexican sector. The lack of cointegration of Mexico's agricultural sector can be attributed to its structural lags —*i.e.*, the lack of technology and the absence of long-term strategies that have hindered its productivity— as well its disadvantage with regard to other competitors, such as the USA —resulting from the subsidies that the American government has invested on its farmers, while in Mexico such supports have decreased (Infante and López, 2019; Chávez *et al.*, 2019; Infante *et al.*, 2021).

The objective of this research was to determine if the Mexican, Canadian, and American agricultural industries are co-integrated. For this purpose, six cointegration tests were applied to the agricultural sectors of the three countries. In the case of Mexico, the agriculture, animal husbandry and exploitation, forestry, fishing, and hunting heading was evaluated; in the case of Canada and the United States, the agriculture, forestry, fishing, and hunting heading was used. The analysis period ranged from January, 2012 to December, 2022. The results showed that the Mexican sector, with an α of 5% (with and without trend), is not co-integrated with the USA and Canada, whereas Canada and the USA, with an alpha of 5% (with and without trend), are co-integrated. Therefore, the agriculture, animal husbandry and exploitation, forestry, fishing, and hunting sector in Mexico was not co-integrated with the agriculture, forestry, fishing, and hunting sectors of the United States and Canada from January 2012 to december 2022.

CONCLUSIONS

The Mexican sector is not influenced and does not have the same long-term behavior (with delays) as the equivalent sectors in the US and Canada. The Canadian agricultural, forestry, fishing and hunting sector is cointegrated with its American counterpart; That is, the Canadian sector is influenced and has the same long-term behavior (with delays) as the US sector. Although some Mexican agricultural products have entered the United States and Canada, it has not been enough. This situation opens areas of improvement for the export of more products from Mexico to the United States and Canada. NAFTA and the USMCA/CUSMA have not cointegrated the Mexican, Canadian and US sectors.

Mechanisms, such as avoiding subsidies, should be explored and proposed to achieve the objectives of these treaties. Possible lines of research could include the disaggregation of the sectors and the application of the Engle and Granger causality test, as well as the short-term adjustment mechanism (in the case of Canada and the United States).

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Colegio de Postgraduados

Technological status of prickly pear (*Opuntia ficus-indica* Mill.) fermentation to increase the protein value of its fodder

Cortés-Chamorro, Marco A.[1]; **Rössel Kipping, Erich D.**[1]; **Loera Alvarado, Gerardo**[1*]; **Calzada Lara, Gabriel**[1]; **Ortiz Laurel, Hipólito**[2]; **Durán-García, Héctor M.**[3]; **Morales Flores, Francisco J.**[1]

[1] Colegio de Postgraduados, Campus San Luis Potosí, Iturbide 73, Salinas de Hidalgo, San Luis Potosí. México, C. P. 78620.
[2] Colegio de Postgraduados - Campus Córdoba. Amatlán de los Reyes, Veracruz, México, C. P. 94946.
[3] Universidad Autónoma de San Luís Potosí, Álvaro Obregón #64, Col. Centro, San Luis Potosí, San Luis Potosí, México, C. P. 78000.
* Correspondence: gerardo.loera@colpos.mx

ABSTRACT

Objective: To analyze the state-of-the-art technology and the application of the fermentation process, in order to increase the protein value of prickly pear (*Opuntia ficus-indica*), as a source of fodder, in three technological levels.

Design/Methodology/Approach: The technological parameters and mechanization level of three fermenters —employed to increase the protein value of prickly pear fodder— were characterized. The conceptual technical analysis was determined through quantitative and qualitative indicators, based on mathematical expressions and equations.

Results: Quantitative indicators showed a fermentation technology with limited efficiency, which provides an opportunity for technical improvements.

Findings/Conclusions: The application of fermentation technology results in significant savings in water, soil, and fertilizer resources. Furthermore, it provides an alternative for fodder production and food security for livestock in the semi-arid region of Mexico.

Keywords: Fermenters, technological parameters, *Opuntia* spp.

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INTRODUCTION

The scarcity of rainfall during the year limits fodder production in northern Mexico (Murillo *et al.* 2012). Therefore, supplementing diets with fodders and commercial concentrate feeds is fundamental to meet the animals' protein and energy requirements. Adding concentrates to animal feed increases food costs by 40%; consequently, producers must develop technological alternatives to produce fodder (Herrera *et al.*, 2014), such as the use of prickly pear (*Opuntia ficus-indica*) biomass (Gutiérrez *et al.*, 2009).

Image by Ave Aventin at Pixabay

The scarcity of fodder and protein sources led to the development of a technology that fortifies the protein content of prickly pear. This technology is based on the semisolid fermentation of prickly pear through the application of *Saccharomyces cerevisiae* yeast (1%). Additionally, an external nitrogen source (made up of 1% of urea and 0.1% of ammonium sulfate) was used. The aerobic condition, the temperature (25±3 °C), and acid pH (4.35) were maintained for 8-10 h. The fermentation was subjected to 45-minute mixing, with 30-minute breaks (Flores *et al.*, 2011; Flores *et al.*, 2014).

The implementation of fermentation technology to enhance the nutritional value of solid or semi-solid prickly pear —extracting biomass from the existing carbohydrates (Peláez *et al.*, 2011)— was carried out using fermenters with different mechanization levels. However, the technological parameters of this equipment have not been analyzed. According to Rebollar-Rebollar *et al.* (2011), a low technological and mechanization level in agroindustry results in poor quality products.

Technological parameters such as grinding, cutting power, and particle size are crucial for the fermentation process of green waste, because the biochemical changes involved can impact the process (Pinos *et al.*, 2008). According to Fuentes-Rodríguez (1997), 1-3 cm particles facilitate air elimination during mixing, while Durán-García *et al.* (2012) determined that the cutting and working tools consume most of the energy during the development of technological processes. Therefore, the state-of-the-art technology and technique implemented in the fermentation process —aimed to increase the protein value of prickly pear fodder— were evaluated at three technological levels.

MATERIALS AND METHODS

A technical assessment was carried out to determine the consumption of resources (fertilizer, water, and land) used to produce animal feed proteins, through the cultivation of alfalfa (*Medicago sativa*). Subsequently, this process was compared with the implementation of the prickly pear fermentation process. In addition, a technical analysis was carried out to evaluate the technological parameters of the fermentation process, in three mechanization levels, currently used in the Potosino-Zacatecano plateau (Table 1).

The fermentation technology was characterized through field visits to the locations where the technologies were developed. In addition, an experience exchange with developers and users of the technology was conducted. Measurements, physical comparisons, and

Table 1. Technological levels applied to prickly pear fermentation.

Technology level 1 (experimental)	Technology level 2 (applied)	Technology level 3 (experimental)
This technology was developed by the Central North Regional Universitary Center of the Chapingo Autonomous University (CRUCEN), it is an experimental desing and it is aimed at small producers and to carry out field demonstrations and training.	This technology was developed and adapted to the producer needs, to generate cactus forage with high protein content for intensive feeding of sheep, this technology is considered as a success case.	This technology was developed by the Arid Zones Regional Universitary Unit (URUZA) of the Autonomous University of Chapingo and others collaborators, is an experimental desing for small and medium-sized producers. This technology is in the patent process

calculations of some static and dynamic mechanical elements and tools —used to design and construct the fermentation process equipment— were carried out. This process analyzed the parameters and obtained the quantitative indicators required to determine the mechanization index of the fermentation process technological chain. A flexometer and an Extech 461750 contact tachometer were used to measure the structural elements and to quantify the cutting speed during the prickly pear grinding process, respectively.

Technical evaluation of parameters

The mechanization index was calculated using equation 1, which is based on the principle of incorporating machinery, equipment, and tools into the production systems and processes of agroindustry operations, in order to achieve greater technical, economic, and environmental efficiency, increasing production without degrading natural resources (Aristizábal and Cortés, 2012).

$$nt = HPha^{-1} \tag{1}$$

where: *nt* is the number of tractors and HP is the power (Watts).

The efficiency of motors, tools, and mechanisms integrated in a power system is defined as the capacity of the motor to transform electric power into mechanical energy. The electric power and mechanical energy output of a shaft is determined in watts or kilowatts (W, kW) (Quispe, 2002). In this study, equation 2 was used to determine the output.

$$\%EF = \frac{Mechanical\ output\ power}{Electrical\ input\ power} \times 100 \tag{2}$$

where: *EF* is the efficiency.

The tangential speed was determined based on the characteristics of the cutting systems, using equation 3.

$$ts = \omega * r \tag{3}$$

where: *ts* is the tangential speed $m*s^{-1}$; ω is the angular speed ($Rad*s^{-1}$); *r* is the radius of the cutting shaft (mm).

The grinding torque was determined based on equation 4.

$$T = \frac{P(63000)}{S} \tag{4}$$

where: *T* is the torque (Nm); *P* is the required power (HP); and *S* is the rotation speed (rpm).

The electric power of the motor is defined based on the equation 5.

$$Ep = \frac{M_p}{n} \tag{5}$$

where: Ep is the electric power consumed (Watt); M_p is the mechanical power on the shaft (Watt); and n is the efficiency of the motor.

Martínez-Rodríguez, Valdés-Hernández, Diaz-Suárez, and Maturrell Padín (2004) have described the grinding process. They pointed out that its suction and cutting power smooths the flow of material and prevents blockages in the grinding machine. Cutting power was determined with equation 6.

$$Cp = Cf * \omega_r * r \tag{6}$$

where: Cp is the cutting power (HP, CV, Watts); Cf is the cutting force (N); ω_r is the angular speed of the rotor (rad/s); and r is the radius of the rotor (mm).

The system or cutting tool was calculated with equation 7.

$$b = 2\frac{\pi S}{n\omega} \tag{7}$$

where: b is the length of the tooth or blade; S is the feed speed; n is the number of blades; and ω is the angular speed.

Finally, the transmission ratio index was calculated with equation 8.

$$i = \frac{Nm}{n_c} \tag{8}$$

where: i is the gear ratio index; Nm is the drive shaft rotational speed (rpm); and n_c is the lay shaft speed (rpm).

RESULTS AND DISCUSSION

Technical resource evaluation

The application of fermentation technology has enabled the enhancement of crude protein content, consequently consuming less water, soil, and fertilizer resources than traditional fodders, such as alfalfa (Flores-Hernández, 2019). Since a 67 t ha^{-1} alfalfa average yield and a 160 t ha^{-1} prickly pear average yield are obtained with a 180 cm and 60 cm irrigation depth, respectively, the production of 1.0 kg of prickly pear dry matter will require 52% less water than the same amount of alfalfa dry matter (Table 2).

Table 2. Resources required for 1.0 kg of dry matter (MS).

Species	Water consumption $\left(L\,kg_{MS}^{-1}\right)$	Ground surface $\left(m^2\,kg_{MS}^{-1}\right)$	Protein $\left(g\,kg_{MS}^{-1}\right)$	Fertilizer $\left(g\,kg_{MS}^{-1}\right)$
Prickly pear	480.15	0.80	330	***
Lucerne	916.98	0.49	260	9.91

Technical characterization of the implementation of fermentation technology

The cutting method (grinding) has been incorporated directly into the experimental fermentation equipment. In the case of the applied technology, this operation works better with the direct connection to the power take-off of a tractor, obtaining a higher cutting power (64 W) and a larger cutting tool (15 mm). In all cases, a 2-3.5 cm particle size was obtained, applying a 1,000-1,500-rpm cutting speed (Table 3).

Following grinding, the plant material was transferred to the fermenter for further processing. The volume of the experimental formers ranged from 0.2 to 0.3 m^3. Meanwhile, in the implemented technology, volume should be increased up to 1.2 m^3, which requires greater mixer speed, torque, and motor power (Table 4).

Development of the new fermentation technology

The technology analysis indicated that the parameters can be optimized through the advancement of technology and the improved design and redesign of the elements

Table 3. Analysis of parameters applied to prickly pear grinding.

Technical parameters	Technology level 1 (experimental)	Technology level 2 (applied)	Technology level 3 (experimental)
Windmill	Its structure is made with 12 gauge galvanized sheet structure, 1/8 inch iron flat bar and 1 inch iron angle, and it is mounted to fermenter chassis	It uses a Hammer mill No 16 coupled to the tractor's "PTO" through a mechanical transition.	It is made of stainless steel and the mill is integrated into the fermenter. It is fed manually and unloaded by gravity.
Cutting tool	76.2 mm diameter shaft with serrated steel blades	Oscillating hammer system, the nopal is cut by impact	The system has a vertical axis and a horizontal cutting jagged disc.
Cutting power (W)	2	64	2
Cutting tool length (mm)	7.97	15	13
Particle size (cm)	3	3.5	2
cutting speed (rpm)	1500	1000	1500
Moment of torque (Nm)	9.49	843.6	10.9
Transmission type (windmill)	Mechanical transmission with pulley system and "V" belts	Mechanical transmission tractor power take-off shaft (PTO)	Mechanical transmission with pulley system
Ratio index transmission	1.87	1.1	1.6

Table 4. Technical analysis of the fermenters.

Technical parameters	Technology level 1 (experimental)	Technology level 2 (applied)	Technology level 3 (experimental)
Fermenter	Experimental equipment with an integral design, with a plastic hopper, the grinding falls by gravity since the mill placed at the top, its transmission is mechanical with a system of pulleys and bands.	The equipment was adapted to the production needs, the cactus is loaded to the fermeter through a conveyor belt and unloaded by gravity and worm screw. It uses a mechanical transmission with speed variation trough motor-reducer, chain and sprocket.	It has an integral design (structure, tank and mill), and is made of stainless steel. The mill is on the top of the fermenter. The loading and unloading of material are by gravity. It has a mechanical transmission with pulleys.
Fermenter volume (m^3)	0.2	1.2	0.3
Design (mixer)	Stirrer with galvanized blades integrated into a drive shaft	Double helical agitator action.	Simple stainless steel spiral stirrer.
Transmission of fermenter	Mechanics (pulley and V-belt)	Mechanics (chain and sprockets) and 2 output motor-reducer	Mechanics (pulley and V-belt)
Mixer speed (m/s)	0.119	1.9	0.119
Mixer torque (Nm)	316.3	711.8	316.3
Motor power (KW)	1.492	3.73	1.492
Fermentation Time (h)	8	6	10
Mixing intervals	45 min. of mixing and 30 min. rest	30 min of mixing and 30 min of rest	45 min. of mixing and 30 min. rest
Start System	Electronic system (timer)	Electronic system (timer)	Electronic system (timer)
Equipment cost fermenter	$25,000.00	$120,000.00	$250,000.00

of the fermentation process, improving the efficiency of the mechanization chain. This aim can be achieved through the integration of 4.0 generation technological tools and systems, as well as automation and digital systems. Based on the technology analysis, the technological development can include conveying systems, the optimization of parameters in the grinding equipment, and more efficient cutting tools that guarantee a tailored particle size structure and cut. Furthermore, redesigning the fermenting equipment is key to facilitate a large-scale production of fodder to feed larger cattle herds. In order to achieve a larger high-protein fodder production, improving the technical conditions of each parameter —through the implementation of technological innovations in the process, equipment, and/or integral systems— is fundamental.

CONCLUSIONS

The fermentation technology of fresh prickly pear residues is an effective alternative to increase the protein content and, by extent, the nutritional value of this species. This technology can contribute to the conservation of resources, such as water, fertilizers, and land. Although it has already been adopted by producers, fermenters can still be improved and optimized, through automation and digitalization, and integrated into Agriculture 4.0.

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Colegio de Postgraduados

Study of textural properties Frankfurter's sausages modified with candelilla wax oleogel

Avendaño-Vásquez Gilda[2], Chávez-Alfaro Osiel M.[1], Vargas-García Karen A.[1], Varela-Santos Elizabeth del C.[1*], Bustos-Ramírez Karina[1]

[1] TecNM /ITS de Tierra Blanca, Tierra Blanca, Veracruz, México. C.P. 95180 .
[2] CONAHCyT-TecNM/ITS de Tierra Blanca-Maestría en Ciencias de los Alimentos y Biotecnología Estancia posdoctoral, Tierra Blanca, Veracruz, México. C.P. 95180.
* Correspondence: e.varela@itstb.edu.mx

ABSTRACT

Objective: to evaluate the characteristics of Frankfurt-type sausages formulated with soybean and canola oleogel structured with candelilla wax (CW).

Design/methodology/approach: evaluated the physicochemical properties and textural attributes through storage time (1 and 21 days) after a partial or total substitution of the fat phase in the meat emulsion (50% and 100%) in two comercial edible oil [soybean oil (SO) and canola oil (CO)].

Results: the physicochemical characterization showed that retained higher moisture as the animal fat was displaced in the formulation, being 100% SO and 100% CO (43.46% and 40.64% respectively) those that conserved higher humidity, as well as fat and protein values. Additionally, the samples responded incrementally in 50% SO and 100% SO than in CO, after storage this in hardness, elasticity, cohesiveness, stickiness and chewiness profiles. Besides 100% SO and 100% CO were the ones that developed the greater hardness over time.

Limitations on study/implications: limitations in the manufacture of sausages, which implied a longer maturation time of the meat matrix in order to obtain a stable emulsion.

Findings/conclusions: these results are promising to establish processing parameters in order to design an attractive final product for the consumer, helping to reduce the consumption of saturated fats and trans fats.

Keywords: Oleogels, Vegetable wax, edible oil, Frankfurt Sausages, Saturated fat.

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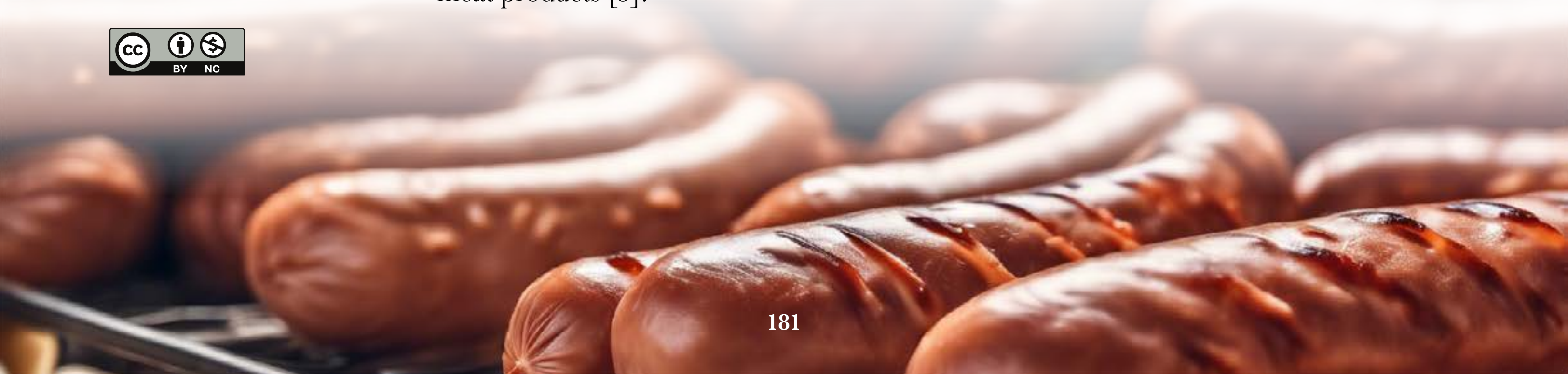
INTRODUCTION

The saturated fats provide functional characteristics in formulation food, however there is evidence of consuming fatty acid saturated are causing of metabolic diseases such as insulin resistance, diabetes, weight gain, adiposity and coronary heart disease [1,3]. This is added to the fact that to produced is necessary to consume or transform inputs as assets and services for example food, medicines, vaccines, direct labor and indirect production costs [4], that increase the cost of production of any meat products, although, animal fat, provide taste and textural properties in meat products, however saturated fatty acids and cholesterol in animal fats are some of the reasons why consumers have avoided meat and meat products [5].



Recently as a strategy to reduce the consumption of saturated fats is increasing the intake of mono and polyunsaturated fatty acid present in edible oils offer advantages such as lower bad cholesterol, as well as reduce the level of triglycerides and control the blood sugar [4,6]. Currently the effort to obtain structured materials without saturated fat or animal fat use, will be translated into oleogels implementation, this strategy provided solid systems, increasing melting point, hardness and chewiness in food and emulsifier food products [7,8]. The oleogels are semisolid system with a continuous phase made of a hydrophobic liquid (vegetable oil), where a self-assembled network, formed by a structuring agent, is responsible for the physical entrapment of the liquid [9], that could provide nutritional benefits and organoleptic attributes, it is required a gelling molecules that can structure the edible oils that contributed to non-covalent interactions such as candelilla wax. Candelilla wax (CW) is obtained from the leaves of a small shrub native to northern Mexico and the southwestern United States, *Euphorbia cerifera* and *Euphorbia antisyphilitica*, from the family Euphorbiaceae. CW is a globally recognized food additive approved by the FDA, can be used as a substitute for carnauba wax and beeswax in different food systems. Reports on the general CW composition indicates the presence of 49-50% n-alkanes with 29 to 33 carbons, 20-9% esters of acids and alcohols with even-numbered carbon chains (C28 to C34), 12-14% alcohols and sterols, and 7-9% free acids. These characteristics are to which its gelling capacity is attributed, in addition to being a great promise to create fat-like materials, using concentrations ranging from 1-4% (w/w) [10]. The study of new alternatives that promote the consumption of polyunsaturated fatty acids is undoubtedly an important advance in this type of materials, after the implementation of cheap and food-grade materials. The objective of this work is to characterize the impact of the partial or total substitution of saturated fat by soybean and canola oleogels structured with candelilla wax (CW) on the production of frankfurter type sausages.

MATERIALS Y METHODS

Oleogel Preparation

The oleogels were made by heating canola oil (Aceites, Grasas y Derivados, SA, Guadalajara - Jalisco, Mexico) and 2.5% CW (p/p) (Alkento Ingredientes, Monterrey - N. L., México), on a grill heating with constant stirring at 100 °C, for a period of 20 min, stored for at least five days at 4 °C covered with aluminum foil, to promote complete crystallization of the resulting oleogel, as previously reported (Wolfer *et al.*, 2018).

Preparation of the Frankfurter type sausage

The pork leg muscle (the initial chemical composition of the meat was 70% moisture, 1% ash, 6.6% fat and 20.3% protein, determined on a dry base) was obtained from the Frigorifico de la Cuenca del Papaloapan SA de CV (Tierra Blanca-Veracruz, Mexico), removing the superficial fat, connective tissue and subcutaneous skin for subsequent grinding in a mill, MGB-120 with 280 W of power (Zimtown, Dayton-NJ, USA), and packed in hermetic bags of 1 kg each, frozen for processing. As stated [11], formulations substituting 50 and 100% of the fat phase were realized with help of a food processor

Cuisinard Model CFP-800 (Corporación Milenium, S. de R.L. de C.V. ("Conair Mexico"), processing the meat with the condiments, adding half of the crushed ice and half of the fat phase, to add the other half afterwards leaving ∼3 min of time of processing between proportions and denominating them as canola (CO or soy (SO) according to the nature of the oil. Once the emulsion was obtained, it was covered by sticky paper and stored to 4 °C for a 24 h period before the stuffing process. The sausages were made in synthetic casing, to then have a blanching process in hot water of 70-75 °C, for a period of 15 min, after which it is cooled down to 4 °C for 24 h before the vacuum packing process. The already packed samples are stored refrigerated until their subsequent analysis.

Proximal chemical analysis

They were determined on days 1 and 21 after their elaboration, the determination of proteins was carried out by the modified Biuret method with a pre-digestion of the sample in a 50/50 mixture of 10% NaOH and distilled water, after which the sample was homogenized and centrifuged (Centrificient IV, CRM Globe Int, CDMX, Mexico) for 20 min at 4000 rpm. After separation, the supernatant is decanted and is brought to a 1:10 solution for its preparation according to the method. Additionally, NMX-F-083 was used for moisture determination in food products, NMX-F-066-S-1978 for ash determination and NMX-F-089-S for determination of ethereal extract in food. All samples were analyzed in duplicate.

Mechanical evaluation

TPA tests are a well-established method for textural analysis. For these tests, 2.0 cm long samples were placed in a texture analyzer (TA. XTPlus, Stable Micro Systems, UK) at room temperature (T=22 °C). The texture parameters were obtained by means of double compression in order to simulate human chewing at 50% deformation and a speed of 2 mm/s with a 100 mm diameter stainless steel compression plate, 10 replicates were evaluated of each formulation to carry out the tests, where the force/time curve was obtained. The average of their measurements is reported.

Statistical analysis

The experiments were randomized, and the evaluations were taken 1 and 21 days after their preparation. For the statistical analysis, the Statistica V10 software (StatSoft, Inc. (2011) was used. Performing a one-line ANOVA with a 95% confidence level, the graphs indicate the standard error.

RESULTS AND DISSCUSION

The physicochemical characterization of the samples from day 1 are expressed in Table 1, where the effect on the humidity parameter is observed with respect to the formulation, with the 50% CO sample being the one with the lowest humidity, and 100% SO. the one that presented the highest humidity of all the samples analyzed, this probably due to the greater structural affinity of the emulsion, when 50% of the pork fat is replaced than when 100% fat is replaced, which could influence the results texture parameters.

Table 1. Means for effect of treatment on moisture, lipid, protein and ashes.

Treatment	Humidity	Ashes	Protein	Lipid
50% CO	37.48 [a]	2.82 [a]	14.43 [a]	26.42 [a]
50% SO	40.48 [b]	2.49 [b]	21.55 [b]	28.98 [a]
100% CO	40.64 [b]	2.78 [a]	23.60 [c]	39.97 [b]
100% SO	43.46 [c]	2.56 [c]	18.05 [d]	43.25 [b]
S. E. M	0.220	0.033	0.285	1.086

S.E.M.: standard error of mean
Mean values within a column with different letters are significantly different ($P \leq 0.05$).

Additionally, and according to the NMX-F-065-1984 standard, said products should not exceed the limit of 30% added fat, which is complied with according to the characterization by observing that the meat presented 6.6±0.43% fat since in the samples of 50% CO and 50% SO they presented values of 26.42% and 28.98% respectively, however, a considerable increase was observed in 100% SO and 100% CO, this due to the mathematical impossibility of obtaining the same moisture and fat content due to the use of lipid sources of different composition [12], coupled with the effect of the composition of the wax, since being mainly composed of alkanes, these are more similar to the polarity of the extraction solvent, so there is a greater dragging capacity, increasing the value of the ethereal extract at a higher concentration of oleogel. In the sausage, which in turn can explain the moisture content since in products with a higher fat-protein ratio moisture is lower (50% SO and 50% CO), on the contrary in products where it was present had higher fat content with a lower proportion of protein the moisture is higher (100% SO and 100% CO), due to the hydrophobic nature of the fat [13], which would directly impact the shelf life, as well as the texture of the products.

Effect of fat replacement on the textural properties of sausage

In the analysis of texture (Table 2), showed the effect of storage time in the sausage's hardness, observing in every case an increase in this parameter, which suggests a good balance between the forces that contribute to hardness, product of the interaction between canola oil oleogel and soybean oil-candelilla wax and protein. It is observed that sausages formulated with 50% of the animal fat replacement obtained less hardness depending on the type oil, in addition the sausages with higher hardness contained 100% of oleogel in both oils., being the formulation of oleogel of soybean oil-candelilla wax resulting in higher hardness of all the segment that was consistent to the 21 days of storage which is demonstrated in other studies [12, 28, 29] to replace from a 20% of animal fat by vegetable oil or oleogels, which are attributable to the specific properties of each oil, incorporation systems used and differences in product formulation, processing, composition and the proportion of animal fat replaced by the oleogel system [17], as well as the formation of small fat globules that shown to have greater resistance to compression [18].

Furthermore, the sausages not presented differences in adhesiveness, cohesiveness and elasticity and resilience, indicating that there is no effect of the type oil, concentration of oleogel and storage time on these attributes, the same reported by other authors [15, 32].

Table 2. Effect of fat replacement of texture profile analysis parameters of Frankfurt sausage containing oleogel bean soy oil (SO) and oleogel canola oil (CO).

Treatment	Hardness (g)	Adhesiveness (g*s)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilence
Day 1							
50%SO	1009.24[c]	−32.78[d]	0.77[b]	0.55[b]	566.78[c]	436.92[d]	0.26[b]
50% CO	1124.36[c]	−15.14[b]	0.78[b]	0.59[b]	675.07[b]	531.69[c]	0.28[b]
100% CO	1586.15[b]	−9.48[a]	0.84[a]	0.69[a]	1085.76[a]	914.31[b]	0.33[a]
100%SO	1702.23[a]	−24.99[c]	0.86[a]	0.69[a]	1199.31[a]	1029.41[a]	0.33[a]
S. E. M	56.35	12.55	0.01	0.01	24.91	20.54	0.01
Day 21							
50%SO	1799.97[b]	−29.05[b]	0.80[b]	0.57[b]	1035.70[c]	825.73[c]	0.27[b]
50% CO	1522.15[c]	−19.27[a]	0.80[b]	0.59[b]	900.62[d]	721.57[d]	0.28[b]
100% CO	1827.00[b]	−28.75[b]	0.85[a]	0.67[a]	1222.93[b]	1041.86[b]	0.32[a]
100%SO	2435.04[a]	−26.28[b]	0.86[a]	0.65[a]	1587.90[a]	1364.82[a]	0.30[a]
S. E. M	40.38	6.15	0.01	0.01	33.33	36.32	0.01

S.E.M.: standard error of mean.
Mean values within a column with different letters are significantly different ($P \leq 0.05$)

However, gumminess and chewiness are affected by time and concentration, especially in soybean oil, observing a greater effect in the substitution of 100% SO with candelilla wax that the 100% CO, since when the proportion of saturated fat replaced is increased, it causes an increase in the chewiness referred to the energy required to chew a solid and disintegrate it until it can be swallowed as a product of hardness*elasticity*cohesiveness, therefore, by decreasing the proportion of oleogel in the meat emulsion, softer products can be generated and therefore with less chewiness, as well as the composition of the oil itself due to the fact that a greater number of unsaturation's present impacts the hardness of the organogels, as well as of the formulated products [21], which could allow the design of products with the desired composition and texture profile.

CONCLUSION

The potential of oleogels to provide an alternative to saturated fat (animal fat) in meat products without high production costs, contributing to enrichment in polyunsaturated fatty acids using edible vegetable oils, which would imply that the consumption of this type of products could prevent the development of chronic degenerative diseases. Soybean oil oleogels had a greater impact on the textural properties of the formulated sausage than canola oil, which would allow the design of the final product with the desired nutritional characteristics. The use of candelilla wax as a structuring agent for oleogels in meat matrices resulted in an economical and food grade alternative to replace animal fat present in formulated sausages, providing better opportunities in the development of meat products, showing physicochemical and textural characteristics similar to the products available in the market, same that were modified by refrigerated storage, however evaluating acceptability of the final product is necessary to evaluate the sensory characteristics of products obtained therefore being able to modify parameters in formulation.

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