

AGRO PRODUCTIVIDAD

Diversity of by-products used in
fish diets
in the aquaculture industry:
a systematic review

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Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.

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From fermentation to function: The hidden antioxidants inside fermented milks

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ABSTRACT

Objective: To perform a literature review on antioxidant compounds present in fermented milks, highlighting their potential role in mitigating oxidative stress.

Design/methodology/approach: This review analyzes scientific literature addressing the antioxidant components of fermented milks, including native milk constituents, microbial metabolites, and bio-transformed compounds generated during fermentation.

Results: Fermented milks contain a wide spectrum of antioxidant compounds arising from milk components, microbial metabolism, and LAB-mediated bio-transformations. These compounds collectively contribute to scavenging free radicals and reducing reactive oxygen species (ROS).

Limitations on study/implications: Further *in vivo* studies and clinical evidence are needed to substantiate health claims and clarify dose-response relationships.

Findings/conclusions: The available evidence supports fermented milks as promising functional foods with substantial antioxidant potential. Their regular consumption may contribute to protection against oxidative stress-related damage and associated chronic degenerative diseases, positioning fermented milks as a potential first line of dietary defense.

Keywords: Fermented milks; antioxidants; lactic acid bacteria; probiotics; oxidative stress; functional foods.

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INTRODUCTION

Fermentation is the oldest biotechnological method used to extend the shelf life of milk and has been practiced for myriads of years across diverse cultures (Hill *et al.*, 2017). Nowadays, fermented milks are consumed worldwide and are very popular products because of their distinctive sensory properties, with more than 400 names used to describe traditional and commercial varieties (Yerlikaya, 2023). Beyond their cultural and gastronomic importance, these fermented foods have played a significant role in human nutrition, serving as sources of energy and nutrients



and being associated with health-promoting effects since medieval times (Hill *et al.*, 2017; Yerlikaya, 2023).

Early production of fermented milk relied on spontaneous fermentation by native microflora present in raw milk. In contrast, present methods use selected starter cultures, allowing control over fermentation parameters and consistent product quality. These starter cultures contribute to food safety and influence sensory, technological, nutritional, and health-related properties of the final product (Bintsis & Papademas, 2022).

At biochemical level, lactic acid bacteria (LAB) play multiple essential roles in fermented milks, contributing to both product quality and nutritional value. They are primarily responsible for the production of lactic acid and various antimicrobial compounds, which enhance preservation and microbiological safety (Sharma *et al.*, 2023; Yerlikaya, 2023). In addition, LAB synthesize key flavor compounds, such as acetaldehyde, that are characteristic of fermented dairy products. These microorganisms also produce exopolysaccharides, which improve texture and mouthfeel. In addition, fermentation promotes changes in lipid fractions, mineral bioavailability, and the stability and synthesis of vitamins. Collectively, these activities lead to an overall improvement of the nutritional value of fermented milks (Anumudu *et al.*, 2024).

The health benefits of fermented milks arise from the biologically active components naturally present in milk, the metabolites produced and those biotransformed by LAB during fermentation, resulting in a biogenic effect that enhances the functional value of these products (Sharma *et al.*, 2023). During fermentation, microorganisms drive complex biochemical transformations, including lactose fermentation and proteolysis of milk proteins, leading to the release of peptides, free amino acids, and other low-molecular-weight compounds with potential biological activity. In addition to generating beneficial compounds, certain LAB strains (probiotics) exert direct effects on the host, including improved lactose digestion, modulation of immune responses, maintenance of gut microbiota balance, reduction of serum cholesterol levels, among others (Gao *et al.*, 2025; Kaur *et al.*, 2022). More recently, several LAB strains have also been shown to possess intrinsic bioactive properties, further expanding their significance as functional microorganisms and reinforcing the potential of fermented milks as health-promoting foods (Bryukhanov *et al.*, 2022).

The scientific interest in the health effects of fermented milk dates back to the early 20th century, when Metchnikoff proposed that the consumption of yogurt containing LAB could positively influence gut microbiota composition, contributing to improved intestinal health and longevity (Bintsis & Papademas, 2022). These early hypotheses placed the foundation for contemporary research exploring the biochemical and physiological mechanisms through which fermented milks exert health-promoting effects, including their antioxidant benefits.

On the other hand, oxidative stress is recognized as a key factor in the development of various diet-related chronic diseases, underlining the importance of dietary antioxidant as a key nutritional property of foods (Fardet & Rock, 2018). In this sense, it has been reported that fermented milks exhibited antioxidant activity, which is attributed to multiple biomolecules, including the release of antioxidant peptides during protein hydrolysis and the

formation of microbial metabolites with redox activity (*e.g.* vitamins, exopolysaccharides). These antioxidant properties not only add functional value to fermented dairy products but also position them as promising dietary components for promoting overall health.

Antioxidants and Their Relevance in Fermented Milks

Antioxidants are molecules capable of preventing or reducing oxidative damage caused by reactive oxygen species (ROS). Epidemiological evidence suggest that an adequate consumption of dietary antioxidants may contribute to the prevention of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Muscolo *et al.*, 2024). ROS can react with lipids, proteins, and DNA, generating cellular damage that contributes to disease progression; however, it is important to note that certain reactive species also play essential physiological roles in cell signaling and immune responses (Sies *et al.*, 2022).

Diet plays a crucial role in modulating oxidative stress and maintaining redox homeostasis (Zujko & Witkowska, 2023). In this sense, antioxidant-rich foods, including fermented milks, as key components of a health-promoting diet. Beyond supplying essential nutrients, dietary patterns influence the balance between pro-oxidant and antioxidant processes, thereby affecting the capacity of biological systems to counteract free radical generation. Fermented milks, in particular, have attracted growing attention because their fermentation process enhances the bioavailability and bioactivity of antioxidant compounds naturally present in milk. Lactic acid bacteria not only preserve nutritional quality but can release bioactive peptides, synthesize antioxidant metabolites, and modulate host antioxidant defenses. As a result, the regular consumption of fermented dairy products may contribute to mitigating oxidative damage, supporting immune function, and promoting overall metabolic resilience, thereby reinforcing their potential role in the prevention of oxidative stress-related disorders and in the promotion of overall health (Stobiecka *et al.*, 2022).

Native Milk Constituents as Sources of Antioxidants

Milk and dairy products naturally contain a diverse array of antioxidant systems that contribute to their capacity to counteract oxidative stress. Among these, enzymatic antioxidants including superoxide dismutase (SOD), catalase, and glutathione peroxidase, which play an important role in protecting against oxidative damage. Although the activity of these enzymes may be partially reduced during processing, a fraction remains functional in the final products (Khan *et al.*, 2019; Stobiecka *et al.*, 2022). Moreover, during fermentation, their antioxidant capacity may be complemented or enhanced by microbial enzymes produced by lactic acid bacteria, thereby contributing to the overall antioxidant potential of fermented dairy products (Khan *et al.*, 2019).

Beyond enzymatic defenses, milk and dairy products contain a wide range of non-enzymatic antioxidants that contribute significantly to their overall antioxidant capacity. Fat-soluble vitamins such as vitamins A and E play a crucial role in protecting milk lipids from peroxidation, while water-soluble vitamin C contributes to the scavenging of aqueous-phase free radicals and the regeneration of other antioxidants. In addition, carotenoids present in milk fat exhibit potent antioxidant activity and may also contribute

to anti-inflammatory effects. Conjugated linoleic acid (CLA), a bioactive lipid found in dairy fat, has been reported to exert antioxidant effects through modulation of oxidative pathways and lipid metabolism (Khan *et al.*, 2019; Zaitsev, 2025). Reduced glutathione (GSH) represents another key component of the milk antioxidant system, acting both as a direct scavenger of reactive species and as a substrate for glutathione peroxidase, thereby maintaining cellular redox homeostasis. Furthermore, essential trace minerals such as zinc and selenium contribute indirectly to antioxidant defense by serving as structural or catalytic cofactors for antioxidant enzymes, including SOD and glutathione peroxidase (Zaitsev, 2025).

Collectively, these native milk constituents form a multifaceted antioxidant network that provides a biochemical foundation for the antioxidant properties of fermented dairy products. When combined with fermentation-derived bioactive compounds and LAB-mediated metabolic activities, these intrinsic antioxidants substantially enhance the functional value of fermented milks as dietary sources of redox-active components.

Microbial-Derived Antioxidant Metabolites in Fermented Dairy Products

Traditionally, dietary proteins have been regarded as sources of energy and essential amino acids. However, accumulating evidence indicates that milk proteins also contain encrypted bioactive peptides that exert physiological functions in cardiovascular, immune, digestive, and nervous systems. These peptides are inactive within the parent proteins and are released during fermentation, storage, ripening, or gastrointestinal digestion (Auestad & Layman, 2021; Chourasia *et al.*, 2021).

Fermented dairy products, including yogurt, kefir, sour milk, and other LAB-fermented milks, are recognized as important sources of antioxidant peptides. During fermentation, LAB proteolytic systems cleave caseins and whey proteins, generating low-molecular-weight peptides with antioxidant potential. The antioxidant activity of these peptides is strongly dependent on their amino acid composition and sequence, particularly the presence of residues such as histidine, tyrosine, cysteine, and methionine, which contribute to free radical scavenging, proton donation, and metal ion chelation. In addition, peptide hydrophobicity, molecular size, and overall structural conformation play critical roles in determining their effectiveness as antioxidants (Peres Fabbri *et al.*, 2024; Wróblewska *et al.*, 2023).

Beyond fermentation, the antioxidant potential of fermented dairy products may be further enhanced during gastrointestinal digestion. Digestive enzymes such as pepsin, trypsin, chymotrypsin, and brush-border peptidases can release additional antioxidant peptides from both native milk proteins and fermentation-derived peptides. This sequential hydrolysis increases peptide bioaccessibility and may amplify their capacity to neutralize reactive oxygen species, chelate pro-oxidant metal ions, and inhibit lipid peroxidation, thereby extending the functional relevance of fermented milks beyond the food matrix (Quintieri *et al.*, 2024; Singh & Gaur, 2024).

Several LAB strains are capable of synthesizing or enhancing the bioavailability of antioxidant-related vitamins during milk fermentation, particularly B-group vitamins. Among these, folic acid (vitamin B9) plays a pivotal role in nucleotide biosynthesis and

one-carbon metabolism, being essential for DNA synthesis and methylation reactions. Although folate does not act as a direct radical scavenger, it exerts indirect antioxidant effects by regulating homocysteine metabolism, thereby reducing oxidative stress associated with hyperhomocysteinemia (Abdul Hakim *et al.*, 2023). Certain LAB strains, such as *Lactobacillus* and *Lactococcus* species, have been reported to synthesize folate during milk fermentation, contributing to the nutritional and functional enhancement of fermented dairy product (Mahara *et al.*, 2021). In addition, LAB-mediated production of riboflavin (vitamin B2) and other cofactors involved in redox reactions may further support cellular antioxidant defenses (Zhang *et al.*, 2022).

On the other hand, organic acids produced during fermentation, including lactic, acetic, and propionic acids, also contribute indirectly to antioxidant activity. These compounds lower the pH of the dairy matrix, which can inhibit oxidative reactions and enhance the stability of antioxidant peptides and vitamins (Anumudu *et al.*, 2024; Erem & Kilic-Akyilmaz, 2024). Moreover, certain organic acids exhibit metal-chelating properties, reducing the availability of pro-oxidant metal ions such as iron and copper, thereby limiting the formation of reactive oxygen species via Fenton-type reactions (Mussio *et al.*, 2025).

In contrast, exopolysaccharides synthesized by LAB are high-molecular-weight polymers that play important roles in the technological properties of fermented milks, such as viscosity and texture. Beyond these functions, EPS have been increasingly recognized for their biological activities, including antioxidant effects (Jurášková *et al.*, 2025; Zhang *et al.*, 2025). LAB-derived EPS can scavenge free radicals, chelate metal ions, and modulate cellular antioxidant pathways. Their antioxidant capacity is influenced by monosaccharide composition, molecular weight, and branching degree, as well as by fermentation conditions and bacterial strain specificity (Hernández-Figueroa *et al.*, 2025; Zhang *et al.*, 2023).

Collectively, the generation of antioxidant peptides and microbial-derived metabolites during fermentation underscores the importance of LAB strain selection and fermentation conditions in maximizing the antioxidant potential of dairy products. Understanding these microbial-driven biochemical processes is essential for the rational design of fermented milks with enhanced functional and health-promoting properties.

Bio-Transformed Antioxidant Compounds Generated During Fermentation

Although milk itself is not a primary source of phytochemicals, fermented milks can act as effective carriers and transformation matrices for phytochemicals introduced through plant-derived ingredients, such as fruits, cereals, legumes, herbs, or plant extracts. During fermentation, lactic acid bacteria (LAB) mediate the bio-transformation of these phytochemicals through enzymatic and metabolic activities, often enhancing their bioavailability and antioxidant capacity (Dixit *et al.*, 2023; Wróblewska *et al.*, 2023).

Polyphenols, including phenolic acids, flavonoids, and tannins, are among the most extensively bio-transformed phytochemicals in fermented milks. LAB enzymes such as β -glucosidases, esterases, and phenolic acid decarboxylases hydrolyze glycosylated or polymeric phenolic into simpler aglycone forms, which generally exhibit higher antioxidant activity and improved intestinal absorption. Similarly, bound phenolic compounds can

be released from plant matrices during fermentation, increasing their functional efficacy (Dissanayake *et al.*, 2025; Kumar *et al.*, 2025).

Other phytochemicals, such as carotenoids and phytosterols, may undergo structural modifications during fermentation that improve their stability or bioaccessibility within the dairy matrix (Dissanayake *et al.*, 2025; Kanimozhi & Sukumar, 2025; Kumar *et al.*, 2025). LAB metabolism can also reduce molecular complexity or alter redox properties, contributing to enhanced radical-scavenging or metal-chelating activity (Arslan *et al.*, 2025; Mussio *et al.*, 2025). Additionally, interactions between phytochemicals and milk proteins or peptides generated during fermentation can protect these compounds from degradation and modulate their release during digestion (Fitsum *et al.*, 2025; Kumar *et al.*, 2025).

Overall, the bio-transformation of phytochemicals in fermented milks is driven by LAB enzymatic activity, changes in pH, and matrix interactions, resulting in fermented dairy products with improved antioxidant potential and functional properties. This highlights the relevance of fermented milks as versatile platforms for delivering bioactive plant compounds and developing innovative functional foods.

CONCLUSIONS

Fermented milks are valuable functional foods containing natural and microbial derived antioxidant compounds. LAB contribute to antioxidant activity through production of bioactive metabolites, release of peptides, synthesis of vitamins such as folate, and the intrinsic antioxidant properties of their cells and intracellular components. Although a substantial body of *in vitro* research demonstrates strong antioxidant potential, more *in vivo* studies are required to fully understand the mechanisms and health implications. Given their global consumption, sensory acceptability, and proven functional properties, fermented milks represent a promising dietary strategy for mitigating oxidative stress and promoting human health.







REFERENCES

- Abdul Hakim, B. N., Xuan, N. J., & Oslan, S. N. H. (2023). A Comprehensive Review of Bioactive Compounds from Lactic Acid Bacteria: Potential Functions as Functional Food in Dietetics and the Food Industry. *Foods*, *12*(15). <https://doi.org/10.3390/foods12152850>
- Anumudu, C. K., Miri, T., & Onyeaka, H. (2024). Multifunctional Applications of Lactic Acid Bacteria: Enhancing Safety, Quality, and Nutritional Value in Foods and Fermented Beverages. *Foods*, *13*(23), 3714.
- Arslan, N. P., Azad, F., Orak, T., Budak-Savas, A., Ortucu, S., Dawar, P.,...Taskin, M. (2025). A review on bacteria-derived antioxidant metabolites: their production, purification, characterization, potential applications, and limitations. *Archives of Pharmacal Research*, *48*(4), 253-292. <https://doi.org/10.1007/s12272-025-01541-5>
- Auestad, N., & Layman, D. K. (2021). Dairy bioactive proteins and peptides: a narrative review. *Nutr Rev*, *79*(Suppl 2), 36-47. <https://doi.org/10.1093/nutrit/nuab097>
- Bintsis, T., & Papademas, P. (2022). The Evolution of Fermented Milks, from Artisanal to Industrial Products: A Critical Review. *Fermentation*, *8*(12), 679.
- Bryukhanov, A. L., Klimko, A. I., & Netrusov, A. I. (2022). Antioxidant Properties of Lactic Acid Bacteria. *Microbiology*, *91*(5), 463-478. <https://doi.org/10.1134/S0026261722601439>
- Chourasia, R., Abedin, M. M., Chiring Phukon, L., Sahoo, D., Singh, S. P., & Rai, A. K. (2021). Biotechnological approaches for the production of designer cheese with improved functionality. *Comprehensive Reviews in Food Science and Food Safety*, *20*(1), 960-979. <https://doi.org/https://doi.org/10.1111/1541-4337.12680>

- Dissanayake, I. H., Tabassum, W., Alsherbiny, M., Chang, D., Li, C. G., & Bhuyan, D. J. (2025). Lactic acid bacterial fermentation as a biotransformation strategy to enhance the bioavailability of phenolic antioxidants in fruits and vegetables: A comprehensive review. *Food Research International*, 209, 116283. <https://doi.org/https://doi.org/10.1016/j.foodres.2025.116283>
- Dixit, V., Joseph Kamal, S. W., Bajrang Chole, P., Dayal, D., Chaubey, K. K., Pal, A. K.,...Bachheti, R. K. (2023). Functional Foods: Exploring the Health Benefits of Bioactive Compounds from Plant and Animal Sources. *Journal of Food Quality*, 2023(1), 5546753. <https://doi.org/https://doi.org/10.1155/2023/5546753>
- Erem, E., & Kilic-Akyilmaz, M. (2024). The role of fermentation with lactic acid bacteria in quality and health effects of plant-based dairy analogues. *Comprehensive Reviews in Food Science and Food Safety*, 23(4), e13402. <https://doi.org/https://doi.org/10.1111/1541-4337.13402>
- Fardet, A., & Rock, E. (2018). In vitro and in vivo antioxidant potential of milks, yoghurts, fermented milks and cheeses: a narrative review of evidence. *Nutrition Research Reviews*, 31(1), 52-70. <https://doi.org/10.1017/S0954422417000191>
- Fitsum, S., Gebreyohannes, G., & Sbhatu, D. B. (2025). Bioactive compounds in fermented foods: Health benefits, safety, and future perspectives. *Applied Food Research*, 5(2), 101097. <https://doi.org/https://doi.org/10.1016/j.afres.2025.101097>
- Gao, Y., Liu, Y., Ma, T., Liang, Q., Sun, J., Wu, X.,...Mu, G. (2025). Fermented Dairy Products as Precision Modulators of Gut Microbiota and Host Health: Mechanistic Insights, Clinical Evidence, and Future Directions. *Foods*, 14(11), 1946.
- Hernández-Figueroa, R. H., López-Malo, A., & Mani-López, E. (2025). Lactic Acid Bacteria-Derived Exopolysaccharides: Dual Roles as Functional Ingredients and Fermentation Agents in Food Applications. *Fermentation*, 11(9), 538.
- Hill, D., Sugrue, I., Arendt, E., Hill, C., Stanton, C., & Ross, R. P. (2017). Recent advances in microbial fermentation for dairy and health. *F1000Res*, 6, 751. <https://doi.org/10.12688/f1000research.10896.1>
- Jurášková, D., Pires, V. C., Ribeiro, S. C., Ferreira, S. S., Bernardo, F., Evtuyugin, D.,...Silva, C. C. G. (2025). Exopolysaccharide (EPS)-Producing *Streptococcus thermophilus*: Functional and Probiotic Potential. *Foods*, 14(17), 3013.
- Kanimozhi, N. V., & Sukumar, M. (2025). Harnessing probiotic fermentation to enhance the bioavailability and health impact of dietary phytochemicals. *Food Wellness*, 1(1), 100018. <https://doi.org/https://doi.org/10.1016/j.foodw.2025.100018>
- Kaur, H., Kaur, G., & Ali, S. A. (2022). Dairy-Based Probiotic-Fermented Functional Foods: An Update on Their Health-Promoting Properties. *Fermentation*, 8(9), 425.
- Khan, I. T., Bule, M., Ullah, R., Nadeem, M., Asif, S., & Niaz, K. (2019). The antioxidant components of milk and their role in processing, ripening, and storage: Functional food. *Vet World*, 12(1), 12-33. <https://doi.org/10.14202/vetworld.2019.12-33>
- Kumar, A., Saranyadevi, S., Thirumalaisamy, S. K., Dapana Durage, T. T., Jaiswal, S. G., Kavitate, D., & Wei, S. (2025). Phenolic acids in fermented foods: microbial biotransformation, antioxidant mechanisms, and functional health implications. *Front Mol Biosci*, 12, 1678673. <https://doi.org/10.3389/fmolb.2025.1678673>
- Mahara, F. A., Nuraida, L., & Lioe, H. N. (2021). Folate in Milk Fermented by Lactic Acid Bacteria from Different Food Sources. *Prev Nutr Food Sci*, 26(2), 230-240. <https://doi.org/10.3746/pnf.2021.26.2.230>
- Muscolo, A., Mariateresa, O., Giulio, T., & Mariateresa, R. (2024). Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. *International Journal of Molecular Sciences*, 25(6), 3264.
- Mussio, C., Garcia-Perez, P., Moret, E., Catena, S., & Lucini, L. (2025). The role, mechanisms and evaluation of natural chelating agents in food stability: a review. *Food Chemistry*, 496, 146682. <https://doi.org/https://doi.org/10.1016/j.foodchem.2025.146682>
- Peres Fabbri, L., Cavallero, A., Vidotto, F., & Gabriele, M. (2024). Bioactive Peptides from Fermented Foods: Production Approaches, Sources, and Potential Health Benefits. *Foods*, 13(21), 3369.
- Quintieri, L., Fanelli, F., Monaci, L., & Fusco, V. (2024). Milk and Its Derivatives as Sources of Components and Microorganisms with Health-Promoting Properties: Probiotics and Bioactive Peptides. *Foods*, 13(4), 601.
- Sharma, H., Ozogul, F., Bartkiene, E., & Rocha, J. M. (2023). Impact of lactic acid bacteria and their metabolites on the techno-functional properties and health benefits of fermented dairy products. *Critical Reviews in Food Science and Nutrition*, 63(21), 4819-4841. <https://doi.org/10.1080/10408398.2021.2007844>
- Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E.,...Winterbourn, C. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature Reviews Molecular Cell Biology*, 23(7), 499-515. <https://doi.org/10.1038/s41580-022-00456-z>

- Singh, N., & Gaur, S. (2024). New insights into multifunctional aspects of milk derived bioactive peptides: A review. *Food Chemistry Advances*, 4, 100628. <https://doi.org/https://doi.org/10.1016/j.focha.2024.100628>
- Stobiecka, M., Król, J., & Brodziak, A. (2022). Antioxidant Activity of Milk and Dairy Products. *Animals*, 12(3), 245.
- Wróblewska, B., Kuliga, A., & Wnorowska, K. (2023). Bioactive Dairy-Fermented Products and Phenolic Compounds: Together or Apart. *Molecules*, 28(24). <https://doi.org/10.3390/molecules28248081>
- Yerlikaya, O. (2023). A review of fermented milks: potential beneficial effects on human nutrition and health. *Afr Health Sci*, 23(4), 498-507. <https://doi.org/10.4314/ahs.v23i4.54>
- Zaitsev, S. Y. (2025). Major Antioxidants and Methods for Studying Their Total Activity in Milk: A Review. *Methods and Protocols*, 8(6), 139.
- Zhang, J.-R., Ge, Y.-Y., Liu, P.-H., Wu, D.-T., Liu, H.-Y., Li, H.-B.,...Gan, R.-Y. (2022). Biotechnological Strategies of Riboflavin Biosynthesis in Microbes. *Engineering*, 12, 115-127. <https://doi.org/https://doi.org/10.1016/j.eng.2021.03.018>
- Zhang, J., Hu, T., Ma, Y., Ma, Y., & Sun, Q. (2025). Characterisation and bioactivity analysis of exopolysaccharides from *Lactiplantibacillus plantarum* L3. *Carbohydrate Polymer Technologies and Applications*, 10, 100830. <https://doi.org/https://doi.org/10.1016/j.carpta.2025.100830>
- Zhang, J., Xiao, Y., Wang, H., Zhang, H., Chen, W., & Lu, W. (2023). Lactic acid bacteria-derived exopolysaccharide: Formation, immunomodulatory ability, health effects, and structure-function relationship. *Microbiological Research*, 274, 127432. <https://doi.org/https://doi.org/10.1016/j.micres.2023.127432>
- Zujko, M. E., & Witkowska, A. M. (2023). Dietary Antioxidants and Chronic Diseases. *Antioxidants*, 12(2), 362.

Functionality, stability and technological challenges of natural polyphenols in food matrices

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ABSTRACT

Objective: To examine the presence, stability, and functionality of natural polyphenols in commercial food matrices. The effects of industrial processing and the emerging technological benefits for their protection were also considered.

Design/Methodology/Approach: A review of natural polyphenols in fruits, vegetables, cereals, and beverages (alcoholic and non-alcoholic) was conducted. The oxidation mechanisms present in food matrices and the stability of the molecules during processing were described. Emerging technologies, such as encapsulation, for preserving the antioxidant functionality of natural polyphenols were analyzed.

Results: Natural polyphenols are present in a variety of foods; their composition is linked to the type of food, the plant tissue, and the growing and post-harvest handling conditions. Fruits are notable for their phenolic acids, anthocyanins, and flavonoids. Vegetables are predominantly composed of hydroxycinnamic acids and flavonoids, cereals contain lignans and resorcinols, and beverages contain flavonols, catechins, and resveratrol. Food processing reduces the bioavailability and functionality of polyphenols through oxidation, heat, pH, and exposure to oxygen. Techniques combined with heat treatments, such as microwaves, ultrasound, and bioprocesses, particularly encapsulation, help preserve natural polyphenols and improve their stability and solubility.

Limitations/Implications of the study: The loss of natural polyphenols during industrial processing reduces their antioxidant activity. Emerging technological alternatives applicable to the production of functional foods are needed.

Findings/Conclusions: Natural polyphenols are molecules with varied bioactivity in different food matrices. Their stability depends on the food and the processing applied. Emerging technologies exist to preserve food functionality, including encapsulation, an effective strategy.

Keywords: Natural polyphenols, functional stability, Food processing, encapsulation.

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INTRODUCTION

Natural polyphenols (NPs) are a group of molecules produced by plants; they are secondary metabolites formed in response to internal or external stressors (such as UV radiation, insects, fungi, and animals). Chemical processes produce synthetic polyphenols; they exhibit high stability and efficacy but can cause toxicity (Delgado-Arenas, 2019), which is why NPs are preferred for consumption. Currently, more than 8,000 NPs are registered, in which an aromatic group forms the basis of their structure and is linked to one or more hydroxyl groups (Di Lorenzo *et al.*, 2021; Rana *et al.*, 2022). Polyphenols

can be grouped into flavonoids (anthocyanins, flavonols, flavones, isoflavones, flavanones, and stilbenes) and non-flavonoids, which include phenolic acids, tannins, stilbenes, and lignans, among others. Previously, polyphenols were categorized as antinutritional components, without an essential function in human nutrition; however, various studies have recognized that polyphenols are molecules with antioxidant, anti-inflammatory, anti-allergic, and anti-hyperglycemic functions, as well as a direct connection with favorable responses in metabolic pathways and the gut microbiota (Ramaiah *et al.*, 2024). The antioxidant functionality of polyphenols has been demonstrated in several studies where they can eliminate reactive oxygen species (ROS) and chelate transition metals (Zhong *et al.*, 2020); they have been considered as potentially useful in the control of degenerative diseases, specifically due to their ability to inhibit cyclooxygenase and lipoxygenase, enzymes responsible for the inflammatory process, and acetylcholinesterase, associated with the development of Alzheimer's or Parkinson's diseases (Nwidu *et al.*, 2019).

Antioxidant capacity is a functionality that can be measured in *in vitro* and *in vivo* assays. In particular, food matrices are evaluated using *in vitro* assays; these quickly measure bioactive compounds, and results are known within product batches. The most commonly used assays are those that employ a free radical to neutralize electron and hydrogen transfer mechanisms or the chelation of transition metals, such as the DPPH method (using the 2,2-diphenyl-1-picrylhydrazyl radical), the ABTS method (using the 2,2'-azino-bis-(3-ethylthiazolinebenzenesulfonic acid-6) radical), the FRAP assay (using ferric reduction), and the ORAC assay (measuring oxygen radical absorption capacity), among others (Munteanu & Apetrei, 2021; Lang *et al.*, 2024). Natural particles have been a focus of interest for the functional food industry. However, the presence of natural particles in food does not guarantee that functionality will remain intact throughout the agricultural chain, post-harvest, and processing. It has been observed that factors such as growing conditions, post-harvest practices, and the presence of Oxygen levels, hours of sunlight, chemical composition, and heat treatments can cause the degradation, oxidation, or imbalance of NPs in the food matrix (Tian *et al.*, 2025), directly affecting their stability and functionality. Therefore, it is necessary to study how NPs behave in food matrices to ensure their preservation and leverage their functionality in food processing. This review analyzes the presence and characteristics of NPs in various food matrices, the oxidation mechanisms that can affect their stability and functionality, and the limitations encountered during processing. The second section addresses emerging technologies proposed as alternatives to improve and/or preserve bioactivity in processed foods. Overall, the aim is to understand the current challenges and opportunities for maintaining NP functionality in the food industry.

Natural polyphenols in foods

The concentration and composition of polyphenols in plant-based foods vary according to genetic, environmental, and physiological factors specific to each crop. Maturity level, climatic conditions, and post-harvest handling influence polyphenol biosynthesis. The type of plant tissue, such as peel, leaves, pulp, and seed, varies in the content and structural diversity of polyphenols (Aneklaphakij *et al.*, 2021). Several studies have reported

that plants of the same species exhibit significant variation in phenolic content due to overexposure to solar radiation or to nutrient availability, which affects enzymatic activity in the phenylpropanoid pathways (Aneklaphakij *et al.*, 2021; Rienth *et al.*, 2021). Therefore, it can be explained that polyphenols are distributed in complex ways across different food matrices, from fruits and vegetables to cereals and alcoholic and non-alcoholic beverages, which constitutes a line of work to understand their behavior during food processing.

Fruits are recognized sources of NPs, with berries, citrus fruits, drupes, pomes, and tropical fruits containing the highest levels. Berries are reported to contain 30 to 2000 mg/100g of total polyphenols (TPs), exhibiting a broad polyphenol profile characteristic of each species, notably anthocyanins, ellagitannins, and proanthocyanidins (Arena *et al.*, 2023). The polyphenols in citrus fruits are primarily flavone glycosides, followed by polymethoxylated flavones, anthocyanins, and traces of hydroxycinnamic acids. The NPs found in bottled orange juice are hesperidin, narirutin, and didymin (65, 13, and 15 mg/250 mL of TPs, respectively) (Sanches *et al.*, 2022). Pome fruits (pear, apple, and quince) and drupes (cherry, peach, plum, and nectarine) primarily contain a mixture of TPs compounds, including chlorogenic acids, anthocyanins, flavonols, catechins, and proanthocyanidins. Specifically, hydroxycinnamic acid and anthocyanins predominate in drupes, while neochlorogenic acid is the most prevalent in other pomes (Lujan *et al.*, 2023). The variation in TPs content among tropical fruits is significant, ranging from 15 to 143 mg/100 g. Guava contains the highest amount (126 mg/100 g), followed by kiwifruit (116 mg/100 g), mango (104 mg/100 g), and pomegranate juice (44 mg/100 ml) (Domínguez-Rodríguez *et al.*, 2021).

Hydroxycinnamic acids predominate in leafy vegetables; lettuce contains 5-caffeoylquinic acid and quercetin glycosides; spinach is high in flavonols derived mainly from quercetagenin. Swiss chard contains up to 1320 mg/100 g, including vitexin 2''-O-xyloside and kaempferol 3-O-gentiobioside. Onions have a higher quercetin content (3.2, 12, and 17 mg/100g in white, yellow, and red onions, respectively). Root vegetables, specifically the cell wall of carrots, contain 5-caffeoylquinic acid, coumaroylquinic acids, and esterified feruloyl acids; black carrots contain anthocyanins (xylosylglucosylgalactoside). Celery contains up to 14 mg/100g of TPs, notably furanocoumarins (8-methoxypsoralen and isopimpinelin), while Swiss chard has 410 mg/g of TPs. Colored potatoes contain twice as many NPs as white potatoes; the main polyphenol is chlorogenic acid (14. mg/100 g) (Kuppusamy *et al.*, 2020; Yuan *et al.*, 2020).

The NPs in the pericarps of cereals such as barley, buckwheat, maize, oats, rice, rye, sorghum, and wheat mainly contain phenolic acids (free or bound: 4-hydroxybenzoic acid and ferulic acid), flavonoids, and resorcinols. Barley flour has been quantified at up to 73 mg/100 g of PCs (Deng *et al.*, 2021), while buckwheat reports 37 and 8 mg/100 g of PCs for whole and refined flours, respectively. Maize kernels have a PC content of 179 mg/100 g, notably containing ferulic acid, synaptic acid, p-coumaric acid, 2-hydroxybenzoic acid, caffeic acid, and syringic acid. Specifically, red, blue, purple, and orange corn varieties are predominantly composed of anthocyanins, while whole grain flours have been identified as containing lignans such as syringaresinol, oxomatairesinol, and hydroxymatairesinol (Suriano *et al.*, 2021). Oats, consumed as rolled oats, whole grain oats, and whole grain

oat flour, have a protein content of 26, 39, and 82 mg/100 g, respectively. The polyphenol mixture includes phenolic acids (ferulic acid, vanillic acid, 4-hydroxybenzoic acid, caffeic acid, and vanillin), avenanthramides (N-caffeoyl-5-hydroxyanthranilic acid, N-feruloyl-5-hydroxyanthranilic acid, and N-p-coumaroyl-5-hydroxyanthranilic acid), and lignans (hydroxymatairesinol, larii-resinol, matairesinol, pinoresinol, and syringaresinol) (Raguindin *et al.*, 2021). Rice grain contains ferulic acid (23 mg/100 g of refined flour and 91 mg/100 g of whole grain flour), followed by p-coumaric acid and resorcinols (5-heneicosylresorcinol, 5-heptadecylresorcinol, and 5-nonadecylresorcinol). Colored varieties such as black rice contain anthocyanins (cyanidin 3-glucoside) (Ramos *et al.*, 2023). Sorghum contains up to 413 mg/100g of protein, mainly proanthocyanidins, phenolic acids, and lignans (Kumari *et al.*, 2021). Wheat contains protein in the endosperm and bran, with concentrations 15 times higher in the endosperm. Protein contains phenolic acids (ferulic acid, p-coumaric acid, synaptic acid, caffeic acid, vanillic acid), alkylresorcinols (5-heneicosenylresorcinol and 5-heneicosylresorcinol), lignans, anthocyanins, and flavones (Tian *et al.*, 2021).

Beers and wines are rich in TPs (quantified by the Folin method). Beer contains xanthohumol, isoxanthohumol, 6-prenylnaringenin, ferulic acid, gallic acid, vanillic acid, p-coumaric acid, and synaptic acid, with TPs concentrations of 52, 42, and 28 mg/100 mL for ale, dark beer, and light beer, respectively. The concentration and type of polyphenols depend on the ingredients, processing, aging, and storage (Cortese *et al.*, 2020; Šibalić *et al.*, 2021). Grape polyphenols are highest in the seed (60%) and skin (30%), followed by the pulp and stem (<10%). During winemaking, NPs are obtained through vinification; the quantity and type of NPs depend on the grape species, the process, time and type of aging; including phenolic acids, styrbenes, flavonols, dihydroflavonols, anthocyanins (malvidin 3-O-glucoside, malvidin 3-O-(6-acetyl-glucoside)), catechins and proanthocyanidins, in TPs concentrations of 216, 32 mg/100 ml in red wine and white wine, respectively. Wine resveratrol has high biological activity; it occurs as aglycones (trans- and cis-resveratrol), glycosides (trans- and cis-piceid), and dimers (viniferin and pallidol) (Dos Santos *et al.*, 2022; Pereira-Coelho *et al.*, 2023).

Coffee and tea are the non-alcoholic beverages with the highest TPs content. Green coffee beans contain approximately 45 chlorogenic acids. When making the beverage, roasted beans are used, and this process influences the variability of TPs composition, as do bean maturity, storage, and brewing methods. The main components are phenolic acids, including 5-caffeoylquinic, ferulic, p-coumaric, 4-hydroxybenzoic, and vanillic acids. The only lignan identified was secoisolariciresinol, with TPs concentrations of 87-212 mg/mL (Mehari *et al.*, 2021; Alnsour *et al.*, 2022). Brewed tea contains up to 40% TPs; its content varies depending on the serving size. Green tea contains 7 catechins ((+)-gallocatechin, (+)-gallocatechin 3-O-gallate, (-)-epicalocatechin, (-)-epicalocatechin 3-O-gallate, (-)-epicatechin and (-)-epicatechin 3-O-gallate), phenolic acids, flavonoids and proanthocyanidins (65.7, 12.5, 5.3 and 5.5 mg/100 mL, respectively) (Abdullah & Mazlan, 2020), and for black tea a fermentation must be carried out in which thearubigins and theaflavins are formed by the oxidation of polyphenols. Cocoa beans store up to 2% w/w of protein in the cotyledon. When the beans are fermented, and cocoa powder is obtained, the protein composition is 5624 mg/100 g, consisting mainly of flavonols (catechins and

proanthocyanidins). During chocolate processing, epicatechins and phenolic acids are formed, while clovamide and deoxyclovamide predominate in cocoa liquor (see Figure 1).

Effects of industrial processing on food matrices

The food industry uses technology to process food to provide microbiologically safe edible products, improve the digestibility of some nutrients, and develop the organoleptic characteristics of smell and texture. Processing induces structural changes within the food matrix and affects the polyphenols present naturally or added to prevent oxidation in easily degraded foods (Cao *et al.*, 2021). Food processing can include thermal and mechanical treatments, refrigeration, drying, and fermentation (Conte *et al.*, 2023), which cause significant changes in structure and composition, with effects that may be desirable or undesirable (Arfaoui, 2021). In particular, exposure to pro-oxidant metals and/or oxygen-rich atmospheres, heat, and pH changes promotes oxidation reactions that lead to the loss of molecules and their functionality (Cao *et al.*, 2021). Food quality is also affected by oxidation, compromising the functionality of native proteins, lipids, carbohydrates, and polyphenols. Oxidation influences the bioavailability, functionality, and stability of bioactive components during processing and storage (Conte *et al.*, 2023; Dini & Grumetto, 2022).

Protein oxidation is another cause of food spoilage, resulting in the loss of thousands of pesos' worth of products that are not consumed as intended. Amino acid side chains

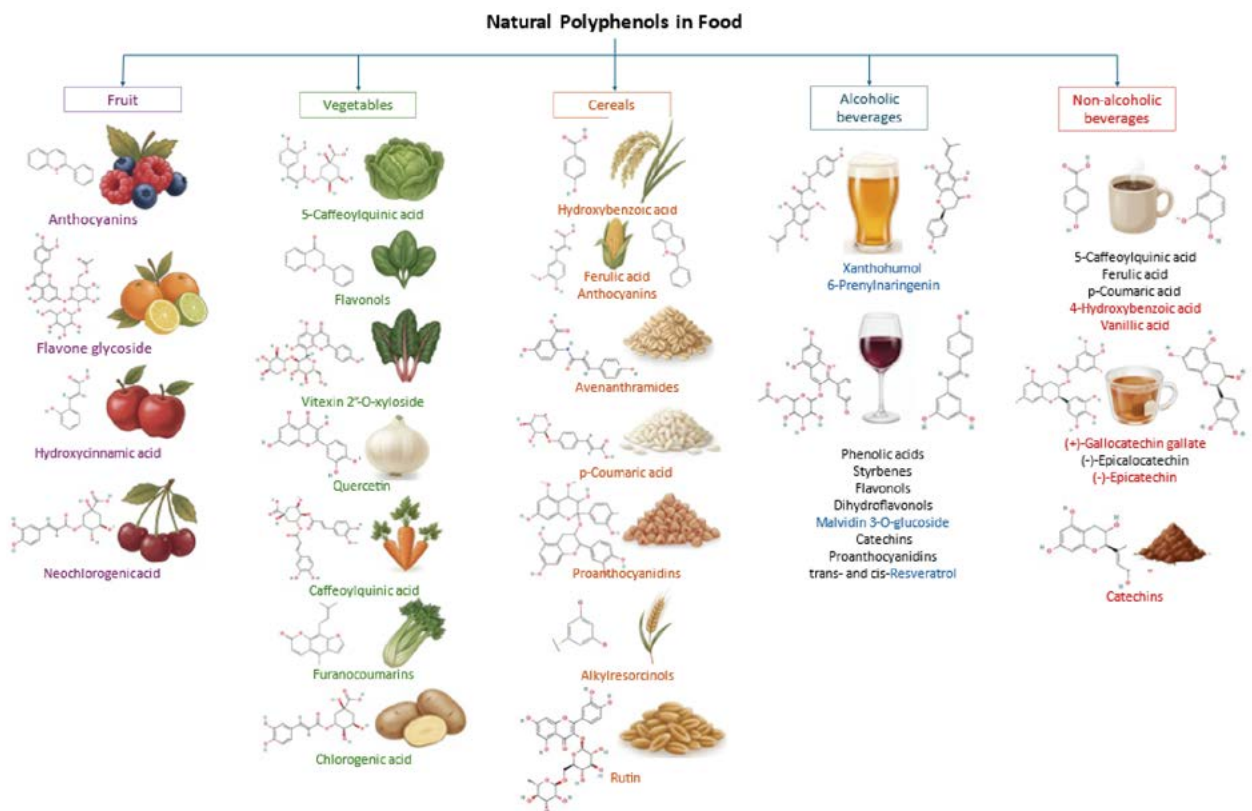


Figure 1. Chemical structure and food source of natural polyphenols.

can undergo oxidation reactions, altering protein function and compromising food quality. Protein oxidation is primarily a consequence of the presence of free radicals, heat treatments that modify protein structure, and myoglobin or metal catalysts (Davies, 2016). Protein oxidation promotes the generation of hydroxyperoxides, carbonyls, and sulfoxides, and the solubilization of proteins through the breaking of peptide bonds. Carbonylation of protein groups is similar to lipid oxidation. In the first stage, a hydrogen atom is removed from the protein structure by a reactive oxygen species, generating a protein free radical ($P\bullet$) in the presence of molecular oxygen. The $P\bullet$ transforms into a peroxy radical, which can react with another protein and trigger oxidative reactions. In the second stage, an alkoxy radical is formed (Domínguez *et al.*, 2021). In meat products, the protein carbonylation process results in loss of juiciness and tenderness, color changes, decreased protein digestibility, and impaired functionality.

Lipid oxidation occurs in three stages: the first is initiation, in which a hydrogen atom is removed from an unsaturated fatty acid (RH) by a reactive species, generating an alkyl radical ($R\bullet$). Endogenous enzymes, metalloproteins, light, and high temperatures are recognized as catalysts for this first stage, generating radicals such as the hydroxyl radical, the superoxide anion, singlet oxygen, the lipid radical, or the peroxy-lipid radical. In the second stage, atmospheric oxygen reacts with the alkyl radical, forming a peroxy radical. This promotes the removal of another hydrogen atom from a neighboring fatty acid, continually forming alkyl radicals. In this phase, it is common for products to develop a rancid odor, resulting from lipid peroxidation and the formation of aldehydes, ketones, and malondialdehyde (Hennebelle *et al.*, 2024). In the third stage, termination and direct interaction between two radicals result in dimers, trimers, or fatty acid polymers. Especially in food matrices of animal origin, polyoxygenated cholesterol derivatives are produced, which are potentially toxic (Wu *et al.*, 2024).

In the case of fried foods with high fat and carbohydrate content, deterioration occurs due to the generation of lipid-free radicals, resulting from thermal oxidation or auto-oxidation (Zeb, 2019). Protein-rich foods subjected to heat treatments above 250 °C and in the presence of free radicals form heterocyclic amines (Fernández *et al.*, 2012). The presence of these compounds has been linked to chronic degenerative diseases caused by the consumption of processed and ultra-processed foods. Processed food matrices are susceptible to oxidation but can self-regulate when they contain native antioxidants, such as vitamins, carotenoids, and polyphenols, which can neutralize reactive oxygen species and reduce deterioration. This self-regulation depends on the stability and concentration of the antioxidants. However, when intrinsic antioxidant capacity is lost, the addition of antioxidant-rich extracts, such as polyphenols, provides stability to the processes. The changes that polyphenols undergo during processing are, in many cases, desirable, even if they involve some loss, due to the desired sensory quality of the final product. In the case of grape fermentation, aged wines degrade anthocyanins, thereby achieving sensory stability. In barley malting, a pretreatment for beer production, up to 60% of flavonoids are lost; however, polyphenol derivatives are generated that give each type of beer its characteristic profile. The decaffeination process eliminates 9% of chlorogenic acid, and roasting the grain reduces polyphenols by 67% to 90%.

Packaged tea contains fewer polyphenols than tea consumed directly due to pasteurization. In the case of grains, most of the NPs on the outside of the seeds are lost during the mechanical refining process; however, some NPs can be retained in whole-grain flours. Tortillas made with corn in Latin American countries use nixtamalization (an alkaline-thermal process), in which the presence of Ca^{2+} and the temperature reduce the total polyphenol content by 78-90%. Pearling oats reduces avenanthramides by 58-83%. Flaked sorghum loses up to 90% of its proanthocyanidin polymers. Products made with whole-wheat flour retain 80% of their total polyphenol content, while those made with refined flour have a low NPs content. Baked potatoes contain 80% less 5-caffeoylquinic acid, microwaved potatoes retain 55% of the same polyphenol, and fried potatoes do not contain 5-caffeoylquinic acid. (Dao & Friedman, 1992; Peaterson *et al.*, 2001; Rothwell *et al.*, 2013).

Emerging technologies for polyphenol protection

Foods selected to preserve polyphenol functionality employ technologies that reduce polyphenol loss, such as vacuum thermal processing, microwaves, and ultrasound. Alternatives to thermal treatment also exist, such as high-pressure, freeze-drying, and bioprocesses, which reduce polyphenol loss and improve bioaccessibility (Conte *et al.*, 2023). Encapsulation is a preferred bioprocess due to its high specificity and ease of implementation. Polyphenol encapsulation can involve the use of proteins, lipids, polysaccharides, or mixtures thereof as carriers. This preserves the antioxidant activity of the polyphenol because the carriers prevent it from interacting with oxygen and water. The advantages of polyphenol encapsulation, beyond stabilizing them, include preventing the development of unwanted odors or flavors, improving aqueous solubility, controlling release, and increasing bioavailability. Encapsulation techniques depend on the desired physical state, core compound, required particle size, and sensitivity to high temperatures. Notable techniques include freeze-drying, fluidized bed coating, spray drying, cocrystallization, extrusion, spray cooling, coacervation, emulsification, nanoencapsulation, and molecular inclusion.

Freeze-drying and spray-drying are commonly used techniques for removing water from food matrices with encapsulation effects; they require expensive equipment with limited operating capacity (Ramírez *et al.*, 2015). Fluidized bed coating is a technique that involves coating particles suspended in air; it uses various coating materials, such as starch derivatives, gums, proteins, and cellulose in solution, among others (Lipin & Lipin, 2022). Co-crystallization modifies the crystalline structure of sucrose to an irregular structure with pores into which molecules of interest can be incorporated (Pawar *et al.*, 2021). Extrusion is a technique that forces a polymer solution, along with the molecules to be encapsulated, through a small orifice into a gelling solution. This technique uses sodium alginate as the polymer, and chlorine solution is commonly used as the gelling agent. The disadvantage of this encapsulation method is the loss of encapsulated molecules, and its industrial application is very expensive (Nurhudan *et al.*, 2021). Spray cooling is similar to spray drying, except that a cooling chamber is used instead of a drying chamber; the coating materials employed are lipid-based, such as waxes, fats, fatty alcohols, and

polyethylene glycols. Coacervation uses the principle of phase separation of a hydrocolloid from a solution, followed by the deposition of the coacervate phase formed around the molecule of interest suspended in a medium; this technique is mainly used with high-value-added compounds due to their high cost (Sing & Perry, 2020). Emulsification involves dispersing one liquid in another as small droplets; it requires a stabilizing emulsifier, and drying is used to obtain encapsulated powders.

Nanoencapsulation is a constantly evolving technique that generates particles ranging from 1 to 1000 nm. It includes nanospheres and nanocapsules. Nanospheres are matrices in which the molecule of interest is adsorbed to the surface. At the same time, nanocapsules contain the molecule of interest within a cavity with an internal liquid core surrounded by a polymer network. This results in greater bioavailability, solubility, and controlled release (Naskar *et al.*, 2022). Molecular inclusion is a supramolecular technology that confines molecules of interest, called guests, within a polymer matrix. Interactions between the interior and exterior can be controlled because the guest fits within a circularly surrounding molecule through specific hydrophobic and/or hydrophilic interactions. The main encapsulating polymer used for molecular inclusion is cyclodextrin (Arce-Vazquez *et al.*, 2016). This technique offers significant advantages, including achieving desirable biocompatibility, high targeting and safety, a unique molecular structure, and easy functionalization. Inclusion complexes overcome the limitations of nanoparticle encapsulation.

CONCLUSION

This review confirms that natural polyphenols are an abundant group of molecules with antioxidant properties and are of interest to human health. However, their stability depends on agronomic factors and the mechanical conditions during processing. Polyphenol groups in food are closely associated with factors of genetic variability, environmental stress, and physiological maturity. Historically considered antinutritional, NPs are now recognized for their antioxidant, anti-inflammatory, and regulatory functions in metabolic processes, which are associated with consumer health. Therefore, NPs can be used in the development of functional foods; however, their sensitivity to oxidation, heat, pH, and light limits their direct application.

Furthermore, it is emphasized that food matrices possess self-regulatory mechanisms that balance oxidation via compounds such as carotenoids, vitamins, and natural polyphenols. This capacity can be affected when processing conditions favor oxidative reactions, leading to degradation or reduced functionality. The loss of NPs during processes such as cooking, roasting, nixtamalization, or refining can be excessive. Still, it is sometimes desirable due to its contribution to improving sensory characteristics. NPs preservation can be achieved through emerging technologies, such as minimal-intensity processes, bioprocesses, and, especially, encapsulation strategies. These represent alternatives for improving the stability and bioavailability of NPs. The appropriate integration of these technologies can foster the development of processed foods with effective functionality for the contemporary food industry.








REFERENCES

- Abdullah, S. S. S., & Mazlan, A. N. (2020). Quantification of polyphenols and antioxidant activity in several herbal and green tea products in Malaysia. *Materials Today: Proceedings*, 31, A106-A113. doi: 10.1016/j.matpr.2020.12.1083.
- Alnsour, L., Issa, R., Awwad, S., Albals, D., & Al-Momani, I. (2022). Quantification of total phenols and antioxidants in coffee samples of different origins and evaluation of the effect of degree of roasting on their levels. *Molecules*, 27(5), 1591. doi:10.3390/molecules27051591.
- Aneklaphakij, C., Saigo, T., Watanabe, M., Naake, T., Fernie, A. R., Bunsupa, S., & Tohge, T. (2021). Diversity of chemical structures and biosynthesis of polyphenols in nut-bearing species. *Frontiers in Plant Science*, 12, 642581.
- Arce-Vázquez, M. B., Ponce-Alquicira, E., Delgado-Fornué, E., Pedroza-Islas, R., Díaz-Godínez, G., & Soriano-Santos, J. (2016). Integral use of Amaranth starch to obtain cyclodextrin glycosyltransferase, by *Bacillus megaterium*, to produce β -cyclodextrin. *Frontiers in microbiology*, 7, 1513. doi:10.3389/fmicb.2016.01513.
- Arena, K., Cacciola, F., Miceli, N., Taviano, M. F., Cavò, E., Murphy, R. E., Dugo, P. & Mondello, L. (2023). Determination of the polyphenolic content of berry juices using focusing-modulated comprehensive two-dimensional liquid chromatography coupled to mass spectrometry detection. *Analytical and Bioanalytical Chemistry*, 415(13), 2371-2382. doi: doi.org/10.1007/s00216-022-04216-y.
- Arfaoui, L. (2021). Dietary plant polyphenols: Effects of food processing on their content and bioavailability. *Molecules*, 26(10), 2959. doi:10.3390/molecules26102959.
- Cao, H., Saroglu, O., Karadag, A., Diaconeasa, Z., Zoccatelli, G., Conte Junior, C. A., González-Aguilar, G. A., Ou, J., Bai, W., Zamarioli, C. M., Freitas, P. A., Shpigelman, A., Campelo, P., Capanoglu, E., Hii, C.L., Jafari, S.M., Qi, Y., Liao, P., Wang, M., Zou, L., Bourke, P. Simal-Gandara, J. & Xiao, J. (2021). Available technologies on improving the stability of polyphenols in food processing. *Food Frontiers*, 2(2), 109-139. doi: 10.1002/fft2.65.
- Conte, A., Martini, S., & Tagliacuzzi, D. (2023). Influence of Processing and Digestion on the Stability, Bioaccessibility and Bioactivity of Food Polyphenols. *Foods*, 12(4), 851. doi: 10.3390/foods12040851
- Cortese, M., Gigliobianco, M. R., Peregrina, D. V., Sagratini, G., Censi, R., & Di Martino, P. (2020). Quantification of phenolic compounds in different types of craft beers, worts, starting and spent ingredients by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 1612, 460622. doi:10.1016/j.chroma.2019.460622.
- Davies, M. J. (2016). Protein oxidation and peroxidation. *Biochemical journal*, 473(7), 805-825. doi:10.1042/BJ20151227.
- Dao, L. & Friedman, M. (1992). Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. *Journal of Agricultural and Food Chemistry*, 40, 2152-2156.
- Delgado-Arenas, H. F., Rodríguez-López, A., Rivera, F., Ramos, K. J., Reséndiz-Ramírez, R., & Antano-Lopez, R. (2019). Effect of electrode geometry on the electrolyte resistance measurement over the surface of a skin phantom in a noninvasive manner. *Bioelectrochemistry*, 130, 107337. doi: 10.1016/j.bioelechem.2019.107337.
- Deng, J., Xiang, Z., Lin, C., Zhu, Y., Yang, K., Liu, T., Xia, C., Chen, J., Zhang, W., Zhu, B. & Zhu, B. (2021). Identification and quantification of free, esterified, and insoluble-bound phenolics in grains of hullless barley varieties and their antioxidant activities. *Lwt*, 151, 112001. doi:10.1016/j.lwt.2021.112001.
- Di Lorenzo, C., Colombo, F., Biella, S., Stockley, C., Restani, P. (2021). Polyphenols and Human Health: The Role of Bioavailability. *Nutrients*, 13, 273. doi:10.3390/nu13010273.
- Dini, I., & Grumetto, L. (2022). Recent advances in natural polyphenol research. *Molecules*, 27(24), 8777. doi: 10.3390/molecules27248777.
- Domínguez-Rodríguez, G., Plaza, M., & Marina, M. L. (2021). High-performance thin-layer chromatography and direct analysis in real time-high resolution mass spectrometry of non-extractable polyphenols from tropical fruit peels. *Food Research International*, 147, 110455. doi:10.1016/j.foodres.2021.110455.
- Domínguez, R., Pateiro, M., Munekata, P. E., Zhang, W., Garcia-Oliveira, P., Carpena, M., Prieto, M. A., Bohrer, B. & Lorenzo, J. M. (2021). Protein oxidation in muscle foods: A comprehensive review. *Antioxidants*, 11(1), 60. doi:10.3390/antiox11010060.
- Dos Santos, I., Bosman, G., Aleixandre-Tudo, J. L., & du Toit, W. (2022). Direct quantification of red wine phenolics using fluorescence spectroscopy with chemometrics. *Talanta*, 236, 122857. doi:10.1016/j.talanta.2021.122857.
- Fernández, A. M. C., Jiménez, M. R., Gallego, Á. J., Navarro, I. M. M., & Sánchez, S. P. (2012). Tóxicos formados durante el procesado, preparación y almacenamiento de los alimentos: Toxicología alimentaria. Ediciones Díaz de Santos. Madrid, España. 453-462. ISBN 978-84-9969-208-1.

- Hennebelle, M., Villeneuve, P., Durand, E., Lecomte, J., Van Duynhoven, J., Meynier, A., Yesiltas, B., Jacobsen, C. & Berton-Carabin, C. (2024). Lipid oxidation in emulsions: New insights from the past two decades. *Progress in Lipid Research*, 101275. doi:10.1016/j.plipres.2024.101275.
- Kumari, P. K., Umakanth, A. V., Narsaiah, T. B., & Uma, A. (2021). Exploring anthocyanins, antioxidant capacity and α -glucosidase inhibition in bran and flour extracts of selected sorghum genotypes. *Food Bioscience*, 41, 100979. doi:10.1016/j.fbio.2021.100979.
- Kuppusamy, S., Venkateswarlu, K., & Megharaj, M. (2020). Examining the polyphenol content, antioxidant activity and fatty acid composition of twenty-one different wastes of fruits, vegetables, oilseeds and beverages. *SN Applied Sciences*, 2, 1-13. doi:10.1007/s42452-020-2441-9.
- Lipin, A. A., & Lipin, A. G. (2022). Prediction of coating uniformity in batch fluidized-bed coating process. *Particuology*, 61, 41-46. doi:10.1016/j.partic.2021.03.010.
- Lujan, P., Dura, S., Guzman, I., Steiner, R., & Sanogo, S. (2023). Comparison of Proanthocyanidin-and Phenolic-Rich Extracts Derived from Pecan Shell and Husk as Elicitors of Induced Resistance Against *Phytophthora capsici* on Chile Pepper. *Plant Health Progress*, 24(3), 369-374. doi:10.1094/PHP-12-22-0123-RS.
- Mehari, B., Chandravanshi, B. S., Redi-Abshiro, M., Combrinck, S., McCrindle, R., & Atlabachew, M. (2021). Polyphenol contents of green coffee beans from different regions of Ethiopia. *International Journal of Food Properties*, 24(1), 17-27. doi:10.1080/10942912.2020.1858866.
- Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International journal of molecular sciences*, 22(7), 3380. doi:10.3390/ijms22073380.
- Naskar, A., Roy, S. R., Chakraborty, I., & Bhattacharya, T. (2022). Advancement of Nanotechnology in Developing Functional Foods: Nanotechnology in Functional Foods. *Nanotechnology in Functional Foods*, 1-24. doi:10.1002/9781119905059.ch1.
- Nurhudan, A. I., Supriadi, S., Whulanza, Y., & Saragih, A. S. (2021). Additive manufacturing of metallic based on extrusion process: A review. *Journal of Manufacturing Processes*, 66, 228-237. doi: 10.1016/j.jmapro.2021.04.018.
- Nwidu, L. L., Alikwe, P. C. N., Elmorsy, E., & Carter, W. G. (2019). An Investigation of potential sources of nutraceuticals from the Niger Delta Areas, Nigeria for attenuating oxidative stress. *Medicines*, 6(1), 15. doi:10.3390/medicines6010015.
- Pawar, N., Saha, A., Nandan, N., & Parambil, J. V. (2021). Solution cocrystallization: A scalable approach for cocrystal production. *Crystals*, 11(3), 303. doi:10.3390/cryst11030303.
- Pereira-Coelho, M., da Silva Haas, I. C., Reinke, C. K., Dognini, J., Amboni, R. D. D. M. C., Vitali, L., & dos Santos Madureira, L. A. (2023). A green analytical method for the determination of polyphenols in wine by dispersive pipette extraction and LC-MS/MS. *Food Chemistry*, 405, 134860. doi:10.1016/j.foodchem.2022.134860.
- Peterson, D. M., Emmons, C. L. & Hibbs, A. H. (2001). Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. *Journal of Cereal Science*. 33, 97-103. doi:10.1006/jcrs.2000.0347.
- Raguindin, P. F., Itodo, O. A., Stoyanov, J., Dejanovic, G. M., Gamba, M., Asllanaj, E., Minder, B., Bussler, W., Metzger B., Muka, T., Glisic, M. & Kern, H. (2021). A systematic review of phytochemicals in oat and buckwheat. *Food chemistry*, 338, 127982. doi:10.1016/j.foodchem.2020.127982.
- Ramaiah, P., Baljon, K. J., Hjazai, A., Qasim, M. T., Salih Al-ani, O. A., Imad, S., Hussien B. M., Alsalamy A. & Garousi, N. (2024). Dietary polyphenols and the risk of metabolic syndrome: a systematic review and meta-analysis. *BMC Endocrine Disorders*, 24(1), 26. doi:10.1186/s12902-024-01556-x.
- Ramírez, M. J., Giraldo, G. I., & Orrego, C. E. (2015). Modeling and stability of polyphenol in spray-dried and freeze-dried fruit encapsulates. *Powder Technology*, 277, 89-96. doi:10.1016/j.powtec.2015.02.060.
- Ramos, M., Laveriano, E., San Sebastián, L., Perez, M., Jiménez, A., Lamuela-Raventos, R. M., Garrigos M. C. & Vallverdú-Queralt, A. (2023). Rice straw as a valuable source of cellulose and polyphenols: Applications in the food industry. *Trends in Food Science & Technology*, 131, 14-27. doi: 10.1016/j.tifs.2022.11.020.
- Rienth, M., Vigneron, N., Darriet, P., Sweetman, C., Burbidge, C., Bonghi, C., & Castellarin, S. D. (2021). Grape berry secondary metabolites and their modulation by abiotic factors in a climate change scenario—a review. *Frontiers in Plant Science*, 12, 643258.
- Rothwell JA, Pérez-Jiménez J, Neveu V, Medina-Ramon A, M'Hiri N, Garcia Lobato P, Manach C, Knox K, Eisner R, Wishart D, Scalbert A. (2013). Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. Database, 10.1093/database/bat070.
- Sanches, V. L., Cunha, T. A., Viganó, J., de Souza Mesquita, L. M., Faccioli, L. H., Breikreitz, M. C., & Rostagno, M. A. (2022). Comprehensive analysis of phenolics compounds in citrus fruits peels by

- UPLC-PDA and UPLC-Q/TOF MS using a fused-core column. *Food Chemistry: X*, 14, 100262. doi: 10.1016/j.fochx.2022.100262.
- Šibalić, D., Planinić, M., Jurić, A., Bucić-Kojić, A., & Tišma, M. (2021). Analysis of phenolic compounds in beer: from raw materials to the final product. *Chemical Papers*, 75, 67-76. doi:10.1007/s11696-020-01276-1.
- Sing, C. E., & Perry, S. L. (2020). Recent progress in the science of complex coacervation. *Soft Matter*, 16(12), 2885-2914. doi:10.1039/D0SM00001A.
- Suriano, S., Balconi, C., Valoti, P., & Redaelli, R. (2021). Comparison of total polyphenols, profile anthocyanins, color analysis, carotenoids and tocopherols in pigmented maize. *Lwt*, 144, 111257. doi:10.1016/j.lwt.2021.111257.
- Tian, X. Y., Bai, J. W., Fang, Q., Wang, M., Wu, X., & Aheto, J. H. (2025). Polyphenols in Modern Nutrition: Green Extraction Technologies, Encapsulation, and Promissory Applications. *Food and Bioprocess Technology*, 1-31. doi:10.1007/s11947-025-03876-9
- Tian, W., Chen, G., Gui, Y., Zhang, G., & Li, Y. (2021). Rapid quantification of total phenolics and ferulic acid in whole wheat using UV-Vis spectrophotometry. *Food Control*, 123, 107691. doi: 10.1016/j.foodcont.2020.107691.
- Wu, H., Tatiyaborworntham, N., Hajimohammadi, M., Decker, E. A., Richards, M. P., & Undeland, I. (2024). Model systems for studying lipid oxidation associated with muscle foods: Methods, challenges, and prospects. *Critical Reviews in Food Science and Nutrition*, 64(1), 153-171. doi: 10.1080/10408398.2022.2105302
- Zeb, A. (2019). *Food frying: Chemistry, biochemistry and safety I* (1), 1-469. London, UK: John Wiley & Sons. doi:10.1002/9781119468417.
- Zhong, Y., Li, W., Ran, L., Hou, R., Han, P., Lu, S., Wang, Q., Zhu, Y. & Dong, J. (2020). Inclusion complexes of tea polyphenols with HP cyclodextrin: Preparation, characterization, molecular docking, and antioxidant activity. *Journal of food science*, 85(4), 1105-1113. doi:10.1111/1750-3841.15083.

Cry proteins from *Bacillus thuringiensis*: From natural insecticide to biotechnological tool for agricultural pest control

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ABSTRACT

Objective: The Cry protein family from *Bacillus thuringiensis* (Bt) exhibits biocidal activity against various insect orders, with Lepidoptera being among their primary targets. These insects often evolve into major agricultural pests, leading to significant crop losses. Consequently, research efforts have focused on the identification and cloning of the genes encoding these proteins, as well as elucidating their mechanism of action.

Design/Methodology/Approach: Methods for the identification, isolation, and purification of Cry proteins, along with the current state of the art, were explored, described, and discussed by reviewing relevant literature using scientific databases for bibliographic research.

Results: Cry proteins constitute a diverse group of molecules with demonstrated activity for combating agricultural insect pests, primarily Lepidoptera. These proteins and their corresponding genes have been widely studied and employed in the genetic transformation of several crop plant species.

Limitations/Implications of the study: Although several models have been proposed to explain the mode of action of Cry proteins, molecular mechanism remain unresolved to fully understand their insecticidal activity.

Findings/Conclusions: Bt encodes multiple groups of proteins with insecticidal activity, among which the Cry protein family is the most extensively studied. The identification of its first members, along with the elucidation of their three-dimensional structure, has been essential for proposing their mechanism of action. This has enabled the targeted control of several Lepidopteran pests in agriculture.

Keywords: Pests, insect, toxins.

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INTRODUCTION

In contemporary agriculture, one of the most pressing challenges is pest management, particularly of insect species that have developed resistance to chemical insecticides. Excessive use of these compounds accelerates resistance, diminishes their efficacy, and



results in significant global economic losses (Walsh *et al.*, 2022; Daraban *et al.*, 2023; Gula, 2023).

Moreover, conventional chemical pesticides generally exhibit a broad spectrum of activity, affecting not only pests but also pollinators, other beneficial insects, animals, and even humans (Devine & Furlong, 2007; Walsh *et al.*, 2022; Daraban *et al.*, 2023). These products also contribute to soil, water, and air pollution. Consequently, global policies are increasingly oriented toward sustainable alternatives to synthetic insecticides (Devine & Furlong, 2007; Jurat-Fuentes *et al.*, 2021; Daraban *et al.*, 2023).

Given this context, it is imperative to design pest-control strategies that minimize environmental impact while preserving biodiversity. Biotechnology has played a pivotal role in this endeavor by providing scientifically grounded solutions based on the use of biological agents with pest-control potential (Agaïsse & Lereclus, 1995; Jurat-Fuentes *et al.*, 2021; Pacheco *et al.*, 2023). Compared to chemical pesticides, more favorable alternatives include biochemical control, which employs essential oils, pheromones, hormones, plant metabolites, and enzymes, as well as biological control using microorganisms such as bacteria (*Brevibacillus laterosporus*, *Dickeya dadantii*, *Yersinia* spp., among others), fungi (*Beauveria*, *Metarhizium*, *Paecilomyces*, *Verticillium*, *Trichoderma* and many more), beneficial insects, entomopathogenic nematodes, parasitoids, and viruses such as baculoviruses and entomopoxviruses (Loth *et al.*, 2015; Springer *et al.*, 2018; Glare *et al.*, 2020; Kim *et al.*, 2024; de Sousa *et al.*, 2025; Mitsuhashi, 2025; Ng & Zuharah, 2025).

Nevertheless, several of these strategies face limitations such as reduced biocidal efficacy, restricted specificity, or limited commercial availability, with many still in early research stages. Therefore, the present work focused on analyzing scientific advances related to Bt Cry proteins, which have been extensively studied for insect pest control.

***Bt*, the Bacterium that Synthesizes Insecticidal Compounds**

Bt is a Gram-positive, facultative anaerobic bacterium predominantly found in soil, stagnant water, insects, plants, and other habitats from which it has been isolated (de Maagd *et al.*, 2003). Historical records indicate that it was first isolated at the dawn of the 20th century by the Japanese scientist Shigetane Ishiwata, from silkworms (*Bombyx mori*) that had developed the sotto disease (Roh *et al.*, 2017; Fernández-Chapa *et al.*, 2019; Li *et al.*, 2024). Subsequently, in 1915, Berliner again isolated the bacterium from the flour moth in a storage facility located in Thuringia, Germany, the site from which its name was derived (Valtierra *et al.*, 2020; Li *et al.*, 2024). During the 1950s and 1960s, it was reported that certain *Bt* strains produced intracellular crystal-like inclusions that proved toxic to specific insect orders such as Lepidoptera, Coleoptera, Diptera, Hemiptera and Hymenoptera (Figure 1).

The ability of this bacterium to act selectively on harmful insects without affecting other living organisms made it a valuable tool for biological pest control. In 1938, France introduced a commercial product called Sporine, formulated with *Bt*, intended to eradicate the flour moth (Roh *et al.*, 2017; Li *et al.*, 2024). Since 1961, in the United States of America (USA), the first *Bt*-based products designed for agricultural use have been marketed (Baum *et al.*, 1999). The potential of this bacterium as an alternative to chemical insecticides



Figure 1. Insecticidal activity of proteins Cry. Cry toxins have insecticidal activity against different orders of insects (Lepidoptera, Diptera, Coleoptera, Hymenoptera and Hemiptera). Adapted from Berry *et al.*, 2025.

stimulated the emergence of multiple research lines, which have since expanded to identify new genes enabling the development of innovative insecticidal products.

At present, numerous *Bt*-derived products are available, which may include spores containing several insecticidal proteins or genetically modified bacteria harboring engineered pesticidal toxins to enhance efficacy (Sanahuja *et al.*, 2011). Some formulations incorporate enzymes that potentiate the insecticidal activity of Cry proteins. These products are applied either as powders or aqueous suspensions sprayed onto plants, allowing insects to ingest the bacterium or its spores through the consumption of plant tissue (Jurat-Fuentes *et al.*, 2021; Sanahuja *et al.*, 2011).

Bt Pesticidal Toxins

Bt pesticidal toxins exhibit insecticidal activity against various Lepidoptera that are major agricultural pests of diverse crops, including: *Spodoptera frugiperda* (fall armyworm, affecting maize), *Trichoplusia ni* (cabbage looper, attacking broccoli and cauliflower), *Manduca sexta* (tobacco hornworm, damaging tobacco, tomato, and pepper), *Plutella xylostella* (diamondback moth, affecting cabbage, radish, watercress, and cauliflower), among many others. In addition, there are Cry proteins toxic to Coleoptera (beetles) and Diptera (mosquitoes), among other insect orders.

In the USA, various *Bt* subspecies are employed to control different insect classes, including strains such as *kurstaki*, *aizawai*, and *galleriae* (Lepidoptera), *morrisoni* biovar *tenebrionis* (Coleoptera), and *israelensis* (Diptera) (Karabörklü *et al.*, 2018).

Furthermore, the use of *Bt* can be integrated as a resource within integrated pest management (IPM) systems, encompassing strategies such as crop rotation, biological control through natural enemies, the use of plant-derived metabolites, and the implementation of appropriate agricultural practices to prevent pest outbreaks. All these

measures aim to minimize reliance on synthetic insecticides, thereby contributing to the production of safer food for human health (Sanahuja *et al.*, 2011). For these reasons, *Bt* continues to be widely used worldwide, not only for agricultural pest control but also for combating disease-vector insects affecting humans, such as mosquitoes, since certain strains produce proteins toxic to them.

Growth and Sporulation

Bt undergoes a vegetative developmental stage during which it reproduces by binary fission. When nutrients become limited, the bacterium initiates a differentiation process that culminates in sporulation. During this phase, it produces spores and proteinaceous parasporal crystals composed mainly of Cry proteins and others, whose presence depends on the strain (Pinos *et al.*, 2021). These crystals can exhibit diverse morphologies, including bipyramidal, cubic, circular, rhomboidal, and amorphous forms (Prieto *et al.*, 1997; Sauka & Benintende, 2008; Li *et al.*, 2024). It is important to emphasize that not all Cry proteins are synthesized during sporulation. For example, Cry3A is produced during the vegetative phase (Agaisse & Lereclus, 1995).

During the vegetative phase, *Bt* undergoes exponential growth in synthetic culture media containing a carbohydrate source such as glucose, a nitrogen source such as peptone or soybean extract, and mineral salts including magnesium, zinc, calcium, and manganese, among others. The optimal pH is approximately 7.0, the optimal growth temperature is 30 °C, and aerobic conditions are required. Upon nutrient depletion, the molecular machinery is activated to initiate sporulation—a process extensively studied in *B. subtilis* (Agaisse & Lereclus, 1995). Throughout sporulation, the cell generates a sporangium that divides into two compartments: the mother cell (where parasporal bodies containing Cry proteins accumulate) and the forespore that ultimately matures into the spore. When environmental conditions become favorable, the spore germinates, and the growth cycle is reinitiated (Lereclus, 2000; Cho & Chung, 2020).

***Bt* and Its Insecticidal Proteins**

Several *Bt* strains have been identified that exhibit toxicity against a wide spectrum of insects and invertebrates, including both agricultural pests and disease vectors such as mosquitoes. This lethality is attributable to the production of insecticidal proteins that form pores in the insect midgut or activate the alternative G-protein mediated apoptotic signalling pathway ultimately leading to death (Jurat-Fuentes *et al.*, 2021; Best *et al.*, 2023; Pacheco *et al.*, 2023).

Bt has emerged as the most widely used bacterium worldwide in the control of insect pests. This success is largely due to the diversity of pesticidal toxins it produces, which display high specificity, strong efficacy, and reduced environmental impact, as previously noted. Importantly, these pesticidal toxins are biodegradable through sunlight and environmental microorganisms, significantly reducing environmental residues (Jurat-Fuentes *et al.*, 2021; Li *et al.*, 2024; Soberón & Bravo, 2025).

In addition, *Bt* synthesizes other bioactive molecules such as cytolytic proteins (Cyt), vegetative insecticidal proteins (Vip), α - and β -exotoxins, enterotoxins, chitinases,

bacteriocins, hemolysins, phospholipases, among others. These compounds can be applied in both pest management and the neutralization of disease vectors, parasites, bacteria, and fungi (Gillis, 2013; Hernández, 2016; Syed *et al.*, 2020; Gupta *et al.*, 2021). Therefore, *Bt* produces a wide array of molecules that are lethal to insects, and their synergistic activity enhances its overall efficiency as an insecticide.

Cry Protein Family

The Cry protein family consists of nearly 800 genes, which, based on amino acid sequence homology, have been classified into 74 families (Cry1-Cry74; Crickmore *et al.*, 2021). Cry genes are predominantly located on plasmids, although some are also chromosomal.

Wild-type *Bt* strains exhibit variability in the number of plasmids; for example, the HD1 strain belonging to the kurstaki subspecies carries 12 plasmids (Faust *et al.*, 1979). One of these plasmids contains the gene encoding Cry1Ab, while another encodes four proteins: Cry1Aa, Cry1Ac, Cry2A, and Cry2B. This capacity to produce multiple toxins accounts for the high efficacy and broad applicability of *Bt* in commercial formulations since its discovery.

Mechanism of Action of Cry Proteins

Cry proteins, which are characterized by a three-domain (3d) structural organization, have been extensively investigated, particularly Cry1Ab. When *Bt* is ingested by susceptible insect larvae, the parasporal crystal inclusions are also consumed. Once released, these crystals are solubilized in the alkaline environment of the insect midgut (Höfte & Whiteley, 1989). Subsequently, the solubilized Cry protoxins require proteolytic activation to exert their function, a process that converts them into active pesticidal toxins (Höfte & Whiteley, 1989).

Model of Action: Receptor Binding and Pore Formation

Pesticidal toxins possess the ability to bind to specific receptors located in the membrane of the intestinal epithelium, such as aminopeptidase-N (APN) and alkaline phosphatase (ALP), both anchored via glycosylphosphatidylinositol (GPI). A significant affinity has also been reported for cadherins. This interaction promotes the oligomerization of 4 to 8 monomers, facilitating the formation of lytic pores in the membranes of midgut epithelial cells. The generation of these pores induces an osmotic imbalance that triggers deregulated ion fluxes, bacterial translocation, septicemia and ultimately leading to cell lysis (Pardo-López *et al.*, 2013; Gómez *et al.*, 2014; Bravo *et al.*, 2023; Soberón & Bravo, 2025).

Experimental approaches such as ligand blotting, surface plasmon resonance, and pulldown assays have demonstrated that the C-terminal region of Cry1Ab binds to APN and ALP but not to cadherins. This suggests that complementary receptors may interact with the region cleaved during protoxin activation, a subject of ongoing investigation (Peña-Cardena *et al.*, 2018). In addition to the aforementioned receptors, integral membrane proteins belonging to the ATP-binding cassette (ABC) transporter family have also been identified as receptors for Cry pesticidal toxins (Sato, 2024).

Three-Dimensional Structure of Cry Proteins

Cry proteins, with an estimated molecular weight of approximately 130 kDa, are composed of seven domains. During the activation process, however, the protoxin undergoes proteolytic processing, as previously described (Höfte & Whiteley, 1989). In this process, the C-terminal region comprising nearly 500 amino acids—including domains 4 through 7—is cleaved, resulting in an active pesticidal toxin of approximately 60 kDa that retains only three domains. These domains are responsible for receptor binding and pore formation. In laboratory practice, proteolytic processing is typically carried out with trypsin, yielding truncated versions analogous to those isolated from the midguts of susceptible insects (Soberón *et al.*, 2000; Peña-Cardena *et al.*, 2018).

Elucidation of the three-dimensional configuration of the active portion of Cry1Aa and Cry3A revealed the presence of three structural domains. Consequently, proteins with a similar architecture are referred to as three-domain (3d) Cry proteins (Li *et al.*, Loseva *et al.*, 2001; 1991; Grochulski *et al.*, 1995; Pacheco *et al.*, 2023).

Domain I consists of seven α -helices, six amphipathic helices arranged around a central hydrophobic helix ($\alpha 5$). In contrast, Domain II is composed of two short α -helices and three antiparallel β -sheets terminating in loop regions. Domain III is organized as a β -sandwich structure (Li *et al.*, 1991; Grochulski *et al.*, 1995).

Confirmation of the Three-Domain Structure in Cry Proteins

Subsequent investigations have corroborated this three-domain architecture in other proteins such as Cry3Bb1 (Galitsky *et al.*, 2001), Cry4Ba (Boonserm *et al.*, 2005), Cry4Aa (Boonserm *et al.*, 2006), Cry8Ea1 (Guo *et al.*, 2009), Cry5B (Hui *et al.*, 2012; Li *et al.*, 2022), Cry2Aa (Morse *et al.*, 2001), Cry7Ca1 (Jing *et al.*, 2019), Cry1Da (Wang *et al.*, 2019), and Cry11Aa and Cry11B (Tetreau *et al.*, 2022). Despite sequence diversity, all converge on the three-domain structure. This suggests a conserved folding pattern that supports the hypothesis of an analogous mode of action, the ultimate relevance of which lies in the induction of insect mortality. Achieving a comprehensive understanding of how these proteins function constitutes a critical step toward the development of strategies for insect pest management.

Several approaches—including site-directed mutagenesis, crystallographic and structural analyses, among others—have enabled the proposal of multiple models to explain the mechanism by which these proteins bind to receptors located in the midgut of lepidopterans. Among these are the “umbrella,” “buried dragon,” “knife,” and “folding cane” models, which suggest possible interaction sites with gut receptors, ultimately leading to insect toxicity. An excellent review of these models can be found in Pacheco *et al.*, 2023.

Strategies for Obtaining Insecticidal Proteins from *Bt*

Isolation of New Strains

The most widely used methodology for the detection of novel genes with insecticidal potential relies on the search for *Bt* strains in a free-living state. For this purpose, samples are collected from diverse habitats where entomopathogenic bacteria are likely to proliferate. Sampling sites include soil, foliage, dead insects, water from various sources, and organic

residues from different ecosystems. These samples are subsequently preserved under appropriate laboratory conditions until strain isolation, after which they are subjected to a range of analyses to facilitate identification (Figure 2). These procedures include Gram staining, a variety of biochemical assays, and microscopy for the detection of parasporal bodies (Bravo *et al.*, 1998).

With respect to the molecular identification of isolated strains, amplification is performed using bacterial DNA to target the 16S rRNA gene with universal oligonucleotides via polymerase chain reaction (PCR). The resulting sequences enable taxonomic identification at the genus and species levels (López *et al.*, 2003). Given that the majority of cry genes are typically plasmid-borne, their purification and amplification with universal oligonucleotides is essential. The obtained sequences are analyzed against databases such as the National Center for Biotechnology Information (NCBI), as well as other gene and protein repositories, with the aim of identifying novel genes encoding pesticidal toxins.

Purification of Cry Proteins

Initially, bacterial cultures are grown in liquid media until sporulation occurs. Cry proteins are accumulated within the cell and account for approximately 20-30% of the dry cell mass. In the laboratory, sporulated cultures are harvested by centrifugation to concentrate the bacterial biomass. The pellets are then subjected to sonication to achieve cell lysis and protein release. Fractionation of the cell lysate is performed using sucrose gradients through ultracentrifugation (Soberón *et al.*, 2000). Cry proteins are localized within one of the gradient phases, allowing their separation from the remaining cellular components.

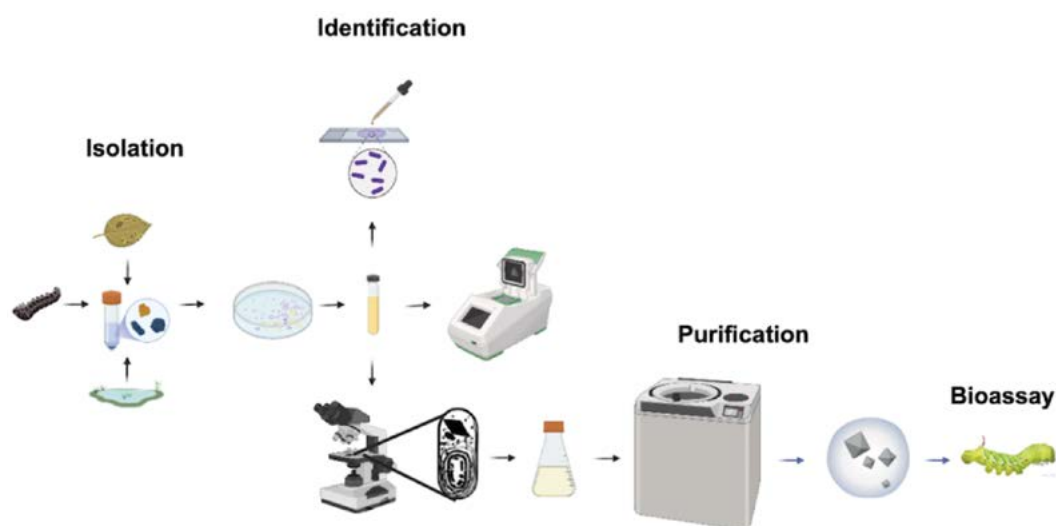


Figure 2. Isolation and identification of bioinsecticidal activity strains. To find new strains, they are isolated from environments where bacteria is likely to be found, such as water, dry leaves, dead insects and soil. The samples are then taken to the laboratory to isolate colonies, which are subjected to various tests such as Gram staining, molecular and microscopic analyses to find bacilli with inclusion bodies, which are then purified and fed to insects to determine their lethality.

The degree of purity is typically assessed using electrophoretic methods, which may be one-dimensional (1D), two-dimensional (2D), or capillary electrophoresis. In most cases, samples still exhibit contamination with other bacterial proteins, necessitating further purification using various chromatographic techniques, including affinity chromatography, ion-exchange chromatography, or size-exclusion chromatography, among others. These methodologies enable the isolation of Cry proteins in quantities and purity levels suitable for subsequent experimental studies.

Detection and Quantification of Cry Proteins

Conventional procedures for protein detection may include immunoassays such as ELISA and Western blot (Bravo *et al.*, 1998; Soberón *et al.*, 2000), both of which rely on antigen-antibody interactions. In the case of Cry proteins, certain assays may yield false positives due to cross-hybridization, an aspect that must be carefully considered. For protein quantification, spectrophotometric techniques such as the Lowry and Bradford assays can be employed. However, some limitations arise: spectrophotometric measurements provide an estimate of the total protein content in a sample rather than quantifying each individual protein present. Accurate determination of the specific Cry proteins within a sample requires highly efficient methodologies capable of precise estimation of each protein. This need has led to the development of reproducible and robust proteomic methods — such as liquid chromatography coupled with mass spectrometry— that allow the relative quantification of each protein in a given sample (Caballero *et al.*, 2020).

Genomics

The advent of next-generation sequencing (NGS) technologies has enabled rapid determination of complete genomic nucleotide arrangements. At present, this procedure is relatively straightforward and cost-effective, allowing accurate elucidation of the nucleotide sequences of genes of interest, whether located on plasmids or chromosomes. This, in turn, facilitates the comprehensive characterization of strains with biotechnological potential (Yamamoto, 2022). Availability of such sequences also allows for the detection of variants and comparative analyses across gene databases. The identification of nucleotide sequences of novel cry genes constitutes essential information used in genetic engineering tools to clone genes into heterologous systems —whether bacteria or plants— with the purpose of enhancing production or synthesizing proteins in more controlled systems (Baum *et al.*, 1999).

Mutagenesis and Protein Engineering

Several tools enable the generation of site-specific mutants for the study of Cry proteins, with site-directed mutagenesis being among the most widely used. Once the DNA sequences are identified, they can be altered through specific mutations incorporated during oligonucleotide design to achieve the desired modification. The oligonucleotides are annealed to the bacterial template DNA and amplified via PCR. The amplified product is then sequenced to confirm the mutation, followed by cloning into expression vectors (Yamamoto, 2022).

Another approach is DNA shuffling, a technique used to restructure and recombine cry genes in order to generate multiple point mutants. This allows researchers to study the effects of discrete amino acid changes. In this method, a gene of interest is amplified and subsequently fragmented using DNase I, generating random fragments that are recombined and re-amplified. Sequences with point mutations differing from the original gene are then selected for cloning (Yamamoto, 2022).

These tools enable modifications such as single amino acid substitutions, facilitating the study of structural changes that affect protein function and the creation of optimized variants to enhance insecticidal efficacy. They have also allowed the generation of diverse mutants that clarify which amino acids are critical for efficient toxin-receptor interactions. Additionally, chimeric proteins—created by exchanging entire domains between different Cry proteins—have been employed to elucidate the roles of specific domains in the mode of action.

In parallel, with experimental approaches, *in silico* methods such as structural modelling molecular docking, and computational mutagenesis have been employed to engineer Cry proteins and accelerating the developments of optimized variants.

Bioassays

When a strain with insecticidal potential is identified, one of the most critical evaluations for establishing its biocidal activity is performing bioassays to assess efficacy. This involves maintaining insect colonies under controlled conditions (temperature, humidity, and light) with a diet tailored to the developmental stage of the insects. Rearing insects in the laboratory is labor-intensive and costly but is indispensable for obtaining reliable results regarding protein toxicity. Bioassays allow the direct administration of bacteria or purified proteins for testing in live insects (Soberón *et al.*, 2000; Yamamoto, 2022).

Additionally, maintaining insect colonies facilitates the collection of midgut vesicles, which are essential for *in vitro* experiments that indirectly evaluate pore formation induced by Cry proteins, linking this activity to the observed insecticidal toxicity.

Transgenic Plants with cry Genes

Since the creation of the first transgenic plants in 1983 using *Agrobacterium tumefaciens*, genetic modification of agronomically important plant species has become feasible. This modification involves transferring genes from other species to confer novel traits that provide advantages over their wild-type counterparts (Herrera-Estrella *et al.*, 1983; Sanahuja *et al.*, 2011). In addition to this methodology, biolistics (gene gun technology) was developed, enabling the introduction of genes into plants and is widely used today. Using biolistics, the first genetically modified maize lines were created by incorporating the Cry1AB gene, which is lethal to lepidopterans. This allowed the generation of plants capable of producing their own insecticidal proteins, referred to as *Bt* maize.

This innovation represented a significant step toward the gradual replacement of conventional synthetic pesticides. In 1998, Monsanto introduced the first transgenic maize to the market. These plants constitutively express the *Bt*-encoded Cry1Ab protein, which

is lethal to susceptible lepidopterans that consume plant tissues, thereby enhancing plant protection and growth.

Molecular detection of cry gene expression in plants is typically performed using quantitative reverse transcription PCR (RT-qPCR) to expedite assay processing. This technique also allows relative quantification of cry gene expression and is more efficient than endpoint PCR, which requires longer processing times.

Transgenic plants incorporating *Bt* genes are collectively referred to as *Bt* plants. Currently, several transgenic *Bt* species exist, including maize, cotton, soybean, canola, tomato, potato, and rice, among others (Sanahuja *et al.*, 2011). These plants have been widely adopted in several agricultural systems worldwide; more than 50% of the global cultivated area of genetically modified organisms (GMOs) consists of crops expressing cry genes. The implementation of this technology has been associated with reduced reliance on chemical insecticides and agronomic benefits in many production contexts; however, the magnitude of these effects depends on crop species, regional management practices, and regulatory and ecological considerations.

Resistance and Defense Strategies of Pests against Cry Proteins

The progressive resistance of insects to Cry proteins mirrors that observed with synthetic insecticides. Continuous exposure to these toxins exerts selective pressure, promoting the emergence of resistant individuals. Understanding the mechanisms involved is essential to develop strategies that delay resistance and enable the design of more effective pesticidal proteins (Fabrick & Wu, 2023).

Key mechanisms by which insects acquire resistance include:

1. **Weak toxin-receptor interactions:** Site-directed mutagenesis studies on cry genes have demonstrated the critical role of amino acids in receptor binding and pore formation (Pacheco *et al.*, 2023). Amino acid substitutions in the laboratory provide insights into processes leading to insect mortality. Mutations can also occur in genes encoding the receptors themselves, which may disrupt toxin-receptor interactions and hinder toxin action.
2. **Insufficient solubilization and poor protoxin digestion:** Altered expression or reduced activity of midgut proteases can impair protoxin activation, preventing optimal function. Disruptions in the alkaline conditions of the insect midgut can also reduce solubility, limiting pesticidal activity (Pinos *et al.*, 2021).
3. **Sequestration or immobilization of activated toxins:** Upon reaching midgut cells, toxins may be sequestered. Molecules such as esterases and glycolipids have been documented to perform this role (Gunning *et al.*, 2005; Pinos *et al.*, 2021). This process may function as a detoxification mechanism, reducing effective toxin-receptor interactions.
4. **Alteration of membrane components:** At sublethal toxin concentrations, pore formation may occur but without causing severe damage, allowing membrane repair. Endocytic processes, in which the membrane invaginates to form vesicles that are degraded or recycled, facilitate repair (Cytrynska *et al.*, 2016). These

membrane repair mechanisms enhance insect survival and may have evolved over time.

5. **Cumulative selective pressure:** Continuous exposure to a specific pesticidal toxin exerts persistent selection on a population, ultimately promoting resistance development. Resistant insects emerge, complicating pest management and eradication in agricultural areas (Sanahuja *et al.*, 2011).

The simultaneous occurrence of several of these mechanisms in a single insect can generate super-resistant pests, adding an additional layer of complexity to pest control (Wu *et al.*, 2014; Tabashnik *et al.*, 2021; Fabrick & Wu, 2023).

The resistance mechanisms described above are the focus of study in numerous laboratories worldwide, driven by the search for strategies to prevent the emergence of resistant insects. This research enables the optimization of pesticidal toxins to enhance their affinity and specificity, thereby helping to slow the progression of resistance (Fabrick & Wu, 2023).

It is also important to highlight that alternative strategies are being investigated to counteract insect resistance. For example, synergistic combinations of different Cry toxins, or of Cry toxins with other bioactive molecules, are being explored to increase efficacy. However, as noted previously, certain Cry protein receptors—such as APN, ALP, cadherin, and ABC transporters—exhibit variations in their interactions depending on the specific Cry protein studied. Molecular-level investigations are providing insights into how these interactions occur (Pacheco *et al.*, 2023; Bravo & Soberón, 2025).

The accumulation of experimental evidence has revealed the complexity of toxin-receptor binding, which is highly specific and dependent on the particular protein involved. Moreover, although numerous potential receptors have been identified, not all interactions result in insect mortality. This underscores the challenges in analyzing resistance mechanisms and designing effective countermeasures, as the full process is not yet completely understood (Sato, 2024).

CONCLUSION

Cry proteins produced by *Bt* were initially identified as insecticidal molecules. This led to their structural and functional characterization, which resulted in advances understanding their mechanism of action at a molecular level, as well as revealing the range of their diversity and laying the foundations for their application as biological insecticides.

Through the study of Cry protein diversity and using *in vivo* and *in silico* experimental strategies, the efforts to improve the insecticidal efficacy of these proteins continue, while also considering the management of insect resistance.

An important applied aspect of Cry proteins has been the generation of transgenic crops to combat specific pests in certain agricultural regions of the planet, where their role as a component of modern agricultural systems is important, particularly outside the centers of origin and diversification of cultivated plants.

Finally, the activity of *Bt* toxins against insects that are vectors of human diseases highlights the relevance of research beyond agricultural applications.

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REFERENCES









- Agaisse, H. & Lereclus, D. (1995) How does *Bacillus thuringiensis* produce so much insecticidal crystal protein?. *Journal of Bacteriology*. 177(21), 6027-6032. Doi: 10.1128/jb.177.21.6027-6032.1995
- Best, H.L., Williamson, L. J., Heath, E.A., Waller-Evans, H., Lloyd-Evans, E. and Berry, C. (2023). The role of glycoconjugates as receptors for insecticidal proteins, *FEMS Microbiology Reviews*, 47:4. Doi.org/10.1093/femsre/fuad026
- Berry, C., Valby, V, Mishra, R, Bryony, C.B., Palma, L. and Crickmore, N. (2025). Specificity database for bacterial pesticidal proteins against invertebrate targets. *Journal of Invertebrate Pathology*. 211, 108319. Doi.org/10.1016/j.jip.2025.108319
- Baum, J.A., Johnson, T.B. & Carlton, B.C. (1999). *Bacillus thuringiensis*: Natural and recombinant bioinsecticide products: Use and Delivery. 189-209. Methods in Biotechnology, vol. 5: Biopesticides: Use and Delivery Edited by: F. R. Hali and J. J. Menn Humana Press Inc., Totowa, NJ
- Boonserm, P., Davis, P., Ellar, D.J. & Li, J. (2005). Crystal structure of the mosquito-larvicidal toxin Cry4Ba and its biological implications. *Journal of Molecular Biology*. 348, 363-382. Doi: 10.1016/j.jmb.2005.02.013
- Boonserm, P., Mo, M., Angsuthanasombat, C. and Lescar, J (2006). Structure of the functional form of the mosquito larvicidal Cry4Aa toxin from *Bacillus thuringiensis* at a 2.8-angstrom resolution. *Journal of Bacteriology*. 188, 3391-340. Doi: 10.1128/JB.188.9.3391-3401.2006
- Bravo, A., Sarabia, S., Lopez, L., Ontiveros, H., Abarca, C., Ortiz, A., Ortiz, M., Lina, L., Villalobos, F. J., Peña, G., Nuñez-Valdez, M. E., Soberón, M., & Quintero, R. (1998). Characterization of cry genes in a Mexican *Bacillus thuringiensis* strain collection. *Applied and Environmental Microbiology*, 64(12), 4965-4972. Doi:10.1128/AEM.64.12.4965-4972.1998.
- Bravo, Alejandra., Pacheco, S., Gómez, I., & Soberón, M. (2023). Mode of action of *Bacillus thuringiensis* Cry pesticidal proteins. En *Advances in Insect Physiology* (pp. 55-92). Elsevier.
- Caballero, J., Jiménez-Moreno, N., Orera, I., Williams, T., Fernández, A.B., Villanueva, M., Ferré, J., Caballero, P. & Ancín-Azpilicueta, C. (2020). Unraveling the composition of insecticidal crystal proteins in *Bacillus thuringiensis*: a proteomics approach. *Applied and Environmental Microbiology*. 86:e00476-20. Doi.org/10.1128/AEM .00476-20.
- Cho, W.I. & Chung, M.S. (2020). Bacillus spores: a review of their properties and inactivation processing technologies. *Food Science Biotechnology* 29, 1447-1461. Doi.org/10.1007/s10068-020-00809-4
- Crickmore, N., Berry, C., Panneerselvam, S., Mishra, R., Connor, T.R & Bonning, B.C. (2021). A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. *Journal of Invertebrate Pathology*. 186:107438. Doi: 10.1016/jip.2020.107438.
- Cytrynska, M., Wojda, I. & Jakubowicz, T. (2016). How insects combat infections, p 117-128. In *Lessons in immunity*. Academic Press, Inc, New York, NY.
- Daraban, G.M., Hlihor, R.M. & Suteu, D. (2023). Pesticides vs. Biopesticides: From Pest Management to Toxicity and Impacts on the Environment and Human Health. *Toxics*. 17(12):983. Doi: 10.3390/toxics11120983.
- de Maagd, R.A., Bravo, A., Berry, C., Crickmore, N. & Schnepf, H.E. (2003). Structure, diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria. *Annual Review of Genetics*. 37:409-33. Doi: 10.1146/annurev.genet.37.110801.143042.
- de Sousa, T.K., Silva, A.T.D. & Soares, F.E.F. (2025). Fungi-Based Bioproducts: A Review in the Context of One Health. *Pathogens*. 14(5):463. Doi: 10.3390/pathogens14050463.
- Devine, F. & Furlong, M. (2007). Insecticide use: Contexts and ecological consequences. *Agriculture and Human Values*. 24. 281-306. doi:10.1007/s10460-007-9067-z
- Fabrick, J.A. and Wu, Y. (2023). Mechanisms and molecular genetics of insect resistance to insecticidal proteins from *Bacillus thuringiensis*. *Advances in Insect Physiology*, Academic Press. 65:123-183. ISBN 9780323954662
- Faust, R.M., Spizizen, J., Gage, V. & Travers, R.S. (1979). Extrachromosomal DNA in *Bacillus thuringiensis* var. *kurstaki*, var. *finitimus*, var. *sotto*, and in *Bacillus popilliae*. *Journal of Invertebrate Pathology*. 33:2, 233-238. Doi:10.1016/0022-2011(79)90158-7.

- Fernández-Chapa, D., Ramírez-Villalobos, J. and Galán-Wong, L. (2019). Toxic Potential of *Bacillus thuringiensis*: An Overview. 10.5772/intechopen.85756. Doi: 10.5772/intechopen.85756
- Galitsky, N., Cody, V., Wojtczak, A., Ghosh, D., Luft, J.R., Pangborn, W. & English, L. (2001) Structure of the insecticidal bacterial δ -endotoxin Cry3Bb1 of *Bacillus thuringiensis*. *Acta Crystallographica*. 57, 1101-1109. Doi: 0.1107/s0907444901008186.
- Gillis, A. (2013). El arsenal de *Bacillus thuringiensis*: su uso en manejos agrícolas y en la industria alimentaria. (The arsenal of *Bacillus thuringiensis*: its use in agricultural management and in the food industry). *Entomotropica*. 28:106-107.
- Gómez, I., Sánchez, J., Muñoz-Garay, C., Matus, V., Gill, S.S., Soberón, M. & Bravo, A. (2014). *Bacillus thuringiensis* Cry1A toxins are versatile proteins with multiple modes of action: two distinct pre-pores are involved in toxicity. *Biochemical Journal*. 459(2):383-96. Doi: 10.1042/BJ20131408.
- Grochulski, P., Masson, L., Borisova, S., Pusztai-Carey, M., Schwartz, J.L., Brousseau, R. & Cygler, M. (1995). *Bacillus thuringiensis* CryIA(a) insecticidal toxin: Crystal structure and channel formation. *Journal of Molecular Biology*. 254, 447-464. Doi: 10.1006/jmbi.1995.0630
- Gula, L.T. (2023.) Researchers Helping Protect Crops from Pests. Available online: <https://www.nifa.usda.gov/about-nifa/blogs/researchers-helping-protect-crops-pests> (Accessed November 23, 2025).
- Gunning, R.V., Dang, H.T., Kemp, F.C., Nicholson, I.C. & Moores, G.D. (2005). New Resistance Mechanism in *Helicoverpa armigera* Threatens Transgenic Crops Expressing *Bacillus thuringiensis* Cry1Ac Toxin. *Applied of Environmental Microbiology*. 71: Doi.org/10.1128/AEM.71.5.2558-2563.2005
- Guo, S., Ye, S., Liu, Y., Wei, L., Xue, J., Wu, H., Song, F., Zhang, J., Wu, X., Huang, D., et al. (2009). Crystal structure of *Bacillus thuringiensis* Cry8Ea1: An insecticidal toxin toxic to underground pests, the larvae of *Holotrichia parallela*. *Journal of Structural Biology*. 168, 259-266. Doi: 10.1016/j.jsb.2009.07.004.
- Gupta, M., Kumar, H. & Kaur, S. (2021). Vegetative insecticidal protein (Vip): A potential contender from *Bacillus thuringiensis* for efficient management of various detrimental agricultural pests. *Frontiers in Microbiology*. 12: 659736. Doi: 10.3389/fmicb.2021.659736.
- Hernández Fernández, J. (2016). *Bacillus thuringiensis*: a Natural Tool in Insect Pest Control. *The Handbook of Microbial Bioresources*. CABIS. doi.org/10.1079/9781780645216.0121
- Herrera-Estrella, L., Block, M.D., Messens, E., Hernalsteens, J.P., Montagu, M.V. & Schell, J. (1983). Chimeric genes as dominant selectable markers in plant cells. *The EMBO Journal* 6:987-95. Doi: 10.1002/j.1460-2075.1983.tb01532.x.
- Höfte, H. & Whiteley, H. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews*. 53:242-255. Doi: 10.1128/mr.53.2.242-255.1989.
- Hui, F., Scheib, U., Hu, Y., Sommer, R.J., Aroian, R.V. & Ghosh, P. (2012). Structure and glycolipid binding properties of the nematocidal protein Cry5B. *Biochemistry*. 51:9911-9921. Doi: 10.1021/bi301386q.
- Jing, X., Yuan, Y., Wu, Y., Wu, D., Gong, P. & Gao, M. (2019). Crystal structure of *Bacillus thuringiensis* Cry7Ca1 toxin active against *Locusta migratoria manilensis*. *Protein Science*. 28: 609-619. Doi: 10.1002/pro.3561.
- Jurat-Fuentes, J.L., Heckel, D.G. & Ferré, J. (2021) Mechanisms of Resistance to Insecticidal Proteins from *Bacillus thuringiensis*. *Annual Review Entomology*. 66:121-140. Doi: 10.1146/annurev-ento-052620-073348.
- Karabörklü, S., Azizoglu, U., & Azizoglu, Z.B. (2018). Recombinant entomopathogenic agents: a review of biotechnological approaches to pest insect control. *World Journal of Microbiology and Biotechnology*. 34:14. Doi:10.1007/s11274-017-2397-0.
- Kim, J.C., Hwang, I.M., Kim, H.M., Kim, S., Shin, T.S., Woo, S.D. & Park, H. W. (2024). Rapid analysis of insecticidal metabolites from the entomopathogenic fungus *Beauveria bassiana* 331R using UPLC-Q-Orbitrap MS. *Mycotoxin Research*. 40(1):133. Doi: 10.1007/s12550-023-00516-z.
- Lereclus, D., Agaisse, H., Grandvalet, C., Salamitou, S. and Gominet, M. (2000). Regulation of toxin and virulence gene transcription in *Bacillus thuringiensis*. *International Journal of Medical Microbiology*. 295-9. Doi: 10.1016/S1438-4221(00)80024-7
- Li, J., Carroll, J. & Ellar, D.J. (1991). Crystal structure of insecticidal δ -endotoxin from *Bacillus thuringiensis* at 2.5 Å resolution. *Nature*. 353, 815-821. Doi:10.1038/353815a0.
- Li, J., Wang, L., Kotaka, M., Lee, M.M. & Chan, M.K. (2022). Insights from the Structure of an Active Form of *Bacillus thuringiensis* Cry5B. *Toxins* 2022, 14, 823. Doi: 10.3390/toxins14120823
- Li, K., Chen, M., Shi, J. & Mao, T. (2024). An overview of the production and use of *Bacillus thuringiensis* toxin. *Open Life Science*. 19(1):20220902. Doi: 10.1515/biol-2022-0902.
- López, I., Ruiz-Larrea, F., Cocolin, L., Orr, E., Phister, T., Marshall, M. & Mills, D.A. (2003). Design and evaluation of PCR primers for analysis of bacterial populations in wine by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*. 69(11), 6801-6807. Doi: 10.1128/AEM.69.11.6801-6807.2003

- Loseva, I., Tiktopulo, E.I., Vasiliev, V.D., Nikulin, A.D., Dobritsa, A.P., Potekhin, S.A. (2001). Structure of Cry3A d- endotoxin within phospholipid membranes. *Biochemistry* 40:14143-51451. Doi: 10.1021/bi010171w
- Loth, K., Costechareyre, D., Effantin, G., Rahbe, Y., Condemine, G., Lando, C. & da Silva P. (2015) New Cyt-like delta-endotoxins from *Dickeya dadantii*: structure and aphicidal activity. *Scientific reports* 5, 8791. Doi: 10.1038/srep08791
- Mitsuhashi, W. (2025). Studies on insect virus-producing proteins as potential synergists for microbial insecticides: status and prospects. *Virus Genes*. Doi: 10.1007/s11262-025-02162-2.
- Morse, R.J., Yamamoto, T. & Stroud, R.M (2001). Structure of Cry2Aa suggests an unexpected receptor binding epitope. *Structure*. 9, 409-417. Doi: 10.1016/s0969-2126(01)00601-3.
- Ng, K.H. & Zuharah, W.F. (2025). Effectiveness and the synergism effects of *Ipomoea cairica* leaf plant extract and *Metarhizium antsopliae* fungi (Meta-G4) against larvae of *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse (Diptera: Culicidae). *Journal of Insect Science* 5(3):2. Doi: 10.1093/jisesa/ieaf044.
- Pacheco, S., Gómez, I., Peláez-Aguilar, A. E., Verdusco-Rosas, L. A., García-Suárez, R., do Nascimento, N. A., Rivera-Nájera, L. Y., Cantón, P. E., Soberón, M., & Bravo, A. (2023). Structural changes upon membrane insertion of the insecticidal pore-forming toxins produced by *Bacillus thuringiensis*. *Frontiers in Insect Science*, 3, 1188891. Doi.org/10.3389/finsc.2023.1188891
- Pardo-López L., Soberón, M. & Bravo, A. (2013). *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. *FEMS Microbiology Reviews*. 37(1):3-22. Doi: 10.1111/j.1574-6976.2012.00341.x.
- Peña-Cardena, A., Grande, R., Sánchez J, Tabashnik, B.E., Bravo, A., Soberón, M. & Gómez, I. (2018). The C-terminal protoxin region of *Bacillus thuringiensis* Cry1Ab toxin has a functional role in binding to GPI-anchored receptors in the insect midgut. *Journal of Biological Chemistry*. 293(52):20263-20272. Doi: 10.1074/jbc.RA118.005101.
- Pinos, D., Andrés-Garrido, A., Ferré, and Hernández-Martínez, P. (2021). Response Mechanisms of Invertebrates to *Bacillus thuringiensis* and Its Pesticidal Proteins. *Microbiology and Molecular Biology Reviews*. 85(1):e00007-20. Doi: 10.1128/MMBR.00007-20.
- Prieto, D., Vázquez, R., Ayra, C., González, J. & de la Riva, G. A. (1997). *Bacillus thuringiensis*: from biodiversity to biotechnology. *Journal of Industrial Microbiology & Biotechnology* 19, 202-219. Doi: 10.1038/sj.jim.2900460
- Roh, J.Y., Choi, J.Y., Li, M.S., Jin, B.R. & Je, Y.H. (2017). *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. *International Journal of Microbiology. Biotechnology*. 17:547.
- Sanahuja, B., Banakar, R., Twyman, R.M., Capell, T. & Christou, P. (2011), *Bacillus thuringiensis*: a century of research, development and commercial applications. *Plant Biotechnology Journal*, 9: 283-300. Doi:10.1111/j.1467-7652.2011.00595.x
- Sato, R. (2024). Utilization of Diverse Molecules as Receptors by Cry Toxin and the Promiscuous Nature of Receptor-Binding Sites Which Accounts for the Diversity. *Biomolecules*. 425. Doi.org/10.3390/biom14040425
- Sauka, D. & Benintend, G. (2008). *Bacillus thuringiensis*: generalidades. Un acercamiento a su empleo en el biocontrol de insectos lepidópteros que son plagas agrícolas. *Revista Argentina de Microbiología*. 40, 124-140.
- Soberón M, & Bravo A. (2025). The Future of Bt Proteins: From Pore Formation and Insect Resistance to the Next Generation of Pest Control. *Toxins*. Doi: 10.3390/toxins17110522.
- Soberón, M., Pérez, R.V., Nuñez-Valdéz, M.E., Lorence, A., Gómez, I., Sánchez, J. & Bravo, A. (2000) Evidence for intermolecular interaction as a necessary step for pore-formation activity and toxicity of *Bacillus thuringiensis* Cry1Ab toxin. *FEMS Microbiology Letters*. 191(2):221-5. Doi: 10.1111/j.1574-6968.2000.tb09343.x
- Springer, K., Sängler, P.A., Moritz, C., Felsl, A., Rattei, T. & Fuchs, T.M. (2018). Insecticidal Toxicity of *Yersinia frederiksenii* Involves the Novel Enterotoxin YacT. *Frontiers in Cellular and Infection Microbiology*. 8:392. Doi: 10.3389/fcimb.2018.00392.
- Syed, T., Askari, M., Meng, Z., Li, Y., Abid, M.A., Wei, Y., Guo, S., Liang, C. & Zhan, R. (2020). Current Insights on Vegetative Insecticidal Proteins (Vip) as Next Generation Pest Killers. *Toxins*. 13, 200. Doi: 10.3390/toxins12080522.
- Tabashnik, B.E., Liesner, L.R., Ellsworth, P.C., Unnithan, G.C., Fabrick, J.A., Naranjo, S.E., Li, X., Dennehy, T.J., Antilla, L., Staten, R.T. & Carrière, Y. (2021). Transgenic cotton and sterile insect releases synergize eradication of pink bollworm a century after it invaded the United States. *Proceedings of the National Academy of Sciences U S A*. 118(1):e2019115118. Doi: 10.1073/pnas.2019115118

- Tetreau, G., Sawaya, M.R., De Zitter, E., Andreeva, E.A., Banneville, A.-S., Schibrowsky, N.A., Coquelle, N., Brewster, A.S., Grünbein, M.L., Kovacs, G.N., *et al.* (2022). De novo determination of mosquitocidal Cry11Aa and Cry11Ba structures from naturally occurring nanocrystals. *Nature Communications*. 13, 4376. Doi: 10.1038/s41467-022-31746-x.
- Valtierra-de-Luis, D., Villanueva, M., Berry, C. & Caballero, P. (2020). Potential for *Bacillus thuringiensis* and other bacterial toxins as biological control agents to combat dipteran pests of medical and agronomic importance. *Toxins*. 12, 773. Doi: 10.3390/toxins12120773.
- Walsh, T.K., Heckel, D.G., Wu, Y., Downes, S., Gordon, K.H.J. & Oakeshott, J.G. (2022). Determinants of Insecticide Resistance Evolution: Comparative Analysis Among Heliothines. *Annual Review of Entomology*. 67:387-406. Doi: 10.1146/annurev-ento-080421-071655.
- Wang, Y., Wang, J., Fu, X., Nageotte, J.R., Silverman, J., Bretsnyder, E.C., Chen, D., Rydel, T.J., Bean, G.J., Li, K.S., *et al.* (2019). *Bacillus thuringiensis* Cry1Da₇ and Cry1B.868 Protein Interactions with Novel Receptors Allow Control of Resistant Fall Armyworms, *Spodoptera frugiperda* (J.E. Smith). *Applied and Environmental Microbiology*. 85, e00579-19. Doi: 10.1128/AEM.00579-19
- Wu, Y. (2014). Chapter Six - Detection and Mechanisms of Resistance Evolved in Insects to Cry Toxins from *Bacillus thuringiensis*. *Advances in Insect Physiology*. 47:297-342, Editor(s): Tarlochan S. Dhadialla, Sarjeet S. Gill, Academic Press.
- Yamamoto, T. (2022). Engineering of *Bacillus thuringiensis* insecticidal proteins. *Journal of Pesticide Science*. 47(2):47-58. Doi: 10.1584/jpestics.D22-016.

Physicochemical evaluation of the meat of the American Bullfrog (*Lithobates catesbeianus*)

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ABSTRACT

Objective: To determine the physicochemical characteristics of bullfrog (*Lithobates catesbeianus*) meat.

Design/methodology/approach: Fifteen samples of bullfrog legs and fifteen samples of chicken meat were used. Subsequently, 50% of the meat was cooked in a water bath.

Results: Bullfrog meat had less protein and more fat ($P < 0.05$) than chicken meat. The percentage of moisture and collagen was higher in bullfrog meat ($P < 0.05$). The L*, a*, and b* values showed significant differences, with greater luminosity in cooked bullfrog meat, while a* showed a variation of 2.36 units in raw vs. cooked meat. b* was higher in cooked meat ($P < 0.05$) compared to raw meat. The parameters water activity, water-holding capacity, and pH showed significant differences ($P < 0.05$) between the two treatments analyzed.

Limitations/Implications of the study: The number of bullfrog samples was limited due to their cost and producers' distrust of institutional and governmental organizations.

Findings and Conclusion: Bullfrog meat is lean. The meat presented water activity values below the established limit for meat. This species is a viable alternative in the food sector.

Keywords: Meat, Bullfrog, raw meat, cooked meat, evaluation, physicochemical.

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INTRODUCTION

The introduction of species outside their natural habitat occurs intentionally or accidentally as a result of human activities. This process generates adaptation mechanisms that allow species to survive in new environments. The absence of natural predators favors rapid population growth. This population increase facilitates the establishment of these species as pests and causes negative ecological effects (Casas *et al.*, 2001). In this context, the American bullfrog (*Lithobates catesbeianus*) is among the main drivers of the decline in amphibian populations worldwide (Laufer *et al.*, 2008).



Consuming frog meat is an age-old practice. In ancient Greece, frog meat was considered a delicacy. In Asian countries such as China, consumption of frog meat dates back more than 4,000 years. Currently, this meat remains widely recognized as a regional food. *Rana catesbeiana* is a species native to North America. Intensive frog farming began in the United States around 1915. Subsequently, during the 19th century, countries such as Mexico, Canada, Japan, and Brazil incorporated their production and consumption. Since then, frog meat has been introduced as an alternative food in industrialized Western countries and in various Latin American nations, such as Chile, Venezuela, and Argentina (Aragón, 2010). In Mexico, frog farming has gained importance in recent years. This activity responds to growing domestic demand and the export of specimens for research, teaching, and food. National production is concentrated mainly on the domestic market. Tourist areas account for the largest share of production (Ojeda, 2020). Likewise, population growth increases demand for food for human consumption. In this context, frog farming represents an activity with high productivity and economic potential. This potential is especially evident in the species *Lithobates catesbeianus* (Méndez, 2014).

Limited statistical data is available on bullfrog production and consumption. In 1980, aquaculture supplied approximately 3% of the global bullfrog market. By 2002, this proportion had increased to nearly 15%. Taiwan, Mexico, and Brazil led global production of this species during that period (Islas *et al.*, 2020; Islas *et al.*, 2021). In Mexico, the main bullfrog-producing states are the State of Mexico, Sinaloa, Nayarit, and Jalisco. Production in Jalisco reached approximately 20 tons in 2015. In 2016, production increased by 3.5 tons. In 2017, total production reached 26 tons. Bullfrogs have been successfully introduced to at least 16 states in the country. The State of Mexico stands out among the regions with a significant presence of this species. Official records indicate a sustained increase: national production rose from 5 tons in 2009 to 71 tons in 2014. This change represented a total increase of 66 tons over five years. The region's climate favors the development of the American bullfrog. The geographic location of the State of Mexico contributes to the establishment of the species. Physical and biological heterogeneity characterizes this region due to the diversity of physiographic, geographic, and hydrological regions, which increases the area's biotic richness (Aguilar *et al.*, 2009).

In the State of Mexico, the American bullfrog has gradually become established in various municipalities. Records indicate the species' presence in 39 municipalities. These include Almoloya de Juárez and Almoloya del Río. It is also found in Amatepec and Capulhuac, specifically in the town of San Miguel Almaya. Additionally, the species is present in Chicoloapan, in the town of San Vicente Chicoloapan, and in Chimalhuacán, Coatepec de Harinas, Ecatzingo, and Huehuetoca, in the town of Jorobas. The distribution includes Ixtapaluca, Ixtapan de la Sal and Jilotepec, Lerma, Malinalco, Metepec, Naucalpan, Nepantla, Ocoyoacac, Oztolotepec, San Antonio La Isla, San Mateo Atenco, San Simón de Guerrero, Santa María Rayón, Santo Tomás de los Plátanos, Sultepec, Tejupilco, Temascaltepec, Tenancingo, Tenango, Tepetlixpa, Tepotzotlán, Texcoco, Tlatlaya, Toluca, Valle de Bravo, Villa Victoria and Zumpahuacán (Casas *et al.*, 2001).

The American bullfrog adapts to temperate and warm zones with suitable aquatic systems. The species can thrive at altitudes up to 2,600 meters above sea level. Some

reports indicate the species' ability to adapt to colder climates. In the Lerma region and along its river, a government-run breeding facility was established during the 1960s. The facility was located in the community of San Pedro Tlaltizapán, in the municipality of Tianguistenco. At this site, the American bullfrog exhibited a period of hibernation during the winter. This behavior was associated with the organism's poikilothermic nature. Generally speaking, the species thrives across a wide altitudinal range. Development occurs when water is available. It also requires adequate relative humidity. The species develops at ambient temperatures between 20 and 30 °C (Méndez Béjar, 2014). However, aquaculture activities in the region have declined. This decrease was due to the need to allocate the drinking water resource to supply other areas surrounding the Toluca Valley (Pillado and Viesca, 2013).

The culinary tradition of frog meat consumption has declined in recent years. Overexploitation of these species has contributed to this reduction. This process has led to frog meat currently being considered a high-value commercial product. In this context, frog farming presents significant production potential. This activity generates various byproducts for human consumption. The meat and its derivatives are the main products of interest. Frog legs represent the product with the highest export volume. Restaurants and supermarkets require this product to prepare gourmet dishes (Rodríguez, 2014; Vargas, 2015). The skin is another relevant byproduct; this material is mainly used in the manufacture of leather goods, such as wallets and belts. The byproduct represents approximately 11% of the animal's total weight. After harvesting, the skin must undergo sterilization before distribution. Other byproducts of interest include the viscera and liver. The viscera are used in the production of suture thread. The liver is used in the production of pâté. Additionally, the frog's body is used in cosmetic applications, such as the production of moisturizing creams (Rodríguez, 2014; Vargas, 2015).

The objective of this study was to perform a physicochemical evaluation of bullfrog meat. The meat of this species appears to have a high protein, mineral, and essential amino acid content for human consumption. It also contains mineral salts and is low in fat. The net meat yield represents approximately 60% of the animal's live weight. Frog legs constitute between 33% and 35% of the live weight (Vásconez and Rodríguez, 2003). However, the available scientific literature on the quality of bullfrog meat and the benefits associated with its consumption is limited.

MATERIALS AND METHODS

Meat quality is defined by its physicochemical characteristics. These characteristics include organoleptic and instrumental properties. Organoleptic properties include tenderness, juiciness, color, and flavor, among others (Guerrero, 2002). For the physicochemical analysis, the study used 15 bullfrog leg samples and 15 chicken meat samples, the latter serving as a positive control. The procedure involved defleshing the samples beforehand, followed by cooking 50% of the meat in a water bath at 60 °C for 20 minutes. Proximate chemical analysis was performed using a FoodScan™ Lab meat analyzer (Figure 1). Once the equipment was calibrated, the analysis was performed on 180 g samples placed in sample holders. The procedure avoided the formation of air

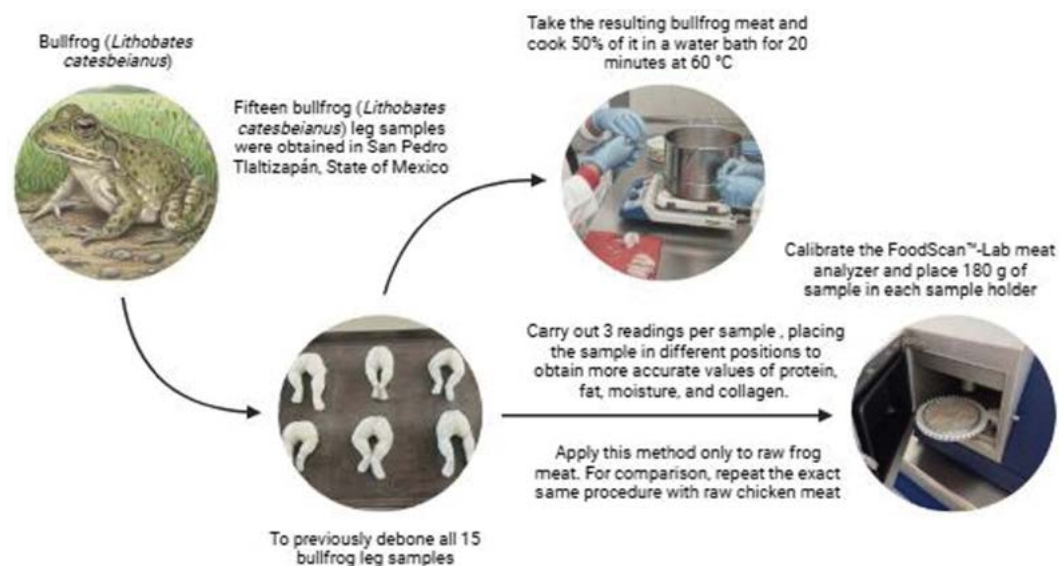


Figure 1. Procedure for the Proximate Analysis and Comparison of Raw Meat Using the FoodScan™-Lab Analyzer.

pockets and bubbles in the meat, thereby preventing alterations in the sample readings. The analysis included three readings per sample, taken at different positions. This procedure allowed for greater precision in determining the percentages of protein, fat, moisture, and collagen. The method was applied exclusively to raw meat. The study was compared to a positive control. The analysis used the same procedure for both treatments.

pH analysis was performed using potentiometry. The study employed a Beckman pH Φ 50 potentiometer (Palo Alto, California, USA). Color determination was performed using a Minolta portable colorimeter (Chroma Meter CR-400, Tokyo, Japan). The procedure used black-and-white standards for calibration. The analysis evaluated the color coordinates L^* , a^* , and b^* . The meat was placed in the instrument's sample holder (Figure 2). The procedure involved rotating the sample 90°, allowing for four readings per repetition. Water activity (A_w) was determined using an Aqualab psychrometric instrument (Decagon CX-1, Washington, USA). The instrument exhibited a sensitivity of 0.001. A_w was expressed as $a_w = P/P_0$. In this equation, P represents the vapor pressure of the solution and P_0 the vapor pressure of the solvent, corresponding to pure water.

Finally, the study determined the meat's water-holding capacity (WHC). The procedure used 10 g samples. The analysis involved placing the samples in duplicate into centrifuge tubes. During the procedure, 16 mL of a 0.6 M NaCl solution was added to each tube. The samples were then incubated for 30 min in an ice bath. Subsequently, the samples were centrifuged at 5,000 rpm for 30 min. The study reported water-holding capacity as the milliliters of water retained per gram of meat (Figure 3).

RESULTS AND DISCUSSION

The chemical composition of bullfrog and chicken meat is shown in Table 1. The analysis identified differences between both treatments ($P < 0.05$).

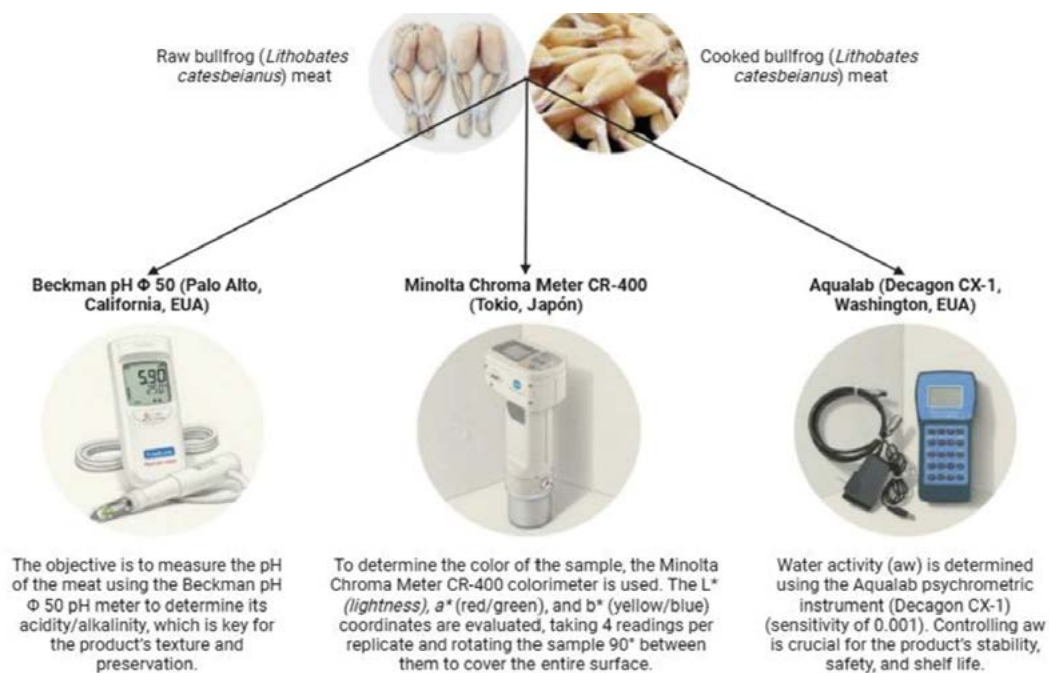


Figure 2. Determination of pH, color, and water activity in raw and cooked bullfrog (*Lithobates catesbeianus*) meat samples.

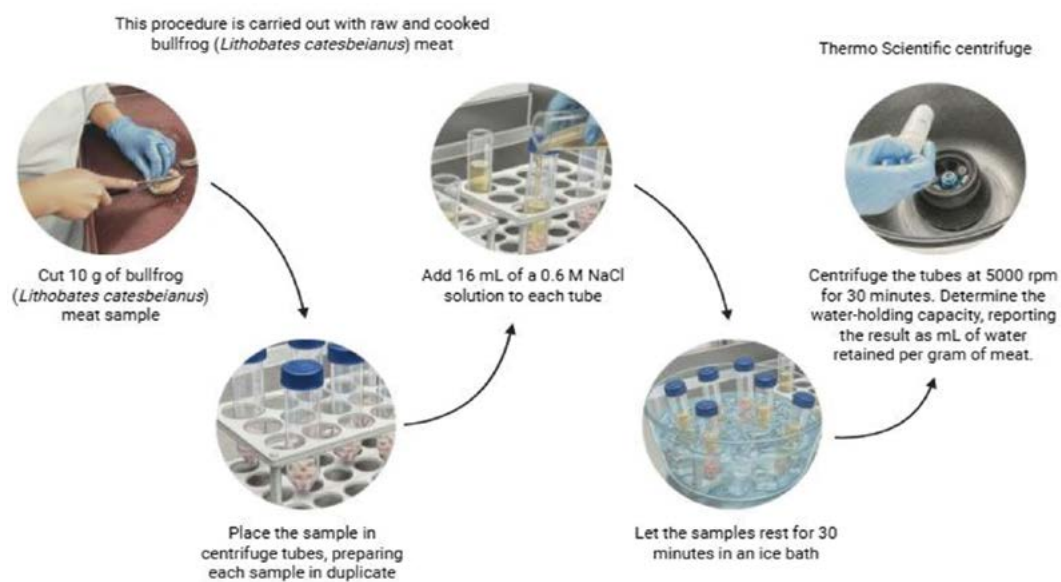


Figure 3. Analysis of water-holding capacity (WHC) by centrifugation method.

Table 1. Chemical composition of bullfrog meat (mean ± SD).

Meat	Protein	Fat	Moisture	Collagen
Bullfrog	16.84 ± 0.34 ^b	0.71 ± 0.02 ^b	81.39 ± 0.28 ^a	1.03 ± 0.10 ^a
Chicken	23.62 ± 0.03 ^a	1.59 ± 0.01 ^a	73.41 ± 0.06 ^b	0.75 ± 0.24 ^b

^{a,b} Different letters in the same column indicate significant differences (p<0.05).

The protein content differed between bullfrog meat and chicken meat. Chicken meat showed a higher protein content than bullfrog meat. The values were 23.62% for chicken meat and 16.84% for bullfrog meat. This difference represented an approximate variation of 6.0 units. A study by Zhu *et al.* (2021) evaluated the nutritional composition of bullfrog meat in different parts of the animal. The authors reported protein values similar to those observed in the present study. The protein contents were $15.32 \pm 1.08\%$ in the foreleg, $21.17 \pm 1.72\%$ in the thigh, and $17.66 \pm 0.98\%$ in the calf. The same study analyzed the amino acid content in different parts of the meat. The results identified glutamic acid, aspartic acid, and lysine as the most abundant amino acids. These results are consistent with those reported by Özden and Erkan (2011), who indicate that these amino acids predominate in aquatic organisms. The analysis also identified alanine, valine, glycine, arginine, and methionine in smaller proportions. Coura *et al.* (2016) evaluated the protein content of raw frog meat. They reported a value of 17.09%, which was lower than that observed in other species analyzed in the same study. Raw beef accounted for 21.09%. Indriana *et al.* (2023) described frogs and salamanders as the most common edible amphibians. They noted that frog legs are considered a culinary delicacy in Europe, the United States, Asia, Australia, and Africa. These authors also indicated that frog meat is consumed as an alternative to chicken. The flavor of frog meat was similar to that of poultry. Fat content showed statistically significant differences between the samples ($p < 0.05$). Chicken meat had a higher fat content than bullfrog meat. Fat values were 1.59% in chicken meat and 0.71% in frog meat. Statistical analysis confirmed significant differences between the two treatments ($p < 0.05$). Moisture content showed an opposite trend. Bullfrog meat had a higher moisture content than chicken meat. This difference represented an increase of 7.98 units ($p < 0.05$). Collagen content showed a similar trend to that observed in moisture. Bullfrog meat had a higher collagen content than chicken meat ($p < 0.05$). Quinto Mina (2021) indicated that frog meat has low levels of fat and carbohydrates. This nutritional profile is associated with higher protein and water content. These characteristics contribute to greater juiciness and lower acidity. These properties differentiate frog meat from that of other species, such as chicken, pork, and beef.

The results of the bullfrog meat color parameters are shown in Table 2. Statistical analysis identified significant differences between treatments ($p < 0.05$). Meat color plays an important role in product quality. Color also influences consumer acceptance. This study evaluated bullfrog meat color using the parameters L^* (lightness), a^* (+red/−green), and b^* (+yellow/−blue) in both raw and cooked meat. The L^* parameter was higher in cooked meat than in raw meat ($p < 0.05$). The L^* value increased by 9.38 units after cooking. The a^* parameter was lower in cooked meat than in raw meat ($p < 0.05$). The difference between the two treatments was 2.36 units. The b^* parameter showed a significant increase in cooked meat ($p < 0.05$). The b^* value was higher in cooked meat (14.15) than in raw meat (8.38).

Meat color results depend on the slaughter method used for bullfrogs. Ramos *et al.* (2005) evaluated meat color and muscle pigment levels in frogs subjected to electrical or thermal stunning, with and without exsanguination. The study reported lower myoglobin content in the muscles of electrically stunned frogs. This myoglobin content was similar to

that observed in birds' white muscle. The analysis identified a correlation between muscle pigments and color parameters. Oxymyoglobin, reduced myoglobin, and metmyoglobin contents correlated with lightness (L^*), redness (a^*), and yellowness (b^*). These results are consistent with those reported by Cori *et al.* (2014), who indicated that meat lightness depends primarily on the availability of hemoglobin and myoglobin. Additionally, Ramos *et al.* (2005) reported a lower content of muscle hemoglobin in the meat of frogs subjected to bleeding. This content was significantly lower than that observed in non-bleeding frogs ($p < 0.05$).

A study by Padilla *et al.* (2022) evaluated the color parameters of raw frog legs from Jalisco and Morelos. The study reported L^* values between 68 and 71, a^* values between -0.80 and 0.90 , and b^* values between 8.00 and 9.40 . All parameters showed statistically significant differences ($p < 0.05$). These results indicated greater luminosity than that obtained for the raw meat analyzed. The production and consumption of bullfrog meat have increased steadily in recent years. However, the available information on its physicochemical properties remains limited. The composition of the meat is a relevant physicochemical property. Water loss during cooking is another important physicochemical property. These factors determine the intrinsic quality attributes and functional properties of the meat (Ssepuyaya *et al.*, 2019).

Table 2 presents the results of pH, Aw, and WHC measured in bullfrog meat, both raw and cooked. The pH value was lower in raw meat than in cooked meat ($p < 0.05$). The cooking process increased the pH by 0.14 units.

These results are consistent with those reported in the scientific literature. Several studies indicate that the pH values of meat increase during cooking; this increase is associated with the breaking of bonds involving imidazole, sulfhydryl, and hydroxyl groups. Other studies demonstrate that cooking processes at temperatures above $80\text{ }^\circ\text{C}$ favor the formation of free hydrogen sulfide (Vasanthi *et al.*, 2007; Oz *et al.*, 2016).

Aw exhibited a similar behavior to that observed for pH. The Aw value was higher in cooked meat than in raw meat ($p < 0.05$). The difference between the two treatments was 0.027 units. Increasing cooking temperature increases the energy of free water, thereby favoring an increase in water activity in food. Mendoza (2014) indicated that the Aw value in chicken meat should not fall outside the range of 0.98 to 0.99. The scientific literature establishes that Aw values in food range from 0.0 to 1.0. The values obtained in this study were 0.93 and 0.95. These values correspond to the ranges reported for meat products and for foods with high water availability, such as fresh meats, cured

Table 2. Analysis of color, pH and water functionality in bullfrog meat (mean \pm SD).

Type of meat	L^*	a^*	b^*
Cooked frog	75.97 ± 2.93^a	2.41 ± 0.46^b	14.15 ± 1.06^a
Raw frog	66.14 ± 2.29^b	4.77 ± 1.03^a	8.38 ± 1.69^b
Type of meat	pH	Water activity	water-holding capacity
Cooked frog	7.14 ± 0.05^a	0.957 ± 0.007^a	14.97 ± 0.04^b
Raw frog	7.00 ± 0.15^b	0.930 ± 0.008^b	15.87 ± 0.69^a

^{a,b} Different letters in the same column indicate significant differences ($p < 0.05$).

meats, fresh fish, fermented sausages, soft cheeses, evaporated milk, fruits in syrup, and some vegetables. Foods with high A_w values are more likely to undergo microbiological spoilage. Consequently, the application of preservation technologies is necessary to control decomposition processes. These technologies help extend the product's shelf life (Inungaray and Reyes, 2013).

WHC showed significant differences between raw and cooked meat ($p < 0.05$). Cooked meat exhibited a lower water-holding capacity than raw meat. The WHC value decreased from 15.87 to 14.97 units after cooking. This decrease represented a 0.9-unit reduction. Rengifo (2010) evaluated the water-holding capacity of chicken meat, reporting a value of 22.5% in fresh meat and 6.62% in cooked meat. León *et al.* (2017) analyzed the water-holding capacity of chicken meat and reported a value of $17.603 \pm 2.587\%$. These studies indicate that pH influences WHC, increasing as pH moves away from the proteins' isoelectric point.

The WHC of frog meat showed statistically significant differences compared to chicken meat. This difference was observed in both raw and cooked frog meat. However, the available scientific evidence on the physicochemical analysis of frog meat is limited. For this reason, the study used chicken meat as a reference. Both species share similarities in their organoleptic characteristics, including taste, color, and aroma. Nóbrega *et al.* (2007) analyzed the volatile compounds present in pressure-cooked bullfrog legs. The study used steam distillation and simultaneous solvent extraction. The authors identified compounds with high odorant activity, including (E,E)-2,4-decadienal, (E,Z)-2,4-decadienal, (E,Z)-2,6-nonadienal, 1-octanol, and (E)-2-nonenal. These compounds are commonly associated with the characteristic aroma of chicken, which explains the sensory similarity between bullfrog and chicken meat.

CONCLUSION

This study revealed differences in the nutritional quality of bullfrog meat compared to chicken meat. However, the scientific literature reports similarities in the organoleptic characteristics of the two species, including flavor, color, and aroma. Bullfrog meat is considered lean due to its low-fat content. This characteristic suggests that this species is more commonly associated with aquatic organisms than with terrestrial ones. Furthermore, bullfrog meat presented water activity values below the established limit for meat. However, these values approached 1.0, increasing the risk of microbiological spoilage. In this context, the application of preservation methods is necessary to guarantee product stability. Brine is a viable alternative, as it reduces the meat's water activity and limits the growth of microorganisms responsible for spoilage. The available scientific information on bullfrog meat in the State of Mexico is limited. This lack of evidence restricts a comprehensive evaluation of its quality and the benefits associated with its consumption. Therefore, this study provides relevant information from a general perspective and contributes to the physicochemical knowledge of bullfrog meat. The results obtained highlight the need to continue research into the nutritional, functional, and technological properties of this species to further strengthen its use as a viable alternative in the food sector.

REFERENCES

- Aguilar, X., Casas, G., Cárdenas, P., & Castellano de Rosas, E. (2009). Análisis espacial y conservación de los anfibios y reptiles del Estado de México. *CIENCIA ergo-sum, Revista Científica Multidisciplinaria de Prospectiva*, 16(2), 171-180.
- Aragón Domínguez, C. H. (2010). Estudio de factibilidad de un proyecto para la crianza y comercialización nacional de ranas toro en la provincia de Zamora Chinchipe (Bachelor's thesis, Quito; 2010). <http://dspace.ups.edu.ec/handle/123456789/4553>
- Casas Andreu, Gustavo, Aguilar-Miguel, X., & Cruz Aviña, J. R. (2001). La introducción y el cultivo de la rana toro (*Rana catesbeianus*). ¿Un atentado a la biodiversidad de México?.
- Coura, O. T., Lopes, L. S., & Bressan, J. 2016. Influences of different thermal processings in milk, bovine meat and frog protein structure. *Nutrición hospitalaria*, 28(3), 896-902. Disponible en https://scielo.isciii.es/scielo.php?pid=S0212-16112013000300047&script=sci_arttext&tlng=pt
- Cori, M. E., Michelangeli, C., De Basilio, V., Figueroa, R., & N, R. (2014). Solubilidad proteica, contenido de mioglobina, color y ph de la carne de pollo, gallina y codorniz. *Scielo*, 63, Artículo 241.
- Indriani, S., Karnjanapratum, S., Prakash, N. N., & Nalinanan, S. 2023. Amphibian skin and skin secretion: an exotic source of bioactive peptides and its application. *Foods*, 12(6), 1282. <https://doi.org/10.3390/foods12061282>
- Inungaray, M. L. C., & Reyes, A. (2013). Vida útil de los alimentos. *Revista Iberoamericana de las Ciencias Biológicas y Agropecuarias: CIBA*, 2(3), 3.
- Islas-Ojeda, E., García-Munguía, A., Chávez-González, L., López-Gutiérrez, M., Hernández-Valdivia, E., & García-Munguía, C. (2021). Producción sostenible de rana toro (*Lithobates catesbeianus*) con agua de reúso de un sistema Biofloc. *Abanico veterinario*, 11.
- Laufer, G., Canavero, A., Núñez, D., Maneyro, R. (2008). Bullfrog (*Lithobates catesbeianus*) invasión en Uruguay. *Biological Invasions*, 10, 1183-1189.
- León C, M., Orduz C, A., & Velandia, M. (2017). Composición fisicoquímica de la carne de ovejo, pollo, res y cerdo. *@Limentech ciencia y tecnología alimentaria*, 15(2), 62-75.
- Méndez Béjar, L. (2014). Evaluación de dos alimentos balanceados, peletizado y extruido en el crecimiento de la rana toro.
- Mendoza Parada, M. D. (2014). Determinación microbiológica de la carne de pollo que se expende en el mercado el guarda ciudad de Guatemala [Licenciatura en Zootecnia inédita]. Universidad de San Carlos de Guatemala.
- Nobrega, I. C., Ataíde, C. S., Moura, O. M., Livera, A. V., & Menezes, P. H. (2007). Volatile constituents of cooked bullfrog (*Rana catesbeiana*) legs. *Food Chemistry*, 102(1), 186-191. <https://doi.org/10.1016/j.foodchem.2006.05.047>
- Ojeda, E. I., García-Munguía, A. M., Munguía, C. G., Gutiérrez, M. L., Huerta, A. C., Valdivia, E. H. (2020). Dinámicas de consumo de rana toro (*Lithobates catesbeianus*) en segmentos de la población mexicana. *Abanico Agroforestal*, 2,1- 13.
- Oz, F., Aksu, M. I., & Turan, M. 2016. The effects of different cooking methods on some quality criteria and mineral composition of beef steaks. *Journal of Food Processing and Preservation*, 41(4), e13008. <https://doi.org/10.1111/jfpp.13008>
- Özden, Ö, & Erkan, N. 2011. A preliminary study of amino acid and mineral profiles of important and estimable 21 seafood species. *British Food Journal*, 457-469. <https://doi.org/10.1108/00070701111123943>
- Pillado Albarrán, K. V., Viesca González, F. C. (2013). Efectos en las actividades económicas en los pueblos Ribereños del curso alto del Río Lerma por la extracción del Agua de las Lagunas (1942-2011).
- Quinto Mina, R. A. (2021). La comercialización de las ancas de rana toro y su valor nutricional para la sociedad de Guayaquil (Bachelor's thesis, Facultad de Ciencias Económicas. Universidad de Guayaquil).
- Rangel Ramírez, M. T. (2017). Protocolo para la reproducción, incubación y crecimiento de rana toro americana (*Lithobates catesbeianus* SHAW, 1802), en condiciones controladas en Mexico.
- Ramos, E. M., Gomide, L. A. M., Fontes, P. R., Ramos, A. L. S., & Peternelli, L. A. (2005). Meat color evaluation and pigment levels in bullfrog (*Rana catesbeiana*) slaughtered by different methods. *Aquaculture*, 245(1-4), 175-182.
- Rengifo, L. I. (2010). Capacidad de retención de agua y pH en diferentes tipos de carnes y en embutido [Ingeniería en industrias alimentarias inédita]. Universidad Nacional Agraria de la Selva.
- Rodríguez Briones, M. A. (2014). Proyecto de factibilidad para la creación de una empresa comercializadora de ancas de rana toro en el Cantón Yantzaza, provincia de Zamora Chinchipe para el periodo 2014. [Ingeniería en finanzas inédita]. Escuela Superior Politécnica de Chimborazo.

- Ssepuyya, G., Smets, R., Nakimbugwe, D., Van Der Borght, M., & Claes, J. 2019. Nutrient composition of the long-horned grasshopper *Ruspolia differens* Serville: Effect of swarming season and sourcing geographical area. *Food Chemistry*, 301, 125305. <https://doi.org/10.1016/j.foodchem.2019.125305>
- Vargas Palacios, J. P., & Moreno Agui, I. (2015). Proyecto Para La Constitución De Una Empresa Productora De Ancas De Rana Tipo Toro, En La Ciudad De Guayaquil (Doctoral dissertation, ESPOL. FCSH). <http://www.dspace.espol.edu.ec/handle/123456789/54583>
- Vasanthi, C., Venkataramanujam, V., & Dushyanthan K. 2007. Effect of cooking temperature and time on the physico-chemical, histological and sensory properties of female carabeef (buffalo) meat. *Meat Science*, 76(2), 274-280. <https://doi.org/10.1016/j.meatsci.2006.11.018>
- Vasconez Tenezaca, J. R., & Rodriguez Rodriguez, J. G. (2003). Desarrollo técnico, investigación de mercado y comercialización de la rana toro en las ciudades de Quito y Guayaquil (thesis, ESPOL. FIMCM: Acuicultura).
- Zhu, Y., Bao, M., Chen, C., Yang, X., Yan, W., Ren, F., Wang, P., & Wen, P. 2021. Comparison of the nutritional composition of bullfrog meat from different parts of the animal. *Food Science of Animal Resources*, 41(6), 1049-1059. <https://doi.org/10.5851/kosfa.2021.e56>



Biol, nitrogen fertilization, and topological arrangement effects on the yield and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions

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ABSTRACT

Objective: To evaluate the effect of planting arrangements and different fertilization treatments on biomass production and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions in Veracruz, Mexico.

Design/Methodology/Approach: A randomized complete block design with split plots was used. Planting density (40, 60, and 80 cm) was the main factor. Three biol formulas and two doses of urea fertilization (50 and 100 kg ha⁻¹) were the secondary factor. The following variables were measured: plant height, biomass yield, and leaf and stem weight. Data were analyzed through an ANOVA and Tukey's test ($\alpha=0.05$).

Results: The 40-cm planting arrangement and Biol 1 (manure+water) treatment combination reached the highest biomass yield (123.11 t ha⁻¹) and plant height (2.44 m). Denser planting improved biomass production, while chemical fertilization increased leaf ratio. The interaction between planting arrangement and fertilization only had a significant impact on stem weight.

Study Limitations/Implications: The study was conducted under specific climatic conditions, which limited the generalizability of the results. Further research is recommended to assess long-term economic viability and sustainability.

Findings/Conclusions: High planting density combined with organic fertilization optimizes biomass yield and the efficient use of resources. Biols are a sustainable alternative that enhances forage productivity and soil health.

Keywords: Maralfalfa grass, organic fertilization, planting density, biomass, sustainable agriculture.

INTRODUCTION

The growing demand for high quality forages in intensive livestock systems has driven the search for sustainable alternatives to optimize biomass production, without compromising soil or environmental health (Karasawa *et al.*, 2023). Maralfalfa grass (*Pennisetum* spp.) is one of the most promising foraging species, given its fast growth, high biomass yield, and nutritional value (Fuller *et al.*, 2021; Condori *et al.*, 2018). Consequently, maralfalfa could become a strategic resource to improve livestock productivity in tropical and subtropical regions. In addition, the climate in these regions favors its development (Kebede *et al.*, 2023).

Biomass production and composition of tropical grasses are directly influenced by several factors, including planting density and fertilization type (Syam *et al.*, 2021; Ledea-Rodríguez *et al.*, 2023). Planting density impacts the competition for light, water, and nutrients, modifying the growth and morphology of crops (Tilus *et al.*, 2022). In addition, fertilization plays a key role, supplying essential nutrients for plant development. Although chemical fertilizers are widely applied, due to their fast availability, their excessive use have negatively impacted soil health and water quality (Pereira *et al.*, 2022; Bacarrillo-López *et al.*, 2021). Consequently, sustainable alternatives, such as liquid biofertilizers (biols), have been developed. Organic waste is fermented to prepare biols (Jara-Samaniego *et al.*, 2021). These products not only gradually provide nutrients, but also improve the physical, chemical, and biological structure of soils (Mupambwa and Mnkeni, 2018). Recent studies have proven that biols significantly increase forage production and the resistance of agricultural systems to climate change (Moreno-Sandoval *et al.*, 2022). Biol application promotes sustainability in production systems, reducing their dependency on chemical fertilizers and favoring beneficial biological processes in soils (Mupambwa and Mnkeni, 2018). The appropriate planting density combined with organic fertilization can enhance the efficient use of resources, sustainably increasing biomass yield (Gaudio *et al.*, 2019; Wang *et al.*, 2023). Meanwhile, other studies about foraging systems have shown the positive effects of planting geometry (Umesh *et al.*, 2022) and spacing between the plants on key morphological variables (Wang *et al.*, 2025). For their part, Oliveira *et al.* (2025) reported that nitrogen biological fixation significantly contributed to the biomass production of elephant grass grown in low-fertility soils, without synthetic nitrogen fertilizers. Nevertheless, information about the interaction between topological planting arrangements and biols in *Pennisetum* spp. crops is very limited, particularly in the case of maralfalfa grown under subhumid conditions. This lack of information provides an opportunity to further research these factors. Understanding the impact of these agronomic elements on the yield and morphological composition of the biomass is fundamental to design more efficient and sustainable strategies, aimed to improve forage productivity in tropical systems (Fang *et al.*, 2021). Therefore, the objective of this study was to evaluate the effect of different topological planting arrangements and fertilization treatments, as well as their interaction, on the production and composition of maralfalfa (*Pennisetum* spp.) biomass grown under subhumid conditions, in the state of Veracruz, Mexico.

MATERIALS AND METHODS

Location

This study was conducted at the Instituto Tecnológico Superior de Juan Rodríguez Clara (ITSJRC), located in the municipality of Juan Rodríguez Clara, Veracruz, Mexico, at 133 m.a.s.l. (INEGI, 2010). Based on the Mexican version of the Köppen system, this region is classified as AW_0 —i.e., a subhumid warm climate. It has a 1,100 mm mean annual precipitation and a 24.5 °C mean annual temperature (García, 2004). The region mainly has a dystric cambisol soil, with a strongly acid pH. This condition directly impacts the development of foraging crops (Morales Rivera *et al.*, 2015; Tosquy-Valle *et al.*, 2020).

Establishment of the crop

The evaluation was conducted in a 525 m² area. The crops were established on July 5, 2023. The plant material chosen for the experiment was maralfalfa grass (*Pennisetum* spp.). Land preparation included a double harrowing and 80 cm separation between furrows. The grass was sown by hand during the early spring-summer season, following the method for tropical forages suggested by Cerdas-Ramírez *et al.* (2021). A randomized complete block design with an arrangement of split plots was used. The main factor was topological planting arrangement (PTA) with a 40, 60, and 80 cm distance between furrows. The secondary factor was the nitrogen fertilization systems (NFS) with three biol formulas and two doses of urea chemical fertilization (50 and 100 kg N ha⁻¹) (Cerdas-Ramírez *et al.*, 2021). Three repetitions per treatment were established. In order to minimize environmental variability, each block was randomized, guaranteeing the accuracy and reliability of the results (Villanueva-Avalos *et al.*, 2022).

Preparation of the biols

Biols were prepared through the anaerobic fermentation of organic wastes. This technique improves nutrient availability and promotes plant growth (Cabos *et al.*, 2019; Jara-Samaniego *et al.*, 2021). Table 1 shows the three formulated types of biols.

Measurement of morphological and physiological variables

Following the research protocols established by Botero-Londoño *et al.* (2021) and Mengistu *et al.* (2023), the morphological and physiological variables were evaluated to

Table 1. Composition and characteristics of the fertilization systems applied in the experiment.

Treatment	Main Composition	Objective
BIOL 1	Bovine manure + water	Provide a balanced supply of N, P, and K (anaerobic fermentation).
BIOL 2	Bovine manure + whey + molasses + yeast	Increase microbial activity and micronutrient availability
BIOL 3	Whey + molasses + mountain microorganisms + minerals (Ca, Mg, Zn)	Optimize nutrient absorption with a comprehensive profile
Chemical Fertilization Treatments		
CF1	Urea (50 kg N ha ⁻¹)	Compare efficiency with biols (low nitrogen dose)
CF2	Urea (100 kg N ha ⁻¹)	Compare efficiency with biols (high nitrogen dose)

* BIOL=biol; CF (FQ)=chemical fertilization, N=nitrogen.

quantify maralfalfa grass yield. A Just Home MKZ-BAS-ACS209 digital scale was used to estimate weight.

Total biomass yield (TBY)

Three lineal meters of useful plots were harvested and weighted. Subsequently, leaves and stems were manually divided according to their morphology. Green leaf weight (GLW) was evaluated to determine the biomass of photosynthetic tissue.

Stem weight (SW)

Stems were weighted to determine structural biomass.

Plant height (PH)

Plant height was measured from the base of the stem to the flag leaf, using a 5-m aluminum topographic sight (Nedo).

$$\text{Leaf percentage (LP)} \quad LP(\%) = \left(\frac{GLW}{TBY} \right) \times 100$$

$$\text{Stem percentage (SP)} \quad SP(\%) = \left(\frac{SW}{TBY} \right) \times 100$$

Climatic variables

In order to evaluate the direct effect of weather on crop development, the experiment was conducted under rainfed conditions. Temperature and humidity were recorded with an RCW-800-TH Datalogger, while precipitation was measured with a Tenite 110672 pluviometer. Base temperature (BaseT) was determined with the following equation:

$$MediumT = \frac{(MaxT + MinT)}{2}$$

Meanwhile, two focuses were used to calculate BaseT.

Calculation of accumulated degrees-day:

$$\text{Degrees-day (DeD): } DeD = MediumT - BaseT$$

In addition, the methodology proposed by Dawson *et al.* (2004) and Huang *et al.* (2019) for forage grasses was used.

Statistical analysis

A two-way analysis of variance (ANOVA) was used to analyze the data in SAS v. 9.4 (SAS, 2009). The differences between means were evaluated with the Tukey's Test ($\alpha=0.05$), guaranteeing the statistical rigor between treatments (Gaudio *et al.*, 2019; Cerdas-Ramírez *et al.*, 2021).

RESULTS AND DISCUSSION

Influence of climatic conditions

The humidity and warm conditions during the June-July crop cycle (153- and 176.7-mm rainfall, with 23-30 °C temperatures) favored the establishment and initial growth of maralfala grass. These results matched the reports of Calzada-Marín *et al.* (2014) and Morales Rivera *et al.* (2015). Meanwhile, the decrease of rainfall during August and September (34 and 43 mm) resulted in a moderated water stress that mainly impacted low density plantings. Similar results were registered by Mengistu *et al.* (2023). High density plantings were better at maintaining soil moisture and showed a higher physiological activity. The recovery of rainfall levels during October and November (195 and 140 mm) enabled a vigorous final growth. The gradual accumulation off degrees-days⁻¹ (Figure 1) indicated favorable temperature conditions for continuous plant development, particularly for plants grown under an organic fertilization system (Dawson *et al.*, 2004; Huang *et al.*, 2019). Meanwhile, the highest yields obtained with a high planting density and the application of boils (Figure 2) confirm the influence of climatic factors on the agronomic development of the crop.

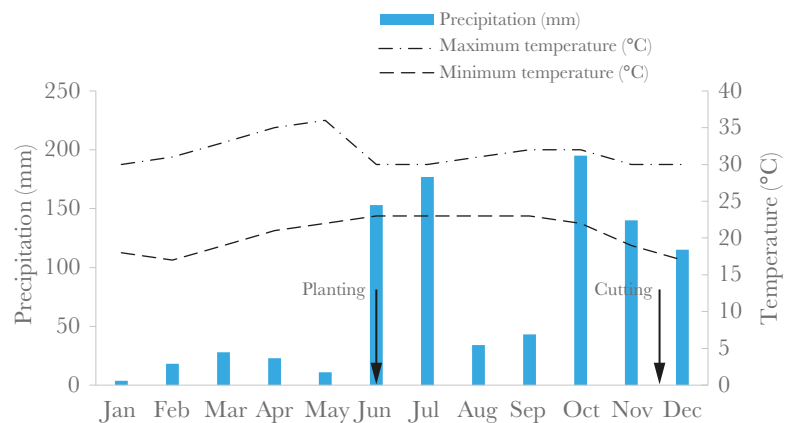


Figure 1. Environmental conditions associated with the growth of maralfalfa grass, under subhumid conditions.

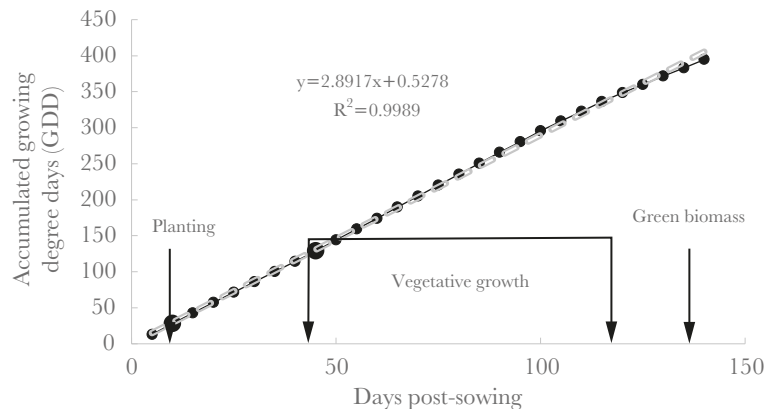


Figure 2. Ratio between days after sowing and the accumulated heat units in maralfalfa grass crops established in the Instituto Tecnológico Superior de Juan Rodríguez Clara.

Effect of the topological planting arrangements

The ANOVA showed statistically significant differences ($P < 0.05$) in the main effects of the topological planting arrangements on plant height (PH), total biomass yield (TBY), green leaf weight (GLW), and stem weight (SW).

Table 2 shows that planting topology at 40 cm (T40) recorded the highest plant height (2.4 m), biomass yield (123.1 t ha^{-1}), green leaf weight (39.3 t ha^{-1}), and stem weight (83.7 t ha^{-1}). In addition, T40 recorded statistical differences from T60 and T80. Although leaf percentage (38.8%) was higher in T60, this treatment recorded a lower total yield. In contrast, T80 obtained the lowest values in all the production variables.

These results indicate that the narrower planting topology (40 cm) favors plant development and biomass accumulation, while larger distances significantly reduce forage yield under subhumid conditions.

Effect of the fertilization treatments

Table 3 shows that the fertilization treatments significantly influence plant height (PH) ($P < 0.0001$), stem weight (SW), and the morphological composition of maralfalfa. The Biol 1 (manure+water) treatment recorded the greatest height (2.7 m) and the highest stem weight (SW) (65.2 t ha^{-1}), suggesting a gradual release of nutrients. Meanwhile, total biomass yield (TBY) and green leaf weight (GLW) did not record statistical differences. However, leaf percentage obtained higher results with CF2 (40.3%). High nitrogen doses had a positive effect on leaf development.

Table 2. Effect of topological planting arrangement and nitrogen fertilization on the yield and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions in Veracruz.

Topologías	PH	TBY	GLW	SW	LP	SP
T40 cm	2.4 ^{a*}	123.1 ^a	39.3 ^a	83.7 ^a	31.9 ^b	68.0 ^a
T60 cm	2.1 ^a	66.7 ^b	25.7 ^b	41.0 ^b	38.8 ^a	62.7 ^b
T80 cm	2.0 ^a	51.7 ^b	19.0 ^b	32.6 ^b	37.2 ^a	61.1 ^b

*Means with different letter in a column are statistically different (Tukey $P = 0.05$). T40=planting topology at 40 cm; T60=planting topology at 60 cm; T80=planting topology at 80 cm; PH (ALTP)=plant height (cm); TBY (REN)=total biomass yield (t ha^{-1}); GLW (PVH)=green leaf weight (t ha^{-1}); SW=stem weight (t ha^{-1}); LP (PHOJ)=leaf percentage (%); and SP=stem percentage (%).

Table 3. Effect of nitrogen fertilization and biols on the yield and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions.

Fertilization Treatment	PH	TBY	GLW	SW	LP	SP
Biol 1	2.7 ^a	95.8	30.5	65.2 ^a	33.4 ^b	66.5 ^a
Biol 2	2.3 ^{ab}	74.4	25.0	49.4 ^{ab}	33.7 ^b	66.2 ^a
Biol 3	2.1 ^b	70.9	24.8	46.1 ^b	35.7 ^b	64.2 ^a
CF1	1.9 ^b	86.5	30.6	55.8 ^{ab}	36.7 ^{ab}	63.2 ^{ab}
CF2	1.8 ^b	74.8	29.2	45.6 ^b	40.3 ^a	59.6 ^b

*Means with different letter in a column are statistically different (Tukey $P = 0.05$). Biol 1: manure+water; Biol 2: manure, serum, treacle, yeast, and micronutrients; Biol 3: serum, treacle, microorganisms, and minerals; CF1 (FQ1) (urea): 50 kg N ha^{-1} ; CF2 (FQ2) (urea): 100 kg N ha^{-1} .

Interaction between planting arrangements and fertilization

Table 4 shows the interaction between topological arrangements and fertilization treatments. Biol 1 combined with T40 recorded the highest biomass yield (123.1 t ha^{-1}), followed by Biol 2 at the same density. These combinations also stood out in green leaf weight (GLW) and stem weight (SW). In contrast, T80 and CF2 recorded the lowest values, particularly regarding SW (32.6 t ha^{-1}).

Influence of the climatic conditions on the development of maralfalfa grass

Climatic conditions are important for the establishment and development of maralfalfa grass. Abundant rainfall and warm temperatures during the first months of the crop (June and July) favored a fast rooting and a strong plant growth. These results match the findings of Calzada-Marín (2014), who pointed out that maralfalfa grass has a positive response to subhumid warm climates, as a result of its high-water demand during the initial stages. Nevertheless, the reduction of rainfall during August and September had a higher impact on low density plantings (T60 and T80), perhaps due to the lower competition for resources. Similar results were reported by Morales Rivera *et al.* (2015) in their analysis of crops under water stress.

Table 4. Effect of the planting topology combination, nitrogen fertilization, and biols on the yield and composition of maralfalfa grass (*Pennisetum* spp.) grown under subhumid conditions.

Topological Arrangement	Fertilization Treatment	PH	TBY	GLW	SW	LP	SP
T40 cm	Biol 1	2.4	123.1	39.3	83.7 ^a	31.9	68.0
	Biol 2	2.3	95.8	30.5	65.2 ^a	33.7	66.5
	Biol 3	2.1	86.5	25.0	55.8 ^b	35.7	64.2
	CF1	1.9	86.5	30.6	55.8 ^b	36.7	63.2
	CF2	1.8	74.8	29.2	45.6 ^b	40.3	59.6
T60 cm	Biol 1	2.1	74.4	25.7	49.4 ^b	33.7	66.2
	Biol 2	2.3	70.9	24.8	46.1 ^b	35.7	64.2
	Biol 3	2.1	86.5	30.6	55.8 ^b	36.7	63.2
	CF1	1.9	74.8	29.2	45.6 ^b	40.3	59.6
	CF2	1.8	70.9	30.5	49.4 ^b	35.7	64.2
T80 cm	Biol 1	2.0	74.8	19.0	32.6 ^b	40.3	59.6
	Biol 2	1.9	70.9	25.7	41.0 ^b	38.8	62.7
	Biol 3	1.8	74.8	19.0	32.6 ^b	37.2	62.7
	CF1	1.8	70.9	25.7	41.0 ^b	38.8	62.7
	CF2	1.8	70.9	19.0	32.6 ^b	37.2	62.7

* Means with different letters in a column are statistically different (Tukey $P=0.05$). T40=planting topology at 40 cm; T60=planting topology at 60 cm; T80=planting topology at 80 cm; Biol 1: manure+water; Biol 2: manure, serum, treacle, yeast, and micronutrients; Biol 3: serum, treacle, microorganisms, and minerals; CF1 (FQ1) (urea): 50 kg N ha^{-1} ; CF2 (FQ2) (urea): 100 kg N ha^{-1} ; PH (ALTP)=plant height (cm); TBY (REN)=total biomass yield (t ha^{-1}); GLW (PVH)=green leaf weight (t ha^{-1}); SW=stem weight (t ha^{-1}); LP (PHOJ)=leaf percentage (%), and SP=stem percentage (%).

Effect of topological planting arrangements

Planting density had a significant effect on total biomass yield and morphological composition of maralfalfa grass. The treatments where furrows were located closer to each other (40 cm) recorded higher yields than those where furrows were 60 and 80 cm apart. These results prove that a higher plant population per area unit favors plant development and biomass accumulation. In physiological terms, denser plantings promote a higher competition for light, promoting longer stems and wider canopy area. This competition also intensifies root exploration, improving water and nutrient absorption and resulting in a more efficient use of resources. In addition, these results match the reports of Botero-Londoño *et al.* (2021), who recorded a higher biomass production and nutrient uptake in *Pennisetum purpureum* grown at higher densities. For their part, Villanueva-Ávalos *et al.* (2022) indicated that a wider canopy cover in dense planting enables a more efficient interception of active photosynthetic radiation, reducing evapotranspiration and favoring the microclimate of the crops. Meanwhile, more separation between furrows (T60 and T80) reduced the competition for light and promoted a higher leaf ratio in the biomass. These results are similar to the findings of Mengistu *et al.* (2023), who pointed out that low densities increased leaf development in *Pennisetum* spp. The highest stem development took place in denser plantings. This phenomenon reveals the preference for biomass assignation in the support structures required to maintain a more competitive architecture. According to Dawson *et al.* (2003), who studied the competition among perennial grasses, this response is associated with a higher meristematic activity and transport of photo-assimilates towards structural tissues.

Impact of the fertilization treatments

The application of organic biofertilizers, particularly Biol 1 (manure + water), promoted taller plants and a significant accumulation of structural biomass of *Pennisetum* spp. This effect could be the result of a gradual release of essential nutrients during the crop cycle, enabling a constant improvement of plant nutrition. According to Dubey (2019), this type of formulas favors photosynthetic activity and nutrient absorption, maintaining a stable soil fertility. Likewise, Rosabal *et al.* (2021) reported that the use of biols increases yield without dependence on synthetic fertilizers. In addition, Monge-Pérez *et al.* (2022) proved that biols improve soil moisture retention, which is a key factor for an ongoing physiological activity under subhumid conditions. These benefits show how organic treatments have a better performance than chemical treatments, particularly regarding plant height and stem weight.

Interaction between topological arrangements and fertilization

The combination of a high-density planting (40 cm between furrows) and the application of Biol 1 significantly favored the production of structural biomass of maralfalfa grass, particularly regarding stem development. This synergy suggests that the topological arrangement influences the efficient uptake of nutrients from biofertilizers, because a higher competition for light and resources promotes a preferred assignation towards support tissues. Hermitaño *et al.* (2022) reported similar results with *Pennisetum* spp. In

their experiment, the appropriate management of organic fertilization in dense planting increased structural biomass. For their part, Caballero *et al.* (2023) reported that the adjustment of density and fertilization type improved nutrient uptake. Finally, Harish *et al.* (2022) and Kiruba and Saeid (2022) pointed out that the combination of biofertilizers and appropriate agricultural practices significantly increases the productivity of foraging crops. Under subhumid conditions, this joint strategy was more efficient than the use of chemical fertilization. Although treatments such as CF2 (100 kg N ha⁻¹) increased leaf percentage (Pereira *et al.*, 2022), they did not reach the total biomass yield recorded by Biol 1. These results are similar to those reported by Dubey (2019) and Hermitaño *et al.* (2022), who pointed out that biofertilizers gradually release nutrients, maintaining a steady growth of the crop, even under water stress. In addition, Maldonado-Quiñones *et al.* (2021) and Fayos-Fever *et al.* (2023) pointed out that organic fertilization increases yield in warm and subhumid environments. Finally, Rosabal *et al.* (2021) and Pereira *et al.* (2022) mentioned that the use of biols not only improves the efficient use of nutrient and water, but also contributes to the productive stability of the system and reduces the dependency on synthetic fertilizers, proving its value as a sustainable practice in tropical livestock systems.

CONCLUSIONS

In order to achieve a better morphological development and to improve yield under the subhumid conditions of Veracruz, the use of topological arrangements (high density plantings and the application of fertilizers) to provide nutrients for *Pennisetum* spp. is fundamental.

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REFERENCES

- Bacarrillo-López R, Pedroza-Sandoval A, Inzunza Ibarra MA, Flores-Hernández A, Macías-Rodríguez FJ (2021). Productividad de forraje de variedades de nopal (*Opuntia* spp.) bajo diferentes regímenes de humedad del suelo. *Ecosistemas y Recursos Agropecuarios*, 8(3): 1-10.
- Botero-Londoño JM, Celis-Celis EM, Botero-Londoño MA (2021). Nutritional quality, nutrient uptake and biomass production of *Pennisetum purpureum* cv. King grass. *Scientific Reports*, 11(13799). <https://doi.org/10.1038/s41598-021-93301-w>
- Caballero EMC, Durango EMP, Pérez-Polo DJ (2023). Nutritional content of pastures with phosphate fertilization in 2 calcareous soils. *Revista Caatinga*, 36(1): 87-95. <https://doi.org/10.1590/1983-21252023v36n110rc>
- Calzada-Marín, Jesús Miguel, Enríquez-Quiroz, Javier Francisco, Hernández-Garay, Alfonso, Ortega-Jiménez, Eusebio, & Mendoza-Pedroza, Sergio I. (2014). Análisis de crecimiento del pasto maralfalfa (*Pennisetum* sp.) en clima cálido subhúmedo. *Revista mexicana de ciencias pecuarias*. 5(2): 247-260. Recuperado en 15 de enero de 2025, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-11242014000200009&lng=es&tlng=es
- Cerdas-Ramírez R, Vega-Villalobos EV, Vargas-Rojas JC (2021). Productividad del pasto Cuba OM-22 (*Pennisetum purpureum* × *Pennisetum glaucum*) con distintas dosis de fertilización nitrogenada. *InterSedes*, 22(45): 136-161. <https://doi.org/10.15517/isucr.v22i45.47069>

- Cabos Sánchez J, Bardales Vásquez CB, León Torres CA, Gil Ramírez LA (2019). Evaluación de las concentraciones de Nitrógeno, Fósforo y Potasio del biol y biosol obtenidos a partir de estiércol de ganado vacuno en un biodigestor de geomembrana de policloruro de vinilo. *Arnaldoa*, 26(3): 1165-1176. <https://doi.org/10.22497/arnaldoa.263.26321>
- Condori Vargas S, Ruiz Huanca P, Ticona Guanto O, Chipana Mendoza GJ (2018). Eficiencia del uso del agua y características bromatológicas de maralfalfa (*Pennisetum* sp.) bajo la aplicación de biol bovino en la Estación Experimental Choquenaira. *Revista de Investigación e Innovación Agropecuaria y de Recursos Naturales*, 5(2): 68-80.
- Dawson LA, Thornton B, Pratt SM, Paterson E (2003). Morphological and topological responses of roots to defoliation and nitrogen supply in *Lolium perenne* and *Festuca ovina*. *New Phytologist*, 161(3): 811-818. <https://doi.org/10.1046/j.1469-8137.2003.00979.x>
- Dubey M, Verma V, Barpete R, Verma N (2019) Effect of biofertilizers on growth of diferent crops: a review. *Plant Arch* 19:1083-1086
- Fang M, Si G, Yu Q, Huang H, Huang Y, Liu W and Guo H (2021). Study on the relationship between topological characteristics of vegetation ecospatial network and carbon sequestration capacity in the Yellow River Basin, China. *Remote Sensing*, 13(4926): 1-27. <https://doi.org/10.3390/rs13234926>
- Fayos-Febre J, Juan-Vicedo J, Rodríguez-Mengod A, Mazón J, Gardón JC (2023). Impact of Harvest Time on the Dry Matter Content, and Nutritional Parameters Related to Forage Quality of Maralfalfa (*Cenchrus purpureus* (Schumach.) Morrone, Poaceae) under Mediterranean Climate. *Plants*. 12: (4045):1-12. <https://doi.org/10.3390/plants12234045>
- Fuller DQ, Barron A, Champion L, Dupuy C, Commelin D, Raimbault M, Denham T (2021). Transition from wild to domesticated pearl millet (*Pennisetum glaucum*) revealed in ceramic temper at three Middle Holocene sites in northern Mali. *African Archaeological Review*, 38: 211-230. <https://doi.org/10.1007/s10437-021-09428-8>
- García AE (2004) Modificaciones al Sistema de Clasificación Climática de Köppen para Adaptarlo a las Condiciones de la República Mexicana. Offset Larios. México. 246p.
- Gaudio N, Escobar-Gutiérrez AJ, Casadebaig P, Evers JB, Gérard F, Louarn G, Colbach N, Munz S, Launay M, Marrou H, Barillot R, Hinsinger P, Bergez J-E, Combes D, Durand J-L, Frak E, Pagès L, Pradal C, Saint-Jean S, Van Der Werf W, Justes E (2019). Current knowledge and future research opportunities for modeling annual crop mixtures: A review. *Agronomy for Sustainable Development*, 39(20):1-20. <https://doi.org/10.1007/s13593-019-0562-6>
- Harish MN, Choudhary AK, Kumar S, Dass A, Singh VK, Sharma VK, Varatharajan T, Dhillon MK, Sangwan S, Dua VK, Nitesh SD, Bhavya M, Sangwan S, Prasad S, Kumar A, Rajpoot SK, Gupta G, Verma P, Kumar A, George S (2022). Double zero tillage and foliar phosphorus fertilization coupled with microbial inoculants enhance maize productivity and quality in a maize-wheat rotation. *Scientific Reports*. 12(3161):1-23 <https://doi.org/10.1038/s41598-022-07148-w>
- Hermitaño Osorio FM, Bernal Marcelo AR, Tantauhuilca Landeo FC, Trillo Zárate F, Calderón Montes M, Quiroga Espilco P, Nuñez Delgado J, Rodríguez Vargas AR (2022). Dinámica de crecimiento y valor nutricional del *Pennisetum* spp (cv. Maralfalfa) en tres edades de corte y dos épocas en el trópico peruano. *Revista de Investigación Veterinaria del Perú*. 33(4): 1-15. <https://doi.org/10.15381/rivep.v33i4.23337>
- Huang X, Xu X, Liu S, Song S, Chang S, Liu C, Yu D (2019). Impact of eutrophication on root morphological and topological performance in free-floating invasive and native plant species. *Hydrobiologia*. 836(1): 123-139. <https://doi.org/10.1007/s10750-019-3946-3>
- INEGI (Instituto Nacional de Estadística y Geografía). (2010). Compendio de información geográfica municipal 2010: Juan Rodríguez Clara, Veracruz de Ignacio de la Llave (Clave geoestadística 30094). Instituto Nacional de Estadística y Geografía. <https://www.inegi.org.mx>
- Jara-Samaniego J, Gallegos-Núñez J, Cruz-Torres A (2021). Elaboración y caracterización de bioles de residuos orgánicos. *InterSedes*, 22(45): 189-203. <https://doi.org/10.15517/isucr.v22i45.46013>
- Karasawa MMG, Botega VT, Pinto JEBP, Lédo EJS, Pereira AV, Pinto JC (2023). Effects of thermotherapy and meristem culture on forage production and nutrition value in elephant grass cultivars. *Agronomy Science and Biotechnology*, 9: 1-24. <https://doi.org/10.33158/ASB.r176.v9.2023>
- Kebede G, Feyissa F, Faji M, Mohammed K, Dejene M, Mengistu G, Geleti D, Assefa G, Alemayehu M, Mengistu S, Mengistu A, Tsegahun A (2023). Dry matter accumulation dynamics, morphological characteristics and nutritive value of desho (*Pennisetum glaucifolium*) grass varieties in the central Highlands of Ethiopia. *Journal of Agriculture and Environmental Sciences*, 8(1): 1-15. <https://dx.doi.org/10.4314/jaes.v8i1.8>

- Kiruba JM and Saeid A (2022). An Insight into Microbial Inoculants for Bioconversion of Waste Biomass into Sustainable “Bio-Organic” Fertilizers: A Bibliometric Analysis and Systematic Literature Review. *International Journal of Molecular Sciences*. 23(13049):1-33 <https://doi.org/10.3390/ijms232113049>
- Ledeza-Rodríguez JL, Benítez-Jiménez DG, Nuviola-Pérez Y, Wright-Ramírez JV, Rubio-Sanz L (2023). Producción de semilla de variedades de *Moringa oleifera* Lam según densidad de siembra y cosechas sucesivas. *Agronomía Mesoamericana*. 34(1): Artículo 50528. <https://doi.org/10.15517/am.v34i1.50528>
- Maldonado-Quiñones H, Carrete-Carreón FO, Reyes-Estrada O, Sánchez-Arroyo JF, Murillo-Ortiz M, Araiza-Rosales EE (2021). Rendimiento y valor nutricional del pasto maralfalfa (*Pennisetum* sp.) a diferentes edades de rebrote. *Revista Fitotecnia Mexicana*. 44(2): 143-149. <https://doi.org/10.35196/rfm.2021.2.143>
- Mengistu G, Kebede G, Faji M, Feyissa F, Mohammed K, Kehaliew A, Geleti D, Minta M, Balehegn M, Rios EF, Adesogan AT, Dubeux JCB Jr, Boote KJ (2024). Morphological characteristics, dry matter yield, and nutritive value of maralfalfa grass (*Pennisetum* spp.) grown under different planting densities in the central highlands of Ethiopia. *Frontiers in Animal Science*. 4(1308911):1-11. <https://doi.org/10.3389/fanim.2023.1308911>
- Monge-Pérez, J.E., Loria-Coto, M. y Oreamuno-Fonseca, P. (2022). Efecto de un biol sobre las características del suelo y la producción de brotes en pitahaya (*Hylocereus* sp.). *UNED Research Journal*, 14(1): 1-9 <https://doi.org/10.22458/urj.v14i1.3836>
- Morales-Rivera A, López-Castañeda C, Kohashi-Shibata J, Miranda-Colín S y García-Esteva A (2015). Comparación de los componentes del rendimiento en variedades de frijol en condiciones de acidez y humedad residual del suelo en el sur de Veracruz. *Terra Latinoamericana*, 33(4): 309-319.
- Moreno-Sandoval JA, Bernal-Ovalle MC, Acosta-Urrego LM, Riveros-Esteva DA (2022). Evaluación de bioles en pasto estrella (*Cynodon nlemfluousis*), para el subsector ganadero. Caso Fusagasugá-Colombia. *Revista Ciencias Técnicas Agropecuarias*, 31(4): 1-9. <https://cu-id.com/2177/v31n4e02>
- Mupambwa HA and Mnkeni PNS (2018). Optimizing the vermicomposting of organic wastes amended with inorganic materials for production of nutrient-rich organic fertilizers: a review. *Environmental Science and Pollution Research*, 25: 10543-10561. <https://doi.org/10.1007/s11356-018-1328-4>
- Oliveira CAGd, Monteiro EdC, Souza WdS, Pio PVA, Machado JC, Alves BJR, Boddey RM, Urquiaga S (2025). Contribution of Biological Nitrogen Fixation to the Biomass Productivity of Elephant Grass Grown in Low-Fertility Soil for Energy Purposes. *Agronomy*, 15(3): 605. <https://doi.org/10.3390/agronomy15030605>
- Pereira LET, Herling VR, Tech ARB (2022). Current scenario and perspectives for nitrogen fertilization strategies on tropical perennial grass pastures: A review. *Agronomy*, 12(9): 1-19. <https://doi.org/10.3390/agronomy12092079>
- Rosabal Ayan L, Macías Coutiño P, Maza González M, López Vázquez R, Guevara Hernández F (2021). Microorganismos del suelo y sus usos potenciales en la agricultura frente al escenario del cambio climático. *Magna Scientia UCEVA*. 1(1): 103-116. <https://doi.org/10.54502/msuceva.v1n1a14>
- SAS (2009) SAS Institute. SAS/STAT[®]9.2. User Guide Release. Cary, NC: SAS Institute Inc. USA. 111p.
- Syam N, Hasan S, Rusdy M (2021). Dry matter production and quality of *Pennisetum purpureum* cv. Taiwan applied different fertilizer. *IOP Conference Series: Earth and Environmental Science*, 788(012162):1-7. <https://doi.org/10.1088/1755-1315/788/1/012162>
- Tilus G, Zinn RA, Joseph M, Chay-Canul AJ, Santillano-Cázares J, Galicia-Juárez M, Tilus ML, Tilus D, Estrada-Delgado EE, Montaña-Gómez MF (2022). Forage yield, elongation rate and botanical composition of *Lolium multiflorum* LAMB. in response to different grazing intervals and intensities. *Tropical and Subtropical Agroecosystems*, 25(063):1-14. <http://doi.org/10.56369/tsaes.3664>
- Tosquy-Valle OH, Zetina-Lezama R, López-Salinas E, Ibarra-Pérez EJ, Villar-Sánchez B. y Rodríguez-Rodríguez JR. 2020. Comparación de genotipos de frijol negro opaco en suelos ácidos del sur de Veracruz. *Terra Latinoamericana*. 38: 91-102. <https://doi.org/10.28940/terra.v38i1.411>
- Umesh MR, Angadi S, Begna S, Gowda P (2022). Planting density and geometry effect on canopy development, forage yield and nutritive value of sorghum and annual legumes intercropping. *Sustainability*, 14(8): 4517. <https://doi.org/10.3390/su14084517>
- Villanueva-Avalos JF, Vázquez-González A, Quero-Carrillo AR (2022). Atributos agronómicos y producción de forraje en ecotipos de *Cenchrus purpureus* en condiciones de trópico subhúmedo. *Revista Mexicana de Ciencias Agrícolas*, publicación especial núm. 27: 1-14.

- Wang F, Guo H, Zhang Q, Yu Q, Xu C and Qiu S (2023). Optimizing ecological spatial network topology for enhanced carbon sequestration in the ecologically sensitive middle reaches of the Yellow River, China. *Remote Sensing*, 15(2308):1-29. <https://doi.org/10.3390/rs15092308>
- Wang X, Wang J, Li E, Guo Y, Li W (2025). Plant height is the main factor driving forage yield of Poa species under different row spacings and seeding rates in the Qilian Mountains. *Frontiers in Plant Science*, 16(1535937):1-10. <https://doi.org/10.3389/fpls.2025.1535937>



Production of dry matter and nutritional value of tropical forage shrub species in a biodiverse system

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ABSTRACT

Objective: To estimate production, chemical composition, and ruminal degradation kinetics of dry matter from *Guazuma ulmifolia* and *Gliricidia sepium*.

Design/Methodology/Approach: *Guazuma ulmifolia* and *Gliricidia sepium* were studied at the Germplasm Bank of the Rosario Izapa Experimental Field of INIFAP, estimating dry matter production per plant and per hectare, chemical composition, in situ degradability of dry matter, and ruminal degradation parameters of dry matter.

Results: Both species recorded >19% crude protein content at 30, 45, and 60 days of regrowth. The *in situ* degradability of dry matter at 45 days of regrowth was higher in *G. sepium* at all incubation times, while dry matter production per plant and per hectare was higher in *G. ulmifolia* in all three regrowth times.

Study Limitations/Implications: Data collection and sampling followed manual defoliation. Evaluating the defoliation caused by grazing is still a pending matter.

Findings/Conclusions: The species *G. ulmifolia* and *G. sepium* have nutritional and productive characteristics that make them suitable for inclusion in biodiverse forage production systems. *G. ulmifolia* can be defoliated at 45 days of regrowth, while the defoliation of *G. sepium* requires 60 days.

Keywords: feeding, cattle, grazing, native species.

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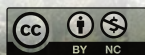
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INTRODUCTION

In tropical regions, low nutritional quality and limited availability of forage cause nutritional deficiencies in livestock, negatively impacting average daily weight gain, reproductive performance, milk production, and immune function (Solorio *et al.*, 2017). To enhance forage production and quality for livestock feeding, sustainable technologies must be developed. Additionally, those technologies must be economically competitive and appealing to producers.

Biodiverse forage production for livestock feeding under intensive rotational grazing systems is based on native grasses, legumes (Fabaceae), and forage shrubs. The most outstanding of those species include *Gliricidia sepium* (Jacq.) Walp. and *Guazuma ulmifolia* Lam., given their natural adaptation to tropical ecosystems. These forage species contribute to the sustainability of the system, because they reduce production costs and dependency on external supplies to provide forage and protein to livestock, moderate both macro- and microclimatic conditions, improve soil fertility, and mitigate the impact of droughts (Aguirre *et al.*, 2021). *G. ulmifolia*, a species native to Central America, is recognized for its rapid regrowth after defoliation, an >18% foliage protein content, and its ability to produce forage during the dry season. Therefore, it could be a valuable alternative for high-quality forage production (Manríquez *et al.*, 2011).

Gliricidia sepium belongs to the Fabaceae family. This tree species is native to Central America and Mexico, but has spread to other tropical regions, due to its versatility and multiple uses. It has a remarkable capacity to produce foliage during the dry season, with a >20% crude protein content and a 50-70% dry matter digestibility, depending on growth conditions and management practices (Cuervo *et al.*, 2013; Canul *et al.*, 2018). Therefore, the objective of this study was to evaluate the forage production potential and nutritional quality of *G. ulmifolia* and *G. sepium* in a biodiverse forage production system, through the analysis of chemical composition and *in situ* digestibility.

MATERIALS AND METHODS

Study area and climate

The study was conducted at the Rosario Izapa Experimental Field of INIFAP, located in the municipality of Tuxtla Chico, Chiapas, Mexico (latitude 14.961864, longitude -92.153272, 398.41 m.a.s.l.). The climate is classified as warm humid (INEGI, 2025). Mean, maximum, and minimum monthly temperatures recorded 26.2 °C, 27.0 °C, and 25.6 °C, respectively. The average annual precipitation is 3,894.6 mm. Most of the rainfall took place during the summer and the highest rainfall was recorded from June to September. Winter rains accounted for 5 to 10.2% of the annual total. The total annual evaporation is 1,384.2 mm, with an annual average of 115.35 mm, and maximum and minimum monthly values of 155.0 mm and 111.5 mm, respectively. Solar radiation intensity is approximately 7 ± 1 h of sunshine per day (CONAGUA, 2024). The soil type is classified as Eutric Sideralic Cambisol (Clayic, Humic) (INEGI, 2025). Soil texture varies between horizons, ranging from clay to silty clay and clay loam. Soil pH ranges from 5.7 to 5.9, organic matter content from 1.2% to 1.3%, and total nitrogen content from 0.05% to 0.35% (Gómez *et al.*, 2018).

Establishment of the biodiverse system

In May 2023, a *Guazuma ulmifolia* and *Gliricidia sepium* plantation was established in a 2,500 m² plot. The plants were arranged in double rows, with 1.5 m between them and 1.5 m between rows, resulting in a planting density of 1,600 plants per hectare. *Panicum maximum* grass was sown in a 10-meter alley between the rows. The three-month-old plants with an average height of ≈ 0.5 m were established in the plot. Four months after

field establishment, a uniformity cut was performed at a 1.1 m height, ensuring that ≈ 0.30 -m branches were left on the plants. Subsequently, evaluations were conducted at 30, 45, and 60 days of regrowth. For this purpose, three rows were randomly selected and 10 plants were randomly measured per row, totaling 30 plants per forage species and evaluation period. To determine dry matter production per plant, all regrowth was clipped with scissors 30 cm away from the main stem; the regrowth was placed in plastic bags and weighed using a 20-kg scale with a 10 g precision. To estimate dry matter production, samples were transferred to paper bags and dried in a forced-air oven at 60 °C for 48 h. After drying, the final weight was recorded and the dry matter percentage was calculated. Dry matter (DM) production per hectare (MS ha^{-1}) was estimated with the following equation:

$$DM \text{ per plant (kg)} \times 1,600 \text{ plants}$$

The dried samples were ground using a mill with a 2-mm sieve and placed in labeled plastic bags for their subsequent analysis.

Proximate chemical analysis

The sample was analyzed in a laboratory located in Aguascalientes, Mexico. Total dry matter and crude protein (CP) were determined using the Kjeldahl method. Ether extract was measured following the Soxhlet method. Crude fiber and nitrogen-free extract (NFE) were calculated according to the Weende method, using the following formula:

$$NFE = 100 - (\text{ash} + \text{ether extract} + \text{protein} + \text{fiber})$$

Ash content was determined using the techniques described by AOAC (2012). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions were analyzed based on the methodology described by Van Soest *et al.* (1991).

In situ degradability of dry matter

This trial was conducted using two heifers, which were allowed to graze freely on *Digitaria swazilandensis* pastures and had free access to mineral salt. Both heifers had been fitted with ruminal fistulas. Degradability was determined through the methodology proposed by Ørskov (2000), using six incubation times. Approximately 5 g of *G. ulmifolia* or *G. sepium* dry samples were collected from the experimental materials at 45 days of regrowth, placed in 10 × 15 cm ANKOM™ nylon bags (ANKOM Technology, Fairport, NY, USA) with 53- μm pores. They were incubated in rumen for 0, 6, 12, 24, 48, and 72 h, with three repetitions per incubation time. The bags were sequentially introduced into the rumen and subsequently washed according to the recommendations of Nocek and Grant (1987).

Rumen kinetics were analyzed using the model proposed by Ørskov and McDonald (1979):

$$P = a + b(1 - e^{-cet})$$

Where: P =proportion (%) of material that disappeared from the bag at time t ; a =ruminal solubility or the fraction disappearing at time zero (%); b =insoluble but potentially digestible fraction (%); c =rate of disappearance of fraction b ; t =incubation time.

Statistical analysis

A completely randomized design was used, with two treatments and 10 and 3 repetitions, for dry matter production per plant and per hectare and for *in situ* degradability, respectively. The data were subjected to an analysis of variance (ANOVA) using the PROC GLM procedure (SAS, 2011). Mean comparisons between treatments were performed with Tukey's test, reporting statistically significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Chemical composition and dry matter production

Table 1 shows the chemical composition of *Guazuma ulmifolia* and *Gliricidia sepium*. At 30 days, the crude protein (CP) content of *G. ulmifolia* was 25.2%, while *G. sepium* recorded 23.4%. At 45 days, CP content decreased to 22.7% and 23.02% in *G. ulmifolia* and *G. sepium*, respectively. By day 60, both species continued to show a decline in CP content: *G. ulmifolia* decreased to 19.16%, while *G. sepium* recorded 22.49%. From day 30 to 60, *G. ulmifolia* and *G. sepium* decreased by 5.6% and 0.91%, respectively. These values are higher than those reported by Jiménez *et al.* (2008) —who recorded 13.6% in *G. ulmifolia*— and Castrejón *et al.* (2016) —who reported 12.25% in *G. ulmifolia* and 16.32% in *G. sepium*. The differences with previous studies may be attributed to variations in the age of the plant at the time of sampling, which neither research team specified.

In tropical regions, the low crude protein (CP) content of grasses limits both productivity and reproductive performance in cattle. To maximize forage digestibility, the protein available in the rumen for microbial activity must exceed 8%, with the ultimate purpose of enhancing nutrient use and the overall energy value of the diet (Rayas *et al.*, 2012; Kichel *et al.*, 2018). In this study, the CP content of *G. ulmifolia* and *G. sepium* remained above 19% even at 60 days of regrowth, indicating that both species are an important protein source for livestock feeding. Their high CP content, even during the dry season, makes them viable alternatives for the mitigation of protein deficiencies in tropical cattle production systems.

Table 1. Chemical composition of *Guazuma ulmifolia* and *Gliricidia sepium* at three regrowth ages.

Variable	<i>G. ulmifolia</i>			<i>G. sepium</i>		
	30 days	45 days	60 days	30 days	45 days	60 days
Crude protein (%)	25.20	22.73	19.16	23.45	23.02	22.49
Crude fat (%)	2.68	2.30	2.44	3.17	2.39	2.25
Crude fiber (%)	29.55	35.47	41.67	26.14	22.19	24.34
Nitrogen-free extraction (%)	32.56	28.22	27.37	37.30	42.92	41.41
Neutral detergent fiber (%)	57.91	63.07	67.19	36.23	32.34	35.19
Acid detergent fiber (%)	33.80	38.20	44.20	28.65	25.76	27.37
Ash (%)	10.02	11.28	9.36	9.94	9.48	9.52

Crude fat (ether extract: EE) content at 30 days was 2.68% for *G. ulmifolia* and 3.17% for *G. sepium*. At 45 and 60 days, both species showed a slight decrease in fat content, but their levels remained above 2.2% (Table 1). These results are similar to those reported for *G. sepium* by Santana *et al.* (2019), who recorded a 2.33% EE, and by Winarti *et al.* (2022), who reported 4.47% EE. In the case of *G. ulmifolia*, Pereira *et al.* (2019) reported 2.57% EE—a similar value to the results of this study.

Regarding crude fiber (CF) content, *G. ulmifolia* registered 29.55% at 30 days, increasing to 41.67% at 60 days. In contrast, *G. sepium* showed a 26.14% and 24.34% CF content at 30 and 60 days, respectively (Table 1). These values are lower than those reported in leaf samples by Cabrera *et al.* (2018), who observed 54.3% CF for *G. ulmifolia* and 59.2% CF for *G. sepium*. CF has a positive effect on rumen function, helping to regulate pH and serving as a primary energy source for rumen microorganisms, particularly when livestock diets are forage-based (Dijkstra *et al.*, 2012). As the grass matures, both CF and lignin contents increase, while the ruminal digestibility of dry matter decreases (Van Soest, 1994). This disadvantage of grasses can be mitigated through the incorporation of forage trees with low fiber content into grazing diets.

At 30 days, the nitrogen-free extract (NFE) content of *G. ulmifolia* and *G. sepium* was 32.56% and 37.30%, respectively. At 45 days, NFE in *G. ulmifolia* decreased to 28.22%, while in *G. sepium* it increased to 42.92%. By day 60, *G. ulmifolia* further decreased to 27.37%, while *G. sepium* slightly decreased to 41.41% (Table 1). These results are similar to those reported by Okoruwa *et al.* (2018) (39.02%) and Julius *et al.* (2016) (44.53%). Higher values have also been reported, including the 54.51% NFE content recorded by Guna *et al.* (2023) and the 58.3% content found by Aye *et al.* (2012). In the case of *G. sepium*, the NFE contents in this study are lower than those reported by López *et al.* (2008) (45.14%) and Cabrera *et al.* (2018) (45.90%).

Nitrogen-free extract (NFE) is an estimate of the content of soluble carbohydrates (Apráz *et al.*, 2020)—readily fermentable compounds, such as starch and sugars. They are more easily available than cellulose and therefore support better microbial growth in the rumen (Bach *et al.*, 2005). Grazing cattle diets that contain an adequate concentration of soluble carbohydrates (NFE) significantly improves weight gain (Gaviria *et al.*, 2015).

The neutral detergent fiber (NDF) content at 30 days was 57.91% in *G. ulmifolia* and 36.23% in *G. sepium*. At 45 and 60 days, NDF in *G. ulmifolia* increased to 63.07% and 67.19%, respectively; meanwhile, it slightly decreased (35.19%) in *G. sepium* at 60 days (Table 1). For their part, Cortez *et al.* (2016) recorded 63.88% NDF for *G. ulmifolia*. Meanwhile, Ayala *et al.* (2006) reported 43.2% NDF and 27.2% acid detergent fiber (ADF) for *G. ulmifolia* and 44.2% NDF and 30.2% ADF for *G. sepium*. Finally, Pinto *et al.* (2010) observed 37.10% NDF and 30.17% ADF in *G. ulmifolia* and 46.26% NDF and 32.32% ADF in *G. sepium*.

Neutral detergent fiber (NDF) is primarily composed of cellulose, hemicellulose, and lignin (Van Soest *et al.*, 1991). Its concentration in the diet of ruminants is inversely related to voluntary dry matter intake. Meanwhile, the increase in acid detergent fiber (ADF)—which includes cellulose and lignin—is associated with the decrease of cellulose digestibility (Mertens, 2002). Therefore, the integration of forage trees with low NDF

and ADF content into biodiverse forage systems can enhance both digestibility and forage intake in grazing cattle.

Ash content at 30 days was 10.02% and 9.94% in *G. ulmifolia* and *G. sepium*, respectively. At 45 days, ash content in *G. ulmifolia* increased to 11.28%, while in *G. sepium* it decreased to 9.48%. By day 60, ash content was 9.36% in *G. ulmifolia* and 9.52% in *G. sepium* (Table 1). Other authors report variable ash content: Cortez *et al.* (2016) recorded 12.65% in *G. ulmifolia*, while Molina *et al.* (2013), Pacheco *et al.* (2013), and Mayren *et al.* (2018) reported 9.02%, 9.36%, and 11.3% content in *G. sepium*.

Meanwhile, dry matter production (DMP) (kg plant^{-1} and t ha^{-1}) showed higher values in *G. ulmifolia* at 30-, 45-, and 60-days post-cutting ($P < 0.05$). At 30 days, DMP for *G. ulmifolia* was 0.489 t ha^{-1} , which was higher than the DMP for *G. sepium* (0.328 t ha^{-1}). At 45 days, the production of *G. ulmifolia* increased to 0.835 t ha^{-1} , reaching 1.082 t ha^{-1} at 60 days. Meanwhile, *G. sepium* produced 0.451 t ha^{-1} . The DMP (t ha^{-1}) of *G. ulmifolia* was 0.161, 0.498, and 0.631 higher than that of *G. sepium* at 30, 45, and 60 days, respectively.

Several authors have reported different dry matter (DM) yields under different conditions. Vargas (2017) reported a density of 10,000 plants per hectare with 45-day cutting intervals, in a *G. ulmifolia* forage bank in Colombia, recording 5.8 t ha^{-1} of leaf DM per year, 82 g DM per plant, and 0.8 t ha^{-1} per cut. In Yucatán, Mexico, Casanova *et al.* (2014) reported a 3.7 t DM ha^{-1} and 5.3 t DM ha^{-1} yield during the dry season and the rainy season, respectively. Their experiment was conducted in a plantation with $2 \times 0.5 \text{ m}$ between plants, harvested every three months. Meanwhile, in Colima, Mexico, Palma (1997) reported the highest dry matter (DM) levels in *G. sepium* (1.456 t ha^{-1}), based on an average of five monthly harvests. Rangel (2023) obtained leaf DM yields ranging from 10.5 to 14.8 t ha^{-1} per year in a Brazilian plantation, with 1 m between rows and 50 cm between plants.

The results show that, at 45 days of regrowth, *G. ulmifolia* reaches a dry matter production level of 0.835 t ha^{-1} , with a high crude protein (CP) content and low neutral detergent fiber (NDF) content. Given these characteristics, this forage species could be potentially included in biodiverse forage production systems.

Table 2. Dry matter production of *G. ulmifolia* and *G. sepium* per plant and per hectare, at different regrowth ages.

Days post-cutting	Dry matter production per plant (kg)		
	<i>Guazuma ulmifolia</i>	<i>Gliricidia sepium</i>	EEM
30	0.306a	0.205b	0.026
45	0.522a	0.211b	0.055
60	0.670a	0.282b	0.086
	Dry matter production per hectare (t)		
30	0.489a	0.328b	0.187
45	0.835a	0.337b	0.281
60	1.082a	0.451b	0.269

a, b: Means with different letters in different rows are statistically different, according to Tukey's test ($P < 0.05$). SEM (EEM): standard error of the mean.

At all incubation times, *G. sepium* showed greater degradability than *G. ulmifolia* ($P < 0.05$). Degradability in *G. sepium* exceeded 60% and 91% at 6 and 72 h, respectively. Meanwhile, it reached 58.55% at 24 h in *G. ulmifolia* (Table 3). *In situ* degradability is related to the chemical composition of the forage and the ability of ruminants to degrade it. Higher degradability is associated with a greater availability of forage nutrients for animal functions, including production (Krizsan *et al.*, 2012). In this study, *G. sepium* showed a lower percentage of crude fiber and neutral detergent fiber, which contributes to higher degradability and increased ruminal propionate production. Propionate acts as a hydrogen sink, resulting in a lower proportion of enteric methane (Jun *et al.*, 2023).

The degradation parameters and dry matter degradation rate showed a higher soluble fraction in *G. sepium* than in *G. ulmifolia*, indicating that a greater proportion of dissolved nutrients is available for microbial fermentation (Ørskov and McDonald, 1979).

Meanwhile, both forages recorded similar degradable fraction and degradation rate (Table 4), indicating that microbial enzymes in the rumen can efficiently degrade the nutrients in these forages at a moderate rate, which benefits microbial protein production and volatile fatty acid synthesis (Bach *et al.*, 2005).

The indigestible fraction of dry matter was higher in *G. ulmifolia* than in *G. sepium*; this situation is related to the higher NDF content and may limit digestion (Table 4). According to the results, *G. sepium* has better ruminal degradation parameters: a high soluble and degradable fraction, a low indigestible fraction, and a moderate degradation rate. These characteristics may allow the animal to make better use of other dietary nutrients, such as proteins, carbohydrates, and fats (Bach *et al.*, 2005).

Table 3. *In situ* dry matter degradability (%) of tropical forage shrubs at 45 days of regrowth.

Incubation time (h)	<i>Guazuma ulmifolia</i>	<i>Gliricidia sepium</i>	EEM
72	78.27b	91.24a	2.24
48	73.22b	81.90a	2.95
24	58.55b	76.27a	3.05
12	36.77b	64.03a	2.44
6	29.13b	63.00a	2.06
0	9.12b	39.78a	1.44

a, b: Means within different letters in different rows are statistically different according to Tukey's test ($P < 0.05$). SEM (EEM): standard error of the mean.

Table 4. Dry matter fractions and degradation rate of the evaluated forages (%).

Fraction	<i>Guazuma ulmifolia</i>	<i>Gliricidia sepium</i>
Soluble (a)	34.21b	42.64a
Degradable (b)	43.72b	45.33a
Indigestible	22.07a	12.03b
Degradation rate (k d/h)	0.066a	0.062a

a, b: Means within different letters in different rows are statistically different, according to Tukey's test ($P < 0.05$).

CONCLUSIONS

The crude protein (CP) content in both species was >19% at 30, 45, and 60 days of regrowth, while the in situ dry matter degradability at 45 days of regrowth exceeded 58% after a 24-hour incubation. *Gliricidia sepium* stands out for its high proportion of soluble fraction and low indigestible fraction, which make it a better forage than *Guazuma ulmifolia* in terms of nutritional quality. However, the latter has a greater dry matter production at all three regrowth periods, showing a remarkably high production capacity starting from day 45. In conclusion, *G. ulmifolia* and *G. sepium* have a significant potential to produce high-quality forage in biodiverse forage production systems.

REFERENCES

- Aguirre, M. L., La, O. L. O., González, G. H. y Ladea, R. J. (2021). Producción forrajera de especies de arbóreas en ecosistemas frágiles, salinos y de alta sequía, del Oriente Cubano. *Bosques Latitud Cero*, 11(1), 148-154.
- AOAC. (2012). Official Methods of Analysis of AOAC international. 19th Edition. Washington DC.
- Apráez, G. J. E. (2020). Análisis Químico de Alimentos para Animales.- 1ª. Ed. San Juan de Pasto. Editorial Universidad de Nariño.
- Ayala, B. A. J., Capetillo, L. C. M., Cetina, G. R. H., Zapata, C. C. y Sandoval, C. C. A. (2006). Composición química-nutricional de árboles forrajeros. Compilación de los análisis de laboratorio de nutrición animal. Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán.
- Aye, P. A. (2012). Production of *Gliricidia* and *Leucaena* – based multi-nutrient blocks as supplementary ruminant feed resource in South Western Nigeria. *Agriculture and Biology Journal of North America*, 3(5), 213-220. Doi:10.5251/abjna.2012.3.5.213.220.
- Bach, A., Calsamiglia, S. and Stern, M. D. (2005). Nitrogen Metabolism in the Rumen. *J. Dairy Sci.*, 88(E. Suppl.), E9-E21. [https://doi.org/10.3168/jds.S0022-0302\(05\)73133-7](https://doi.org/10.3168/jds.S0022-0302(05)73133-7).
- Cabrera, N. A., Daniel, R. I., Del C., Lammoglia, V. M. A., Martínez, S. C. E., Alarcón, P. S. A. and Rojas, R. R. (2018). Effect of *Guazuma* Leaves (*Guazuma ulmifolia*) Inclusion as Protein Source on Growing Calves. *Journal of Agricultural Science and Technology*, A8, 48-53. Doi: 10.17265/2161-6256/2018.01.006.
- Canul, S. J. R., Castillo, S. L., E., Escobedo, M. J. G., López, H. M. A. & Lara, L. P. E. (2018). Rendimiento y calidad forrajera de *Gliricidia sepium*, *Tithonia diversifolia* y *Cynodon nlemfuensis* en monocultivo y sistema agroforestal. *Agrociencia*, 52(6), 853-862.
- Casanova, L. F., Solorio, S. F. J., Ramírez, A. L., Caamal, M. J. A. and Ku, V. J. C. (2014). Forage yield and quality of *Leucaena leucocephala* and *Guazuma ulmifolia* in tropical silvopastoral systems. *Tropical Grasslands – Forrajes Tropicales*, 2, 24-26. [https://doi.org/10.17138/tgft\(2\)24-26](https://doi.org/10.17138/tgft(2)24-26).
- Castrejón, P. F. A., Martínez, P. P., Corona, L., Valle, C. J. L. & Mendoza, G. D. (2016). Partial substitution of soybean meal by *Gliricidia sepium* or *Guazuma ulmifolia* leaves in the rations of growing lambs. *Trop Anim Health Prod*, 48(1), 133-137. Doi: 10.1007/s11250-015-0932-2.
- CONAGUA, Comisión Nacional del Agua (2024). Información Estadística Climatológica. Servicio Meteorológico Nacional. <https://smn.conagua.gob.mx/es/climatologia/informacion-climatologica/informacion-estadistica-climatologica> (13 de marzo de 2025).
- Cortez, E. J. G., Uribe, G. M., Cruz, L. A., Lara, B. A. y Romo, L. J. L. (2016). Árboles nativos para el diseño de tecnologías silvopastoriles en la Sierra de Huautla, Morelos. *Revista Mexicana de Ciencias Agrícolas*, 16, 3371-3380.
- Cuervo, J. A., Narváez, S. W. & Von, H. C. H. (2013). Características forrajeras de la especie *Gliricidia sepium* (Jacq.) Stend, Fabaceae. *Boletín Científico Centro de Museos, Museo de Historia Natural*, 17(1): 33-45.
- Dijkstra, J., Ellis, J. L., Kebreab, E., Strathe, A. B., López, S., France, J. and Bannink, A. (2012). Ruminant pH regulation and nutritional consequences of low pH. *Animal Feed Science and Technology*, 172(1), 22-33. <https://doi.org/10.1016/j.anifeeds.2011.12.005>
- Gaviria, X., Rivera, J. E. y Barahona, R. (2015). Calidad nutricional y fraccionamiento de carbohidratos y proteína en los componentes forrajeros de un sistema silvopastoril intensivo. *Pastos y Forrajes*, 38(2), 194-201.
- Guna, D. I., Jayanegara, A., Sofyan, A., Budiartilaconi, E., Ridla, M. and Herdian, H. (2023). Evaluation of nutritional values of tree-forage legume leaves from Gunungkidul District, Indonesia. *Biodiversitas*, 24(5), 2733-2745. DOI:10.13057/biodiv/d240527.

- INEGI, Instituto Nacional de Estadística y Geografía. (2025). Geografía y Medio Ambiente. Climatología. <https://www.inegi.org.mx/temas/climatologia/> (13 de marzo de 2025).
- Jiménez, F. G., López, C. M., Nahed, T. J., Ochoa, G. S. y Jong, B. de (2008). Fodder trees and shrubs of the north-tzotzil region of Chiapas, Mexico. *Vet. Méx.*, 39(2), 199-213.
- Julius, O. O., Olajide, A. V. and Mercy, O. S. (2016). Nutrient Digestibility and Growth Performance of West African Dwarf (WAD) Goats Fed Foliage Combinations of *Moringa oleifera* and *Gliricidia sepium* with Equal Proportions of A Low-cost Concentrate. *Journal of Natural Sciences Research*, 6(18), 202-29.
- Jun, F. A. G., Bestil, L. C. and Obitsu, T. (2023). Ruminant volatile fatty acid concentration and methane gas production in sheep (*Ovis aries*) fed two types of basal diets with or without *Gliricidia sepium* legume forage supplementation. *Annals of Tropical Research*, 45(2), 89-101. <https://doi.org/10.32945/art4525.2023>.
- Kichel, A. N., Souza, L. C. F. de, Almeida, R. G. de, & Costa, J. A. A. da. (2018). Productivity and nutritional value of tropical grasses in monoculture and intercropping with interseason corn. *Ciências Agrárias*, 39(6), 2517-2530. <https://doi.org/10.5433/1679-0359.2018v39n6p2517>.
- Krizsan, S. J., Nyholm, L., Nousiainen, J., Karl, H. S. and Pekka, H. (2012). Comparison of *in vitro* and *in situ* methods in evaluation of forage digestibility in ruminants. *Journal of animal science*, 90, 3162-3173. Doi:10.2527/jas2011-4347.
- Gómez, G. R., Palma, L. D. J., Obrador, O. J. J. y Ruiz, R. O. (2018). Densidad radical y tipos de suelos en los que se produce café (*Coffea arabica* L.) en Chiapas, México. *Ecosistemas y recursos agropecuarios*, 5, 203-215.
- López, H. M. A., Rivera, L. J. A., Ortega, R. L., Escobedo, M. J. G., Magaña, M. M. A., Sanginés, G. J. R. and Sierra, V. A. C. (2008). Nutritional composition and antinutritional factor content of twelve native forage species from northern Quintana Roo, Mexico. *Téc. Pec. Méx.*, 46(2), 205-215.
- Manríquez, M. L. Y., López, O. S., Pérez, H. P., Ortega, J. E., López T. Z. G. and Villarruel, F. M. (2011). Agronomic and forage characteristics of *Guazuma Ulmifolia* Lam. *Tropical and Subtropical Agroecosystems*, 14, 453- 463.
- Mayren, M. F. J., Rojas, G. A. R., Maldonado, P. M. A., Ramírez, R. O., Herrera, P. J., Torres, S. N., Sánchez, S. P., Bottini, L. M. B. and Hernández, G. A. (2018). Productive behavior of pelibuey sheep in grazing supplemented with *Guazuma ulmifolia* Lam. *Agroproductividad*, 11(5), 29-33.
- Mertens, D. R. (2002). Measuring fiber and its effectiveness in ruminant diets. *Journal of Dairy Science*, 85(12), 2873-2890.
- Molina, B. I. C., Cantet, J. M., Montoya, S., Londoño, G. A. C. and Rosales, R. B. (2013). Producción de metano *in vitro* de dos gramíneas tropicales solas y mezcladas con *Leucaena leucocephala* o *Gliricidia sepium*. *Ces. Med. Vet. Zootec.*, 8(2), 15-31.
- Nocek, J. E. and Grant, A. L. (1987). Characterization of *in situ* nitrogen and fiber digestion and bacterial nitrogen contamination of hay crop forages preserved at different dry matter percentages. *J. Anim. Sci.*, 64, 552-564.
- Okoruwa, M. I., Edobor, A. E., Ogbeide, F. O. and Ikhimiyo, I. (2018). Predicting nutrient retention for body weight gain in ewe-lambs fed diets containing gliricidia (*Gliricidia sepium*) and neem (*Azadirachta indica*) leaves supplement. *Nigerian J. Anim. Sci.*, 20(1), 106-114.
- Ørskov, E. R. and McDonald, I. (1979). The estimated of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science*, 92(2), 499-503.
- Ørskov, E. R. (2000). The *in situ* technique for the estimation of forage degradability in ruminants. In D. I. Givens, E. Owen, R. F. E. Axford & H. M. Omed (Eds.), *Forage Evaluation in Ruminant Nutrition* (pp. 175-198). CAB International.
- Pacheco, W. F., Carneiro, M. S. de S., Edvan, R. L., Arruda, P. C. L. de e Carmo, A. B. do. (2013). Valor nutritivo de silagens de capim-elefante (*Pennisetum purpureum*, Shum) com feno de gliricídia (*Gliricidia sepium* (Jacq.) Walp). *Revista Verde de Agroecologia e Desenvolvimento Sustentável*, 8(2), 240-246.
- Palma, J. M. (1997). Establishment of *Gliricidia sepium* in the dry tropic with high density. *Archivos Latinoamericanos de Producción Animal*, 5(S1), 5-6.
- Pereira, G. A., Peixoto Araujo, N. M., Arruda, H. S., Farias, D. P., Molina, G. and Pastore, G. M. (2019). Phytochemicals and biological activities of mutamba (*Guazuma ulmifolia* Lam.): A review. *Food Research International*, 126, 108713. DOI: 10.1016/j.foodres.2019.108713
- Pinto, R. R., Hernández, D., Gómez, H., Cobos, P. M. A., Quiroga, R. y Pezo, D. (2010). Árboles forrajeros de tres regiones ganaderas de Chiapas, México: usos y características nutricionales. *Universidad y Ciencia*, 26(1), 19-31.
- Rangel, J. H. de A., Muniz, E. N., Souza, S. F. de, Santos, R. D. dos and Piovezan, U. (2023). *Gliricidia sepium*: a promising legume tree for the Brazilian semiarid zone. *The Journal of the International Legume Society*, (17), 36-38.

- Rayas, A. A., Estrada, F. J. G., Lawrence, M. F. and Castelán, O. O. (2012). Nutritional value of forage species from the Central highlands region of Mexico at different stages of maturity. *Ciencia Rural*, 42(4), 705-712.
- Santana, A. A., Cheng, L., Verdecia, D. M., Ramírez, J. L., López, S., Cisneros, M. V., Rugoho, I., Maxwell, T. M. R. and Al, M. O. (2019). Effect of a mixed ilage of king grass (*Cenchrus purpureus*) and forage legumes (*Leucaena leucocephala* or *Gliricidia sepium*) on sheep intake, digestibility and nitrogen balance. *Animal Production Science*, 59(12), 2259-2264. DOI:10.1071/AN18559.
- SAS. (2011). User, s Guide: Statistics, version 9.3. Ed. SAS Institute, Inc., Cary N.C.
- Solorio, F., Wright, J., Franco, J., Basu, S., Sarabia, L., Ramírez, L., Ayala, A., Aguilar, C. and Ku, J. (2017). Silvopastoral Systems: Best Agroecological Practice for Resilient Production Systems under Dryland and Drought Conditions. In M. Ahmed & C. O. Stockle (Eds.), *Quantification of Climate Variability, Adaptation and Mitigation for Agricultural Sustainability* (pp. 233-250). Springer.
- Van-Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Van-Soest, P. J. (1994). *Nutritional ecology of the ruminants*. Comstock Publishing Associates, Cornell University Press.
- Vargas, J., A. F., & Quintero, B. D. (2017). Evaluación de bancos forrajeros de Guácimo (*Guazuma ulmifolia*) en el Piedemonte Llanero colombiano. Retrieved from <https://ciencia.lasalle.edu.co/zootecnia/> 330.
- Winarti, E., Gunawan, A., Sofyan, C.A., Wirasti, C.T., Noviandi, P. A., Agus, K.J., and Poppi, D. P. (2022). Improving live weight gain in Ongole crossbred bulls through processing of *Gliricidia sepium* leaf meal and cassava in a supplement concentrate. *Animal Feed Science and Technology*, 292, 115401. <https://doi.org/10.1016/j.anifeedsci.2022.115401>.

Effect of coffee pulp silage on productive and microbiological variables in New Zealand rabbits

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ABSTRACT

Objective: To determine the optimal inclusion level of coffee pulp in the diet of New Zealand rabbits based on their productive and microbiological parameters.

Methodology: Thirty (n=30) weaned male rabbits, with an average age of 30 days and an average weight of 589 g, were randomly distributed into individual cages across three treatments with inclusion levels of 0%, 25%, and 45% coffee pulp silage fermented for 60 days. The animals were fed for eight weeks with isoenergetic and isoproteic diets.

Results: Feed intake did not show significant differences, suggesting that the inclusion of 45% coffee pulp silage does not affect the diet's acceptability for rabbits. Although feed intake was not affected, daily weight gain showed significant differences among treatments. On the other hand, hot carcass yield presented significant differences between treatments, with the 0% coffee pulp inclusion showing the highest carcass yield. Regarding cecal total bacterial concentration, an increasing trend was observed in treatments with higher inclusion levels of coffee pulp silage.

Implications/Limitations: No significant limitations were observed.

Conclusions: The use of coffee pulp silage remains a viable alternative for rabbit feeding, showing excellent acceptability by the animals and promoting cecal bacterial growth.

Keywords: Tannins, Carcass, Cecum, Nutrition.

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INTRODUCTION

Over the years, various agricultural by-products have been used in animal feeding with the aim of improving productive performance and reducing feed costs. The inclusion of coffee pulp in animal diets is not recent, as its use has been documented for more than 30 years in species such as sheep (Ferreira *et al.*, 2003), poultry (Florez and Rosales, 2018), pigs (Braham and Bressani, 1978), fish (Moreau *et al.*, 2003), cattle (Vargas *et al.*, 1977),



and rabbits (Bautista *et al.*, 1999). The presence of high concentrations of tannins and caffeine in coffee pulp limits its inclusion in animal diets (Salinas *et al.*, 2015), since, by acting as enzyme inhibitors, they reduce the availability of consumed protein (Noriega *et al.*, 2008). However, these concentrations can be reduced through silage, improving its nutritional value (Ferreira *et al.*, 2000). It has been demonstrated that, at optimal levels, these compounds can provide antioxidant properties beneficial to the animal (Sroka and Cisowski, 2003). Currently, the inclusion of coffee pulp in animal diets is mainly applied to ruminants; however, it represents an excellent alternative for rabbit feeding, where inclusion levels of up to 85% have been reported (Bautista *et al.*, 1999). The dietary fiber content of coffee pulp favors the digestive function of rabbits (Zhu *et al.*, 2014), since it is known that a low intake of dietary fiber leads to a decrease in cecal bacterial biomass, associated with reduced fermentative activity and consequently lower bacterial fibrolytic activity (Bellier and Gidenne, 1996), which may result in decreased nutrient absorption and utilization. Based on the above, the optimal inclusion level of coffee pulp in the diet of New Zealand rabbits was evaluated through their productive and microbiological parameters.

MATERIALS AND METHODS

This study was conducted at the Rabbit Production Module of the Educational Program in Agricultural Engineering and Animal Science of the Benemérita Universidad Autónoma de Puebla.

Coffee Pulp Silage

The silage process of the coffee pulp was carried out in 1,100-liter airtight containers, with a fermentation period of 60 days. It is important to note that the silage was drained and dried prior to its incorporation into the diets.

Animals

Thirty ($n=30$) weaned male New Zealand rabbits, with an average age of 30 days and an average weight of 589 ± 39 g, were randomly distributed into individual cages across three treatments with inclusion levels of 0% (T1), 25% (T2), and 45% (T3) coffee pulp silage (Table 1).

Feeding

The animals were fed isoenergetic and isoproteic diets for eight weeks, formulated according to the requirements for growing rabbits established by the NRC (1985), with free access to feed and water (Table 2).

Productive Variables

Feed intake (FI) was calculated as the difference between final and initial body weight divided by the number of days in the period. Daily weight gain (DWG) was measured periodically, considering the amount of feed offered and refused. Hot carcass yield (HCY) was obtained by the ratio of hot carcass weight to slaughter weight, multiplied by 100.

Table 1. Composition of the experimental diets.

Ingredients	Treatments		
	T1	T2	T3
Coffee pulp	0	25	40
Alfalfa	20	8	0
	13	9	10
Corn stubble	20	15	20
	24	25	24
Corn kernels	10	12	0
	7	0	0
Sorghum kernels	5	5	5
	1	1	1

Table 2. Chemical analysis of the diets.

Component (%)	Treatments		
	T1	T2	T3
CP	14.17	15.24	14.70
ADF	42.00	45.35	46.46
NDF	34.23	34.00	35.00
Ash	5.52	5.60	6.33

T1: 0% control, T2: 25% coffee pulp, T3: 45% coffee pulp.

CP: Crude protein; ADF: Acid detergent fiber; NDF: Neutral detergent fiber.

Cecal Bacteria

Cecal bacterial counts were performed using the direct count technique with a Petroff-Hausser counting chamber by capillarity, with an area of 0.0025 mm² and a depth of 0.02 mm. Counting was carried out using an optical microscope model WF10X-18MM at 100X magnification. Total bacterial concentration was calculated using the following formula described by Ley de Coss *et al.* (2016):

$$\text{Total bacteria mL}^{-1} = (\text{mean})(\text{dilution factor})(2 \times 10^7)$$

Carcass Temperature and pH

Temperature and pH data were obtained at the time of slaughter for all animals. The selected region for measurement was the Biceps Femoris at 0- and 20-min post-mortem, using a Hanna potentiometer, model HI9811-5.

Statistical Analysis

A completely randomized design with three treatments was used. Data for productive variables, pH, and temperature were analyzed by analysis of variance (ANOVA). Hot carcass yield data were subjected to an arcsine transformation. Mean comparisons were performed using Tukey's test.

RESULTS AND DISCUSSION

Productive Parameters

Feed intake (Table 3) did not show significant differences ($P>0.05$), suggesting that inclusion of up to 45% coffee pulp silage does not affect diet acceptability in rabbits. This behavior is similar to that reported in other species (Salinas *et al.*, 2015; Noriega *et al.*, 2008; Bautista *et al.*, 1999; Romero *et al.*, 1995), where no significant differences in feed intake were observed with the inclusion of coffee pulp silage in the diet. On the other hand, Yoplak *et al.* (2017) evaluated inclusion levels of 5, 15, 25, and 35% coffee pulp meal in diets for Peruvian guinea pigs, finding differences between treatments, with the control and the 5% coffee pulp meal inclusion showing the best response in terms of feed intake. This behavior may be more related to diet presentation than to coffee pulp inclusion, as feed intake depends on multiple factors, including feed texture (Tarazona *et al.*, 2012), with very fine particle diets reducing voluntary feed consumption.

Although feed intake was not affected, daily weight gain showed significant differences between treatments ($P<0.05$). This effect was observed in animals fed with coffee pulp silage and may be related to the amount of tannins present in the silage, as they can bind proteins, reducing their absorption and utilization, thereby decreasing the biological availability of this nutrient (Vázquez *et al.*, 2012). This condition limits the supply of amino acids for protein synthesis, which is necessary for various physiological functions, including growth. When adequate protein intake is not achieved under chronic conditions, the body may develop protein hypercatabolism, leading to continuous depletion of protein and energy reserves, resulting in loss of fat and muscle mass (García *et al.*, 2014), reflected in a reduction in body weight.

It should not be overlooked that one of the processes to reduce tannins in coffee pulp is silage (Ferreira *et al.*, 2000). However, this process depends on the fermentation time to which the pulp is subjected during ensiling; that is, the shorter the fermentation period, the higher the tannin content.

Regarding hot carcass yield, it is directly proportional to the final body weight of the animals, showing significant differences between treatments ($P\leq 0.05$), with the control group (0% inclusion) exhibiting the highest carcass yield percentage. However, all three treatments performed optimally in terms of hot carcass yield for New Zealand rabbits, according to Vázquez *et al.* (2007), who reported a hot carcass yield of 55% for this breed. Similarly, Hernández *et al.* (2015) mentioned that rabbit carcass yield ranges between 50 and 65%, depending on various factors such as diet, breed, management, visceral weight, and others.

Table 3. Productive response of rabbits fed with varying levels of coffee pulp.

Variable	T1	T2	T3
Feed intake (g day^{-1})	97.45 ^a	96.98 ^a	108.95 ^a
Daily weight gain (g day^{-1})	29.25 ^a	10.77 ^b	8.73 ^b
Carcass yield (%)	72.02 ^a	61.15 ^{ab}	59.58 ^b

T1: 0% coffee pulp, T2: 25% coffee pulp, T3: 45% coffee pulp.

^{a,b,c} Different letters in the same row indicate significant differences ($P 0.05$).

Temperature and pH

Regarding carcass temperature, significant differences were observed between treatments ($P \leq 0.05$), with a marked decrease at 20 minutes post-mortem for each treatment (Table 4). These values are consistent with Flores (2009), who reported temperatures of 38.0 ± 5.8 °C at 0 minutes and 23.0 ± 7.2 °C at 20 minutes post-mortem using the stunning by neck dislocation method, which was the same method applied in this study. The relationship between pH and temperature is very close, and a primary response of this relationship is meat shortening, an irreversible effect that negatively affects meat quality. This occurs when cooling drops below 10 °C before rigor mortis, causing contractions of muscle tissues (sarcomeres) and increasing meat toughness.

pH is one of the main variables for determining meat quality, as color, water-holding capacity, and tenderness depend directly on this parameter (Cornejo *et al.*, 2016). The results obtained in this study regarding pH did not show significant differences ($P \geq 0.05$) at 0 and 20 min post-mortem, with a minimal decrease compared to expected values. It should be noted that normal pH values for rabbit meat range from 5.71 to 6.0 (Ouhayoun and Dalle, 1993).

This pH response is influenced by several factors, such as the amount of glycogen present in the muscle during stunning, the type of muscle analyzed (in glycolytic muscles, pH can drop to 5.6, while in oxidative muscles it ranges up to 6.4) (Cabanes, 1996), sex, management, species (Mota *et al.*, 2012), breed (Smitzis *et al.*, 2014), and certain hormones present in the bloodstream. Hernández *et al.* (2015) analyzed carcass quality in New Zealand and California breeds, reporting a sharp decrease in pH in New Zealand rabbit carcasses during the first 20 minutes post-mortem, stabilizing after 12 hours with a final pH of 6.1.

Total Cecal Bacteria Count

Total cecal bacterial concentration showed significant differences between treatments ($P \leq 0.05$), as shown in Table 5. Although the diet analyses revealed similar fiber contents (ADF and NDF), a growing trend was observed in treatments containing coffee pulp silage. This suggests that, even when fiber percentages were similar, the nature of the fiber allowed greater cecal fermentation and, consequently, an increase in bacterial development, as these microorganisms are responsible for degrading approximately 75% of the fiber consumed by the animal (Hernández and Cobos, 2001).

Table 4. Post-mortem carcass variables of rabbits fed with varying levels of coffee pulp.

Variable	Time (min)	T 1	T 2	T 3
Temperature (°C)	0	34.15 ^a	31.50 ^b	29.31 ^c
	20	28.20 ^a	23.85 ^b	24.90 ^c
pH	0	7.83 ^a	7.65 ^a	7.50 ^a
	20	7.64 ^a	7.46 ^a	7.19 ^a

T1: 0% coffee pulp, T2: 25% coffee pulp, T3: 45% coffee pulp.

^{a,b,c} Different letters in the same row indicate significant differences ($P \leq 0.05$).

Table 5. Total cecal bacteria results.

Treatments	Total bacteria in cecal appendix
T1	1.7×10^{11a}
T 2	1.9×10^{11b}
T 3	2.2×10^{11c}

T1: 0% coffee pulp, T2: 25% coffee pulp, T3: 45% coffee pulp.
^{a,b,c} Different letters in the same row indicate significant differences ($P \leq 0.05$).

This is important because it has been demonstrated that cecal bacteria directly participate in immunoglobulin synthesis, thereby enhancing the animal's immune system and contributing to the presence of beneficial bacteria in the digestive tract. Additionally, increased lignin content in rabbit diets has been associated with a lower incidence of digestive transit problems (Caïsin *et al.*, 2020).

CONCLUSIONS

The use of coffee pulp silage remains a viable alternative in rabbit feeding, showing excellent acceptability by the animals and promoting cecal bacterial growth. However, further research is needed on optimal inclusion levels and ensiling times, as inclusion levels of 25% and 45% of coffee pulp silage fermented for 60 days negatively affected carcass yield and daily weight gain. It is worth noting the potential for studying the quality of fiber provided by coffee pulp silage and its role in managing intestinal microbiota, which could help elucidate the prebiotic effects it may have on the animal.

REFERENCES

- Animal Feed Science and Technology, 262 1-9 <https://doi.org/10.1016/j.anifeeds.2020.114396>
- Bautista, E.O., Useche, M., Pérez, P., & Linares, F. (1999). Utilización de la pulpa de café ensilada y deshidratada en la alimentación de Cachamay (*Colossoma × Piaractus*). En Ramírez J. (Ed) Pulpa de Café Ensilada. Producción, Caracterización y Utilización en la Alimentación Animal. Consejo de Desarrollo Científico y Humanístico, Universidad Central de Venezuela, pp. 109-135.
- Bellier R. & Gidenne T. (1996). Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbit. *British Journal of Nutrition*. 75(3):353-63. <https://doi.org/10.1079/bjn19960139>
- Braham, J. & Bressani, R. (1978). Coffee Pulp. Composition, Technology and Utilization. Institute of Nutrition of Central America and Panama. Ottawa Research and Development Centre, Canada.
- Cabanes A. (1996). Qualités de la viande de lapin facteurs de variation des qualités organoleptiques et caractères corrélés. *Viandes & Produits Carnés*, (17): 10-16.
- Cornejo, J.G.E., Rodríguez, O.L.T., Pro, A.M., González, F.C., Conde, M.V.F., Ramírez, M.E.G., López, P.E., & Hernández, A.S. (2016). Efecto del ayuno ante mortem en el rendimiento de la canal y calidad de la carne de conejo. *Archivos de Zootecnia*. 65(250): 171-175
- Ferreira I., Olalquiaga, J., & Teixeira, J. 2003. Componentes de carcaça e composição de alguns cortes de cordeiros Texel × Bergamácia, Texel × Santa Inês e Santa Inês puros, terminados em confinamento, com casca de café como parte da dieta. *Revista Brasileira de Zootecnia*. 32(6):178-199. <https://doi.org/10.1590/S1516-35982003000800025>
- Ferreira I., Olalquiaga, J., Teixeira, J. & Pacheco, C. (2000). Desempenho de cordeiros Texel × Bergamácia, Texel × Santa Inês e Santa Inês Puros, terminados em confinamento, alimentados com casca de café como parte da dieta. *Revista Brasileira de Zootecnia*, 29(2): 89-100. <https://doi.org/10.1590/S1516-35982000000200033>

- Flores, P. S. 2009. Efecto del periodo de ayuno y método de aturdimiento sobre el bienestar y características físico-químicas de la carne de conejo. Universidad Nacional Autónoma de México. Maestría de ciencia de la producción animal. 56 p
- Flórez, D.D.F. & Rosales, A.E. (2018). Uso del ensilaje de pulpa de café en alimentación animal. *Mundo* 15(1):73-82. <https://doi.org/10.61799/2216-0388.254>
- Gracia, C.I., González, P.E., Barril, C.E., Sánchez, R.G.E., Ortiz, A.A.J., & Carrero, J.J.(2014). Definiendo el síndrome de desgaste proteico energético en la enfermedad renal crónica: prevalencia e implicaciones clínicas. *Nefrología (Madrid)*, 34(4), 507-519.https://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0211-69952014000400011#:~:text=https%3A//dx.doi.org/10.3265/Nefrologia.pre2014.Apr.12522%C2%A0
- Hernández, S.D., & Cobos P.M.A. (2012). Digestibilidad *in vitro*, población de bacterias celulolíticas y totales del apéndice cecal, ciego y colon del conejo. *Revista Mexicana De Ciencias Pecuarias*, 39(3). 1-8. <http://dx.doi.org/10.24275/uam/izt/dcbn/nacameh/2015v9n2/Hernandez>
- Jorge Hernández Bautista, Jesica Leticia Aquino López*, Amós Palacios Ortiz (2015). Rendimiento de la canal, color de la carne y evolución del pH muscular de conejos. *NACAMEH* Vol. 9, No. 2, pp. 66 76.
- Jorge Hernández Bautista, Jesica Leticia Aquino López, Amós Palacios Ortiz. (2015). Rendimiento de la canal, color de la carne y evolución del pH muscular de conejos. *NACAMEH*. 9(2):66 76.
- L. Caísín, E. Martínez-Paredes, L. Ródenas, V.J. Moya, J.J. Pascual, C. Cervera, E. Blas, M. Pascual (2020). Effect of increasing lignin in isoenergetic diets at two soluble fibre levels on digestion, performance and carcass quality of growing rabbits,
- Ley de Coss, A.L., Guerra, W.M., Cándido, E., Espino, C.A., Ruiz, R.P. (2016). Crecimiento de bacterias ruminales en un medio de cultivo a base de pasta de *Jatropha curcas* L. sin detoxificar. *Agrociencia*, 50(8), 1001-1011.
- Moreau Y., Arredondo, &, Perraud, I. y Roussos. S. (2003). Utilización dietética de la proteína y de la energía de la pulpa de café fresca y ensilada por las tilapias del Nilo (*Oreochromis niloticus*). *Brazilian Archives of Biology and Technology*, 46(2): 35-347. <http://dx.doi.org/10.1590/S1516-89132003000200014>
- Mota, D.R, Bolanos, D.L., Concepcion, M.M., Ramirez, T.J., Roldan, S.P., Flores, P.S., Mora, M.P. (2012). CO₂ gas stunning pigs: controversies in animal welfare and behaviour. *International Journal of Pharmacology*. (8): 141-151.<http://dx.doi.org/10.3923/ijp.2012.141.151>
- N R C, (1985) Nutrient requirements of sheep. 6th Edition, National Academy of Sciences, National Research Council, Washington, D.C.
- Noriega, S., Silva, A.A.R., & García de Salcedo, M. (2008). Revisión: Utilización de la pulpa de café en la alimentación animal. *Zootecnia Tropical*, 26(4): 411-419.
- Ouhayoun, J., Dalle, Z.A. (1993). Muscular energy metabolism and related traits in rabbit: A review. *World rabbit science*. 01(3): 97-108.<http://dx.doi.org/10.4995/wrs.1993.201>
- Salinas, R.T., M.E. Ortega, M.T. Sánchez, J. Hernández, A. Díaz, J.L. Figueroa, R. Guinzberg, & J.L. Cordero. 2015. Productive performance and oxidative status of sheep fed diets supplemented with coffee pulp. *Small Ruminant Research*. 123:17-21<https://www.sciencedirect.com/science/article/abs/pii/S0921448814002697?via%3Dihub#:~:text=https%3A//doi.org/10.1016/j.smallrumres.2014.09.008>
- Smitzis P.E., C. Babaliaris, M.A. Charismiadou, G. Papadomichelakis, M. Goliomytis, G.K. Symeon, S.G. Deligeorgis (2014). Effect of hesperidin dietary supplementation on growth performance, carcass traits and meat quality of rabbits. *World Rabbit Science*; 22: 113 12.<https://doi.org/10.4995/wrs.2014.1760>
- Sroka, Z & Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some fenolic acid. *Food and chemical toxicology*. 41:753-758.[https://doi.org/10.1016/S0278-6915\(02\)00329-0](https://doi.org/10.1016/S0278-6915(02)00329-0)
- Tarazona, A.M. Ceballos, M.C., Naranjo, J.F., Cuartas, C.A. (2012). Factores que afectan el comportamiento de consumo y selectividad de forrajes en rumiantes. *Revista Colombiana de Ciencias Pecuarias*. 25(3):473-487.
- Vargas E., Cabeza, M. & Bressani. R. (1977). Pulpa de café en la alimentación de rumiantes. Absorción y retención de nitrógeno en novillos alimentados con concentrados elaborados con pulpa de café deshidratada. *Agronomía Costarricense*, 1(2): 101-106.
- Vásquez, R.M., Manrique, C., Rodríguez, Y. (2007). Evaluación genética del comportamiento productivo y reproductivo en núcleos de conejos de las razas Nueva Zelanda y Chinchilla. *Ciencia y Tecnología Agropecuaria Genética Animal y Biodiversidad*. (15):24-27 https://doi.org/10.21930/rcta.vol8_num1_art:86
- Vázquez, F.A.A, Alvarez, P.E., López, D.J.A., Wall, M.A & De la Rosa, L.A. (2012). Taninos hidrolizables y condensados: naturaleza química, ventajas y desventajas de su consumo. *TECNOCENCIA Chihuahua* 6(2): 84-93.<https://doi.org/10.54167/tch.v6i2.678>

- Yoplac, I., Juan, Y., Vásquez, H.V., & Maicelo, J.L. (2017). Efecto de la alimentación con pulpa de café (*Coffea arabica*) en los índices productivos de cuyes (*Cavia porcellus* L) Raza Perú. *Revista de Investigaciones Veterinarias del Perú*, 28(3): 549-560.<http://dx.doi.org/10.15381/rivep.v28i3.13362>
- Zhu, L., Liu, W., Alkhouri, R., Baker, RD., Bard, JE., Quigley, EM., and Baker, SS. (2014). Structural changes in the gut microbiome of constipated patients. *Physiological Genomics* 46: 679-686.<https://doi.org/10.1152/physiolgenomics.00082.2014>



Impact of 22-Year Rotational Grazing on the Soil Cover of a Grassland in Northern-Central Mexico

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ABSTRACT

Objective: To evaluate the impact of 22-year rotational grazing on the soil cover change of a grassland in the northern-central region of Mexico.

Design/Methodology/Approach: In 2000, two transects were established in a grassland to measure soil and vegetation properties. The transects were established at the same time than the rotational grazing. Subsequently, additional information corresponding to 2002, 2003, 2004, 2005, and 2022 was registered. Each variable was subjected to an analysis of variance to identify the effect of rotational grazing.

Results: Rotational grazing increased the plant base, leaf litter cover, and soil cover, while it reduced bare soil and mature crust.

Study Limitations/Implications: Only few ranchers use rotational grazing in this region; consequently, no more ranches were included in this study.

Findings/Conclusions: Rotational grazing was fundamental to improve soil cover in the grassland. The increase of the plant base of perennial plants was associated with a precipitation increase.

Keywords: Plant base, leaf litter, soil cover.

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INTRODUCTION

Native plants from grasslands mainly consist of grasses, grass-like plants, weed, and shrubs. Grasslands provide different ecosystem services and benefits to humans, including forage for cattle (Goodwin *et al.*, 2023).

Natural grasslands cover 4.9% (9,578,430 ha) of the Mexican territory (SEMARNAT, 2019) and are mainly located in its northern-central region. The cow-calf production system predominates in this area. This system consists of a continuous grazing, where animals have constant and unrestricted access to grasslands during a given period or the whole year. This system can make excessive use of certain areas and can potentially lead to overgrazing (Teague *et al.*, 2011).



In large paddocks, cattle graze selectively and tend to use preferred areas, consequently, increasing the animal load in those areas. When this type of grazing pattern is repeated year after year, a progressive deterioration of grassland takes place. This situation worsens during drought periods (Teague *et al.*, 2011; Wang *et al.*, 2020). Worldwide, grasslands have suffered a notorious degradation and desertification, as a consequence of the inappropriate use by humans (Teague and Dowhower, 2022).

In Mexico, deteriorated grasslands cover 3,794,283 ha, accounting for 39.61% of the natural grasslands (CONABIO, 2021). This situation leads to an increase of bare soils, erosion, and runoffs. In addition, it reduces infiltration, forage capacity, and ecosystem resilience (Teague *et al.*, 2013; Wang *et al.*, 2020).

Rotational grazing is an alternative to continuous grazing (Lawrence *et al.*, 2019, Wang *et al.*, 2022). This system consists of using fences to divide grasslands into several paddocks. Afterwards, cattle are moved between paddocks on a scheduled basis (Sandhage-Hofmann, 2023). The aim is to follow short grazing periods which involve a large animal load with long break periods in which plants and soil can rest and recover (Lawrence *et al.*, 2019).

Rotational grazing efficiently improves the use of resources from grasslands and prevents the deterioration caused by overgrazing (Xiaoyan *et al.*, 2019). In some countries, the benefits of rotational grazing in commercial cattle ranches have been clear for many years (Teague *et al.*, 2008). In the United States of America, particularly in California and Wyoming states, two thirds of the surveyed cattle-raisers used rotational grazing and reported more ecological, economic, and social benefits than cattle-raisers that used continuous grazing (Roche *et al.*, 2015). Likewise, cattle-raisers from the northern-central region of Texas that used eight or more paddocks reported more ecological improvements in their grasslands than cattle-raisers that used continuous grazing (Becker *et al.*, 2017). However, some studies suggested that, unlike continuous grazing, rotational grazing—which uses multiple paddocks— does not improve vegetation and animal production (Briske *et al.*, 2008; Chowdhury *et al.*, 2020). The debate on the superiority of rotational grazing over continuous grazing is still on. The champions of both systems agree that carrying out comprehensive research is required to optimize management decisions regarding grazing (Harmel *et al.*, 2021). In addition, Rouquette *et al.* (2023) pointed out that grazing systems must be considered as “a work in progress”. Consequently, this research focused on the evaluation of the impact of 22-year rotational grazing on the soil cover change of a grassland in the northern-central region of Mexico.

MATERIALS AND METHODS

Study Area

The research was conducted in La Copa Ranch, located in the municipality of Sombrerete, Zacatecas, México (23° 39' 09" N and 103° 27' 49" W, 23° 39' 36" N and 103° 26' 59" W, 23° 40' 15" N and 103° 27' 23" W, and 23° 39' 42" N and 103° 28' 18" W, and 2,261 m.a.s.l.). The ranch has an irregular topography, with small plains and low hills. The ranch has shallow limestone soils, with a sandy texture. The region has a medium dry climate, with summer rains (BS₁kw), a mean annual precipitation of 462.9

mm, and a mean annual temperature of 15.2 °C (CNA, 2023; García, 1981). This natural grassland mainly consists of foraging species (Poaceae) of the genera *Bouteloua* and *Aristida*. In addition, the presence of huizache (*Acacia farnesiana*) increased in the area, during the 22 years that lasted the research.

Grazing and Cattle Management

La Copa Ranch has 406 ha (390 ha for grasslands and 16 ha for agriculture). Its grasslands were used for continuous grazing for many years. Rotational grazing started in 2000, with an Angus herd. The ranch had a grazing area of 11 paddocks and each paddock had an average of 35 ha. Cattle grazed for four days in each paddock during the fast-growing season of grass, followed by a 40-45-day break period. Meanwhile, during the low-growing season, cattle grazed 6-8 days, followed by a 60-80-day break period. The herd grazed in the grassland all year long. All the calves and some heifers were sold during weaning, when they were seven months-old. In addition, cull cows were commercialized to adjust the animal load to the forage production of each year, which changed depending on precipitation. The animal load of the ranch was flexible, recording 47, 51, 53, 69, 65, and 55 animals units during 2000, 2002, 2003, 2004, 2005, and 2022, respectively. During the dry season, feed was complemented with oat (*Avena sativa* L.) forage produced at the ranch.

Sampling Procedure

Two monitoring transects were strategically distributed in the grassland. Monitoring sites were selected at random for each transect. The properties of several plant and soil were measured at each site. These properties included different percentages, including bare soil, leaf litter area, rock covered areas, perennial plant base, and soil cover area. In addition, on the bare soil area, the percentages of mature, immature, recent, and broken crust were recorded. The first measurement was conducted in 2000, the same year that rotational grazing started in the ranch. Subsequently, measurements were carried out in 2002, 2003, 2004, 2005, and 2022. In the Results section, these periods are mentioned as year zero (0), two (2), three (3), four (4), five (5), and twenty-two (22) of rotational grazing, respectively. In 2000, 2002, and 2004, 66 monitoring points were randomly selected for both transects, while in 2003, 2005, and 2022, 100 monitoring points were selected per transect. Monitoring was performed at the beginning of the autumn of all the years, using the Early Warning Biological Monitoring-Rangelands and Grasslands method (ASCHM, 1999).

Precipitation

Precipitation values were analyzed to determine the annual surplus or deficit regarding the annual mean (462.9 mm) of the last 49 years of observation. The precipitation data were obtained from El Arenal weather station, managed by the Comisión Nacional del Agua (CNA, 2023). The weather station is located at 200 m from the northern border of the study area.

Rotational grazing in the ranch started in 2000. During the first two years (2000-2001) of the implementation of rotational grazing, annual precipitation was lower than the mean.

However, between 2002 and 2010, annual precipitation was higher than the mean. From 2011 to 2022 —except for 2015 and 2018—, annual precipitation was lower than the mean (Figure 1).

Statistical Analysis

The response variables of the rotational grazing included the percentages of bare soil area, leaf litter area, rock covered area, perennial plant base, and soil cover area. In addition, the percentages of mature, immature, recent, and broken crust were determined. Each variable was subjected to an analysis of variance, taking into account the year as source of variation. The statistical analysis was conducted with the Minitab® 16 software, using the General Linear Model procedure. The Tukey's range test ($\alpha=0.05$) was used to compare means, when significant effects were identified in any variable.

RESULTS AND DISCUSSION

Significant differences ($p<0.01$) were recorded in the rotational grazing (RG) implemented in La Copa Ranch (LCR), including bare soil area, leaf litter area, perennial plant base, soil cover area, mature crust area, immature crust area, recent crust area, and broken crust area (Figures 2 and 3). Rock covered area did not record significant differences (Figure 3).

When the RG started, bare soil covered 87.9% of the LCR area. Four years later, that percentage decreased by 25 percentage points. This percentage recorded minimal differences from year 4 (62.9%) to year 22 (66%) (Figure 2). The reduction of bare soil area is the result of the increase of plant base area and leaf litter area during the study. The decrease of bare soil in the LCR was significant; however, the extension of bare soil remained significant. Teague et al. (2011, 2022) reported similar results. They pointed out that the RG implementation led to a bare soil area reduction.

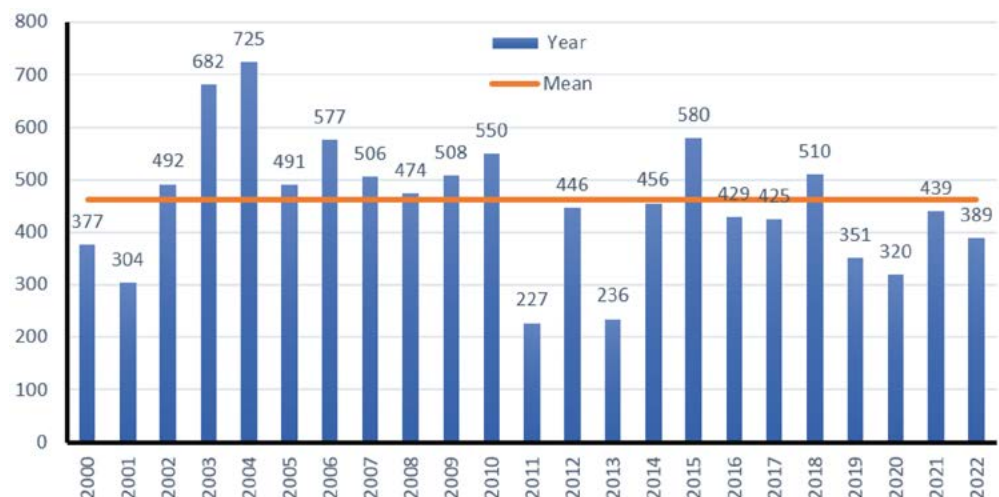


Figure 1. Annual mean precipitation (mm) in the study area during the 2000-2022 period (CNA, 2023). The orange horizontal line (annual mean precipitation: 462.9 mm) is the climatological normal of the last 49 years.

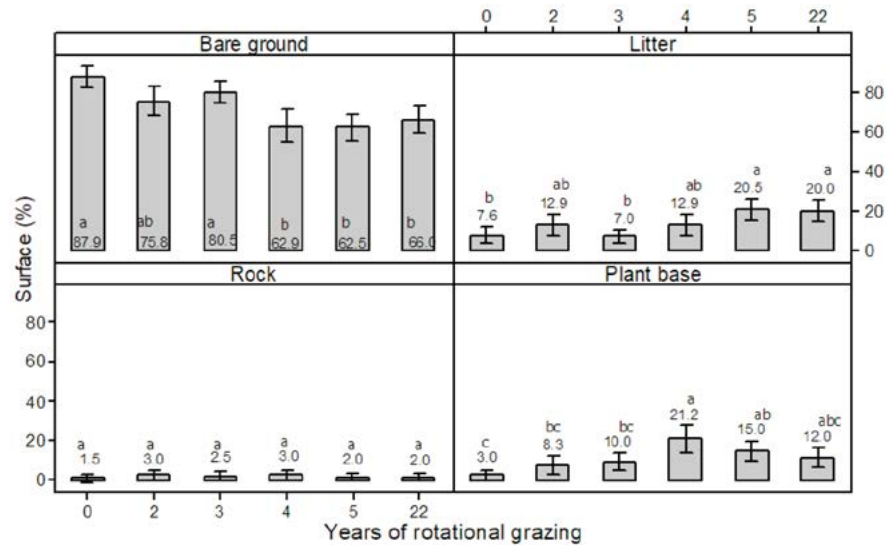


Figure 2. Percentages of bare soil area, leaf litter area, rock covered area, and perennial plant base area in the LCR grassland, during 22 years of rotational grazing. Means with different letters are significantly different, based on Tukey’s test ($p < 0.01$). The bars represent the mean \pm 1 standard error.

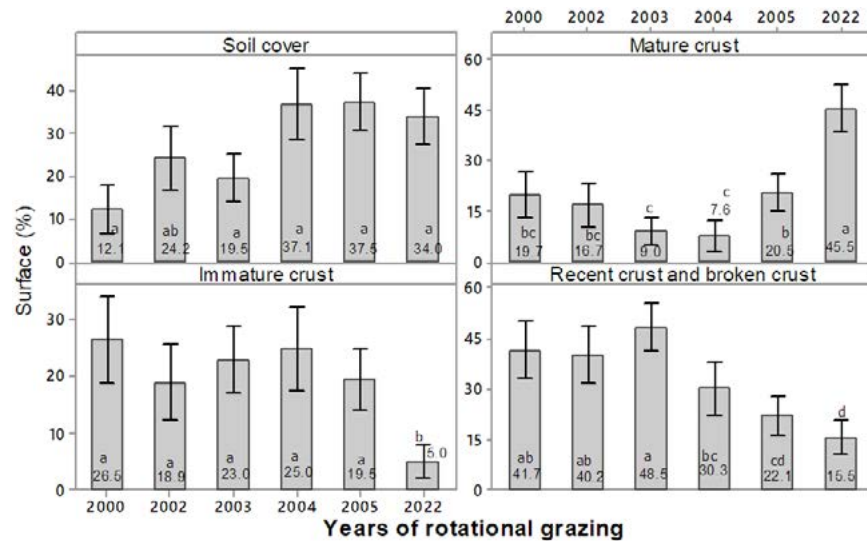


Figure 3. Soil cover area, mature crust, immature crust, recent crust, and broken crust percentages on the grassland of La Copa Ranch, after 22 years of rotational grazing. Means with different letters are significantly different, based on the Tukey’s test ($p < 0.01$). The bars represent the means \pm 1 standard error.

The objective is to reduce the bare soil area. Bare soils increase the risk of erosion, as a consequence of the lack of coverage that softens the impact of rain drops (Teague *et al.*, 2011). This situation can lead to the formation of superficial crusts, which negatively affect infiltration rate and speed up the loss of organic matter (Smith *et al.*, 2012). In addition, the increase or loss of temperature can negatively impact infiltration and evaporation rates, nutrient retention, and biological functions essential for the correct functioning of the ecosystems (Teague *et al.*, 2011).

During this study, the area covered by leaf litter showed a growing trend. Five years after the RG started, the leaf litter cover had increased by 12.9%, changing from 7.6% to 20.5%. This percentage did not change until year 22 of the RG, recording only a slight reduction of 0.5% (Figure 2). Teague *et al.* (2011) recorded similar results, identifying the positive effect of rotational grazing on the increase of leaf litter area.

The impact of RG on the increase of leaf litter area is the result of the short grazing periods and the slight to moderate defoliation of the plants during the fast-growing season. This situation allows plants to remain in their best vegetative conditions, favoring a higher production level (Teague *et al.*, 2011). Consequently, the forage available surpasses the demand, resulting in a higher forage accumulation (Teague *et al.*, 2011). Meanwhile, during the low-growing season, cattle consume the accumulated forage and stamp on part of the leaves that will become leaf litter.

Leaf litter is fundamental for the grassland ecosystems. High leaf litter levels improve soil moisture retention and enhance and stabilize plant production (Deutsch *et al.*, 2010). Döbert *et al.* (2021) pointed out that rotational grazing helps leaf litter to improve the ecological processes of the grasslands, as it increases water infiltration by facilitating a more efficient incorporation of leaf litter into the soil surface, resulting in a better soil structure and a higher organic matter content.

The rock covered area of the grasslands was small ($\leq 3\%$) and did not significantly change during the research (Figure 2). This area is a physical indicator that is hardly influenced by the management of the grassland or rainfall patterns.

At the beginning of the RG, only a small part (3%) of RLC was covered in plant base. After four years, it significantly increased, reaching a maximum of 21.2%. However, during year 5, the cover decreased by 6.2 percentage points and, after 22 years of RG, it decreased again by 3 percentage points (Figure 2).

During the first two years of RG, precipitation was lower than the annual mean (Figure 1). Along with the final impact of continuous grazing, it resulted in a small plant base area; however, the area covered by plants increased 21.2% during year four of RG. This situation suggests that both RG and the higher precipitation favored this increase.

Although precipitation during both periods was lower than the mean, the plant base area was notably greater after 22 years of RG than during the first two years. The plant base area reported in year 22 was the result of a long period of moisture deficit (2016-2022). Overall, precipitation recorded lower values than the annual mean, except for 2018 (Figure 1 and 2). These results match the findings of Teague *et al.* (2011, 2022), who reported that RG increased plant base.

An increase in the covered areas of foraging plants efficiently reduces soil erosion and improves rainfall infiltration (Teague and Kreuter, 2020). In addition, cover protects the soil from the impact of raindrops and influences plant production (Smith *et al.*, 2012). Areas covered with vegetation and leaf litter reduce temperature and decrease evaporation, improving growing conditions for plants and adding organic matter to the soil (Teague *et al.*, 2011).

The soil cover area showed a growing trend during the study period (Figure 3). At the beginning of the experiment, the soil cover area in the grassland was small (12.1%); after four years, a significant increase took place and, during year five, the highest percentage (37.5%) of soil cover area was recorded. However, during year 22 of RG, a 3.5 percentage points reduction was observed (Figure 3).

The increase of soil cover area could be the result of the increase of leaf litter and of the plant base area. These results are similar to those reported by Sanjari *et al.* (2009), who recorded an increase in soil cover with RG. These authors pointed out that this improvement in soil cover was the result of the effect of the long break periods of the RG, which provided a recovery and sprout opportunity during the humid seasons. In addition, McDonald *et al.* (2024, 2018) reported that the soil cover area increased after the adoption of rotational grazing. Furthermore, McDonald *et al.* (2024) recorded a higher soil cover area (57.9%) in plots where the annual mean precipitation was similar to the study area. These results could indicate that the soil cover area in the research can potentially increase. For their part, Leys *et al.* (2023) mentioned that soil cover areas reduce wind erosion.

Meanwhile, the physical crusts of the soil showed an irregular trend (Figure 3). At the beginning of the RG system, the soil of the grassland had 19.7% mature crust. However, four years later, it was smaller (7.6%). Nevertheless, a significant increase was recorded on year 22. The 12.1 percentage points reduction recorded during the first four years of RG are important, because mature crusts reduce the efficiency of hydrological cycles and mineral content in grasslands (Savory, 2005).

Rotational grazing includes small grazing periods with large densities of cattle, causing the herd effect that can break physical crusts and facilitate the integration of dead plant material into the soil (Savory, 2005). The immature crust area did not record significant changes during the first five years of RG (Figure 3). The smallest percentage (5.0%) was recorded on year 22 of RG.

The recent and broken crust area recorded higher percentages during the first years of RG. The maximum percentage was 48.5% and was recorded during year 3 (Figure 3). From that moment on, a significant decrease was reported, until a 15.5% crust cover was recorded on year 22 of RG (Figure 3).

The irregular trend recorded in the bare soil area condition —particularly regarding the presence of mature crust— matched the results reported by Döbert *et al.* (2023), who pointed out that stamping has a short-lived effect on soil crust.

Overall, the results of this research indicate a positive effect of rotational grazing on the soil of the grassland. Significant changes ($p < 0.01$) were recorded regarding the bare soil area, plant base area, and soil cover variables from year 4 of RG; meanwhile, significant changes were reported for leaf litter in year 5.

The improved performance of the plant soil cover could be due to precipitation level that surpassed the annual mean. These results match the findings of Bartley *et al.* (2023), who pointed out that rotational grazing can improve vegetation, soil, and land condition, although it takes about 3-5 years or even longer to observe statistically significant improvements, particularly in degraded areas.

CONCLUSIONS

Rotational grazing proved to be an important management practice to change the soil in the grassland of the study area. Rotational grazing increased covered by the plant base area, leaf litter area, and soil cover. At the same time, it decreased bare soil and mature crust. The positive changes in the soil cover area of the grassland were clear after 3-5 years of the implementation of rotational grazing. Subsequently, they kept improving. The increase of the perennial plant base area was associated with precipitation levels.

REFERENCES

- Allan Savory Center for Holistic Management (ASCHM). 1999. Early Warning Biological Monitoring- Rangelands and Grasslands- Albuquerque, New Mexico. USA. Center for Holistic Management. 31 p.
- Bartley, R., Abbott, B. N., Ghahramani, A., Ali, A., Kerr, R., Roth, C. H., & Kinsey-Henderson, A. (2023). Do regenerative grazing management practices improve vegetation and soil health in grazed rangelands? Preliminary insights from a space-for-time study in the Great Barrier Reef catchments, Australia. *The Rangeland Journal*, 44(4), 221-246. <https://doi.org/10.1071/RJ22047>.
- Becker, W., Kreuter, U., Atkinson, S., & Teague, R. (2017). Whole-ranch unit analysis of multipaddock grazing on rangeland sustainability in North Central Texas. *Rangeland Ecology & Management*, 70(4), 448-455. <http://dx.doi.org/10.1016/j.rama.2016.12.002>.
- Briske, D. D., Derner, J. D., Brown, J. R., Fuhlendorf, S. D., Teague, W. R., Havstad, K. M., Gillen, R.L., Ash, A.J & Willms, W. D. (2008). Rotational grazing on rangelands: reconciliation of perception and experimental evidence. *Rangeland Ecology & Management*, 67(1), 3-17. doi: 10.2111/06-159R.1.
- Comisión Nacional del Agua (CNA). (2023). Base de datos de la precipitación pluvial (1970-2022) de la estación climatológica 'El Arenal'. Compartida por el Departamento de Hidrometeorología de la Dirección Local Zacatecas. Comisión Nacional del Agua.
- Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). 2021. Ecosistemas de México. Pastizales. Consultado en línea 30/08/ 2023. <https://www.biodiversidad.gob.mx/ecosistemas/pastizales>.
- Chowdhury, I. U. A., Wang, T., Jin, H., & Smart, A. J. (2020). Exploring the Determinants of Perceived Benefits of Rotational Grazing in the US Great Plains.
- Deutsch, E. S., Bork, E. W., & Willms, W. D. (2010). Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. *Agriculture, Ecosystems & Environment*, 135(1-2), 1-9. doi:10.1016/j.agee.2009.08.002.
- Döbert, T. F., Bork, E. W., Apfelbaum, S., Carlyle, C. N., Chang, S. X., Khatri-Chhetri, U., Silva Sobrino, L., Thompson, R. & Boyce, M. S. (2021). Adaptive multi-paddock grazing improves water infiltration in Canadian grassland soils. *Geoderma*, 401, 115314. [tps://doi.org/10.1016/j.geoderma.2021.115314](https://doi.org/10.1016/j.geoderma.2021.115314).
- García, E. 1981. Modificaciones al sistema de clasificación climática de Köppen: para adaptarlo a las condiciones de la República Mexicana. UNAM.
- Goodwin, J., Porensky, L. M., Meiman, P., Wilmer, H., Derner, J. D., Iovanna, R., Monlezun, A. C., Vandever, M. W., Griggs, J., Price, F., Spiegel, S.A., Padilla, N., Voth, D., maher, A., O'Connor, R., Hoover, D. L., Pluhar, J., Estep, C. & Fox, W. (2023). Rangeland Ecosystem Services: Connecting nature and people. Society for Range Management. Rangeland Ecosystem Services. 42 p. <https://rangelands.org/rangelands-provide-five-ecosystem-services/>
- Harmel, R. D., Smith, D. R., Haney, R. L., Angerer, J., Haile, N., Grote, L., Grote, S., Tiner, K., Goodwin, J., Teague, R. & Derner, J. (2021). Transitioning from conventional continuous grazing to planned rest-rotation grazing: A beef cattle case study from central Texas. *Journal of Soil and Water Conservation*, 76(6), 534-546. <https://doi.org/10.2489/jswc.2021.00159>.
- Lawrence, R., Whalley, R. D. B., Reid, N., & Rader, R. (2019). Short-duration rotational grazing leads to improvements in landscape functionality and increased perennial herbaceous plant cover. *Agriculture, Ecosystems & Environment*, 281, 134-144. doi: 10.1016/j.agee.2019.04.031.
- Leys, J., Heidenreich, S., White, S., Guerschman, J., & Strong, C. (2023). Dust-storm frequencies, community attitudes, government policy and land management practices during three major droughts in New South Wales, Australia. *The Rangeland Journal*, 44(5-6), 343-355. <https://doi.org/10.1071/RJ22059>.
- McDonald, S. E., Reid, N., Waters, C. M., Smith, R., & Hunter, J. (2018). Improving ground cover and landscape function in a semi-arid rangeland through alternative grazing management. *Agriculture, Ecosystems & Environment*, 268, 8-14.

- McDonald, S. E., Simmons, A. T., Harden, S., Orgill, S. E., Guerschman, J., & Strong, C. (2024). Managing grazing to increase ground cover in rangelands: using remote sensing to detect change. *The Rangeland Journal*, 46(4). <https://doi.org/10.1071/RJ24021>.
- Minitab (2016). Software estadístico, versión 16. Minitab Incorporation, State College.
- Roche, L. M., Cutts, B. B., Derner, J. D., Lubell, M. N., & Tate, K. W. (2015). On-ranch grazing strategies: context for the rotational grazing dilemma. *Rangeland Ecology & Management*, 68(3), 248-256. doi: 10.1016/j.rama.2015.03.011.
- Rouquette Jr, F. M., Sollenberger, L. E., & Vendramini, J. M. (2023). Grazing management and stocking strategy decisions for pasture-based beef systems: experimental confirmation vs. testimonials and perceptions. *Translational Animal Science*, 7(1), txad069. <https://doi.org/10.1093/tas/txad069>.
- Sandhage-Hofmann, A. (2023). Rangeland Management. pp. 88-101. In: Goss, M. J., Oliver, M. (Eds.). *Encyclopedia of Soils in the Environment* (Second Edition). Academic Press. ISBN 9780323951333. <https://doi.org/10.1016/B978-0-12-822974-3.00117-8>.
- Sanjari, G., Ghadiri, H., Yu BoFu, Y. B., & Ciesiolka, C. A. (2010). Increase in ground cover under a paddock scale rotational grazing experiment in south-east Queensland. <http://hdl.handle.net/10072/37511>.
- Savory, A. 2005. Manejo holístico. Un nuevo marco para la toma de decisiones. Secretaría del Medio Ambiente y Recursos Naturales. Instituto Nacional de Ecología. Fondo Mexicano para la Conservación de la Naturaleza.
- Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). 2019. Informe de la Situación del Medio Ambiente en México, edición 2018. SEMARNAT. México. 490 p.
- Smith, L., Ruyle, G., Dyess, J., Meyer, W., Barker, S., Williams, S. M., Maynar, J. L., Bell, D. & Stewart, D. (2012). Guide to rangeland monitoring and assessment: Basic concepts for collecting, interpreting and use of rangeland data for management planning and decisions. Arizona Grazing Lands Conservation Association, Tucson, Arizona, USA.
- Teague, W.R., S.L. Dowhower and J.A. Waggoner. 2004. Drought and grazing patch dynamics under different grazing management. *Journal of Arid Environments* 58: 97-117. doi.org/10.1016/S0140-1963(03)00122-8.
- Teague, R., Provenza, F., Norton, B., Steffens, T., Barnes, M., Kothmann, M., & Roath, R. (2008). Benefits of multi-paddock grazing management on rangelands: limitations of experimental grazing research and knowledge gaps. *Grasslands: ecology, management and restoration*. Hauppauge, NY, USA: Nova Science Publishers, 41-80.
- Teague, W. R., Dowhower, S. L., Baker, S. A., Haile, N., DeLaune, P. B., & Conover, D. M. (2011). Grazing management impacts on vegetation, soil biota and soil chemical, physical and hydrological properties in tall grass prairie. *Agriculture, Ecosystems & Environment*, 141(3-4), 310-322. doi: 10.1016/j.agee.2011.03.009.
- Teague, R., Provenza, F., Kreuter, U., Steffens, T., & Barnes, M. (2013). Multi-paddock grazing on rangelands: why the perceptual dichotomy between research results and rancher experience? *Journal of Environmental Management*, 128, 699-717. doi: 10.1016/j.jenvman.2013.05.064.
- Teague, R., & Kreuter, U. (2020). Managing grazing to restore soil health, ecosystem function, and ecosystem services. *Frontiers in Sustainable Food Systems*, 4, 534187. doi: 10.3389/fsufs.2020.534187.
- Teague, R., & Dowhower, S. (2022). Links of microbial and vegetation communities with soil physical and chemical factors for a broad range of management of tallgrass prairie. *Ecological Indicators*, 142, 109280. doi.org/10.1016/j.ecolind.2022.109280
- Wang, T., Jin, H., Kreuter, U., Feng, H., Hennessy, D. A., Teague, R., & Che, Y. (2020). Challenges for rotational grazing practice: views from non-adopters across the Great Plains, USA. *Journal of Environmental Management*, 256, 109941. doi: 10.1016/j.jenvman.2019.109941.
- Wang, T., Jin, H., Kreuter, U., & Teague, R. (2022). Understanding producers' perspectives on rotational grazing benefits across US Great Plains. *Renewable Agriculture and Food Systems*, 37(1), 24-35. doi: 10.1017/S1742170521000260.
- Xiaoyan, G., Zirui, J., Yingying, Y., Dengsheng, W., & JianPing, L. (2019). Research on Dynamic Rotational Grazing Assignment Model based on Grass Resource Leveling. *Procedia Computer Science*, 162, 696-703.

Profitability of Pitahaya as an Alternative Crop in the Southern Region of the State of Mexico

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ABSTRACT

Objective: The objective of this study was to evaluate the economic profitability of pitahaya (*Hylocereus* spp.) production in southern State of Mexico through a financial analysis of representative production units, under the hypothesis that this crop represents a viable regional agricultural alternative by generating positive and sustainable financial indicators.

Design/methodology/approach: We utilized a financial analysis approach focusing on 0.25-hectare units, which is the average area managed by producers in the region. This scale was chosen to avoid distortions caused by economies of scale and to provide a more accurate reflection of local conditions. The analysis focused on the third year after crop establishment, when production stabilizes, and income and expense flows become clearer. Costs, revenues, and key profitability indicators such as Net Present Value (NPV), Internal Rate of Return (IRR), Break-even Point, and Benefit-Cost Ratio (B/C) were integrated.

Results: The study yielded a Net Present Value (NPV) of \$127,850 pesos, an Internal Rate of Return (IRR) of 27.1%, and a Benefit-Cost Ratio (B/C) of 2.175, indicating solid profitability. The break-even point was reached with the sale of 1.17 tons per cycle. The sensitivity analysis showed that the project remains viable even with a 27% reduction in income or a 38% increase in costs.

Limitations on study/implications: The analysis is based on data collected from a specific region, which may limit its applicability to other contexts.

Findings/conclusions: Pitahaya cultivation represents a profitable, resilient, and sustainable alternative for diversifying agricultural production in the southern region of the State of Mexico.

Keywords: viability, production, financial analysis.

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INTRODUCTION

Pitahaya (*Hylocereus* spp.), commonly known as dragon fruit, is a cactus species native to the Americas that has gained relevance in Mexican agriculture due to its adaptability to adverse climatic conditions and the growing demand in national and international markets (Ortega-Hernández, 2015; Ricalde & Andrade, 2009). Its cultivation is concentrated in



arid and semi-arid regions of the country, including Quintana Roo, Yucatán, Puebla, Aguascalientes, Tabasco, Guerrero, Nuevo León, and Sinaloa, where agroclimatic conditions favor its development and have consolidated these states as the main national producers (SIAP, 2023). The species is characterized by low water requirements, tolerance to soils of limited quality, and resistance to both drought and high temperatures, making it a strategic option for agricultural diversification (Wu, 2005). In addition, its nutritional value and antioxidant properties have increased its market acceptance and strengthened its economic potential (Montesinos-Cruz, 2015).

At the international level, interest in crops of the genus *Hylocereus* has increased steadily. Currently, production takes place in countries such as Vietnam, Mexico, Colombia, Nicaragua, Ecuador, the United States, Israel, Thailand, Japan, and Australia (Tel-Zur, 2015). Vietnam has consolidated its position as the main exporter of fresh fruit, while China, Indonesia, Taiwan, and Malaysia have increased their participation in production (Chen & Paull, 2019; Mercado-Silva, 2018). Global production is concentrated in tropical regions of the Americas and Asia, with an estimated output exceeding 2.1 million tonnes cultivated on approximately 116,836 hectares (MIDAGRI, 2021). Market dynamism has been enhanced by the approval, in 2012, of an irradiation protocol in the United States that facilitated compliance with phytosanitary import requirements (Chen & Paull, 2019). Although the fruit is often considered exotic, its flavor has sometimes been described as bland. This perception has led to the importation of sweeter varieties from Ecuador and Colombia into North America and Europe. In this context, Vietnam plays a significant role in Asian and European markets, as well as at times in the United States, where larger red pitahayas have gained strong consumer preference (Wakchaure *et al.*, 2020).

Profitability analyses of this crop have also been the subject of research in different countries. In Ecuador, it is recognized as a driver of development in producing areas, despite existing structural challenges (Farez-Jaramillo *et al.*, 2025). In Nepal, a benefit-cost ratio of 1.87 has been documented, while in regions of India values of 2.04 have been reported, along with an Internal Rate of Return (IRR) of 11.78% or benefit-cost ratios ranging between 1.75 and 1.86 (Mehta *et al.*, 2024; Sharma *et al.*, 2021; Kikon *et al.*, 2021). In Mexico, Michel-Lara *et al.* (2022) reported a benefit-cost ratio of 2.6 in Tolimán, Jalisco. Additional studies in Campeche and Puebla have also shown favorable results, with positive indicators of Net Present Value (NPV), IRR, and Benefit-Cost Ratio (BCR), supporting the viability of the crop under appropriate management and marketing conditions (Ku-Caamal, 2019; Trejo-López, 2012; Flores-Miranda, 2011; Méndez-Ku, 2003). These findings highlight the usefulness of financial indicators, such as total costs, revenues, BCR, and IRR, for assessing economic viability.

Although comprehensive profitability studies are not available for traditional crops such as maize and beans in the southern region of State of Mexico, approximate gross income and expenditure per hectare allow comparisons with commercial crops for which economic analyses are available, such as cucumber and tomato, and subsequently with pitahaya, which represents the emerging crop under consideration. For example, in recent years maize, cucumber, and tomato have shown benefit-cost ratios of 1.6, 1.27, and 1.6,

respectively (Amaya-Pérez *et al.*, 2025; Rebollar-Rebollar *et al.*, 2022; Morales-Hernández *et al.*, 2018). These values suggest that pitahaya, given its economic potential, represents an attractive alternative to traditional and commercial crops in the study area.

Despite these advantages, the expansion of the crop faces constraints, including limited access to technical information, the absence of historical cost and yield records, and reliance on intermediaries for marketing. These conditions reduce profitability and compromise the sustainability of production, particularly in the southern region of the State of Mexico and in certain areas of Puebla. In this context, it is essential to generate techno-financial evaluations that integrate these factors and provide evidence of profitability to strengthen regional agricultural development.

MATERIALS AND METHODS

The research was conducted in the southern and central regions of State of Mexico, as well as in selected producing areas of the state of Puebla, where a representative number of small-scale pitahaya producers are concentrated. These regions were selected due to their similar production systems and scales, their growing interest in the crop, and the presence of conditions comparable to those of southern State of Mexico.

Structured surveys and semi-structured interviews were applied to active pitahaya producers. In State of Mexico, two producers from the southern region and three from the eastern region were included, given that this is an emerging crop and the number of producers in these areas remains limited. To complement the information, five producers from the Mixteca region of Puebla were considered, an area that hosts more than 100 production units dedicated to this crop. The sampling was classified as non-probabilistic and purposive, as priority was given to including units with production conditions, levels of technological adoption, and scales similar to those observed in southern State of Mexico. The study design was cross-sectional, with data collected at a single point in time. It also included a five-year financial projection to assess the economic viability of the crop.

The questionnaires made it possible to estimate production costs, revenues, sale prices, inputs used, harvest volumes, and technological practices. Considering that producers primarily manage 0.25-hectare plots, the economic analysis was conducted at this scale in order to accurately represent real production conditions and avoid biases derived from scale-related variations. Based on the information obtained, a five-year financial projection was developed and key financial indicators were calculated: Net Present Value (NPV), Internal Rate of Return (IRR), Benefit-Cost Ratio (BCR), and the producer break-even point (BEP). Data processing and the development of the financial projection were carried out using Microsoft Excel, with annual cash flows recorded and both operating expenses and residual value at the end of the evaluation horizon taken into account.

The use of Microsoft Excel was justified by its versatility in constructing financial cash flows and estimating economic indicators. To ensure the validity of the results, the formulas were verified through manual calculations and were based on the *Guidelines for Traditional Quantitative Techno-Financial Evaluation* of the FIRA Evaluation Procedure- Bank of Mexico (n.d.). This methodological framework enabled consistent indicators and mitigated the

limitations of the exclusive use of spreadsheets, in accordance with technical standards commonly applied to the evaluation of agricultural projects in Mexico.

A Minimum Acceptable Rate of Return (MARR) of 18% was considered, supported by three components: (i) a risk-free rate of approximately 8%, based on the historical yields of CETES and the Interbank Equilibrium Interest Rate (TIEE) reported by the Bank of Mexico; (ii) a country risk premium of around 6%, derived from the EMBI+ indicator for Mexico; and (iii) a sectoral premium of 4%, reflecting the emerging nature of the crop, the small scale of production units, and uncertainty in marketing channels. This value is consistent with methodological recommendations from international organizations, which suggest using discount rates between 12% and 20% for agricultural and rural projects in Latin America (FAO, 2015; IDB, 2019).

The break-even point was estimated as the level of sales at which total revenues equal total costs. Finally, a sensitivity analysis was conducted to simulate the impact of revenue and cost variations on project profitability.

RESULTS AND DISCUSSION

Pitahaya cultivation in the southern region of the State of Mexico exhibits variable productivity, determined by technical and environmental factors such as plant age, agronomic management, access to irrigation, planting density, and local agroecological conditions.

The area managed by pitahaya producers ranges from 300 m² to 3,120 m², with a median of 2,500 m². Most producers operate on a small scale, using their own land and combining pitahaya cultivation with other agricultural or non-agricultural activities. The average age of the plantations evaluated was 3 years, suggesting that most are in the commercial development stage.

Regarding production, the estimated average yield in the study area was 10 tonnes per hectare per cycle, with a range from 6 to 12 tonnes depending on agricultural management conditions. This value falls within the parameters reported for other regions of the country, such as Sinaloa, where, according to the study by Osuna-Enciso *et al.* (2016), yields in the third, fourth, and fifth years of the orchard ranged between 10 and 13.4 tonnes per hectare, depending on crop age.

Harvesting is concentrated mainly between June and October, with slight variations among municipalities. Producers indicated that production becomes significant from the second year onward, reaching peak productivity between the fourth and fifth years.

In this context, Figure 1 presents a comparative analysis of pitahaya's relative profitability compared with other crops and regions. In the left panel, the benefit-cost ratio of pitahaya (2.2) is contrasted with traditional crops in the region, such as maize (1.6) and black beans (1.7), as well as with predominant commercial crops such as tomato (1.6) and cucumber (1.3). In the right panel, pitahaya produced in the southern region of the State of Mexico (2.2) is compared with that reported for Jalisco (2.44), Guanajuato (3.0), and Campeche (2.35), evidencing favorable regional competitiveness. These results show that pitahaya not only exceeds traditional and well-established commercial crops in terms of profitability in the study area, but also exhibits economic performance

comparable to that of regions with greater productive experience. The evidence suggests that adopting pitahaya represents an economically attractive alternative and a viable diversification strategy for producers in the southern region of the State of Mexico.

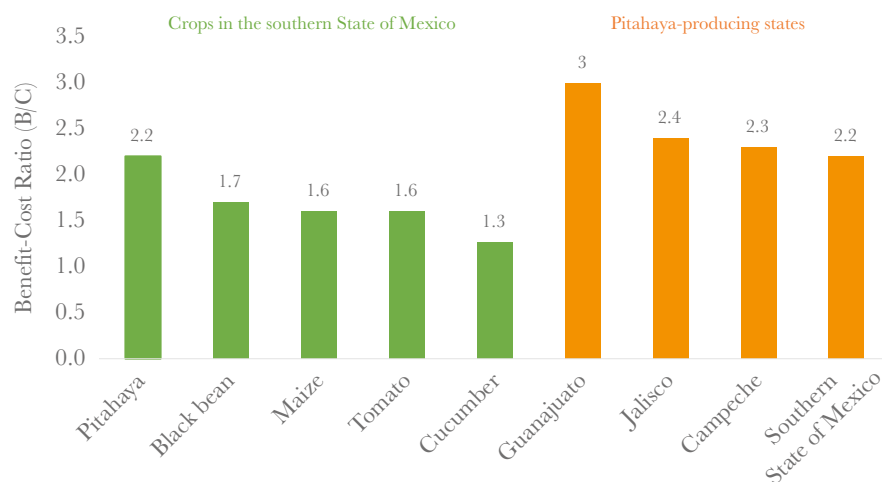


Figure 1. Benchmarking of the benefit-cost ratio (B/C) of pitahaya in the southern region of the State of Mexico.

Source: Authors' own elaboration based on data from Amaya-Pérez *et al.* (2025); ICAMEX (2024); SIAP (2024); López-Chávez (2024); Rebollar-Rebollar *et al.* (2022); Michel-Lara *et al.* (2022); Ku-Caamal (2019); Carrillo-Martínez *et al.* (2019); Morales-Hernández *et al.* (2018).

Costs and revenues

Important note: All costs and revenues presented in this section correspond to an area of 0.25 hectares, in order to accurately reflect the local production scale and avoid distortions associated with economies of scale.

The most representative variable costs include labor (planting, pruning, fertilization, harvesting), organic and/or chemical fertilizers, irrigation water, pest control, and fruit transportation. On average, the estimated variable cost for 0.25 ha was MXN 60,018 per production cycle, while annualized fixed costs (including depreciation) amounted to MXN 30,326 for the same area.

Based on an average yield of 2.5 tonnes on 0.25 hectares and an average farm-gate selling price of MXN 50 per kilogram, gross revenues amounted to MXN 125,000 per production cycle. This income varies depending on the marketing channel used. Some producers sell to local intermediaries, while others can access specialized markets or engage in direct sales (as in the case of the producers interviewed in Puebla), where prices may increase.

When revenues were compared with total costs, a net profit of MXN 34,655 per quarter hectare per cycle was estimated, reflecting positive margins even under traditional management scenarios. This profit represents a 28% return on total costs, positioning the crop as a viable alternative to other crops in the region with lower margins. It is worth noting that revenues show strong correlations with price and crop age, with coefficients of $r=0.99$ and $r=0.73$, respectively, based on the data. Producers with more mature

plantations (≥ 4 years) reported higher net profits due to greater production and lower per-plant expenditures associated with establishment activities.

The summary table of average costs and revenues per production cycle is presented in Table 1.

Table 1. Average costs and revenues per 0.25 hectares of pitahaya.

Concepts	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5
Annualized fixed costs (\$)	30,326	30,326	30,326	30,326	30,326	30,326
Variable costs (\$)	47,893	46,144	60,018	74,740	95,116	117,748
Total cost per cycle (\$)	78,219	76,470	90,345	105,066	125,442	148,075
Gross yield (t)	0	0.432	2.5	4	6	8
Average farm-gate price (\$)	-	45,000	50,000	55,000	60,000	65,000
Estimated net profit (\$)	-78,219	-57,030	34,655	114,934	234,558	371,925

This economic analysis establishes the basis for projecting the crop's financial performance in the medium term, which is developed in the following section.

Financial analysis of the project

To assess profitability, a financial projection was prepared based on an estimated cash flow for a quarter of a hectare. The analysis considered:

1. Initial investment
2. Annual operating costs
3. Expected revenues from fruit sales
4. A residual value at the end of the time horizon

The initial investment (Table 2) includes expenses for the water pump, brush cutter, land preparation, installation of the irrigation system, the PTR structure (metal tubing), and cuttings, with a total estimated cost of MXN 271,631. This investment was depreciated over six years using the straight-line method, recognizing that, from an accounting perspective, some fixed assets are not fully exhausted within that period.

Table 2. Initial investment.

Fixed assets	Quantity	Amount (\$)	Depreciation rate (%)	Residual value (\$)
Water pump	1	5,500	10	2,200
Brush cutter	1	3,000	10	1,200
Land preparation and irrigation installation	1	40,631	20	32,505
PTR structure	1	135,000	5	94,500
Cuttings	2500	87,500	10	35,000
Total		271,631		165,405

Note: Rates according to the Income Tax Law (LISR), Ministry of Finance and Public Credit (SHCP, 2024).

Annual revenues were calculated based on average production by crop age and on prices reported by producers, depending on fruit quality and origin, resulting in increasing gross cash flows as the plants reach their full productive capacity (Table 3).

Table 3. Net cash flow, NPV, and IRR.

Concepts	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5
Sales (\$)	-	19,440	125,000	220,000	360,000	520,000
Costs (\$)	53,743	51,994	65,868	80,590	100,966	123,598
Operating profit (\$)	-53,743	-32,554	59,132	139,410	259,034	396,402
Fixed and deferred assets (\$)	271,631					40,631
Change in working capital (\$)	11,733	-262	2,081	2,208	3,056	
Total investment (\$)	283,364	-262	2,081	2,208	3,056	40,631
Residual values (\$)						165,405
Recovery of working capital (\$)						18,816
Net cash flow (\$)	-337,106	-32,554	57,050	137,202	255,978	539,992
Minimum acceptable rate of return (MARR)				18.0%		
Internal Rate of Return (IRR)				27.1%		
Net Present Value (NPV)				\$128,072.22		

Under these conditions, the project showed a positive Net Present Value (NPV) of MXN 128,072.22 at a 18% discount rate, indicating that the present value of benefits exceeds the present value of costs. Likewise, an Internal Rate of Return (IRR) of 27.1% was obtained, which exceeds the opportunity cost of capital, thereby confirming the project's financial viability (Table 3). For this study, a Minimum Acceptable Rate of Return (MARR) of 18% was used, reflecting the opportunity cost of capital in the Mexican agricultural sector, along with an additional risk margin associated with the productive and commercial conditions of pitahaya cultivation.

Benefit-cost ratio (B/C)

The benefit-cost ratio (B/C) obtained was 2.175, indicating that for every peso invested in the project, MXN 2.175 in net benefits is generated at present value. This result reflects strong economic efficiency, as the present value of benefits (MXN 653,127) far exceeds that of costs (MXN 300,263) (Table 4).

Table 4. B/C ratio.

Concepts	Value
Present value of benefits (\$)	653,127
Present value of costs (\$)	300,263
Benefit-cost ratio (B/C)	2.175

Producers' break-even point

The break-even point was reached in the third year, when total costs were fully covered by revenues, a period in which the plantation began to stabilize its yields.

Figure 2 shows that the break-even point is approximately 1.17 tonnes or MXN 58,336.15, implying that an average producer in the study area must sell at least this amount to cover both fixed costs (MXN 30,326.20) and variable costs associated with crop management. Beyond this level, the yellow-shaded area indicates the profit margin, while the blue area indicates an operating loss.

This visualization provides a clear understanding of the relationship between production and income, helping producers make strategic decisions regarding minimum sales volume, target prices, and profitability levels. It also highlights the importance of optimizing costs and securing marketing channels that allow this threshold to be exceeded with confidence.

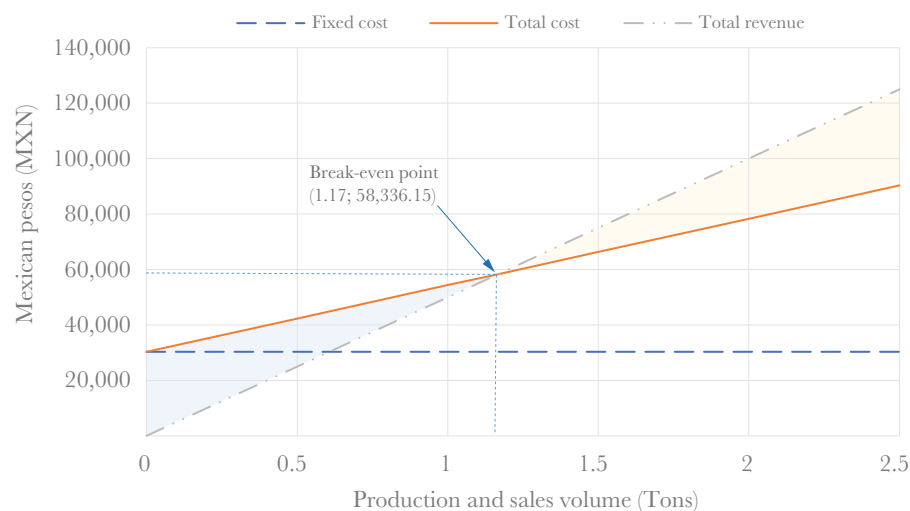


Figure 2. Break-even point of pitahaya production.

Sensitivity analysis

A sensitivity analysis is presented under a first combined scenario that considers progressive reductions in revenues (-5% , -10% , and -15%) together with increases in production costs (between $+5\%$ and $+35\%$). The results show that crop profitability remains positive only when revenue reductions are below 10% and cost increases are below 20% . Beyond a 20% increase in costs and a 10% decrease in revenues, profitability becomes negative, revealing the production system's vulnerability to simultaneous market shocks. More adverse scenarios, such as a 15% reduction in revenues and cost increases above 30% , result in significant losses, highlighting the importance of strategies aimed at stabilizing prices and controlling production costs (Table 5).

In the second scenario (decline in sales), the results show that the project remains profitable with reductions of up to 27% , although costs are barely covered and margins become much tighter. In contrast, under scenario 3 (cost increase), the project withstands cost increases of up to 38% without incurring losses. Beyond 39% , costs exceed revenues, resulting in an operating loss (Table 6).

Table 5. Combined effect of variations in revenues and costs.

Adjusted NPV		Reduction in sales			
80,402.68		-5.00%	-10.00%	-15.00%	-20.00%
Cost increase	5.00%	80402.679	47746.3186	15089.9581	-17566.402
	10.00%	65389.533	32733.1725	76.8120455	-32579.548
	15.00%	50376.387	17720.0265	-14936.334	-47592.694
	20.00%	35363.2409	2706.88046	-29949.48	-62605.84
	25.00%	20350.0949	-12306.266	-44962.626	-77618.987
	30.00%	5336.94887	-27319.412	-59975.772	-92632.133
	35.00%	-9676.1972	-42332.558	-74988.918	-107645.28

Table 6. Analysis of sales decline and cost increase.

Scenarios	Reduction in sales			Increase in costs		
Variation en %	0	27	28	0	38	39
Sales (\$)	125,000	91,250	90,000	125,000	125,000	125,000
Costs (\$)	90,345	90,345	90,345	90,345	124,676	125,579
Difference (\$)	34,655	905	-345	34,655	324	-579

CONCLUSIONS

The present study confirms that pitahaya (*Hylocereus* spp.) constitutes a profitable and viable productive alternative for small-scale producers in the southern region of the State of Mexico from the third year after establishment onward. The financial analysis showed a positive Net Present Value, an Internal Rate of Return exceeding the opportunity cost of capital, and a Benefit-Cost Ratio of 2.175, indicating the economic soundness of the evaluated system. The break-even point was located at 1.17 tonnes, which is attainable under appropriate management conditions. The sensitivity analysis indicated that, under adverse scenarios—such as a 15% decrease in revenues combined with cost increases above 30%—the project may incur significant losses, underscoring the need for strategies to stabilize prices and control production costs.

The results suggest that the evaluated model could be replicated in areas with similar agroecological conditions, such as the Rural Development Districts of Tejupilco, Valle de Bravo, and Coatepec Harinas. According to Sotelo-Ruiz *et al.* (2006), these districts were identified through climate and soil requirement modeling using a Geographic Information System (GIS), showing very high agroclimatic potential for pitahaya. Consistently, observations from the present study indicate that certain areas of the Mixteca region of Puebla exhibit comparable conditions and production experience, allowing the application of the same model. This reinforces the crop's viability in these regions and its potential as an alternative for agricultural diversification, capable of generating sustainable income in the medium and long term.

The findings provide relevant information for decision-making in public policy and productive planning. The design of accessible financing schemes that promote an

agricultural credit culture is recommended, along with support programs for technological upgrading and financial education strategies that enable producers and investors to make informed decisions regarding costs, revenues, and risks. Complementarily, integrating market studies would improve production planning and price control, thereby reducing vulnerability to volatility. These actions would not only consolidate the economic viability of pitahaya cultivation but also strengthen regional agricultural diversification and producers' economic security.

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REFERENCES

- Ortega-Hernández, A., León Andrade, M., & Rosas Vargas, R. (2015). Producción de pitahaya para promover el desarrollo regional y sustentable. https://ru.iiec.unam.mx/4299/1/2-Vol3_Parte1_Eje5_Cap2-123-Ortega-Leon-Rosas.pdf
- Ricalde, M. F., & Andrade, J. L. (2009). La pitahaya, una delicia tropical. *Ciencia*, 60(3), 36-42. https://www.amc.edu.mx/revistaciencia/images/revista/60_3/PDF/05-488-La-pitahaya.pdf
- SIAP. (2023). Producción agrícola por cultivo. Secretaría de Agricultura y Desarrollo Rural.
- Wu, J.C.S. (2005). Manual del cultivo de la pitaya. Instituto de Ciencia y Tecnología Agrícola (ICTA). MAGA. Mision de Taiwan.
- Montesinos-Cruz, J. A., Rodríguez-Larramendi, L., Ortiz-Pérez, R., Fonseca-Flores, M. D. L. Á., Ruíz-Herrera, G., & Guevara-Hernández, F. (2015). Pitahaya (*Hylocereus* spp.) Un recurso fitogenético con historia y futuro para el trópico seco mexicano. *Cultivos Tropicales*, 36(), 67-76. <https://www.redalyc.org/articulo.oa?id=193243640007>
- Tel-Zur, N. (2015). Research and development of pitahayas dragonfruit - vine cacti: limitations and challenges and the current global market. *Acta Hort.* 1067, 365-370. <https://doi.org/10.17660/ActaHortic.2015.1067.50>
- Chen, N. J., & Paull, R. E. (2019). Overall dragon fruit production and global marketing. FFTC Agricultural Policy Platform. <https://ap.ffc.org.tw/article/1596>
- Mercado-Silva, E.M. (2018). Pitaya—*Hylocereus undatus* (Haw). Exotic Fruits, *Academic Press*, p 339-349, <https://doi.org/10.1016/B978-0-12-803138-4.00045-9>.
- Ministerio de Desarrollo Agrario y Riego (MIDAGRI). (2021). Análisis de mercado: Pitahaya 2015-2020. *Sierra y Selva Exportadora*. 51 p.
- Wakchaure, G.C., Kumar, S., Meena, K.K., Rane, J., & Pathak, H. (2020). Dragon Fruit Cultivation in India: Scope, Marketing, Constraints and Policy Issues. Technical Bulletin No. 46. ICAR-National Institute of Abiotic Stress Management, Baramati, Pune, Maharashtra, India, p. 54. https://www.researchgate.net/publication/348844675_Dragon_Fruit_Cultivation_in_India_Scope_Marketing_Constraints_and_Policy_Issues
- Farez-Jaramillo, H. A., Mitiap-Vargas, D. L., Cambisaca-Ortiz, C. E., & López-Jara, A. A. (2025). Impacto del Sistema de Costeo por Procesos en la Rentabilidad de la Producción de Pitahaya en Palora, Morona Santiago. *Pacha. Revista De Estudios Contemporáneos Del Sur Global*, 6(17), e250360. <https://doi.org/10.46652/pacha.v6i17.360>
- Mehta, A. , Lamichhane, P. , Khatiwada, R. , Mandal, A. , Chaudhary, P. K., & Aryal, S. (2024). Dragon Fruit Cultivation, Profitability, and Production Efficiency in Southern Terai, Nepal. *International Journal of Horticultural Science and Technology*, 11(4), 469-480. <https://doi.org/10.22059/ijhst.2024.366181.703>
- Sharma, S. C., Mittal, R., Sharma, A., & Verma, V. (2021). Dragon fruit: A promising crop with a growing food market that can provide profitable returns to farmers. *Int. J. Agric. Sci. Res.*, 11, 1-14.

- Kikon, P., Kashyap, D., Choudhury, J., Devi, H., & Rameshori, R. (2021). Investment feasibility analysis of dragon fruit farming. *International Journal of Agricultural Sciences*, 17, 265-270. <https://doi.org/10.15740/HAS/IJAS/17.2/265-270>
- Michel-Lara, R.S., Mancilla-Villa, O. R., Preciado-Ortiz, C., & Jardel-Peláez, E. J. (2022). Indicadores de rentabilidad en la producción y comercialización de pitaya en Huisichi, Tolimán, Jalisco. *Idesia (Arica)*, 40(4), 125-135. <https://dx.doi.org/10.4067/S0718-34292022000400125>
- Ku-Caamal, E. A. 2019. Evaluación técnico-financiero de producción de pitahaya (*Hylocereus undatus*) en la localidad Chunchintok, Hopelchen, Campeche. Chapingo, Edo. de México.
- Trejo-López, M. P. (2012). Evaluar las posibilidades de inversión en una red de valor de pitahaya (*Hylocereus undatus*) como alimento funcional. Colegio de postgraduados, México.
- Flores-Miranda, L. (2011). Indicadores de rentabilidad en la producción de pitahaya (*Hylocereus undatus*) en San Juan Ixcaquixtla, Puebla. Colegio de Postgraduados, México.
- Méndez-Ku, P. 2003. Evaluación financiera de producción de pitahaya (*Hylocereus undatus*) orgánica en Sihochac, Champotón, Campeche. Universidad Autónoma Chapingo, México.
- Amaya-Pérez, D. B., Martínez-Damián, M. Ángel, & Sangerman-Jarquín, D. M. . (2025). Análisis de paquetes tecnológicos para la producción de maíz en el Estado de México. *Revista Mexicana De Ciencias Agrícolas*, 16(4), e3583. <https://doi.org/10.29312/remexca.v16i4.3583>
- Rebollar-Rebollar, S., Ramírez-Abarca, O., & Hernández-Martínez, J. (2022). Competitiveness and value added in Persian cucumber (*Cucumis sativus* L.) in contract farming: case study. *Revista Terra Latinoamericana*, 40. <https://doi.org/10.28940/terra.v40i0.952>
- Morales-Hernandez, J. L., González-Razo, F. d. J., & Hernandez-Martinez, J. (2018). Análisis de rentabilidad del cultivo de jitomate bajo invernadero en San Simón de Guerrero, Estado de México. Universidad Autónoma del Estado de México.
- FIRA. (s. f.). Instructivo para la Evaluación Técnico-Financiera Cuantitativa Tradicional del Procedimiento de Evaluación de FIRA – Banco de México.
- Banco Interamericano de Desarrollo (BID). (2019). Evaluación de proyectos de inversión: Guía práctica para América Latina y el Caribe. Washington, D.C.: BID. <https://publications.iadb.org/es>
- Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO). (2015). Manual para la evaluación de proyectos de inversión agrícola y rural. Roma: FAO. <http://www.fao.org/documents>
- Osuna-Enciso, T., Valdez-Torres, J. B., Sañudo-Barajas, J. A., Muy-Rangel, M. D., Hernández-Verdugo, S., Villarreal-Romero, M., & Osuna-Rodríguez, J. M. (2016). Fenología reproductiva, rendimiento y calidad del fruto de pitahaya (*Hylocereus undatus* (How.) Britton and Rose) en el valle de Culiacán, Sinaloa, México. *Agrociencia*, 50(1), 61-78. Recuperado en 04 de abril de 2025, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-31952016000100061&lng=es&tlng=es.
- SIAP. (2024). Producción agrícola por municipios. Secretaría de Agricultura y Desarrollo Rural.
- López-Chávez, A., Alvarado-Raya, H.E., Estrada-Chavira, M.E., Altamirano-Cárdenas, J. 2019. Diseño y evaluación técnico-financiera de módulos productivos de pitahaya adaptables en el estado de Guanajuato. Chapingo, México.
- Carrillo-Martínez, C. J., Álvarez-Fuentes, G., Aguilar-Benítez, G., García-López, J. C., & Contreras-Servín, C. (2019). Rentabilidad de la producción de frijol (*Phaseolus vulgaris* L.), maíz (*Zea mays* L.) y chile (*Capsicum annuum*) en el municipio de Morelos, Zacatecas. *Acta universitaria*, 29, e1984. Epub 01 de diciembre de 2019. <https://doi.org/10.15174/au.2019.1984>
- Instituto de Investigación y Capacitación Agropecuaria, Acuícola y Forestal (ICAMEX). 2024. cultivo de frijol. Gobiernos de México. <https://icamex.edomex.gob.mx/frijol>
- Secretaría de Hacienda y Crédito Público (SHCP). (2024). Ley del Impuesto sobre la Renta (LISR). Diario Oficial de la Federación. <https://www.dof.gob.mx>
- Sotelo-Ruiz, E. D., Ortiz Trejo, C., & Rizo-arellano, M.I. (2006). Áreas potenciales para el cultivo de pitahaya (*Hylocereus undatus* (haw.) britt. & rose) en el sur del Estado de México. *Revista Mexicana de Ciencias Forestales*, 30(98), 11. <https://cienciasforestales.inifap.gob.mx/index.php/forestales/article/view/850/2125>

Retention of H_3PO_4 and NH_4OH by the fibrous components of oat (*Avena sativa* L.) forage

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ABSTRACT

Objective: To evaluate the structural modification of oat straw through chemical treatment with phosphoric acid (H_3PO_4) and ammonium hydroxide (NH_4OH), focusing on fiber retention and hydrolysis over time, due to its relevance as a forage resource.

Design/Methodology/Approach: Oat straw samples (500 g) were treated with 1 M aqueous solutions of H_3PO_4 and NH_4OH separately and exposed for 0, 8, 16, 24, 32, 40, and 48 hours. Samples were not washed prior to analysis to preserve any residual chemical retention on the fiber surface. The following variables were evaluated: neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, lignin (ADL), silica, and hemicellulose. Data were statistically analyzed using ANOVA and Tukey's test ($p < 0.05$). Results: After 8 hours of treatment, H_3PO_4 showed higher retention on structural components, with NDF, ADF, ADL, cellulose, and silica retaining 9.98%, 8.84%, 1.87%, 7.65%, and 2.00%, respectively. In contrast, NH_4OH treatments resulted in lower retention: 4.01% in NDF, 4.84% in ADF, and 4.11% in cellulose. Significant reductions in NDF, ADF, and cellulose content were observed with H_3PO_4 after 16-48 hours, indicating a hydrolytic effect. NH_4OH treatments did not result in significant degradation.

Study Limitations/Implications: The study did not consider the effects of post-treatment washing or neutralization, which could influence the practical application of the treated forage. Nevertheless, the findings highlight the relevance of chemical selection and exposure time in modifying lignocellulosic structures.

Findings/Conclusions: Phosphoric acid (H_3PO_4) exhibited a greater affinity and reactivity with the lignocellulosic matrix of oat straw compared to ammonium hydroxide (NH_4OH), as evidenced by higher initial retention and significant hydrolysis of structural fibers. These results suggest that H_3PO_4 may be a more effective agent for enhancing the nutritional accessibility of oat straw in ruminant diets. Further research is needed to evaluate its practical viability and safety in forage treatment systems.

Keywords: retention, phosphoric acid, ammonium hydroxide, oat straw, cell walls.

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INTRODUCTION

In Mexico, oats rank fifth among the crops with the largest cultivated area (Espitia *et al.*, 2007). It is a winter crop cultivated both for forage (De la Garza-Caballero &

Garza-Cedillo, 2013) and grain production (Villaseñor *et al.*, 2019; Rodríguez-Herrera *et al.*, 2020). As a food source for humans, oat grain is notable for its high dietary fiber content, primarily composed of non-starch polysaccharides, with hemicelluloses being predominant (Nowakowski *et al.*, 2008; Miller & Fulcher, 2011; Sargautiene *et al.*, 2018). These fiber fractions contribute functional and health-promoting properties, largely due to their arabinoxylan content, which can reach up to 21% (Couture *et al.*, 2022). From a forage perspective, oats are characterized by a high proportion of fibrous components such as neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, and crude protein (Mendoza-Pedroza *et al.*, 2021). Reported values vary according to cutting stage and environmental conditions; on average, NDF ranges from 50 to 65%, and ADF from 30 to 45% (Martínez *et al.*, 2010; Coblenz *et al.*, 2013; Mendoza-Pedroza *et al.*, 2021). These lignocellulosic fractions play a key role in determining forage digestibility and nutrient retention capacity. Several studies have shown that certain plant species can absorb inorganic ions such as ammonium (NH_4^+) and phosphate (PO_4^{3-}). For instance, *Eichhornia crassipes*, *Lemna minor*, and *Saccharum officinarum* have demonstrated ion retention capabilities and are commonly employed in wastewater remediation (Raimbekov *et al.*, 2021; De Marchi *et al.*, 2022). In oats, the xylan fraction can also bind phosphate groups, forming phosphorylated polysaccharides with varying degrees of substitution (Dace *et al.*, 1997; Salama *et al.*, 2014; Laffargue *et al.*, 2023). Under acidic conditions, phosphate ions readily penetrate barley and maize roots, promoting complex formation within the cell wall (Barrow, 2017). However, limited information is available regarding the capacity of oat Straw an inert lignocellulosic residue to retain or absorb ammonium ions post-harvest. This knowledge gap is particularly relevant, as ammonium may interact with functional groups in lignin and cellulose, potentially modifying the fiber's structure and affecting its suitability for animal feed or nutrient recycling. Investigating these interactions is essential for determining whether chemical treatments with NH_4OH or H_3PO_4 differentially alter fibrous fractions, thereby influencing their composition and reactivity. Previous studies have reported that both phosphoric acid and ammonium hydroxide can act as hydrolytic agents, capable of altering structural components of the plant cell wall (Berger *et al.*, 1981; Fithri *et al.*, 2022). Prior to hydrolysis, H_3PO_4 may penetrate plant tissues and form covalent bonds with polysaccharides, whereas NH_4OH may form reversible ionic interactions (Laffargue *et al.*, 2023). Based on this, it is hypothesized that the application of H_3PO_4 and NH_4OH to oat straw results in the initial retention of phosphate or ammonium ions within fibrous fractions, followed by a progressive hydrolysis of lignocellulosic components over time.

It is expected that oat straw treated with H_3PO_4 will retain a greater amount of phosphate ions compared to NH_4OH treatment and that phosphoric acid will induce a more pronounced hydrolytic effect on lignocellulosic fractions. Therefore, this study aims to evaluate the changes in the fibrous composition of oat straw treated with H_3PO_4 or NH_4OH over a 48-hour period and to determine the retention and hydrolytic effects of both compounds.

MATERIALS AND METHODS

Experimental site

The study was conducted at the Animal Nutrition Laboratory, Department of Zootechnics, located on Peripheral 5 of the Universidad Autonoma Chapingo, Texcoco de Mora, State of Mexico (19° 29' 23" N, 98° 53' 30" W). According to García (2004), the regional climate is classified as C(w2)(w)b(i)g a temperate sub-humid climate, the driest among the sub-humid types with a mean annual temperature of 15.2 °C and an average annual precipitation of 636.5 mm, primarily occurring in the summer.

Biological material and handling

The experiment was conducted between April and July 2023 using oat straw (*Avena sativa* L.), Chihuahua variety, harvested at the pre-flowering stage from experimental plots at the Colegio de Postgraduados (COLPOS), Montecillo Campus, Texcoco, Mexico (19° 27' 51" N, 98° 54' 21" W; 2,252 masl). The straw was baled (average weight ≈36 kg) and stored under a corrugated galvanized metal roof on a concrete floor to prevent moisture exposure. A 2 kg subsample was taken to the Animal Nutrition Laboratory for immediate analysis.

Chemical analysis

All analyses were performed at the Animal Nutrition Laboratory, Department of Zootechnics, Universidad Autónoma Chapingo. The fibrous components of the lignocellulosic matrix were determined following the methodology proposed by Van Soest (1991). Each sample constituted one experimental unit and was analyzed in triplicate.

Solutions and treatments

An untreated control (0 h) was included as a baseline. Analytical-grade ammonium hydroxide (27.3% purity, density 0.88 g/cm³) and phosphoric acid (85% purity, density 1.88 g/cm³) were used. Both solutions were diluted to a concentration of 1 M using distilled water. For each treatment, 500 g of oat straw was placed in plastic bags to ensure uniform mixing and manually sprayed with the respective solution for homogeneous distribution. The treated samples were sealed and incubated at room temperature for 0, 8, 16, 24, 32, 40, and 48 hours before analysis (Van Soest, 1991).

Evaluated variables

Based on the Van Soest (1991) methodology, the following response variables were measured: neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, silica, and hemicellulose (Hem).

Determination of NDF and its fractions

Crude fiber determination underestimates the cell wall content in forages; therefore, Van Soest's method uses a neutral detergent solution to isolate the cell wall (NDF), which comprises lignin, cellulose, hemicellulose, and silica. ADF is the residue after acid detergent digestion, where hemicellulose is removed. Subsequent treatment of ADF with sulfuric

acid (H_2SO_4) dissolves cellulose, leaving lignin and silica, which are separated via ashing at approximately 500 °C. The difference between 100 and NDF represents the cell contents, which are considered 98% digestible. NDF is partially digestible by ruminants, and its digestibility can be improved with acid or alkaline treatments.

Experimental design

A completely randomized design with a factorial treatment structure was employed: two levels of chemical treatment (H_2PO_4 and NH_4OH) \times seven exposure times (0, 8, 16, 24, 32, 40, and 48 hours). In addition to the two-way factorial analysis, each chemical compound was also analyzed independently using a one-way completely randomized design. The 48-hour maximum exposure period was selected based on preliminary trials and prior studies, indicating that measurable hydrolytic or retention effects of H_2PO_4 and NH_4OH occur within this timeframe (Krongtaew *et al.*, 2010; Sabir *et al.*, 2020). Longer durations may lead to excessive fiber degradation or reagent loss through evaporation, potentially compromising data consistency.

Statistical analysis

Data were analyzed using the general linear model procedure (SPSS, 2011). Significance was assessed at $p < 0.05$ for main effects and interactions within the factorial arrangement. The main effect of chemical compound and the interaction term were evaluated using the F-test ($p < 0.05$), while differences in the time factor were analyzed using Tukey's test ($p < 0.05$). Additionally, the effect of time for each chemical treatment was analyzed separately using Tukey's test ($p < 0.05$). No data transformations were required. Each experimental unit consisted of the mean of three replicates derived from a single straw batch per treatment and time point.

RESULTS AND DISCUSSION

Main effects and interaction

The results of the factorial analysis, including the main effects of time and chemical compound, as well as their interaction, are presented below. The time factor consisted of seven levels (0, 8, 16, 24, 32, 40, and 48 hours), while the chemical compound factor included two levels (H_3PO_4 and NH_4OH). These effects are summarized in Tables 1 and 2, with their interaction illustrated in Figure 1.

Table 1. Main effects of the chemical compounds (H_3PO_4 and NH_4OH) on fiber fractions of oat straw (% dry matter).

Chemical compound	NDF	ADF	ADL	Cellulose	Silica	Hem
H_3PO_4	64.05	38.55b	4.42b	31.43b	4.68b	25.50a
NH_4OH	64.18	42.84a	5.38a	34.21a	5.69a	21.33b
EE	0.2081	0.3273	0.0713	0.2535	0.0754	0.3467
P value	0.6670	<0.01	<0.01	<0.01	<0.01	<0.01

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error.

Means for each chemical compound, irrespective of exposure time, were compared using an F-test ($p < 0.05$). Different letters within the same column indicate significant differences.

Table 2. Main effects of exposure time (0, 8, 16, 24, 32, 40, and 48 h) on fiber fractions of oat straw (% dry matter), regardless of chemical compound.

Time (h)	NDF	ADF	ADL	Cellulose	Silica	Hem
0	58.48c	35.14b	3.81b	28.55c	4.02b	23.34
8	65.98a	42.43a	5.19a	34.43a	5.46a	23.55
16	65.50a	42.48a	5.27a	34.24ab	5.55a	23.02
24	64.70ab	41.26a	4.83a	33.49ab	5.11a	23.44
32	65.19ab	41.84a	5.34a	33.48ab	5.66a	23.36
40	65.45a	41.18a	4.89a	33.31ab	5.19a	24.27
48	63.51b	40.54a	4.98a	32.25b	5.29a	22.96
EE	0.3893	0.6123	0.1334	0.4742	0.1411	0.6486
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.8430
0	58.48c	35.14b	3.81b	28.55c	4.02b	23.34

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error. All data were compared across time using Tukey’s test ($p < 0.05$), regardless of chemical compound. Different letters within the same column indicate significant differences.

NDF remained unchanged ($p > 0.05$), while Hem content was higher and the other variables were lower with phosphoric acid (H_3PO_4) compared to ammonium hydroxide (NH_4OH). According to the results presented in Table 1, ADF, ADL, cellulose, and silica retained more NH_4OH than H_3PO_4 , whereas Hem retained more H_3PO_4 than NH_4OH .

The Hem variable remained unaffected by the time factor, while NDF and cellulose increased up to 8 h (indicating a retention effect) and decreased by 48 h, with this decline interpreted as a hydrolytic effect. The remaining response variables increased until 8 h and then remained stable through 48 h (Table 2). These results are further examined below, and Tables 3 and 4 detail the effects of time for each level of the chemical compound factor (H_3PO_4 and NH_4OH). Figure 1 illustrates the interaction between compound and time, revealing two distinct phenomena: (1) an initial retention phase (increase at 8 h),

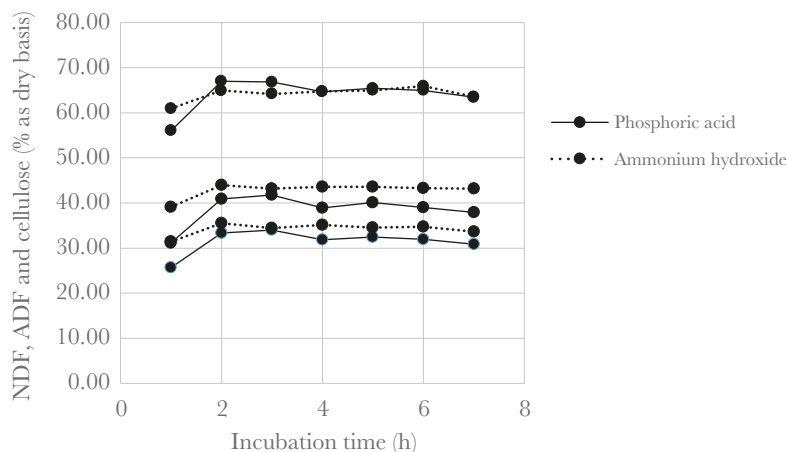


Figure 1. Interaction between chemical compound (H_3PO_4 and NH_4OH) and exposure time on neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose content of oat straw. Solid lines represent H_3PO_4 and dashed lines represent NH_4OH . Significant compound \times time interactions were detected ($p < 0.05$).

during which H_3PO_4 binds to the fiber matrix, and (2) a hydrolytic phase (decline from 16 to 48 h), where phosphate progressively cleaves glycosidic and ester bonds. In contrast, NH_4OH exhibits only the retention phase, indicating a limited hydrolytic capacity. This distinction holds practical significance, as it suggests that acid and alkaline treatments can be strategically applied depending on whether the goal is nutrient enrichment or fiber degradation.

Subsequently, no further changes were observed, as the trend lines for each variable remained parallel. The greater initial increase with phosphoric acid suggests that NDF, ADF, and cellulose exhibit a higher affinity for phosphate retention compared to ammonium.

The interaction between the factors time and chemical compound was significant for neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose ($p < 0.05$). No significant interactions were observed for the other response variables ($p > 0.05$). In Figure 1, the upper pair of lines corresponds to NDF: solid line (H_3PO_4) and dashed line (NH_4OH); the middle pair to ADF: solid line (H_3PO_4) and dashed line (NH_4OH); and the lower pair to cellulose: solid line (H_3PO_4) and dashed line (NH_4OH). *Eichhornia crassipes* and *Lemna minor* are known to absorb phosphates (Pavlidis *et al.*, 2022), which is why they are commonly used in wastewater treatment. Sugarcane also takes up phosphate ions, with De Marchi *et al.* (2022) reporting peak phosphate absorption in sugarcane leaves at 49 days post-transplantation. Sabir *et al.* (2020) demonstrated that cellulose phosphate or cellulose/hydroxyapatite can form at room temperature. According to Barrow (2017), phosphate uptake by isolated barley or maize roots is notably high at pH 4. Hilt *et al.* (2016) observed that oats can absorb excess phosphate from manure. Furthermore, sodium trimetaphosphate has been shown to crosslink starch and carboxymethyl cellulose, forming hydrogels (Cagnin *et al.*, 2021; Marim *et al.*, 2024). Therefore, it is considered that in the present study, the phosphate ion reacted with the oat straw sample under low pH conditions and was not removed by the neutral detergent reagent at pH 7. Consequently, NDF values at 8 h were higher than at 0 h, as were those of ADF, ADL, cellulose, and silica (Table 2). A greater retention of phosphate than ammonium has also been reported for rice straw. This higher phosphate retention suggests that rice straw carries more positive than negative charges (Sooksawat *et al.*, 2021). Additionally, the lignocellulosic matrix of straw, characterized by its porous structure and surface charges, allows for electrostatic interactions that enhance ion retention (Marim *et al.*, 2024). Polysaccharides such as cellulose and hemicellulose, rich in hydroxyl groups, can form hydrogen bonds with these ions, while lignin, with its complex and heterogeneous polymeric structure, offers additional adsorption sites. These combined interactions contribute to the capacity of oat straw to retain phosphate and ammonium ions.

Effect of phosphoric acid on oat straw over time

With H_3PO_4 , NDF was higher at 8 h of treatment compared to 0 h, and consequently, ADF, ADL, cellulose, and silica also increased. After 8 h, NDF decreased, and when comparing 48 h and 16 h, NDF, ADF, and cellulose were lower at 48 h (Table 3), indicating a tendency toward hydrolysis as the exposure time to phosphoric acid increased. It is likely

Table 3. Effects of phosphoric acid (H_3PO_4) exposure time on fiber fractions of oat straw (% dry matter).

Time (h)	NDF	ADF	ADL	Cellulose	Silica	Hem
0	56.02c*	31.16c	2.88d	25.68c	3.05d	24.86
8	66.00a	40.90ab	4.75abc	33.33ab	5.05abc	26.10
16	66.83a	41.76a	4.95ab	34.00a	5.22ab	25.06
24	64.67ab	38.94ab	4.37c	31.84ab	4.64c	25.73
32	65.41ab	40.11ab	4.96a	32.41ab	5.25a	25.30
40	64.97ab	39.06ab	4.47bc	31.92ab	4.72bc	25.91
48	63.45b	37.89b	4.55abc	30.82b	4.80abc	25.56
EE	0.5255	0.7183	0.1013	0.6064	0.1071	0.8757
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.9431

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error. Means within each column were compared using Tukey's test ($p < 0.05$). Different letters within the same column indicate significant differences.

that H_3PO_4 is retained primarily by cellulose and NDF, rather than by other fractions. Krongtaew *et al.* (2010) reported that the total polysaccharides in oat and wheat straw increased after 4 h of acid (pH 3, 25 °C) or alkali (pH 11.5, 90 °C) treatment. In oat straw, the total polysaccharides rose from 51% to 71.5% with acid and to 74% with alkali treatment. When oat and wheat straws were treated with sulfuric acid (pH 3, 25 °C, 4 h), Krongtaew *et al.* (2010) observed increases in total polysaccharides of 20.5% and 4.4%, respectively. These findings align with those of Sabir *et al.* (2020), who demonstrated that at room temperature, cellulose phosphate or cellulose/hydroxyapatite complexes can form. Sodium trimetaphosphate is also capable of crosslinking starch and carboxymethyl cellulose to produce hydrogels (Cagnin *et al.*, 2020; Marim *et al.*, 2024). It is important to note that the studies by Krongtaew *et al.* (2010) and Sabir *et al.* (2020) employed more extreme conditions (acidic pH 3-4, temperatures up to 90 °C, and short reaction times of 4 h), whereas the present study was conducted at room temperature and moderate acidity (1 M H_3PO_4). These methodological differences account for the milder hydrolytic effects observed in this experiment. No additional data were found regarding phosphate retention by the other lignocellulosic fractions. While the uptake of ammonium and phosphate ions by living plants has been well documented due to the relevance of fertilization (Sharma & Chetani, 2017), the retention of these ions by post-harvest inert materials such as oat straw has received limited attention. Research has instead focused on the hydrolysis of lignocellulosic components. This hydrolytic effect is addressed in the following discussion. After 8 and 16 h of treatment, phosphoric acid begins to exhibit hydrolytic activity, resulting in lower levels of NDF, ADF, and cellulose at 48 h compared to 16 h (Table 3). However, the other variables remained unchanged. It is possible that beyond 48 h, this reduction could become more pronounced. Since the present study was limited to a maximum of 48 h, any further decline beyond this point was not observed in comparison to the original (0 h) sample. These findings suggest that phosphate ions infiltrate the cell wall and form stable phosphate esters with the hydroxyl groups of cellulose and hemicellulose, leading to increased apparent retention. In contrast, ammonium ions (NH_4^+) interact primarily

through ionic exchange with negatively charged sites, which are relatively scarce in lignocellulosic materials, thereby explaining their lower retention capacity.

Effect of ammonium hydroxide on oat straw over time

The lack of significant changes in most variables with NH_4OH may be attributed to the lower reactivity of ammonium ions compared to phosphate ions. Ammonium tends to interact electrostatically with lignin and phenolic groups, forming weak, reversible bonds that do not disrupt the fiber matrix. Thus, under the tested conditions (1 M, room temperature), NH_4OH acts primarily as a mild alkalizing agent rather than a potent hydrolytic compound.

With NH_4OH , an increase in NDF, ADF, and cellulose levels was observed between 0 and 8 h, with no further changes ($p > 0.05$). The variables ADL, silica, and Hem were unaffected by NH_4OH treatment. Not a single variable showed a decrease after 8 or 16 h of treatment (Table 4). However, at extended treatment durations, NH_4OH could exhibit hydrolytic effects. For instance, Berger *et al.* (1981) reported increased digestibility of oat kernels treated with 6% NaOH and 6% NH_4OH over three weeks, attributing the improvement to hemicellulose solubilization. Similarly, Zhang *et al.* (2018) demonstrated high glucose yields from sugarcane bagasse through sulfuric acid hydrolysis followed by ethanol/NaOH treatment. When oat and wheat straws were treated with sodium hydroxide (pH 11.5, 90 °C, 4 h), Krongtaew *et al.* (2010) observed increases in total polysaccharides of 11.1% and 23%, respectively. Bachmann *et al.* (2023) also reported a 6.3% increase in ash content when barley straw was treated with NaOH (6 g/kg DM, 5 min), rewetted to 40% dry matter, and stored for 14 days. In Krongtaew *et al.*'s study, the increases were likely due to a concentration effect following distilled water washing a step not performed in the present study. An increase in NDF content over time was observed, with phosphoric acid resulting in a 9.98% rise (Table 3), significantly greater ($p > 0.05$) than the 4.01% increase with ammonium hydroxide (Table 4). This was not due to a concentration effect, as no sample washing occurred before analysis. Hence,

Table 4. Effects of ammonium hydroxide (NH_4OH) exposure time on fiber fractions of oat straw (% dry matter).

Time (h)	NDF	ADF	ADL	Cellulose	Silica	Hem
0	60.94b*	39.12b	4.73	31.41b	4.98	21.82
8	64.95a	43.96a	5.62	35.52a	5.88	21.00
16	64.18a	43.20ab	5.59	34.48ab	5.88	20.97
24	64.72a	43.57ab	5.29	35.15a	5.58	21.15
32	64.97a	43.56ab	5.72	34.54ab	6.07	21.41
40	65.93a	43.30ab	5.32	34.70ab	5.66	22.63
48	63.56ab	43.19ab	5.41	33.68ab	5.78	20.36
EE	0.5744	0.9919	0.2468	0.7293	0.2612	0.9569
P value	<0.01	0.0520	0.1760	0.0252	0.1630	0.7476

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error. Means within each column were compared using Tukey's test ($p < 0.05$). Different letters within the same column indicate significant differences.

phosphoric acid was more strongly retained by NDF than ammonium hydroxide. Since NDF includes cellulose, this result aligns with findings by Marim *et al.* (2024), who showed that sodium trimetaphosphate can bind to cellulose chains. From a practical standpoint, understanding the differential retention and hydrolysis patterns of H_3PO_4 and NH_4OH in oat straw can inform strategies to enhance forage digestibility. Mild acid pretreatments such as H_3PO_4 may partially solubilize hemicellulose and improve fiber accessibility to microbial enzymes, thereby promoting ruminal degradation. Conversely, NH_4OH treatments could be more suitable when the objective is to boost nitrogen content without causing significant structural breakdown.

CONCLUSIONS

The evaluation of the retention and hydrolytic effects of phosphoric acid (H_3PO_4) and ammonium hydroxide (NH_4OH) on the fibrous fractions of oat straw was successfully carried out. The results showed that both compounds interacted with the lignocellulosic matrix, though with differing intensities and mechanisms. Phosphoric acid exhibited significantly greater retention and hydrolytic activity ($p < 0.05$) than ammonium hydroxide, as demonstrated by the more pronounced reductions in NDF, ADF, and cellulose contents after 16-48 h of treatment. These findings confirm that phosphate ions form stronger chemical bonds with polysaccharides, while ammonium ions primarily engage in weak electrostatic interactions. The retention observed at 8 h and the hydrolysis noted at 48 h represent two distinct chemical processes: initial ion adsorption followed by partial depolymerization of the fiber matrix. From an applied perspective, these results suggest that mild phosphoric acid treatments could enhance the digestibility of oat-based forages through partial disruption of lignocellulosic bonds, while ammonium hydroxide could serve to enrich nitrogen content without damaging fiber structure. Future studies should examine the *in vivo* effects of these treatments on ruminal fermentation and nutrient bioavailability.

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REFERENCES

- Bachmann M, Martens SD, Le Brech Y, Kervern G, Bayreuther R, Steinhöfel O & Zeyner A. (2022). Physicochemical characterisation of barley straw treated with sodium hydroxide or urea and its digestibility and *in vitro* fermentability in ruminants. *Scientific Reports*, 12(1), 20530. DOI: 10.1038/s41598-022-24738-w
- Barrow, NJ. (2017). The effects of pH on phosphate absorption from soil. *Plant and Soil*, 410, 401-410. DOI: 10.1007/s11104-016-3008-9
- Berger LL, Anderson GD & Fahey Jr GC. (1981). Alkali treatment of cereal grains. I. *In situ* and *in vitro* evaluation. *Journal of Animal Science*, 52(1), 138-143. DOI: 10.2527/jas1981.521138x
- Cagnin C, Simões BM, Yamashita F, Andrello AC, de Carvalho GM & Grossmann MVE. (2021). Hydrogels of starch/carboxymethyl cellulose crosslinked with sodium trimetaphosphate via reactive extrusion. *Journal of Applied Polymer Science*, 138(15), 50194. DOI: 10.1002/app.50194

- Coblentz WK, Nellis SE, Hoffman PC, Hall MB, Weimer PJ, Esser NM & Bertram MG. (2013). Unique interrelations between fiber composition, water-soluble carbohydrates, and *in vitro* gas production for fall-grown oat forages. *Journal of Dairy Science*, 96(11), 7195-7209. DOI: 10.3168/jds.2013-6889
- Couture G, Luthria DL, Chen Y, Bacalzo Jr NP, Tareq FS, Harnly J & Lebrilla CB (2022). Multi-Glycomic Characterization of Fiber from AOAC Methods Defines the Carbohydrate Structures. *Journal of Agricultural and Food Chemistry*, 70(45), 14559-14570. <https://doi.org/10.1021/acs.jafc.2c06191>
- Dace R, McBride E, Brooks K, Gander J, Buszko M & Doctor VM (1997). Comparison of the anticoagulant action of sulfated and phosphorylated polysaccharides. *Thrombosis Research*, 87(1), 113-121. DOI: 10.1016/S0049-3848(97)00110-2
- De la Garza-Caballero M & Garza Cedillo RD. (2013). Tecnología para la producción de avena forrajera en el altiplano de Tamaulipas. INIFAP
- De Marchi Soares T, Raniro HR & Pavinato PS. (2022). Sugarcane Byproduct Influence on Mineral Fertilizer Solubility and Phosphorus Dynamics in the Soil. *Journal of Soil Science and Plant Nutrition*, 22(2), 1458-1467. DOI: 10.1007/s42729-021-00745-0
- Espitia Rangel E, Villaseñor Mir HE, Huerta Espino J, Salmerón Zamora JJ, González Iñiguez RM & Osorio Alcalá L. (2007). Obsidiana, variedad de avena para la producción de grano y forraje en México. *Agricultura técnica en México*, 33(1), 95-98.
- Fithri L, Damayanti M & Ayu AK. (2022). Isolation and Enzymatic Degradation of Hemicellulose from Corncoobs Waste. *Journal of Bio-Molecule Research and Engineering*, 1(1), 14-21. DOI: 10.20473/jbiome.v1i1.35855
- García E. (2004) Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía-UNAM. México. 90p
- Hilt K, Harrison J, Bowers K, Stevens R, Bary A & Harrison K. (2016). Agronomic response of crops fertilized with struvite derived from dairy manure. *Water, Air, & Soil Pollution*, 227(10), 388. DOI: 10.1007/s11270-016-3093-7
- Krongtaew C, Messner KT & Fackler K. (2010). Characterization of key parameters for biotechnological lignocellulose conversion assessed by FT-NIR spectroscopy. Part 1. Qualitative analysis of pretreated straw. *BioResources*. 5(4), 2063-2080. DOI: 10.15376/biores.5.4.2063-2080
- Laffargue T, Moulis C & Remaud-Simeon M. (2023). Phosphorylated polysaccharides: Applications, natural abundance, and new-to-nature structures generated by chemical and enzymatic functionalisation. *Biotechnology Advances*, 65, 108140. DOI: 10.1016/j.biotechadv.2023.108140
- Marim BM, Mantovan J, Pereira JF, Debiagi F & Mali S. (2024). Sustainable process based on reactive extrusion to modify cellulose from oat hull with sodium trimetaphosphate and tartaric acid. *Polymer Bulletin*, 81(3), 2345-2364. DOI: 10.1007/s00289-023-04825-2
- Martínez MF, Arelovich HM & Wehrhahne LN. (2010). Rendimiento de grano, contenido de nutrientes y perfil lipídico de genotipos de avena cultivados en un ambiente semiárido. *Investigación de cultivos extensivos*, 116(1-2), 92-100.
- Mendoza-Pedroza SI, Sosa-Montes E, Rodríguez-Ortega LT, Vasquez-Gomez IL, Ramírez-Bribiesca JE & Hernández-Guzmán FJ. (2021). Composición química de los componentes del cultivo de avena (*Avena sativa* L.), variedad Chihuahua. *Ecosistemas y Recursos Agropecuarios*, 8(II). DOI: 10.19136/era.a8nII.2966
- Miller SS & Fulcher RG. (2011). Microstructure and chemistry of the oat kernel. In F.H. Webster & P.J. Wood (Eds.) Oats: Chemistry and technology, (Ed. 2), 77-94. AACCC International.
- Nowakowski DJ, Woodbridge CR & Jones JM (2008). Phosphorus catalysis in the pyrolysis behaviour of biomass. *Journal of Analytical and Applied Pyrolysis*, 83(2), 197-204. DOI: 10.1016/j.jaap.2008.08.003
- Pavlidis G, Zotou I, Karasali H, Marousopoulou A, Bariamis G, Tsihrintzis VA & Nalbantis I. (2022). Performance of pilot-scale constructed floating wetlands in the removal of nutrients and pesticides. *Water Resources Management*, 36, 1-18. DOI: 10.1007/s11269-021-03033-9
- Raimbekov KT, Absatarov RR & Apyev DB. (2021). Possibilities of using *Eichhornia crassipes* Solms and *Lemna minor* L. In Wastewater Phytoremediation. *NVEO-Natural Volatiles & Essential Oils Journal*, 8(4), 7950-7966.
- Rodríguez-Herrera SA, Salgado-Ramírez O, García-Rodríguez JG, Cervantes-Ortiz F, Figueroa-Rivera MG & Mendoza-Elos M. (2020). Fertilización química y orgánica en avena: rendimiento y calidad de la semilla. *Agronomía Mesoamericana*, 31(3), 564-576.
- Sabir M, Ali A, Siddiqui U, Muhammad N, Khan AS, Sharif F, Iqbal F, Shah AT, Rahim A & Rehman IU. (2020). Synthesis and characterization of cellulose/hydroxyapatite based dental restorative composites. *Journal of biomaterials science. Polymer edition*, 31(14), 1806-1819. DOI: 10.1080/09205063.2020.1777827

- Salama A, Neumann M, Günter C & Taubert A. (2014). Ionic liquid-assisted formation of cellulose/calcium phosphate hybrid materials. *Beilstein Journal of Nanotechnology*, 5, 1553-1568. DOI: 10.3762/bjnano.5.167
- Sargautiene V, Nakurte I & Nikolajeva V. (2018). Broad prebiotic potential of non-starch polysaccharides from oats (L.): An study. *Polish journal of microbiology*, 67(3), 307-313. DOI: 10.21307/pjm-2018-036
- Sooksawat N, Santibenchakul S, Kruatrachue M & Inthorn D. (2021): Recycling rice husk for removal of phosphate and nitrate from synthetic and swine wastewater: Adsorption study and nutrient analysis of modified rice husk, *Journal of Environmental Science and Health, Part A*, 56(10), 1080-1092. DOI: 10.1080/10934529.2021.1962165
- Sharma A & Chetani R. (2017). A review on the effect of organic and chemical fertilizers on plants. *International Journal for Research in Applied Science & Engineering Technology*, 5(2), 677-680. DOI: 10.22214/ijraset.2017.2103
- SPSS, Statistical Package for the Social Science. (2011). Institute. SPSS-X. User's Guide. Version 8, Chicago IL. USA.
- Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583-3597. DOI: 10.3168/jds.S0022-0302(91)78551-2
- Villaseñor Mir HE, Espitia Rangel E, Rodríguez García MF, Martínez Cruz E, Huerta Espino J, Hortalano Santa Rosa R & Osorio Alcalá L. (2019). Jade: new oat variety for grain production under rainfed conditions in Mexico. *Mexican Journal of Agricultural Sciences*, 10(5), 1183-1188. DOI: 10.29312/remexca.v10i5.1872
- Zhang H, Wei W, Zhang J, Huang S & Xie J. (2018). Improvement of enzymatic saccharification of sugarcane bagasse by combined pretreatment and Tween 80. *Biotechnology for Biofuels*, 11, 1-12.

Study of backyard poultry farming in the Sierra and Valley of Villaflores, Chiapas, Mexico

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ABSTRACT

Objective: to analyze the current status and factors that constraint backyard poultry farming in rural communities in the state of Chiapas, Mexico.

Design/Methodology/Approach: this study was conducted in four ejidos (a Mexican form of collective landholding) in the Sierra and Valle areas of the municipality of Villaflores in the Frailesca Region, Chiapas (Mexico) through a participatory diagnosis that considered a population of 1600 households. A correlational-causal cross-sectional design was used, along with systematic sampling determined by using the appropriate formula for finite populations.

Results: results showed that while 77% of households still breed birds, production has fallen 41% in the last five years. This activity persists especially in ejidos with greater marginalization, where interest in conserving aviculture is maintained. Despite the negative trend, backyard poultry farming continues to be an activity of interest in the ejidos considered to have a certain degree of marginalization, where a constant commitment to this practice is observed.

Limitations/Implications of the study: the distrust of the people surveyed in the face of the problems of social insecurity and paternalism that prevail in the study area, negatively influenced to obtain information that would have helped to comprehensively analyze backyard poultry farming.

Findings/Conclusions: despite the potential as an economic alternative to food insecurity, the decline in participation and production per household threatens the long-term sustainability of backyard poultry farming. Therefore, this study underscores the need to implement effective strategies to preserve this vital practice for rural communities.

Keywords: food security, poultry farming, rural production, social property.

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INTRODUCTION

Poultry farming plays a crucial role in feeding the Mexican population. According to SIAP (2025), per capita consumption is 24.4 kg of eggs and 37.3 kg of chicken meat; followed in descending order by pork, 21.8 kg and beef, 16.0 kg. In the first quarter of 2025, the growth of poultry meat (2.7%) and pork (1.5%) were relevant, as well as the supply of eggs, which grew 0.5%; in contrast, beef consumption contracted (SIAP, 2025). Regarding chicken meat and eggs, it is logical that there is an increasingly greater demand because their price is lower per kilogram. This also makes the growth in demand and in consumption greater, in addition to more availability in time and place.



However, an obstacle to the growth of poultry farming, especially backyard poultry farming, is the lack of technical assistance, the presence of diseases, lack of feed, predators, and the lack of organizational structures in the market (Mata *et al.*, 2023). In this regard, INEGI (2018) observed that, at the national scale, only a small fraction of poultry receives vaccines, deworming or technical assistance. Proof of this is that the inclusion of backyard poultry farming in government programs, such as the Mexico's strategic program for food security (called PESA, in Mexico), has had a limited impact and beneficiary families show a 20% continuity with the project after two years of implementation (Cruz *et al.*, 2016).

Despite these challenges, some studies suggest considering family poultry farming as a way to address food insecurity in rural communities through alternative models. These also preserve the environment and the knowledge transmitted from generation to generation (Becerra *et al.*, 2023). For these reasons, it was proposed to explore the current state and the factors that condition backyard poultry farming in the Sierra and Valley areas of the municipality of Villaflores, Chiapas (Mexico).

MATERIALS AND METHODS

Study location

The research implemented during 2024, in the ejidos Dr. Domingo Chanona (ChN), Mexican Agronomists (AM), Ricardo Flores Magón (RFM) and Los Ángeles (LA) in the Valle and Sierra region of the Frailesca region, Chiapas. These ejidos are located respectively 27 km, 42 km, 56 km and 66 km apart from the seat of the municipality, which is Villaflores, Chiapas. This region is in the Pacific coastal plain and the central lowlands of Chiapas. It is characterized by a high production of maize, also it is part of the physiographic regions Sierra Madre and central lowlands of Chiapas, with a terrain relief composed of sierras (hills or mountains) and valleys (INEGI, 2023).

Experimental design. The 'correlational-causal cross-sectional' experimental design described by Hernández & Mendoza (2018) was used. This design seeks to describe the relationships between two or more categories, concepts, or variables at a specific time, either in correlational terms or as a function of the cause-effect relationships among the variables.

Sample. For the selection of the sample, the systematic sampling described by Otzen & Manterola (2017) was used. Also, a population base of 1600 households was taken as the analysis framework, from which a calculated sample of 70 households was obtained. Sample size was determined using the appropriate formula for finite populations (Tillé, 2020);

$$n = \frac{N * Z_{\alpha}^2 * p * q}{d^2 * (N - 1) + Z_{\alpha}^2 * p * q}$$

where; N : total population; Z_{α}^2 : 1.96 squared with 95% confidence; d : accuracy at 95%, $\alpha=0.05$; $p*q$: binomial distribution; p : probability of success; q : probability of failure, each at 0.5%, which corresponds to $(0.5)(0.5)=0.25$.

A questionnaire was applied consisting of a set of questions suggested by Hernández & Mendoza (2018). This questionnaire included qualitative and quantitative variables related to poultry production, as well as management and development factors of poultry farming. The questionnaire also included variables for maize production, because this is the main resource used in poultry feed.

Variables evaluated. Different categories of households were evaluated in relation to poultry production; a) households that currently produce poultry; b) households that did so at some point; c) households that have never been involved in production; d) households that stopped producing poultry; and, e) households that are willing to resume activity. Information was collected on the average number of birds produced per household, the total number of birds per household, those causes that led to the abandonment of the activity, and the level of knowledge about poultry production and breeding.

Statistical analysis. A descriptive statistical analysis and a correlation analysis were performed with the IBMTM SPSS[®] - Statistical Package for Social Sciences (IBM, 2017).

RESULTS AND DISCUSSION

Poultry production, poultry consumption and maize production

Currently, 77% of households produce birds, 20% have had previous experience in this activity, but not today; and 3% have not tried poultry farming. In addition, it was observed that 52% of households that stopped producing birds are willing to resume the activity. Regarding the average number of birds per household, there has been a decrease of 41% compared to that number five years ago; this is, it has gone from 37 to 22 birds per household. One of the possible reasons is what Guevara *et al.* (2023) indicated, this is, that the abandonment of the backyard, as a productive and utilitarian space, is also linked to subsidiary policies that contravene the principles of sustainable access to food.

These results acquire greater relevance when considering the differences between the ejidos; especially, as a function of their proximity to the municipal seat (Table 1).

Except Los Angeles ejido, the other ejidos showed a certain positive relationship between the average number of birds per household and the distance to the municipal seat. That

Table 1. Status of poultry production by Ejido in the municipality of Villaflores, Chiapas, Mexico.

Ejid ^{os}	Distance (km)	(% of housewives that:				Average number of birds produced per household	
		They are now producing	They produced once before	They have never produced	They would produce again	5 years ago	Now
ChN	27	47	40	13	40	33	20
LA	66	77	23	0	100	40	27
AM	42	83	17	0	67	42	17
RFM	56	100	0	0	0	34	23

ChN: Ejido Dr. Domingo Chanona; LA: Ejido Los Angeles; AM: Ejido Agrónomos Mexicanos; RFM: Ejido Ricardo Flores Magón † Distance to Villaflores (Chiapas), the seat of the municipality.

is, there are more birds per household the farther away the ejidos are from Villaflores, the municipal seat. This indicator could be influenced because higher population density leads to the atomization of plots. García *et al.* (2020) also observed that the reduction of the space of the plots increased the precariousness of poultry activity. In this study, among the variables that were important for poultry farming, more than the distance from the ejido to the municipal seat, the most important reason was the population density of the communities.

Poultry meat production and consumption. It was found that 4 out of every 6 kg consumed per month come from commercial strains, while the other 2 kg come from backyard birds. Of those who choose to consume commercial strains, 41.5% do so because they consider them cheaper. On the other hand, 43% of those who prefer to consume backyard poultry do so because they consider those healthier and with better flavor, because producers feed them maize. However, the preference for commercial birds may also be due to some health concerns in backyard poultry farming. In some places this is critical because of the poverty and marginalization that prevail in certain regions of Chiapas and in other states of the country (Medina *et al.*, 2018; Ramírez *et al.*, 2024).

Faced with these challenges, backyard poultry farming emerges as a strategy that allows rural families to generate alternative means of self-consumption and economic income from women's work to promote family well-being (Jaramillo *et al.*, 2018; Romero, 2021). If this strategy is not strengthened, backyard poultry meat consumption could decrease further; as shown by the downward trend in backyard poultry production over the past five years and the increase in preference for commercial poultry due to their lower cost.

Although the percentage of preference for creole birds was declared as higher, with the argument of a better taste and a favorable health response, this did not translate into a higher consumption, rather was it the expression of a communicated preference. In this sense, it is notable that the most populated ejido (ChN), the closest to the municipal seat, had fewer households with poultry production, only 30% of these, and 24% less maize production than the average produced in the four ejidos. This result could be due to the smaller plots. In contrast, in the ejido RFM, the least populated and also far from the municipal seat, all households reported that they have poultry and maize production. Although both ejidos show a similar number of birds per household, 81% of the producers are dedicated to the breeding of native birds, valued for their better flavor, greater health and resistance to diseases.

Maize production. Forty-six percent of households do not plant maize, but 77% of households do produce poultry, suggesting that some housewives continue to breed poultry even when the husband has stopped planting the grain. This is despite the fact that in the last five years, although maize yield has been maintained between 5 and 5.8 tons per hectare, the area planted per producer has decreased from 6 to 3 ha. This trend was influenced by the disinterest of older producers in providing accurate information during interviews. Especially if you consider that 45% of producers are between 35 and 65 years old and show a higher level of distrust.

Relationship between the average number of birds per household and the distance to the municipal seat

Only in three of the four ejidos studied (ChN, AM and RFM) was a moderately significant positive correlation (0.676) found between the number of birds per household and the distance to the municipal seat. It also turned out that the farthest ejido (LA) had the lowest ratio between the number of birds per household and the distance from the municipal seat (Table 1), possibly due to a higher percentage of extra-agricultural workers (15.4%), which implies a lower dependence on productive activities within the ejido. Although health issues account for 40% of the 11 reasons identified for bird population decline, it is not considered a major cause of aviculture abandonment.

Knowledge and decrease in poultry farming

The reasons for abandoning the activity can be visualized in a grouped mode (Figure 1). It is highlighted that the lack of space and economic resources are the most significant causes, respectively in 40% and 50% of cases. Regarding the reasons for abandonment, Kumar *et al.* (2021) noted that the main limitation of backyard poultry farming was the high mortality rate due to a combination of diseases. If such a reason were in accordance with this study, it would be partly due to lack of knowledge (Correia-Gomes & Sparks, 2020).

Although 72.4% of housewives indicated that the number of laying months per year is four to six, which is considered technically acceptable, the rest of the indicators varied between 56.7% and 66.6%. This means that 33.4 to 43.3% of responses were outside the range considered acceptable (Figure 2).

Health. The main diseases affecting birds are respiratory diseases (27%), white diarrhea (26%), fowl pox (25%) and Newcastle (22%). However, 77% of housewives who are dedicated to poultry production reported that they vaccinate their birds. Thus, the diseases may be due to poor sanitary management by housewives (Toapanta *et al.*, 2019; Kumar *et al.*, 2021). In addition, variability was observed in vaccination dates, July is the predominant month (42%), followed by January (28%), December (14%), and March (11%). Also, 14% of the people surveyed responded that they vaccinate the birds every six months, usually in January and July.

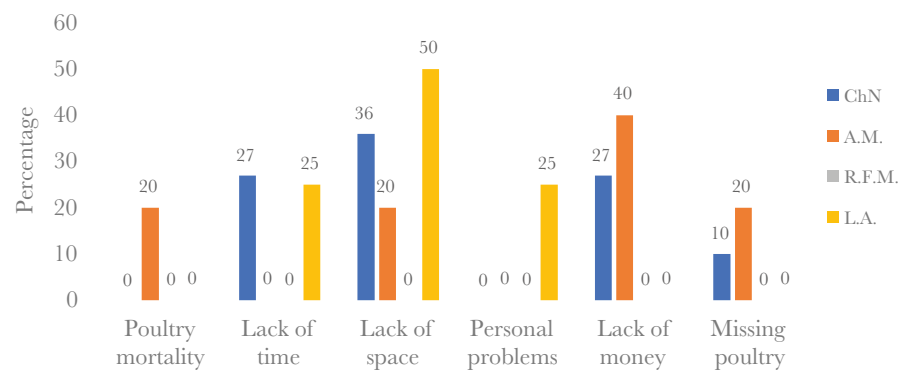


Figure 1. Reasons for abandonment (%) of backyard poultry farming in ejidos in the state of Chiapas, Mexico.

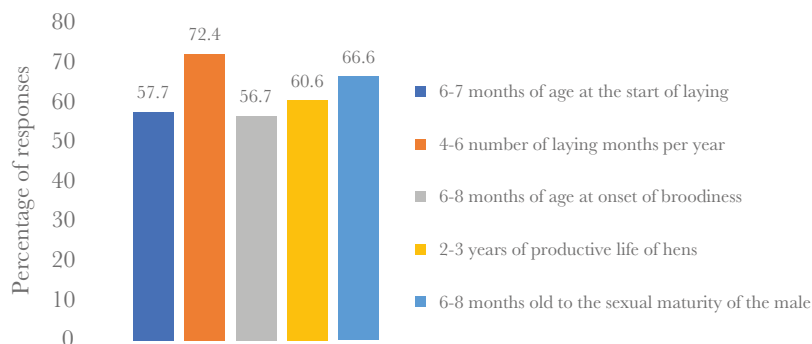


Figure 2. Knowledge of the technical indicators (%) of backyard poultry farming in ejidos of Chiapas, Mexico.

Parasitosis is another health problem, especially due to consuming contaminated water from domestic service, or by ingesting food from the ground or waste containers, an aspect that is not considered a problem by housewives. These health challenges may be one of the reasons why 81% of housewives choose to breed creole landraces, as they are not only valuable to ethnic communities, but also serve as an important genetic reservoir for breed development globally (Becerril *et al.*, 2024). Although the genetic diversity and conservation of the genes of native hens are threatened by the distribution of exotic hens (Senbeta & Keyata, 2024).

In this context, the existence and physical and hygienic conditions of the facilities and equipment are of paramount importance. All the housewives who raise birds claimed to have a shed for chicken with a roof and close to their homes. Of the total, 46% women have had sheds for approximately 12 years on average, while the others allow birds to be free during the day and confine them at dusk. This is similar to what was reported by Thomas *et al.* (2025) about there is a preference and willingness to breed birds outdoors, even with a shed available, although nighttime housing practices are variable.

A 70% of housewives mentioned that the reason why they choose to have their hens locked up is because of the risk of loss due to theft or attacks by predatory animals. As for the feeders and drinkers that are used, most are improvised with waste containers, only 5% are of industrial origin for that purpose. These are placed outdoors or in the shade of trees, and only 7% are located in the household backyard, under a roof top.

Extra-agricultural activities. A 35% of the families that are dedicated to the production of maize, get additional income with extra-agricultural activities. Of those, 61% are dedicated to the provision of services and commerce, 16% work as professionals, 15% are involved in livestock and coffee production, and only 8% work as laborers or factory workers. This shows that extra-agricultural work is a strategy used by the population to supplement their income, and in some cases, it constitutes the only available economic source. These activities are done both inside and outside the ejido, the municipality and even out of the state of Chiapas. This supports what was reported by Morett & Cosío (2017), who found that only 34.3% of young people, in at least 58.8% of the ejidos in Mexico, remain in their lands and join some productive activity.

Of the extra-agricultural workers, 67% work within the community, 17% outside the community but within the municipality, and 16% outside the municipality but within the state. Regarding the number of months dedicated to those activities, 75% do those during the 12 months of the year, while the remaining 25% work for 10 to 11 months, with average daily income ranging from \$100 to \$500 Mexican pesos (MXN) in 73% of cases. In addition, 41.2% migrate outside the country, 10.7% to urban areas (big cities); and only 2.6% are employed in rural activities, possibly agriculture-related, since a non-agricultural work wage is a better alternative for many young people (Gutiérrez *et al.*, 2019).

In the case of families that have stopped producing maize, 27% pointed to lack of land, or scarcity of economic resources (27%) and low profitability (18%) as the main reason. These families have opted for various trades, such as blacksmithing, masonry or carpentry (56%), day laborers or factory workers (22%) and commercial activities or poultry farming (22%). The average number of years dedicated to these new activities is 9 years, with a working duration of 12 months a year in 89% of cases, and only 11% work 6 months a year. The average daily income is \$183 MXN, with a mode of \$200 MXN. The 77% of these activities is done within the community, and 23% outside, but within the municipality.

In the specific case of the Los Angeles ejido, despite its farthest location from the municipal seat, it has 1.9% more extra-agricultural workers, this is 15.4% in total, compared to the nearest ejido (ChN), which has 13.3%; this situation can be attributed to the migration that characterizes the LA ejido. It is likely that the migration of the inhabitants of the ejidos most distant from the municipal seat is mainly directed to places outside the municipality. This is due to the low dynamism of agricultural activities and the scarce supply of labor alternatives for the young population. This is consistent with the findings of Morett & Cosío (2017), who indicated that four out of ten young people leave their community in search of employment; and they are mainly directed towards the U.S.

CONCLUSIONS

Despite the challenges of backyard poultry farming, such as health issues, space shortages, and other factors, poultry farming remains a source of income and food for people living in rural communities. In the willingness of households to continue or resume the activity, together with the high value attributed to native bird landraces in the local market, their economic and cultural relevance is highlighted.

Coordinated intervention between government and communities is essential to strengthening backyard poultry farming, addressing key issues such as animal health, access to financial resources, and technical capacity building. These actions can contribute to the improvement of productivity and sustainability of the poultry production sector in the state of Chiapas.

REFERENCES

- Becerra A., J.L., Flores P., F.C., Villamar, F. y Hernández, A. (2023). Avicultura de traspatio: modelo alternativo de producción avícola que contribuye a la seguridad alimentaria y nutricional en Honduras. *Innovare: Revista de ciencia y tecnología*, 12(1): 39-41. DOI: <https://doi.org/10.5377/innovare.v12i1.15962>
- Becerril, P.C.M., Chico, J.L. y Zárate, C.D. (2024). El papel vital de las gallinas criollas. *Agro-Divulgación*, 4(4). DOI: <https://doi.org/10.54767/ad.v4i4.351>

- Correia-Gomes, C., & Sparks, N. (2020). Exploring the attitudes of backyard poultry keepers to health and biosecurity. *Preventive Veterinary Medicine* 174, e104812. DOI: <https://doi.org/10.1016/j.prevetmed.2019.104812>
- Cruz, S.B., Muñoz R.M., Santoyo C.V.H., Martínez G.E.G. y Aguilar G.N. (2016). Potencial y restricciones de la avicultura de traspatio sobre la seguridad alimentaria en Guerrero, México. *Agricultura, Sociedad y Desarrollo*, 13(2): 257-275. DOI: <https://doi.org/10.1371/journal.pgph.0004929>
- García, N.M.J., Ramírez, V.B., Cesín, V.A., Juárez, S.J.P. y Martínez, C.D.C. (2020). Funciones agroalimentarias y socioculturales del traspatio en una comunidad Totonaca de Huehuetla, Puebla, México. DOI: <https://doi.org/10.15174/au.2020.2456>
- Guevara, H.F., O-Arias, M.A.L., Aguilar, V.E.H., Ruiz, S.E., Martínez, A.F., y Aguilar, J.C.E. (2023). Backyards: typology and contribution to food security in Mexico. *Ciência Rural*, 53(8), e20220397. DOI: <https://doi.org/10.1590/0103-8478cr20220397>
- Gutiérrez, C.M.G., Magaña, M.M.A., Zizumbo, V.D. y Ballina, G.H. (2019). Diversidad agrícola y seguridad alimentaria nutricional en dos localidades Mayas de Yucatán. *Acta universitaria*, 29, e1996. DOI: <https://doi.org/10.15174/au.2019.1996>
- Hernández, S.R. & Mendoza, C.P. (2018). Metodología de la Investigación: Las rutas cuantitativa, cualitativa y mixta. McGrawHill Interamericana Editores.
- IBM (IBM Corporation). (2017). SPSS Statistics 25 for Windows, Statistics Program for Social Sciences, version 25.0 (IBM Cloud), Armonk, NY: IBM. <https://www.ibm.com/support/pages/>
- INEGI (Instituto Nacional de Estadística y Geografía) (2018). Panorama sociodemográfico de Chiapas 2015. c2016.
- INEGI (Instituto Nacional de Estadística y Geografía) (2023). Aspectos geográficos de Chiapas: compendio 2023, México: INEGI, c2023.
- Jaramillo, A.J.G., Peña, O.B.V., Hernández, S.J.H., Díaz, R.R. y Espinosa, C.A. (2018). Caracterización de productores de maíz de temporal en Tierra Blanca, Veracruz. *Revista Mexicana de Ciencias Agrícolas*, 9(5): 911-923. DOI: <https://doi.org/10.29312/remexca.v9i5.1501>
- Kumar, M., Dahiya, S.P. y Ratwan, P. (2021). Backyard poultry farming in India: A tool for nutritional security and women empowerment. *Biological Rhythm Research*, 52(10), 1476-1491. DOI: <https://doi.org/10.1080/09291016.2019.1628396>
- Mata, E.A. M., Cerón, F.G., Martínez, A.P., Hernández, G.T., Ortega, J.B., Galicia, A.J.V. y Montes, E.S. (2023). Caracterización del sistema de producción avícola de traspatio en el estado de Campeche, México. *Agricultura, Sociedad y Desarrollo*, 20(2), 125-138. DOI: <https://doi.org/10.22231/asyd.v20i2.893>
- Medina, S.L., Guevara, H.F., Nahed, T.J. y Vázquez, P.D. (2018). Aspectos sociales sobre la problemática sanitaria observada en gallinas domésticas dentro del área natural protegida “La Pera”, Chiapas. *CIENCIA Ergo-Sum*, 25(3). DOI: <https://doi.org/10.30878/ces.v25n3a6>
- Morett, S.J.C. & Cosío, R.C. (2017). Panorama de los Ejidos y Comunidades Agrarias en México. *Agricultura, Sociedad y Desarrollo* 14(1).
- Otzen, T., & Manterola, C. (2017). Técnicas de muestreo sobre una población en estudio. *International Journal of Morphology*, 35(1), 227-232. DOI: <https://doi.org/10.4067/S0717-95022017000100037>
- Ramírez, E.P., Martínez, D.G., Ruiz, R.D., Garrido, J.S.E., Ramos, J.C. y Massiotti, R.D.A. (2024). Avicultura de traspatio en las familias participantes del programa pesa (FAO) en Cuetzalan del Progreso, Puebla. *Agricultura, Sociedad y Desarrollo*, 21(1), 64-83. DOI: <https://doi.org/10.22231/asyd.v21i1.1595>
- Romero, A.R. (2021). Las funciones de las aves en la producción avícola de pequeña escala: el caso de una comunidad rural en Hidalgo, México. *Revista Mexicana de Ciencias Pecuarías*, 12(1):217-237. Epub 20 de septiembre de 2021. DOI: <https://doi.org/10.22319/rmcp.v12i1.5088>
- Senbeta, D. H. & Keyata, E. O. (2024). Genetic diversity, gene conservation and indigenous-chicken gene erosion in Ethiopia: A review. *Journal of agriculture, food and natural resources*, 2(2), 49–54. DOI: <https://doi.org/10.20372/afnr.v2i2.970>
- SIAP (Servicio de Información Agroalimentaria y Pesquera) (2025). Reporte trimestral del producto interno bruto agroalimentario. SIAP-Secretaría de Agricultura. Primer trimestre de 2025.
- Thomas, E.D., et al. (2025). Who has sheds? Exploring practices and determinants of overnight housing for backyard poultry in rural Bangladesh to inform an intervention to limit exposure to poultry and poultry feces. *PLOS Global Public Health*, 2025, vol. 5, no 7, p. e0004929.
- Tillé, Y. (2020). Sampling and estimation from finite populations. John Wiley y Sons.
- Toapanta, M., Avilés, E.D.F., Montero, R.M. y Pomboza, P. (2019). Caracterización del sistema de producción de aves de traspatio del cantón Cevallos, Ecuador. *Actas Iberoamericanas de Conservación Animal AICA*, 13:1-5.

Effect of electrolyzed water supplementation in the liquid diet on the growth performance of Holstein calves

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ABSTRACT

Objective: To evaluate the effect of electrolyzed water (EW) supplementation in the liquid diet on energy intake, growth performance, and health of lactating Holstein calves.

Design/Methodology/Approach: Forty calves were randomly assigned to two treatments: T1=control and T2=5 mL of EW (Supra[®]) per liter of milk. Calves received colostrum after birth and were individually housed in disinfected metal hutches. Liquid feeding lasted for 60 days, and growth was monitored until 100 days of age. Body weight was recorded every 10 days. Milk and starter intake were measured to estimate metabolizable energy intake. Health was evaluated by recording the incidence of diarrhea, pneumonia, and mortality. Data were analyzed using Student's t-tests, repeated-measures mixed models, and Pearson's χ^2 tests ($p < 0.05$).

Results: Average daily gain, weight gain, feed conversion, and metabolizable energy intake were similar between treatments ($p > 0.05$). The incidence of diarrhea and pneumonia did not differ, and no mortality was recorded.

Limitations on study/Implications: The study was conducted under controlled management and hygiene conditions, which may limit the extrapolation of results to herds with higher sanitary challenges.

Findings/Conclusions: Electrolyzed water supplementation in the liquid diet of Holstein calves did not affect growth, feed efficiency, or health.

Keywords: Holstein calves, starter feed, growth performance, health.

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INTRODUCTION

Neonatal calves are a fundamental component of dairy production systems and must be reared under conditions that preserve their health and allow them to express their full genetic potential. During the first weeks of life, calves face critical periods in which any health impairment may have long-term consequences on productivity, including



growth and overall efficiency (Cangiano *et al.*, 2020). In this stage, neonatal diarrhea and respiratory diseases are the main causes of morbidity and mortality, often associated with pathogens such as *Escherichia coli*, rotavirus, and *Cryptosporidium parvum*, as well as with deficiencies in management, hygiene, and colostrum supply (Silva *et al.*, 2024).

Different technologies have been developed to reduce the prevalence of neonatal diseases (Sedky *et al.*, 2025). In the pharmaceutical sector, citric acid and its salts are widely used as antioxidants, antimicrobials, and anticoagulants (Ibeagha-Awemu *et al.*, 2025). The use of *Moringa oleifera* for controlling infections caused by microorganisms has also been documented, showing effective antimicrobial activity (Allam *et al.*, 2024). In the case of water, treatment and disinfection are critical for animal rearing. Electrolyzed water (EW), rich in hypochlorous acid, has demonstrated antimicrobial efficacy in sanitizing facilities and equipment, and it is widely applied in countries such as Japan and Italy in the food and dairy industries (Rebezov *et al.*, 2022; Suárez-Zuñiga *et al.*, 2023; Meghwar *et al.*, 2024).

The increasing global demand for dairy products has forced producers to improve the efficiency of production systems (Brkić & Puvača, 2024). Successful calf rearing is essential to ensure productive replacement animals; therefore, achieving healthy development in early life is a priority (Battaglini *et al.*, 2023). Proper nutrition plays a determining role: milk, colostrum, and quality starter feed promote both growth and rumen development. Starter feed fermentation in the rumen produces volatile fatty acids (VFA), particularly butyrate, which stimulate epithelial proliferation and accelerate functional maturity of the rumen (Elizondo-Salazar, 2013; Diao *et al.*, 2019; Palczynski *et al.*, 2020). Inadequate energy intake may delay growth and puberty, and compromise fertility (Wathes *et al.*, 2014).

In this context, it has been hypothesized that EW, in addition to its potential as a disinfectant agent, could contribute to calf performance and health when incorporated into the liquid diet. Therefore, the objective of this study was to evaluate the effect of EW supplementation in the liquid diet on energy intake, growth performance, and health of Holstein calves during the rearing stage.

MATERIALS AND METHODS

Study area

The study was conducted from November 5, 2020, to January 30, 2021, in a dairy farm located in the municipality of Delicias, Chihuahua, Mexico. The region is classified as semi-arid and is located at an altitude of 1170 m above sea level, between parallels 28° 11' N and meridians 105° 28' W (INEGI, 2009).

Colostrum management

Colostrum was collected from primiparous and multiparous Holstein Friesian cows within the first 24 h after calving. Immediately after collection, density was determined using a Brix refractometer (Model-1221, DeltaTrak[®]). Colostrum with $\geq 24.5\%$ Brix (corresponding to 80 mg mL⁻¹ of Ig) was pooled until reaching 15 L (one batch). The colostrum was pasteurized at 60 °C for 60 min in a commercial pasteurizer (Rudder[®]).

After pasteurization, it was packed in vacuum-sealed plastic bags (20×30 cm, 2 L each) and stored at $-20\text{ }^{\circ}\text{C}$ until feeding.

Treatments and liquid feeding

Forty calves were randomly selected and separated from their dams at birth. Calves were individually housed in metal hutches previously cleaned and disinfected. Two treatments were applied: T1=control and T2=5 mL of EW (Supra[®]) per liter of milk. Both treatments were provided until 60 days of age. Colostrum feeding was given in two doses: the first within two hours after birth, and the second between four and seven hours later. Each feeding consisted of 2 L. Each calf represented an experimental unit (n=20 per treatment). During the rearing period, 500 L of pasteurized whole milk were supplied per calf, divided into two daily meals (08:00 and 15:00 h). Fresh water was offered *ad libitum* throughout the study.

Starter feed management

Starter feed (Table 2) was offered *ad libitum* from the first day of life until weaning. It was provided every morning, and additional feed was offered in the afternoon if necessary. Intake was calculated daily as the difference between the amount offered and the refusals. Refusals were weighed each morning using a digital electronic scale (L-EQ 5, Torrey[®]).

Body weight recording

Calf body weight was recorded every 10 days from birth until 100 days of age using a livestock scale (PG-2000, Torrey[®]).

Health records

Calf health was evaluated from birth until 60 days of age by recording cases of diarrhea, pneumonia, and mortality. Diarrhea was diagnosed based on fecal consistency: calves

Table 1. Volume of milk offered to Holstein calves supplemented with EW (Supra[®]).

Trial days	1-7	8-15	16-40	41-56	57-60
Control	6	8	10	8	4
Supra [®]	6	8	10	8	4

Source: own elaboration with results from the RStudio output. Values expressed as liters offered per calf per day.

Table 2. Ingredients of the starter feed used in calf feeding.

Ingredient	Value	%*
Moisture	Max.	13
Crude protein	Min.	21.50
Crude fat	Min.	3.00
Crude fiber	Max.	8.00
Ash	Max.	7.00

Source: own elaboration according to the manufacturer's label.

with normal feces were considered healthy, whereas those with semi-pasty to liquid feces were classified as diarrheic. Respiratory problems were identified in calves showing nasal discharge, tearing, coughing, and body temperature above 39.5 °C; calves without these signs were classified as healthy.

Metabolizable energy calculation

Metabolizable energy (Mcal) intake was estimated considering whole milk and starter feed consumption. For milk, an energy value of 0.69 Mcal/L was used, based on the average total solids analyzed during the study period. For starter feed, an energy value of 2.83 Mcal/kg of dry matter was applied, according to the manufacturer's formulation. Individual energy intake was calculated by multiplying the volume or mass consumed by these factors

Statistical analysis

Productive performance (average daily gain, weight gain, total energy intake, and feed conversion ratio) was analyzed using Student's t-tests for mean comparisons between treatments. The effect of time on body weight was assessed with a repeated-measures mixed model (lme, AR(1) structure, random intercept by calf). The incidence of diarrhea and pneumonia was compared using Pearson's χ^2 test. All analyses were performed at a significance level of $p < 0.05$ using R software (v4.4.3).

RESULTS AND DISCUSSION

Body weight

Calf body weight increased progressively from birth to 100 days of age in both treatments (Figure 1). Growth trends were parallel between groups, with no evident differences in the trajectory of the curves. In general, calves reached body weights above 100 kg at the end of the observation period.

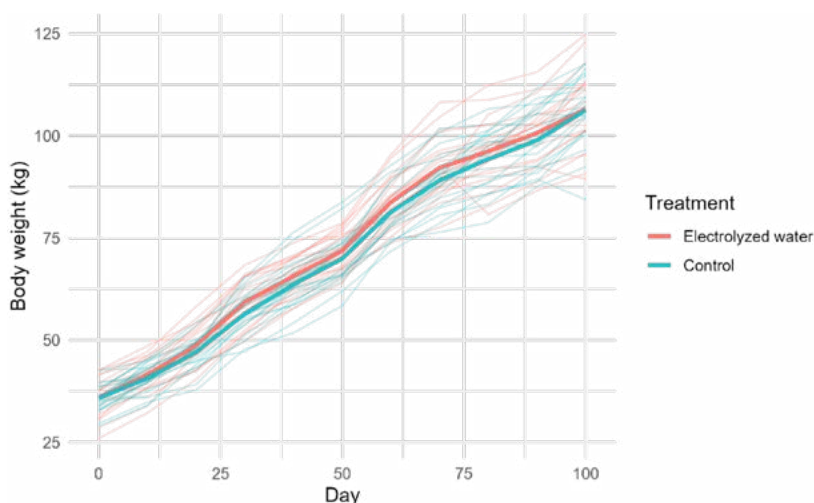


Figure 1. Growth curve of Holstein calves from birth to 100 days of age. Mean values by treatment with standard errors are shown.

At 100 days of age, productive performance was similar between treatments (Table 3). Average daily gain was 0.70 ± 0.10 kg/day, with no statistical difference ($p=0.897$). Total weight gain (~ 70 kg) and metabolizable energy intake (~ 71 Mcal) were also statistically equivalent between groups ($p>0.05$). These findings agree with Elizondo-Salazar (2013), who explained that calf energy requirements are directly related to body weight and gain rate, and that adequate intake allows normal growth patterns without compromising health or structural development. Furthermore, early starter intake promotes rumen development and continuous growth. McCurdy *et al.* (2019) demonstrated that consumption of fermentable feed increases volatile fatty acid production, which stimulates rumen epithelial development and facilitates more efficient growth.

Repeated-measures analysis confirmed that body weight increased significantly with time ($p<0.0001$). However, no overall differences were detected between treatments ($p=0.259$), nor a Treatment \times Day interaction ($p=0.715$), indicating that growth curves were parallel between groups (Table 4). The lack of interaction suggests that EW supplementation did not alter growth trajectories compared to the control under the feeding conditions of this study. In calves, growth curves during lactation are strongly influenced by milk allowance and the transition to starter feed; higher milk intake often reduces starter consumption before weaning but is compensated after weaning, without affecting overall growth when management is appropriate (Welk *et al.*, 2023). Postnatal rumen development depends on starter fermentation (VFA production and epithelial stimulation), which explains the similar weight curves between groups under equal nutritional supply (Jafari *et al.*, 2020). These results do not support our a priori hypothesis that incorporating EW into milk would enhance growth; with equal milk allowance and starter availability, growth is largely driven by energy intake and rumen development, which were comparable between groups.

Table 3. Productive performance of Holstein calves supplemented with electrolyzed water (EW) or control up to 100 days of age.

Variable	EW	Control	p-value
ADG (kg day ⁻¹)	0.706 ± 0.110	0.705 ± 0.087	0.961
Weight gain (kg)	70.6 ± 11.02	70.5 ± 8.71	0.961
Total Mcal intake	71.6 ± 7.41	70.9 ± 7.43	0.752

Source: own elaboration with results from RStudio output. Values expressed as mean \pm SD. ADG=average daily gain (0-100 days, n=20).

Table 4. Repeated-measures variance analysis for body weight of Holstein calves supplemented with EW or control during 100 days.

Source of variation	Num df	Den df	F
Treatment	1	38	1.315
Day	1	398	10,819.169
Treatment \times Day	1	398	0.134

Source: own elaboration with results from RStudio output. Linear mixed model with repeated measures (AR(1) structure, random intercept by calf).

Starter feed intake

During the first 60 days of life, starter feed intake increased progressively in both treatments (Figure 2). The consumption curves were very similar between groups, with no significant differences at any measurement point. At 60 days of age, calves reached average intakes close to 950 g/day.

The absence of differences agrees with reports indicating that, under adequate liquid feeding conditions, starter intake depends more on feed quality and consistency of feeding management than on external factors such as the type of water provided (Gelsing *et al.*, 2016; Zhang *et al.*, 2019; Nikkhah & Alimirzaei, 2022).

Feed conversion

Feed conversion, expressed as metabolizable energy (Mcal) consumed per kilogram of weight gain, was similar between treatments (Table 5). Calves supplemented with EW showed an FCR of $\approx 1.02 \pm 0.10$ Mcal/kg, while the control group reached $\approx 1.01 \pm 0.09$ Mcal/kg, with no statistical differences ($p > 0.05$).

The efficiency observed indicates that both groups transformed the consumed energy into growth with equivalent yields. These results suggest that EW supplementation did not modify overall energy utilization during rearing. Therefore, this suggests limited room for EW to influence nutrient utilization under these feeding conditions. Similar findings have been reported in studies where liquid diet and starter intake, rather than water source, determine feed efficiency in dairy calves (Wickramasinghe *et al.*, 2019; Jafari *et al.*, 2020).

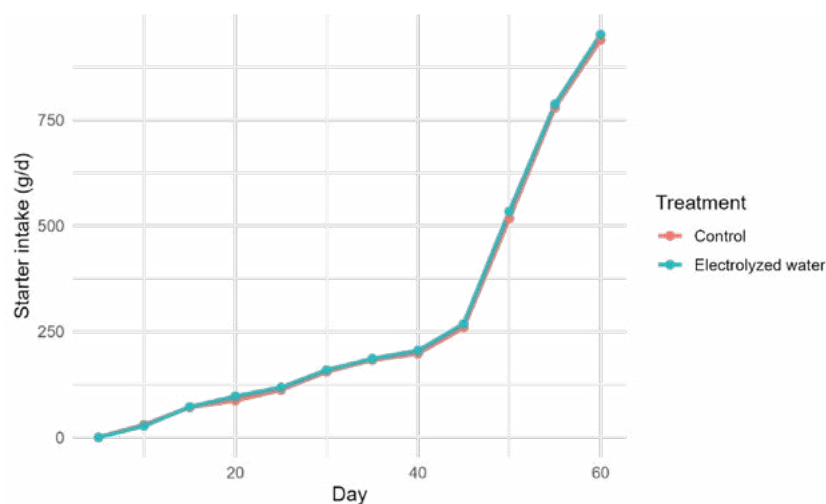


Figure 2. Average starter feed intake (g/day) of Holstein calves supplemented with EW or control during the first 60 days of age.

Table 5. Feed conversion (Mcal kg^{-1} of weight gain) in Holstein calves up to 100 days of age.

Variable	EW	Control	p-value
Feed conversion (Mcal/kg gain)	1.04 ± 0.20	1.04 ± 0.20	1.04 ± 0.20

Source: own elaboration with results from the RStudio output. Values expressed as mean \pm SD. FCR=total metabolizable energy intake (Mcal)/weight gain (kg) from 0-100 days.

Likewise, recent studies emphasize that milk feeding consistency and feed quality are the main determinants of FCR, while external factors such as water type show no clear effects on conversion efficiency (Senevirathne *et al.*, 2018; Wickramasinghe *et al.*, 2019).

Health

Health evaluation up to 60 days of age showed a diarrhea incidence of 30.0% in the EW group and 35.0% in the control group, with no statistical differences ($p=0.736$). Pneumonia occurred in 5.0% of control calves, while no cases were detected in the EW group ($p=0.311$). No mortality was recorded in either treatment (Table 6).

Table 6. Incidence of health problems in Holstein calves up to 60 days of age.

Variable (%)	EW	Control	p-value
Diarrhea	6 (30.0)	7 (35.0)	0.736
Pneumonia	0 (0.0)	1 (5.0)	0.311
Mortality	0 (0.0)	0 (0.0)	NA

Source: own elaboration with results from RStudio output. Data expressed as frequency (percentage), p-value: Pearson's χ^2 test.

These results indicate that EW supplementation did not significantly reduce the incidence of diarrhea or pneumonia, although a favorable trend was observed in the absence of respiratory cases among treated calves. Neonatal diarrhea remains one of the main health problems in calves, mainly associated with *Escherichia coli*, rotavirus, and *Cryptosporidium parvum*, and with management factors such as hygiene, colostrum quality, and stress rather than the type of water provided (Brunauer *et al.*, 2021). Furthermore, improvements in water microbiological quality can contribute to reducing infection pressure, although effects depend on the initial contamination level and herd management practices (Kamal *et al.*, 2024). The absence of mortality in both groups is consistent with reports from systems where adequate colostrum feeding and hygienic management reduce the impact of enteric and respiratory diseases (Keller *et al.*, 2024).

CONCLUSIONS

Electrolyzed water supplementation in the liquid diet of Holstein calves did not significantly affect growth performance, energy intake, or health indicators during the rearing period. Under adequate management conditions, the use of EW can be implemented without negative effects on calf development; however, it does not provide additional benefits in weight gain or disease reduction during the pre-weaning stage.

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REFERENCES

- Allam, S. A., Elnomrosy, S. M., & Mohamed, S. M. (2024). Virulent-MDR-ESBL *E. coli* and *Klebsiella pneumoniae* report from North Sinai calves diarrhea and *in vitro* antimicrobial by *Moringa oleifera*. *BMC Veterinary Research*. 20(1): 259.
- Battaglini, L. M., Miretti, I., Giammarino, M., Rastello, L., Audisio, A., & Renna, M. (2023). Effect of the feeding system on the growth performance of Holstein-Friesian calves in the pre-weaning period. In *Italian Journal of Animal Science*. 22: 202-202.
- Brkić, I., & Puvača, N. (2024). Economic and Ecological Sustainability of Dairy Production. *Journal of Agronomy Technology and Engineering Management*. 7: 1088-1104.
- Brunauer, M., Roch, F. F., & Conrady, B. (2021). Prevalence of worldwide neonatal calf diarrhoea caused by bovine rotavirus in combination with bovine coronavirus, *Escherichia coli* K99 and *Cryptosporidium* spp.: A meta-analysis. *Animals*. 11(4): 1014. <https://doi.org/10.3390/ani11041014>
- Cangiano, L. R., Yohe, T. T., Steele, M. A., & Renaud, D. L. (2020). Invited review: Strategic use of microbial-based probiotics and prebiotics in dairy calf rearing. *Applied Animal Science*. 36(5): 630-651.
- Diao, Q., Zhang, R., & Fu, T. (2019). Review of strategies to promote rumen development in calves. *Animals*. 9(8): 490. <https://doi.org/10.3390/ani9080490>
- Elizondo-Salazar, J. A. (2013). Energy requirements for dairy calves. *Agronomía Mesoamericana*. 24(1): 209-214. <https://doi.org/10.15517/am.v24i1.9801>
- Gelsing, S. L., Heinrichs, A. J., & Jones, C. M. (2016). A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. *Journal of Dairy Science*. 99(8): 6206-6214.
- Ibeagha-Awemu, E. M., Omonijo, F. A., Piché, L., & Vincent, A. T. (2025). Alternatives to antibiotics for sustainable livestock production in the context of the One-Health-Approach: Tackling a common foe. *Frontiers in Veterinary Science*. 12: 1605215.
- Jafari, A., Azarfar, A., Ghorbani, G. R., Mirzaei, M., Khan, M. A., Omid-Mirzaei, H., ... & Ghaffari, M. H. (2020). Effects of physical forms of starter and milk allowance on growth performance, ruminal fermentation, and blood metabolites of Holstein dairy calves. *Journal of Dairy Science*. 103(12): 11300-11313. <https://doi.org/10.3168/jds.2020-18252>
- Kamal, M. A., Khalf, M. A., Ahmed, Z. A., Eljakee, J. A., Alhotan, R. A., Al-Badwi, M. A., ... & Saleh, A. A. (2024). Effect of water quality on causes of calf mortality in cattle-farm-associated epidemics. *Archives Animal Breeding*. 67(1): 25-35.
- Keller, S., Donat, K., Söllner-Donat, S., Wehrend, A., & Klassen, A. (2024). Immediate dam-sourced colostrum provision reduces calf mortality: Management practices and calf mortality in large dairy herds. *Acta Veterinaria Scandinavica*. 66(1): 61.
- McCurdy, D. E., Wilkins, K. R., Hiltz, R. L., Moreland, S., Klanderma, K., & Laarman, A. H. (2019). Effects of supplemental butyrate and weaning on rumen fermentation in Holstein calves. *Journal of Dairy Science*. 102(10): 8874-8882. <https://doi.org/10.3168/jds.2019-16652>
- Meghwar, P., Saeed, S. M. G., Forte, L., Smaoui, S., Khalid, N. I., De Palo, P., & Maggiolino, A. (2024). Electrolyzed Water: A Promising Strategy for Improving Food Quality and Safety of Fruits, Vegetables, and Meat. *Journal of Food Quality*. (1): 3272823.
- Nikkhah, A., & Alimirzaei, M. (2022). Boosting concurrent intakes of milk, solid starter and water: The ultimate preweaning calf management success triangle. *Journal Clinical Research and Reports*. 11(3).
- Palczynski, L. J., Bleach, E. C. L., Brennan, M. L., & Robinson, P. A. (2020). Appropriate dairy calf feeding from birth to weaning: It's an investment for the future. *Animals*. 10(1): 116. <https://doi.org/10.3390/ani10010116>
- Rebezov, M., Rebezov, Y., Khayrullin, R., Okuskhanova, E., & Gorelik, O. (2022). Electrolyzed water and its applications in agriculture and food industry: A review. *Food Science and Technology*. 42: e83620. <https://doi.org/10.1590/fst.83620>
- Sedky, D., Ghazy, A. A., & Abou-Zeina, H. A. (2025). Advances in diagnosis of diseases causing diarrhea in newborn calves. *Veterinary Research Communications*. 49(5): 1-17.
- Senevirathne, N. D., Anderson, J. L., & Rovai, M. (2018). Growth performance and health of dairy calves given water treated with a reverse osmosis system compared with municipal city water. *Journal of Dairy Science*. 101(10): 8890-8901.
- Silva, F. G., Silva, S. R., Pereira, A. M., Cerqueira, J. L., & Conceição, C. (2024). A comprehensive review of bovine colostrum components and selected aspects regarding their impact on neonatal calf physiology. *Animals*. 14(7): 1130.
- Suárez-Zúñiga, O., Contreras-Morales, G. E., Melo-Sabogal, D. V., & Hernández-Pimentel, V. M. (2023). Tendencias recientes en aplicaciones de agua electrolizada: áreas de estudio y perspectivas. *Revista especializada en ciencias químico-biológicas*. 26.

- Wathes, D. C., Pollott, G. E., Johnson, K. F., Richardson, H., & Cooke, J. S. (2014). Heifer fertility and carry-over consequences for lifetime production in dairy and beef cattle. *Animal*. 8(1): 91-104. <https://doi.org/10.1017/S1751731114000755>
- Welk, A., Otten, N. D., & Jensen, M. B. (2023). Invited review: The effect of milk feeding practices on dairy calf behavior, health, and performance —A systematic review. *Journal of Dairy Science*. 106(9): 5853-5879. <https://doi.org/10.3168/jds.2022-22900>
- Wickramasinghe, H. K. J. P., Kramer, A. J., & Appuhamy, J. A. D. R. N. (2019). Drinking water intake of newborn dairy calves and its effects on feed intake, growth performance, health status, and nutrient digestibility. *Journal of Dairy Science*. 102(1): 377-387.
- Zhang, R., Zhang, W. B., Bi, Y. L., Tu, Y., Beckers, Y., Du, H. C., & Diao, Q. Y. (2019). Early feeding regime of waste milk, milk, and milk replacer for calves has different effects on rumen fermentation and the bacterial community. *Animals*. 9(7): 443. <https://doi.org/10.3390/ani9070443>



Sensing Parameters Related to the Cultivation Environment and Hydroponic Solution for Automation Purposes

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ABSTRACT

Objective: Automation in various industrial sectors has proven to be a relevant alternative, enhancing speed and reliability of activities. It reduces task execution time, ensures greater operational efficiency, minimizes operator interventions, cuts production costs, and optimizes the production chain. This work aims to present an engineering solution regarding the sensing of environmental parameters in a hydroponic greenhouse for automation and real-time monitoring through a Human-Machine Interface developed in collaboration with another research project.

Design/methodology/approach: An ATmega 2560 microcontroller on the Arduino Mega 2560 platform, along with sensors for flow, temperature, humidity, luminosity, solution level, and flow presence, served as climate and greenhouse system readers. The open-source ScadaBR software was employed as the supervisory system for online monitoring.

Results: The developed system enabled tracking the state of the hydroponic system through a web interface with graphs illustrating changes in system parameters over time.

Findings/conclusions: The implementation of an automated monitoring and control system in a hydroponic greenhouse demonstrated the potential to improve operational efficiency, data accessibility, and crop management. By integrating low-cost sensors, open-source hardware, and ScadaBR supervisory software, it was possible to monitor temperature, humidity, luminosity, flow rate, and solution level in real time, with responsive actuators triggered when thresholds were exceeded.

Keywords: Real-time simulation, Transmitters, Distributed control, Control systems.

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INTRODUCTION

The automation of production processes has increased the safety and agility of activities in different industrial sectors and can reduce the time it takes to carry out a given task, thus ensuring operational efficiency, and even reducing production costs. In automation, a central processing unit with peripherals, such as a personal computer, programmable logic controller (PLC) or even a microcontroller, is programmed to read sensors and act on the process, when necessary, to stabilize it. In the agricultural field, the automation

of horticultural crops has generated positive advances, which is why it is becoming increasingly widespread in small, medium, and large-scale crops, Barauskas *et al.* (2022); Naveena *et al.* (2024). Linked to this, there is a continuous search by producers for advances in technologies that allow production to be automated, thus generating high quality products with reduced costs and greater profitability, reducing time, and providing freedom to the producer, Untoro and Hidayah (2022).

Automation not only reduces the risk of losing the crop but also makes it possible to improve the quality of products by providing optimum production conditions, allowing autonomy in cultivation and, consequently, human labor to be directed towards activities that require greater intellectual dedication. Hydroponic cultivation has been shown to be an alternative for producers who want to produce products without the application of chemical pesticides, with high added value, close to urban centers and with restricted surface area for cultivation. Other reasons that have encouraged this type of cultivation are the difficulties encountered in conventional production due to various factors, including temperature control, humidity, wind, soil fertility and pest control, making it necessary to implement more advanced technological resources, such as automation, to assist in daily activities, Alselek *et al.* (2022); Untoro and Hidayah (2022).

Maintaining a hydroponic system usually requires the recurring presence of the operator to keep the system in good working order. One way of reducing the presence of the operator is to monitor the operation of the hydroponics system and enabling him to carry out other activities while keeping an eye on the operation of the greenhouse is to monitor it online. Hydroponics is a technique for growing plants without soil, in a water environment, where the roots receive a balanced nutrient solution made up of water and the nutrients needed for the plant to develop properly, Rouphael and Colla (2019). It should also be noted that this is a new technique in Brazil, and the trend is for it to be used more in places where the climate is unstable, hindering agricultural development. In this context, automation is important for reducing losses by controlling multiple variables in crop shelters, Lopes *et al.* (2014); Kurniasari *et al.* (2025).

Despite these advances, most studies in hydroponic automation have focused on specific parameters such as pH and electrical conductivity (EC), often using high-cost commercial systems, Domingues *et al.* (2012); Rouphael & Colla (2019); Untoro and Hidayah (2022). Few works address the integration of multiple low-cost sensors into an open-source supervisory system, especially one like ScadaBR, which enables remote, web-based monitoring with accessibility for small and medium-sized producers. This study contributes by implementing and evaluating such a system under real greenhouse conditions, highlighting its scalability, affordability, and potential for democratizing access to automation technologies in protected cultivation, Kurniasari *et al.* (2025). Unlike most studies that focus only on pH and EC monitoring, this work integrates multiple low-cost sensors into an open-source supervisory platform (ScadaBR), enabling real-time web-based monitoring accessible to small and medium-scale producers. Therefore, the objective of this study was to develop and evaluate a low-cost automation system for hydroponic greenhouses, integrating multiple sensors with the open-source ScadaBR platform. Based on this, it is hypothesized that the integration of low-cost

sensors into an open-source supervisory platform (Arduino Mega 2560 + ScadaBR) can ensure reliable real-time monitoring of hydroponic cultivation parameters (temperature, humidity, luminosity, flow rate, and solution level), providing performance comparable to commercial high-cost systems, while maintaining affordability and accessibility for small and medium-sized producers. This combination of Arduino Mega 2560 and ScadaBR represents a unique contribution, since most previous works relied on commercial controllers or simulations, Penjor *et al.* (2022). By using a fully open-source and low-cost platform, the present study expands automation accessibility for small and medium-scale farmers, democratizing technologies that are often restricted to large-scale production. This practical validation under tropical greenhouse conditions also distinguishes the present work from most studies that remain at a simulation or laboratory stage.

MATERIALS AND METHODS

Figure 1 shows an overview of the applied approach, such as the Arduino Mega2560 development platform and the sensors, to monitor the operation of the greenhouse as well as the actuators, which act to stabilize some variables of the process, the human-computer interface developed with the software (ScadaBR), responsible for developing all the online interfaces used in this work, and the procedures used above each stage. To validate this hypothesis, the proposed system was implemented and tested under real hydroponic greenhouse conditions, monitoring critical environmental and solution parameters.

The project was carried out in a hydroponic greenhouse at the Federal University of Recôncavo da Bahia-UFRB, located in the Trio-Elétrico residence on the Cruz das Almas Campus, and data collection for system evaluation took place between June and August 2019. The municipality is in the Recôncavo Baiano physiographic region, with geographical coordinates of 12° 40' 19" south latitude, 39° 06' 23" west longitude from Greenwich and an average altitude of 220 m. The local climate is subsumed, with an average temperature of 24.1 °C and average rainfall of 1170 mm; Almeida & Ferreira (2022); Fabiano and Borges-Filho (2024). The greenhouse is 48 m in size 2, with a 4 m high roof and 3 m high sides with two roofs. It is covered by a transparent tarpaulin and a 50% screen on all sides of the greenhouse. Inside, there are three growing benches: The first growing level was 1m high at the head, 6m long with a 10% slope at the end and containing 7 70 mm tubes spaced 20 cm apart; the second was 1.70 m high at the head and had a 10% slope at the end with 5 0.70 m × 6 m tubes spaced 30

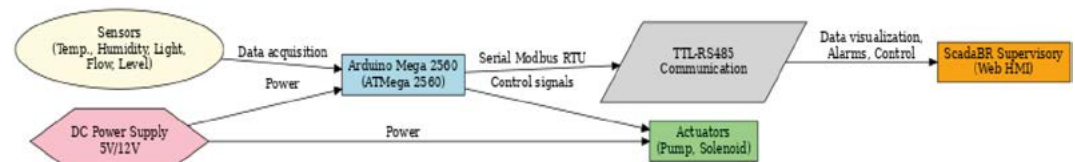


Figure 1. Workflow diagram of the hydroponic monitoring and control system, showing data acquisition from sensors, processing by the Arduino Mega 2560, communication with the ScadaBR supervisory system, and actuation through pump and solenoid.

cm apart. The two levels have the capacity to grow 480 coriander plants. For system automation, the Arduino Mega 2560 platform was adopted, based on the ATmega2560 microcontroller, due to its versatility and multiple input/output ports, which make it suitable for connecting several sensors and actuators simultaneously. The integration between sensors, actuators, the microcontroller, and the supervisory interface is illustrated, Soetedjo and Hendriarianti (2023).

To automate the project, hardware had to be built to monitor the meteorological parameters of the greenhouse's internal environment, such as temperature, humidity, and light. As for the nutrient solution used in hydroponic cultivation, the temperature, reservoir level, flow in the cultivation channels and a solenoid actuator to add water to the reservoir when the level is low were evaluated. For online monitoring, a partnership was made with the work of, where a SCADABR supervisory system was used in his work, which aimed to develop a low-cost supervisory system. All the greenhouse's parameters were monitored using the operation and control system developed in ScadaBR. In addition, it gives the user daily access to all the data recorded and the option of creating alarms if something unexpected (out of the ordinary) happens in the system. It also allows the operator to access it from any electronic device such as a desktop, notebook, tablet, or smartphone, if it has internet access, Almeida and Ferreira (2022); Sulaiman *et al.* (2025).

In order to be able to access the monitoring, it was necessary to keep a fixed computer in the hydroponic greenhouse, connected to the network 24 hours a day, so that it was possible to receive the data from the TTL-RS485 connected to the Arduino board inside the hydroponic greenhouse and transmit it via cable to a USB-RS485 serial converter connected to the computer, through which the operator had access to the data and graphs, which could also be accessed from another location via a telephone with the address and password pre-defined in the system, Almeida and Ferreira (2022); Soetedjo and Hendriarianti (2023).

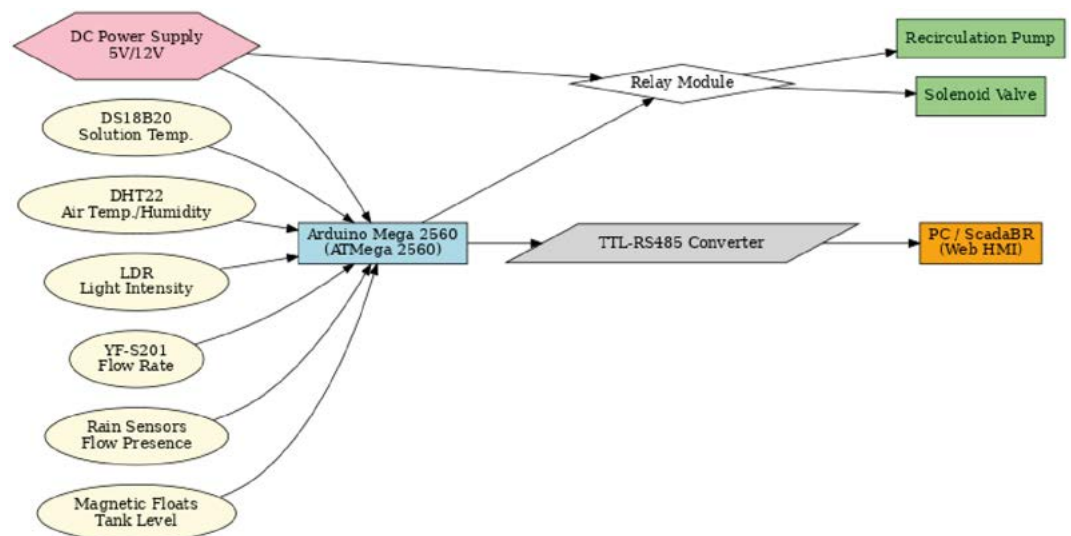


Figure 2. Simplified scheme showing the interconnection between sensors, actuators, and the Arduino Mega 2560 microcontroller, with RS485 communication to the ScadaBR supervisory software.

Sensors for data Collection

Determining which sensors would be used in the system was only possible after studying how hydroponic cultivation works and the main parts needed for operation and monitoring, Resh (2001). After this initial study, a bench with two cultivation levels was separated inside the greenhouse to carry out the project with the following components: two 90° side water level floats magnetic switch type, a DS18B20 temperature sensor, a solenoid valve for water model A147B coil voltage 127v (NC- normally closed), flow sensor water flow 1/2" model Yf-s201-30l min⁻¹, two rain sensors (detector) used as flow sensors, a DHT22 (humidity and temperature), a 5mm photoresistor LDR, a 4-channel 5v relay module, a TTL - RS485, USB- RS485 module, an Arduino Mega 2560 and two 5V DC supplies.

Recirculation tank water level sensor

The sensor installed to monitor the water level is a horizontal magnetic type with a 90° float. This mini float is an instrument used to monitor the water level in a box or similar. The sensor does not report the amount of water in analog form, but whether water is present in a certain position. It can be used in conjunction with another float, which, with the help of a relay, will trigger a solenoid when the water level is lower than the second

Table 1. Sensors and actuators are used in the hydroponic monitoring and control system.

Device	Model/Type	Measurement range	Accuracy	Function in the system
Water level float switch	Magnetic, 90° side type	Presence/absence (binary)	–	Detects high/low nutrient solution levels
Temperature sensor	DS18B20 (digital)	–55 to 125 °C	±0.5 °C	Monitors nutrient solution temperature
Humidity & temp. sensor	DHT22 (digital)	–40 to 125 °C; 0-100% RH	±0.5 °C; ±2-5 %RH	Monitors ambient temperature and humidity
Flow sensor	YF-S201 (Hall effect)	1-30 L/min	±10%	Measures nutrient solution flow rate
Flow presence sensors	Rain sensors (detectors)	Presence/absence (binary)	–	Detects nutrient solutions at each cultivation level
Light sensor	LDR 5 mm photoresistor	Relative light intensity	–	Measures luminosity inside the greenhouse
Solenoid valve	A147B, 127 V (NC)	0.2-8 kgf/cm ² (operating)	–	Automatic refilling of the nutrient tank
Relay module	4-Channel, 5 V	Up to 10 A load	–	Controls solenoid valve and pump
Real-time clock (RTC)	DS1302	Timekeeping functions	–	Provides time/date for logging and scheduling

Authorial

float, as was done in the project, Sulaiman *et al.* (2025). A 220 Ohm resistor was used in the graduated neutral density filter (GND) terminal and float 1 was connected to digital port 03 and float 2 to port 07. The DS18B20 digital sensor was used to monitor nutrient solution temperature (range -55 to 125 °C, accuracy ± 0.5 °C). Flow was measured with a YF-S201 Hall-effect sensor (range 1-30 L/min, $\pm 10\%$ accuracy).

The sensor has 3 wires for connection to the system: red (vcc), black (GND) and yellow (data). An important detail is the 4.7K pull-up resistor between VCC and the data wire. Without it, the sensor will not be detected by the program, and it is connected to digital port 11. The DS18B20 digital sensor was used to monitor nutrient solution temperature (range -55 to 125 °C, accuracy ± 0.5 °C). A normally closed solenoid valve (127 V, 0.2 - 8 kgf/cm²) was used for automatic refilling of the nutrient tank. Flow rate was measured with a YF-S201 Hall-effect sensor (range 1-30 L/min, accuracy $\pm 10\%$). Rain sensors were repurposed as flow detectors to identify nutrient solution presence at each cultivation level. Ambient temperature and humidity were monitored with a DHT22 sensor (range -40 to 125 °C, ± 0.5 °C; 0-100% RH, ± 2 -5%), Sulaiman *et al.* (2025). Light intensity was measured using a 5 mm LDR photoresistor, with resistance inversely proportional to incident light. A 4-channel 5 V relay module was used to control the solenoid and pump, supporting loads up to 10 A and electrically isolating the Arduino from actuators. Communication between Arduino and ScadaBR was established using a TTL to RS-485 converter with Modbus RTU protocol, ensuring reliable long-distance

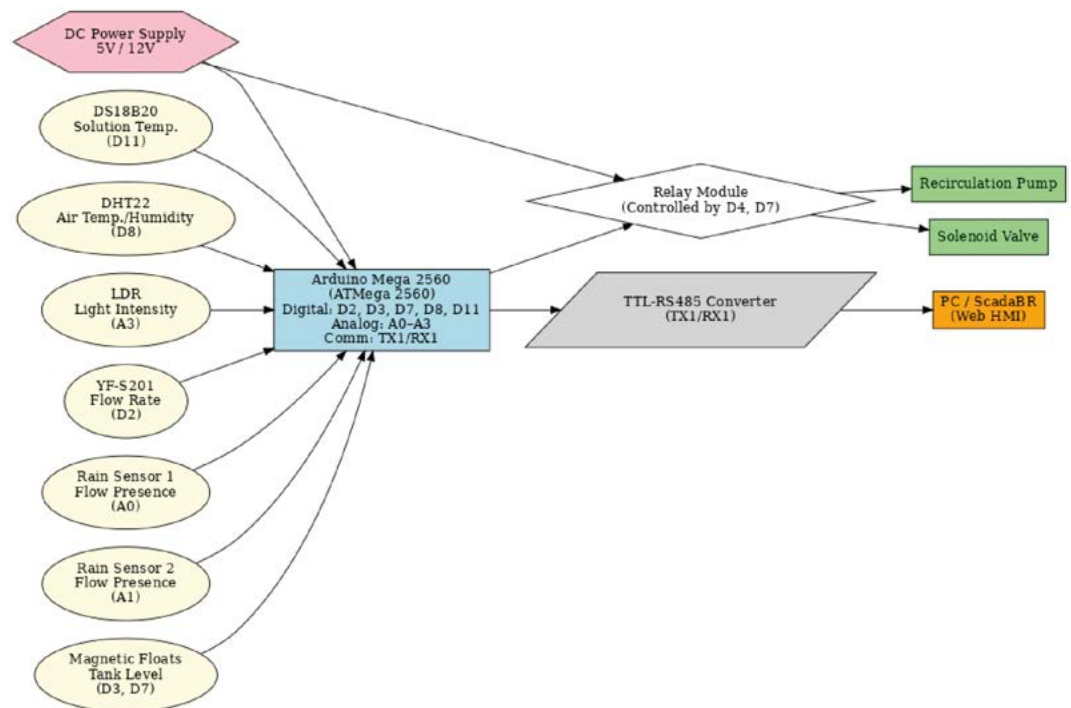


Figure 3. Expanded simplified electrical/signal scheme showing the interconnection between sensors, actuators, Arduino Mega 2560 microcontroller (with main pins), and RS485 communication to the ScadaBR supervisory software.

data transmission. A DS1302 real-time clock (RTC) provided timekeeping functions (hours, date, leap year adjustment) for data logging and scheduling, Kushawaha (2024); Austria *et al.* (2023).

Firmware Development

After assembling the hardware, an open-source code was developed within Arduino's own Integrated Development Environment (IDE). The environment allows you to group together a set of step-by-step information about what you want to do using a computer language, and then these programs, also known as sketches, are downloaded via a Universal Serial Bus (USB) cable to the microcontroller (Arduino Mega2560), which is responsible for reading the electrical pulses coming from the sensors, transforming them into a high-level programming language (C and C++), processing the computer logic and transforming it into graphic data that can be viewed on the screen by the ScadaBR software via the RS-485 module.

Development and Interconnection of the System to Sensors and Actuators

The microcontroller programming stage was completed, containing the implementation of all the sensors and actuators already mentioned, Austria *et al.* (2023). The list with the "Pin out" and the positioning of each sensor and actuator has been identified. In the following sections, the execution (hardware) of each component will be reported step by step, as illustrated by the architecture.

Sensor Installation

During the installation of the sensor modules, the procedures described in this section of the text were of great value, because as these sensors are easy to acquire and low cost, it is necessary to take certain precautions with them. In this study, mechanical protection was created using 75mm PVC pipes to prevent humidity, rain, and direct contact with the sun's rays from damaging the sensors.

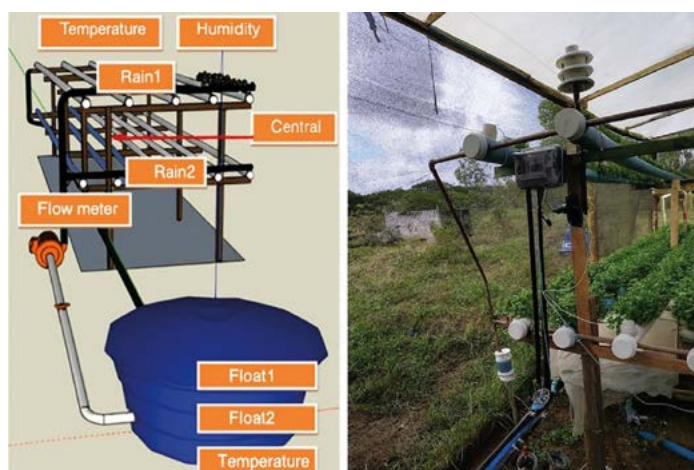


Figure 4. Schematic (left) and real installation (right) showing the location of sensors and actuators in the hydroponic bench system.

Float and DS18B20 (Temperature)

The sensors (float and temperature) were mounted in the solution tank according to the procedure below: In the solution tank with a capacity of 500 l, two level buoys were mounted 20 cm apart to check and maintain the solution level, as this distance keeps the level safe for the solution pump, and a DS18B20 temperature sensor to monitor the temperature of this solution. At first, the cables had to be spliced due to the distance, and they were placed in a 20 mm conduit pipe from the solution box to the control center inside the greenhouse to protect and make the data collected more reliable.

All sensors and actuators were installed in the greenhouse with mechanical protection to ensure measurement reliability and durability. Float switches and the DS18B20 temperature sensor were placed in the nutrient solution tank, with conduits protecting cables from humidity, Azmi (2024). The solenoid valve and flow meter were protected with PVC casings, while rain sensors (used as flow detectors at each cultivation level) and the DHT22 (temperature and humidity) received customized housing to minimize the effect of direct sunlight and rain, Azmi (2024). The LDR was positioned inside the acrylic control box, which provided sufficient protection without interfering with light measurement. These precautions ensured long-term sensor performance under greenhouse conditions.

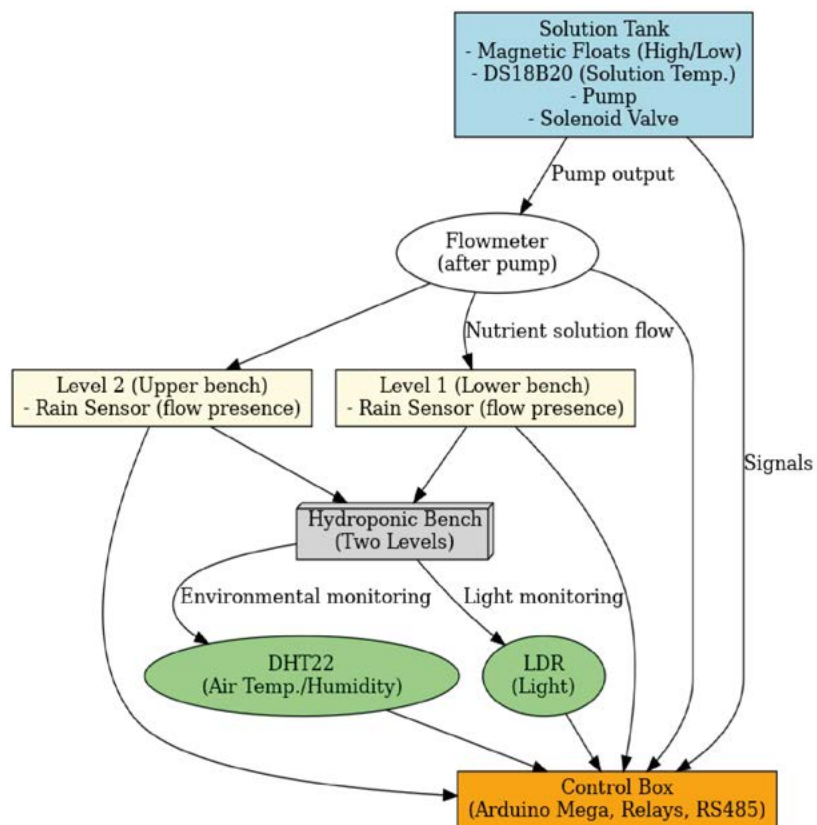


Figure 5. Layout of the hydroponic greenhouse bench showing the installation of sensors (DS18B20, DHT22, LDR, rain sensors, flowmeter, magnetic floats) and actuators (pump and solenoid valve).

TLL-RS-485 Human-Computer Interface

This session will show the screens (interfaces) created in ScadaBR, showing all the greenhouse-operator interaction in real time and the benefits that this tool can provide. The details of installation, software configuration and each screen are described step by step in the work by. Firstly, to access the hydroponic greenhouse's supervisory system, the user must enter their login and password at the "localhost:8080/ScadaBR/" link. In the software (ScadaBR), some screens were created for interaction with the user via the web interface to visualize all the climate parameters with the operation of the hydroponic greenhouse actuators in real time, with values updated every minute. The main screen (Watch List menu) on entering the software contains information on all the variables present in the project. The ScadaBR supervisory system allowed real-time monitoring of climate parameters and actuators, with alarms and data logging accessible via web interface.

Alarm Screen

The alarm was developed to signal to the owner sensor readings that are outside the parameters recommended for growing coriander in hydroponic conditions. Once the alarm detects values outside the recommended range for the plant's development or the hydroponic system's programmed operation, it will send an online signal to the mobile device and light up a Red, Green, Blue (RGB) LED in the central monitoring box located inside the greenhouse, remaining on until the sensors detect the ideal conditions pre-established in the project. As there are several sensors taking readings, only one RGB LED was installed as an alarm for all the sensors, so as not to fill the central box with LEDs by placing one LED for each sensor. Once the RGB LED, which contains three colors, is lit (Blue for the flow and solution presence sensors on both levels, Green for luminosity and humidity, Red for the ambience and solution box temperature), the owner must access the online page to identify in more detail which parameters are outside the recommended range and take corrective action, Nikolov *et al.* (2023).

The parameters used to light the LED were determined as follows: Humidity <30%, luminosity <200, ambience and solution temperature >29 °C and flow meter together with the other two solution presence sensors >15 min without detecting nutrient solution. Any measurement outside these ranges will cause the LED to remain on until it normalizes.

Signal LEDs

Its purpose is to signal adverse situations in the solution box. The green LED will light up when it is full and as the solution level drops, the green LED will turn off and the red LED will light up, signaling that the solution is at a level between the two floats installed in the box. As soon as this level reaches the lower float, the next red LED will turn on, indicating that the solenoid has been triggered to fill the box with water. The two red LEDs will remain on until the water level reaches the upper float to turn them off and the green LED will turn on again, Foster *et al.* (2010); Rajaseger (2023). At this stage, the aim of the project was to monitor all the operating conditions of the hydroponic bench from a long distance, giving the operator more freedom and convenience. To this end, interfaces were created in the software to better meet the project's proposal.

RESULTS AND DISCUSSION

Flow Meter

The average flow rate measured was 4.7 L/min during each pump activation. This value is consistent with recommended flow rates for leafy vegetable hydroponics, typically ranging from 4 to 6 L/min to ensure uniform nutrient distribution, Resh (2001); Mwaniki *et al.* (2024). The agreement with literature supports the hypothesis that low-cost flow sensors can provide reliable measurements comparable to commercial monitoring systems. This parameter is critical in hydroponic systems, as the solution pump acts as the system's heart, ensuring proper flow and pressure for nutrient distribution across the cultivation benches, Kurniasari *et al.* (2025). The pump operated in 15-minute on/off cycles during the day, and every two hours at night, consistent with best practices for energy efficiency and crop physiology.

An important system feature was the alarm, which was triggered (blue LED and web notification) when the pump failed to operate for more than 15 minutes. Prompt operator intervention prevented crop loss, demonstrating the value of automated supervision, Aselek *et al.* (2022); Kurniasari *et al.* (2025).

Flow Presence Sensors

Rain sensors were used as tactile flow sensors at each cultivation level to detect the presence of the nutrient solution, Sail (2024); Kurniasari *et al.* (2025). The system alarm was programmed to activate if no flow was detected for more than 15 minutes, alerting the operator to potential issues such as pressure drops or leaks.

Temperature and Humidity

Ambient temperature inside the greenhouse, measured by the DHT22 sensor, showed minimums of 17 °C at night and maximums between 27 °C and 32 °C during late morning and early afternoon, which are suitable for coriander cultivation. For temperatures above 29 °C, a red LED was triggered both locally and on the web interface, Nikolov *et al.* (2023).

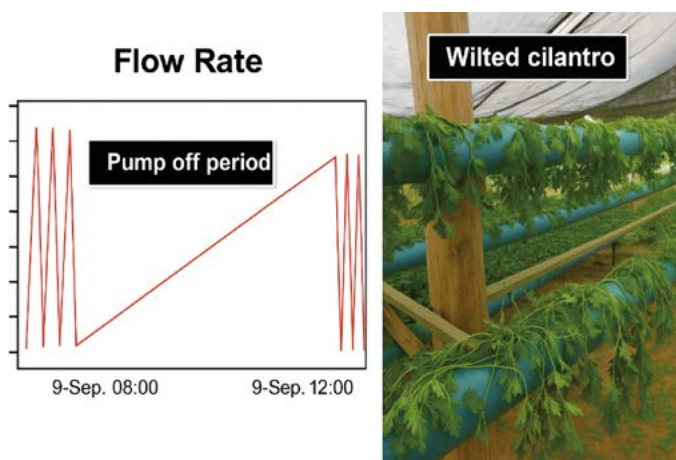


Figure 6. Flow rate monitoring during a pump failure event (left), indicating irrigation interruption, and visual evidence of cilantro wilting as a consequence (right).

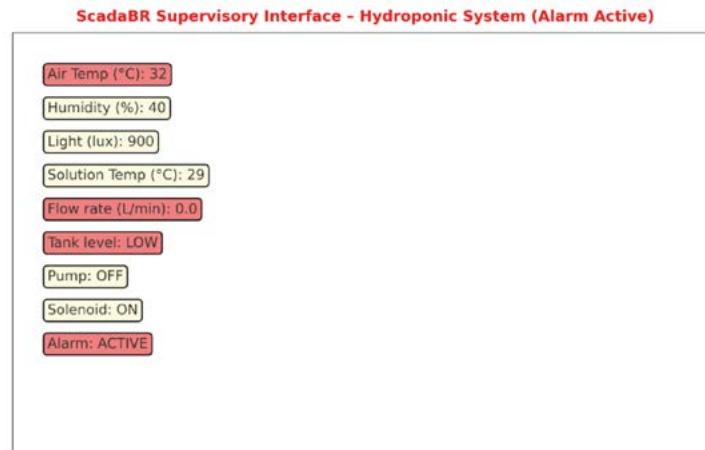


Figure 7. ScadaBR HMI screen during an alarm event, showing critical values (air temperature above 30 °C, zero flow rate, low tank level), with pump OFF and solenoid ON. The alarm status is highlighted as ACTIVE.

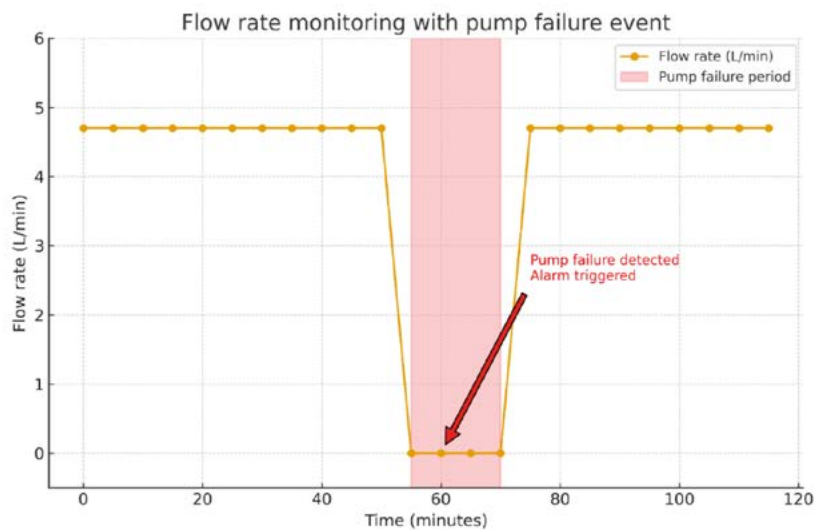


Figure 8. Temporal flow record (L/min) showing a pump failure event between 55 and 70 minutes, during which the alarm was triggered.

Relative humidity remained above 50% throughout the monitoring period, favorable for crop development and reducing excessive evapotranspiration. According to Rouphael and Colla (2019), coriander and similar leafy crops show optimal growth between 18 and 30 °C with relative humidity above 50%. In this study, 92% of recorded values remained within this recommended range, indicating adequate system sensitivity and control. These findings reinforce the reliability of low-cost sensors for environmental monitoring in greenhouses, Kushawaha (2024).

Solution Temperature

The temperature of the nutrient solution, monitored by the DS18B20 sensor, ranged from 16 °C at night to 25 °C during the hottest periods of the day. This stability was

aided by the solution tank being buried 20 cm underground and covered, minimizing direct solar heating.

Solution Level

Two level sensors spaced 20 cm apart in the solution tank enabled reliable detection of high and low water levels. When the solution dropped below the lower float, the system automatically activated the solenoid to refill the tank, maintaining operational safety for the pump and ensuring adequate solution supply, Uddin *et al.* (2022); Mwaniki *et al.* (2024). Nutrient consumption could be calculated based on the number of solenoid activations, as each refill volume was known and logged by the ScadaBR system.

Luminosity

The LDR sensor provided data on light intensity, with highest levels observed between 9:00 and 14:00. This information can support future integration of artificial lighting or shading strategies.

System Performance

The system enabled real-time visualization of all monitored parameters and status of actuators via a web interface, including graphical trends. The alarm system, through RGB LEDs and notifications, efficiently alerted the operator to abnormal conditions, minimizing risks, Mwaniki *et al.* (2024). Despite minor hardware failures (a faulty RTC timer and DHT22 sensor, both replaced), the platform performed robustly, providing continuous automated monitoring at low cost. In addition to qualitative observations, quantitative assessment of system performance indicated that alarms were triggered within an average of 40 seconds after sensor thresholds were exceeded. The overall uptime of the monitoring platform was 97% during the test period, despite minor hardware replacements. These metrics demonstrate that the developed system can achieve operational performance comparable to higher-cost commercial platforms. Compared with previous works that focused on a limited set of parameters such as pH and EC only (Domingues *et al.* (2012); Untoro and Hidayah (2022), the present system integrates six environmental and solution-related variables into a single supervisory platform. This broader coverage improves crop safety, since failures in temperature, luminosity, or solution flow can also be detected in real time. This broader scope of monitoring demonstrates a higher degree of integration than previously reported in literature, highlighting the novelty of combining environmental and solution-related parameters in a unified low-cost platform.

System Reliability and Response

Real-time feedback and alarms significantly reduced risks associated with pump failure or low nutrient solution levels. The operator could act promptly to prevent crop losses. This highlights the importance of redundant sensors and clear alarm signals in critical agricultural automation. While the rapid detection of pump failures and abnormal solution levels provides strong evidence in favor of the hypothesis, occasional hardware malfunctions (*e.g.*, RTC timer, humidity sensor) highlight potential vulnerabilities of relying solely on low-

cost devices. This suggests that redundancy and sensor calibration protocols are necessary to further improve long-term reliability, Domingues *et al.* (2012); Mwaniki *et al.* (2024). Another innovative aspect is that, unlike many IoT-based prototypes reported only under laboratory or simulated conditions, this system was tested under real greenhouse operation with coriander, demonstrating practical feasibility in a tropical environment.

Environmental and Resource Management

Continuous monitoring of temperature, humidity, and solution parameters ensured that the greenhouse environment remained within optimal conditions for coriander growth. Automated actuation based on thresholds (such as activating solenoids or LEDs) minimized the need for manual interventions, as supported by previous research, Costa *et al.* (2012); Domingues *et al.* (2012). The logging of water consumption through solenoid activations enabled precise calculation of nutrient needs, reducing waste and improving sustainability.

Scalability and Adaptability

The open-source and low-cost design proved scalable and adaptable. With minor modifications, the system can integrate additional sensors (pH, EC) or be applied to other agricultural contexts, such as poultry or aquaculture farming. The modularity of the platform and ease of web-based monitoring democratize access to advanced crop automation for small and medium-sized producers, Almeida & Ferreira (2022).

Limitations and Future Prospects

While the system showed robust performance, timely maintenance of sensors is required, and the software could benefit from more advanced analytics and decision-making capabilities (*e.g.*, predictive alerts, automated pH/EC control). Future expansion may include mobile app notifications and integration with machine learning algorithms for predictive crop management, Almeida & Ferreira (2022). This study demonstrates that accessible automation platforms can significantly improve hydroponic crop management, reduce labor requirements, and enhance system reliability. The results provide a solid foundation for broader application and continued technological advancement in sustainable agriculture. Another limitation is that the system was tested with coriander in one greenhouse only; future studies should test scalability in different crops and environments.

CONCLUSIONS

The implementation of an automated monitoring and control system in a hydroponic greenhouse demonstrated the potential to improve operational efficiency, data accessibility, and crop management. By integrating low-cost sensors, open-source hardware (Arduino Mega 2560), and ScadaBR supervisory software, it was possible to monitor temperature, humidity, luminosity, flow rate, and solution level in real time, with responsive actuators triggered when thresholds were exceeded. The system maintained nutrient solution flow at an average of 4.7 L/min, kept solution temperature between 16 and 25 °C, and successfully activated alarms in cases of pump malfunction and low tank levels. These results support

the proposed hypothesis, showing that affordable automation solutions can provide reliable data acquisition and supervision under real greenhouse conditions. In addition, the web-based human-machine interface offered remote access and control, increasing operator autonomy and optimizing labor use. A key contribution of this study lies in demonstrating that a fully open-source, low-cost solution can achieve reliable monitoring and control under real cultivation conditions, offering a practical alternative to high-cost commercial systems. The originality of this study lies in the integration of multiple variables into an open-source supervisory platform tested under real cultivation conditions, providing empirical evidence that such systems can bridge the gap between academic prototypes and practical agricultural adoption.







Although minor hardware issues (*e.g.*, RTC timer and humidity sensor malfunctions) occurred, they did not compromise overall system functionality. However, these events highlight the need for redundancy and calibration protocols when employing low-cost devices. Moreover, since the study was limited to one crop and one greenhouse, the conclusions should be considered within this experimental scope. Overall, the findings indicate the feasibility of using open-source and low-cost supervisory systems for hydroponic automation, particularly for small and medium-sized producers. Future research should include long-term testing, integration of additional parameters such as pH and EC, and evaluation across different crops and environments to strengthen evidence and confirm scalability.

REFERENCES

- Almeida, M. L. S. de., & Ferreira, A. F. (2022). Simulação baseada em Arduino e supervisor ScadaBR de acionamento e monitoramento sob carga de disjuntor de média tensão em subestações blindadas. *Revista Eletrônica TECCEN*, 15(1), 87-93. doi: 10.21727/teccen.v15i1.2613
- Alselek, M., Alcaraz Calero, J. M., Segura Garcia, J., & Wang, Q. (2022). Water IoT Monitoring System for Aquaponics Health and Fishery Applications. *Sensors*, 22(19), 7679. doi: 10.3390/s22197679
- Austria, A. C. H., Fabros, J. S., Sumilang, K. R. G., Bernardino, J., & Doctor, A. C. (2023). Development of IoT Smart Greenhouse System for Hydroponic Gardens.
- Azmi, K. H. M. (2024). The future of sustainable agriculture: A review of IoT and autonomous control in vertical hydroponic farming. *Advances*, 22.
- Barauskas, R., Kriščiūnas, A., Čalnerytė, D., Pilipavičius, P., Fyleris, T., Daniulaitis, V., & Mikalauskis, R. (2022). Approach of AI Based Automatic Climate Control in White Button Mushroom Growing Hall. *Agriculture*, 12(11), 1921. doi: 10.3390/agriculture12111921
- Costa A.G., Chagas J.H., Pinto J.E.B.P., Bertolucci S.K.V. Crescimento vegetativo e produção de óleo essencial de hortelã-pimenta cultivada sob malhas. *Pesquisa Agropecuária Brasileira*, v. 47, n. 4, p. 534-540, 2012. doi: 10.1590/S0100-204X2012000400009
- Domingues D.S., Takahashi H.W., Camara C.A.P., Nixdorf S.L. (2012). Automated system developed to control pH and concentration of nutrient solution evaluated in hydroponic lettuce production. *Computers and electronics in agriculture*, Elsevier, v. 84, p. 53-61. doi: 10.1016/j.compag.2012.02.006
- Fabiano, U. C., & Borges Filho, M. Q. (2024). Proteção social no Brasil: desafios do neoliberalismo e perspectivas de equidade. *Serviço Social & Sociedade*, 150, e024002. doi: 10.1590/0101-6628.378
- Foster, J. T., Allan, G. J., Chan, A. P., Rabinowicz, P. D., Ravel, J., Jackson, P. J., & Keim, P. (2010). Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). *BMC Plant Biology*, 10, 13. doi: 10.1186/1471-5402-10-13
- Kurniasari, A. A., Puspitasari, P. S. D., Perdanasari, L., Mei Yuana, D. B., & Jumiatun (2025). An intelligent fuzzy logic controlled IoT system for efficient hydroponic plant monitoring and automation. *Jurnal ELTIKOM: Jurnal Teknik Elektro, Teknologi Informasi dan Komputer*, 9(1), 47-56. doi: 10.31961/eltikom.v9i1.1475
- Kushawaha, A. (2024). Urban small-scale hydroponics: A compact, smart home-based hydroponics system.

- Lopes, G. E. M., Vieira, H. D., & Partelli, F. L. (2014). Evaluation of castor bean genotypes sown in winter and summer at a low altitude. *Genetics and Molecular Research*, 13(4), 8807-8814. doi: 10.4238/2014.October.27.22
- Mwaniki, G. W., Mansoor, M., Teli, S. H., Piyush, C., & Sabir, H. T. (2024). IoT-based hydroponics system. *International Journal of Electrical and Computer Engineering*, 10(6), 6389-6398. doi: 10.11591/ijece.v10i6.pp6389-6398
- Naveena, A., Saheb, S. N., Mamidi, R., & Murthy, G. L. N. (2024). Automated hydroponic nutrient control system for smart agriculture. *Indonesian Journal of Electrical Engineering and Computer Science*, 33(2), 839-846. doi: 10.11591/ijeecs.v33.i2.pp839-846
- Nikolov, N. V., Atanasov, A. Z., Evstatiev, B. I., Vladut, V. N., & Biris, S.-S. (2023). Design of a small scale hydroponic system for indoor farming of leafy vegetables. *Agriculture*, 13(6), 1191. doi: 10.3390/agriculture13061191
- Penjor, T., Dorji, L., Wangmo, D., Yangzom, K., & Wangchuk, T. (2022). Automation of Hydroponics System using Open-source Hardware and Software with Remote Monitoring and Control. *Bhutanese Journal of Agriculture*, 5(1), 95-108. doi: 10.55925/btagr.22.5108
- Rajaseger, G. (2023). Hydroponics: Current trends in sustainable crop production. *Archives of Current Research International*, 24(11), 317-328.
- Resh, H. M. (2001). Hydroponic food production: A definitive guidebook for the advanced home gardener and the commercial hydroponic grower (6th ed.). CRC Press. doi: 10.1201/9780849331671
- Rouphael, Y. and Colla, G. (2019). Hydroponic solutions for soilless production systems: Issues and opportunities in plant mineral nutrition. *Frontiers in Plant Science*, 10, Article 923. doi: 10.3389/fpls.2019.00923
- Soetedjo, A., & Hendriarianti, E. (2023). Development of an IoT-Based SCADA System for Monitoring of Plant Leaf Temperature and Air and Soil Parameters. *Applied Sciences*, 13(20), 11294. doi: 10.3390/app132011294
- Sulaiman, H., Yusof, A. A., & Mohamed Nor, M. K. (2025). Automated hydroponic nutrient dosing system: A scoping review of pH and electrical conductivity dosing frameworks. *AgriEngineering*, 7(2), 43. doi: 10.3390/agriengineering7020043
- Uddin, S. U., Baig, M. J. A., & Iqbal, M. T. (2022). Design and implementation of an open-source SCADA system for a community solar-powered reverse osmosis system. *Sensors*, 22(24), 9631. doi: 10.3390/s22249631
- Untoro, M. C., & Hidayah, F. R. (2022). IoT based hydroponic plant monitoring and control system to maintain plant fertility. *INTEK: Jurnal Penelitian*, 9(1), 33-41. doi: 10.31963/intek.v9i1.3407

Development and characterization of a liqueur from discarded 'Ataulfo' baby mango (*Mangifera indica* (L.) Wall) as an upcycling option

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ABSTRACT

Objective: To develop and characterize a fruit liqueur by upcycling mango cv. 'baby Ataulfo' (discarded fruit) blended with *Agave cupreata* mezcal.

Design/methodology/approach: Two ripeness stages —physiological (PM) and consumption (CM)— were evaluated. Mango concentrates (PM: 26 °Brix, 1:2 pulp:water; CM: 23 °Brix, 1:3 pulp:water) were blended with mezcal. Physicochemical traits, proximate composition, and volatile compounds were determined by gas chromatography. Shelf life was estimated at 35 °C through first-order color kinetics. A 5-point hedonic test ($n=79$; 18-29 years) assessed sensory traits.

Results: CM showed higher TSS (26.86 °Brix) and flavour index (FI=87.5) than PM (21.23 °Brix; FI=41.10). Both complied with NOM-142-SSA1/SCFI-2014: methanol 200.66-206.41 mg 100 mL⁻¹ AA; acetaldehyde 11.57-12.56; furfural 1.75-2.29; higher alcohols 64.44-65.30; ABV 14.95-16.57% (v/v). LPM exhibited greater color stability ($k=2.60 \times 10^{-3} \text{ day}^{-1}$; $t_{1/2}=266.59$ days) than LMC ($k=4.90 \times 10^{-3}$; $t_{1/2}=141.45$ days). Sensory analysis revealed significant differences ($p<0.05$): LPM scored higher in mouthfeel, sweetness, acidity, aftertaste, and overall impression, while LMC was preferred for colour and odour.

Limitations/implications: The narrow panel age (18-29 years) and accelerated storage conditions limit extrapolation. Nonetheless, the study illustrates a viable upcycling strategy with socio-economic benefits for mango and mezcal producers.

Conclusions: Upcycling 'baby Ataulfo' into a mezcal-based liqueur is feasible, compliant with volatile-compound regulations, and sensorially acceptable. The PM-based formulation (LPM) showed superior stability and consumer acceptance, highlighting its industrial potential.

Keywords: mango liqueur; mezcal; volatile compounds; shelf-life.

INTRODUCTION

The mango cultivar (cv.) 'Ataulfo' is a fruit native to Mexico, recognised for its high pulp content (69%), with the peel representing 19% and the seed representing 8.5% of its total weight. This fruit stands



out for being an excellent source of vitamins A and C, as well as for its high sugar content. Compared to other varieties, ‘*Ataulfo*’ mango has a longer shelf life and is highly appreciated for its sensory characteristics: fleshy pulp, juicy texture, elongated shape and an attractive yellow colour when ripe for consumption (Mendoza-Hernández *et al.*, 2020; Rivas-Robles *et al.*, 2024).

However, one of the main problems associated with this cultivar is the production of small-sized fruits, known as ‘*baby Ataulfo*’ mangoes, whose weight is less than 118 grams. This characteristic renders them unattractive to both domestic and international markets, resulting in economic losses for producers. In addition, the proportion of these small fruits tends to increase with each production cycle (Escalera-Mota *et al.*, 2022; Hernández-Guerrero *et al.*, 2015).

This problem originated in the 1980s. In the state of Chiapas, orchards that previously produced up to 15 tons per hectare reduced their yield to only 5 tons per hectare. In the state of Nayarit, losses ranging between 80% and 90% are reported due to this same incidence. In that entity, adult trees older than 10 years present an incidence of 47%, in contrast to young trees, whose affliction is only 1.7% (Hernández-Guerrero *et al.*, 2015; Gehrke Vélez, 2007). In the state of Guerrero, particularly in the municipalities of the Costa Grande region, all municipalities producing common mangoes have this problem, with an incidence of nearly 45%, which translates into significant losses (García Osuna *et al.*, 2002).

Mezcal is a distilled Mexican alcoholic beverage that has a Denomination of Origin (DO) in nine states of the Republic, including the state of Guerrero, which has this designation in full (López, 2018). This beverage is made from the fermentation of sugars contained in the cooked heads (pineapples) of the agave and is subsequently subjected to a distillation process. However, mezcal faces serious problems, including the clandestine nature of much of its production due to the lack of certification. This situation poses a significant health risk, as many of these beverages originate from processes of dubious origin (Salinas-García *et al.*, 2023). According to data from the Government of Mexico (2025), in the state of Guerrero, more than 90 % of the annual mezcal production is marketed irregularly, with high levels of volatile compounds that exceed the limits allowed by the official standard (NOM-070-SCFI-2016).

The production of liqueurs from tropical fruits acquires commercial relevance when it is proposed as an alternative for the use of fruits at risk of waste, allowing the transformation of these resources into products with higher added value (Leonarski *et al.*, 2021; Pinto *et al.*, 2017). There is a growing trend in the development of new fruit-based liqueurs, driven by the demand for minimally processed products. Today’s consumers are increasingly demanding and show a clear preference for innovative beverages with low preservative content that maintain the sensory characteristics typical of the raw material used in their production (Petrović *et al.*, 2021). Likewise, the market presents an inclination towards products compatible with vegetarian lifestyles, which implies the exclusion of ingredients of animal origin, such as dairy bases, in the formulation of alcoholic beverages (Pinto *et al.*, 2022). Therefore, the objective of the following work is the elaboration and characterization of a liqueur from mango cultivar ‘*baby Ataulfo*’ to mezcal as a strategy to take advantage of a commercially wasted fruit. Its transformation into liquor allows for the rescue of this

resource, giving it added value. Additionally, the sustainable use of agricultural resources is encouraged. This can generate new economic opportunities for mango and mezcal-producing communities.

MATERIALS AND METHODS

Biological material

For the present investigation, fruits of mango cv. '*baby Ataulfo*' at two stages of maturity, physiological (PM) and consumption (CM), were harvested in an orchard in the town of San Jerónimo de Juárez, in the municipality of Benito Juárez, Guerrero, Mexico, with the coordinates 17° 08' 09" north latitude and 100° 28' 08" west longitude and taken to the laboratory. On the other hand, an artisanal mezcal made from *Agave cupreata* (*Agave papalote*), with an alcohol content of 38% by volume, was used. The sample was obtained in the town of Mazatlán, which belongs to the municipality of Chilpancingo de los Bravo, located in the central region of the state of Guerrero. Its geographical coordinates are 17° 37' 34" north latitude and 99° 27' 53" west longitude.

Physical-chemical analysis

The morphology, colour and firmness of the fruits used for the preparation of the liqueurs were evaluated, as well as total soluble solids (TSS), total titratable acidity (TTA), pH and flavour index (FI) according to the methodologies proposed by Maldonado-Astudillo *et al.* (2016) and Maldonado-Astudillo *et al.* (2019).

For the determination of proximate chemical composition (PCA), the official methods proposed by the Association of Official Analytical Chemists (AOAC, 1997) were used. Moisture content was determined using AOAC 925.10, ash content by AOAC 923.03, fat by AOAC 920.39, protein by AOAC 984.13, and carbohydrate was calculated by difference.

Liquor formulation

Two concentrates were developed from mango with the two maturity grades: one for consumption maturity (CM), with a pulp-water ratio of 1:3 and adjusted to 23 °Brix, and another for physiological maturity (PM), with a ratio of 1:2 and 26 °Brix. Both concentrates were supplemented with 10% citric acid and 5% sodium benzoate.

To prepare the final liquor in a one-liter presentation, 580 mL of the corresponding concentrate and 420 mL of *Agave cupreata* mezcal (38% alc. vol.) were mixed, obtaining two products: consumption maturity liquor (LMC) and physiological maturity liquor (LPM).

Determination of alcohol content and volatile components

After formulation, 250 mL of liquor from both formulations was collected at 20 °C and transferred to a distillation flask with 100 mL of water and boiling beads. The system was connected to a steam trap cooler. The distillate was collected in a volumetric flask that had previously contained 20 mL of distilled water, which was kept in an ice bath. On approaching the final volume, distillation was stopped, and the volumetric flask was topped up with water until a temperature of 20 °C was reached. The mixture was homogenized

and transferred to a graduated cylinder, and the alcohol strength was measured with an alcoholmeter.

Samples were analyzed using a gas chromatograph (PerkinElmer model 8600) equipped with an ionization detector. 1 μL of the sample was injected into the chromatograph. The chromatographic column used was 4 m \times 0.32 mm i.d. and was filled with Carbowax 1500 at 15% in Chromosorb W (80-100 mesh). The gas used in the mobile phase is Nitrogen at a flow rate of 20 mL min^{-1} as a carrier; the oven, injector, and detector temperatures were maintained at 93, 150, and 200 $^{\circ}\text{C}$, respectively. Variations resulting from the integration of chromatographic peaks and the width of the data observation window were checked. Once the optimal working conditions were established, each sample was analyzed. The chromatographic peaks used in the analyses corresponded to the following individual standards: acetaldehyde, methyl acetate, ethyl acetate, methanol, ethanol, sec-butanol (2-butanol), n-propanol, iso-butanol (2-methyl-1-propanol), iso-amyl (3-methyl-1-butanol), and n-amyl (1-pentanol). This methodology was proposed by de Oliveira *et al.* (2022) and Reyes Galindo (2020).

Shelf life

The prepared formulations were analyzed for 30 days in a forced convection oven (Luzeren model DHG-9070a, Beijing, China) at a constant temperature of 35 $^{\circ}\text{C}$. During this time, colour changes (brightness) were evaluated using a spectrophotometer (X-Rite Model Ci62 X-Rite Inc., Grandville, MI, USA) by measuring 10 mL of liquor every three days. At the end of this period, reaction rate constants (k) were determined. Finally, using first-order chemical kinetics to calculate the half-life ($t_{\left(\frac{1}{2}\right)}$), the time required for half the initial concentration of a reagent to react, using a mathematical model described below:

$$t_{\left(\frac{1}{2}\right)} = \frac{\ln 2}{k}$$

Sensory analysis

A hedonic scale was applied to 79 affective judges, selected based on the following criteria: age between 18 and 29 years, habitual consumption of alcoholic beverages, liking for mango and absence of allergies or medical conditions that would prevent their participation. The two liquor formulations, LPM (543) and LMC (926), were evaluated using a five-point scale, where five corresponds to “I like it a lot” and 1 to “I dislike it a lot”. The attributes evaluated were colour, odour, mouthfeel, sweetness, acidity, aftertaste and overall impression.

Statistical analysis

Physical, chemical, volatile compounds and sensory analyses were evaluated using a Student's t-test for two groups, with a significance level of $p \leq 0.05$. The results were expressed as the average of three replicates \pm standard deviation, using GraphPad Prism version 9.4 statistical software.

RESULTS

The liqueurs made with mango cv. '*baby Ataulfo*' were well evaluated in sensory tests and presented good physical and chemical properties, thanks to the quality of the fruit and the differences in its maturity at the time of formulation. Despite its smaller size, the mango showed a pulp rich in sugars, good firmness and a balanced flavour between sweetness and acidity. These attributes, together with adequate processing, resulted in liqueurs with pleasant organoleptic profiles. Both formulations (LPM & LMC) retained their sensory qualities during their shelf life, with LPM standing out for its greater stability and overall sensory preference, attributed to its firm texture, greater aromatic intensity and taste balance.

Physical characterization of the fruit and pulp of mango cv. '*baby Ataulfo*'

Table 1 shows the morphometric and biomass analyses of mango cv. '*baby Ataulfo*', where significant differences in DL, weight, pulp proportion and firmness were observed among the samples analyzed. This cultivar, smaller in size compared to its commercial version, maintains a proportionality between DL, TD and total mass. The CM sample shows a lower firmness, which translates into a softer flesh, while the PM sample shows greater resistance to deformation and, therefore, a firmer texture. Significant differences were also observed in flesh colour parameters, such as brightness, chromaticity and hue, with a general tendency towards yellow tones. As maturity advances, these values decrease, leaning towards more orange tones in CM.

Table 2 shows significant differences between the samples analyzed for TTA, pH, TSS and FI. Mango, rich in organic acids, presents a high concentration of citric acid, which contributes to its acidity. In PM, higher TTA and lower pH values are observed. Mango cv. '*baby Ataulfo*' is characterized by its sweetness, which is reflected in its high TSS content.

Regarding the proximal chemical composition of the pulp, significant differences were detected in moisture, ash and carbohydrates. These parameters are key to the nutritional and sensory quality of the fruit. Moisture influences texture, juiciness and palatability; ash content indicates the presence of minerals; and carbohydrate variation directly impacts

Table 1. Physical parameters mango cv. '*baby Ataulfo*'.

Variable	PM	CM	P value
DL (mm)	63.4±1.2	67.6±1.04	0.0004*
TD (mm)	42.82±2.03	41.38±1.6	0.250
Weight (g)	58.6±5.5	70.6±2.1	0.025*
Pulp (g)	28.7±1.81	35.23±1.7	0.010*
Firmness (N)	9.6±0.07	5.7±0.1	<0.0001*
Brightness (L*)	37.20±1.7	31.98±0.5	<0.0001*
Chromaticity	41.12±1.5	39.08±2.0	0.0025*
°Hue	75.12±1.6	68.41±0.8	0.0011*

PM: physiological maturity, CM: consumer maturity, DL: longitudinal diameter, TD: transverse diameter. Average of three repetitions ± standard deviation. A Student's t-test was performed ($p < 0.05$). Significant differences are indicated by (*).

Table 2. Chemical parameters of mango pulp cv. '*baby Ataulfo*'.

Variable	PM	CM	P value
TTA	0.51±0.05	0.30±0.02	0.0025*
pH (-)	3.54±0.03	3.87±0.03	0.0004*
TSS (°Brix)	21.23±0.05	26.86±0.05	<0.0001*
FI (-)	41.10±4.07	87.5±5.74	0.0003*
Moisture (%)	75.68±0.4	77.09±0.3	0.0132*
Ash (%)	0.86±0.09	0.66±0.0	0.0324*
Ethereal extract (%)	0.28±0.01	0.46±0.01	0.3844
Protein (%)	2.77±0.3	3.31±0.2	0.0792
Carbohydrates (%)	20.38±0.3	18.45±0.4	0.0055*

PM: physiological maturity, CM: consumer maturity, TTA: total titratable acidity (% citric acid), pH: the potential of hydrogen, TSS: total soluble solids, FI: flavour index (TSS/TTA). Average of three repetitions ± standard deviation. A Student's t-test was performed ($p < 0.05$). Significant differences are indicated by (*).

flavour, especially sweetness, factors that may depend on the stage of maturity and growing conditions.

Volatile components and shelf life in mango liquor from mango cv. '*baby Ataulfo*'

Table 3 shows significant differences in the concentrations of aldehyde and furfural. The presence of these compounds, along with the higher alcohols, has an important influence on the aromatic and taste profile of the liquor. These volatile compounds provide distinctive sensory characteristics, and their concentration can vary according to various factors, such as fruit ripeness, processing and storage conditions.

Likewise, shelf life was determined by colour kinetics based on the brightness parameter. The rate constant (k) indicates that the LMC formulation exhibits higher degradation, as its k value is 4.90E-03, whereas LPM shows a lower value of 2.60E-03. In terms of half-life, the LPM formulation reaches 266.59 days, compared to 141.45 days for LMC. In comparative terms, LPM has twice the shelf life: eight months versus four months for LMC, counted from the date of manufacture. This period represents the time during

Table 3. Ethanol, methanol, aldehyde, furfural and higher alcohols in mango liqueurs.

Variable	LPM	LMC	P value
Ethanol	16.57±0.0	14.95±0.0	Nd
Methanol	200.66±0.03	206.41±5.7	0.29
Aldehyde	12.56±0.2	11.57±0.04	0.033*
Furfural	2.29±0.02	1.75±0.02	0.001*
Hig. Alc.	65.30±0.6	64.44±1.9	0.7

LPM: liquor physiological maturity, LMC: liquor maturity of consumption. Ethanol (% Alc. Vol.). The results of methanol, aldehyde (acetaldehyde), furfural and Hig. Alc.: higher alcohols are shown in mg 100 mL⁻¹ of anhydrous alcohol. Average of three repetitions ± standard deviation. A Student's t-test was performed ($p < 0.05$). Significant differences are indicated by (*). Nd: not determined.

which the liquor retains its organoleptic properties, such as colour, flavour, odour and texture, as well as its physical and chemical stability, without undergoing alterations or oxidation processes that compromise its suitability for consumption. To maintain product stability, it is advisable to store it in sealed containers, shielded from direct sunlight, kept at ambient temperature, and protected from abrupt temperature fluctuations.

Sensory analysis of mango liqueur cv. ‘*baby Ataulfo*’

The results of the hedonic scale (level of liking) in mango liqueurs are shown in Figure 1. Significant differences were observed between these two samples in the attributes of colour, odour, flavour, aftertaste and overall impression. Sample 543 (LPM) presented the best acceptance in terms of mouthfeel, sweetness, acidity, flavour, and aftertaste and obtained the best overall impression from the judges. Sample 926 (LMC) showed the best level of liking in the attributes of colour and odour attributed to the state of maturity with which it was made.

DISCUSSION

Physical characterization of the fruit and pulp of mango cv. ‘*baby Ataulfo*’

The main problem for mango cv. ‘*Ataulfo*’ is the appearance of the so-called ‘*baby Ataulfo*’, a phenomenon that has been present since the 1980s, affecting the central producing states, including Chiapas, its place of origin (Gehrke Vélez, 2007). In Nayarit, losses reach 80-90% of production, with a trend of increasing each season (Hernández-Guerrero *et al.*, 2015).

Fruit morphological analyses in this study reveal smaller dimensions in DL and TD compared to the values reported by Maldonado-Astudillo *et al.* (2016), who recorded 119 mm for the longitudinal diameter and 80 mm for the transverse diameter for the standard cv. ‘*Ataulfo*’ mangos are grown in the Costa Grande of Guerrero.

According to NOM-188-SCFI-2012, mango cv. ‘*baby Ataulfo*’ should not exceed 118 g in weight, and the fruits analyzed in this study are below that limit. In contrast Maldonado-

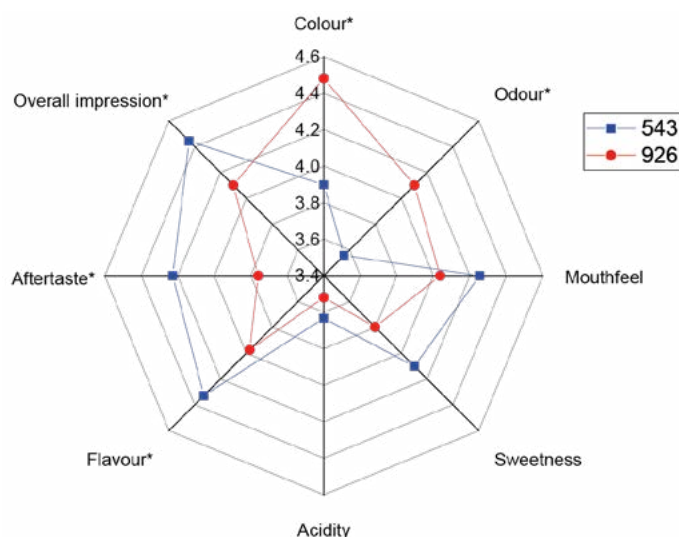


Figure 1. Sensory analysis of mango liqueurs (level of liking). Formulations: LPM (543) and LMC (926).

Astudillo *et al.* (2019) report an average weight of 387.8 g for commercially accepted mangoes, which evidences a considerable difference. In addition, although SIAP (2024) establishes an average of 69% pulp, the results obtained were lower, possibly due to the orchard's edaphic and climatic conditions.

Firmness is a key indicator of mango maturity and quality. As it matures, it decreases due to the enzymatic degradation of polysaccharides, such as starch, pectins, cellulose, and hemicellulose, which are essential for cell wall structure (Chomba *et al.*, 2025; Hor *et al.*, 2020). Previous studies by Maldonado-Astudillo *et al.* (2016, 2019) confirm that mangoes at eating maturity (CM) show lower firmness than those at physiological maturity (PM), which reinforces the relationship between firmness and degree of ripening.

Colour is a fundamental sensory attribute in the perception of food since it directly influences expectations about its flavour (Siller-Cepeda *et al.*, 2009). In this study, fruits at physiological maturity (PM) presented greater luminosity and chromaticity compared to those at consumption maturity (CM); this is in agreement with Diop *et al.* (2021), as mangoes advance in their ripening process, their luminosity decreases, giving rise to more intense, but less bright colour. According to Maldonado-Astudillo *et al.* (2016) mango cv. 'Ataulfo' reaches a hue angle of 88.7°, higher than the values observed in this analysis for 'baby Ataulfo': 75.12° in PM and 68.41° in CM. This suggests that the latter presents a less pure and more opaque yellow hue. As a climacteric fruit, mango continues to ripen after harvest, resulting in a loss of brightness and the development of yellow-orange hues, which is attributed to chlorophyll degradation and increased carotenoids (García Osuna *et al.*, 2002; López García & Trejo Duran, 2015; Wei *et al.*, 2021). These changes make the colour a reliable visual indicator of fruit maturity and quality.

Chemical characterization of mango pulp cv. 'baby Ataulfo'

In the chemical analysis of the pulp, it was observed that fruits at physiological maturity (PM) presented a higher concentration of total titratable acidity (TTA). This parameter decreases as mango ripening progresses, as organic acids are decomposed and transformed into sugars during this process (Dieye *et al.*, 2022). In contrast, the pH was higher in fruits at consumption maturity (CM), which is also associated with the advancement in the maturity stage, as the increase in pH is typical of sweeter and less acidic fruits.

A similar pattern was observed for total soluble solids (TSS), which increased with fruit maturity (Quintero *et al.*, 2013). In our analysis, TSS values in CM were within the range reported by Maldonado-Astudillo *et al.* (2019) for mango cv. 'baby Ataulfo', which ranged from 25.6 to 33.5 °Brix. According to NMX-FF-058-SCFI-2006, the minimum acceptable value for considering a quality mango is 8 °Brix, which is widely exceeded in both maturity stages evaluated. López García & Trejo Duran (2015) explain that the decrease in TTA in advanced ripening stages is due to the degradation of organic acids, which are transformed into sugars, reflected in the increase of TSS. This dynamic directly influences the flavour index (FI), which results from the relationship between sugars and acidity, and has a determining impact on the sensory perceptions of fruit flavour and aroma (Briceño *et al.*, 2005). According to Soto *et al.* (2004), an FI higher

than 50 is perceived as sweet, a value close to 50 is considered balanced and pleasant, while an SI lower than 50 is interpreted as acidic. In this study, with these criteria, CM is considered sweet, and PM is acidic.

The proximal chemical analysis (PCA) of the pulp of mango cv. '*baby Ataulfo*' is characterized by high water content, ranging from 75 to 78%, in agreement with Rivas-Robles *et al.* (2020), who reported 77.2% for discarded mangoes. In contrast Maldonado-Astudillo *et al.* (2016) report higher moisture (82.4%) in standard-size mangoes. Regarding ash and fat, our values were lower than those reported by Rivas-Robles *et al.* (2020), who recorded 2.58 and 0.77%, respectively. However, the protein content was higher than that reported by Pérez-Meza *et al.* (2024) in standard-size mangoes, who reported 0.34% in MF and 0.6% in MC. These differences may be due to maturity, storage conditions and fruit damage. Carbohydrates, after water, were the most abundant component, contributing sweetness to the pulp (Rivas-Robles *et al.*, 2024). In summary, the '*baby Ataulfo*' mango is distinguished by its high moisture and carbohydrates, low-fat content, and moderate levels of protein and ash.

Volatile components and shelf life in mango liquor from mango cv. '*baby Ataulfo*'

The LPM and LMC liquor formulations comply with the limits established by NOM-142-SSA1/SCFI-2014 or volatile compounds: methanol ($\leq 300 \text{ mg } 100 \text{ mL}^{-1}$), aldehydes ($\leq 40 \text{ mg } 100 \text{ mL}^{-1}$), furfural ($\leq 5 \text{ mg } 100 \text{ mL}^{-1}$) and higher alcohols ($\leq 500 \text{ mg } 100 \text{ mL}^{-1}$). Both products fall within these ranges, ensuring regulatory compliance. In comparison, studies such as Giménez-López *et al.* (2025) reported on mango liquors cv. '*Tommy Atkins*' reports $99.76 \text{ mg } 100 \text{ mL}^{-1}$ methanol and $0.3 \text{ mg } 100 \text{ mL}^{-1}$ furfural, but higher levels of acetaldehyde ($99.76 \text{ mg } 100 \text{ mL}^{-1}$) and higher alcohols ($529.25 \text{ mg } 100 \text{ mL}^{-1}$). Other values for higher alcohols reported by de Oliveira *et al.* (2022) were $121.9 \text{ mg } 100 \text{ mL}^{-1}$ in '*Kent*' and $112.3 \text{ mg } 100 \text{ mL}^{-1}$ in '*Alphonso*', all higher than those obtained in this study. In addition, both formulations comply with the range allowed by Mexican alcohol regulations (13.5-55% alc. vol.), placing them within the category of beverages with a medium content (6.1-20.0% alc. vol.). Compliance with these specifications is essential to ensure the quality and safety of the final product.

The reaction rate constant (k) allows estimating the rate of thermal degradation: low values indicate higher stability. According to Loypimai *et al.* (2016), the LPM formulation showed twice the stability of LMC. Feitosa *et al.* (2020) found that, among various fruit liqueurs, guava liqueur was the most stable in terms of colour, while mango liqueur was the least. This degradation is primarily associated with oxidation processes (Patras *et al.*, 2010). The higher stability of LPM is attributed to its lower water content, intermediate alcohol content, and the use of preservatives and pH regulators. In addition, mango pulp provides nutrients with antioxidant properties, such as carotenoids, flavonoids, polyphenols, and tocopherols, whose concentration depends on the variety, maturity, climate, soil, and postharvest handling (Corrales-Bernal *et al.*, 2014). In particular, polyphenols, which are more abundant at physiological maturity, are the predominant antioxidants (Li *et al.*, 2014). An extended shelf life offers logistical and commercial advantages, allowing the product

to be stored for longer without compromising its safety or quality before consumption (Ekmeiro Salvador & Arevalo Vera, 2023).

Sensory analysis of mango liqueurs prepared from cv. ‘baby Ataulfo’

Both the LPM and LMC formulations received high acceptance ratings from sensory panellists, surpassing initial expectations. LPM achieved the highest scores in key sensory attributes including sweetness, acidity, flavour, aftertaste, mouthfeel, and overall impression indicating a well-balanced and appealing sensory profile. Conversely, the LMC formulation was particularly appreciated for its visual and olfactory characteristics, especially colour and aroma, which strongly influence consumers’ initial perceptions, as noted by Macedo *et al.* (2021) and Silva *et al.* (2021).

The dominant fruity aroma of LMC, paired with its colour resembling that of fresh mango, played a critical role in its favourable evaluation. Meanwhile, LPM was distinguished by its equilibrium between sweetness and acidity, which made it especially palatable. This finding supports the conclusions of Pina *et al.* (2018), who emphasize that consumers prefer liqueurs with clearly defined fruit flavours and minimal alcoholic aroma.

Mouthfeel was another decisive factor in sensory preference. LPM was rated higher in this dimension due to its thicker, more viscous consistency, resulting from a greater concentration of mango pulp an observation consistent with Leite *et al.* (2019), who underscore the role of texture in liqueur sensory evaluation.

Both formulations contained a moderate to low level of alcohol, a feature that, as noted by Carolyn *et al.* (2020), improves consumer acceptance by reducing the intensity of ethanol related sensations. Finally, while sensory evaluation is inherently subjective and influenced by individual perception—as emphasized by Urán (2022) the observed pattern consistently reflected a strong preference for both LPM and LMC.

CONCLUSIONS

Mango cv. ‘baby Ataulfo’ has proven to be a suitable raw material for the production of fruit liqueurs, as it complies with the established regulatory parameters. Both formulations evaluated are within the permissible limits of volatile compounds and offer particular sensory properties. The formulation made with physiologically mature fruit was the best evaluated by the judges. This process facilitates the reintegration of otherwise discarded fruit by assigning it functional utility and presenting a novel route for its inclusion in value-added manufacturing chains. Similarly, mezcal—frequently lacking official certification and marketed through informal means—finds in this proposal a practical and structured use scenario. In addition, it incorporates a sustainability component by using an undervalued resource, promoting its responsible use within a more efficient and conscious production chain.

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REFERENCES

- AOAC. (1997). Official methods of analysis (16th ed.). Gaithersburg, MD, USA: AOAC International.
- Briceño, S., Zambrano, J., Materano, W., Quintero, I., & Valera, A. (2005). Calidad de los frutos de mango 'bocado', maduros en la planta y fuera de la planta. *Agronomía Trop.*, 55(4), 461-473.
- Caroliny, J., Almeida, D., & Gherardi, S. (2020). Elaboração, Caracterização Físico-química e Aceitabilidade de Licor de Acerola. *Revista de Engenharias Da Faculdade Salesiana*, 11, 15-19. <https://www.researchgate.net/publication/344818505>
- Chomba, G., Ambuko, J., Onyango, C., & Ouko, J. R. (2025). Effect of different 1-methylcyclopropene formulations and dosing on the ripening profile of Tommy Atkins mango fruits. *Frontiers in Horticulture*, 4. <https://doi.org/10.3389/fhort.2025.1509989>
- Corrales-Bernal, A., Maldonado, M. E., Amparo-Urango, L., Franco, M. C., & Rojano, B. A. (2014). Sugar mango (*Mangifera indica*), variety from Colombia: antioxidant, nutritional and sensorial characteristics. *Rev Chil Nutr.*, 3(3), 312-318.
- de Oliveira, P. N., Alcarde, A. R., Bortoletto, A. M., Abud, A. K. de S., & de Oliveira Júnior, A. M. (2022). Production and characterization of a new mango distilled beverage. *Scientia Agricola*, 79(1), 1-7. <https://doi.org/10.1590/1678-992x-2020-0149>
- Dieye, M., Ndiaye, N. D., Bassama, J., Mertz, C., Bugaud, C., Diatta, P., & Cissé, M. (2022). Storage Time as an Index for Varietal Prediction of Mango Ripening: A Systemic Approach Validated on Five Senegalese Varieties. *Foods*, 11(23). <https://doi.org/10.3390/foods11233759>
- Diop, A., Méot, J. M., Léchaudel, M., Chiroleu, F., Ndiaye, N. D., Mertz, C., Cissé, M., & Chillet, M. (2021). Impact of preharvest and postharvest on color changes during convective drying of mangoes. *Foods*, 10(3), 1-14. <https://doi.org/10.3390/foods10030490>
- Ekmeiro Salvador, J. E., & Arevalo Vera, C. R. (2023). Fecha de caducidad de alimentos perecederos: evaluación del cumplimiento de su normativa legal vigente en grandes supermercados venezolanos. *RESPYN Revista Salud Pública y Nutrición*, 22(2), 12-25. <https://doi.org/10.29105/respyn22.2-724>
- Elena Hernández-Guerrero, S., Balois-Morales, R., & Bello-Lara, J. E. (2015). *Acta Agrícola y Pecuaria*, 1(3): 86-92 Julio-octubre de 2015.
- Escalera-Mota, A. A., Noriega-Cantú, D. H., González-Mateos, R., Hilda Perez-Barraza, M., Álvarez-Bravo, A., Maldonado-Astudillo, Y. I., & Jiménez-Hernández, J. (2022). Mango partenocárpico y su relación con la temperatura Parthenocarpic mango and its relationship with temperature. <https://doi.org/10.30973/aap/2022.8.0081018>
- Feitosa, B. F., Oliveira, E. N. A. de, Oliveira Neto, J. O. de, Farias, A. M. T., & Feitosa, R. M. (2020). Processamento de licores tipo creme como alternativa para o aproveitamento de resíduos agroindustriais. *Revista Brasileira de Gestão Ambiental e Sustentabilidade*, 7(16), 995-1010. [https://doi.org/10.21438/rbgas\(2020\)071633](https://doi.org/10.21438/rbgas(2020)071633)
- García Osuna, A. J., Guzmán Robles, M. L., Tovar Gómez, B., Montes De Oca, M. M., & Vidal Martínez, V. A. (2002). Quality of Ataulfo mango grown in Nayarit, México. *Rev. Fitotec. Mex.*, 25(4), 367-374.
- Gehrke Vélez, M. R. (2007). Vista de Reflexiones sobre problemas de biología reproductiva del mango Ataulfo en el Soconusco, Chiapas. *Tecnología En Marcha*.
- Giménez-López, B. C., Mayuri, A. A. Q., Giménez-Medina, M. A., Morales, C. R., & Sheron, H. B. B. (2025). Production of a distilled mango drink: volatile compounds and heavy metal. *Revista de La Facultad de Agronomía*, 42(1). [https://doi.org/10.47280/RevFacAgron\(LUZ\).v42.n1.VI](https://doi.org/10.47280/RevFacAgron(LUZ).v42.n1.VI)
- Gobierno de México. (2025). Guerrero, y su tradicional bebida el Mezcal, Segundo Lugar en Producción | Representación Agricultura Guerrero | Gobierno | gob.mx. <https://www.gob.mx/agricultura/guerrero/articulos/guerrero-y-su-tradicional-bebida-el-mezcal-segundo-lugar-en-produccion?idiom=es>
- Hor, S., Léchaudel, M., Mith, H., & Bugaud, C. (2020). Fruit density: A reliable indicator of sensory quality for mango. *Scientia Horticulturae*, 272. <https://doi.org/10.1016/j.scienta.2020.109548>
- Leite, P. B., Machado, W. M., Guimarães, A. G., De Carvalho, G. B. M., Magalhães-Guedes, K. T., & Druzian, J. I. (2019). Cocoa's Residual Honey: Physicochemical Characterization and Potential as a Fermentative Substrate by *Saccharomyces cerevisiae* AWRI726. *Scientific World Journal*, 2019. <https://doi.org/10.1155/2019/5698089>
- Leonarski, E., Fernando Dos Santos, D., Kuasnei, M., Lenhani, G. C., Quast, L. B., & Zanella Pinto, V. (2021). Development, Chemical, and Sensory Characterization of Liqueurs from Brazilian Native Fruits. *Journal of Culinary Science and Technology*, 19(3), 214-227. <https://doi.org/10.1080/15428052.2020.1747035>
- Li, L., Wang, S., Chen, J., Xie, J., Wu, H., Zhan, R., & Li, W. (2014). Major antioxidants and in vitro antioxidant capacity of eleven mango (*Mangifera Indica* L.) cultivars. *International Journal of Food Properties*, 17(8), 1872-1887. <https://doi.org/10.1080/10942912.2012.687798>

- López García, S. D., & Trejo Duran, M. (2015). Análisis del estado de madurez de mango (*Mangifera indica*) mediante espectroscopía UV-VIS-NIR. *Jóvenes en la Ciencia*, 1(2), 1206-1210.
- López, J. de J. H. (2018). El mezcal como patrimonio social: de indicaciones geográficas genéricas a denominaciones de origen regionales. Em *Questão*, 404-433. <https://doi.org/10.19132/1808-5245242.404-433>
- Loypimai, P., Moongngarm, A., & Chottanom, P. (2016). Thermal and pH degradation kinetics of anthocyanins in natural food colorant prepared from black rice bran. *Journal of Food Science and Technology*, 53(1), 461-470. <https://doi.org/10.1007/s13197-015-2002-1>
- Macedo, L. L., Vimercati, W. C., Da Silva Araújo, C., Maradini Filho, A. M., Saraiva, S. H., & Quintão Teixeira, L. J. (2021). Banana liqueur made with yacon syrup: Evaluation of stability during maturation. *Brazilian Journal of Food Technology*, 24. <https://doi.org/10.1590/1981-6723.03120>
- Maldonado-Astudillo, Y. I., Cantú-Guzmán, Á., Salazar, R., & Jiménez-Hernández, J. (2019). Análisis de perfil de textura de frutos partenocarpicos de mango cv. 'Ataulfo.' *Revista Mexicana de Ciencias Agrícolas*, 10(5), 1175–1181.
- Maldonado-Astudillo, Y. I., Navarrete-García, H. A., Ortiz-Morales, Ó. D., Jiménez-Hernández, J., Salazar-López, R., Alia-Tejagal, I., & Álvarez-Fitz, P. (2016). Physical, chemical and antioxidant properties of mango varieties grown at the Guerrero coast. *Rev. Fitotec. Mex.*, 39, 207-214.
- Mendoza-Hernández, C., Rosas-Quijano, R., Vázquez, A., & Didiana Gálvez-López, O. (2020). Challenges and controversies of the Ataulfo mango. In *Revista Mexicana Ciencias Agrícolas* (Vol. 11, Issue 7).
- NMX-FF-058-SCFI-2006. Norma Mexicana-FF-058-SCFI-2006 Productos alimenticios no industrializados para consumo humano-fruta fresca-mango (*Mangifera indica* L.)- Especificaciones.
- NOM-070-SCFI-2016. Norma Oficial Mexicana NOM-070-SCFI-2016, Bebidas alcohólicas-Mezcal-Especificaciones. Retrieved June 11, 2025, from https://www.dof.gob.mx/normasOficiales/6437/secco11_C/secco11_C.html
- NOM-142-SSA1/SCFI-2014. Norma Oficial Mexicana NOM-142-SSA1/SCFI-2014, Bebidas alcohólicas. Especificaciones sanitarias. Etiquetado sanitario y comercial. Retrieved June 8, 2025, from https://www.dof.gob.mx/nota_detalle.php?codigo=5386313&fecha=23/03/2015#gsc.tab=0
- NOM-188-SCFI-2012. Norma Oficial Mexicana NOM-188-SCFI-2012, Mango Ataulfo del Soconusco, Chiapas (*Mangifera caesia* Jack ex Wall)-Especificaciones y métodos de prueba. Retrieved May 26, 2025, from https://dof.gob.mx/nota_detalle_popup.php?codigo=5280541
- Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. In *Trends in Food Science and Technology* (Vol. 21, Issue 1, pp. 3-11). <https://doi.org/10.1016/j.tifs.2009.07.004>
- Pérez-Meza, N. B., Ayala-Tafoya, F., Vélez-de la Rocha, R., López-Orona, C. A., Martínez-Gallardo, J. Á., Muy-Rangel, M. D., & San-Martín-Hernández, C. (2024). The Nutritional, Mineral, and Nutraceutical Quality Is Differentially Affected by the Mango Cultivar. *Horticulturae*, 10(10). <https://doi.org/10.3390/horticulturae10101082>
- Petrović, M., Veljović, S., Tomić, N., Zlatanović, S., Tosti, T., Vukosavljević, P., & Gorjanović, S. (2021). Formulation of novel liqueurs from juice industry waste: Consumer acceptance, phenolic profile and preliminary monitoring of antioxidant activity and colour changes during storage. *Food Technology and Biotechnology*, 59(3), 282–294. <https://doi.org/10.17113/ftb.59.03.21.6759>
- Pina, C. A., Silva, P. P. M. da, Spoto, M. H. F., & Da Cruz, S. H. (2018). Desenvolvimento de licor de caju utilizando cajuína e aroma de caju. *Brazilian Journal of Food Research*, 9(3), 82. <https://doi.org/10.3895/rebrapa.v9n3.6882>
- Pinto, T., Vilela, A., & Cosme, F. (2022). Chemical and Sensory Characteristics of Fruit Juice and Fruit Fermented Beverages and Their Consumer Acceptance. *Beverages*, 8(2). <https://doi.org/10.3390/beverages8020033>
- Pinto, V. Z., Rodrigues, V. N., Dos Santos, D. F., Dos Santos, G. H. F., & Bitencourt, T. B. (2017). Market research, elaboration and characterization of pineapple liqueur. *Revista Produção e Desenvolvimento*, 3, 34–42. <http://revistas.cefet-rj.br/index.php/producaoedesenvolvimento>
- Quintero, V., Giraldo, G., Lucas, J., & Vasco, J. (2013). Article in Biotecnología en el Sector Agropecuario y Agroindustrial. *Biotecnología En El Sector Agropecuario y Agroindustrial*, 11(1), 10-18. <https://www.researchgate.net/publication/264976016>
- Reyes Galindo, G. (2020). Mango Wine Production, by an Easy Fermentation and Distillation Procedure, Using the Surplus or Rejected Fruit, Produced in Sinaloa, Mexico. *Journal of Advances in Biology & Biotechnology*, 40-48. <https://doi.org/10.9734/jabb/2020/v23i330147>
- Rivas-Robles, E., Cruz-Espinosa, V., Posada-Toledo, A., Esponda-Pérez, J. A., Caballero-Roque, A., & Vargas-Ortiz, M. A. (2024). Influence of crop age on the production and physicochemical composition of

- Ataulfo mango. *Revista Mexicana de Ciencias Agrícolas*, 15(6). <https://doi.org/10.29312/remexca.v15i6.3237>
- Rivas-Robles, E., Posada-Toledo, A., Vela-Gutiérrez, G., & Vargas-Ortiz, M. A. (2020). Evaluación fisicoquímica y determinación sensorial de frutos descartados de *Mangifera indica* L. variedad Ataulfo en Acapetahua, Chiapas, México. *Revista Iberoamericana de Tecnología Postcosecha*, 21(1), 1-10. <https://www.redalyc.org/articulo.oa?doi.org/https://doi.org/10.22231/asyd.v21i1.1583>
- Salinas-García, E., Alcaraz-Vera, J. V., Durán-Tovar, E. S., & Chávez-Rivera, R. (2023). Alcances y limitaciones de la denominación de origen del mezcal en México. *Agricultura, Sociedad y Desarrollo*, 1(1), 1-3. <https://doi.org/https://doi.org/10.22231/asyd.v21i1.1583>
- SIAP. (2024). Panorama-Agroalimentario-2024. <https://www.gob.mx/agricultura/dgsiap/acciones-y-programas/panorama-agroalimentario-258035>
- Siller-Cepeda, J., Muiy-Rangel, D., Báez-Sañudo, M., Araiza-Lizarde, E., & Ireta-Ojeda, A. (2009). Calidad poscosecha de cultivares de mango de maduración temprana, intermedia y tardía postharvest quality of mango cultivars of early, middle and late seasons. In Artículo Científico *Rev. Fitotec. Mex* (Vol. 32, Issue 1).
- Silva, R. C. da, Filgueira Amorim, A. B., Feitosa, R. M., Oliveira, E. N. A. de, Feitosa, B. F., & Amadeu, L. T. S. (2021). Licor fino de casca de tangerina: processamento e caracterização. *Arquivos Brasileiros de Alimentação*, 2(3), 164-173. <https://doi.org/10.53928/aba.v2i3.1669>
- Soto, E., Avilán, L., Unai, E., Rodríguez, M., & Ruiz, J. (2004). Comportamiento y características de algunos cultivares promisorios de mango. *Agronomía Tropical*, 54(2), 179-201. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0002-192X2004000200004&lng=es&nrm=iso&tlng=es
- Urán, J. I. (2022). Diseño de un Licor de Café y su Evaluación Técnica-Sensorial. *Department of Chemical & Food Engineering*, 1, 1-24.
- Wei, S., Mei, J., & Xie, J. (2021). Effects of edible coating and modified atmosphere technology on the physiology and quality of mangoes after low-temperature transportation at 13 °C in vibration mitigation packaging. *Plants*, 10(11). <https://doi.org/10.3390/plants10112432>

Diversity of by-products used in fish diets in the aquaculture industry: a systematic review

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ABSTRACT

Objective: The purpose of this systematic review was to identify and analyze studies evaluating the use of agro-industrial by-products as alternative ingredients in farmed fish diets, synthesizing the evidence on their impact on productive and nutritional performance, as well as contextualizing the temporal, geographic, and multidisciplinary trends in this line of research.

Methodology: A systematic search of scientific databases (1999-2024) was conducted using terms related to aquaculture and agro-industrial by-products. Of 1,015 articles identified, 32 met the inclusion criteria, which required experimental studies in farmed fish. Information on species, by-product type, inclusion level, and production parameters was extracted from each study.

Results: The temporal analysis showed sustained growth in the number of publications, with a notable increase starting in 2013, reflecting a growing global interest in sustainable alternatives to fishmeal. Geographically, Nigeria accounted for most of the studies, followed by Egypt, Mexico, and the United States. By-products were classified into six categories: vegetables, terrestrial animals, fish, hydrolyzed meals, bioprocessed meals, and microbial biomass. Ingredients such as brewery waste, poultry meal, and fish silage reported favorable results, with $FCR \leq 1.1$ and $SGR \geq 3.5\%$. Bioprocessed meals and microbial additives showed consistent improvements in digestibility and yield.

Limitations/Implications: Practical application depends on regional availability, the presence of antinutritional factors, processing requirements, and producer acceptance, emphasizing the need for comprehensive assessments that include economic, environmental, and health aspects.

Conclusions: The findings demonstrate that various agro-industrial by-products can partially or totally replace fishmeal without compromising production performance, favoring the economic viability and sustainability of aquaculture.

Keywords: sustainability, aquaculture nutrition, feed conversion, alternative ingredients, digestibility.

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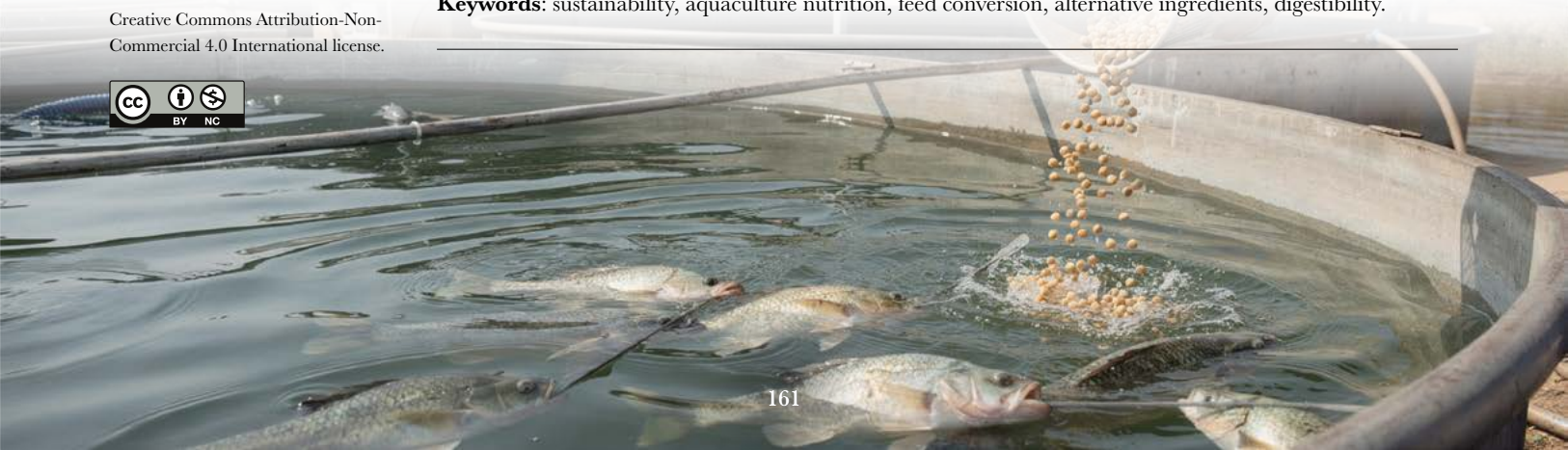
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INTRODUCTION

Aquaculture has become a fundamental pillar of global food security, offering a sustainable alternative to the challenges posed by the overexploitation of wild fish stocks and the impacts of climate change [1]. Over the last three decades, it has established itself as the main driver of growth in fisheries and aquaculture production, with an average annual increase of 5.0% between 2000 and 2021. This upward trend is expected to continue in the coming decades, mainly due to advances in nutrition, management, reproduction, and technological innovation in farmed species, all of which contribute to their success and sustainability [2,3]. Nevertheless, aquaculture remains heavily dependent on unsustainable resources. For more than 50 years, the industry has relied on fishmeal (FM), a highly valued ingredient due to its exceptional nutritional profile. FM contains 60-72% highly digestible protein with a complete essential amino acid profile (notably lysine and methionine), 5-12% lipids rich in omega-3 fatty acids (EPA and DHA), 3,000-4,000 kcal/kg of metabolizable energy, and a wide range of vitamins and minerals, including bioavailable phosphorus critical for bone development and energy metabolism [4,5]. Although FM is highly palatable and digestible (85-95%), its use raises sustainability concerns due to reliance on small pelagic fisheries and high production costs [6]. In response, the aquaculture sector has intensified the search for innovative alternatives that ensure nutritional quality while reducing dependence on FM. Agro-industrial by-products have emerged as a promising option, allowing for optimized raw material use and promoting circular economy practices [7]. By-products are defined as whole bodies or parts of plant and/or animal origin derived from food production and processing for human consumption. Their generation is inevitable and difficult to modify without affecting final product quality [8,9]. Globally, more than 1.3 billion tonnes of by-products are produced annually, equivalent to 13.8% of all food production [10]. This enormous volume has profound environmental, economic, and social implications [11]. If not properly managed, it not only increases disposal costs but can also generate aesthetic problems and serious public health risks. In aquaculture, their incorporation into formulated diets has been proposed as a sustainable and cost-effective alternative [8]. Several sources of animal protein have been evaluated, including by-products from the poultry and swine industries, aquaculture processing residues, and agricultural by-products [12,13]. Such approaches contribute to reducing waste, recycling raw materials, and improving the sustainability of feed production. Therefore, the main objective of this study was to conduct a systematic review of the use of agro-industrial by-products as alternative protein sources in diets for farmed fish, to identify the types of by-products used, analyze their effects on production performance, and compare their effectiveness with diets based on fishmeal.

MATERIALS AND METHODS

A systematic review of the scientific literature was conducted using the Scopus and Web of Science databases, covering the period from 1999 to 2024. A combined search string was applied in English and Spanish: (“By-products” OR “Subproductos”) AND (“Aquaculture” OR “Acuicultura”). Only original research articles evaluating the use of

agro-industrial by-products as ingredients in fish diets were included. Reviews, editorials, book chapters, conference proceedings, duplicate records, and studies assessing alternative ingredients not derived from by-products were excluded. The selection process was carried out in three stages. First, titles, abstracts, and keywords were screened, identifying 491 potentially eligible publications. In the second stage, studies were excluded if the experimental organisms were not fish or if diets contained more than four different by-products. Finally, full-text articles were assessed, and only those reporting at least four of the following five performance parameters were retained: initial weight, final weight, specific growth rate (SGR), feed conversion ratio (FCR), and survival. Reporting of the standard error was required as a criterion for statistical validity.

Data from the selected studies were compiled in an Excel spreadsheet, including type of by-product used as an alternative protein source, fish species evaluated, inclusion level (%), experimental duration (days), feeding frequency (times/day), and production performance outcomes.

RESULTS AND DISCUSSION

A total of 1,015 articles were identified through the selected databases, with no additional records retrieved from other sources. After applying all eligibility criteria, 32 articles were included in the systematic review (Figure 1). Figure 2 presents the distribution of publications across scientific journals. Research on the use of agro-industrial by-products as alternative ingredients in fish diets has been published in a wide variety of specialized journals, reflecting the technical, productive, and multidisciplinary scope of the field. Aquaculture was the leading journal, with four publications, followed by the Journal of the World Aquaculture Society with three. Aquaculture Research, Global Advanced Research Journal of Agricultural Science, and the Journal of Applied Aquaculture each published two studies. The remaining journals contributed a single article, illustrating thematic dispersion from aquaculture-focused outlets to those dedicated to animal science, physiology, or agricultural sciences. The temporal evolution of publications is shown in Figure 3, which depicts both annual (blue) and cumulative (red) outputs between 1999 and 2024. Overall, there was sustained growth in cumulative publications, although the annual output remained relatively low and variable. During the first decade (1999-2009), production was sporadic, with no more than two articles per year and several years without records. From 2013 onwards, publication became more regular, with a modest increase between 2013 and 2015 (up to three studies per year). The most pronounced cumulative growth occurred between 2015 and 2022, when more than 60% of all identified studies were published, suggesting a growing research interest in sustainable aquaculture diets. The year 2020 marked a peak with three publications, followed by stabilization in subsequent years. In 2024, output declined to a single publication, although the cumulative total reached 32 articles. Figure 4 shows the geographical distribution of publications. While there was moderate global dispersion, most studies originated from developing countries with strong aquaculture activity and availability of agro-industrial by-products. Nigeria led with five publications, highlighting the emphasis on local by-products to enhance aquaculture sustainability.

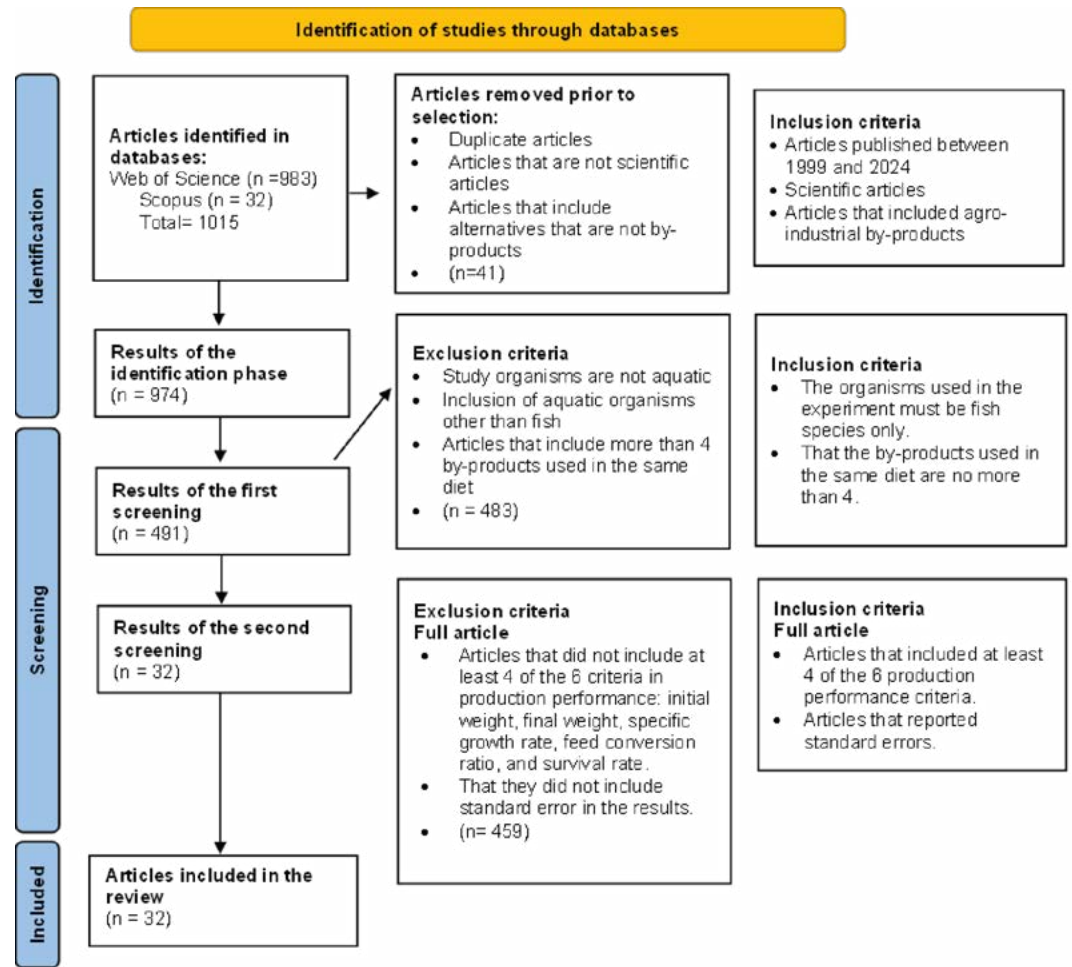


Figure 1. Flow chart of the study selection process for systematic review of animal protein sources as a substitute for fishmeal in aquaculture diets.

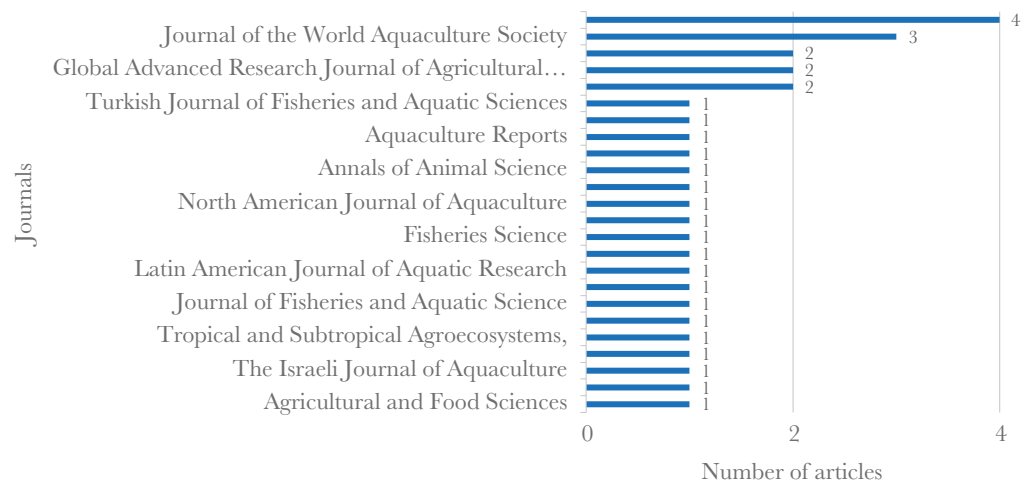


Figure 2. Scientific journals that have published research on the use of agro-industrial by-products as alternative ingredients in fish diets.

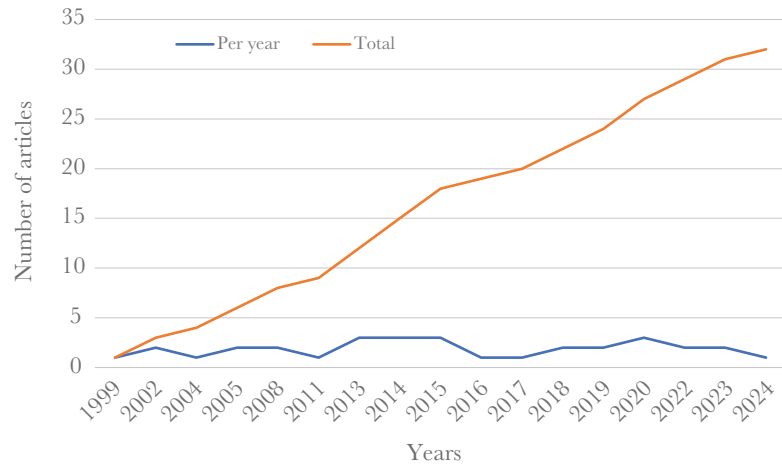


Figure 3. Annual (blue) and cumulative (red) evolution of the number of publications between 1999 and 2024 on the use of by-products in fish diets, compared to fishmeal-based diets.

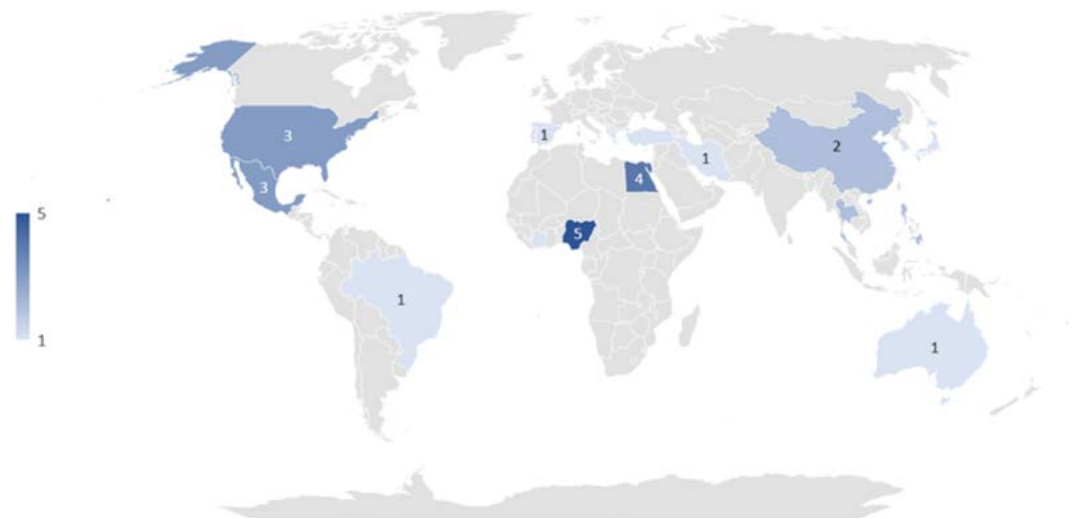


Figure 4. Number of articles and countries that publish most on this topic.

Egypt followed with four studies, while Mexico and the United States each contributed three, reflecting North America’s active role in evaluating protein alternatives. China, despite its global leadership in aquaculture production, contributed only two studies, which is nevertheless notable. Other countries, including Brazil, the Philippines, Iran, Spain, and Australia, published one article each, demonstrating a growing but still incipient global diversification of research efforts. This distribution also reflects that the development of alternative ingredients depends largely on the availability of local resources and the production models implemented in each country. Figure 5 presents a word cloud of the most frequent terms found in the studies included in this review, with “diet” and “protein” being the most predominant.

Table 1.

Protein sources that replace fishmeal	Fish species	Optimal level (%)	Duration of experiment (days)	Feeding frequency (times/day)	Initial weight (g)	Results for recommended levels				Citation
						Final weight (g)	Survival (%)	Specific growth rate (%)	Feed conversion ratio	
Vegetable by-products										
Corn bran	<i>Sarotherodon melanoheron</i>	NR	180	1	1.2±0.0	17.2±1.8	59	NR	7.8±0.6	[20]
Brewery waste	<i>Oreochromis niloticus</i>	5	70	NR	23.8±0.9	76.9±2.3	100	NR	1.9±0.0	[15]
Peanut shell (<i>Arachis hypogaea</i> L.)	<i>Oreochromis niloticus</i>	20	56	2	4.2±0.2	16.2±0.8	90	2.3±0.1	2.3±0.2	[21]
Lemon peel (<i>Citrus lemon</i>)	<i>Oreochromis niloticus</i>	1	45	2	28.9±0.3	31.7±1.3	72	0.2±0.0	19.6±4.6	[14]
By-products of <i>Gracilaria</i> sp.	<i>Sparus aurata</i>	2.5	59	2	10.2±0.2	27.83±0.6	NR	1.6±0.0	1.0±0.0	[22]
Wine by-products	<i>Liza aurata</i>	10	42	5	65.4±0.0	87.7±1.1	NR	1.0±0.0	NR	[23]
Land animal by-products										
Poultry by-product meal	<i>Scophthalmus macoticus</i>	2	60	2	18.0±0.0	29.3±0.7	100	0.8±0.0	0.9±0.0	[24]
Poultry by-product meal	<i>Morone chrysops</i> × <i>saxatilis</i>	1	246	1	5.6	499±0.0	81	1.8±0.0	2.5±0.0	[25]
Poultry by-product meal	<i>Lutjanus guttatus</i>	25	84	3	11.0±0.0	36.1±2.5	100	1.4±0.0	1.2±0.0	[26]
Poultry by-product meal	<i>Oreochromis niloticus</i>	10	120	2	1.5±0.0	54.3±1.5	NR	2.9±0.3	1.3±0.1	[27]
Poultry by-product meal	<i>Dicentrarchus labrax</i>	6	70	3	0.7±0.0	8.2±0.1	94	3.5±0.0	2.2±0.1	[28]
Poultry by-product meal	<i>Centropristis striata</i>	8	56	2	1.2±0.0	15.9±0.3	100	2.4±0.0	1.0±0.0	[29]
Poultry by-product meal	<i>Tilapia obsoletus</i>	7	56	2	17.1±1.83	57.9±2.7	95	2.19±0.1	0.9±0.0	[30]
Feather meal	<i>Clarias gariepinus</i>	20	30	2	NR	NR	88	7.7±0.3	±	[31]
Feather meal	<i>Oncorhynchus mykiss</i>	25	72	2	569.8±9.5	1299.0±91.6	100	1	0.87±0.0	[32]
Blood meal	<i>Clarias gariepinus</i>	5	86	2	8.8±2.9	66.5±13.4	100	1.0±0.0	0.8±0.1	[33]
Meat and bone meal	<i>Ophiocephalus argus</i>	2	70	3	12.1±0.0	138.6±2.3	94	3.4	1.24±0.0	[34]
Meat and blood meal	<i>Epinephelus coioides</i>	8	60	2	6.1±0.5	NR	99	2.9±0.3	1.04±0.0	[35]
By-products of the aquaculture industry										
Silage of <i>Pterygoplichthys multiradiatus</i>	<i>Oreochromis niloticus</i>	15	50	3	1.5±0.7	11.4±1.4	98	4.0±0.2	1.7±0.1	[13]

Table 1. Continues...

Protein sources that replace fishmeal	Fish species	Optimal level (%)	Duration of experiment (days)	Feeding frequency (times/day)	Initial weight (g)	Results for recommended levels				Citation
						Final weight (g)	Survival (%)	Specific growth rate (%)	Feed conversion ratio	
Silage of <i>Pseudophrynosoma</i> sp.	<i>Oreochromis niloticus</i>	12	30	4	4.2±0.1	NR	10	NR	1.3±0.1	[36]
Fish waste silage	Red tilapia (<i>Oreochromis mossambicus</i> × <i>Oreochromis niloticus</i> × <i>Oreochromis aureus</i>)	50	84	NR	2.1±0.0	28.0	NR	3.0±0.1	1.3±0.1	[16]
Tuna by-product hydrolysate	<i>Pagrus major</i>	50	56	2	0.8±0.1	28.5±0.3	95	NR	1.1±0.0	[37]
Shad by-product hydrolysate	<i>Epinephelus fuscoguttatus</i>	15	56	2	2.9±0.2	31.2±1.4	100	NR	1.4±0.0	[38]
Lophius vomerinus protein hydrolysate	<i>Clarias gariepinus</i>	2	70	3	0.9±0.0	32.0±0.3	10	5.0±0.0	0.8±0.0	[39]
Stickwater	<i>Epinephelus fuscoguttatus</i> × <i>Epinephelus lanceolatus</i>	20	56	4	5 g	672	100	NR	0.802	[40]
Flour mix										
Poultry by-product meal and soybean meal	<i>Morone chrysops</i> × <i>saxatilis</i>	10	56	2	15	71.9±4.0	100	2.8±0.1	2.1±0.1	[41]
Cottonseed meal, soybean meal and animal by-product mixture	<i>Oncorhynchus mykiss</i>	100	112	3	0.9±0.0	NR	NR	2.3±0.0	0.9±0.0	[42]
Tuna by-product meal, poultry by-product meal and meat meal	<i>Paralichthys olivaceus</i>	50	56	2	18.0±0.0	91.6±0.2	100	2.9±0.0	NR	[43]
Bioprocessed flours										
Bioprocessed poultry by-product meal with <i>Sacharomyces cerevisiae</i> and <i>Lactobacillus casei</i>	<i>Lates calcarifer</i>	75	42	NR	3.7±0.1	33.7±1.2	86	5.1±0.0	1.1±0.0	[44]
Fermented poultry by-product meal with <i>Sacharomyces cerevisiae</i>	<i>Cyprinus carpio</i>	20	90	2	4.9±0.0	14.5±0.1	98	1.8±0.0	1.5±0.0	[45]
Microbial biomass										
<i>Rubrivivax gelatinosa</i>	<i>Oreochromis niloticus</i>	0.5	72	3	27.4±1.3	244.0±10.6	NR	NR	1.1±0.0	[19]

Other animal-derived meals, including blood meal, meat and bone meal, and feather meal, also demonstrated favorable results, characterized by low FCA values (up to 0.8) and high TCE (up to 3.5%).

This systematic review demonstrates the growing interest and potential of using by-products as alternative ingredients in aquaculture diets, reflecting both economic and environmental pressures to reduce reliance on fishmeal, one of the most expensive and environmentally sensitive inputs in aquaculture [4]. The increasing number of publications since 2013, particularly between 2015 and 2022, highlights a rising awareness among researchers and producers of the need to adopt sustainable practices in line with global circular economy goals. The geographical distribution of studies, with notable contributions from developing countries such as Nigeria and Egypt, underscores the role of locally available by-products in reducing production costs and supporting emerging aquaculture systems. From a nutritional perspective, the efficacy of by-products is largely determined by their origin, composition, processing method, and inclusion level [12].

Land animal by-products, particularly poultry by-product meal, consistently demonstrate high efficiency and compatibility with the dietary requirements of carnivorous and omnivorous fish, even at complete replacement levels [3]. However, their widespread use may be constrained by regional availability, regulatory restrictions, or competition with other livestock industries. Additionally, although the use of animal by-products supports circular economy objectives, the environmental benefits may be limited if the source originates from intensive poultry or pig production systems [1]. Vegetable by-products offer advantages in terms of cost, accessibility, and environmental perception, but their high fiber content, anti-nutritional factors, and limited essential amino acid profile can restrict their use [5]. The poor performance of lemon peel, with an extremely high FCR (19.6), illustrates the risks of incorporating ingredients without adequate evaluation of digestibility, toxicity, or palatability [14].

Conversely, ingredients such as brewer's waste demonstrate potential if inclusion levels respect the physiological limits for protein utilization in fish [15]. Fishery by-products, including silages and hydrolyzed meals, are highly compatible with fish digestive physiology due to their provision of readily digestible peptides [16]. These results reinforce the value of post-harvest processing for enhancing the utility of by-products, particularly in regions with active fisheries. Nevertheless, logistical challenges such as cold storage requirements, microbiological stability, and producer acceptance remain barriers to large-scale adoption [17]. Emerging strategies, such as bioprocessed flours and microbial biomass, show promise in improving digestibility, functional properties, and even nutraceutical value. Fermentation with beneficial microorganisms enhances growth and feed conversion efficiency, sometimes exceeding the performance of traditional fishmeal-based diets [18]. Moreover, bacterial biomass, such as *Rubrivivax gelatinosa*, offers the potential to deliver bioactive compounds with immunostimulatory or antioxidant effects. However, these approaches require further validation under commercial conditions and comprehensive cost-benefit analyses before they can be widely implemented [19]. Overall, the evidence suggests that a diversified approach combining animal, plant, fishery, and bioprocessed by-products may offer the most viable strategy for sustainable aquaculture, balancing

nutritional adequacy, economic feasibility, and environmental responsibility. Future research should focus on optimizing inclusion levels, processing techniques, and functional benefits while addressing logistical and regulatory constraints to facilitate the practical adoption of these alternative ingredients.

CONCLUSION

In conclusion, the findings highlight a clear shift towards a more diversified and sustainable aquaculture sector using by-products that have been underutilized in recent decades. The successful adoption of these alternative ingredients will depend on achieving a balance between technical feasibility, cost-effectiveness, supply stability, and market acceptance. Advancing processing technologies, optimizing diet formulation, and conducting comprehensive evaluations that consider not only production performance but also environmental, health, and economic aspects will be crucial to fully realize the potential of by-products in sustainable aquaculture.

REFERENCES

- [1] R.L. Naylor, R.W. Hardy, A.H. Buschmann, S.R. Bush, L. Cao, D.H. Klingler, D.C. Little, J. Lubchenco, S.E. Shumway, M. Troell, A 20-year retrospective review of global aquaculture, *Nature* 591(2021) 551-563. <https://doi.org/10.1038/s41586-021-03308-6>.
- [2] FAO, World Food and Agriculture – Statistical Yearbook 2023, FAO, 2023. <https://doi.org/10.4060/cc8166en>.
- [3] S.M. Hussain, F. Khurram, A. Naeem, S.Z.H. Shah, P.K. Sarker, E. Naeem, M.Z.-H. Arsalan, D. Riaz, Z. Yousaf, M. Faisal, M. Amjad, A review on the prospects and potentials of fishmeal replacement with different animal protein sources, *International Aquatic Research* 16 (2024) 7-16. <https://doi.org/10.22034/iar.2024.1996466.1517>.
- [4] G.J. Tacon, M. Metian, Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects, *Aquaculture* 285(2008) 146-158. <https://doi.org/10.1016/j.aquaculture.2008.08.015>.
- [5] D.M. Gatlin, F.T. Barrows, P. Brown, K. Dabrowski, T.G. Gaylord, R.W. Hardy, E. Herman, G. Hu, Å. Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E.J. Souza, D. Stone, R. Wilson, E. Wurtele, Expanding the utilization of sustainable plant products in aquafeeds: A review, *Aquaculture Research* 38(2007) 551-579. <https://doi.org/10.1111/j.1365-2109.2007.01704.x>.
- [6] D. Jannathulla, V. Rajaram, M.R. Kalanjiam, A. Kondusamy, M. Moturi, J. Dayal, Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources, *Aquaculture Research* 50(2019). <https://doi.org/10.1111/are.14324>.
- [7] N. Daniel, A. p. Muralidhar, P. p. Srivastava, K. k. Jain, K. Pani Prasad, J. Manish, T. Sivaramakrishnan, Dietary ascorbic acid requirement for growth of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) juveniles, *Aquaculture Nutrition* 24(2018) 616-624. <https://doi.org/10.1111/anu.12596>.
- [8] K. Jayathilakan, K. Sultana, K. Radhakrishna, A.S. Bawa, Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review, *Journal of Food Science and Technology* 49(2011) 278. <https://doi.org/10.1007/s13197-011-0290-7>.
- [9] R. Thakur, R. Santhosh, Y. Kumar, V.R. Suryavanshi, H. Singhi, D. Madhubabu, S. Wickramarachchi, K. Pal, P. Sarkar, Characteristics and application of animal byproduct-based films and coatings in the packaging of food products, *Trends in Food Science & Technology* 140(2023) 104143. <https://doi.org/10.1016/j.tifs.2023.104143>.
- [10] V. Amicarelli, G. Lagioia, C. Bux, Global warming potential of food waste through the life cycle assessment: An analytical review, *Environmental Impact Assessment Review* 91(2021) 106677. <https://doi.org/10.1016/j.eiar.2021.106677>.
- [11] P. Roy, A.K. Mohanty, P. Dick, M. Misra, A Review on the Challenges and Choices for Food Waste Valorization: Environmental and Economic Impacts, *ACS Environ. Au* 3(2023) 58-75. <https://doi.org/10.1021/acsenvironau.2c00050>.

- [12] C. Arunlertaree, C. Moolthongnoi, The use of fermented feather meal for replacement fish meal in the diet of *Oreochromis niloticus*, *Environment and Natural Resources Journal* 6(2008) 13-24.
- [13] S. Rueda-López, M.T. Viana, D. Badillo-Zapata, C.A. Martínez-Palacios, G. Correa-Reyes, Sustainable Tilapia Production in a Backyard System for Rural Areas Using Fish by-Products in Formulated Diets, *Tropical and Subtropical Agroecosystems* 16(2013) 475-482.
- [14] A.N. Abdel Rahman, M. ElHady, S.I. Shalaby, Efficacy of the dehydrated lemon peels on the immunity, enzymatic antioxidant capacity and growth of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*), *Aquaculture* 505(2019) 92-97. <https://doi.org/10.1016/j.aquaculture.2019.02.051>.
- [15] D.B. Zerai, K.M. Fitzsimmons, R.J. Collier, G.C. Duff, Evaluation of Brewer's Waste as Partial Replacement of Fish Meal Protein in Nile Tilapia, *Oreochromis niloticus*, Diets, *J World Aquaculture Soc* 39(2008) 556-564. <https://doi.org/10.1111/j.1749-7345.2008.00186.x>.
- [16] S.S.K. Madage, Medis, W. U. D., Y. and Sultanbawa, Fish Silage as Replacement of Fishmeal in Red Tilapia Feeds, *Journal of Applied Aquaculture* 27(2015) 95-106. <https://doi.org/10.1080/10454438.2015.1005483>.
- [17] T.F. Bernardes, J.L.P. Daniel, A.T. Adesogan, T.A. McAllister, P. Drouin, L.G. Nussio, P. Huhtanen, G.F. Tremblay, G. Bélanger, Y. Cai, Silage review: Unique challenges of silages made in hot and cold regions, *Journal of Dairy Science* 101(2018) 4001-4019. <https://doi.org/10.3168/jds.2017-13703>.
- [18] A. Oliva-Teles, P. Enes, H. Peres, 8 - Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish, in: D.A. Davis (Ed.), *Feed and Feeding Practices in Aquaculture*, Woodhead Publishing, Oxford, 2015: pp. 203-233. <https://doi.org/10.1016/B978-0-08-100506-4.00008-8>.
- [19] T.L.M. Grassi, N.M. Paiva, D.L. Oliveira, F. Taniwaki, J.F. Cavazzana, G.C.R. Da Costa Camargo, J.C.P. Diniz, R. Bermejo-Poza, R. Borghesi, M. Villarroel, E.H.G. Ponsano, Growth performance and flesh quality of tilapia (*Oreochromis niloticus*) fed low concentrations of *Rubrivivax gelatinosus*, *Saccharomyces cerevisiae* and *Spirulina platensis*, *Aquacult Int* 28(2020) 1305-1317. <https://doi.org/10.1007/s10499-020-00527-y>.
- [20] N. Ouattara, V. N'Douba, G. Teugels, J. Philippart, Effects of three agricultural by-products on cage culture growth performances of a landlocked population of *Sarotherodon melanotheron* (Teleostei: Cichlidae) in man-made Lake Ayame, Côte d'Ivoire, *African Journal of Aquatic Science* 30(2005) 125-129. <https://doi.org/10.2989/16085910509503846>.
- [21] N.W. Agbo, D. Adjei-Boateng, K. Jauncey, The Potential of Groundnut (*Arachis hypogaea* L.) By-Products as Alternative Protein Sources in the Diet of Nile Tilapia (*Oreochromis niloticus*), *Journal of Applied Aquaculture* 23(2011) 367-378. <https://doi.org/10.1080/10454438.2011.627780>.
- [22] F. Silva-Brito, F.A. Guardiola, T. Cavalheri, R. Pereira, H. Abreu, A. Kijjoa, L. Magnoni, Dietary supplementation with *Gracilaria* sp. by-products modulates stress response, antioxidant and immune systems of gilthead seabream (*Sparus aurata*) exposed to crowding, *J Appl Phycol* 32(2020) 4347-4359. <https://doi.org/10.1007/s10811-020-02268-0>.
- [23] F.P. Martínez-Antequera, L. Molina-Roque, V. de las Heras, J.M. Mancera, J.A. Martos-Sitcha, F.J. Moyano, Feed supplementation with winery by-products improves the physiological status of juvenile *Liza aurata* during a short-term feeding trial and hypoxic challenge, *Aquaculture Reports* 31(2023). <https://doi.org/10.1016/j.aqrep.2023.101667>.
- [24] A. Turker, M. Yiğit, S. Ergün, B. Karaali, A. Erteken, Potential Of Poultry By-Product Meal As A Substitute For Fishmeal In Diets For Black Sea Turbot *Scophthlmus Maeoticus*: Growth And Nutrient Utilization In Winter, *The Israeli Journal of Aquaculture=Bamidgeh* 57(2005) 49-61. <https://doi.org/10.46989/001c.20392>.
- [25] H.J. Pine, W.H. Daniels, D.A. Davis, M. Jiang, C.D. Webster, Replacement of Fish Meal with Poultry By product Meal as a Protein Source in Pond raised Sunshine Bass, *Morone chrysops* ♀ × *M. saxatilis* ♂, Diets, *J World Aquaculture Soc* 39(2008) 586-597. <https://doi.org/10.1111/j.1749-7345.2008.00202.x>.
- [26] C. Hernández, M.A. Olvera-Novoa, R.W. Hardy, A. Hermosillo, C. Reyes, B. Gonzã Lez, Complete replacement of fish meal by porcine and poultry by-product meals in practical diets for fingerling Nile tilapia *Oreochromis niloticus*: digestibility and growth performance, *Aquaculture Nutrition* 16(2010) 44-53. <https://doi.org/10.1111/j.1365-2095.2008.00639.x>.
- [27] A.M. Yones, A.A. Metwalli, Effects of Fish Meal Substitution with Poultry By-product Meal on Growth Performance, Nutrients Utilization and Blood Contents of Juvenile Nile Tilapia (*Oreochromis niloticus*), *Journal of Aquaculture Research & Development* 07(2015). <https://doi.org/10.4172/2155-9546.1000389>.
- [28] T. Srour, M. Essa, M. Abdel-Rahim, M. Mansour, Replacement of Fish Meal with Poultry By-product Meal (PBM) and its effects on the Survival, Growth, Feed Utilization, and Microbial Load of European Seabass, *Dicentrarchus Labrax* fry, *Global Advanced Research Journal of Agricultural Science* 5(2016) 2315-5094.

- [29] M.R. Dawson, M.S. Alam, W.O. Watanabe, P.M. Carroll, P.J. Seaton, Evaluation of Poultry By-Product Meal as an Alternative to Fish Meal in the Diet of Juvenile Black Sea Bass Reared in a Recirculating Aquaculture System, *North American Journal of Aquaculture* 80(2018) 74-87. <https://doi.org/10.1002/naaq.10009>.
- [30] X. Cui, M. Liang, Y. Wei, P. Li, X. Meng, M. Duan, H. Xu, Application of poultry by-product meal in diets of obscure pufferfish (*Takifugu obscurus*), *Aquaculture Research* 53(2022) 2354-2365. <https://doi.org/10.1111/are.15754>.
- [31] C. Wei-Kang, L. Leong-Seng, S. Rossita, Evaluation of Feather Meal as a Dietary Protein Source for African Catfish Fry, *Clarias gariepinus*, *Journal of Fisheries and Aquatic Science* 8(2013) 697-705. <https://doi.org/10.3923/jfas.2013.697.705>.
- [32] H. Salehi, S. Reiser, M. Pourkazemi, U. Focken, Complete Replacement of Fish Meal with Potential Aquafeed Ingredients for Rainbow Trout in Iran, *Turkish Journal of Fisheries and Aquatic Sciences* 24 (2024). <https://doi.org/10.4194/TRJFAS24254>.
- [33] H.A. Adewole, V.F. Olaleye, Growth performance in *Clarias gariepinus* Burchell fingerlings fed blood meal–bovine rumen digesta blend diets, *Ife Journal of Science* 16(2014) 495-503.
- [34] H. Yu, Q. Zhang, H. Cao, T. Tong, G. Huang, W. Li, Replacement of fish meal by meat and bone meal in diets for juvenile snakehead *Ophiocephalus argus*, *Fish Sci* 81(2015) 723-729. <https://doi.org/10.1007/s12562-015-0871-x>.
- [35] O.M. Millamena, Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*, *Aquaculture* 204 (2002) 75-84. [https://doi.org/10.1016/S0044-8486\(01\)00629-9](https://doi.org/10.1016/S0044-8486(01)00629-9).
- [36] H. Hisano, P.S.D. Pietro, Growth performance and digestibility of juvenile Nile tilapia fed diets containing acid silage viscera of surubim catfish, *Acta Sci. Anim. Sci.* 35(2013) 1-6. <https://doi.org/10.4025/actascianimsci.v35i1.14470>.
- [37] S. Koshio, R.E. Mamauag, S. Yokoyama, M. Ishikawa, J.A. Ragaza, Hydrolyzed Tuna Meat By-Product Supplement for Juvenile Red Sea Bream, *Pagrus major*, and its Effect on Growth, Enzyme Activity, Plasma Parameters, and Apparent Nutrient Digestibility, *Israeli Journal of Aquaculture - Bamidgeh* 66 (2014). <https://doi.org/10.46989/001c.20778>.
- [38] R.E.P. Mamauag, J.A. Ragaza, Growth and feed performance, digestibility and acute stress response of juvenile grouper (*Epinephelus fuscoguttatus*) fed diets with hydrolysate from milkfish offal, *Aquac Res* 48(2017) 1638-1647. <https://doi.org/10.1111/are.12999>.
- [39] J.C. Swanepoel, N.J. Goosen, Evaluation of fish protein hydrolysates in juvenile African catfish (*Clarias gariepinus*) diets, *Aquaculture* 496(2018) 262-269. <https://doi.org/10.1016/j.aquaculture.2018.06.084>.
- [40] Y. Zhang, L. Zhang, L. Huang, Z. Dong, Q. Lu, Y. Zou, F. Tang, S. Zhao, T. Storebakken, Evaluation of conventional or hydrolyzed stickwater from food-grade skipjack tuna by-product in diet for hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*), *Aquaculture* 548(2022) 737714. <https://doi.org/10.1016/j.aquaculture.2021.737714>.
- [41] C.D. Webster, L.G. Tiu, A.M. Morgan, A. Gannam, Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone chrysops* × *M. saxatilis* fed practical diets, *Journal of the World Aquaculture Society* 30(1999) 443-453. <https://doi.org/10.1111/j.1749-7345.1999.tb00992.x>.
- [42] K. J. Lee, K. Dabrowski, J.H. Blom, S.C. Bai, P.C. Stromberg, A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*), *Animal Physiology Nutrition* 86(2002) 201-213. <https://doi.org/10.1046/j.1439-0396.2002.00375.x>.
- [43] H.S. Jeong, S.H. Cho, Inclusion effect of jack mackerel meal as feed stimulants in diets replacing different levels of fish meal with various animal protein sources on growth performance of olive flounder (*Paralichthys olivaceus*), *Aquaculture Reports* 28(2023). <https://doi.org/10.1016/j.aqrep.2022.101450>.
- [44] M.A.B. Siddik, P. Chungu, R. Fotedar, J. Howieson, Bioprocessed poultry by-product meals on growth, gut health and fatty acid synthesis of juvenile barramundi, *Lates calcarifer* (Bloch), *PLoS ONE* 14(2019) e0215025. <https://doi.org/10.1371/journal.pone.0215025>.
- [45] M.A.O. Dawood, F.I. Magouz, M. Essa, M. Mansour, Impact of Yeast Fermented Poultry by-Product Meal on Growth, Digestive Enzyme Activities, Intestinal Morphometry and Immune Response Traits of Common Carp (*Cyprinus carpio*), *Annals of Animal Science* 20(2020) 939-959. <https://doi.org/10.2478/aoas-2020-0021>.

Genetic gain in grain yield across the historical serie of forage triticale cultivars released by the Universidad Autónoma Agraria Antonio Narro

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ABSTRACT

Objective: To evaluate genetic progress in grain yield and associated agronomic traits in forage triticale (\times Triticosecale Wittmack) cultivars developed by the Universidad Autónoma Agraria Antonio Narro (UAAAN) between 1992 and 2022.

Design/Methodology/Approach: Seven winter-habit cultivars, classified as older, intermediate, and modern, were evaluated at 19 locations in northern Mexico (2019-2023) for grain yield (GY) and at three locations (2023-2024) for yield components (YC). A randomized complete block design with three replications was used. Analysis of variance and linear regression were performed to estimate absolute and relative genetic gains.

Results: Significant differences were detected between release periods for GY and YC. Modern cultivars obtained the highest average (6.105 t ha^{-1}), with absolute genetic gains of $70 \text{ kg ha}^{-1} \text{ year}^{-1}$ and relative gains of $2.1\% \text{ year}^{-1}$. A sustained increase in grains m^{-2} and a reduction in plant height without affecting biomass production were observed.

Limitations on study/Implications: The study focused solely on UAAAN winter cultivars under conditions in northern Mexico. Extrapolation to other programs should be done with caution.

Findings/Conclusions: The UAAAN breeding program has achieved significant genetic advances in forage triticale, combining increases in GY and YC with height reduction. The results provided key information for strengthening the institutional program, with a focus on GY and productive stability, while maintaining forage potential and adapting to current systems.

Keywords: triticale, genetic gain, grain yield, yield components, breeding.

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INTRODUCTION

Triticale (\times Triticosecale Wittmack), a synthetic hybrid derived from the cross between wheat (*Triticum* spp.) and rye (*Secale cereale*), combines the high yield and grain quality of wheat with the hardiness and tolerance to adverse conditions of rye (Mergoum *et al.*, 2009). Since its development, it has shown outstanding potential as a forage crop and, to a lesser extent, as a grain source for animal and human consumption. Its adaptability to different

agroclimatic conditions, efficient use of nitrogen, and high biomass production have positioned it as a strategic alternative in regions with water and soil limitations (Varughese *et al.*, 1987; Plana *et al.*, 2016).

In Mexico, the area planted with triticale has grown in forage production areas, particularly in the Laguna Region, due to its ability to produce quality forage and seed at relatively low costs. However, although genetic improvement of triticale in the country has historically prioritized forage production, the demand for high-yield, high-quality seed has increased the need to develop cultivars that optimize both production objectives.

The Universidad Autónoma Agraria, Antonio Narro (Mexico) UAAAN has promoted the development of winter-habit forage cultivars, registering 10 cultivars between 1992 and 2022. Initially, these genotypes yielded more than 45 t ha⁻¹ of green matter but with grain yields lower than 2.5 t ha⁻¹. In response, the breeding program incorporated crosses with spring and facultative cultivars, increasing grain yield without sacrificing forage potential. This approach is consistent with global trends in cereal breeding, which seek to maximize harvest index and stability under different environments (Royo *et al.*, 2007; Fischer *et al.*, 2014).

Estimating genetic gain from historical series of cultivars is a key tool for evaluating the effectiveness of breeding programs. This method, widely used in crops such as wheat, maize, and rice, allows quantifying annual progress in yield and associated components, separating genetic from environmental effects through statistical analyses such as linear regression and mixed models (Valvo *et al.*, 2018; Stoyanov & Baychev, 2023). In triticale, studies on genetic gain are scarce, especially in forage production contexts. In this sense, the present study had the following objectives: 1) to evaluate the genetic progress in grain yield in a historical series of seven winter-habit triticale forage cultivars released by the UAAAN between 1992 and 2022, and 2) to determine the agronomic characteristics associated with these changes in order to generate information that will contribute to guiding future genetic improvement strategies for the crop.

MATERIALS AND METHODS

Genetic material

Seven forage cultivars of triticale with winter and intermediate-winter habits, developed by UAAAN between 1992 and 2022, were evaluated. The cultivars were classified into three release periods, Modern: AN330 and AN388 (2022); Intermediate: ANPELÓN, AN66, and AN184 (2014); and Older: AN31 and AN34 (1992).

Study locations

The experiments were conducted under irrigation in 19 production environments located in northern Mexico (Aldama, Chihuahua; Miguel Auza, Zacatecas; Matamoros, San Pedro de las Colonias; and La Soledad, Coahuila) during the 2019-2023 agricultural cycles. For the evaluation of yield components, three representative locations in the Laguna Region (Soledad, Campanario, and Centenario) were considered during the 2023-2024 autumn-winter cycle.

Experimental design and agronomic management

A randomized complete block design with three replications was used. Experimental plots consisted of five rows, each 5 m long, spaced 30 cm apart (7.5 m^{-2} per plot), sown at a seeding rate of 140 kg ha^{-1} . Fertilization was applied in two stages: at planting and during tillering, with a dose equivalent to 38-0-0 (N) plus 11-52-0 (P_2O_5). Phytosanitary control consisted of preventative applications of fungicide against yellow rust (*Puccinia striiformis* W.) and pre- and post-emergence herbicides. No significant pest problems were recorded.

Variables evaluated

At physiological maturity, plant height (cm) was measured from the base of the stem to the tip of the spike (without awns), as well as the number of spikes m^{-2} (NSM2). At harvest maturity, the number of grains per spike (NGPS), the number of grains m^{-2} (NGM2) (calculated as $\text{NSM2} \times \text{NGPS}$), and the grain yield (GY), estimated after harvesting and threshing a 5 m row and expressed in kg ha^{-1} , were quantified.

Estimated genetic gain

Absolute genetic gain (AGG, $\text{kg ha}^{-1} \text{ yr}^{-1}$) and relative genetic gain (RGG, $\% \text{ yr}^{-1}$) were calculated based on the differences between means for each release period, divided by the number of years elapsed, following the methodology of Royo *et al.* (2007).

Statistical Analysis

A combined analysis of variance was performed for grain yield and yield components, considering locations and release periods as fixed factors, and replicates nested within locations as a random factor. Means were compared using the Tukey test ($p \leq 0.05$).

A simple linear regression analysis was also applied between the year of release and the agronomic variables to estimate genetic progress. Analyses were performed using the Statistical Analysis System (SAS) version 9.4 (SAS Institute, 2013).

RESULTS AND DISCUSSION

Grain Yield

The combined analysis of variance showed highly significant effects ($p \leq 0.01$) of location, release period, and their interaction on grain yield, with a coefficient of variation (CV) of 7.4% (Table 1), indicating high experimental precision. The variability between locations confirms the strong influence of soil and climate factors on triticale yield, consistent with reports for winter cereals (Cooper *et al.*, 2022).

Modern cultivars (AN330 and AN388) recorded the highest average yield (6.105 t ha^{-1}), significantly outperforming intermediate (4.223 t ha^{-1}) and older (3.914 t ha^{-1}) cultivars (Table 2). These increases are associated with improvements in components such as the number of spikes per m^2 , grains per spike, and grain density, traits that have been identified as key drivers of cereal yield (Fischer *et al.*, 2014; Slafer *et al.*, 2015).

Table 1. Mean squares and significance of the combined analysis of variance between locations and release periods for the grain yield variable, cycles 2019 to 2023.

SV	DF	Mean square (t ha ⁻¹)
Location	18	15.66 **
Location × Replication	38	0.56 **
RP	2	80.09 **
Location × RP	36	1.48 **
Error	76	0.12
CV (%)		7.4

SV=Source of variation; DF=Degrees of freedom; CV=Coefficient of variation; RP=Release periods. ** Significant at $p \leq 0.01$.

Table 2. Results of the mean comparison test for the grain yield variable for the release period factor.

Release period	Cultivar	Mean (t ha ⁻¹)
Modern	AN330	6.105 ^a
	AN388	
Intermediate	ANPELÓN	4.223 ^b
	AN66	
	AN184	
Older	AN31	3.914 ^b
	AN34	

Different letters indicate significant differences according to Tukey's multiple comparison test ($p \leq 0.05$).

Yield components

In the 2023-2024 crop cycle, modern cultivars had higher values of spikes per m⁻² (464), grains per spike (61), and grains per m² (27,559), followed by intermediate and older cultivars (Table 3). These differences reflect the effectiveness of genetic improvement in increasing spike fertility and reproductive organ density, patterns also documented in wheat and barley (Sadras & Slafer, 2012; Han *et al.*, 2020).

Table 3. Results of the mean comparison test of yield components between release periods. 2022-2023 cycle.

Release period	Cultivar	NSM2 (Spikes m ⁻²)	NGPS (Grains spike ⁻¹)	NGM2 (Grains m ⁻²)
Modern	AN330	464 ^a	61 ^a	27559 ^a
	AN388			
Intermediate	ANPELÓN	381 ^b	49 ^b	18773 ^b
	AN66			
	AN184			
Older	AN31	350 ^c	42 ^c	15366 ^c
	AN34			

NSM2=Number of spikes m⁻²; NGPS=Number of grains per spike; NGM2=Number of grains m⁻². Different letters indicate significant differences according to Tukey's multiple comparison test ($p \leq 0.05$).

The number of spikes m^{-2} was mainly influenced by the location, demonstrating the sensitivity of this trait to management and environmental factors, while the number of grains per spike and grains m^{-2} were more consistent between locations, indicating greater genetic stability (Table 4).

Plant height

Significant differences in plant height (Table 5) were detected between groups of triticale cultivars (release periods), demonstrating a genetic shift toward more moderate plant heights, consistent with trends reported for triticale and wheat (Trini *et al.*, 2021).

A progressive reduction in plant height was observed (Table 6) from the older cultivars (128 cm) to the modern ones (113 cm). This change is related to the incorporation of dwarfing genes (*Rht*) and selection toward more compact phenotypes to reduce lodging and improve the partitioning of assimilated nutrients into the grain, without affecting forage potential (Reynolds *et al.*, 2009; Velasco-López *et al.*, 2025).

Genetic Gain

Linear regression analysis between year of release and yield (Figure 1) indicated a genetic gain of 70 to 236 $\text{kg ha}^{-1} \text{ year}^{-1}$, depending on the comparison between the periods, ($R^2=0.49$). The components most associated with the increase were the number

Table 4. Results of the analysis of variance for the yield components of 7 triticale cultivars classified by release periods, evaluated at three locations. Autumn-winter cycle 2023-2024.

SV	DF	NSM2 (Spikes m^{-2})	NGPS (Grains spike $^{-1}$)	NGM2 (Grains m^{-2})
Location	2	16771.90 **	11.37	9550836.50
Location × Replication	6	1399.40	25.21 *	3840150.90
RP	2	1133.40 **	1351.21 **	1542287771 **
Location × RP	4	2010	11.16	362370
Error	12	4981.20	5.63	19447541.60
CV (%)		6.30	5.60	9.50

SV=Source of variation; DF=Degrees of freedom; RP=Release periods; CV=Coefficient of variation; NSM2=Number of spikes m^{-2} ; NGPS=Number of grains per spike; NGM2=Number of grains m^{-2} .
** Significant at $p \leq 0.01$.

Table 5. Results of the analysis of variance for the plant height (PH) variable of triticale cultivars released by the UAAAN between 1992 and 2023.

SV	DF	PH (cm)
Release period	2	282.18 *
Replication	2	29.12
Error	4	12.14
CV (%)		2.8

SV=Source of variation; DF=Degrees of freedom; CV=Coefficient of variation; * Significant at $p \leq 0.05$.

Table 6. Results of the comparison of means between release periods for the plant height (PH) variable.

Release period	Cultivar	PH (cm)
Modern	AN330	113 c
	AN388	
Intermediate	ANPELÓN	123 b
	AN66	
	AN184	
Older	AN31	128 a
	AN34	

Different letters indicate significant differences according to Tukey's multiple comparison test ($p \leq 0.05$).

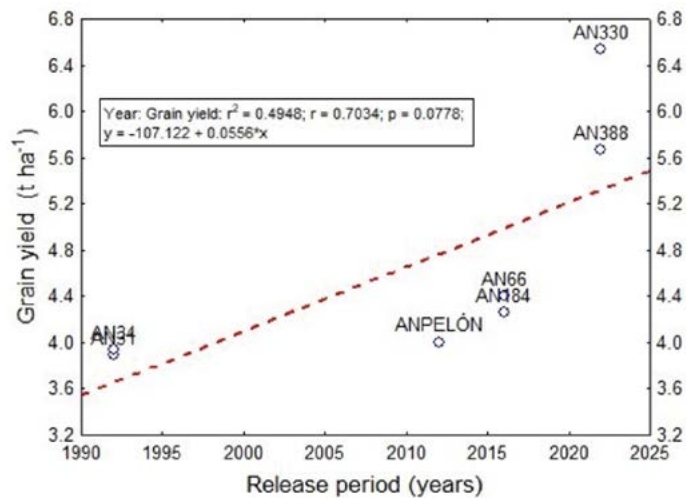


Figure 1. Relationships between the release period (1992–2022) of winter triticale cultivars developed by UAAAN and grain yield.

of grains m^{-2} ($R^2=0.62$) and grains per spike ($R^2=0.61$) (Figure 2), confirming that the increase in grain number has been the main driver of genetic progress in triticale, as is the case with other small-grain cereals (Ferrante *et al.*, 2013).

The decrease in height showed a negative relationship with the year of release ($\beta = -7.02$ $cm\ year^{-1}$), consistent with trends in cereal breeding aimed at improving stability and reducing lodging losses.

Recent studies have reported that yield regression over the year of release is a practical tool for quantifying the effectiveness of breeding schemes in cereals (Fischer *et al.*, 2014).

Tables 7 and 8 show the absolute genetic gain (AGG) and relative genetic gain (RGG) by year in winter-habit triticale cultivars, evaluating key variables such as GY, NSM2, NGPS, NGM2, and plant height (PH). Overall, a positive trend in genetic gains for yield and its components was observed when comparing modern cultivars with older and intermediate cultivars, which is consistent with current reports on genetic improvement in triticale and other cereals.

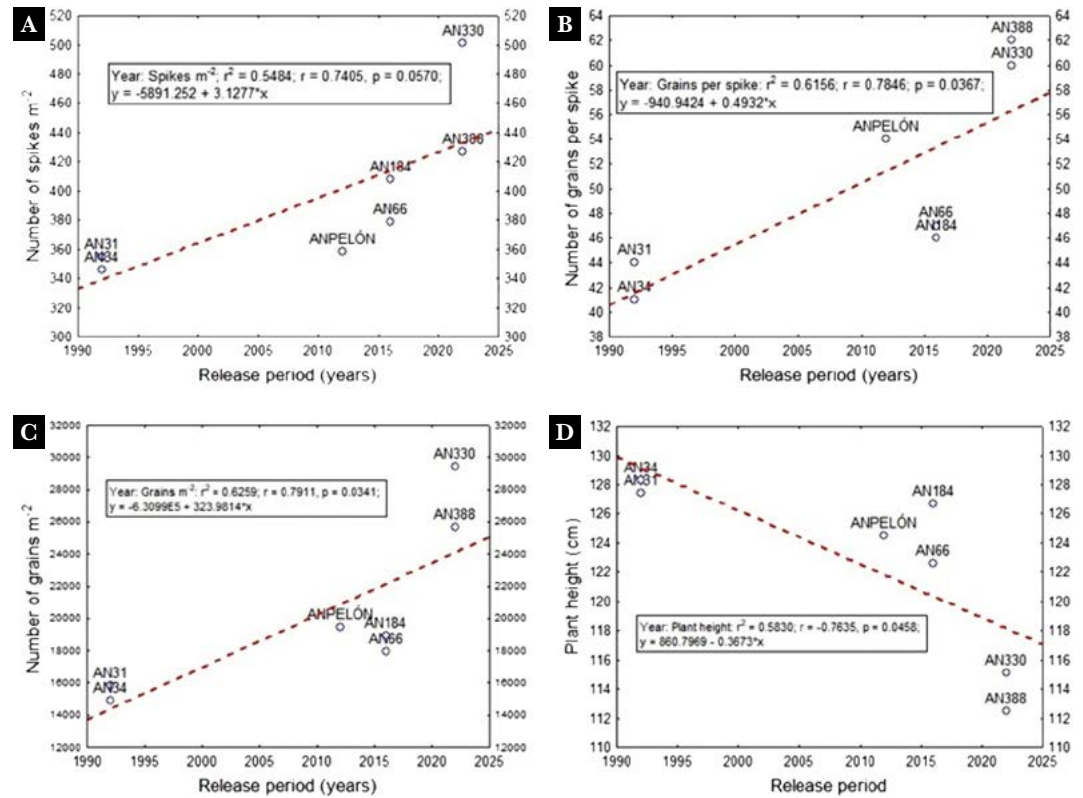


Figure 2. Relationships between the release period (1992-2022) of winter triticale cultivars developed by UAAAN and: A) number of spikes m^{-2} , B) number of grains per spike, C) number of grains m^{-2} , D) plant height.

Table 7. Absolute genetic gains per year for grain yield (GY) and its components of the cultivars under study according to their release period.

	GY ($kg\ ha^{-1}\ yr^{-1}$)	NSM2 (Spikes $m^{-2}\ yr^{-1}$)	NGPS (Grains spike $^{-1}\ yr^{-1}$)	NGM2 (Grains $m^{-2}\ yr^{-1}$)	PH ($cm\ yr^{-1}$)
M vs. O	70	3.80	0.60	499.60	-0.46
M vs. Int	236.20	10.10	1.50	1832.60	-1.36
Int vs. O	9.50	1.50	0.27	14.80	-0.14

M vs. O=Modern vs. Older; M vs. Int=Modern vs. Intermediate; Int vs. O=Intermediate vs. Older; NSM2=Number of spikes m^{-2} ; NGPS=Number of grains per spike; NGM2=Number of grains m^{-2} .

Table 8. Relative genetic gains (%) per year for grain yield (GY) and its components of the cultivars under study according to their release period.

	GY	NSM2	NGPS	NGM2	PH
M vs. O	2.10	2.50	2.30	1.60	-3.70
M vs. Int	8.50	10.30	9.90	6.30	-13.60
Int vs. O	4.30	4.10	3.90	4.40	-4.60

M vs. O=Modern vs. Older; M vs. Int=Modern vs. Intermediate; Int vs. O=Intermediate vs. Older; NSM2=Number of spikes m^{-2} ; NGPS=Number of grains per spike; NGM2=Number of grains m^{-2} .

The comparison between modern and old cultivars (M *vs.* O) showed an increase in AGG of $70 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in GY (Table 7), with an increase in RGG of $2.10\% \text{ yr}^{-1}$ (Table 8). These values are similar to those reported by Ferrante *et al.*, (2017) in wheat, who observed annual genetic improvements in yield between 1.80% and 2.50% under Mediterranean climate conditions. Furthermore, the increase in NSM2 with $3.80 \text{ spikes m}^{-2} \text{ yr}^{-1}$ of AGG and 2.50% of RGG; as well as NGPS with $0.60 \text{ grains spike}^{-1} \text{ yr}^{-1}$ of AGG and 2.30% of RGG suggests that the improvement has been effective in both the formation of reproductive structures and fertility.

The comparison between modern and intermediate cultivars (M *vs.* Int) revealed even higher gains, with a GY increase of $236.2 \text{ kg ha}^{-1} \text{ yr}^{-1}$ of AGG and a relative increase of $8.5\% \text{ yr}^{-1}$ of RGG (Tables 7 and 8), indicating that recent generations of cultivars have incorporated significant genetic advances. This observation is consistent with that reported by Velasco-López *et al.* (2025) in Mexico, who report that new triticale cultivars show significant improvements in phenotypic and genetic parameters associated with yield, thanks to assisted selection and the incorporation of materials adapted to specific environments. On the other hand, plant height showed an AGG and RGG decrease in all comparisons, with a reduction of up to $-13.6\% \text{ yr}^{-1}$ in the case of modern versus intermediate cultivars. This reduction in height is desirable from an agronomic viewpoint, since shorter plants tend to be more resistant to lodging problems and promote better resource utilization, as recent studies by Paccapelo *et al.* (2015) in triticale indicate.

Finally, genetic gains between intermediate and old cultivars (Int *vs.* O) were moderate but positive, reflecting continued progress in genetic improvement over the years, supported by the use of modern statistical and phenotypic tools to evaluate and select superior genotypes (Fischer *et al.*, 2014).

The above findings are consistent with those reported by Perry & D'Antuono (1989), Royo *et al.*, (2007) and Valvo *et al.*, (2018) in wheat and Stoyanov & Baychev, (2023) in triticale, who recorded linear increases in yield in response to genetic improvement over time. Genetic gains in yield were also related to progress in yield components, such as NGPS and NGM2 (Tables 7 and 8). This positive relationship between the aforementioned traits explains the trend observed in Figure 1, given that yield is a complex and multigenic trait, highly dependent on the components that affect it (Slafer *et al.*, 2015).

The above results confirm that the implemented strategies have been effective in increasing and improving yield potential by strengthening precision in the selection stage (Reynolds *et al.*, 2009; Rodríguez-Pérez *et al.*, 2023).

The analysis of the historical series of winter-habit triticale forage cultivars released by the UAAAN between 1992 and 2022 showed significant genetic progress in grain yield, primarily associated with an increase in the number of grains per square meter and grains per spike. The modern cultivars outperformed the older and intermediate cultivars by more than 2 t ha^{-1} , with estimated annual genetic gains of up to 70 kg ha^{-1} , reflecting the effectiveness of the breeding program in optimizing yield without compromising forage production. The progressive reduction in plant height helped minimize the risk of lodging and improve the efficiency of biomass partitioning toward the grain, while maintaining adequate forage production potential.

CONCLUSIONS

Overall, these results demonstrate that the UAAAN triticale breeding program has achieved significant advances in yield and stability, prioritizing key traits such as grain density and height reduction, while maintaining its value as a forage crop. These results confirm that the breeding applied at UAAAN has allowed the development of triticale cultivars that are more productive, stable, and adapted to the conditions of northern Mexico, strengthening its role as a strategic crop in dual-purpose production systems.

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REFERENCES

- Cooper, M., Messina, C. D., Tang, T., Gho, C., Powell, O. M., Podlich, D. W., Hammer, G. L. (2022). Predicting Genotype×Environment×Management (G×E×M) interactions for the design of crop improvement strategies: integrating breeder, agronomist, and farmer perspectives. *Plant Breeding Reviews*, 46, 467-585. <https://doi.org/10.1002/9781119874157.ch8>.
- Ferrante, A., Savin, R., y Slafer, G. A. (2013). Is floret primordia death triggered by floret development in durum wheat? *Journal of Experimental Botany*, 64(10), 2859-2869. <https://doi.org/10.1093/jxb/ert129>.
- Ferrante, A., Cartelle, J., Savin, R., y Slafer, G. A. (2017). Yield determination, interplay between major components and yield stability in a traditional and a contemporary wheat across a wide range of environments. *Field Crops Research*, 203, 114-127. <https://doi.org/10.1016/j.fcr.2016.12.028>.
- Fischer, R. A., Byerlee, D., y Edmeades, G. (2014). Crop yields and global food security. *ACIAR: Canberra, ACT*, 8-11. ISBN 978 1 925133 06 6.
- Han, J., Zhang, Z., Cao, J., Luo, Y., Zhang, L., Li, Z., y Zhang, J. (2020). Prediction of winter wheat yield based on multi-source data and machine learning in China. *Remote Sensing*, 12(2), 236. <https://doi.org/10.3390/rs12020236>.
- Mergoum, M., Singh, P. K., Peña, R. J., Lozano-del Río, A. J., Cooper, K. V., Salmon, D. F., and Macpherson, H. G. 2009. Triticale: a “new” crop with old challenges. In *Cereals* (pp. 267-287). Springer, New York, NY.
- Paccapelo, H., Ferreira, V., Castaño, M., Ferrari, E., Dominguez, R., Grassi, E., y Moreno, O. (2015). Mejoramiento genético de “triticales” (×Triticosecale Wittmack) y “trícepiros” (×Triticosecale Wittmack xx Agrotriticum Ciferri & Giacom) con diferentes aptitudes de uso. *Semiárida*, 25(2), 58-59. <https://ojs.unlpam.edu.ar/index.php/semiarida/article/view/2579>.
- Perry, M. W., y D'Antuono, M. F. (1989). Yield improvement and associated characteristics of some Australian spring wheat cultivars introduced between 1860 and 1982. *Australian Journal of Agricultural Research*, 40(3), 457-472. <https://doi.org/10.1071/AR9890457>.
- Plana Llerena, R. R., González Cañizares, P. J., Rivera Espinosa, R., Varela Nualles, M., y Álvarez Gil, M. A. (2016). Producción de forraje a base de triticale (×Triticosecale Wittmack) en suelo nitisol ferrálico lúxico, con dosis variables de nitrógeno e inoculación con hongos micorrízicos arbusculares. *Cultivos Tropicales*, 37(2), 22-32. <https://doi.org/10.13140/RG.2.1.1583.2567>.
- Reynolds, M., Foulkes, M. J., Slafer, G. A., Berry, P., Parry, M. A., Snape, J. W., y Angus, W. J. (2009). Raising yield potential in wheat. *Journal of Experimental Botany*, 60(7), 1899-1918. <https://doi.org/10.1093/jxb/erp016>.
- Royo, C., Alvaro, F., Martos, V., Ramdani, A., Isidro, J., Villegas, D., y García del Moral, L. F. (2007). Genetic changes in durum wheat yield components and associated traits in Italian and Spanish varieties during the 20th century. *Euphytica*, 155, 259-270. <https://doi.org/10.1007/s10681-006-9327-9>.
- Sadras, V. O., y Slafer, G. A. (2012). Environmental modulation of yield components in cereals: Heritability's reveal a hierarchy of phenotypic plasticities. *Field Crops Research*, 127, 215-224. <https://doi.org/10.1016/j.fcr.2011.11.014>.
- SAS (2013). Version 9.2. SAS Institute Inc SAS. Cary, NC.

- Slafer, G. A., Elia, M., Savin, R., García, G. A., Terrile, I. I., Ferrante, A., y González, F. G. (2015). Fruiting efficiency: an alternative trait to further rise wheat yield. *Food and Energy Security*, 4(2), 92-109. <https://doi.org/10.1002/fes3.59>.
- Stoyanov, H., y Baychev, V. (2023). Achievements and tendencies in the breeding of triticale (\times Triticosecale Wittm.) in Bulgaria. *Bulgarian Journal of Crop Science / Rastenievadni Nauki*, 60(4).
- Trini, J., Maurer, H. P., Neuweiler, J. E., & Würschum, T. (2021). Identification and fine-mapping of quantitative trait loci controlling plant height in central European winter triticale (\times Triticosecale Wittmack). *Plants*, 10(8), 1592. <https://doi.org/10.3390/plants10081592>.
- Valvo, P. J. L., Miralles, D. J., y Serrago, R. A. (2018). Genetic progress in Argentine bread wheat varieties released between 1918 and 2011: Changes in physiological and numerical yield components. *Field Crops Research*, 221, 314-321. <http://dx.doi.org/10.1016/j.fcr.2017.08.014>.
- Varughese, G., Barker, T., y Saari, E. (1987). Triticale. Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). Recuperado de <https://repository.cimmyt.org/bitstreams/727b40c5-050e-4227-b15c-cc0b5a9b69ac/download>.
- Velasco-López, J. L., Ruiz-Torres, N. A., Sosa-Flores, M. A., Aíl-Catzim, C. E., y Sánchez-Ramírez, F. J. (2025). Evaluación del rendimiento de grano de 55 líneas de triticale en el Valle de Mexicali. *Revista Mexicana de Ciencias Agrícolas*, 16(1). <https://doi.org/10.29312/remexca.v16i1.3522>.

Economic performance of the hake (*Merluccius productus* Aires) fishery in the Gulf of California

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Abstract

Objective: Analyze the Pacific hake (*Merluccius productus*) fishery in the northern Gulf of California from 2010 to 2021 to evaluate fleet performance, profitability, and implications for a quota system.

Design/methodology/approach: Information on fleet composition, fishing effort, catch efficiency, and economic indicators was analyzed for small and large vessels. Net cash flow, cost-benefit ratio, and return on investment were calculated. A break-even analysis was conducted to estimate the minimum harvest required to sustain fleet operations.

Results: Small vessels were more numerous and active, while large vessels showed 1.5 times higher efficiency. Average catch per vessel rose from 30 t in 2010 to 108 t in 2021, and CPUE in large vessels increased from 2 t/d to 6 t/d. In 2021, small vessels recorded negative returns (net cash flow: -MX\$176,604; C/B=0.90; ROI=-10%), while large vessels achieved positive outcomes (MX\$337,735; C/B=1.13; ROI=12%). Break-even analysis indicated that sustaining half the fleet required 7,798 t, equal to 75% of the proposed TAC.

Limitations/Implications: Market price variability and environmental fluctuations may affect results beyond the study period.

Findings/Conclusions: Quota systems must consider efficiency disparities. Implementing quotas, monitoring, and stakeholder participation is essential to prevent overfishing and ensure sustainability.

Keywords: Economic performance, cost-benefit ratio, return of investment, Gulf of California, fisheries management.

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INTRODUCTION

Excess fishing capacity, generated by increased fishing effort or technological improvements in fleets, has been recognized as one of the factors threatening fishery sustainability, with ecological, economic, and social consequences (FAO, 2022; Zhang *et al.*, 2018; Pascoe *et al.*, 2012; Coll *et al.*, 2008). Economically, excess fishing capacity within a



fishery increases production costs, affects the prices of landed products, and reduces fishing economic benefits (Pascoe *et al.*, 2012; Arnason, 2005; Hatcher *et al.*, 2005), as fishers increase fishing effort or adopt technological improvements (*e.g.*, number of trips, fishing days, number and size of fishing gears, effective fishing hours) to achieve economically profitable extraction levels (Zhang *et al.*, 2018; Sumaila *et al.*, 2012; Whitmarsh *et al.*, 2000).

To achieve biologically sustainable and economically efficient fisheries, it is necessary to regulate excess fishing capacity (FAO, 2022). For this purpose, different countries have implemented management systems based on catch quotas (Q), which can be total (TAC) or individual vessel quotas (IVQ), with or without transferability (Zhang *et al.*, 2018; Pascoe *et al.*, 2012; Hatcher *et al.*, 2005; Arnason, 2005). Although quota systems have the potential to generate economic rent, eliminate the “race to fish,” and improve fishery management, their implementation and effectiveness depend on the capacities of governmental fishery managers, fisher participation, and the availability of data monitoring and enforce quota compliance, as well as to estimate biological and economic indicators regarding the status of the target resource and the fishing fleet involved (Arnason, 2005, 2007; Hatcher *et al.*, 2005; Whitmarsh *et al.*, 2000).

In principle, the total allowable catch (TAC) establishes limits on the amount of a given species that can be harvested, aiming to prevent overexploitation. However, suppose the fleet grows excessively or becomes technologically more efficient. In that case, technological and eco-technological externalities could rise, potentially increasing the pressure on the resource, and leading to effects such as the “race to fish,” where vessels aim to catch the maximum number of fish in the shortest time before the TAC is exhausted (Pascoe *et al.*, 2012). This primarily occurs in systems where quotas are allocated to the entire fleet rather than to individual vessels (Arnason, 2007; Hatcher *et al.*, 2005). Beyond its impact on the resource, this modification affects potential vessel revenues and profitability (Birkenbach *et al.*, 2017; Grimm *et al.*, 2012; Asche *et al.*, 2009).

The profitability of fishing vessels is influenced by catch quotas, operational efficiency, and conservation policies (Zhang *et al.*, 2018; Pascoe *et al.*, 2012). Fishery management that balances resource sustainability with the economic viability of fleets is essential to secure the future of the fishing sector in emerging economies. This requires analyzing the economic performance of fleets or individual vessels to evaluate whether they can operate profitably and sustainably over time, considering revenues, costs, investments, and regulatory policies in accordance with vessel characteristics, fishing systems, and the spatial-temporal distribution of the fishing effort applied (Anderson & Seijo, 2010).

This is the case for the Pacific hake fishery (*Merluccius productus*), which is conducted in the northern Gulf of California, Mexico (Figure 1). The National Fisheries Charter (CNP) indicates that this fishery is still developing and requires management actions to maintain the resource at biologically sustainable and economically profitable levels for fishers (SADER, 2022).

Therefore, some researchers (Zamora-García *et al.*, 2013; Zamora-García & Stavrinsky Suárez, 2018) have proposed establishing a TAC based on a percentage of the total estimated biomass for each season in the Pacific hake fishery (SADER, 2022), with the

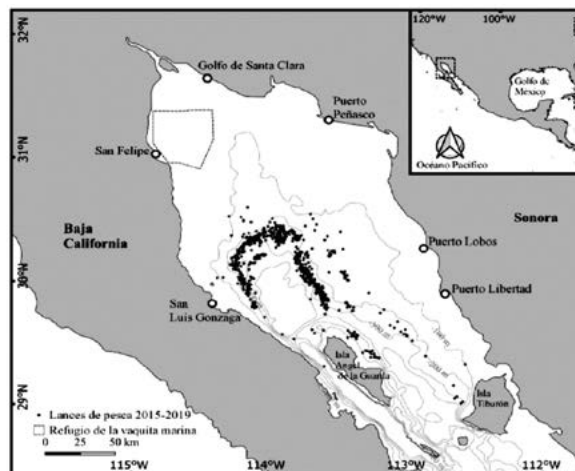


Figure 1. Distribution of the PHF operations in the Gulf of California. (Adapted from Parra-Alaniz & Ramírez-Rodríguez, 2022).

possibility of implementing individual vessel quotas (IVQs) as a means of respecting the TAC. This could ensure profitable vessel operations; however, the information required for a more in-depth analysis is currently insufficient (Ramírez-Rodríguez, 2017).

The CNP provides a summary of information on the status of fishery resources in Mexico, including species-specific data and management recommendations. This document reports that from 2006 to 2014, Pacific hake catches followed a positive trend, reaching 10,800 tonnes in 2014, and remained steady until 2016. Subsequently, and up to 2020, the trend reversed, reaching a minimum of 2,700 tonnes (SADER, 2022).

This study examines the economic performance of the Pacific hake fleet (PHF) in the northern Gulf of California to estimate economic indicators by vessel type and assess possible changes in fleet/vessel profitability. The analysis also aims to estimate the minimum catch required to generate economic benefits and its implications for managing policies, such as implementing an individual vessel quota system.

MATERIALES Y MÉTODOS

The methodology employed to analyze the PHF was conducted as follows: 1) technical and productive elements of the fleet through analysis of catch trends, 2) costs and revenues structures updating by vessel type according to the Representative Production Unit (URP) method, and 3) application of break-even analysis to determine the minimum catch level required to cover operational and management costs for a URP for small and large vessels. To characterize the performance of the PHF, data from vessel landing notices reporting Pacific hake catches from 2010 to 2021 were analyzed at the offices of the National Commission of Aquaculture and Fisheries (CONAPESCA). These data include departure and arrival dates, landing port, vessel name, national registry code, number of fishing days, and landed weight (in kilograms).

Additionally, based on vessel characteristics, a classification label was assigned to categorize vessels as small or large. This classification followed the proposal by Parra-

Alaniz and Ramírez-Rodríguez (2022), considering attributes related to vessel size and cargo capacity.

The characterization of production trends by season and vessel type included the number of vessels, the average number of trips per vessel, the average duration of a fishing trip (in days), the total catch (in tons), the average catch per vessel (in tons), and the catch per day per vessel (in tons). The average catch by vessel type was estimated as follows:

$$C_{avg} = CTvt / NTv \quad (1)$$

Where $CTvt$ is the total catch by vessel type per season, and NTv is the total number of vessels per type per season.

To evaluate whether the performance of small and large vessels differed significantly across fishing seasons, a Kruskal-Wallis test was applied to eight vessel performance indicators: total number of vessels, number of trips, fishing days per trip, number of trips per vessel, total catch by vessel type, catch per vessel, catch per trip, and catch per day. The analysis was conducted using version 8.9.1 of the Real Statistics Resource Pack software (Zaiontz, 2023).

Cost structure and prices of landed products

For the profitability analysis, data for the cost structure were obtained based on the characterization and evaluation of a Representative Production Unit (URP) (Richardson & Nixon, 1985) during the 2013 fishing season (Ramírez-Rodríguez & Almendarez-Hernández, 2014). Cost structure data were updated for vessels operating in the 2021 season, considering two URPs: one representing a small vessel and another representing a large vessel. All values are expressed in Mexican pesos (MXN).

Following the recommendations of Georgianna *et al.* (2001) and Tietze *et al.* (2005), the cost structure was divided into two sections: 1) Fixed costs (do not vary with production level), associated with vessel maintenance and URP administration, *e.g.*, office expenses and fishery services. 2) Variable costs (vary with production level), generally recognized as operating costs, such as fuel, deck supplies, and crew wages.

Fixed costs were divided into administrative and maintenance expenses. Administrative costs of the URPs include those related to office operations and fishing services. Office expenses include salaries and social security contributions for employees, vehicle ownership taxes, insurance, maintenance and fuel for cars, telephone and internet services, water, electricity, and office supplies. Fishing services include third-party vessel insurance, membership fees to the National Chamber of Fisheries and Aquaculture Industries, fishing permits, dispatch fees, maritime safety training, fire extinguishers, fumigation services, and waste management.

The administrative structure is the same for both URPs. Each has an office staffed by the owner, a secretary, a driver, and an assistant. For mobility and product transport, they operate two vehicles. Administrative expenses include salaries, permits, insurance, and accounting services. Fishing permits are paid annually and renewed every four years

(Ramírez-Rodríguez & Almendarez-Hernández, 2014). Vessel insurance only covers third-party damages; vessels are not insured against partial or total loss. Although the vessels remain operational, due to their age (over 24 years), they were considered fully depreciated. Fishing activity in Mexico is not eligible for credit from commercial or development banks.

The maintenance cost of a vessel participating in the hake fishery (Ramírez-Rodríguez & Almendarez-Hernández, 2014) was calculated proportionally to the Pacific hake fishing season. Maintenance items included payments to shipyards, hull repairs, propellers, zinc plates, engines, winches, hake nets, boards, refrigeration systems, navigation equipment, welding, and vessel fumigation. Vessel and fishing gear maintenance costs were considered equal for both types of vessels.

Variable costs include those associated with vessel operations, such as fuel, crew wages, deck and engine supplies, and landing services. Since 90% of hake fishing trips are carried out mainly between January and March of each season, the fixed costs defined in the 2013 URP (Ramírez-Rodríguez & Almendarez-Hernández, 2014) were proportionally adjusted to the hake fishery, considering only three months of activity.

Total costs were calculated by considering the differences in average catch, the number of trips, and the duration of fishing days. Administrative and maintenance costs for the 2021 season were then added. Total costs (TC) per vessel type were estimated as follows:

$$TC = ACk + Sk + MCk + TPCk + Dk \quad (2)$$

Where ACk is the administrative cost ($k=1$ office costs; $k=2$ fishing services), Sk is the fishermen's and captains' wages ($k=1$, fishermen's salary; $k=2$, captain's salary), MCk is the maintenance and fishing gear costs ($k=1$, shipyard; $k=2$, repairs; $k=3$, fishing gear; $k=4$, refrigeration; $k=5$, navigation equipment; $k=6$, fumigation), $TPCk$ is the trip provisioning costs ($k=1$, fishing materials; $k=2$, food; $k=3$, oil; $k=4$, spare parts; $k=5$, tools; $k=6$, landing and ice costs), and Dk is the diesel cost per trip.

Cost Structure Updating

To update the values of the production cost structure and prices per kilogram of hake, the Producer Price Index (PPI) and the Consumer Price Index (CPI) for the fisheries sector were used as adjustment factors. These indices measure the variation in prices for a fixed basket of goods and services, which is representative of a Mexican fishery production unit over time, using the second semester of 2019 as the basis for the index for calculation (INEGI, 2024).

The values for each cost structure component were updated to 2021 by adding the annual inflationary increase from its 2013 reference value, as follows:

$$VE_{cost} = VO_{cost} * (1 + Vi INPP \text{ of the year } t) \quad (3)$$

$$VE_{price} = VO_{price} * (1 + Vi INPC \text{ of the year } t) \quad (4)$$

Where, VE_{cost} is the updated cost value, VE_{price} is the updated price value.

Where, VO_{cost} is the observed value for the cost structure, VO_{price} is the observed value of the price, Vi_{price} and Vi_{cost} is the inflationary value of the index.

$$Vi_{price} = (INPP_{year\ i} - INPP_{base\ year}) / INPP_{base\ year} \quad (5)$$

$$Vi_{cost} = (INPC_{year\ i} - INPC_{base\ year}) / INPC_{base\ year} \quad (6)$$

Revenues

The value of landed catch (VLC) by vessel type is used as a proxy for income (I). This indicator was estimated by multiplying the average catch by vessel type by the market price per ton of hake as follows:

$$I = VLC = Cavg * Pi \quad (7)$$

Where $Cavg$ is the average catch by vessel type in year i , and Pi is the price of hake per kilogram in Mexican pesos in year i .

Economic profitability and break-even point

To evaluate the profitability of the URPs for small and large vessels, three economic indicators were used, based on their income and cost. The first indicator was the Net Cash Benefit before Taxes (NCB), which measures the URP's capacity to generate profits during a fishing season. This indicator is obtained by subtracting total costs (TC) from total income (TI). The greater the positive difference between income and costs, the greater the URP's capacity to generate economic benefits from catches. The formula used was:

$$NCB = Total\ Revenue - Total\ costs (IT - CT) \quad (8)$$

The second indicator was the Return on Investment (ROI), which assesses the URP's capacity to generate profits from the investment made during a fishing season (Tietze *et al.*, 2005). The higher the ROI, the more profitable the fishery. For this calculation, investment was considered as the total expenditure required to ensure vessel functioning, fishing operations, and URP administration. The formula used was:

$$ROI = (Net\ Profit - Investment\ Made / Investment\ Made) \times 100 \quad (9)$$

Where ROI is the return on investment, and the *Investment Made* is assumed to be the total Costs (TC).

The third indicator used was the Benefit-Cost Ratio (BCR). This indicator compares the total net benefits generated by the URP with the total costs incurred during a fishing season (Hernández-Trejo *et al.*, 2014). If $BCR > 1$, it indicates that the net benefits obtained from catches exceed total costs, making the project economically viable. A BCR equal to 1 indicates that benefits equal costs, meaning there is no net gain, but the project covers

its own expenses. Conversely, if $BCR < 1$, it indicates that total costs exceed the economic benefits, making hake fishing unprofitable. The formula used was:

$$BCR = (TR / TC) \quad (10)$$

Break-Even Analysis

The break-even analysis was used to project the minimum catch level of small and large vessels, at the expected price, required to cover both variable costs and fixed costs during the 2021 hake fishing season. This method has been applied for demersal fisheries to assess the effect of changes in fishing operations on equilibrium catch levels (Georgianna *et al.*, 2011).

Based on the identified cost structure, the average catch per vessel type ($Cavg$) and the unit price (Pi , price per ton) were considered. The individual variable cost ($UVC = TVC / Cavg$) and the individual fixed cost ($UFC = TFC / Cavg$) per ton of hake were calculated as indicators to estimate the equilibrium catch for both small and large vessels URP, as follows:

$$Q = TFC / (Pi - UVC)$$

Where Q is the break-even catch (in tons), TFC is the Total fixed costs (in MXN), Pi is the price per ton of hake (MXN), and UVC is the variable cost per ton (MXN). This calculation enables the determination of the minimum catch required for a vessel (or URP) to cover both variable and fixed costs, thereby ensuring the economic sustainability of the fishery.

Finally, based on the latest estimate of Pacific hake biomass in the northern Gulf of California (INAPESCA, 2014), the catch identified to maintain the operation of small boats in economic equilibrium and assuming differences in the number of vessels participating in the same season, we calculate whether the break-even point defined as IVQ does not exceed the TAC and would allow an increase in economic benefits (ROI) for both types of vessels if they maintained their cost and price structure.

RESULTADOS AND DISCUSSION

Fleet performance

During the analyzed period, fleet landings and catch per vessel have exhibited marked variations across fishing seasons. Initially, from 2010 to 2012, catches increased from 903 t to 2,373 t. Between 2013 and 2016, catches rose further, averaging 5,463 t per season, reaching their maximum in 2014 with 6,582 t. From 2017 onwards, landings declined by 43% compared to the average of the previous period. By the first year of the millennium, the trend had shifted to a positive, reaching 4,973 tons (Figure 2a). Regarding catch per vessel, Figure 2b displays the same pattern as landings for the 2010-2021 period. An increase from 2010 to 2012. Five years later, catch per vessel remains almost steady, averaging 86 tons/vessel, with a peak in 2014. By 2020, there is a marked decrease, followed by a sudden rise in 2021.

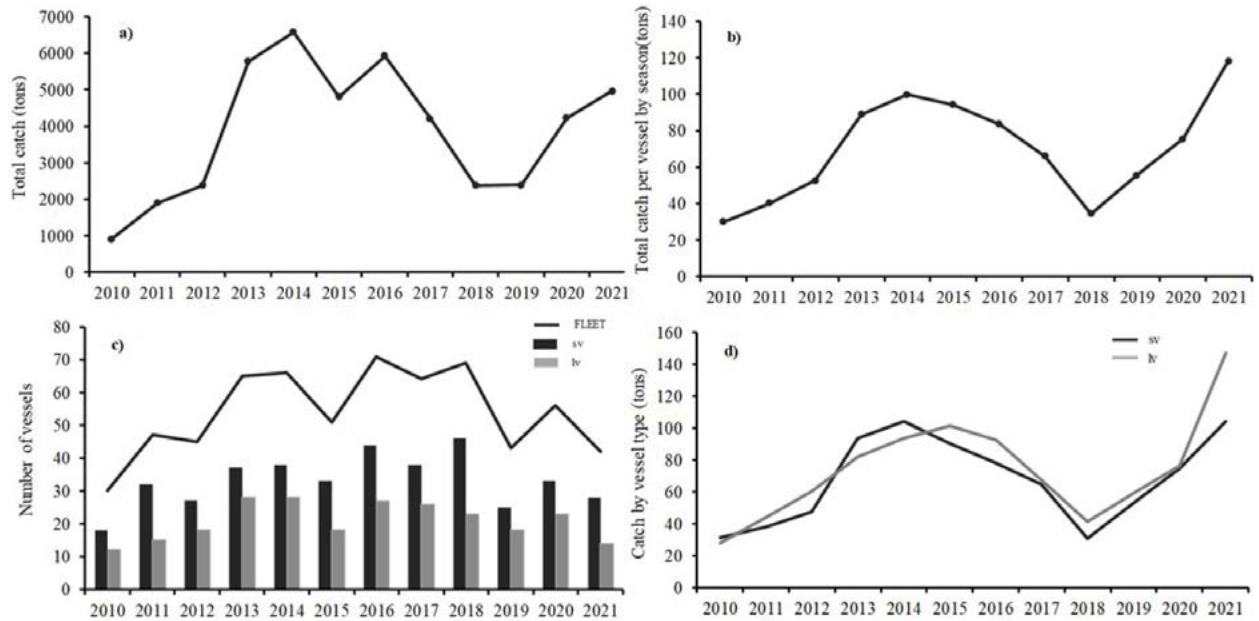


Figure 2. Gulf of California FPM production indicators. a) Total catch by season; b) Total catch per vessel by season; c) Number of vessels per season; d) Catch by vessel type. *sv=small vessel; lv=large vessel.

The fleet is made up of small and large boats. Small vessels are relatively old (average age of 48 years), with an average net tonnage of 45 tons and a storage capacity of 17 tons, an average length of 20.8 meters, and an average engine power of 400 HP. In contrast, large vessels are not so old (average age of 42 years), with higher net tonnage (64 t) and an average storage capacity of 32 tons, greater length (22.3 m), and more powerful engines (508 HP).

A total of 150 different vessels participated in the PHF; however, the total number of vessels varied per season. From 2010 to 2016, the fleet size expanded from 30 to 71 vessels. Furthermore, the fleet has gradually decreased in the number of vessels, from 65 in 2017 to 42 in 2021 (Figure 2c). Although large vessels generally performed better than small vessels, the Kruskal-Wallis test revealed no significant differences in catch per vessel per season ($p < 0.39$) (Figure 2d). However, from 2018 to 2021, catches increased to 104 tonnes for small vessels and 144 tonnes for large vessels. The average monthly catch per season of small boats (643 t) was significantly higher ($p > 0.026$) compared to large boats (408 t) across all seasons (Figure 2d).

The number of fishing trips during the analyzed period for small vessels and large vessels did not show significant differences ($p > 0.057$). Meanwhile, the trend in the number of trips per month per season from 2010 to 2017 for small boats and large boats increased significantly ($p > 0.017$). Both URPs reached their maximum records in 2016 with 61 and 31 trips, respectively. From 2018 to 2019, the number of trips decreased significantly in both cases. However, from 2020 to 2021, the trend reversed, with 41 trips per month for small boats and 24 for large ones (Figure 3a).

Regarding the number of vessels per month in the PHF, an average of eight small and five large vessels has been incurred in the PHF from 2010 to 2021. From 2013 to 2017, the

monthly increase in vessels was significant ($p > 0.00$). In 2018, the number decreased to 14 small and seven large vessels; the number of small vessels remained unchanged until the end of the period, while the number of large vessels increased by 2 (Figure 3b).

Small vessels carry out more trips and are more numerous than large vessels. The average number of trips per season per vessel showed no significant differences for the 2010-2021 period ($p < 0.150$), despite changes in the average number of fishing trips per season per vessel (Figure 3c).

The average catches per vessel show no significant difference ($p = 0.474$). However, from 2010 to 2017, catches per vessel increased significantly ($p = 0.022$). For the period from 2018 to 2019, catches per vessel for both types decreased, and in 2021, average catches by vessel type increased (Figure 4a). Catches per trip (t/v) for vessels improved significantly ($p = 0.022$) in line with the increase in fleet size and trip length (Figure 4b). The catch per day of fishing ($CPUE = t/d$) between small and large vessels is not significantly different ($p = 0.052$) (small vessels 3.2 t/d; large vessels 3.5 t/d). However, CPUE varied significantly across seasons ($p > 0.002$) (Figure 4c). Finally, the fishing trip length decreased from 7 to 5 days for small vessels and from 6 to 5 days for large vessels. Between 2018 and 2019, in both cases, trip duration increased to 7 days, and catches to 10 and 12 t/v, respectively.

Economic Benefit of the URP

The estimated average price per kilogram of hake landed for the 2021 season was MX\$13.84, reflecting a 54% increase compared to the MX\$10.00 observed in 2013. Over the last decade, the average annual inflation rate associated with the INPP was 4.3%. During the 2021 season (January to March), small vessels made six fishing trips and large

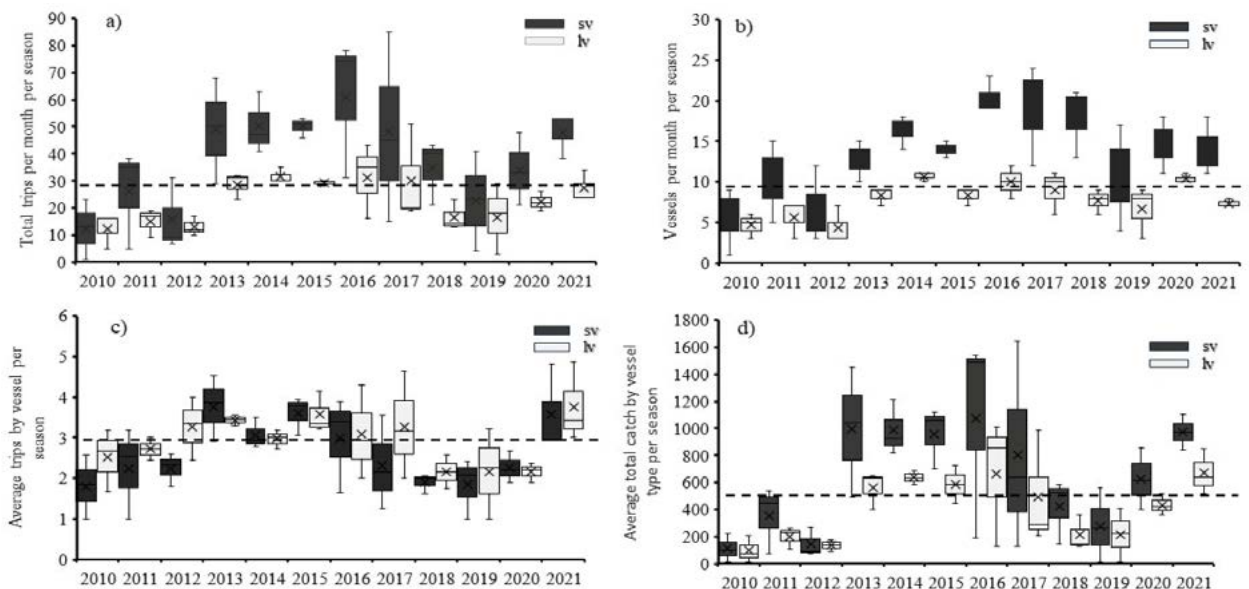


Figure 3. Gulf of California FPM activity indicators by vessel type. a) Total trips per month per season; b) Number of vessels per month per season; c) Average trips per vessel per season; d) Average total catch by vessel type per season *sv: small vessels; lv: large vessels.

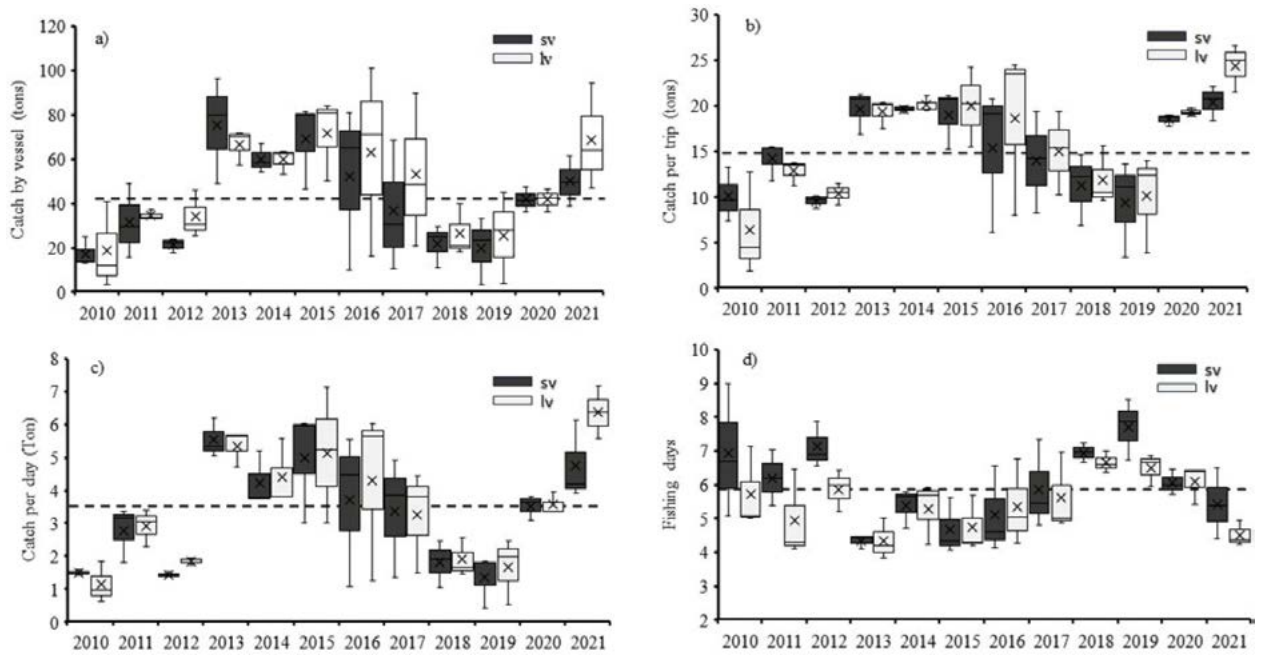


Figure 4. Gulf of California PHF performance indicators by vessel type: a) Catch per vessel per month per season; b) Catch per trip per month per season; c) Catch per day (CPUE) per vessel per season; d) Fishing trip length per season. *sv=small vessels; lv=large vessels.

vessels made nine. It involved six crew members and lasted an average of six days for both small and large vessels. Small vessels caught an average of 20 tons per trip, totaling 120 tons over the three months, which represented a revenue of MX\$1,660,800. Meanwhile, large vessels averaged 24 tons per trip and a total catch of 216 tons, representing a revenue of MX\$2,989,440.

The fixed costs of the URP were estimated at MX\$390,987. These include payments for fishing services (6%), vessel maintenance (24%), office expenses (29%), and salaries (41%). In 2021, the total estimated variable costs of fishing were MX\$1,446,418 for small vessels and MX\$2,244,751 for large vessels. The proportional distribution of variable costs was similar for both vessel types. About 43% corresponded to fuel (diesel), 37% to crew wages, and the remaining was allocated to deck supplies (11%), landing services (5%), and engine provisions (4%). Highlighting that the fuel cost for small vessels was 30% lower than that of large vessels. Crew payments and landing service costs for small vessels were 56% lower than those of large vessels (Table 2).

The updated total cost showed an upward trend from 2010 to 2021, but there was no corresponding increase in revenue. This is associated mainly with an unchanged price (Figure 7b) and seasonal fluctuations in catch. Although the nominal price of hake increased by 38%, its real price showed a slight decrease (Figure 5b). Diesel is the primary input for the vessel's operation; this input increased by 137%, going from MX\$9.12 in 2010 to MX\$21.70 in 2021 (Figure 5c). The total maintenance cost of a vessel that fishes for hake from January to March 2021 was estimated at MX\$99,015.21. Hull, engine, and hake nets maintenance represented 75% of this total.

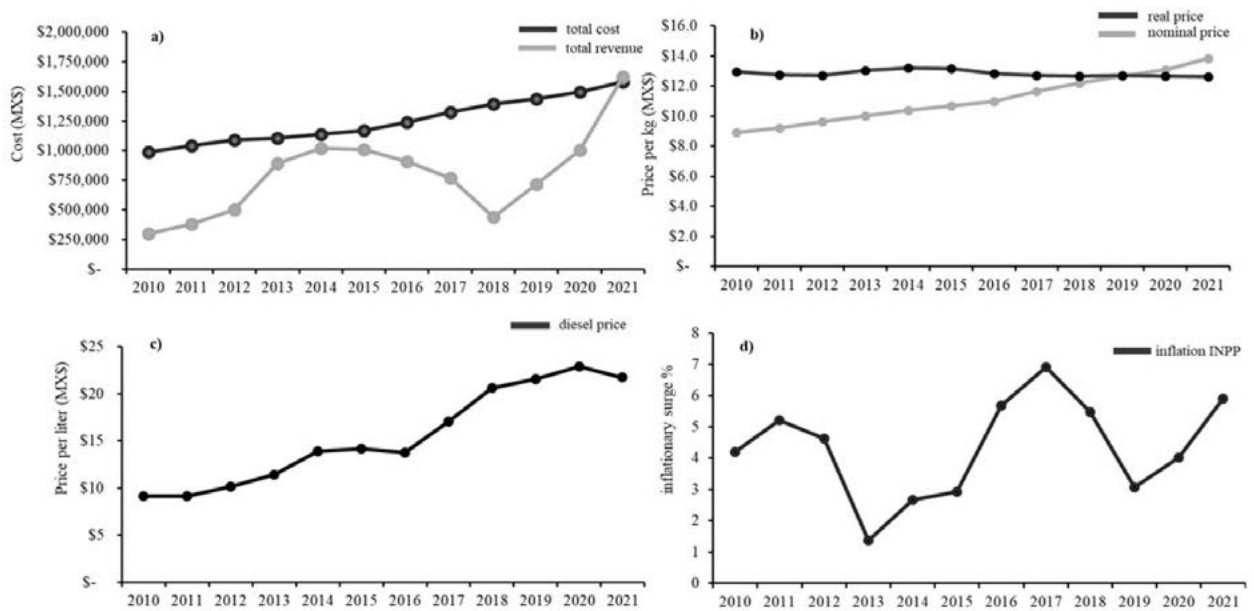


Figure 5. Trends in economic indicators related to the operation of the URP for small and large vessels: a) revenue and total cost; b) nominal and real price of hake; c) diesel price (liter); d) INPP (National Producer Price Index).

Economic Indicators

The total operating costs for a small vessel are considerably lower than those of a large vessel. However, the relationship between total costs and catch efficiency per vessel indicates that the catch cost per ton for small vessels (MX\$12,053) is 15% higher than for large vessels (MX\$10,392) (Table 1). The difference between total revenue and total costs in 2021 generated a gross margin of 12% and 24% for small vessels and 24% for large vessels, representing MX\$214,382 and \$744,689, respectively.

After deducting operating costs from gross profit, the results indicate that in 2021, small vessels experienced a cash flow deficit before taxes of MX\$176,604, with a total cost-income ratio (C/B) of 0.90 and an ROI of -10% . In contrast, large vessels achieved a net revenue of MX\$337,735, with a C/B ratio of 1.13 and an ROI of 12% during the 2021 season.

The overall balance for the 2021 season shows that small vessels did not obtain economic benefits from hake fishing (C/B=0.90). Their costs exceeded total revenues by 10%. Their total cost per ton was MX\$15,312, while the market price was MX\$13,840. Conversely, large vessels generated positive economic returns (C/B=1.13), with a total cost per ton of MX\$12,276, at the same market price.

Break-even

The break-even analysis, considering the selling price per ton of hake and the cost structure, identified that in the 2021 season, small vessels required a catch of 219 tons to reach economic equilibrium. However, their observed catch was only 120 tons, *i.e.*, 99 tons below the threshold, indicating that they failed to cover their total costs and operated at a

Table 1. Revenue and cost figures (MX\$) of the representative production unit of the Pacific hake fishery for small and large vessels.

Concept	Total landed catch		Selling price per ton		small vessels	large vessels
	120	216	\$ 13,840	\$ 13,840		
Landing Income			\$ 13,840	\$ 13,840	\$ 1,660,800	\$ 2,989,440
Total Capture costs			\$ 12,053	\$ 10,392	\$ 1,446,418	\$ 2,244,751
Mechanic supplies					\$ 51,000	\$ 76,500
Deck supplies					\$ 169,920	\$ 254,880
Fuels					\$ 688,474	\$ 946,728
Landing services					\$ 72,000	\$ 129,600
Crew wages					\$ 465,024	\$ 837,043
Gross Profit			\$ 1,787	\$ 3,448	\$ 214,382	\$ 744,689
Total Operating costs			\$ 3,258	\$ 1,884	\$ 390,987	\$ 406,953
Fishing services					\$ 23,615	\$ 23,615
Office expenses					\$ 103,103	\$ 119,070
Maintenance					\$ 99,015	\$ 99,015
Administrative salaries					\$ 165,253	\$ 165,253
Total costs					\$ 1,837,404	\$ 2,651,705
Economic benefit					-\$ 176,604	\$ 337,735
VAT refund					\$ 120,713	\$ 164,941
Net Income					-\$ 55,892	\$ 502,676
Rentabilidad (ROI)					-10%	12.7%
Cost/Benefit					0.90	1.13

Note: RPU=representative production unit. The total catch landed is expressed in tons.

loss (Figure 6a). In contrast, large vessels reached break-even with approximately 119 tons, and their observed catch of 216 tons far exceeded this level, reflecting a more efficient operation, with revenues exceeding costs and, therefore, favorable economic performance (Figure 6b).

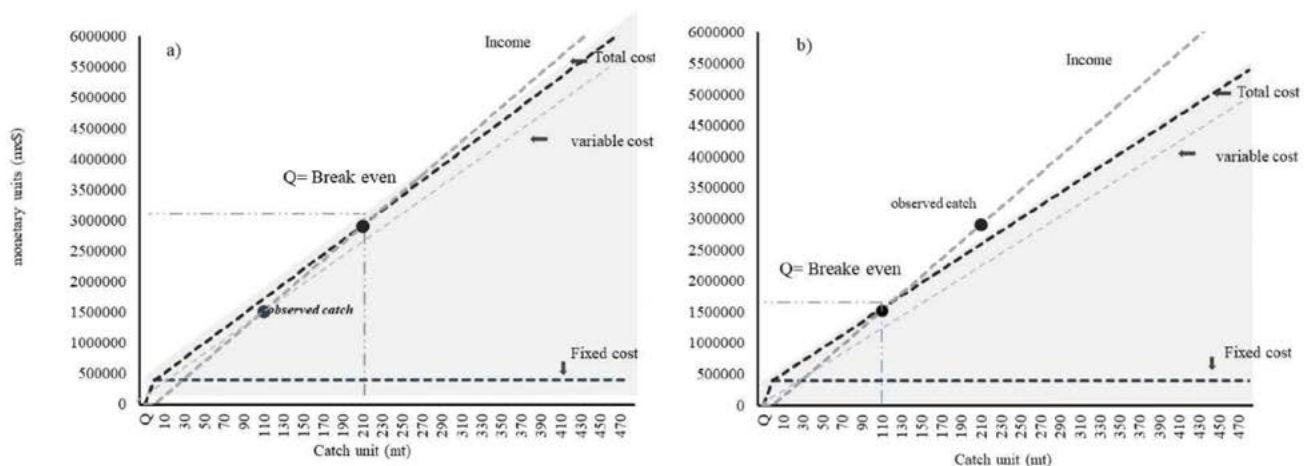


Figure 6. Break-even point in tons of hake for small (a) and large (b) vessels of the FPM in the Gulf of California, Mexico.

Assuming that the TAC corresponds to 25% of the available biomass, that the IVQ is defined at the break-even point for small vessels (219 tons), and that the size and proportion of the fleet remains the same (28 small vessels and 14 large vessels), 9,198 tons would be required, representing 54% of the available TAC, to maintain the fleet in economic equilibrium, with the possibility of generating an ROI of 6.4 and 12.7% for small and large vessels, respectively. If the entire authorized fleet participated with the same IVQ, the entire TAC (17,520 tons) would be required to generate the same cost-benefit values and maintain the fleet in equilibrium (Table 2).

The results indicate that the Pacific hake fleet (FPM) in the Gulf of California experienced a significant increase in the number of trips, fishing days, and vessels per season from 2010 to 2021, with small vessels being more active and numerous. Catch per vessel, per trip, and per day changed significantly across seasons, primarily for large vessels. This is attributed to their higher efficiency, estimated to be 1.5 times greater than that of small vessels, resulting in higher catches in fewer days (Parra-Alaniz & Ramírez-Rodríguez, 2022).

Differences in catch efficiency between small and large vessels must be considered when defining a quota system (total or per vessel), as they differentially affect vessel profitability (Arnason, 2005, 2007; Hatcher *et al.*, 2005; Whitmarsh *et al.*, 2000). Quota allocations should be adjusted to ensure that economic benefits from fishing are achieved while maintaining resource sustainability. Constructing the data required to estimate economic benefits for both vessel types in 2021, based on the 2013 representative production unit (URP) analysis, assumes that the operational cost structure was similar in both periods.

In the 2013 season, it was estimated that the hake URP, without distinguishing vessel types, could generate a 6.98% ROI with 100 t per season, with a CBR equal to 1.07. However, the operational conditions of the hake URP will differ, as all vessels have higher participation in shrimp fishing, and until 2020, fuel for fishing vessels was subsidized (Ramírez-Rodríguez, 2017). According to Sumaila *et al.* (2010), subsidies are considered a “financial transfer created by public entities to benefit a sector, allowing firms to earn more profits than they would otherwise.” Although the FPM generated a positive ROI (6.98%) in 2013, this benefit may have masked the fleet’s real capacity to generate economic returns and maintain its own operations.

Table 2. Unit cost values, return on investment, and benefit-cost ratio under a scenario with two fleet sizes and catch quota per vessel.

Scenario	Vessel type	Q observed (mt)	Number of vessels	TAC estimated (mt)	TAC % (mt)	Total catch (mt)	Total unit cost (\$/mt)	ROI	Q BE (mt)
no-quota	Small vessel	120	28	17,065	20%	3,360	\$ 15,311	-10.0%	219
	Large vessel	216	14		18%	3,024	\$ 12,276	12.7%	119
	Vessel type	BE=IVQ (mt)	Number of vessels	TAC estimated (mt)	TAC % (mt)	Total catch (mt)	Total unit cost (\$/mt)	ROI	Q break-even point (mt)
with quota	Small vessel	219	40	17,065	51%	8,760	\$ 15,311	0%	219
	Large vessel	219	40		51%	8,760	\$ 12,276	12%	219

Subsidies for fuel, equipment, or bait are particularly prone to increasing fishing capacity and contributing to stock depletion, as they reduce production costs and incentivize fishing intensity (Cisneros-Montemayor *et al.*, 2016). Our results indicate that in the 2021 season, during the fleet's three peak months, catch values for both vessel types (120 t for small vessels and 216 t for large vessels) were higher than the 2013 estimates, despite production costs per kilogram of hake being 10% higher than the market price and the absence of fuel subsidies, which were eliminated by the Mexican government starting in 2020 (DOF, 2025), limiting economic benefits, particularly for small vessels.

Some authors suggest that economic losses resulting from the removal of a subsidy can be offset by gains in catches as the harvested population recovers (Cisneros-Montemayor *et al.*, 2016). However, in the case of the FPM, a lack of effort control and strict enforcement of fisheries regulations prevents these gains from being retained, as vessels increase their effort and investment to compensate for lost economic benefits (Hernández-Trejo, 2011).

In 2013, 54 vessels recorded a catch of 4,450 t, with an average of 82 t per vessel (Ramírez-Rodríguez, 2017). In 2021, a total of 48 vessels operated—28 small and 14 large—with a recorded catch of 4,973 t, averaging 118 t per vessel, 104 t for small vessels, and 141 t for large vessels. Our results suggest that nearly half the fleet could be close to exceeding the total catch proportion estimated for 59 vessels, as proposed by Ramírez-Rodríguez (2017), based on the latest biomass estimate (INAPESCA, 2014).

In 2021, the cost-benefit ratio for a small vessel with an average catch of 120 t per season was $RCB=0.9$, with a negative ROI (-10%), requiring an additional 99 t per season to reach the break-even point. The cost-benefit ratio for a large vessel was $RCB=1.3$, with an average seasonal catch of 216 t, achieving an ROI of 12.7%, although its break-even point would be 119 t. These figures indicate that, to maintain the operation of half the authorized fleet (42 vessels) at or slightly above break-even for 14 large and 28 small vessels, a total catch of 7,798 t would be required. This catch represents 75% of the TAC level (11,905 t) estimated by Ramírez-Rodríguez (2017) for 119 vessels.

Álvarez-Trasviña *et al.* (2022) estimated an available biomass of 68,260 t for 2014, which would result in a total allowable quota of 17,065 t. Assuming that the fishery were managed with IVQ considering the break-even point for small vessels, the evidence indicates that the catch would be 4% above the TAC (17,520 t), small vessels would have an $RCB=1.06$ (income=cost) and large vessels would maintain their $RCB=1.13$, which implies that, despite having the entire quota (TAC), the fleet would continue without obtaining considerable economic benefits. This highlights the need to structure a management plan that considers not only biological and vessel efficiency indicators, but also economic indicators to establish catch limits that generate economic benefits and maintain the sustainability of the fishery.

In the Pacific hake fishery in the Gulf of California, labor and fuel costs account for the highest proportion, representing 63% of total costs. Trends in rising inflation and government fiscal and economic policies limit options related to fishing system efficiency, diversity, and the quality of landed products, as well as their prices (Ponce-Díaz *et al.*, 2021; Hernández-Trejo, 2012). The goal is to establish individual vessel quotas that support resource sustainability and vessel profitability, avoiding a “race to fish.” This requires

optimal management with participation from fishery sector stakeholders, focusing on strengthening the value chain (Knútsson *et al.*, 2016).

The fleet's economic results confirm that profitability differs markedly between small and large vessels. While large vessels achieved a positive ROI of 12%, small vessels recorded losses (−10%). This pattern is consistent with findings in other fisheries where scale and efficiency determine the ability to cover costs and generate surpluses (Olukunle, 2017).

Our results indicate that sustaining the operation of at least half of the fleet would require catching approximately 75% of the proposed TAC. This level of exploitation underscores the need to design differentiated quotas by vessel type, so that smaller vessels have access to minimum catch levels that allow them to cover costs without compromising the sustainability of the resource. When quotas are allocated equitably but without considering relative efficiency, this creates economic inequalities and disincentives for the less profitable fleet (Pascoe *et al.*, 2012; Whitmarsh *et al.*, 2000).

A complementary strategy would be to link quotas to strengthening the value chain, improving the quality and value of the landed product. As Knútsson *et al.* (2016) showed in Iceland, effective fisheries management has a positive impact not only on biological sustainability but also on competitiveness in high-value markets. For the FPM, this would involve accompanying quota allocation with technological innovation programs, market diversification, and access to financing.

In short, the evidence confirms that without quota-based management that integrates economic and biological criteria, the hake fishery will hardly achieve a sustainable balance. An IVQ scheme adapted to local conditions could generate incentives for efficiency and ensure the long-term sustainability of economic benefits, avoiding overexploitation and the marginalization of small vessels.

Overall, the results support the need for information that enables fishery managers to assess the current economic benefits of fishing and its potential under individual quota management schemes.

An economic analysis of the hake fishing fleet in the Gulf of California reveals a marked difference in profitability between small and large vessels, with the former operating below the break-even point and with a negative ROI, while the latter achieves positive margins. This asymmetry reflects the fact that scale and catch efficiency are determining factors for the financial viability of production units, which is consistent with what has been observed in other small- and large-scale fisheries (Olukunle, 2017).

The elimination of fuel subsidies and the increase in operating costs have limited the capacity of small vessels to sustain their activities, highlighting the need to rethink fisheries management beyond strictly biological criteria. In this sense, the evidence suggests that an individual catch quota (IVQ) system designed with differentiated criteria by vessel type could help balance profitability between fleet segments and, at the same time, promote the sustainability of the resource. International experiences, such as the Icelandic ITQ model, demonstrate that well-designed use rights schemes are capable of enhancing efficiency, reducing overcapacity, and generating sustainable economic benefits (Arnason, 2006).

However, the allocation of quotas alone does not guarantee benefits. They will need to be complemented with policies that strengthen the value chain, promote technological

innovation, and improve access to higher value-added markets, as has been observed in successful fishery management cases (Knútsson *et al.*, 2016).

CONCLUSIONS

The economic viability and sustainability of the hake fishery depend on the implementation of a comprehensive management scheme that considers both biological and economic indicators. Only under a system that combines fair catch quotas, efficiency incentives, and market strategies will it be possible to guarantee the permanence of the fleet and the long-term conservation of the resource.

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REFERENCES

- Álvarez-Trasviña, E., Arizmendi-Rodríguez, D. J., Marín-Enríquez, E., Salcido-Guevara, L. A., Sánchez-Cárdenas, R., & Rodríguez-Domínguez, G. (2022). Distribution and abundance of the Pacific hake, *Merluccius productus*, and relationship with the environment in the Gulf of California, México. *Ciencias Marinas*, 48, e3256. <https://doi.org/10.7773/cm.y2022.3256>
- Anderson, L. G., & Seijo, J. C. (2010). Bioeconomics of fisheries management. John Wiley and Sons.
- Asche, F., Bjørndal, T., & Gordon, D. V. (2009). Resource rent in individual quota fisheries. *Land Economics*, 85(2), 279-291. <https://doi.org/10.3368/le.85.2.279>
- Arnason, R. (2005). Property rights in fisheries: Iceland's experience with ITQs. *Reviews in Fish Biology and Fisheries*, 15(3), 243-264. <https://doi.org/10.1007/s11160-005-5139-6>
- Arnason, R. (2007). Fisheries self-management under ITQs. *Marine Resource Economics*, 22. <https://doi.org/10.1086/mre.22.4.42629568>
- Birkenbach, A. M., Kaczan, D. J., & Smith, M. D. (2017). Catch shares slow the race to fish. *Nature*, 544, 223-226. <https://doi.org/10.1038/nature21728>
- Cisneros-Montemayor, A. M., Sanjurjo, E., Munro, G. R., Hernández-Trejo, V., & Sumaila, U. R. (2016). Strategies and rationale for fishery subsidy reform. *Marine Policy*, 68, 229-236. <https://doi.org/10.1016/j.marpol.2015.10.001>
- Coll, M., Libralato, S., Tudela, S., Palomera, I., & Pranovi, F. (2008). Ecosystem overfishing in the ocean. *PLoS One*, 3, e3881.
- Food and Agriculture Organization of the United Nations. (2022). The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation. Rome: FAO. <https://doi.org/10.4060/cc0461en>
- Georgianna, D., Thunberg, E., Keiley, E., McAfee, B., & Reed, S. (2011). Break-even analysis of the New England groundfish fishery for FY2009 and FY2010. Massachusetts Division of Marine Fisheries.
- Grimm, D., Barkhorn, I., Festa, D., Bonzon, K., Boomhower, J., Hovland, V., & Blau, J. (2012). Assessing catch shares' effects: Evidence from federal United States and associated British Columbian fisheries. *Marine Policy*, 36, 644-657. <https://doi.org/10.1016/j.marpol.2011.10.014>
- Hatcher, A. (2005). On the microeconomics of quota management in fisheries. *Marine Resource Economics*, 20(1), 77-99. <https://doi.org/10.1086/mre.20.1.42629415>
- Hernández-Trejo, V. (2012). Subsidios al sector pesquero en México. Un panorama general. La Paz: WWF México.
- Hernández-Trejo, V., Ramírez-Rodríguez, M., Ponce-Díaz, G., & Almendarez-Hernández, L. (2014). Rentabilidad de Unidades Representativas de Producción Pesquera del calamar gigante *Dosidicus gigas* en el Golfo de California. *Hidrobiológica*, 24(1), 78-80.

- INAPESCA (Instituto Nacional de Pesca). (2014). Prospección de merluza (*Merluccius* spp.) en aguas marinas del centro y norte del Golfo de California. Instituto Nacional de Pesca, Centro Regional de Investigación Pesquera en Guaymas.
- INEGI (Instituto Nacional de Estadística y Geografía). (2024). Índice Nacional de Precios Productor: documento metodológico: base julio 2019=100. México: Instituto Nacional de Estadística y Geografía.
- Knútsson, Ö., Kristófersson, D. M., & Gestsson, H. (2016). The effects of fisheries management on the Icelandic demersal fish value chain. *Marine Policy*, 63, 172-179. <https://doi.org/10.1016/j.marpol.2015.03.015>
- Olukunle, O. T. (2017). Profitability analysis of small-scale fisheries and value chain in Lagos State, Nigeria. *Ocean & Coastal Management*, 141, 106-113. <https://doi.org/10.1016/j.ocecoaman.2017.03.011>
- Parra-Alaniz, J. C., & Ramírez-Rodríguez, M. (2022). Fleet efficiency in the Pacific hake (*Merluccius productus*) fishery in the Gulf of California, Mexico. *Latin American Journal of Aquatic Research*, 50(1), 99-109.
- Pascoe, S., Coglan, L., Punt, A. E., & Dichmont, C. M. (2012). Impacts of vessel capacity reduction programmes on efficiency in fisheries: The case of Australia's multispecies northern prawn fishery. *Journal of Agricultural Economics*, 63(2), 425-443.
- Ponce-Díaz, G., Vergara-Solana, F. J., & Aranceta-Garza, F. (2021). Análisis bioeconómico de los objetivos de manejo pesquero ante cambios en los precios de venta. *Economía: teoría y práctica* (55), 149-170. <https://doi.org/10.24275/etypuam/ne/552021/ponce>
- Ramírez-Rodríguez, M. (2017). A profitability analysis of catch quotas for the Pacific hake fishery in the Gulf of California. *North American Journal of Fisheries Management*, 37, 23-29.
- Ramírez-Rodríguez, M., & Almendarez-Hernández, L. C. (2014). Unidad Representativa de Producción de camarón-merluza-escama de Puerto Peñasco, Sonora. Environmental Defense Fund de México.
- Richardson, J. W., & Nixon, C. J. (1985). Description of FLIPSIM V: A general firm model policy simulation model. Texas A&M University, Department of Agricultural Economics, Agricultural and Food Policy Center, Bulletin B1528.
- SADER (Secretaría de Agricultura y Desarrollo Rural). (2022). Acuerdo mediante el cual se da a conocer la actualización de la Carta Nacional Pesquera. Diario Oficial de la Federación, 26/07/2022.
- Sumaila, U. R., Rashid, A. S., Khan, A. J., Dyck, R. W., Munro, G., Tydemers, P., & Pauly, D. (2010). A bottom-up re-estimation of global fisheries subsidies. *Journal of Bioeconomics*, 12(3), 201-225. <https://doi.org/10.1007/s10818-010-9091-8>
- Tietze, U., Thiele, W., Lach, R., Thomsen, B., & Rihan, D. (2005). Economic performance and fishing efficiency of marine capture fisheries. FAO Fisheries Technical Paper, 482, 68 pp.
- Whitmarsh, D., James, C., Pickering, H., & Neiland, A. (2000). The profitability of marine commercial fisheries: A review of economic information needs with particular reference to the UK. *Marine Policy*, 63, 257-263.
- Zamora-García, O. G., & Stavrinaky-Suárez, A. (2018). Ficha técnica de merluza del Pacífico norte. Environmental Defense Fund de México.
- Zamora-García, O. G., Díaz-Avalos, C., & Stavrinaky-Suárez, A. (2020). Catch rate, length, and sex ratio of Pacific hake (*Merluccius productus*) in the northern Gulf of California. *Fishery Bulletin*, 118, 365-379.
- Zhang, D., Sokveland, M., & Hermansen, O. (2018). Fishing fleet capacity and profitability. *Marine Policy*, 88, 116-121.
- Zaiontz, C. (2023). Real Statistics Using Excel. www.real-statistics.com

Effects of Groundwater Extractions and Agricultural Irrigation Through Spatial Analysis, Endorheic Watershed Case

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ABSTRACT

Objective: To examine the relationship between human activity and the occurrence of ground fissures in Menonite agricultural soils within the Laguna de Santiaguillo endorheic watershed.

Design/methodology/approach: Earth fissures were geolocated and digitized using satellite imagery. Remote sensing techniques were utilized to calculate the Normal Difference Moisture Index (NDMI) during the 2023-2024 agricultural cycles. Kernel density per Km² was calculated by concession and annual concessioned volume. Moreover, the total of rainfall in the study area was calculated using Thiessen polygons to be compared with the monthly NDMI.

Results: The study area has groundwater extractions throughout the year to meet the needs of hydric crops, which is observed through annual concession volume with an average density of 6.46 wells/Km². While the NDMI value for the agriculture cycle 2023 presents important variations in specific areas that zones containing major moisture are agricultural production areas, making it evident that the moisture patron is strongly related to agricultural activities in parcels that have a groundwater concession.

Limitations on study/implications: The study area presents significant pressure related to groundwater concessions. According to the Kernel analysis density, it was detected that one of the regions with major volume concessioned density coincides with one of the earth fissures detected through spatial images. These results suggest that this zone presents an advanced subsidence phenomenon. Kernel's density analysis was calculated based on the volume of CONAGUA's groundwater concessioned values; it is unknown the exact amount of groundwater extraction from the aquifer.

Findings/conclusions: One of the principal earth fissures coincides with a high Kernel's density estimation per annual volume concessioned, 30% of the total aquifer's concessions are in the study area, showing a high concentration of groundwater extractions.

Keywords: Aquifer, Earth Fissures, GIS Kernel's Density.

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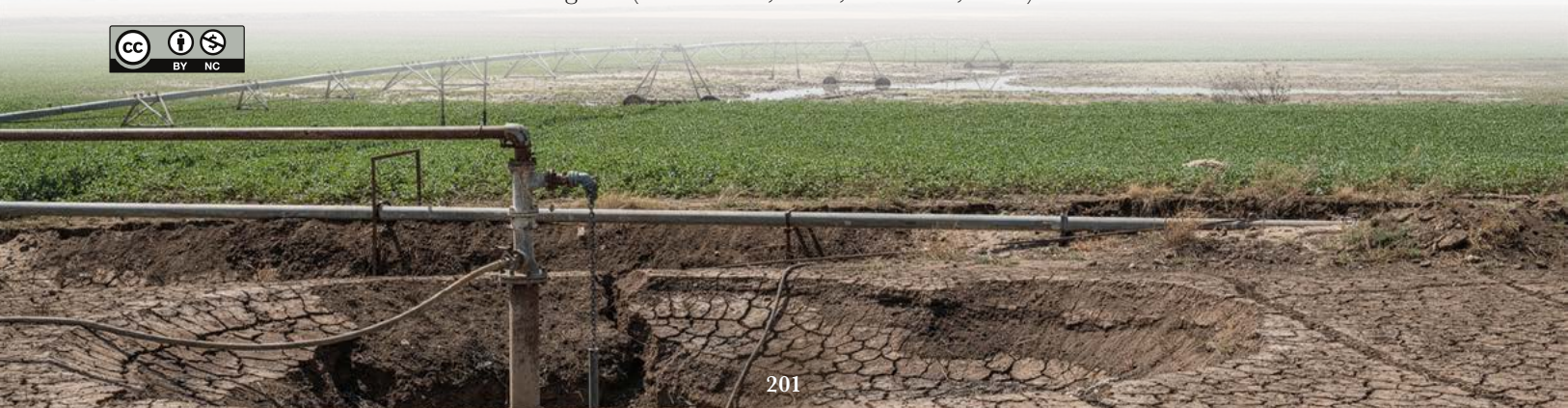
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INTRODUCTION

Groundwater accounts for 98% of the world's freshwater and is crucial to ecosystems, human consumption, industry, and particularly to agricultural irrigation in arid and semiarid regions (Barati *et al.*, 2019; Yan *et al.*, 2025).



Groundwater availability in several aquifers worldwide is decreasing, influenced by a combination of natural factors, including precipitation, slope, and lithology, as well as anthropogenic influences such as the number of wells, urbanization, industry, and agriculture. This phenomenon affects future food production (Redwan and Abdel Moneim, 2016; Whittemore *et al.*, 2023).

If the total of groundwater extracted over many years is close to or exceeds the total recharge, it's considered that there is overexploitation. In other words, an overexploited aquifer is defined as the amount of water extracted being greater than the volume that infiltrates. This term becomes more significant in arid or semi-arid areas due to the limited recharge capacity (Van Camp *et al.*, 2010).

In Mexico, 77% of the volume of water extracted is used for the agricultural sector. However, there are three significant constraints on this use: a) Seasonal distribution of rainfall over four months of the year (June- September) with the rest of the year relatively dry; b) Distribution of rainfall across the country, states like Tabasco (southeast) rainfall is close to 2,095 mm per year, while Baja California Sur (northwest) only 160 mm is received; c) in the northern, central and northwestern regions of the country natural water availability is 31%, and with 77% of total population living there, it generates 87% of GDP. These three limitations result in a heavy dependence on groundwater for agricultural activities, leading to overexploitation of more than 100 aquifers in the country. This lowers water tables, resulting in settlements and landslides (SEMARNAT, 2010).

Agriculture is the primary water consumer worldwide, utilizing approximately 72% of the world's renewable water resources. Its expansion has increased water demand, leading to excessive groundwater extraction and subsidence phenomena. This may trigger earth fissures that affect the surrounding communities' infrastructure, as well as permanently affect aquifer compaction and decrease storage capacity (Fan *et al.*, 2025; Song *et al.*, 2023).

Subsidence is a phenomenon that has been present in recent years. This is a superficial manifestation of overexploitation of natural resources (hydrocarbons, vapor, minerals, or groundwater) that changes the state of soil efforts (Alvarez *et al.*, 2001).

Intensive groundwater extraction reduces pore pressure in granular media, causing land deformation and compaction of unconsolidated sediments (Solano Rojas *et al.*, 2015). When groundwater extraction is greater than recharge, cones of abatement are formed, and it's associated with three deformation stages 1) sinking for water abatement; 2) fissure generation, and 3) fissure growth. Figueroa-Miranda *et al.* (2018) define an Earth fissure as any discontinuity related to subsidence caused by groundwater extraction in alluvial or lacustrine valleys.

The Earth fissure formation process is complex; they are characterized by soil and superficial rock breakage resulting from a combination of various natural geographic phenomena, as well as human activities. Among the leading causes are geological structures and soil sinking from groundwater extraction. Superficial water runoff process and erosion contribute to fissure development, increasing the weight and length of small fissures located under the surface. These superficial processes could be increased fissure impacts, creating severe damage (Jia *et al.*, 2021; Li *et al.*, 2024).

The use of special tools eases the processes, the results of which can be geographically analyzed. Creation of Kernel density maps enables the identification of major pressure areas, either for point concentration or any specific value or attribute of interest (Díaz Caravantes *et al.*, 2013).

During the decade 2010-2020, remote sensors were used to make temporal and spatial monitoring of the superficial hydrological resources' variations and drought in different parts of the world. These methods allowed to get parameters such as Normalized Differential Water Index (NDWI), Normalized Differential Vegetation Index (NDVI), Normalized Differential Moisture Index (NDMI), and Soil-adjusted Vegetation Index (SAVI) (Song *et al.*, 2025; Tibangayuka *et al.*, 2025).

Some research shows that subsidence in agricultural areas is related to hydric resources management in cases where excessive water use might increase land subsidence, directly impacting agricultural productivity (Guzy and Malinowska, 2020).

Territory management keeps a significant relationship with subsidence phenomena, which is why it is essential to analyze how agricultural practices affect these processes (Minderhoud *et al.*, 2018).

Inside Mennonite agricultural areas located in the Laguna de Santiaguillo watershed, Durango, México, fissures related to subsidence processes have been observed. These fissures present a relatively limited extension and a lack of visible soil mass movements, and they are scattered and non-patterned. However, due to the impact that is generated in the agricultural landscape and the region's productive activities, their study results are relevant to this research.

Given the limitations of the use of interferometry techniques described above and the need to establish management planning criteria to prevent significant risk effects, this study proposes to use indicators that integrate the leading causes and subsidence effects.

This study examines the relationship between human activities and the occurrence of earth fissures in Mennonite agricultural soils within Laguna de Santiaguillo watershed, Durango, Mexico, employing a geospatial approach that incorporates territorial management indicators, resources, and soil conditions.

MATERIALS AND METHODS

The research was conducted in Laguna de Santiaguillo endorreic watershed, located in the north-center of Durango State, Mexico, within hydrologic region number 11, north of the San Pedro-Mezquital River. Hydrologic net flows to two lagoons, which are also important migratory bird routes in North America, support about 67 species that reside there in winter, some of them coming from Alaska and Canada. Laguna de Santiaguillo is recognized as an Area of Importance for Bird Conservation (AICA, in Spanish) and was declared a Ramsar site in 2012 (Cassio Madrazo and Sánchez Ortiz, 2018; Corral-Bermudez *et al.*, 2019).

In the 1920s, a series of Mennonite communities' settlements were established in the area. Since their arrival until the present day, the principal economic activities undertaken by the population have been agriculture and cattle raising (Hansen, 2005).

The study area spans 167.9 ha and encompasses the Mennonite communities of the region. Geographically, it is located within extreme coordinates: W -105.07° , S 24.83° , E -104.93° , and N 25.02° (Figure 4).

According to INEGI information, the topofom system comprises an alluvial plain (162.286 ha) and a floodable alluvial plain (5.04 ha). Its climate is semi-dry temperate, with summer rain (BS1kw(w)), and Vertisol is the predominant edaphology.

Mexican Geological Service (SGM) reports that the study area presents a stratigraphy from the Cenozoic, Quaternary period with alluvial sediments (sand and lime) and lacustrine (clays). Geological faults are not reported in the study area; the nearest are Chapala and Morillitos faults located 12 and 11 Km away, respectively. These faults are classified as normal faults provoked by superficial tension that trigger an inclined vertical slide of 60° (Figure 4).

Kernel density analysis

Based on Díaz Caravantes *et al.*, (2013) methodology and using ArGis 10-8 an analysis of Kernel density was made, using a cell size of 30×30 m (Figure 8 and 9), whose principle is the continuous surfaces construction from points, in this case were used the wells distributed in the zone, as well as an analysis including the annual quantity of concessioned volume per REPDA concession, this represented areas of water use density and classified as high, medium and low enabling to establish areas where is concentrated a central pressure per Km^2 to aquifer.

To estimate the calculation of surfaces, the Kernel method uses the calculation of density observations around a pixel (Equation 1). The Kernel's quadratic function used in ArcGIS is:

$$D_{(s)} = \sum_{i=1}^n S_i \left(\frac{3}{\pi \tau^2} \right) \left[1 - \left(\frac{h_i^2}{\tau^2} \right) \right]^2 \quad (1)$$

Where τ is the radius of the circles of nearby points, h_i is the distance between point s and the observed point S_i , n is the number of observed points, and $D_{(s)}$ is the density on that point.

The results achieved allow the creation of maps that spatially distribute areas with greater pressure on the aquifer. In this research, a radius of 569.14 m was delimited to get an influence area of 1 Km^2 .

Given that the results do not have a normal distribution, they were reclassified by Jenks' natural breaks method. This reclassification creates classes where similar values cluster more effectively and increases the difference between classes. In this manner, entities are divided into classes, for which limits are established where considerable differences exist between values and data. A blue-yellow-red color ramp was utilized to represent high, medium, and low-density zones. Blue represents low-density zones, yellow represents medium, and red represents high-density zones.

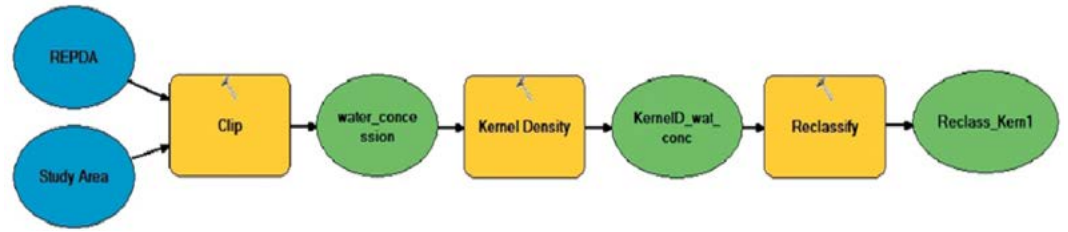


Figure 1. Work diagram to estimate the Kernel's density per Km². Blue ovals indicate that users only have to load the spectral bands of their interest. Yellow squares are geospatial processes, and green ovals are the result of each geoprocess.

Normalized differential moisture index (NDMI)

Monthly multispectral imagery from March 2023 to March 2024 was downloaded from the U.S. Geological Survey (USGS) <http://glovis.usgs.gov/> from the Operation Land Imager (OLI) Landsat 8 and 9 collection 2 level 2 instrument, Path 31, Row 43, and cloud cover less than 30%.

To calculate NDMI, the Bo-Cai Gao (1996) technique was employed, utilizing remote sensors to estimate the moisture content in vegetation, through two narrow channels located at 0.86 μm and 1.24 μm wavelengths (Equation 2). The results obtained are from -1 to 1, describing the crops' hydric stress levels (Table 1).

NDMI is calculated by the quotient of the difference between and the sum of radiation reflected within the near infrared region band (NIR) and the shortwave infrared region, using the equation:

$$NDMI = \frac{NIR - SWIR}{NIR + SWIR} \tag{2}$$

For Landsat 8 and 9 S2 L2 images NIR band corresponds to band 5 and SWIR band corresponds to band 6 (Equation 3)

$$NDMI = \frac{Band\ 5 - Band\ 6}{Band\ 5 + Band\ 6} \tag{3}$$

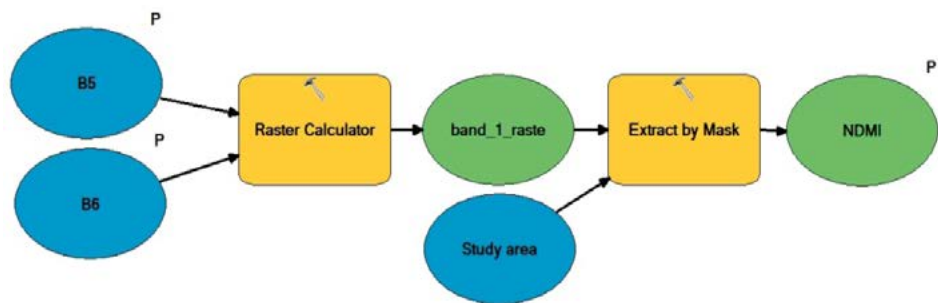


Figure 2. ArcGIS method to calculate NDMI. Blue ovals have a P superscript indicating that users only have to load spectral bands of their interest. Yellow squares are geospatial processes, and green ovals are the result of each geoprocess.

Table 1. Parameters of interpretation of NDMI results.

Value	Interpretation
-1 - -0.8	Bare ground
-0.8 - -0.6	Almost non-existent canopy cover
-0.6 - -0.4	Very low canopy cover
-0.4 - -0.2	Low and dry canopy cover
-0.2 - 0	Medium-low canopy cover and high-water stress
0 - 0.2	Medium canopy cover and high-water stress
0.2 - 0.4	Medium-high canopy cover and high-water stress
0.4 - 0.6	High canopy cover, no water stress
0.6 - 0.8	Very high canopy cover, no water stress
0.8 - 1	Full canopy cover, no water stress/flooding

Calculating this index allows the analysis of soil moisture or hydric stress periods in regional crops. Using this index, the irrigation time and its intensity could be estimated during rainless periods, as well as in extreme or abnormal drought conditions.

Precipitation analysis

National Meteorological Service (SMN) (Servicio Meteorológico Nacional, in Spanish) is the government agency responsible for recording and providing weather information to the national and local level of Mexican Republic, SMN constantly monitors atmospheric variables through 2,400 active meteorological stations distributed throughout the country and has a monthly database rainfall from meteorological station, in this study the information was taken from Tejamén station (10083), Guatimapé station (10137) and Coneto de Comonfort station (10129), using ArcGIS 10.8 algorithms, Thiessen polygons were created to establish the regionalization of rainfall in the study area (Figure 3), the area of influence of each station was calculated and multiplied by the monthly precipitation data, with this information, histograms were created to know the trend of rainfall in the area and identify extreme drought phenomena in the region.

Using a comparative analysis of precipitate volume and NDMI corresponding to the agricultural cycle 2023 was possible to visualize anthropogenic influence on the humidity of the study area caused by periods of low water and drought.

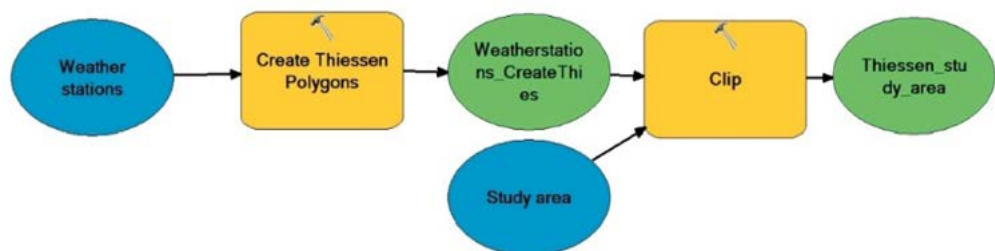


Figure 3. Method utilized to calculate precipitate volume in the study area. Blue ovals indicate that users only have to load the spectral bands of their site of interest. Yellow squares are geospatial processes, and green ovals are the result of each geoprocess.

RESULTS AND DISCUSSION

Seven earth fissures were visually detected using Google Earth (GE) satellite images (Figure 4). Table 2 shows the appearance date and the actual extension of the main fissures according to available information for GE

In accordance with Galloway and Burbey (2011), subsidence caused by groundwater extraction and aquifer compaction is an almost imperceptible phenomenon. The San Joaquin Valley, California, has experienced 9-meter subsidence from 1925 to 1975; well drilling for groundwater use is the principal cause.

Figuroa-Miranda *et al.* (2018) report that Mexico has 99 cities affected by subsidence. Mexico State presents the significant quantities of affected areas, with 17, followed by Jalisco State and Chihuahua State, with 16 and 11, respectively.

The evolution of Earth's fissures is available in the annexes link.

Hydrologic analysis

The weather in the study area indicates that the main precipitation occurs in summer (July, August, and September) with an average annual rainfall of 465.7 mm. Based on meteorological station data and Thiessen polygons, the precipitation regime in the study area over the last few years exhibits a rainfall trend, as illustrated in Figure 5.

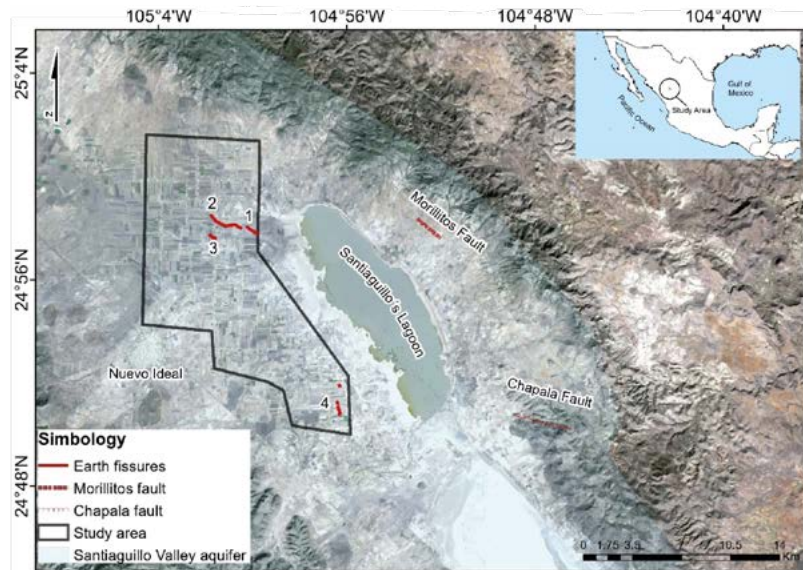


Figure 4. The study area is located inside the Laguna de Santiaguillo watershed, since 2012 is considered a Ramsar Wetland and is cataloged as an Area of Importance for Bird Conservation (AICA).

Table 2. Characteristics of earth fissures.

Fissures	Appearance date	Extension m
1	September 2023	1,002
2	August 2019	2,758
3	September 2019	443
4	January 2021	723

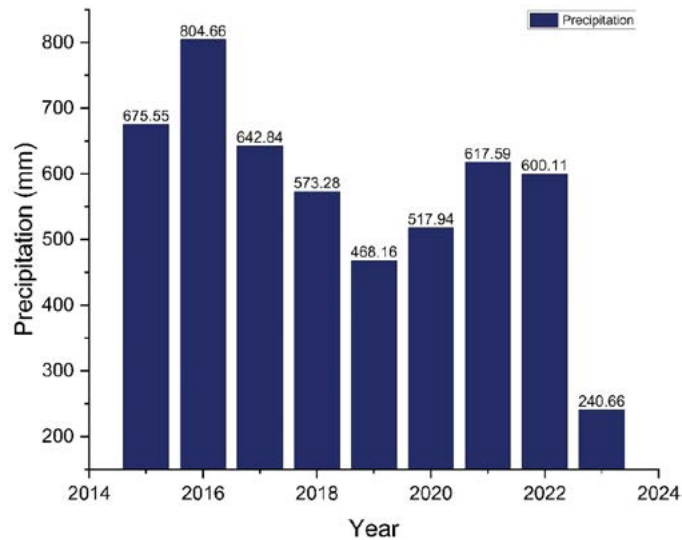


Figure 5. Rainfall volume in the study area over the past 9 years (mm per year).

The aquifer's natural conditions limit the recharge exclusively to precipitation inside the watershed. In the year 2023, rainfall decreased below average, provoking drought conditions in the study area. One of the principal effects of this meteorological phenomenon is the increase in groundwater extraction, which is destined to cover crops' hydraulic needs (Simon Wang *et al.*, 2016).

Due to geological, geophysics, and hydrogeological conditions, Valle de Santiaguillo is defined as a free, heterogeneous, anisotropic aquifer. The upper portion is made of alluvial, fluvial, foothill, lacustrine, and aeolian sediments, as well as polygenic conglomerates (Figure 5), with thicknesses that vary several meters at the center of the valley and gradually decrease towards the vicinity of the mountain range (CONAGUA).

Conditions described by CONAGUA coincide with Zhu *et al.* (2024) definition, which establishes that overexploitation of hydric resources coming from aquifers in compressible alluvial deposits could create subsidence phenomena, which, under specific hydrogeological conditions, are associated with earth fissures. Pacheco-Martínez *et al.* (2013) describe similar geological conditions in Aguascalientes, where earth fissure formation has also occurred.

Groundwater uses

Valle de Santiaguillo aquifer is located inside the endorheic Laguna de Santiaguillo watershed (Figure 6) whose precipitations form runoffs that flow into the lagoon body of the area, and the recharge depends entirely on the rainfalls, CONAGUA, 2020 estimates an annual average recharge of 44.9 hm³ and 115.13 hm³ per year are concessioned, based on this information it is evident an aquifer overexploitation condition.

In the study area, according to REPDA, 1,097 registered wells have concessioned a total of 34.08 hm³ annual volume, with predominance of agricultural concessions in the number of registers. Groundwater concessions located in the study area (16,790 ha)

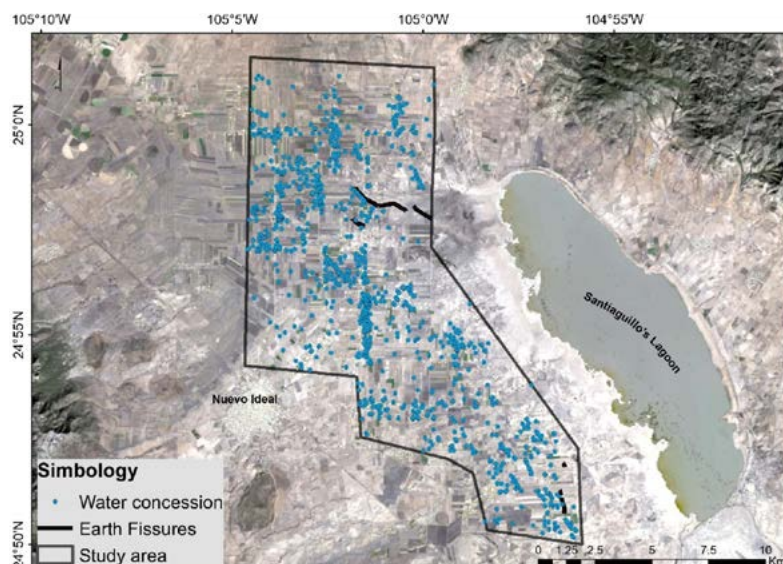


Figure 6. Groundwater concessions within the study area.

represent 30% of the aquifer's (250,400 ha) total volume concessioned, which represents a significant concentration of groundwater extraction in the area.

Kernel density analysis

Number of concessions per Surface unit

Based on REPDA information, the annual concessions volume from the analysis per right was observed to have an average density of 6.46 wells/Km², spatially distributed. 82% (138.40 Km²) of the study area has a low well density with an accumulation from 0-12 wells/Km², 15% (25.10 Km²) has medium density with values from 13-32 wells/Km², and 3% (4.40 Km²) has high density with values from 33-88 wells/Km² (Figure 7).

Concessioned volume per surface unit

It was determined that the lowest volumes per square kilometer are in areas where annual concessions range from 0 to 228,654 m³, corresponding to 70% (117.63 km²). On the other hand, 24% (40.17 Km²) of the study area has medium concessions with annual volume values from 228,655 to 705,846 m³; finally, the areas with higher yearly volume concessions fluctuate from 705,847 to 2'535,084 m³. Showing that the study area is under intense pressure due to groundwater extractions (Figure 8).

Comparing Kernel density by number of concessions and volume concessioned, it's observed that earth fissures are mainly localized in areas where extraction densities and number of wells are from medium to high. This suggests that pressure exerted on the aquifer in these zones has favored the fissures opening directly related to the location of the concentration of groundwater concessions.

Based on the result analysis, the pressure on the aquifer has encouraged the formation of earth fissures in this area. This phenomenon has already been described by other researchers, who mention that high levels of well drilling, prolonged periods

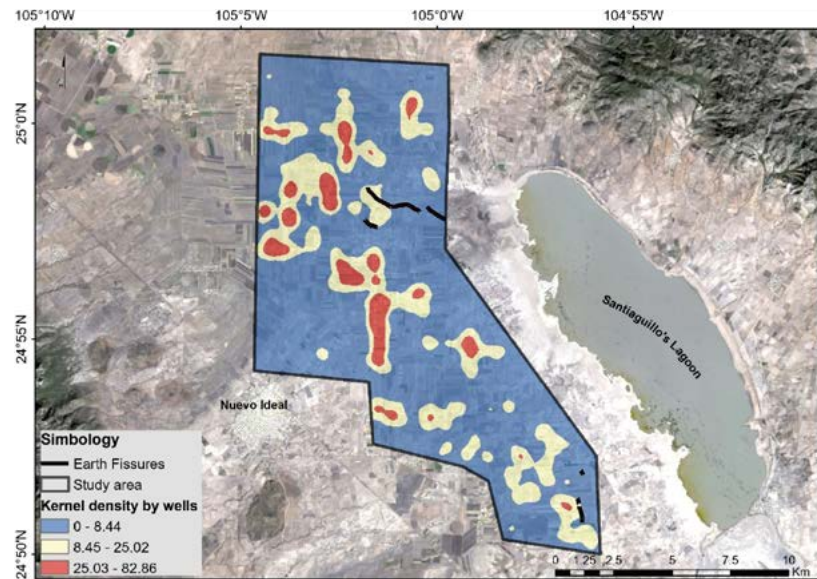


Figure 7. Kernel's density per concession.

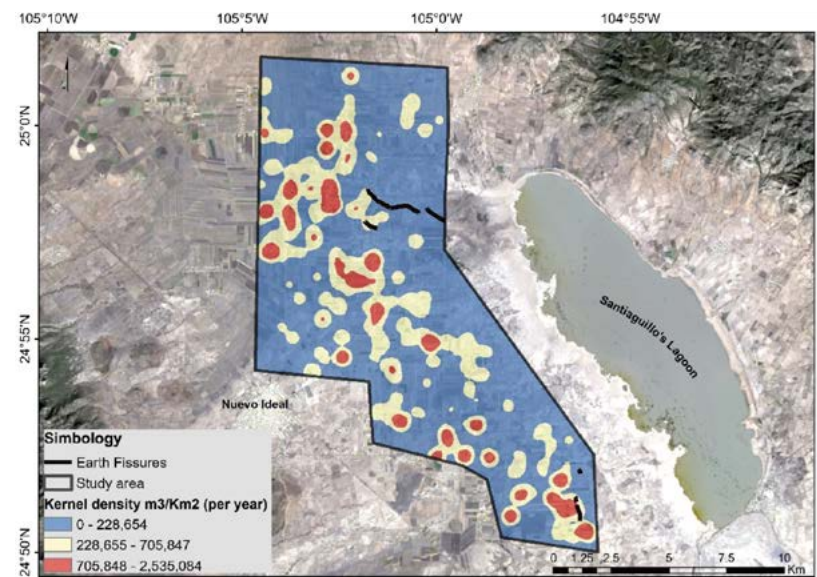


Figure 8. Kernel's density per annual volume concessioned.

of water pumping, an arid or semiarid climate, and a decrease in static levels are factors that facilitate the formation of these failures (Burbey, 2002; Pacheco-Martínez *et al.*, 2013).

Normalized differential moisture index (NDMI)

According to the obtained values for the agriculture cycle 2023, NDMI spatial distribution presents important variations in specific areas during the months of the agriculture cycle; however, it is observed that zones containing major moisture quantities are agricultural production areas, making evident that the moisture patron

is strongly related to agricultural activities in parcels that have groundwater concessions (Figure 9).

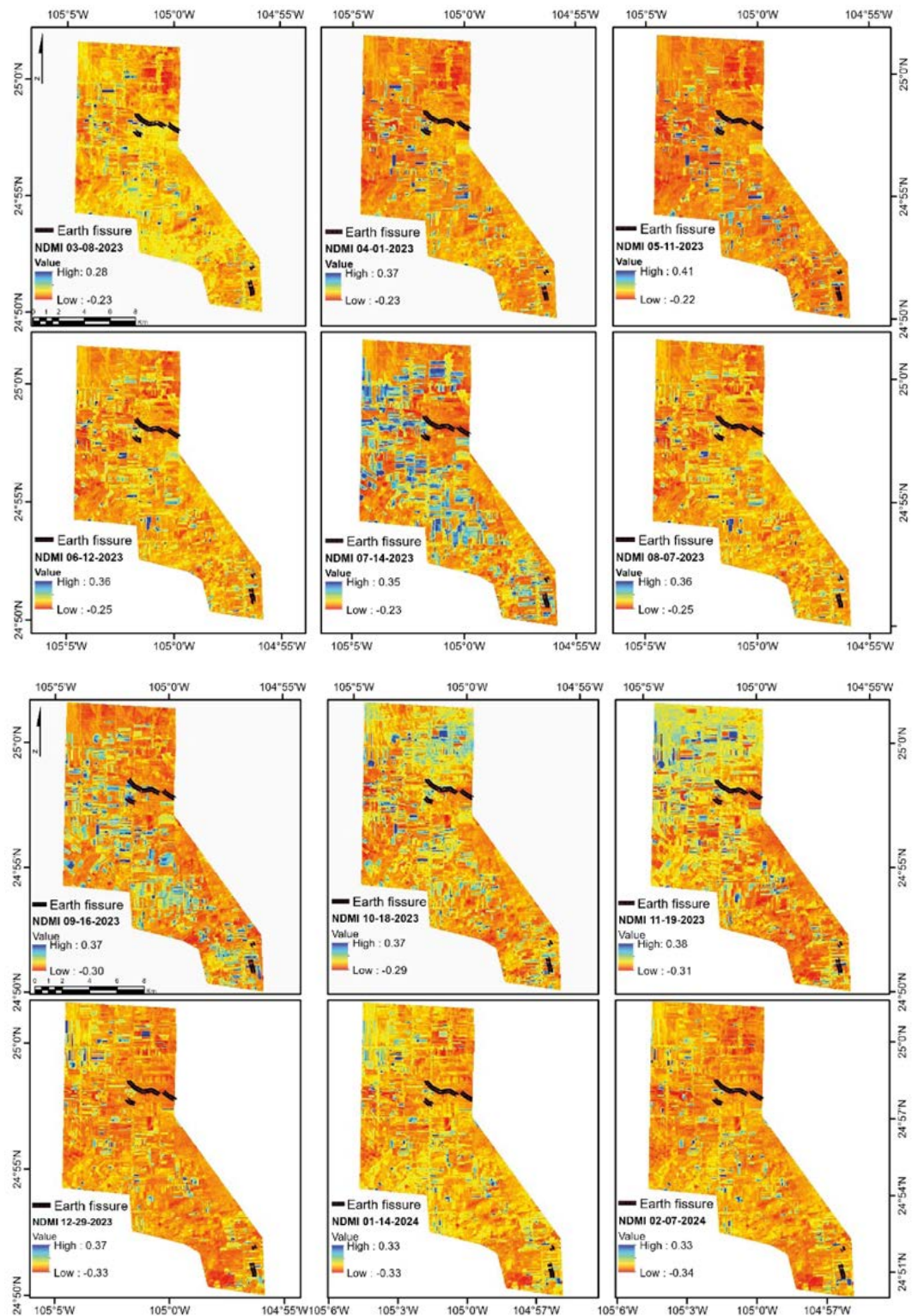


Figure 9. NDMI of the agricultural cycle 2023-2024 in the study area

The average NDMI value for the 2023-2024 agricultural cycle is close to 0, indicating a generalized high hydric stress, with an increase in NDMI value in July, which is attributed to the start of the rainy season in the region (Table 4). However, some parcels present higher moisture levels than others, which is why they have been irrigated even during the rainy season.

The highest NDMI in a parcel (0.41) was observed in May (Table 4), being an abnormal value according to climatological conditions of the region. May is within the drought season, and the main rainfall occurs in the summertime. Thus, the quantity of observed humidity might be related to irrigation activities in parcels

Agricultural activities within the spring-summer cycle, shown in the images (May to September), present a major humidity presence derived from groundwater irrigation. However, winter cycle irrigation activities, although minor, are still present in located parcels with great correspondence, in both cycles, with earth fissure localization, particularly in the south of the study area.

Histograms of NDMI are available in the annexes link.

Comparison analysis between precipitation and NDMI shows a higher humidity level in May and November; this does not coincide with the natural humidity peaks caused by rain.

Sustained hydric stress, evidenced by low average NDMI values, leads to constant groundwater extraction for irrigation, favoring decreasing phreatic levels (van Opstal *et al.*, 2022).

Aquifers overexploitation may lead to land differential subsidence, manifesting through fissure formation (Alvarez *et al.*, 2001), besides, moistening cycles for irrigation followed by intense evapotranspiration generate ground tensions that aggravate the fracturing, thereby, fissures spatial distribution might be related correlated to, not only to groundwater extraction patterns, but also to superficial humidity captured by NDMI (Zang *et al.*, 2019).

Table 4. Monthly comparison of NDMI of the agricultural cycle 2023-2024.

Date	Maximum	Minimum	Average
03/08/2023	0.28	-0.23	-0.07
04/01/2023	0.36	-0.25	-0.06
05/11/2023	0.41	-0.22	-0.06
06/12/2023	0.35	-0.25	-0.07
07/14/2023	0.35	-0.23	-0.02
08/07/2023	0.35	-0.25	-0.07
09/16/2023	0.37	-0.29	-0.04
10/18/2023	0.37	-0.29	-0.02
11/19/2023	0.38	-0.31	-0.008
12/29/2023	0.37	-0.32	-0.07
01/14/2024	0.33	-0.33	-0.08
02/07/2024	0.33	-0.34	-0.09

According to the analysis of the relation of precipitation and NDMI of 2023 agricultural cycle, maximum NDMI values in dry season (January to May) are comparable to the rainy months (July to September), being evident that anthropogenic activities, especially intensive use groundwater for irrigation, allows to maintain crops humidity levels, even in natural hydric deficit conditions (Figure 10).

Based on the results obtained, it's observed that the study area is subject to strong anthropogenic pressure, and the use of both water and soil resources is evident, as agricultural activities are constantly developed throughout the year. This continuous exploitation not only affects water availability but may also contribute to soil degradation processes, such as earth fissures and subsidence, which are related to decreasing phreatic levels and intensive irrigation management (Faunt *et al.*, 2024).

Based on Tukey test, June and August do not present significant difference in their precipitations, it's also observed that during agricultural cycles there are hydric stress conditions in the study area due to NDMI average is close to 0, from the analyzed period a large amount of aberrant data was obtained that increased the NDMI, and making a comparative it's observed that spatial distribution matches to agricultural parcels that have a groundwater source for irrigation supply (Figure 11).

If the tendency of groundwater extractions in the Laguna de Santiaguillo aquifer continues, the consequences could be similar to those of aquifers on Chile, where research has exposed that a constant exploitation of groundwater might provoke severe effects on hydrological systems, such as disconnection between the aquifer and natural surface water runoff, modifying the aquifer's discharge that supplies wetlands or rivers in the dry season. Finally, excessive groundwater extraction from the aquifer storage rather than natural recharge increases the period required to replenish naturally (Jódar *et al.*, 2024).

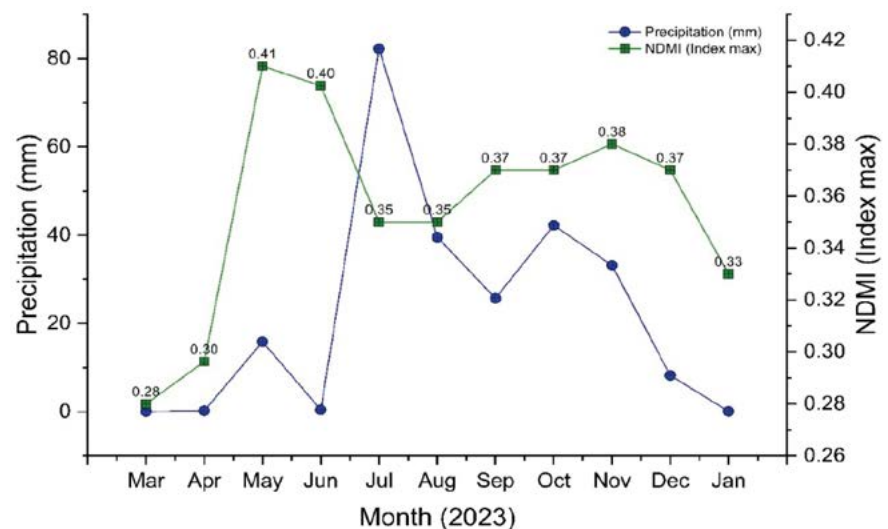


Figure 10. Monthly comparison between NDMI and precipitation

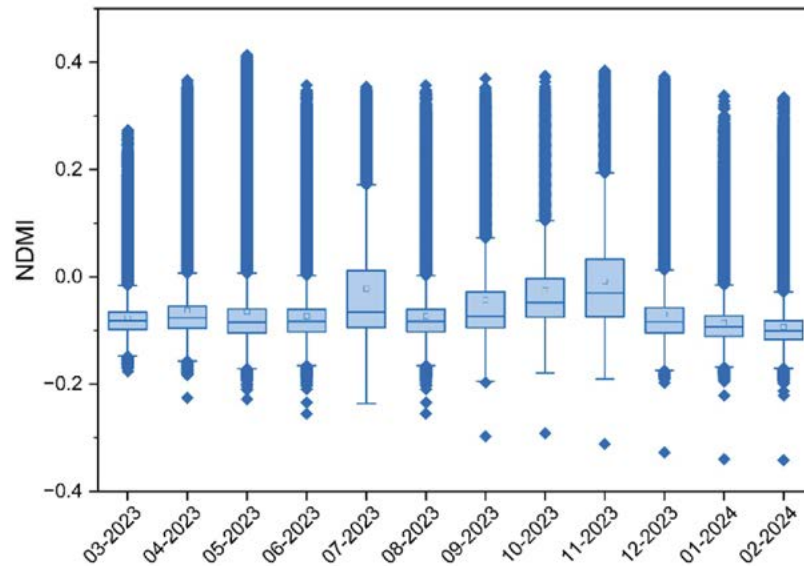


Figure 11. NDMI temporal variation in the spring-summer and autumn-winter agricultural seasons.

CONCLUSIONS

The study area presents significant pressure related to groundwater concessions. According to the Kernel analysis density, it was detected that one of the regions with major volume concessioned density coincides with one of the earth fissures detected through spatial images. These results suggest that this zone presents an advanced subsidence phenomenon.

A constant groundwater extraction throughout the year was detected, as NDMI values surpass monthly natural means, and the areas with major humidity correspond to agricultural parcels that depend on the aquifer. This affects the phreatic levels and may cause a decrease in water volume, pore compression, lower storage capacity, and changes in soil layers.

In 2023, the decrease in rainfall provoked a lower aquifer recharge. This situation, in addition to increasing agricultural groundwater demand, has generated saturation and drying of soil, which in turn has increased the risk of fissure appearance due to the increased efforts on its structure.

The study area's natural characteristics, such as a semiarid climate, the aquifer's lithology characteristics, and the significant groundwater demand destined for agricultural activities, correspond to identified factors in specialized literature that facilitate earth fissure formation.

The location of the longest earth fissure is within a zone that is subject to extensive humidity and drying variations. This condition, in addition to high concession density per square kilometer, can be related to relevant changes in the soil stress state over a considerable area.

ACKNOWLEDGMENT

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ANNEXES

https://docs.google.com/document/d/1IWjf4bsSwpR6zUozK_jN2f6meTvdcH9y/edit?usp=drive_link&oid=113470542075061576459&rtpof=true&sd=true

REFERENCES

- Alvarez, A., Garnica, P., Martínez, G.E.I., 2001. Metodología Para Determinar La Influencia De La Extracción De Agua Subterránea En La Infraestructura Del Transporte. 1-70.
- Barati, A.A., Azadi, H., Scheffran, J., 2019. A system dynamics model of smart groundwater governance. *Agric. Water Manag.* 221, 502-518. <https://doi.org/10.1016/j.agwat.2019.03.047>
- Burbey, T., 2002. The influence of faults in basin-fill deposits on land subsidence, Las Vegas Valley, Nevada, USA. *Hydrogeol. J.* 10, 525-538. <https://doi.org/10.1007/s10040-002-0215-7>
- Chakraborty, A., Basu, P., 2025. Intensive agriculture influences functional diversity, redundancy and trait profile of bee community and interacting plant community in a tropical agricultural landscape. *Agric. Ecosyst. Environ.* 383, 109544. <https://doi.org/10.1016/j.agee.2025.109544>
- CONAGUA, 2020. S Ubdirección G Eeneral T Écnica 32.
- Dash, S.K., Sembhi, H., Langsdale, M., Wooster, M., Dodd, E., Ghent, D., Sinha, R., 2025. Assessing the field-scale crop water condition over an intensive agricultural plain using UAV-based thermal and multispectral imagery. *J. Hydrol.* 655, 132966. <https://doi.org/10.1016/j.jhydrol.2025.132966>
- Fan, B., Shi, X., Luo, G., Hellwich, O., Ma, X., Shang, M., Wang, Y., Ochege, F.U., 2025. Ground subsidence and disaster risk induced by groundwater overexploitation: A comprehensive assessment from arid oasis regions. *Int. J. Disaster Risk Reduct.* 119, 105328. <https://doi.org/10.1016/j.ijdrr.2025.105328>
- Faunt, C.C., Traum, J.A., Boyce, S.E., Seymour, W.A., Jachens, E.R., Brandt, J.T., Sneed, M., Bond, S., Marcelli, M.F., 2024. Groundwater Sustainability and Land Subsidence in California's Central Valley. *Water* 16, 1189. <https://doi.org/10.3390/w16081189>
- Figuroa-Miranda, S., Tuxpan-Vargas, J., Ramos-Leal, J.A., Hernández-Madrigal, V.M., Villaseñor-Reyes, C.I., 2018. Land subsidence by groundwater over-exploitation from aquifers in tectonic valleys of Central Mexico: A review. *Eng. Geol.* 246, 91-106. <https://doi.org/10.1016/j.enggeo.2018.09.023>
- Galloway, D.L., Burbey, T.J., 2011. Review: Regional land subsidence accompanying groundwater extraction. *Hydrogeol. J.* 19, 1459-1486. <https://doi.org/10.1007/s10040-011-0775-5>
- Jódar, J., Urrutia, J., Herrera, C., Custodio, E., Martos-Rosillo, S., Lambán, L.J., 2024. The catastrophic effects of groundwater intensive exploitation and Megadrought on aquifers in Central Chile: Global change impact projections in water resources based on groundwater balance modeling. *Sci. Total Environ.* 914. <https://doi.org/10.1016/j.scitotenv.2023.169651>
- Pacheco-Martínez, J., Hernandez-Marín, M., Burbey, T.J., González-Cervantes, N., Ortíz-Lozano, J.Á., Zermeño-De-Leon, M.E., Solís-Pinto, A., 2013. Land subsidence and ground failure associated to groundwater exploitation in the Aguascalientes Valley, México. *Eng. Geol.* 164, 172-186. <https://doi.org/10.1016/j.enggeo.2013.06.015>
- Redwan, M., Abdel Moneim, A.A., 2016. Factors controlling groundwater hydrogeochemistry in the area west of Tahta, Sohag, Upper Egypt. *J. African Earth Sci.* 118, 328-338. <https://doi.org/10.1016/j.jafrearsci.2015.10.002>
- SEMARNAT, 2010. El agua en México, Academia Mexicana de Ciencias. Ciudad de México.
- Simon Wang, S.-Y., Lin, Y.-H., Gillies, R.R., Hakala, K., 2016. Indications for Protracted Groundwater Depletion after Drought over the Central Valley of California*,+. *J. Hydrometeorol.* 17, 947-955. <https://doi.org/10.1175/JHM-D-15-0105.1>
- Song, M., He, W., An, M., Fang, X., Wang, B., Ramsey, T.S., 2023. Toward better agricultural grey water footprint allocation under economy-resource factors constraint. *Ecol. Indic.* 154, 110806. <https://doi.org/10.1016/j.ecolind.2023.110806>
- Van Camp, M., Radfar, M., Walraevens, K., 2010. Assessment of groundwater storage depletion by overexploitation using simple indicators in an irrigated closed aquifer basin in Iran. *Agric. Water Manag.* 97, 1876-1886. <https://doi.org/10.1016/j.agwat.2010.02.006>
- van Opstal, J.D., Neale, C.M.U., Hipps, L.E., 2022. Evaluating the adaptability of an irrigation district to seasonal water availability using a decade of remotely sensed evapotranspiration estimates. *Agric. Water Manag.* 261, 107383. <https://doi.org/10.1016/j.agwat.2021.107383>
- Whittemore, D.O., Butler, J.J., Bohling, G.C., Wilson, B.B., 2023. Are we saving water? Simple methods for assessing the effectiveness of groundwater conservation measures. *Agric. Water Manag.* 287, 108408. <https://doi.org/10.1016/j.agwat.2023.108408>

- Yan, Y., Shi, H., Miao, Q., Zhao, Y., Nie, X., Li, Z., Pan, M., Feng, W., Gonçalves, J.M., Duarte, I.M., 2025. Evolution of chemical characteristics and irrigation suitability of groundwater in arid and semi-arid regions. *Agric. Water Manag.* 311, 109361. <https://doi.org/10.1016/j.agwat.2025.109361>
- Zang, M., Peng, J., Qi, S., 2019. Earth fissures developed within collapsible loess area caused by groundwater uplift in Weihe watershed, northwestern China. *J. Asian Earth Sci.* 173, 364-373. <https://doi.org/10.1016/j.jseae.2019.01.034>
- Zhu, L., Li, J., Gong, H., Ye, M., Dai, Z., Li, X., Teatini, P., 2024. Simulating earth deformation evolution caused by groundwater pumping through ordinary state-based Peridynamics method. *J. Hydrol.* 634, 131133. <https://doi.org/10.1016/j.jhydrol.2024.131133>



Animal Welfare, Artificial Intelligence and Rural Reality in Mexico

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ABSTRACT

Objective: To analyze the compatibility between existing livestock infrastructure and requirements for digital monitoring technologies in Mexico, proposing an adaptive infrastructure model for gradual technological modernization.

Design/methodology/approach: Documentary analysis of secondary data from production units was conducted. Technical parameters from manufacturer specifications and scientific literature (2015-2024) were synthesized. Bioclimatic design principles from international standards were adapted to meet the specific conditions of Mexico. The Flexible Infrastructure for Animal Welfare (IFBA) conceptual model was developed through the synthesis of technical requirements and contextual constraints.

Results: Analysis revealed that around 70% of Mexican livestock facilities consist of perimeter fences with improvised roofing, presenting fundamental incompatibilities with sensor operation requirements. Temperature fluctuations of 18-22 °C in traditional installations exceed calibration tolerances, while 92% lack adequate ventilation design. The proposed IFBA model structures interventions in three integrated components: passive bioclimatic design, technological pre-installations, and evolutionary flexibility, generating immediate welfare improvements while preparing for future technology adoption.

Limitations on study/implications: Analysis based on secondary data and technical literature synthesis requires field validation before large-scale implementation.

Findings/conclusions: Physical infrastructure constitutes the primary limiting factor for livestock modernization in Mexico, surpassing economic or educational barriers. The principle “precision cannot be monitored in imprecise spaces” synthesizes the fundamental incompatibility that has been identified. Investment in appropriate infrastructure generates immediate returns through improved animal welfare while establishing necessary conditions for successful technological modernization.

Keywords: livestock infrastructure, animal welfare, precision livestock farming, bioclimatic design, tropical livestock

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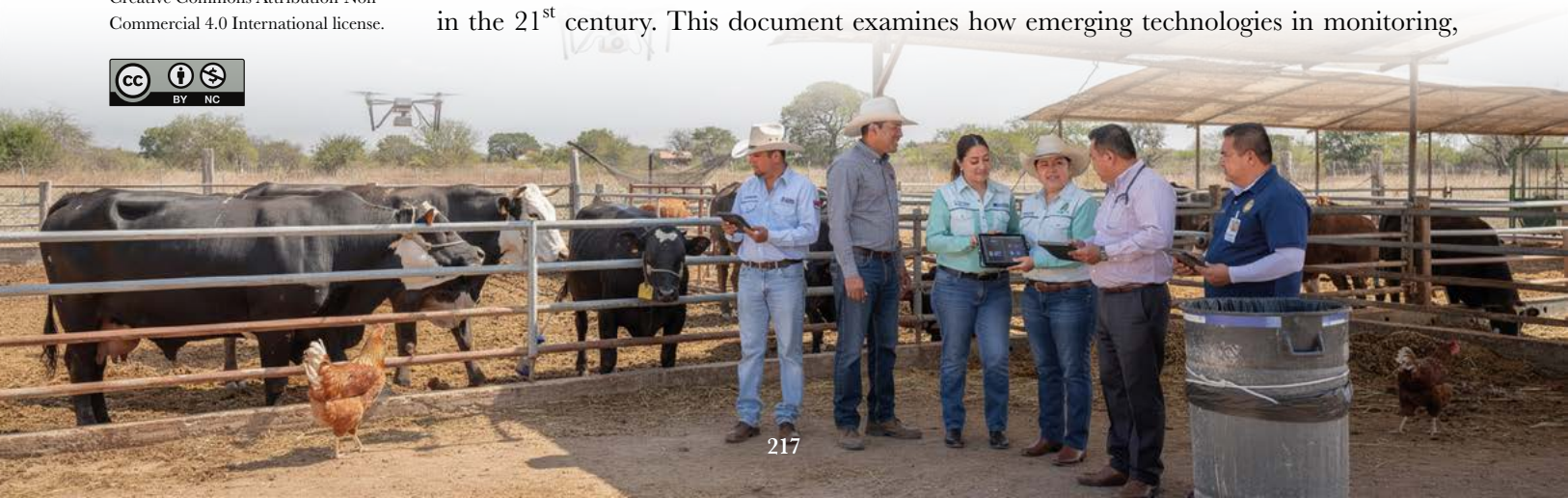
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INTRODUCTION

The convergence of animal welfare, artificial intelligence, and rural development constitutes one of the most complex challenges facing the Mexican agricultural sector in the 21st century. This document examines how emerging technologies in monitoring,



data analysis, and automation can transform animal production systems, simultaneously improving animal welfare, economic productivity, and environmental sustainability, while considering the structural realities of the Mexican countryside.

Mexico faces a fundamental paradox: while precision agriculture technologies and artificial intelligence systems promise to revolutionize livestock production, a high percentage of national production units operate with limited resources, deficient infrastructure, and significant technological gaps. This disparity is not merely technical but profoundly social, economic, and cultural, requiring approaches that transcend simple technology transfer.

A frequently ignored but fundamental aspect is physical infrastructure. Animal welfare assessments in Mexican livestock systems reveal significant infrastructure deficiencies. Academic studies report that small-scale production units—which represent a significant proportion of the sector—present infrastructure levels of 36% relative to the required optimum (Hernández-López *et al.*, 2008), while assessments using international protocols classify 100% of studied units barely at the ‘Acceptable’ level, without reaching higher welfare categories (Mota-Rojas *et al.*, 2017). This situation evidences a gap between international animal welfare standards established by the OIE and actual production conditions, particularly in aspects of thermal comfort, vital space, and expression of natural behaviors.

This analysis integrates three critical dimensions. First, it examines the conceptual evolution of animal welfare from Anglo-European, Latin American, and Mexican perspectives, identifying convergences and specificities that inform the development of regulatory frameworks and production practices. Second, it evaluates the state of the art in animal monitoring technologies, ranging from basic sensors to distributed artificial intelligence ecosystems, and analyzes their transformative potential and practical limitations. Third, it diagnoses the reality of rural Mexico, identifying structural barriers and opportunities for technological modernization that are culturally pertinent, economically viable, and socially inclusive.

The central hypothesis argues that successful implementation of advanced technologies in the Mexican countryside requires not the uncritical importation of external models, but the development of hybrid solutions that integrate traditional knowledge with technological innovation, that recognize the heterogeneity of the national territory, and that prioritize animal welfare as an essential component of productive sustainability. Specifically, this document argues that adaptive infrastructure constitutes the necessary bridge between traditional knowledge and technological innovation in Mexican livestock production.

MATERIALS AND METHODS

A systematic search was conducted following the PRISMA protocol adapted for agricultural sciences. Consulted databases included Web of Science, CAB Abstracts, Scopus, and SciELO México (for the period 2015-2024 for technologies and 2010-2024 for the Mexican context). The strategy employed terms: (“precision livestock” OR “smart farming”) AND (“animal welfare”) AND (“artificial intelligence” OR “IoT”) AND (“Mexico” OR “Latin America”). Studies with field applications were included, excluding

those that were exclusively laboratory-based. Scientific articles, technical reports, and normative documents were analyzed.

Development of Conceptual Models

The economic models presented are theoretical exercises based on parameters reported in the literature, without empirical validation. They should be considered as working hypotheses, not as definitive projections.

Infrastructure Evaluation

Secondary data from documented production units and published literature on livestock design parameters were analyzed.

RESULTS AND DISCUSSION

Conceptual framework of animal welfare-historical evolution and theoretical perspectives

The concept of animal welfare has evolved significantly from the Brambell Report (1965) to current multidimensional conceptualizations. The Anglo-European perspective, developed in post-industrial societies with more than 60 years of evolution, has prioritized theoretical-conceptual refinement and research on positive affective states. This paradigm establishes five fundamental freedoms that have guided global legislation and practice: freedom from hunger and thirst, from discomfort, from pain and disease, to express normal behavior, and from fear and distress.

Fraser (2008) proposes three complementary conceptions of welfare: biological functioning (health and productivity), affective states (subjective experiences), and natural life (expression of species-specific behaviors). This integration recognizes that welfare is not unidimensional, but emerges from the complex interaction among physical health, mental experience, and capacity to express natural behaviors.

Broom and Fraser (2015) define welfare as the state of an individual in relation to its attempts to adapt to its environment, establishing that it is scientifically measurable through physiological, behavioral, and preference indicators. This scientific approach provides objective bases for evaluating and improving the living conditions of animals in production systems.

The Latin American and Ibero-American Perspective

Latin America has developed a pragmatic and adaptive model that integrates traditional knowledge with modern standards. According to Mota-Rojas and Orihuela (2018), this perspective recognizes the region's economic and structural limitations while seeking progressive and contextualized improvements. The Latin American position is characterized by:

- **Integration of traditional knowledge:** Valuation of ancestral management practices that, although not scientifically formalized, have demonstrated effectiveness in maintaining animal health and productivity.

- **Graduality in implementation:** Recognition that improvements must be economically viable and socially acceptable to guarantee sustained adoption.
- **Emphasis on training:** Prioritization of producer training as a more effective strategy than normative imposition.

Gallo and Tadich (2010) document how Latin American countries have adapted universal principles to local realities, generating innovative solutions such as silvopastoral systems that simultaneously improve animal welfare, productivity, and ecosystem services.

The Mexican Paradigm

Mexico has constructed a distinctive technical-normative synthesis that navigates the tensions between cultural tradition and modern standards. Mota-Rojas *et al.* (2016) establish that the Mexican model integrates advanced neuroscience with cultural sensitivity, developing innovative methodologies such as infrared thermography for non-invasive pain evaluation.

The Mexican regulatory framework, articulated through Official Mexican Norms (NOM-033-SAG/ZOO-2014 and NOM-062-ZOO-1999), establishes specific standards while allowing flexibility in their implementation according to production contexts. This approach recognizes the diversity of national production systems, from technified commercial livestock operations to family backyard production.

The convergence of these perspectives reveals that global animal welfare does not require homogenization, but intelligent articulation of diversity. The emerging integrative model maintains universal ethical principles as a non-negotiable core while allowing contextual adaptation in implementation strategies.

Emerging technologies for animal monitoring

Precision Livestock Farming

Precision livestock farming (PLF) constitutes a transformative paradigm that integrates digital technologies for continuous monitoring and individualized management. Berckmans (2017) establishes that this approach represents the transition from periodic observation toward continuous 24/7 surveillance, enabling early detection of problems and individualized optimization.

The revolutionary concept of the “animal as sensor” recognizes that animals themselves, through their behavioral and physiological manifestations, constitute the most reliable indicators of their state. Norton *et al.* (2019) proposed the concept of “digital twins,” which are virtual representations that reflect the state of the real animal through continuous updates with multisensory data.

The evolution toward “Digital Livestock 4.0” (Neethirajan, 2023) integrates the Internet of Things (IoT) for ubiquitous connectivity, Artificial Intelligence for predictive analysis, big data analysis for pattern identification, blockchain for immutable tracing, and augmented reality for complex information visualization.

Sensor Systems

The taxonomy of sensors for animal monitoring is structured in three categories according to their spatial relationship:

- **Sensors on the animal:** wearable devices such as accelerometers for activity monitoring, global positioning systems for grazing patterns, and physiological sensors for vital parameters. These devices face challenges of miniaturization, durability, and energy autonomy.
- **Sensors off the animal:** computer vision systems for morphological evaluation, microphone arrays for vocalization analysis, and environmental sensors for microclimate characterization. The main advantage is avoiding the stress of handling for device placement.
- **Sensors through the animal:** On-line analysis of products (milk composition), sensors in infrastructure (smart feeders), and individual consumption measurement systems. These systems provide valuable information without direct intervention. Schillings *et al.* (2021) propose a comprehensive framework for evaluating welfare through technologies, structured in five measurable domains: nutritional, environmental, behavioral, health, and affective states. This multidimensional approach recognizes the complexity of animal welfare.

Internet of Things in Livestock

Halachmi *et al.* (2019) conceptualize Smart Animal Agriculture as the convergence of real-time sensors, ubiquitous connectivity, intelligent analysis, and automatic actuation. The architecture is structured in layers:

- **Perception layer:** Integrates wearable devices, environmental sensors, fixed infrastructure, and robotic systems that generate massive flows of heterogeneous data.
- **Network layer:** Implements differentiated protocols according to requirements. Bluetooth Low Energy (BLE) for short range with minimal consumption, WiFi for high data throughput, LoRaWAN for extensive livestock operations with ranges up to 15km, and cellular technologies for ubiquitous coverage.
- **Application layer:** Transforms data into actionable intelligence through distributed processing architectures. Local edge computing executes immediate detection algorithms, intermediate fog nodes aggregate data before transmission, and cloud computing services provide massive computational capacity.

The emerging concept of the “Internet of Living Things” (IoLT) transcends technology to focus on animal welfare, creating systems that not only monitor but understand and respond to individual needs.

The massive generation of data by sensor systems requires processing capabilities that transcend traditional statistical analysis. Artificial intelligence in precision livestock operates through deep learning architectures that identify complex patterns imperceptible

to human observers (Neethirajan, 2024). This transformative capacity, however, depends fundamentally on the quality and consistency of input data, establishing a critical dependency on the real conditions of physical capture infrastructure.

Computer vision systems based on convolutional neural networks (CNN) have demonstrated capacity for contactless individual identification, automatic body condition scoring, and early lameness detection through gait analysis (Bezen *et al.*, 2024). These applications require controlled lighting conditions with variation less than 15% to maintain precision above 90%. In contrast, traditional Mexican livestock facilities present light variations of 300-500% between shaded and exposed areas, generating error rates that invalidate the practical utility of these systems.

Machine learning applied to behavioral time series enables prediction of critical events with time horizons useful for intervention. Romadhonny *et al.* (2024) report 85-92% precision in estrus detection through random forest algorithms trained with triaxial accelerometer data. However, these models assume data continuity and stable environmental conditions. The Mexican reality of frequent electrical interruptions and thermal fluctuations of 18-22 °C introduces discontinuities and noise that degrade predictive precision to levels no better than traditional visual observation.

Optimization through reinforcement learning represents the most advanced frontier of AI application in livestock, enabling dynamic adjustment of multiple variables to maximize complex objective functions such as feed efficiency while considering animal welfare (García *et al.*, 2023). These systems require continuous high-fidelity feedback and the capacity to actuate on the environment. Traditional Mexican infrastructure lacks both prerequisites, making even pilot implementations of these approaches impossible.

Infrastructure quality problems

Materials and Construction Standards

In southern Veracruz, 88% of livestock operations function as free-grazing systems in extensive pastures, operating without any infrastructure or investment in intensive management practices (Lazos-Chavero *et al.*, 2024). This represents the predominant construction reality: absence of formal structures rather than low-quality buildings.

Traditional storage structures contribute to grain losses between 5-25% of total production, mainly due to grain moisture and problems related to fungi and pests (World Bank, 2020). These traditional structures demonstrate: inadequate moisture barriers, deficient ventilation systems, vulnerable roofing materials, and permeable wall construction.

Characteristics of Infrastructure in the Mexican Tropics

According to González-Padilla and Dávalos-Flores (2018), in the Mexican tropical region there are more than 500 thousand production units, with more than 80% operating with fewer than 30 cows. This fragmentation is reflected in precarious infrastructure where producers, despite having very limited financial resources, possess enormous wealth in their lands and livestock that does not translate into investment in adequate facilities.

Durability and Service Life Limitations

Extensive and low-infrastructure production systems (C3) in southern states, including Veracruz, Chiapas, and Tabasco, are characterized by markedly deficient conditions and limited technology (Villarroel-Molina *et al.*, 2025). The infrastructure disparity manifests in:

- **Persistence of Regional Infrastructure:** Despite the economic efficiency of technologically advanced systems in northern states, extensive southern systems demonstrate resilience to economic crises while maintaining deficient infrastructure conditions (Villarroel-Molina *et al.*, 2025).
- **Structural Inadequacy:** Current cattle slaughter facilities operate with 46% inefficiency, suggesting not only underutilization but potentially inadequate maintenance of existing installations (Hernández-Martínez *et al.*, 2021).
- **Underutilized Biological Capacity:** Herd productivity is below 50% of its biological capacity, reflecting structural limitations that prevent optimizing productive potential (González-Padilla and Dávalos-Flores, 2018).

Maintenance and Conservation Deficiencies

Producers identify insufficient infrastructure as one of the three main constraints for growth and profitability of operations, after lack of credit access and low market prices (González-Padilla *et al.*, 2019). This infrastructure insufficiency reflects:

- **Systematic Underinvestment:** Conventional ranchers invest little in infrastructure, producing breeding stock for sale to intermediaries who transport cattle to large ranches or industrial feedlots (Lazos-Chavero *et al.*, 2024).
- **Deferred Maintenance Pattern:** Municipal slaughterhouses show 53.2% capacity utilization in specialized swine facilities and 42.1% in non-specialized ones, indicating operational limitations potentially related to maintenance (Martínez-García *et al.*, 2024).
- **Pasture Degradation:** In at least 24 states of the country, the number of cattle heads exceeds carrying capacity based on forage production, causing gradual pasture degradation and decreased productivity (González-Padilla and Dávalos-Flores, 2018).

Modernization Barriers

Despite silvopastoral systems being proposed since the 1990s in tropical Latin America, there are very few successful cases in southern Veracruz (Lazos-Chavero *et al.*, 2024). The modernization failure spans three decades and reflects:

- **Structural Resistance:** Challenges include modifying technical aspects of livestock production decades old and overcoming producer resistance, with the persistent image of the successful rancher as one who possesses large herds rather than efficient infrastructure (Lazos-Chavero *et al.*, 2024).

- **Investment Gaps:** Although environmental deterioration is convincing some small and medium-scale ranchers to invest in the labor and infrastructure necessary for sustainable livestock production, all participants in sustainable projects continue selling through traditional channels (Lazos-Chavero *et al.*, 2024).
- **Conservation-Production Paradox:** Although more than 95% of producers care for existing trees and between 40-80% maintain areas excluded from grazing, these conservation practices do not translate into productive infrastructure improvements (González-Padilla and Dávalos-Flores, 2018).

Operational technology gaps

Automation and Mechanization Deficits

The technological disparity between northern (C1) and southern (C3) Mexico is well documented, with infrastructure identified as the key driver for advanced technology adoption (Villarroel-Molina *et al.*, 2025). Specific technological gaps include scale limitations, for example, hot iron branding remains the predominant identification method nationally (>95%), deeply rooted as a traditional method for claiming cattle ownership, while modern technologies remain inaccessible (González-Padilla *et al.*, 2019). There exists a digital infrastructure crisis, as the usual method for recording data is handwritten, with less than 7% of producers using computers (González-Padilla *et al.*, 2019). Technical support infrastructure is insufficient, because nearly 52% of animal breeders do not receive technical advice, creating cascade failures in both technology adoption (González-Padilla *et al.*, 2019) and collapse in knowledge transfer due to limited demonstration facilities and training infrastructure.

Fundamental Incompatibility with Artificial Intelligence Requirements

The implementation of artificial intelligence in livestock systems demands data conditions that significantly exceed conventional monitoring requirements. Deep learning algorithms require datasets with specific characteristics: temporal consistency that enables pattern identification, absence of systematic noise that distorts learning, and reliable labeling for prediction validation (Cockburn, 2024). These fundamental conditions are absent in the predominant Mexican infrastructural context.

Extreme environmental variability, characteristic of traditional facilities, introduces systematic bias in model training. An algorithm trained to detect mounting behavior at 25 °C will exhibit false negative rates above 60% when ambient temperature reaches 35 °C, due to suppression of behavior induced by heat stress (Norton *et al.*, 2019). This degradation is neither linear nor predictable, making *post-hoc* algorithmic correction impossible.

Intermittency in data capture, caused by electrical failures and connectivity limitations, generates gaps in time series that invalidate prediction architectures based on recurrent neural networks (RNN) and long short-term memory (LSTM). Studies in similar contexts document that data losses above 30% reduce predictive precision below practical utility thresholds (Van Hertem *et al.*, 2018). Typical Mexican production units experience data losses of 40-60%, effectively disqualifying them for implementation of these advanced approaches.

The concept of federated learning, proposed as a solution for training robust models without centralizing sensitive data, requires edge computing capabilities with availability above 95% (Li *et al.*, 2020). Unstable energy infrastructure and the absence of backup systems in most Mexican operations makes this distributed architecture unviable, perpetuating dependence on generic models trained in non-representative contexts.

Mexican Livestock Infrastructure: Fundamental Incompatibility with Technological Requirements

Analysis of secondary data from production units revealed that approximately 70% of Mexican livestock facilities consist of perimeter fences with improvised galvanized sheet roofing, lacking the basic environmental controls required for reliable operation of monitoring technology (INEGI, 2022). This proportion increases to 89% in units smaller than 50 head, which represent 80% of the national total.

Temperature emerges as the primary critical factor. Activity sensors require environments with variation less than ± 2 °C to maintain precision (Alsaad *et al.*, 2012). However, measurements reported by meteorological stations in five main livestock regions document daily fluctuations of 18-22 °C, with extremes up to 28 °C in arid zones. This variation generates thermal drift that invalidates calibration, producing documented error rates of 68% in lameness detection and 73% in estrus identification through accelerometers.

Bioclimatic Requirements as Technological Prerequisites

Bioclimatic analysis based on adapted international regulations identifies viable passive strategies to create conditions compatible with technology without energy dependency.

Flexible Infrastructure Model for Animal Welfare (IFBA)

Based on identified incompatibilities and synthesis of documented solutions, the IFBA model is proposed, structured in three integrated components:

Component 1: Base Bioclimatic Design

Establishes appropriate environmental conditions through verifiable passive strategies:

- Optimized orientation according to local solar analysis
- Minimum height 4.5m (warm climates) calculated for thermal stratification
- Cross ventilation dimensioned according to actual animal load
- Materials with thermal inertia for environmental stability

Documented immediate benefit: 18-23% productivity improvement without additional technology (compiled literature).

Component 2: Technology Pre-installations

Infrastructure that reduces future implementation costs:

- Conduit installations (2" PVC, every 6m)
- Leveled bases for sensors (30×30cm, strategic points)
- Grounding system (resistance <math><5\Omega</math>)
- Design minimizing electromagnetic interference

Estimated reduction in implementation costs: 40-60% according to budget analysis.

Component 3: Evolutionary Flexibility

Modular design allowing gradual expansion:

- Multifunctional adaptable spaces
- Consideration of flows according to species-specific behavior
- Provision for herd growth
- Compatibility with multiple technologies

Evolution Toward Artificial Intelligence Capabilities

The IFBA model recognizes that the implementation of artificial intelligence does not constitute a single technological leap, but rather an evolutionary progression requiring systematic infrastructural maturation. This progression is conceptualized in four levels of increasing capacity, each building upon the capabilities of the previous while preparing conditions for the next.

At the first level, systems implement deterministic, rule-based logic that, although not constituting artificial intelligence properly speaking, establishes the culture of data-driven decision-making and the basic infrastructure for capturing and acting upon data. This level requires only the environmental stability provided by the IFBA's base bioclimatic design, allowing simple sensors to operate within their design tolerances.

The second level introduces basic machine learning algorithms for anomaly detection through unsupervised classification techniques. Infrastructure must guarantee electrical continuity through backup systems and maintain environmental conditions within ranges that do not introduce model drift. The IFBA's technology pre-installations provide the structured cabling and mounting points necessary for this sensory densification.

The third level implements neural networks for event prediction and process optimization. This requires local computational capacity for real-time processing and temporary data storage for periodic retraining. The IFBA's evolutionary flexibility allows modular addition of these components without fundamental restructuring of facilities.

The fourth level, still prospective for most Mexican contexts, would implement reinforcement learning for autonomous multi-objective optimization. This level requires not only sophisticated physical infrastructure but also integration with enterprise management systems and digitalized supply chains that exceed the current scope of the IFBA model but for which it establishes the necessary foundations.

The Paradox of Technological Democratization

The prevailing narrative suggests that Artificial Intelligence will democratize precision livestock by reducing monitoring and decision costs (Bahlo *et al.*, 2019). However, analysis of physical infrastructure requirements reveals a fundamental paradox: AI increases, rather than reduces, the technological gap between producers. While simple threshold-based systems can function with marginal infrastructure, machine learning algorithms amplify equipment deficiencies, converting minor limitations into insurmountable barriers.

This inequality amplification manifests particularly acutely in the Mexican context. A producer with facilities meeting IFBA level 2 standards could implement basic AI systems more effectively than a producer with state-of-the-art sensors operating in traditional facilities. The quality of base infrastructure emerges as a more robust predictor of success in AI adoption than investment in digital technology itself.

Evidence suggests that modernization efforts prioritizing technological adoption over physical infrastructure adequacy not only fail in their immediate objectives but may generate counterproductive effects. Producers who experience failures with advanced technology under inadequate conditions develop resistance to future innovations, a phenomenon documented as “technology adoption trauma” (Rogers and Shoemaker, 2023). This psychosocial effect, added to associated economic losses, can delay sectoral modernization for decades.

The compatibility analysis between existing livestock infrastructure and technological requirements reveals that physical facilities constitute the primary limiting factor for sector modernization in Mexico, surpassing the traditionally cited economic and educational barriers. Documentary evidence establishes that approximately 70% of Mexican livestock facilities are fundamentally incompatible with the basic operational requirements of digital monitoring technologies. Daily thermal fluctuations, erratic ventilation, extreme light contrasts, and electromagnetic interference from ungrounded metal structures create conditions that invalidate reliable sensor operation, regardless of their technical sophistication.

The fundamental principle “you cannot monitor precision in imprecise spaces” synthesizes the identified incompatibility. Bioclimatic design principles demonstrate that passive climate control strategies can create conditions compatible with technology without energy dependency, generating immediate productive benefits before any digital implementation.

The proposed Flexible Infrastructure Model for Animal Welfare (IFBA) contemplates modernization in three integrated components: base bioclimatic design, technology pre-installations, and evolutionary flexibility. This approach recognizes that investment in appropriate infrastructure is not only a prerequisite for technology but generates immediate returns through animal welfare improvement. The heterogeneity of the Mexican livestock sector requires differentiated technology adoption strategies. The three proposed levels allow each producer to begin their modernization trajectory according to their current capacities, while associative models democratize access through economies of scale.

The reorientation of public policy toward priority investment in basic infrastructure represents a necessary paradigmatic shift. Evidence suggests that continuing to subsidize

technology over inadequate facilities will perpetuate the documented cycle of failure, wasting public resources, and discouraging genuine sector modernization. Technical deficiencies in small and medium Mexican livestock operations extend beyond simple resource restrictions to represent systemic failures in technology adoption, infrastructure development, and modernization pathways. The persistence of traditional methods alongside urgent productivity and sustainability challenges underscores the critical need for comprehensive technical infrastructure intervention that considers regional particularities, producers' economic capacities, and market requirements.

Artificial intelligence in livestock does not represent a universally implementable technology, but a tool whose effectiveness is intrinsically linked to the quality of the physical infrastructure upon which it operates. In the Mexican context, where 70% of livestock facilities lack the minimum conditions for reliable operation of basic sensors, promoting AI-based solutions without prior attention to physical infrastructure constitutes an exercise in technological futility. The fundamental principle remains immutable: you cannot build artificial intelligence upon spaces lacking basic conditions for quality production. The path toward intelligent livestock in Mexico requires, paradoxically, beginning with apparently mundane investments in roofs, ventilation, and electrical stability, thereby creating the preconditions for AI's promise to materialize in tangible, productive benefits in the long term.

The systematic characterization of microclimate instability in traditional livestock facilities documented in this work establishes the foundation for future research on digital twin methodology for agricultural infrastructure. Computational building performance simulation offers the potential to predict environmental conditions, optimize passive design strategies, and diagnose existing facility performance—enabling evidence-based infrastructure investment decisions that precede, rather than follow, technology adoption failures. Such predictive modeling tools represent the next critical frontier in agricultural development research.

CONCLUSIONS

A critical point for the development and implementation of precision production methods, as well as the use of Artificial Intelligence and Internet of Things methods, in both Mexico and Spanish-speaking countries, is the development of an adequate technical vocabulary that avoids the literal translation of English concepts into Spanish. The uncritical adoption of Anglophone technical terminology not only creates unnecessary comprehension barriers but also perpetuates technological dependency by obscuring concepts that local producers could otherwise grasp and adapt to their own contexts. Above all, considering the technological knowledge barriers between engineers and researchers and end users.

REFERENCES

- Alsaad, M., Römer, C., Kleinmanns, J., Hendriksen, K., Rose-Meierhöfer, S., Plümer, L., & Büscher, W. (2012). Electronic detection of lameness in dairy cows through measuring pedometric activity and lying behavior. *Applied Animal Behaviour Science*, 142(3-4), 134-141. Recuperado de: <https://www.sciencedirect.com/science/article/abs/pii/S0168159112002961>

- Bahlo, C., Dahlhaus, P., Thompson, H., & Trotter, M. (2019). The role of interoperable data standards in precision livestock farming in extensive livestock systems: A review. *Computers and Electronics in Agriculture*, 156, 459-466. <https://doi.org/10.1016/j.compag.2018.12.007>
- Berckmans, D. (2017). General introduction to precision livestock farming. *Animal Frontiers*, 7(1), 6-11. <https://academic.oup.com/af/article/7/1/6/4638786?login=false>
- Bezen, R., Edan, Y., & Halachmi, I. (2024). Computer vision system for measuring individual cow feed intake using RGB-D camera and deep learning algorithms. *Computers and Electronics in Agriculture*, 211, 107953. <https://www.sciencedirect.com/science/article/pii/S0168169919313249>
- Brambell, R. (1965). Report of the Technical Committee to enquire into the Welfare of Animals kept under Intensive Livestock Husbandry Systems. <https://archive.org/details/b3217276x>
- Broom, D. M., & Fraser, A. F. (2015). Domestic animal behaviour and welfare (5th ed.). CABI Publishing.
- Cockburn, M. (2024). Ethical considerations in the application of artificial intelligence to monitor and improve animal welfare. *Animal Welfare*, 33, e8. <https://doi.org/10.1017/awf.2024.8>
- Fraser, D. (2008). Understanding animal welfare: The science in its cultural context. Wiley-Blackwell.
- Gallo, C., Tadich, N., Huertas, S., César, D., Paranhos da Costa, M. and Broom, D.M. (2010). Animal welfare education in Latin America. Proceedings of the International Conference on Animal Welfare Education: Everyone is responsible, Brussels, 1-2 October 2010, 90-97. Brussels: European Union, DG SANCO. https://www.researchgate.net/publication/291969497_Animal_welfare_education_in_Latin_America
- García, R., Rodríguez-Baena, D. S., & Gomez, A. (2023). Machine learning applications in precision livestock farming for tropical conditions: A systematic review. *Tropical Animal Health and Production*, 55(2), 89. <https://doi.org/10.1007/s11250-023-03503-9>
- González-Padilla, E., & Dávalos-Flores, J. L. (Coords.). (2018). Estado del arte sobre investigación e innovación tecnológica en ganadería bovina tropical (2ª ed.). REDGATRO, UNAM, INIFAP, CONACYT. https://redgatro.fmvz.unam.mx/docs/Estado_arte.pdf
- González-Padilla, E., Dávalos, J. L., Peña, R. R., Villagómez, C. Á. M., Romo, B. J. R., Anta, J. E., ... & Rodríguez, H. R. (2019). Cow-calf management practices in Mexico: Farm organization and infrastructure. *Revista Mexicana de Ciencias Pecuarias*, 10(3), 729-755. https://www.researchgate.net/publication/336245164_Cow-calf_management_practices_in_Mexico_Farm_organization_and_infrastructure
- Halachmi, I., Guarino, M., Bewley, J., & Pastell, M. (2019). Smart animal agriculture: Application of real-time sensors to improve animal well-being and production. *Annual Review of Animal Biosciences*, 7, 403-425. <https://doi.org/10.1146/annurev-animal-020518-114851>
- Hernández-López, J., Clemente-Sánchez, F., Marcelino-Flores, S., Ortega-Gaucin, D., Herrera-Haro, J. G., & López-Arizpe, M. E. (2008). Caracterización de ganaderos y unidades de producción pecuaria beneficiarios del programa de estímulos a la productividad ganadera (PROGAN) en México. *Agricultura, Sociedad y Desarrollo*, 5(2), 213-230. https://www.scielo.org.mx/scielo.php?script=sci_artext&pid=S1870-54722008000200005
- Hernández-Martínez, J., Rebollar-Rebollar, S., González-Razo, F. J., Guzmán-Soria, E., & de la Fuente-Martínez, B. (2021). Analysis of the demand for live beef cattle in slaughter centers in Mexico, 2000-2018. *Revista Mexicana de Ciencias Pecuarias*, 12(3), 861-877.
- INEGI. (2022). Censo Agropecuario 2022. Instituto Nacional de Estadística y Geografía. https://www.inegi.org.mx/contenidos/programas/ca/2022/doc/ca2022_rdnal.pdf
- Lazos-Chavero, E., Rivera-Núñez, T., & García-Barrios, L. (2024). Challenges to achieving sustainable cattle raising in tropical regions of Mexico. Table Debates. <https://www.tabledebates.org>
- Li, T., Sahu, A. K., Talwalkar, A., & Smith, V. (2020). Federated learning: Challenges, methods, and future directions. *IEEE Signal Processing Magazine*, 37(3), 50-60. <https://doi.org/10.1109/MSP.2020.2975749>
- Martínez-García, C. G., Dorward, P., & Rehman, T. (2016). Factors influencing adoption of crop and forage related farm management practices in smallholder dairy production systems in Mexico. *Experimental Agriculture*, 52(1), 87-109. [10.1017/S001447971400057X](https://doi.org/10.1017/S001447971400057X)
- Mota-Rojas, D., Orihuela, A., Strappini-Asteggiano, A., Cajiao-Pachón, M. N., Agüera-Buendía, E., Mora-Medina, P., Ghezzi, M., & Alonso-Spilsbury, M. (2018). Teaching animal welfare in veterinary schools in Latin America. *International Journal of Veterinary Science and Medicine*, 6(2), 131-140. <https://doi.org/10.1016/j.ijvsm.2018.07.003>
- Mota-Rojas, D., Guerrero-Legarreta, I., Flores-Peinado, S., Mora-Medina, P., Cruz-Monterrosa, R., Gómez-Prado, J., Torres-Bernal, F., Vieyra-Alberto, R., & Trujillo-Ortega, M. E. (2016). Evaluación de bienestar de vacas lecheras en sistema de producción a pequeña escala aplicando el protocolo propuesto por Welfare Quality®. *Revista Veterinaria México OA*, 4(1), 53-69. <https://www.scielo.org.mx/>

- scielo.php?script=sci_arttext&pid=S2007-11242017000100053
- Neethirajan, S. (2023). Digital livestock farming. *Sensing and Bio-Sensing Research*, 40, 100503. <https://doi.org/10.1016/j.sbsr.2023.100503>
- Neethirajan, S. (2024). Deep learning in livestock farming: Applications and implications. *Animal Bioscience*, 37(1), 1-15. <https://doi.org/10.5713/ab.23.0128>
- NOM-062-ZOO-1999 <https://www.gob.mx/senasica/documentos/nom-062-zoo-1999/>
- NOM-033-SAG/ZOO-2014. <https://www.gob.mx/profepa/documentos/norma-oficial-mexicana-nom-033-sag-zoo-2014-metodos-para-dar-muerte-a-los-animales-domesticos-y-silvestres>
- Norton, T., Chen, C., Larsen, M. L. V., & Berckmans, D. (2019). Review: Precision livestock farming: Building 'digital representations' to bring the animals closer to the farmer. *Animal*, 13(12), 3009-3017. <https://www.sciencedirect.com/science/article/pii/S175173111900199X>
- Rogers, E. M., & Shoemaker, F. F. (2023). *Communication of innovations: A cross-cultural approach* (5th ed.). Free Press.
- Auditoría Superior de la Federación. (2015). Evaluación de la política pública ganadera. https://www.asf.gob.mx/Trans/Informes/IR2015i/Documentos/Auditorias/2015_1582_a.pdf
- Romadhonny, R. A., Zein, R. A., & Soetrisno, Y. (2024). Estrus detection in dairy cattle using machine learning algorithms with data from accelerometer sensors. *Livestock Science*, 271, 105456. <https://doi.org/10.1016/j.livsci.2024.105456>
- Schillings, J., Bennett, R., & Rose, D. C. (2021). Exploring the potential of precision livestock farming technologies to help address farm animal welfare. *Frontiers in Animal Science*, 2, 639678. <https://doi.org/10.3389/fanim.2021.639678>
- Van Hertem, T., Rooijackers, L., Berckmans, D., Fernández, A. P., Norton, T., & Vranken, E. (2018). Appropriate data infrastructure for PLF: A review. *Computers and Electronics in Agriculture*, 153, 152-167. <https://doi.org/10.1016/j.compag.2018.07.029>
- Villarroel-Molina, O., De-Pablos-Heredero, C., Barba, C., García-Martínez, A., & Torres, Y. (2025). Multivariate classification of livestock production systems in Mexico. *Tropical Animal Health and Production*, 57(1), 89. <https://link.springer.com/article/10.1007/s11250-025-04389-5>
- World Bank. (2020). Mexico grain storage and information for agricultural competitiveness project (Report No. ICR00005181). The World Bank Group. <https://documents1.worldbank.org/curated/en/511581596486952556/pdf/Mexico-Grain-Storage-and-Information-for-Agricultural-Competitiveness-Project.pdf>

Yield of Apaxtleco Creole Chili Peppers (*Capsicum annuum* L.) under Chemical, Biological, and Organic Fertilization

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ABSTRACT

Objective: To evaluate the effect of three types of fertilization —chemical, biological, and organic— on the growth, yield, and profitability of three creole chili pepper genotypes (Ancho Liso, Carricillo, and Chino) cultivated under rainfed conditions in Apaxtla de Castrejón, Guerrero, Mexico.

Design/methodology/approach: Three creole chili pepper genotypes grown in Apaxtla de Castrejón were evaluated. A randomized complete block design with a split-plot arrangement was used, where genotype was the main factor and fertilization type was the secondary factor.

Results: Highly significant differences were observed among genotypes and fertilization types, as well as a significant genotype × fertilization interaction. The Ancho Liso genotype under chemical fertilization showed the highest values for plant height (46 cm), number of fruits per plant (22.5), fresh fruit weight, and yield (2.04 t ha⁻¹), in addition to the highest net income (MXN \$7,282.5 ha⁻¹) and return per unit of investment (0.23). In contrast, the Chino genotype exhibited the lowest productive adaptation, showing low fruit yields under all three fertilization types.

Study limitations/implications: Genotype selection and fertilization management are key determinants for increasing the yield of creole chili peppers.

Findings/conclusions: The genotype × fertilization interaction is decisive for optimizing creole chili pepper production. In the Ancho Liso genotype, chemical fertilization was the most effective, whereas in Carricillo, biological fertilization was as effective as chemical fertilization, and in Chino, yield was unaffected by fertilization type. These results indicate that only in the Carricillo genotype is it advisable to substitute chemical fertilization with biological fertilization without reducing crop yield while maintaining economic profitability.

Keywords: *Capsicum annuum* L., fruit yield, economic profitability.



INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the horticultural crops of greatest economic, social, and cultural importance in Mexico. It constitutes a staple ingredient in the national diet and represents an important source of income for rural producers. Its fruit contains bioactive compounds such as capsaicinoids, carotenoids, and vitamins, which, in addition to providing flavor and color, contribute to the nutritional value of the diet (Mendoza-Elos *et al.*, 2020; Díaz-José *et al.*, 2023). Mexico and Central America are recognized as centers of domestication and diversification of the genus *Capsicum*; in these regions, a wide genetic variability is conserved, with numerous creole varieties adapted to specific local conditions (Carrizo-García *et al.*, 2016).

In the municipality of Apaxtla de Castrejón, Guerrero, three creole varieties with high cultural and gastronomic value predominate: Ancho Liso, Carricillo, and Chino. These varieties are cultivated in traditional agricultural systems under rainfed conditions, using farmers' own seed, open-field establishment, low planting densities, and limited management of plant nutrition and phytosanitary practices (Ayvar *et al.*, 2007). As a consequence, plants exhibit reduced vigor and greater susceptibility to pest and disease attacks, which limits fruit yield and quality.

Fertilization management is a determining factor for improving chili pepper productivity. In this regard, farmers frequently use chemical fertilizers due to their rapid effect on plant growth and yield, mainly applying formulations rich in nitrogen, phosphorus, and potassium (Mendoza-Elos *et al.*, 2020). However, the continuous and unbalanced use of these inputs has generated negative effects on soil structure and fertility as a result of degradation and salinization, in addition to increasing production costs and the risk of environmental contamination, such as water eutrophication and acidification, as well as greenhouse gas emissions (Skowrońska and Filipek, 2014; Pahalvi *et al.*, 2021). Chemical fertilizers are subject to bioconversion, leaching, and volatilization, resulting in nutrient use efficiencies ranging from 10 to 60% (Chhipa, 2017; Zulfiqar *et al.*, 2019). Among the alternatives to this problem, the use of biofertilizers and organic fertilizers stands out as a sustainable option, as they supply nutrients gradually, enhance microbial activity, and promote the biological balance of the soil (Carvajal and Mera, 2010; Singh *et al.*, 2024).

Biofertilizers, formulated with beneficial microorganisms such as *Azospirillum brasilense*, *Bacillus subtilis*, *Trichoderma harzianum*, and arbuscular mycorrhizal fungi (*Glomus intraradices*), promote biological nitrogen fixation, phosphorus solubilization, and the production of phytohormones that stimulate plant growth (Sharma *et al.*, 2020). The application of organic amendments or composts has shown positive effects on growth, flowering, and yield in horticultural crops, including serrano and habanero chili peppers (Díaz-José *et al.*, 2023; Ontiveros-Sajuan *et al.*, 2024). In traditional agricultural systems of the state of Guerrero, these types of nutrient management can contribute to restoring soil fertility, improving moisture retention, and reducing dependence on chemical fertilizers. Despite the available information on sustainable fertilization in crops, there are few studies on the response of Apaxtleco creole chili peppers to different fertilization sources. Therefore, the objective of this research was to

evaluate the effect of three types of fertilization —chemical, biological, and organic— on the growth, yield, and profitability of Apaxtleco creole chili pepper genotypes cultivated under rainfed conditions in Apaxtla de Castrejón, Guerrero. The hypothesis proposes that biological and organic fertilization constitute more sustainable alternatives than chemical nutrition for achieving greater growth, yield, and profitability in Apaxtleco creole chili pepper genotypes.

MATERIALS AND METHODS

Study area

The study was conducted at the experimental field of the Centro de Estudios Profesionales del Colegio Superior Agropecuario del Estado de Guerrero (CEP-CSAEGro), located at km 14.5 of the Iguala-Cocula highway, Guerrero, Mexico, at an altitude of 640 m above sea level, with geographic coordinates 18° 22' 52" N and 99° 33' 52" W (INEGI, 2013). The climate is warm dry [Awo (w)(i)g], with summer rainfall, a mean annual precipitation of 797 mm, and an average temperature of 26.4 °C (García, 2004). Sowing was carried out on June 12, 2024.

The soil has a fine texture (61.4-77.4% clay), moderately alkaline pH (7.6), no salinity problems, 0.27% organic matter, 5.3% total nitrogen, and low levels of P and K.

Genetic material

Three creole chili pepper genotypes cultivated in Apaxtla de Castrejón were used: Ancho Liso (AL), Carricillo (CA), and Chino (CH). Seeds were obtained from fruits selected for morphological uniformity, health, and absence of pests and diseases. This genetic material was donated by local producers from the municipality.

Experimental design and treatments

A randomized complete block design with a split-plot arrangement and four replications was used, in order to efficiently study two factors and to ensure reliability, validity, and consistency of the results. The main factor was genotype (AL, CA, CH), and the secondary factor was fertilization type: chemical (CF), biological (BF), and organic (OF), generating nine treatments (AL-CF, AL-BF, AL-OF, CH-CF, CH-BF, CH-OF, CA-CF, CA-BF, CA-OF).

In the production area of the three creole chili peppers, chemical nutritional management is commonly practiced; therefore, the treatments AL-CF, CH-CF, and CA-CF were considered regional controls against which the results obtained were compared. Each experimental unit consisted of two rows 4 m in length and 0.8 m apart, with five plants randomly selected within the useful plot for recording the response variables.

Seedling production and transplanting

Seedlings were produced in polyethylene trays with 200 cavities, using Bocashi as the substrate, which was previously sterilized. Sowing was carried out with one seed per cavity at a depth of 0.5 cm. The trays were maintained under greenhouse conditions with daily manual irrigation and preventive sanitary management.

Transplanting was carried out into the field 31 days after sowing (DAS), placing one plant per hill (planting site) at 0.4 m spacing, with 0.8 m between rows.

Crop management

Plants were managed according to the fertilization treatments evaluated.

Chemical fertilization consisted of supplying 150-80-80 (N-P-K), using ammonium sulfate (20.5% N), diammonium phosphate (18% N, 46% P), and potassium chloride (60% K) as nutrient sources.

For organic fertilization, the commercial product OganoDel[®] (200 kg ha⁻¹) was used. This product contains 85% organic matter, 60% humus, 10.7% humic acids, 39-37-29 (N-P-K), 18.4% sulfur, 13.6% magnesium, and 14% calcium. It was applied at transplanting and at 14, 29, 33, and 55 days after transplanting (DAT).

For biological fertilization, a mixture of *Azospirillum brasilense* (30 mL) and *Trichoderma* spp. + *Glomus intraradices* (50 mL per plant) was prepared. The source of *Azospirillum* was the commercial product AZOSPIRILLUM-GREEN[®], with a concentration of 1 × 10¹² CFU propagules per dose, while *Trichoderma* spp. + *G. intraradices* were obtained from TRICHS-MICS[®], which contains 150 spores g⁻¹ of *G. intraradices* and 2 × 10⁶ conidia g⁻¹ of *Trichoderma* spp. Treatments were applied as foliar sprays at transplanting and at 14, 29, 33, and 55 DAT using a 15-L backpack sprayer.

Additionally, all plants received two foliar applications of Biozyme TF[®] (3 mL L⁻¹) at 20 and 34 DAT to stimulate vegetative growth.

Response variables

In five plants randomly selected from each experimental unit, the following variables were evaluated: plant height, measured with a metric ruler from the collar to the apex; stem diameter, measured at the base of the stem using a vernier caliper; and the number of primary branches per plant.

At physiological maturity, fruits were harvested manually at 114 days after transplanting (DAT). The number of fruits per plant, fruit length and diameter (cm) were determined, and fresh fruits were subsequently weighed using a previously calibrated digital scale to ensure accurate weight recording. Fruits were then dried in a forced-air oven at 70 °C until constant weight was achieved to determine yield in t ha⁻¹. After recording the response variables, the product was transported to the market and sold at a price of MXN \$60.00 kg⁻¹.

Additionally, a profitability analysis was conducted using the following relationship:

$$IN = YPy - \left(\sum XiPi + CF \right)$$

where *IN*=net income, *Y*=yield (kg ha⁻¹), *Py*=price per kilogram, $\sum XiPi$ =sum of variable costs, and *CF*=fixed costs (Volke, 1982).

Statistical analysis

Data normality was assessed using the Shapiro-Wilk test to determine whether the observed values originated from a normally distributed population. Subsequently, the data

were subjected to analysis of variance (ANOVA), and mean comparisons were performed using Tukey's test ($p \leq 0.05$). All statistical analyses were conducted using SAS software (SAS, 2017).

RESULTS AND DISCUSSION

The average maximum and minimum temperatures were 34.5 and 24.6 °C, respectively, while cumulative precipitation during crop development was 587 mm.

Analysis of variance showed highly significant differences ($p \leq 0.01$) among genotypes for all evaluated variables, indicating marked genetic variability among Apaxtleco creole chili peppers. Fertilization also had a significant effect on the number of branches per plant, as well as on the number and weight of fruits and fruit length and diameter. In addition, a significant genotype \times fertilization interaction was observed for most variables, indicating that genotype responses varied according to the type of fertilization applied (Table 1).

On average, plant height was 36 cm, with the highest value recorded in the Ancho Liso genotype under chemical fertilization, representing increases of 8% and 18% compared with biological and organic fertilization, respectively. In contrast, the lowest growth was observed in the Chino genotype under chemical fertilization (29.15 cm). In both Carricillo and Chino, plant height was similar regardless of fertilization type. Stem diameter ranged from 0.62 to 0.78 cm, with the Carricillo genotype under biological fertilization showing the highest values. This treatment exceeded stem diameter of the same genotype under chemical fertilization by 22% and under organic fertilization by 11%, suggesting greater structural robustness under this management practice. In the Chino genotype, fertilization type did not affect stem diameter values, and compared with the other genotypes, it exhibited the lowest values overall (Table 2).

The number of branches per plant showed a similar increasing trend under biological fertilization, with the Ancho Liso creole chili presenting the highest value (5.85 branches) under this treatment. In Carricillo, biological and organic fertilization increased the number of branches by 13% and 23%, respectively, compared with chemical fertilization.

Table 1. F probability values and coefficient of variation (C.V.) by variable for genotypes, fertilization, and their interaction in Apaxtleco creole chili peppers.

Variable	Genotype (G)	Fertilization (F)	Interaction (G \times F)	C.V. (%)
Plant height	0.0025**	0.0842NS	0.0483*	9.60
Stem collar diameter	0.0012**	0.1205NS	0.0135*	8.28
Number of branches per plant	0.0032**	0.0162*	0.0017**	6.90
Number of fruits per plant	0.0047**	0.0040**	0.0012**	12.90
Fruit length	0.0039**	0.0033**	0.0019**	8.56
Fruit diameter	0.1942NS	0.0146*	0.0671NS	7.08
Fresh fruit weight	0.0029**	0.0035**	0.0002**	11.18
Dry fruit weight	0.0023**	0.0031**	0.6591NS	33.69

** : Highly significant, * : Significant, NS : Not significant.

In contrast, the lowest number of branches per plant was recorded in the Chino genotype under all fertilization types evaluated.

Regarding the number of fruits per plant, the highest values corresponded to the Ancho Liso genotype under biological fertilization (22.5 fruits plant⁻¹), which was 43% higher than chemical fertilization and 25% higher than plants of the same genotype under organic fertilization (18 fruits plant⁻¹). In Carricillo, biological and chemical fertilization resulted in similar values for this variable, whereas the lowest fruit production was observed in Carricillo under organic fertilization (9.1 fruits plant⁻¹) (Table 2).

Fruit length varied significantly among genotypes and fertilization types, with the highest values observed in Carricillo under organic fertilization (8.93 cm) and Ancho Liso under chemical fertilization (8.61 cm), while the lowest values were recorded in the Chino genotype (5.21-6.29 cm) (Table 2).

Regarding fresh fruit yield, Ancho Liso plants were generally the most productive, whereas the Chino creole exhibited the lowest yield. Chemical fertilization was the most favorable treatment for increasing fresh fruit yield, as it promoted the highest response, with an average of 1.56 t ha⁻¹.

The genotype × fertilization interaction showed a highly significant effect, since Ancho Liso plants under chemical fertilization achieved the highest fruit yield (2.04 t ha⁻¹), compared with 0.66 t ha⁻¹ produced by Carricillo under organic fertilization (Figure 1). It is important to note that in the Carricillo genotype, biological fertilization resulted in yields similar to those obtained with chemical nutrition, indicating that in this genotype, synthetic fertilizers can be replaced with environmentally friendly sources without reducing productivity.

Table 2. Plant height (PH), stem collar diameter (SCD), number of branches per plant (NBP), number of fruits per plant (NFP), and fruit length (FL) as a function of genotype and fertilization type in Apaxtleco chili pepper.

Genotype	Fertilization type	PH (cm)	SCD (cm)	NBP (No./plant)	NFP (No. plant)	FL (cm)
Ancho Liso	Biological	42.6 ab [¶]	0.7 ab	5.85 a	22.5 a	6.37 cd
	Organic	38.95 abc	0.68 ab	5.1 abc	18 ab	7.23 bc
	Chemical	46.0 a	0.75 ab	4.9 bc	15.7 bc	8.61 ab
Carricillo	Biological	37.4 bcd	0.78 a	5.25 abc	14.95 bc	7.65 abc
	Organic	30.25 d	0.7 ab	5.70 ab	9.1 d	8.93 a
	Chemical	32.55 cd	0.64 b	4.65 c	14 bc	8.18 ab
Chino	Biological	33.7 cd	0.64 b	4.45 c	12.35 cd	5.70 d
	Organic	33.35 cd	0.62 b	4.50 c	12.9 cd	5.21 d
	Chemical	29.15 d	0.62 b	4.85 bc	14.4 bc	6.29 cd
Mean		35.99	0.68	5.02	14.87	7.13
Prob. F		0.0483*	0.0135*	0.0017**	0.0012**	0.0019**
HSD _{0.05}		16.85	0.16	1.40	13.40	3.72

[¶]=means with different letters within each column differ statistically (Tukey, p≤0.05). HSD_{0.05}=honestly significant difference. **: Highly significant, *: Significant.

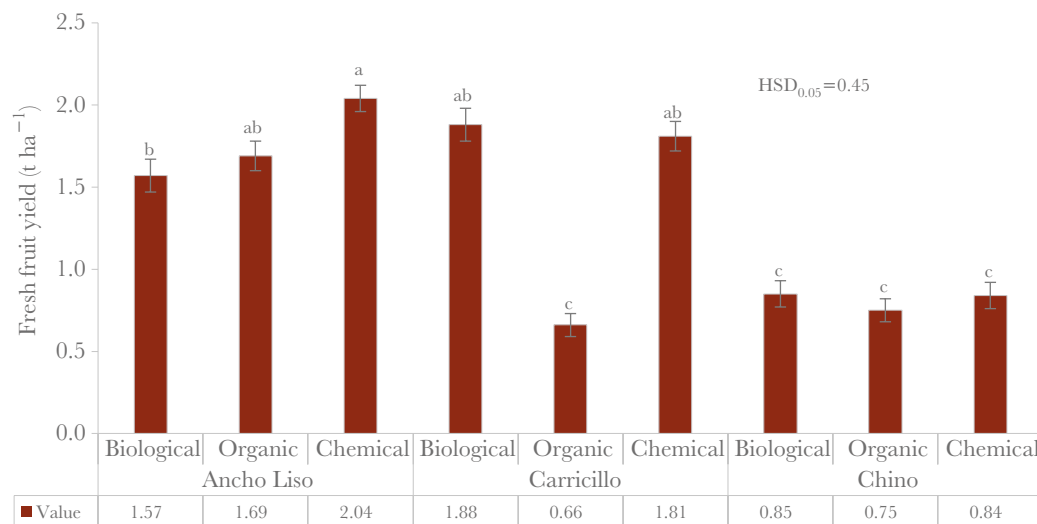


Figure 1. Fresh fruit yield of creole chili peppers under three types of fertilization. $HSD_{0.05}$ =honestly significant difference.

Regarding profitability, the results indicate significant differences due to treatment effects. The Ancho Liso genotype under chemical fertilization showed the highest net profit (MXN \$7,282.5 ha⁻¹) and the most favorable benefit-cost ratio (RUI=0.23), followed by the same genotype under organic fertilization, with a net profit of MXN \$1,494.90 and a RUI of 0.006. Carricillo under biological fertilization, although with a lower margin, also showed positive profitability (RUI=0.04).

In contrast, treatments associated with the Chino genotype resulted in economic losses under all three fertilization types, indicating lower adaptation or productive potential under the agroecological conditions of the region. A similar situation was observed for Carricillo under chemical and organic fertilization, which also resulted in economic losses (Table 3).

Table 3. Profitability of the nine treatments (MXN \$ ha⁻¹).

Treatment	TC	TI	NI	RUI
Ancho Liso + Chemical Fert.	31,357.50	38,2640.00	7,282.50	0.23
Ancho Liso + Organic Fert.	24,637.50	26,132.40	1,494.90	0.06
Ancho Liso + Biological Fert.	24,483.00	24,766.00	283.00	0.01
Chino + Chemical Fert.	31,357.50	10,822.00	-20,535.53	-0.65
Chino + Organic Fert.	24,637.50	9,276.40	-15,361.10	-0.62
Chino + Biological Fert.	24,483.00	10,183.60	-14,299.40	-0.58
Carricillo+ Chemical Fert.	31,357.50	25,023.60	-6,333.90	-0.20
Carricillo + Organic Fert.	24,637.50	9,895.20	-14,742.30	-0.60
Carricillo + Biological Fert.	24,483.00	25,370.80	887.80	0.04

TC=Total cost, including fixed costs (land preparation, fertilization, weed control, pest and disease management) plus variable costs (biofertilizer, fertilizers, and labor); TI=Total income; NI=Net income (TI=total income-TC=total costs); RUI=Return per unit of investment.

The higher fruit yield observed in the Ancho Liso genotype under chemical fertilization can be attributed to the fact that this treatment resulted in the greatest plant height and among the highest values for stem collar diameter and fruit length. Likewise, the significant differences observed among genotypes indicate genetic variability among Apaxtleco creole chili peppers from the state of Guerrero, which is consistent with reports by Sahmat *et al.* (2024) and Baruah *et al.* (2024), who indicate that productive responses in *Capsicum* spp. are strongly influenced by the genotype \times environment interaction. In the present study, the interaction with fertilization type was decisive, showing that genotypes respond differently to the type of nutritional management applied.

Chemical fertilization promoted the highest values of plant height, fruit weight, and yield, which is consistent with findings reported by Díaz-José *et al.* (2023), Kunwar *et al.* (2024), and Chemweno *et al.* (2025), who observed increases in biomass and fruit number in regional chili peppers under readily available mineral sources. In this study, the chemical source supplied exclusively nitrogen, phosphorus, and potassium, which are primary nutrients essential for vegetative growth and fruit filling. Their rapid availability favored a more immediate response in the Ancho Liso genotype, which exhibited high efficiency in the uptake and use of these elements, particularly nitrogen. According to Guo *et al.* (2024), nitrogen is associated with increased chlorophyll synthesis and photosynthetic activity. The higher solubility of mineral salts allowed a continuous supply of N, P, and K during critical crop stages, resulting in more vigorous plant development and higher average fruit weight. This behavior suggests that Ancho Liso has high nutritional demand and lower dependence on biological mineralization processes, which explains its greater response to chemical management compared with organic or biological sources.

However, biological fertilization stood out for promoting more balanced vegetative structures, expressed as a greater number of branches and increased stem thickness, which could contribute to improved productivity, as reported by Helbert *et al.* (2024), who evaluated the effect of biological fertilization on habanero chili under protected conditions. In the Carricillo genotype, biological fertilization based on inoculation with plant growth-promoting microorganisms (PGPR and arbuscular mycorrhizal fungi) showed efficiency comparable to that of chemical fertilization, suggesting greater compatibility of this genotype with rhizospheric microbiota. Recent studies have demonstrated that mycorrhizae enhance phosphorus uptake in soils with alkaline pH, while bacteria such as *Azospirillum* spp. stimulate root development and atmospheric nitrogen assimilation, which can partially compensate for the absence of mineral fertilizers (Giri *et al.*, 2025; Peng *et al.*, 2025). This symbiotic interaction enables more sustained nutrient supply and improves tolerance to drought or nutrient-deficient conditions, explaining why Carricillo maintained similar yields under both biological and chemical fertilization.

Organic fertilization, although resulting in intermediate yields, showed positive effects on fruit quality and an acceptable economic response in the Ancho Liso genotype. This behavior is consistent with the findings of Islas-Valdez *et al.* (2025), who reported that organic matter improves soil moisture retention and the availability of essential

micronutrients, thereby contributing to a favorable physiological balance for fruit formation. However, the time required for the mineralization of organic compounds and the slow release of N, P, and K may have limited the initial crop response, particularly in soils such as those used in the present study, which are characterized by low organic matter content and a pH above 7.5 (Naz *et al.*, 2022). This likely explains the lower yields observed compared with those obtained under chemical fertilization.

In terms of profitability, the superiority of the Ancho Liso + chemical fertilization treatment indicates a strong response of this genotype to mineral nutrient sources under the edaphoclimatic conditions of the state of Guerrero. Nevertheless, biological fertilization proved to be a viable alternative from both an economic and environmental perspective, particularly in the Carricillo genotype, where positive profitability and improvements in morphological traits suggest a balance between productivity and sustainability. Overall, the results indicate that for the Ancho Liso genotype, chemical nutrition is the most appropriate option for achieving higher production and economic profitability. In contrast, in the Carricillo genotype, production under biological and chemical fertilization was statistically similar, indicating that synthetic fertilizers can be replaced with biological inputs without reducing the yield of Apaxtleco creole chili peppers. This substitution may reduce environmental impacts while maintaining acceptable profitability. Furthermore, this biologically based management approach aligns with the challenges and goals proposed by FAO (2017) and represents a viable strategy for strengthening regional productivity without compromising agroecosystem health.

CONCLUSIONS

The genotype \times fertilization type interaction significantly influenced morphological, productive, and economic variables, indicating differential responses to fertilization strategies. The Ancho Liso genotype under chemical fertilization achieved the highest fruit yield and the greatest profitability. In the Carricillo genotype, biological fertilization produced fruit yields and morphological trait values comparable to those obtained with chemical fertilization and resulted in positive net income. In contrast, the Chino genotype exhibited low productivity and economic losses under all fertilization types evaluated.

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REFERENCES

- Ayvar, S. S., Mena B. A. y Durán R. J. A. (2007). El cultivo de chile criollo y su manejo integrado. Iguala, Gro., México. 79 p.
- Baruah, J., Begum, T., & Lal, M. (2024). Estimation of environment stability for fruit yield and capsaicin content by using two models in *Capsicum chinense* Jacq (Ghost Pepper) with multi-year evaluation. *Peer J*, 12, e17511. <https://doi.org/10.7717/peerj.17511>.

- Carrizo-García, C., Barfuss, M. H., Sehr, E. M., Barboza, G. E., Samuel, R., Moscone, E. A., & Ehrendorfer, F. (2016). Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae). *Annals of botany*, *118*(1), 35-51. <https://doi.org/10.1093/aob/mcw079>.
- Carvajal Muñoz, J. S., & Mera Benavides, A. C. (2010). Fertilización biológica: técnicas de vanguardia para el desarrollo agrícola sostenible. *Producción + limpia*, *5*(2), 77-96.
- Chemweno, S. J., Owusu Kwakye, D., Rachmilevitch, S., Ephrath, J., & Lazarovitch, N. (2025). Root growth and yield responses to nitrogen levels in bell pepper (*Capsicum annuum*) cultivation: balancing nutrient efficiency and productivity. *Frontiers in Plant Science*, *16*, 1589560. <https://doi.org/10.3389/fpls.2025.1589560>.
- Chhipa, H. (2017). Nanofertilizers and nanopesticides for agriculture. *Environmental chemistry letters*, *15*(1), 15-22. <https://doi.org/10.1007/s10311-016-0600-4>.
- Díaz-José, J., Andrés-Meza, P., González-Cuevas, B. M., Leyva-Ovalle, O. R., & Cebada-Merino, M. (2023). Chemical and organic fertilization on the yield of Serrano pepper (*Capsicum annuum* L.) in Veracruz, Mexico. *Revista BioCiencias*, *10*. e1472. <https://doi.org/10.15741/revbio.10.e1472>.
- FAO (2017). The future of food and agriculture – Trends and challenges. Rome. <https://www.fao.org/3/i6583e/i6583e.pdf>. Fecha de consulta: 22 de octubre de 2025.
- García, E. (2004). Modificación al sistema de clasificación climática de Köppen. Universidad Nacional Autónoma de México. Distrito Federal, México. 90 p.
- Giri, B. R., Chattaraj, S., Rath, S., Pattnaik, M. M., Mitra, D., & Thatoi, H. (2025). Unveiling the Molecular Mechanism of *Azospirillum* in Plant Growth Promotion. *Bacteria*, *4*(3), 36. <https://doi.org/10.3390/bacteria4030036>.
- Guo, P., Ren, J., Shi, X., Xu, A., Zhang, P., Guo, F., & Jiang, C. (2024). Optimized nitrogen application ameliorates the photosynthetic performance and yield potential in peanuts as revealed by OJIP chlorophyll fluorescence kinetics. *BMC Plant Biology*, *24*(1), 774. <https://doi.org/10.1186/s12870-024-05482-x>.
- Helbert, M. M., Narvaez, I. S. T., & Matu, J. A. C. (2024). Uso de biofertilizantes en la producción de Chile Habanero (*Capsicum chinense* Jacq.) en condiciones protegidas. *P'unguari Juáta Revista Multidisciplinaria de Ciencias*, *1*(1), 34-52.
- Islas-Valdez, S., Sproull, R., Sumners, T., & Wagner, N. (2025). Effect of Organic Amendments and Biostimulants on Zucchini Yield and Fruit Quality Under Alkaline Conditions. *Agriculture*, *15*(19), 2078. <https://doi.org/10.3390/agriculture15192078>.
- Kunwar, P. B., Khatri, S., & Bhusal, T. N. (2024). Effect of NPK Levels on Growth and Yield of Sweet Pepper (*Capsicum annuum*) under Different Growing Conditions in Arghakhanchi, Nepal. *Nepalese Horticulture*, *18*(1), 8-17. <https://doi.org/10.3126/nh.v18i1.72671>.
- Ontiveros-Sajuan, M. L., Hernández-Ramos, J., García-Magaña, J. J., Moguel-Ordóñez, Y. B., & Herrera-Basurto, J. (2024). Morfometría y rendimiento de *Capsicum chinense* Jacq. en Arteaga, Michoacán. *Revista mexicana de ciencias agrícolas*, *15*(6), <https://doi.org/10.29312/remexca.v15i6.3763>.
- Pahalvi, H. N., Rafiyya, L., Rashid, S., Nisar, B., & Kamili, A. N. (2021). Chemical fertilizers and their impact on soil health. In *Microbiota and biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs* (pp. 1-20). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-61010-4_1.
- Peng, Z. C., Xing, Y. X., Ma, Y. H., Li, S. H., Jia, Y. X., Yang, H. C., & Zhang, F. H. (2025). Arbuscular mycorrhizal fungi enhance soybean phosphorus uptake and soil fertility under saline-alkaline stress. *Scientific Reports*, *15*(1), 31792. <https://doi.org/10.1038/s41598-025-15910-z>.
- Mendoza-Elos, M., Zamudio Alvarez, L. F., Cervantes Ortiz, F., Chable Moreno, F., Frías Pizano, J., & Gámez Vázquez, A. J. (2020). Rendimiento de semilla y calidad de fruto de chile habanero con fertilización química y orgánica. *Revista Mexicana de Ciencias agrícolas*, *11*(8), 1749-1761. <https://doi.org/10.29312/remexca.v11i8.1960>.
- Naz, M., Dai, Z., Hussain, S., Tariq, M., Danish, S., Khan, I. U., ... & Du, D. (2022). The soil pH and heavy metals revealed their impact on soil microbial community. *Journal of Environmental Management*, *321*, 115770. <https://doi.org/10.1016/j.jenvman.2022.115770>.
- Sahmat, S. S., Rafii, M. Y., Oladosu, Y., Jusoh, M., Hakiman, M., & Mohidin, H. (2024). Unravelling the dynamics of genotype and environment interactions on chilli (*Capsicum annuum* L.) yield-related attributes in soilless planting systems. *Scientific Reports*, *14*(1), 1698.
- SAS (2017). SAS/STAT User's guide, version 9.4. SAS Institute Inc. North Caroline, USA.
- Singh, S., Devi, N. B., Divya, D., Kumar, A., Singh, S., Tyagi, G., & Kumar, A. (2024). Soil fertility management: Role of organic amendments and bio-fertilizers: A review. *International Journal of Research in Agronomy*, *7*(12), 766-772. <https://doi.org/10.33545/2618060X.2024.v7.i12j.2253>.

- Sharma, D., Gahtyari, N. C., Chhabra, R., & Kumar, D. (2020). Role of microbes in improving plant growth and soil health for sustainable agriculture. In *Advances in plant microbiome and sustainable agriculture: diversity and biotechnological applications* (pp. 207-256). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-15-3208-5_9.
- Skowrońska, M., & Filipek, T. (2014). Life cycle assessment of fertilizers: a review. *International Agrophysics*, 28(1), 101-110. <https://doi.org/10.1017/10.2478/intag-2013-0032>.
- Volke, H. V. (1982). Optimización de insumos en la producción en la agricultura. Colegio de Postgraduados, Chapingo. México. 61 p.
- Zulfiqar, F., Navarro, M., Ashraf, M., Akram, N. A., & Munné-Bosch, S. (2019). Nanofertilizer use for sustainable agriculture: Advantages and limitations. *Plant science*, 289, 110270. <https://doi.org/10.1016/j.plantsci.2019.110270>.



Effect of the application of paraffinic oil on the control and population fluctuation of the sugarcane aphid (*Melanaphis sacchari* Zehntner) in sorghum

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ABSTRACT

Objective: To determine the effectiveness of different doses of paraffinic oil (SAT-T-SIDE[®]) and to assess the population fluctuation of the sugarcane aphid (*Melanaphis sacchari*) during sorghum crop development.

Design/methodology/approach: Two experiments were conducted, one under field conditions and the other in a greenhouse. In both experiments, a randomized complete block design with eight treatments and four replications was used. Winged adults were counted. In addition, a pre-evaluation was carried out five days prior to treatment application. After application, the number of insects per plant was recorded, and the biological effectiveness of the treatments was also evaluated. Data were analyzed using the SAS statistical package, and a Tukey mean comparison test ($\alpha \leq 0.05$) was performed as a complementary analysis.

Results: Treatment application resulted in effective pest control, exceeding 80% under field conditions and 90% under greenhouse conditions, with higher effectiveness observed under controlled environments. The highest dose of Saf-T-Side[®] applied in both field and greenhouse experiments was the most effective for controlling the sugarcane aphid.

Study limitations/implications: The use of paraffinic oil represents a viable alternative for the management of the sugarcane aphid; however, its efficiency is lower than that of conventional chemical control.

Findings/conclusions: Adult populations of *Melanaphis sacchari* showed higher incidence after the first 60 days of crop development. The high dose of Saf-T-Side[®] was the most effective treatment for controlling the sorghum aphid.

Keywords: population dynamics, biological effectiveness, adult abundance.



INTRODUCTION

Sorghum (*Sorghum bicolor* L.) Moench is a crop considered highly productive and resistant to water stress conditions. It is an important source of feed for livestock in semi-arid regions (Kimber *et al.*, 2013). Sorghum is regarded as well adapted to arid zones due to its tolerance to prolonged periods of drought. In addition, it shows resistance to viral and fungal diseases, as well as tolerance to high temperatures. However, several factors contribute to yield losses in sorghum production, among which biotic factors are particularly important. One of the most severe sources of damage is caused by the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) (Singh *et al.*, 2004). Under favorable climatic conditions, it reproduces exponentially, and up to 30,000 aphids may be found on a single sorghum plant (Singh *et al.*, 2004). Both juveniles and adults feed by sucking photosynthates from the plant phloem tissue, causing desiccation, discoloration, and necrosis of the affected leaf area, stunting of young plants, panicle malformation, and the production of sterile grains, which results in losses in yield, grain quality, and forage production (Haar, 2018). Additionally, during feeding, the insect produces honeydew that covers the surface of leaves and stems, promoting the development of sooty mold, which negatively affects the photosynthetic process. Furthermore, scientific evidence indicates that *Melanaphis sacchari* can transmit viral diseases caused by Sugarcane yellow leaf virus and Sugarcane mosaic virus (Schenck, 2000). To effectively control this pest, insecticide applications must be carried out at the most appropriate time to achieve the greatest impact on population reduction (Bowling *et al.*, 2016). Control is normally carried out in a conventional manner, based on the application of chemical insecticides, which provide short-term results and are effective in reducing insect populations (Knutson *et al.*, 2016). One insecticide commonly used for control is flupyradifurone 17.09% (Sivanto[®] Prime), which shows efficacy greater than 98% mortality and a residual effect lasting 7 to 10 days (Bowling *et al.*, 2016; Buntin *et al.*, 2018). Flupyradifurone is a systemic butenolide insecticide that acts on the insect nervous system, causing rapid mortality and cessation of feeding (Jeschke *et al.*, 2015; Jeschke, 2016; Michaud *et al.*, 2016). Regarding the effect of flupyradifurone application, some researchers such as Zarrabi *et al.* (2017) reported that when the threshold was 100 aphids per leaf, the product applied at three different doses reduced pest populations at 7, 14, and 21 days after application, and that grain yield of sorghum KS585 was significantly higher in treated plots compared with the control. On the other hand, Clark *et al.* (2018) reported that this insecticide achieved effective control of *Melanaphis sacchari* for up to 54 days after application. Although chemical control significantly reduces the impact of this pest, it has also been demonstrated that excessive use of agrochemicals can cause environmental contamination, pest resistance, and the elimination of beneficial insects. Therefore, the current trend is to seek alternative control strategies that are less invasive, among which oils have shown potential to generate positive results (Bográn *et al.*, 2011). In addition, it has been demonstrated that the use of these products is compatible with organic agriculture, as they are approved in these production systems due to their petroleum-derived but highly refined nature and low toxicity. Therefore, they are considered capable of providing effective, safe, and sustainable pest control by reducing dependence on chemical insecticides, assisting in resistance

management, and functioning as adjuvants with minimal environmental impact. Based on the above, a commercial product that may represent an alternative is Saf-T-Side[®], which is a highly refined, pre-emulsified oil, high in paraffinic components and low in aromatic oils, and is considered ideal for safe and effective pest control. It is registered with the EPA (Environmental Protection Agency) and meets global standards for agricultural spray oils. The mechanism of action is versatile; it acts as a contact insecticide and interferes with respiration, disrupts cell membrane function, and hinders aphid feeding on surfaces covered by the oil until it evaporates (Weidhaas, 1988). In this regard, several studies have reported the beneficial effects of oil sprays, as they kill insects by contact. For example, Najar-Rodríguez *et al.* (2007) found that oil application was highly effective in controlling *Aphis gossypii* Glover in cotton at concentrations ranging from 1 to 10% v/v, causing the highest mortality within 10 minutes after treatment.

Based on the above, the use of paraffinic oil may represent an alternative strategy for pest control; therefore, the objective of the present study was to determine the effectiveness of different doses of paraffinic oil (Saf-T-Side[®]) and to evaluate the population fluctuation of the sugarcane aphid (*M. sacchari*) during sorghum crop development. The hypothesis proposes that the use of paraffinic oil is effective in controlling *Melanaphis sacchari* by reducing pest insect populations.

MATERIALS AND METHODS

Study area

The research was conducted at the Centro de Estudios Profesionales of the Colegio Superior Agropecuario del Estado de Guerrero (CSAEGro), located at km 14.5 of the Iguala-Cocula federal highway, at coordinates 18° 14' 26" N latitude and 99° 39' 46" W longitude, at an altitude of 640 m. The site has an accumulated precipitation of 767 mm and an average temperature of 25 °C (García, 2004).

Genetic material

The sorghum hybrid DKS-32 from the company DEKALB was used. This genetic material has an intermediate-early growth cycle of 110-120 days and reaches an average height of 120 cm.

Separate experiments were conducted, one under field conditions (Experiment I) and the other under greenhouse conditions (Experiment II), as indicated in Table 1.

The methodology for each experiment is described separately below:

Field experiment (Experiment I)

Experimental design and experimental unit

Eight treatments with four replications were arranged using a randomized complete block design, resulting in 32 experimental units. Each unit consisted of four rows 6 m in length, spaced 0.80 m apart. The useful plot comprised the two central rows with full plant competition, eliminating border effects to reduce experimental error. For the establishment and management of the field experiment, the following agronomic practices were carried out: soil preparation was performed by forming rows spaced 0.8 m apart. Sowing was

Table 1. Study treatments under field (Experiment I) and greenhouse (Experiment II) conditions.

No	Treatment	Applied doses (mL)	Symbol
1	Control	0	T1
2	Saf- T-Side [®]	500	T2
3	Saf- T-Side [®]	750	T3
4	Saf- T-Side [®]	1,000	T4
5	Saf- T-Side [®]	1,250	T5
6	Saf- T-Side [®]	1,500	T6
7	Saf- T-Side [®]	2,000	T7
8	SIVANTO [®] (flupiradyfurone)	1,000	T8

conducted on January 12, 2024, using the drill method, at a population density of 140,000 plants ha⁻¹. The first irrigation was applied one day after sowing, followed by irrigations at 15-day intervals. Nutrient management was carried out according to the recommended rates for the crop. In addition, foliar fertilization with Delfan[®] Plus was applied at a dose of 1.5 mL L⁻¹ of water at 25 days after sowing. Weed control was performed manually.

Treatment application

Treatments were applied twice at eight-day intervals using a backpack sprayer with a 1 L capacity. The commercial products used were Saf-T-Side[®] (paraffinic oil) and SIVANTO[®] (flupyradifurone), which were sprayed over the entire plant foliage.

Study variables

Pre-evaluation was conducted five days before treatment application. After application, the number of *M. sacchari* per plant was recorded every 24 h for the following five days. The biological effectiveness (%) of the treatments was also evaluated, which was calculated using the Abbott (1925) formula as follows:

$$\% \text{ Biological effectiveness} = [(A - B) / A] * 100$$

Where: *A*=Value of the untreated control; *B*=Value of the treatment under evaluation.

To quantify the number of *M. sacchari*, the first evaluation was carried out at 82 days after sowing (DAS), prior to treatment application, during which the number of adults was visually counted on five randomly selected leaves from five plants in the useful plot per treatment. Subsequently, four additional aphid counts were conducted on the dates presented in Table 2.

Statistical analysis

Data from the study variables were subjected to analysis of variance using the Statistical Analysis System (SAS Institute, 2017), according to the experimental design employed. In addition, a Tukey mean comparison test ($p \leq 0.05$) was applied.

Table 2. Schedule of treatment application and aphid counts in Experiments I and II.

Activity	days after sowing (das)
First aphid count	82
1 st application of the treatments	85
Second aphid count	88
2 nd application of the treatments	93
Third aphid count	96
Fourth aphid count	99
Fifth aphid count	102

Greenhouse experiment (Experiment II)

This experiment was established in a greenhouse at the CEP-CSAEGro, constructed with metal structures, covered with white impermeable plastic and anti-aphid mesh.

Study treatments

The same eight treatments previously evaluated under field conditions were used (Table 1).

Experimental design and experimental unit

Eight treatments with four replications were arranged in a completely randomized design. A total of 24 experimental units were used, each consisting of a black polyethylene pot (12×15 cm) with a capacity of 2.5 kg of substrate. The substrate was prepared with silt soil previously disinfected with Anibac[®] (quaternary ammonium + copper sulfate) at a dose of 5 mL L⁻¹ of water. Sowing was carried out using sorghum seed of the hybrid DKS-32 on February 21, 2024.

Inoculation of *M. sacchari* on sorghum plants

Leaves with high infestations of sugarcane aphid were collected from sorghum plants of the untreated control in the field experiment. These aphid-infested leaves were placed among the plants in each pot to induce pest infestation under greenhouse conditions. This procedure was performed at 44 das. Irrigation was carried out manually by applying 250 mL of water per pot at 3-day intervals starting from sowing. Fertilization consisted of a single application of 2 g of urea per pot at 48 das.

Application of study treatments

The commercial products Saf-T-Side[®] and SIVANTO[®] (flupyradifurone), at the doses presented in Table 1, were sprayed twice onto the plant foliage using a manual sprayer, following the schedule indicated in Table 2.

Study variables

A pre-evaluation was conducted five days before treatment application to quantify the number of winged adults of *M. sacchari*. After application, the number of insects per plant

was recorded every 24 h for the following five days. The biological effectiveness (%) of the treatments was obtained using the procedure described for the field experiment.

Statistical analysis

Data from the study variables were subjected to analysis of variance using the Statistical Analysis System (SAS Institute, 2017), according to the experimental design employed. In addition, a Tukey mean comparison test ($p \leq 0.05$) was applied.

RESULTS AND DISCUSSION

Number of *M. sacchari* under field conditions (Experiments I and II) Table 3 presents the analysis of variance for Experiments I and II, showing that treatment effects significantly influenced the reduction in aphid numbers. The mean number of aphids on sorghum leaves of the DKS-32 variety decreased as time progressed, with the lowest values observed during the fourth evaluation.

Table 4 shows the number of aphids in relation to the evaluations conducted for both experiments. In the first evaluation, no significant effects were observed due to treatment application in either experiment. However, in the second evaluation, highly significant differences were detected, in which the untreated control showed the highest values for Experiments I and II, respectively. The greatest level of control was achieved with the chemical insecticide. Nevertheless, the high dose of oil also substantially reduced the number of adult aphids. For Experiment I, control resulted in 299 fewer aphids compared to the untreated control, and 764 fewer aphids for Experiment II. The third, fourth, and fifth evaluations showed the same trend, indicating that the higher oil doses provided the greatest level of pest control by significantly reducing the number of adult aphids in the sorghum crop under both field and greenhouse conditions. Several studies reported by other authors demonstrate the positive effect of oil use for pest control. In this regard, some studies have shown favorable responses to oil application. For example, Díaz-Nájera *et al.* (2019) evaluated the biological effectiveness of Saf-T-Side[®] against *Aphis gossypii* in squash and reported an effectiveness of 82.06%. Likewise, Varela (2013) reported that *Diaphorina citri* nymphs treated with Saf-T-Side[®] showed a mortality rate of 99% at 16 days after application. In the present study, the high dose (2000 mL) of Saf-T-Side[®] in the second evaluation of Experiments I and II showed the lowest population

Table 3. Mean values, calculated frequencies (Fc), and coefficients of variation according to the response variables in Experiments I and II.

	Fc Experiment		C.V (%) Experiment	
	I	II	I	II
Pre-evaluation	1.17 NS	5.06 NS	48.49	26.41
Evaluación 2	0.19**	3.04**	71.15	48.17
Evaluation 3	0.54**	2.41**	64.68	41.13
Evaluation 4	2.71**	0.48**	93.38	86.09
Evaluation 5	0.96**	12.69**	89.14	72.84

** Highly significant differences, NS=Not significant

Table 4. Number of aphids across five evaluations in Experiment I (field) and Experiment II (greenhouse).

Number	Treatment	Evaluation				
		1a	2a	3a	4a	5a
Experiment I						
1	T1	367.11 a*	457.56 a	730.67 a	1062.8 a	864.1 a
2	T2	322.56 a	185.56 bc	570.78 ab	358.7 b	357.7 b
3	T3	480.56 a	292.89 ab	213.78 cd	218.3 b	152.8 cb
4	T4	346.89 a	255.44 ab	319.44 bcd	263.2 b	211.2 cb
5	T5	460.33 a	221.44 abc	165.33 cd	175.7 b	139.0 cb
6	T6	354.33 a	214.89 bc	369.67 cd	221.3 b	187.0 cb
7	T7	456.00 a	157.78 bc	216.00 cd	89.2 b	100.1 cb
8	T8	548.33 a	18.11 c	10.67 d	0.0 b	1.6 c
	HSD _{0.05}	299.03	237.2	310.39	412.37	331.74
Experiment II						
1	T1	544.56 a	542.11 a	864.33 a	872.7 a	843.33 a
2	T2	531.11 a	253.11 b	556.11 b	767.6 a	261.78 b
3	T3	629.33 a	233.56 bc	197.67 de	231.6 b	165.56 b
4	T4	545.56 a	188.89 bc	335.78 cd	132.6 b	171.67 b
5	T5	427.44 a	224.11 bc	281.11 cd	189.6 b	226.44 b
6	T6	444.00 a	205.00 bc	428.11 bc	220.9 b	256.67 b
7	T7	575.33 a	90.33 cd	175.56 de	275.0 b	106.89 b
8	T8	503.56 a	11.22 d	15.78 e	0.0 b	0.22 b
	HSD _{0.05}	205.04	155.66	217.01	428	273.66

*Treatments with the same letter are not statistically different according to Tukey's test. HSD_{0.05}: Honestly Significant Difference. T1: untreated control; T2: mineral oil (Saf-T-Side[®] 500 mL); T3: mineral oil (Saf-T-Side[®] 750 mL); T4: mineral oil (Saf-T-Side[®] 1000 mL); T5: mineral oil (Saf-T-Side[®] 1250 mL); T6: mineral oil (Saf-T-Side[®] 1500 mL); T7: mineral oil (Saf-T-Side[®] 2000 mL); T8: SIVANTO[®] (flupyradifurone).

incidence and, therefore, the highest biological effectiveness against *M. sacchari*, with percentages of 65.52% and 83.34%, respectively. This effect is attributed to the insect's mechanism of action, as described by Porcura (2011), who indicated that the primary cause of mortality in insects produced by mineral oils is anoxia; that is, oils block the insect spiracles, causing suffocation and ultimately death. On the other hand, Najar-Rodríguez *et al.* (2007) evaluated the effect of nC24 oil on *Aphis gossypii* Glover in cotton and found that petroleum spray oils at concentrations between 1 and 10% were highly effective in controlling the pest; they also determined that the oil can kill insects through direct contact.

In the present study, the reduction in population fluctuation was affected from the first application of mineral oil. Najar-Rodríguez *et al.* (2007) reported that mineral oils are effective and remain so up to six days after field application, although most aphid mortality occurs within the first two days after application.

Additionally, Gonzales *et al.* (2006) reported that the mineral oil Saf-T-Side[®] showed a significant effect on *Bemisia* spp. nymphs in eggplant at a dose of 1000 mL ha⁻¹; this effectiveness is comparable to the doses of 500 mL and 1500 mL evaluated in the present

study. Davidson *et al.* (1991) indicated that mineral oil interferes with insect gas exchange, causing asphyxiation and death by forming an impermeable film over the insect's body.

In both Experiments I and II, the product SIVANTO[®] showed high effectiveness, ranging from 82 to 100% across all evaluations. In this regard, Guevara *et al.* (2016) reported that the application of SIVANTO[®] (flupyradifurone) achieved 70.62% effectiveness in the chemical control of aphids in sorghum. However, chemical control of aphids is erratic and unpredictable, and loss of susceptibility has been associated with genetic and agronomic factors of host plants as well as environmental conditions (Godfrey, 2001).

Biological effectiveness of Experiment I (field)

Treatment application resulted in significant differences for the biological effectiveness variable, as shown in Figure 1. The untreated control showed the highest insect incidence, whereas the remaining treatments achieved the following biological effectiveness percentages: T2 (Saf-T-Side[®] 500 mL) 41.74%, T3 (Saf-T-Side[®] 750 mL) 60.61%, T4 (Saf-T-Side[®] 1000 mL) 61.24%, T5 (Saf-T-Side[®] 1250 mL) 64.84%, T6 (Saf-T-Side[®] 1500 mL) 59.41%, T7 (Saf-T-Side[®] 2000 mL) 68.64%, and T8 (SIVANTO[®]) 84.48%, respectively. Although mineral oils act primarily by causing insect asphyxiation, they may also exert a repellent effect and reduce oviposition (Stansly, 2000). The results indicate that the mineral oil Saf-T-Side[®] from the third treatment onward showed a biological effectiveness greater than 60% against *M. sacchari*. Similarly, Sieburth *et al.* (1998) reported that the mineral oil SunSpray[®] Ultra-Fine provided control of *Bemisia tabaci* puparia and nymphs, causing 94-99% and 50-75% mortality, respectively, as well as abnormal development in surviving nymphs, demonstrating that mineral oil is effective for the control of sucking insects.

On the other hand, in another study, Gonzales (2006) reported that mineral oils were effective for controlling *Lepidosaphes gloverii* in Valencia orange and that they are products with lower aggressiveness toward parasitoids, hymenopterans, and entomopathogenic fungi.

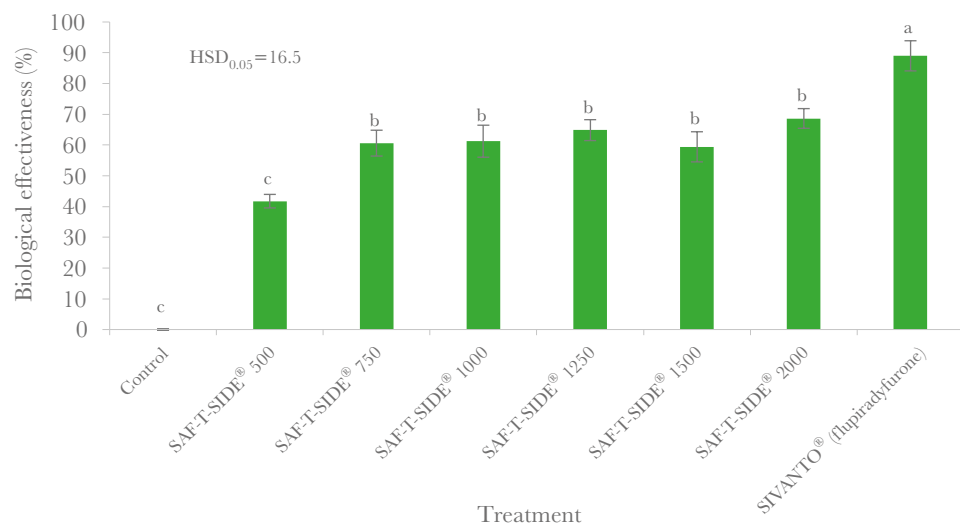


Figure 1. Biological effectiveness of the products used in Experiment I (field). HSD_{0.05}: Honestly Significant Difference.

Biological effectiveness of Experiment II (greenhouse)

Figure 2 shows the biological effectiveness generated by treatment application under greenhouse conditions (Experiment II), where the results indicate the presence of significant differences. The untreated control exhibited the highest aphid abundance and, therefore, 0% biological effectiveness, as no control measure was applied. In contrast, the remaining treatments, starting from treatment 2, produced the following biological effectiveness percentages: T2 (Saf-T-Side[®] 500 mL) 84%, T3 (Saf-T-Side[®] 750 mL) 61%, T4 (Saf-T-Side[®] 1000 mL) 84%, T5 (Saf-T-Side[®] 1250 mL) 74%, T6 (Saf-T-Side[®] 1500 mL) 87%, T7 (Saf-T-Side[®] 2000 mL) 88%, and T8 (SIVANTO[®]) 93%, respectively. Thus, biological effectiveness against *M. sacchari* under controlled conditions was up to 50% higher for all treatments evaluated compared with the results obtained under field conditions. This indicates that, in the greenhouse, where controlled conditions prevailed, treatments were more effective due to the absence of losses caused by volatilization. Stansly *et al.* (2000) indicated that the toxic response of oils is not the only way in which they may protect treated plants from pest damage, as oils can also form a film on the plant surface. Likewise, Butler *et al.* (1993) reported a study conducted in cucumber under heavy infestation of *Bemisia* spp., in which applications of 0.5% soybean oil and Saf-T-Side[®] resulted in captures of 157 and 268 flies per trap, respectively, compared with 522 in the untreated control, indicating that petroleum-derived mineral oils are effective against insect pests. Additionally, Macías *et al.* (2013), in controlling *Diaphorina citri* psyllids, evaluated mineral oil at concentrations of 1 and 2%, showing reductions in nymph populations of 91.6% and 92.8%, respectively. The results of these studies indicate that oils sprayed onto foliage are effective against nymphs and also demonstrate significant mortality of psyllid eggs. Similarly, Cabrera (2006) reported that the mineral oils Rocio Spray[®] and Sun Spray[®] achieved mortality effectiveness of 96% and 95%, respectively, against mobile stages of the citrus rust mite *P. oleivora*, results that are consistent with those found in the present study.

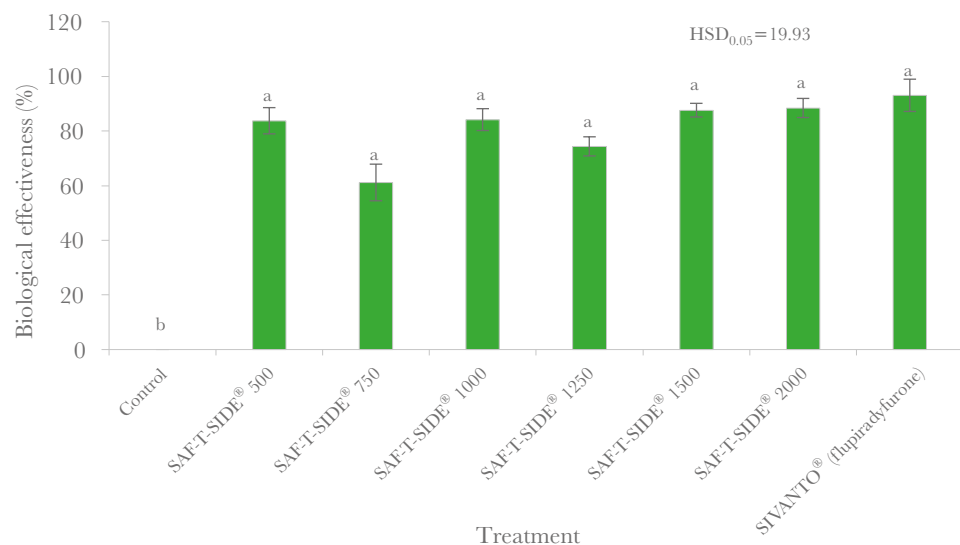


Figure 2. Biological effectiveness of the products used in Experiment II (greenhouse). HSD_{0.05}: Honestly Significant Difference.

CONCLUSIONS

The highest concentration of Saf-T-Side[®] achieved significant control of *Melanaphis sacchari* populations under both field and greenhouse conditions, demonstrating its potential as an ecological alternative to synthetic insecticides.










The treatments evaluated for the control of the sugarcane aphid showed greater control efficiency under greenhouse conditions than under field conditions.

REFERENCES

- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265-267. <http://dx.doi.org/10.1093/jee/18.2.265a>.
- Bográn, C.E., Ludwig, S., & Metz, B. (2011): Using Oils as Pesticides. Department of Entomology Texas A&M University, Agrilife Extension, <http://insects.tamu.edu/extension/publications/epubs/e-419.cfm04/08/2019>.
- Bowling, R. D., M. J. Brewer, D. L. Kerns, J. Gordy, N. Seiter, N. E. Elliot, G. D. Buntin, M. O. Way, T. A. Royer, S. Biles, and E. Maxson. 2016. Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. *J. Integr. Pest Manag.* 7: 12: 1-13. <https://doi.org/10.1093/jipm/pmw011>.
- Buntin, G.D., Toews, M.D., & Roberts. P.M. (2018). Insecticide control of sugarcane aphid on grain sorghum. *Arthropod Management Tests*, 43(1). <https://doi.org/10.1093/amt/tsy057>.
- Butler, G.D., Heneberry, T.J., Stansly, P.A., Stansly, D.J., & Schuster, D.J. (1993). Insecticidal effects of selected soaps, oils detergents on the sweetpotato whitefly (Homoptera: Aleyrodidae). *Floria Entomologist*. 76(1),161-167.
- Cabrera, C.R. I., Gonzales. F.C., & Hernández E. D. (2006). Evaluación de la eficacia de aceites minerales en el control del ácaro del moho *Phyllocoptruta oleivora* Ashmead en cítricos. *Instituto de Investigaciones en Fruticultura Tropical*, 7(1), 30.
- Clark, N., Dahlberg, J.A., Haviland, D., Marsh, B., & Rill, S. (2018). The effects of insecticide treatments on sugarcane aphid, yield and feed quality of forage sorghum in California. University of California Cooperative Extension, Hanford, CA, USA. 11 pp.
- Davidson, N. (1991). The role of spray oils in alternative agriculture. *Components Newsletter* 2:1-7.
- Díaz-Nájera, J. F., Ayvar, S.S., Mena, B. A., Apérez, B. M., Bello, T.S., & Tejeda, R. M. A. (2019). Uso de insecticidas y productos orgánicos en el control de *Aphis gossypii* (hemiptera: aphididae) en calabaza en trópico seco. *Entomología Agrícola*, ISSN: 2448-475X,183-187.
- García, E. (2004). Modificación al sistema de clasificación climática de Köppen. Universidad Nacional Autónoma de México. Distrito Federal, México. 90 p.
- Godfrey, D. L. (2001). Agronomic and environmental factors. influencing control of cotton aphids with insecticides. Proceedings of the Beltwide cotton conference. *National Cotton Council, Memphis TN*, 2(1),1242-1246.
- Gonzales, A.A., Nuñez, P.E., Galvan, P.B., Castro, G.A., & Gonzales, C. J. C. (2006). Extractos vegetales y aceites minerales como alternativa de control de mosca blanca (*Bemisia* spp.) en berenjena (*Solanum melongena* L.) en el Valle de Culiacán, Sinaloa, México. *Revista UDO Agrícola*, 6(1) 84-91.
- Guevara, A.L.P., León, J.C.V., Ochoa, G.S., Cupúl, L.M., Ángel, M.E.G., & Rodríguez, D.M. (2016). Control químico de pulgón amarillo (*Melanaphis sacchari*). Memorias del 3er. Congreso Nacional de Ciencia y Tecnología Agropecuaria. Roque, Celaya, Guanajuato. México. p. 168.
- Haar, P. J. (2018). Integrated pest management systems for sugarcane aphid in grain sorghum. Master of science thesis. The University of Georgia. Athens, Georgia. pp: 1-8.
- Jeschke, P. (2016). Progress of modern agricultural chemistry and future prospects. *Pest Manag Sci.* 72, 433-455. <https://doi.org/10.1002/ps.4190>.
- Jeschke, P., Haas, M., Nauen, R., Gutbrod, O., Beck, M.E., Matthiesen, S., & Velten, R. (2015). Sivanto[®] - a novel insecticide with a sustainable profile. In: Synthesis of Crop Protection Products. Chapter 24344. Maienfisch, P. and Stevenson, T.M. Eds. ACS Symposium Series, American Chemical Society, Washington,DC. USA. 331 p.
- Kimber, C.T., Dahlberg, J.A., & Kresovich, S. (2013).The genepool of Sorghum bicolor and its improvement. In: Paterson, A. H. Ed. Genomics of the Saccharinae. New York: Springer. pp 23-42. https://doi.org/10.1007/978-1-4419-5947-8_2.

- Macias, R. L., Santillán, C., Robles, B. A., Isiordia, N., & Ortiz, C. M. (2013). Insecticidas de bajo impacto ambiental para el control de *Diaphorina citri* (Hemiptera:Psyllidae) en limon persa en “La Fortuna”, Nayarit, México. *Revista BioCiencias*, 2 (3), 154-161. <https://doi.org/10.15741/revbio.02.03.12>
- Michaud, J.P., Whitworth, R.J., Schwarting, H., McCornack, B., & Zukoff, S. (2016). Sorghum insect management. Kansas State University Research and Extension. MF742.
- Najar-Rodríguez, A.J., Walter, G.H., & Mensah, R.K. (2007). The efficacy of a petroleum spray oil against *Aphis gossypii* Glover on cotton. Part 1: mortality rates and sources of variation. *Pest Manag Sci.*, 63(6), 586-95. <https://doi.org/10.1002/ps.1385>.
- Porcura, J. L. (2011). Aceites minerales. Ficha técnica. Disponible en: https://www.agroecologia.net/recursos/Revista_Ae/Ae_a_la_Practica/fichas/n6/ficha-revista-ac-6-aceites-minerales.pdf. (Consultado el 12 de 07 2025).
- SAS (2017). SAS/STAT User’s guide, version 9.4. SAS Institute Inc. North Caroline, USA.
- Schenck, S. (2000). Factors affecting the transmission and spread of sugarcane yellow leaf virus. *Plant Dis*, 84(1), 1085-1088. <https://doi.org/10.1094/PDIS.2000.84.10.1085>.
- Sieburth, P.J., Schroeder, W.J., & Mayer, R.T. (1998): Effects of oil and oil surfactant combination on silverleaf whitefly nymphs (Homoptera: Aleyrodidae) on collars. *The Florida Entomologist*, 81(3), 446-450. <https://doi.org/10.2307/3495935>.
- Singh, B.U., Padmaja, P.G., & Seetharama, N. (2004). Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: A review. *Crop Protection*, 23(1), 739-755. <https://doi.org/10.1016/j.cropro.2004.01.004>.
- Stansly, P, Liu., & D.J. Schuster, T.X. (2000). Effects of horticultural mineral oils on a polyphagous whitefly, its plants hosts and its natural enemies. pp. 120-133.
- Varela, F.S.E., Camacho, C.R., Briones, E.F., & López, S.J.A. (2013). Aceites agrícolas para el control de *Diaphorina citri* (Hemiptera: liviidae) en limón italiano de Tamaulipas. Disponible en: https://swfrec.ifas.ufl.edu/hlb/database/pdf/24_Varela_15.pdf. (Consultado el 11 de julio de 2025).
- Weidhaas, J.A. (1988). Horticultural Oils. Virginia Tech Insect Notes #174. Department of Entomology, Cornell University, 6, 3 pp.
- Zarrabi, A. A., Royer, T.A., Giles, K.L., Seuhs, S.K., & Ghousifam. (2017). Evaluation of Sivanto Prime for Control of Sugarcane Aphid. *Arthropod Management Tests*, 43(1),1. <https://doi.org/10.1093/amt/tsy083>

Production of Embryos by Interspecies Somatic Cell Nuclear Transfer (iSCNT) Between Bovine (*Bos taurus*) and Ovine (*Ovis aries*) Using Handmade Cloning (HMC)

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ABSTRACT

Objective: To determine the ability of the sheep oocyte (*Ovis aries*) to generate cloned bovine embryos (*Bos taurus*), through interspecies Somatic Cell Nuclear Transfer (iSCNT) by Handmade Cloning (HMC).

Design/methodology/approach: For iSCNT, fifth-passage bovine skin fibroblasts were used as karyoplasts, and *in vitro*-matured, manually enucleated ovine oocytes were used as cytoplasts. Cytoplast-karyoplast-cytoplast triplets were formed, which were fused by electrical pulses and activated for the development of cloned bovine embryos, which were cultured in Cleavage medium. Additionally, some ovine oocytes were activated as parthenogenetic embryos to serve as a control group. The *in vitro* development rate (IVD) of the iSCNT group (BOV-OV) *vs.* parthenogenetic ovine embryos (G-OV) was evaluated using a two-way Student t-test for paired data.

Results: It was observed that in the BOV-OV group, bovine cloned embryos produced by iSCNT using HMC only reached the morula stage (20 blastomeres), while in the G-OV blastocysts were observed with significant differences ($p \leq 0.05$).

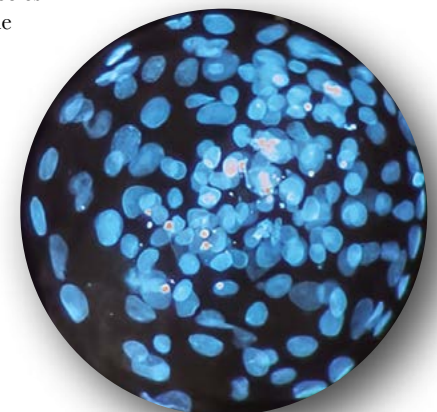
Limitations on study/implications: Limited sample size of slaughterhouse ovaries.

Findings/conclusions: Although ovine oocytes were able to initiate the IVD of cloned bovine embryos produced by iSCNT using HMC, species divergence proved to be a critical limiting factor for reaching the blastocyst stage.

Keywords: bovine, ovine, handmade cloning, interspecies.

INTRODUCTION

Reproductive biotechnologies used in animals enable the growth of populations of species with high genetic value, especially when their numbers are



critically low. Moreover, these technologies support the conservation, preservation, and dissemination of genetic material from individuals with superior traits compared to others of their kind (Loi *et al.*, 2011; Lagutina *et al.*, 2013; Yelissetti *et al.*, 2016; Galli *et al.*, 2021; Mrowiec *et al.*, 2021).

The genetic rescue of selected individuals has led to numerous studies in domestic species, aiming to extrapolate findings to other animal populations or specific individuals. One such approach is Somatic Cell Nuclear Transfer (SCNT), or cloning, which is used to generate embryos with genetic material identical to that of an existing specimen. In 1938, Hans Spemann first reported a technique based on this principle, applying it to amphibians (as cited by Wilmut *et al.*, 1997). Later, in 1997, Wilmut and colleagues achieved the birth of the sheep *Dolly*. This milestone demonstrated that the genome of an adult somatic cell retains all its genetic information, despite being a specialized cell type. In other words, although the genome undergoes restrictions during development and changes during cellular differentiation, these are reversible, as they result from epigenetic modifications rather than permanent alterations to the genome (Verma *et al.*, 2015; Gouveia *et al.*, 2020; Galli *et al.*, 2021).

Among the limitations of this technique is the restricted availability of oocytes from wild animals. As an alternative, iSCNT involves using a somatic cell from the species to be cloned as the nuclear donor (karyoplast) and inserting it into an enucleated oocyte (cytoplast) obtained from a species belonging to a completely different order or taxon. This approach offers the possibility of generating viable and transferable cloned embryos (Beyhan *et al.*, 2007; González-Grajales *et al.*, 2016; Yu *et al.*, 2016; Saini *et al.*, 2018; Ammari *et al.*, 2022). Therefore, iSCNT is employed to conserve wildlife species from which oocytes cannot be collected, species with challenging reproductive management, or those with limited oocyte availability (Saikhun *et al.*, 2002; Pan *et al.*, 2014). Using this method, the gaur (*Bos gaurus*) (Lanza *et al.*, 2000; Srirattana *et al.*, 2012) and the gray wolf (*Canis lupus*) (Oh *et al.*, 2008) have been successfully cloned.

Interspecies Somatic Cell Nuclear Transfer is more efficient when the donor karyoplast and the recipient cytoplast come from species with similar reproductive physiology, pregnancy length, and placental type (Melo *et al.*, 2022).

In species that can naturally hybridize or share a closer phylogenetic relationship, the likelihood of embryos developing and producing live offspring is higher (Lagutina *et al.*, 2013; Mrowiec *et al.*, 2021). However, Dominko *et al.*, (1999) fused ovine somatic cells with enucleated bovine oocytes to study nucleus-cytoplasm interactions in embryos generated via iSCNT, achieving a blastocyst rate of 13.9%.

Even when enucleated, the oocyte retains the ability to reprogram the nucleus of a somatic cell, returning it to an undifferentiated and totipotent state capable of producing a viable embryo (Jiang *et al.*, 2011; Seaby *et al.*, 2013; Saini *et al.*, 2018; Galli *et al.*, 2021), without altering the transplanted genome (Dominko *et al.*, 1999; Gurdon & Wilmut, 2011). In a study published by Hamilton (2004), ovine oocytes were used as recipients for bovine somatic cell nuclei to clone bovine embryos via iSCNT; however, the results were inconclusive, with only four cloned embryos reaching at least the 8-cell stage. The

present study aimed to determine the ability of ovine oocytes (*Ovis aries*) to generate bovine cloned embryos (*Bos taurus*) through iSCNT by Handmade Cloning (HMC).

The hypothesis was that, since both species belong to the same family and mammalian oocytes contain all the necessary information to produce an embryo, using ovine oocytes as cytoplasts will enable the *in vitro* development (IVD) of cloned bovine embryos produced via iSCNT from adult bovine fibroblasts. This approach could serve as an alternative for the reproduction and conservation of individuals with valuable, productive traits.

MATERIALS AND METHODS

This study was carried out at the Assisted Animal Reproduction Laboratory W-210 of the Universidad Autónoma Metropolitana, Iztapalapa Campus, in Mexico City, Mexico. All reagents were purchased from Sigma-Aldrich unless otherwise specified.

Collection of Auricular Skin and Derivation of Bovine Fibroblasts

Following the protocols described by Tovar *et al.*, (2008) and Navarro-Maldonado *et al.*, (2015), skin biopsies were taken from the dorsal side of the ear of an adult female Simbrah bovine, housed at the Agricultural Production Research Center of the Universidad Autónoma de Nuevo León (CIPA-UANL). A 1 cm² area on the back of the ear was shaved, washed with a 2% chlorhexidine solution (Laboratorio Aranda, Mexico), and disinfected with 70% alcohol. Three skin samples, each 0.3 cm² in diameter, were collected using a biopsy punch (Pickett, 2011) and transported in tubes containing 10 mL of Dulbecco's Modified Eagle Medium (DMEM, *In Vitro*, S.A., Mexico), supplemented with 10% Fetal Bovine Serum (FBS, Biowest, Nuaille, France), 2% antibiotic-antimycotic (Antibac-Antifun, *In Vitro*, S.A., Mexico), and kept at 4-8 °C.

Once in the laboratory, the samples were washed in DPBS (Dulbecco's Phosphate-Buffered Saline without calcium or magnesium, *In Vitro*, S.A., Mexico), supplemented with 2% of a 100X antibiotic-antimycotic solution Antibac-Antifun at pH 7 (10,000 IU/mL Sodium Penicillin G, 10,000 µg/mL Streptomycin Sulfate, and 25 µg/mL solubilized Amphotericin B, *In Vitro*, S.A., Mexico) for 3 min, with the wash repeated twice (Santos *et al.*, 2021). The skin samples were then cultured as explants in 35 mm Petri dishes with 3 mL of supplemented DMEM (88% DMEM, 10% FBS, 2% Antibac-Antifun), enriched with 1 ng/mL Epidermal Growth Factor (EGF), and incubated at 38.5 °C with 5% CO₂ in 95% air and saturated humidity for at least 7 days or until a confluent fibroblast layer was obtained (Navarro-Maldonado *et al.*, 2015). Fibroblast confluence (>90%) was assessed, and cells were detached from the extracellular matrix using an enzymatic solution of 0.05/0.05% Trypsin-Versene (*In Vitro*, S.A., Mexico). The fibroblasts were collected, with half of the population subcultured for cell passages and the remainder cryopreserved in a DMSO-based medium (Sigma-Aldrich, USA). These steps were repeated until five cell passages were completed, as required for iSCNT. Cell concentration was determined at each passage using a Neubauer chamber, following Freshney (2010).

Collection and *In Vitro* Maturation (IVM) of Ovine (*Ovis aries*) Cumulus-Oocyte Complexes

Cumulus-oocyte complexes (COC) were obtained from ovine ovaries collected at a local slaughterhouse. The ovaries were transported in physiological saline solution (PSS) supplemented with 2% antibiotics at 30 °C for 1 to 2 h (Martínez-Ibarra *et al.*, 2019). Upon arrival at the laboratory, the ovaries were washed three times with isothermal PSS. The COC were then collected by puncture/aspiration of ovarian follicles (2 to 5 mm in diameter) using a 10 mL syringe with an 18-gauge hypodermic needle in TCM-199 medium with HEPES (*In Vitro*, S.A., Mexico), supplemented with 100 IU/mL of heparin at 30 °C. The COC were evaluated and selected based on quality grades I and II for *in vitro* maturation (IVM) (Yadav *et al.*, 1997).

In vitro maturation was performed in TCM-199 without HEPES, supplemented with 10% FBS, 10 ng/mL Epidermal Growth Factor (EGF), recombinant Follicle-Stimulating Hormone (FSH; 0.1 IU/mL; Gonaf-F, Merck), 5 IU/mL equine Chorionic Gonadotropin (eCG; Gonaforte, Parfarm S.A., Mexico), and 0.6% antibiotics. Groups of 30 COC were then cultured in four-well dishes (Nunc, USA), each containing 500 μ L of IVM medium covered with mineral oil for 20 h (Martínez-Ibarra *et al.*, 2019).

Cloning of Bovine-Ovine (BO-OV) Embryos via iSCNT

Preparation of Bovine Karyoplasts

Bovine fibroblasts from the fifth passage were used as nuclear donors (karyoplasts) for iSCNT (Beltrán & González, 2016; Cortez *et al.*, 2017). Before iSCNT, cells were detached from their extracellular matrix by adding a 0.05/0.05% Trypsin-Versene enzymatic solution (*In Vitro*, S.A., Mexico) for 8-10 min. They were then placed under incubation conditions, followed by enzyme inactivation using DMEM supplemented with 1% FBS.

Preparation of Ovine Cytoplasts

After 20 h of IVM, cumulus cell expansion in the COC was evaluated. The COC were removed from the IVM medium and incubated in a 1.5 mL tube with 500 μ L of hyaluronidase (0.5 mg/mL in TCM-199 without HEPES) for 5 min under the same conditions. The suspension was gently pipetted to disperse the cumulus cells. The contents were transferred to a 35 mm Petri dish, and denuded oocytes were recovered and washed in 500 μ L of TCM-199 supplemented with 2% FBS (T2). Oocytes showing a first polar body (PB), indicating arrest at Metaphase II (MII), were selected. To induce protrusion of the metaphase plate, MII oocytes were incubated in the same IVM medium supplemented with Demecolcine (0.5 μ g/mL) for 1 h. To remove the zona pellucida (ZP) and enucleate the oocytes, a Petri dish was prepared with 30 μ L microdrops of: T2, Pronase (2 mg/mL in TCM-199 supplemented with 10% FBS or T10), TCM-199 supplemented with 20% FBS (T20), T10 (supplemented with 0.05% Cytochalasin B), and T20.

Oocytes were passed through each microdrop and manually enucleated for HMC in T10 with Cytochalasin B using a microblade (Bioniche, USA), as described by Vajta *et al.* (2001). This procedure removed the metaphase plate along with the polar body (PB)

(Cortez *et al.*, 2017; Vazquez-Avendaño *et al.*, 2024). Once enucleated, the cytoplasts were placed in T20 microdrops before fusion with the karyoplasts.

Formation and Fusion of Cytoplast-Karyoplast-Cytoplast Triplets

In a 35 mm Petri dish, 15 μL microdrops were prepared containing: T20, phytohemagglutinin (5 mg/mL in TCM-199 with HEPES), T2, fusion medium (0.3 M D-mannitol, 1 mg/mL polyvinyl alcohol), and T20. Ovine cytoplasts were placed individually into the T20 microdrops. Groups of five cytoplasts were then briefly immersed (3 to 4 s) in the phytohemagglutinin microdrop to facilitate the formation of triplets, composed of ovine cytoplast, bovine karyoplast, and ovine cytoplast. These cellular triplets were equilibrated in the fusion medium and aligned on the positive electrode of the fusion chamber (BTX, 0.5 mm gap, model 450, Holliston, MA, USA). Fusion was induced using a single direct current (DC) pulse of 1.0 kV/cm for 9 μs . The fused structures were then transferred to T20 microdrops for reconstitution into a single cell (reconstructed cloned embryo). This process was repeated with the remaining cytoplasts (Vajta *et al.*, 2001).

In Vitro Development (IVD) of Cloned Bovine (BO-OV) Embryos via iSCNT

The reconstructed cloned bovine (BO-OV) embryos generated through iSCNT via HMC were chemically activated by incubating them in 1 mL of T2 supplemented with 8 $\mu\text{g}/\text{mL}$ of calcium ionophore (A23187) for 5 min, followed by three washes in 1 mL of T20. They were then transferred to 200 μL of Cleavage medium (Cook Medical, IN, USA) containing 2 mM 6-DMAP (6-dimethylaminopurine), covered with mineral oil, to initiate nuclear reprogramming (Vazquez-Avendaño *et al.*, 2024). Each embryo was individually placed in 2 μL microdrops of the same supplemented Cleavage medium and incubated for 4 h under previously described conditions.

For embryo culture, the Well of the Well (WOW) system was used. Microwells were created inside a four-well dish (Nunc, USA) using a punch tool, corresponding to the number of viable reconstructed cloned embryos (Navarro-Maldonado *et al.*, 2015). Each cloned embryo was placed individually into a microwell containing 100 μL of Cleavage medium covered with mineral oil and incubated for 7 days under the same conditions to assess the IVD rate.

Development of Ovine Parthenogenetic Embryos (G-OV)

A group of ovine oocytes at the MII stage were chemically activated following the same protocol used for cloned embryo activation, in order to produce parthenogenetic embryos as a control for the culture system (G-OV).

Determination of Embryo Quality Based on Number of Nuclei Present

To assess embryo quality based on the number of nuclei, DAPI staining, and epifluorescence microscopy were used. This method allows visualization of blastomere nuclei and analysis of cellular fragmentation.

At the end of IVD, embryos were washed in DPBS without antibiotics and fixed in 4% paraformaldehyde for 15 min. They were then incubated in a DAPI (4',6-diamidino-2-

phenylindole) solution at 1 $\mu\text{g}/\text{mL}$ for 10 min, covering the culture dish. Excess stain was removed by washing with DPBS. For evaluation under epifluorescence microscopy, UV-2A filters with a wavelength range of 330-380 nm were used. The presence of nuclei in the blastomeres was observed (Tarnowski *et al.*, 1991). To compare the IVD rate between the BO-OV and G-OV groups, a two-tailed paired Student's t-test was applied using GraphPad Prism version 9.5 (MA, USA), with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Bovine Fibroblast Cultures

After five cell passages, a total fibroblast concentration of 7,892,900 cells was obtained, consistent with the surface area provided for cell growth. These results align with findings reported by Takashima (1998) and Seluanov *et al.*, (2010), who concluded that by the fifth passage, cells reach genetically stable, suitable conditions for use as nuclear donors. Similarly, Baranyi *et al.*, (2019) and Cortez *et al.*, (2021) agree that genetic stability and appropriate phenotypic characteristics are maintained between the fifth and tenth passages. In contrast, exceeding this range may lead to genetic drift, changes in growth rate, and morphological alterations.

In Vitro Maturation of Ovine COC

Five experimental replicates were performed using 364 ovine ovaries, from which a total of 614 oocytes were collected (100% of the initial group), averaging 1.6 oocytes per ovary. Of these, 352 oocytes (57%) were classified as grades A and B according to ASEBIR criteria (2015). The next selection focused on oocytes that successfully reached IVM (MII stage); among the 352 selected, 216 oocytes (61.36%) achieved MII. However, only 200 were used as cytoplasts following enucleation.

Cloning of Bovine-Ovine (BO-OV) Embryos via iSCNT

From the 200 enucleated ovine oocytes, a total of 100 cytoplast-karyoplast-cytoplast triplets were successfully formed.

Table 1 and Figure 1 show the *in vitro* development (IVD) results of bovine-ovine cloned embryos (BO-OV) ($n=100$) versus ovine parthenogenetic embryos (G-OV) ($n=82$), after 5 days of *in vitro* culture, respectively.

Of the 100 reconstructed cloned bovine embryos produced via iSCNT, 49 (49%) underwent cleavage at various stages of IVD, while 51 (51%) exhibited fragmentation. The highest developmental stage reached was the morula, with 15 embryos containing 15 to 20 blastomeres (30.61%). These results surpass those reported by Hamilton (2004), who achieved only 4 cloned embryos (19%) beyond the 8-cell stage using iSCNT. However, they fall short of the outcomes reported by Tecirlioglu *et al.*, (2006), who obtained a blastocyst (4.8%). Notably, those authors used fetal bovine fibroblasts and traditional cloning techniques involving micromanipulators for enucleating ovine oocytes and fusing cytoplast-karyoplast pairs. They also cultured cloned bovine embryos using the WOW system. In contrast, the present study employed adult female bovine fibroblasts and manually

Table 1. *In Vitro* Development (IVD) Rate of Bovine-Ovine Cloned Embryos (BO-OV) (n=100) and Ovine Parthenogenetic Embryos (G-OV) (n=82) at 5 Days of Culture.

Embryo Type	Total (N)	Fragmented (%)	Cleaved (%)	Embryonic Cleavage Stages Based on the Number of Nucleated Cells				
				<6 cells	7 - 10 cells	11 - 14 cells	Morulae 15 - 20 cells	Blastocysts N (%)
				N (%)	N (%)	N (%)	N (%)	
BO-OV	100	51 (51)	49 (49)	11 (22.44)	11 (22.44)	12 (24.48)	15 (30.61)	0 (0) ^a
G-OV	82	27 (32.92)	55 (67.07)	6 (10.90)	7 (12.72)	14 (25.45)	8 (14.54)	20 (36.36) ^b

Different superscript letters (a, b) indicate statistically significant differences (P=0.05).

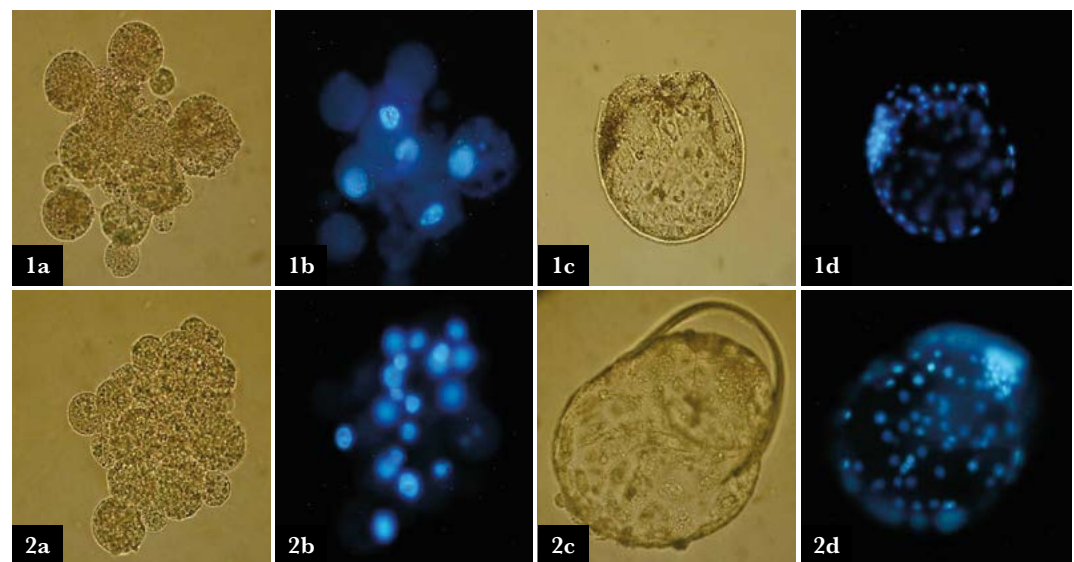


Figure 1. Bright-field and dark-field images of cloned bovine embryos and ovine parthenogenetic embryos produced via iSCNT and stained with DAPI. 1a) Fragmented cloned bovine embryo under bright-field microscopy. 1b) Fragmented cloned bovine embryo under dark-field microscopy, showing blastomeres lacking visible nuclei. 2a) Cloned bovine morula with 20 blastomeres under bright-field microscopy. 2b) Cloned bovine morula with 20 blastomeres under dark-field microscopy. 1c) Ovine parthenogenetic embryo at early blastocyst stage under bright-field microscopy. 1d) Ovine parthenogenetic embryo at the early blastocyst stage under dark-field microscopy. 2c) Ovine parthenogenetic embryo at hatched blastocyst stage under bright-field microscopy. 2d) Ovine parthenogenetic embryo at hatched blastocyst stage under dark-field microscopy. Images were captured using an epifluorescence microscope equipped with a UV-2A filter (wavelength 330-380 nm) at 400X magnification.

enucleated ovine oocytes using the HMC technique, and fused cellular triplets (Vajta *et al.*, 2001). Handmade cloning does not require micromanipulators for enucleation, and the oocytes are stripped of their ZP.

Development of Ovine Parthenogenetic Embryos (G-OV)

To rule out that the low rates of BO-OV clone embryos were due to culture failures, a group of 82 ovine oocytes was activated to produce parthenogenetic embryos (G-OV). After 7 days of IVD, 55 embryos (67.07%) underwent cleavage at various stages, while 27 (32.92%) showed fragmentation. The rate of parthenogenetic blastocyst formation was 20

embryos (36.36%), distributed as follows: 6 early blastocysts (30%), 4 late blastocysts (20%), 4 hatching blastocysts (20%), and 6 hatched blastocysts (30%).

DAPI staining confirmed the presence of blastomere nuclei in the ovine parthenogenetic embryos (G-OV) that reached the blastocyst stage (Figure 2).

It is known that in iSCNT, the degree of embryonic cleavage depends on the mitochondrial genome of one of the two species involved in the cloning process. Therefore, the fusion, activation, and development of cells from genetically distinct species often result in poor embryo development. One possible cause is reduced ATP production, coupled with the failure of interaction between subunits encoded by mitochondrial DNA (mtDNA) and the polypeptides involved in the electron transport chain (Jiang *et al.*, 2011; Selokar *et al.*, 2011).

The condition of heteroplasmy observed in cloned animals—where different mtDNA genotypes coexist within the cloned embryo, originating from both the somatic donor cell and the recipient oocyte—is a factor that leads to metabolic disturbances. This is because the presence of mitochondria from different origins disrupts the respiratory chain and genetic stability, creating mitochondrial incompatibility that affects development and long-term health. Such incompatibility may influence physiological traits and predispose individuals to mitochondrial diseases, ultimately impacting reproductive efficiency (Mrowiec *et al.*, 2021).

On the other hand, although the present study aligns with findings by Jiang *et al.*, (2011), Seaby *et al.*, (2013), Saini *et al.*, (2018), and Galli *et al.*, (2021) who demonstrated that mammalian oocytes contain the necessary information to reprogram donor nuclei by dedifferentiating them to produce cloned embryos, even across unrelated species—this nuclear reprogramming remains incomplete. This is because the donor nucleus can induce abnormal epigenetic modifications that disrupt the expression of key genes involved in early embryonic development, such as: OCT4 (regulator of pluripotency), T-FAM (responsible for mitochondrial DNA stability, maintenance, and transcriptional control), BAX (pro-apoptotic), BCL-2 (anti-apoptotic), and GJA1 (major gap junction protein expressed in cumulus cells) (Ishino *et al.*, 2018; Mrowiec *et al.*, 2021; Adams *et al.*, 2022; Melo *et al.*, 2022).

Additionally, embryo cloning increases the incidence of developmental asynchrony, leading to a higher rate of developmental arrest compared to *in vivo* conditions. This explains the elevated fragmentation rate observed in early stages of *in vitro* development (Mrowiec

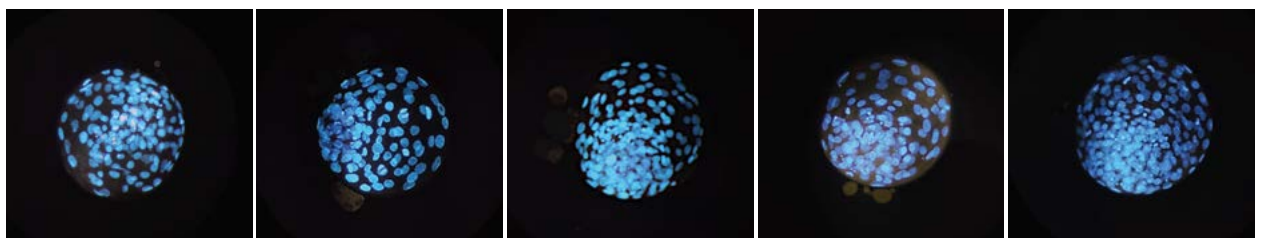


Figure 2. Ovine Parthenogenetic Blastocysts Stained with DAPI. Nuclei and the spatial arrangement of cells within the inner cell mass (ICM) and trophectoderm are clearly visible. Images were captured using an epifluorescence microscope equipped with a UV-2A filter (wavelength 330-380 nm) at 400X magnification.

et al., 2021; Adams *et al.*, 2022). Furthermore, when the taxonomic relationship between cytoplasts and karyoplasts is distant, embryonic development tends to be blocked at early stages. In contrast, development progresses more effectively when donor and recipient cells originate from species with close taxonomic proximity in terms of reproductive physiology, placental type, and gestation duration (Miyamoto, 2019; Malin *et al.*, 2022).

Interspecies Somatic Cell Nuclear Transfer could contribute to the generation of maternal-embryonic cell lines, which may be used for recloning (Loi *et al.*, 1998; Cibelli *et al.*, 2002). This would expand our understanding of embryonic development, as well as the enzymatic and genetic components involved, including incompatibility between mitochondrial DNA (mtDNA) and nuclear DNA, mitochondrial heteroplasmy, challenges in embryonic genome reactivation, and issues related to communication between mtDNA and nuclear genes (Mrowiec *et al.*, 2021).

CONCLUSIONS

Although ovine oocytes were capable of initiating the *in vitro* development of cloned bovine embryos using HMC, species divergence remains a critical factor in achieving the blastocyst stage. Nevertheless, the use of cytoplasts from species different from the nuclear donor opens up possibilities for generating embryos in species that share physiological and reproductive traits, offering a potential strategy for safeguarding valuable genetic material.

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LITERATURE CITED

- Adams, L., Liu, Y., Durrant, B., Ruggeri, E., Young, C., Krisher, R., & Polejaeva, I. (2022). Use of Bisection to Reduce Mitochondrial DNA in the Bovine Oocyte. *Journal of Visualized Experiments: JoVE*, (185), 10.3791/64060. <https://doi.org/10.3791/64060>
- Ammari, A. A., AL Ghadi, M. G., Amran, R. A., Al Malahi, N. M., & Alhimaidi, A. R. (2022). interspecific nuclear transfer blastocysts reconstructed from arabian oryx somatic cells and domestic cow ooplasm. *Veterinary Sciences*, 10(1), 17. <https://doi.org/10.3390/vetsci10010017>
- Asociación para el Estudio de la Biología de la Reproducción (ASEBIR). (2015). Criterios ASEBIR de valoración morfológica de oocitos, Embriones tempranos y blastocistos humanos. *Revista de Embriología Clínica y Biología de la Reproducción*. 20(1): 98.
- Baranyi, U., Winter, B., Gugerell, A., Hegedus, B., Brostjan, C., Laufer, G., & Messner, B. (2019). Primary human fibroblasts in culture switch to a myofibroblast-like phenotype independently of TGF Beta. *Cells*, 8(7), 721. <https://doi.org/10.3390/cells8070721>.
- Beltrán N; & González C (2016). Técnicas de cultivos celulares e ingeniería de tejidos. Universidad Autónoma Metropolitana. México. 199 p.
- Beyhan, Z., Iager, A. E., & Cibelli, J. B. (2007). Interspecies nuclear transfer: implications for embryonic stem cell biology. *Cell Stem Cell*, 1(5), 502-512. <https://doi.org/10.1016/j.stem.2007.10.009>
- Cibelli, J. B., Grant, K. A., Chapman, K. B., Cunniff, K., Worst, T., Green, H. L., Walker, S. J., Gutin, P. H., Vilner, L., Tabar, V., Dominko, T., Kane, J., Wettstein, P. J., Lanza, R. P., Studer, L., Vrana, K. E., & West, M. D. (2002). Parthenogenetic stem cells in nonhuman primates. *Science (New York, N.Y.)*, 295(5556), 819. <https://doi.org/10.1126/science.1065637>

- Cortez, J. V., Murga, N. L., Segura, G. T., Rodríguez, L., Vásquez, H. V., & Maicelo, J. L. (2017). Capacidad de dos líneas celulares para la producción de embriones clonados mediante transferencia nuclear de células somáticas. *Revista de Investigaciones Veterinarias del Perú*, 28(4), 928-938.
- Cortez, J. V., Segura, G. T., & Murga, N. L. (2021). Impacto de la reprogramación celular (células de fibroblastos y cúmulos) en la producción de clones bovinos. *Revista Veterinaria*, 32(2), 216. <https://doi.org/10.30972/vet.3225746>
- Dominko, T., Mitalipova, M., Haley, B., Beyhan, Z., Memili, E., McKusick, B., & First, N. L. (1999). Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. *Biology of reproduction*, 60(6), 1496-1502. <https://doi.org/10.1095/biolreprod60.6.1496>
- Freshney, R.I. (2010). *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*. John Wiley & Sons, Inc. Freshney (Ed). <https://doi.org/10.1002/9780470649367>
- Galli, C., & Lazzari, G. (2021). 25th Anniversary of Cloning by Somatic-Cell Nuclear Transfer: Current applications of SCNT in advanced breeding and genome editing in livestock. *Reproduction (Cambridge, England)*, 162(1), F23-F32. <https://doi.org/10.1530/REP-21-0006>.
- González-Grajales, L. A., Favetta, L. A., King, W. A., & Mastromonaco, G. F. (2016). Lack of effects of ooplasm transfer on early development of interspecies somatic cell nuclear transfer bison embryos. *BMC Developmental Biology*, 16(1), 36. <https://doi.org/10.1186/s12861-016-0137-6>
- Gouveia, C., Huysen, C., Egli, D., & Pepper, M. S. (2020). Lessons learned from somatic cell nuclear transfer. *International Journal of Molecular Sciences*, 21(7), 2314. <https://doi.org/10.3390/ijms21072314>
- Gurdon, J. B., & Wilmot, I. (2011). Nuclear transfer to eggs and oocytes. *Cold Spring Harbor Perspectives in Biology*, 3(6), a002659. <https://doi.org/10.1101/cshperspect.a002659>
- Hamilton, H (2004). The Development and Ultrastructure of Intergeneric Nuclear transfer embryos using ovine ooplasm. Tesis doctoral. Discipline of Animal Sciences School of Agriculture and Wine. Faculty of Sciences. University of Adelaide, Australia. 222p.
- Ishino, T., Hashimoto, M., Amagasa, M., Saito, N., Dochi, O., Kirisawa, R., & Kitamura, H. (2018). Establishment of protocol for preparation of gene-edited bovine ear-derived fibroblasts for somatic cell nuclear trans-plantation. *Biomedical Research (Tokyo, Japan)*, 39(2), 95-104. <https://doi.org/10.2220/biomedres.39.95>
- Jiang, Y., Kelly, R., Peters, A., Fulka, H., & Dickinson A. (2011). Interspecies somatic cell nuclear transfer is dependent on compatible mitochondrial DNA and reprogramming factors. *PLoS ONE* 6(4): e14805. <https://doi:10.1371/journal.pone.0014805>
- Lagutina, I., Fulka, H., Lazzari, G., & Galli C. (2013). Interspecies somatic cell nuclear transfer: advancements and problems. *Cell Reprogram*; 15(5):374-84. <https://doi:10.1089/cell.2013.0036>.
- Lanza, R. P., Cibelli, J. B., Diaz, F., Moraes, C. T., Farin, P. W., Farin, C. E., Hammer, C. J., West, M. D., & Damiani, P. (2000). Cloning of an endangered species (*Bos gaurus*) using interspecies nuclear transfer. *Cloning*, 2(2), 79-90. <https://doi.org/10.1089/152045500436104>
- Loi, P., Ledda, S., Fulka, J., Cappai, P., & Moor, R. M. (1998). Development of parthenogenetic and cloned ovine embryos: effect of activation protocols. *Biology of Reproduction*, 58(5), 1177-1187. <https://doi:10.1095/biolreprod58.5.1177>
- Loi, P., Modlinski, J. A., & Ptak, G. (2011). Interspecies somatic cell nuclear transfer: a salvage tool seeking first aid. *Theriogenology*, 76(2), 217-228. <https://doi.org/10.1016/j.theriogenology.2011.01.016>
- Malin, K., Witkowska-Pilaszewicz, O., & Papis, K. (2022). The many problems of somatic cell nuclear transfer in reproductive cloning of mammals. *Theriogenology*, 189, 246-254. <https://doi.org/10.1016/j.theriogenology.2022.06.030>
- Martínez-Ibarra, J. L., Espinoza-Mendoza, E. A., Rangel-Santos, R., Am-briz-García, D. A., & Navarro-Maldonado, M. D. C. (2019). Effect of resveratrol on the *in vitro* maturation of ovine (*Ovis aries*) oocytes and the subsequent development of handmade cloned embryos. *Veterinaria México OA*, 5(4). <https://doi.org/10.22201/fmvz.24486760e.2018.4.491>
- Melo, L., Silva, S., Magalhaes, L., Cortez, J., Kumar, S., Duarte, J., Rola, L., Chaves, M., & Freitas, V. (2022). The use of somatic cell nuclear transfer to obtain interspecific cloned embryos from brown brocket deer karyoplast and bovine cytoplasm: Embryo development and nuclear gene expression. *Theriogenology Wild* <https://doi.org/10.1016/j.therwi.2022.100001>
- Miyamoto K. (2019). Various nuclear reprogramming systems using egg and oocyte materials. *The Journal of Reproduction and Development*, 65(3), 203-208. <https://doi.org/10.1262/jrd.2019-002>
- Mrowiec, P., Bugno-Poniewierska, M., & Młodawska, W. (2021). The perspective of the incompatibility of nucleus and mitochondria in interspecies somatic cell nuclear transfer for endangered species. *Reproduction in domestic animals. Zuchthygiene*, 56(2), 199-207. <https://doi.org/10.1111/rda.13864>

- Navarro-Maldonado, M. del C., Hernández-Martínez, S., Vazquez-Avendaño, J. R., Martínez-Ibarra, J. L., Zavala-Vega, N. L., Vargas-Miranda, B., & Ambriz-García, D. A. (2015). Deriva de células epiteliales de tejido de piel descongelado de *Ovis canadensis mexicana* para la formación de un banco de germoplasma. *Acta Zoológica Mexicana (N.S.)*, *31*(2), 275-282. <https://doi.org/10.21829/azm.2015.312983>
- Oh, H. J., Kim, M. K., Jang, G., Kim, H. J., Hong, S. G., Park, J. E., Park, K., Park, C., Sohn, S. H., Kim, D. Y., Shin, N. S., & Lee, B. C. (2008). Cloning endangered gray wolves (*Canis lupus*) from somatic cells collected postmortem. *Theriogenology*, *70*(4), 638-647. <https://doi.org/10.1016/j.theriogenology.2008.04.032>
- Pan, X., Zhang, Y., Guo, Z., & Wang, F. (2014). Development of interspecies nuclear transfer embryos reconstructed with argali (*Ovis ammon*) somatic cells and sheep ooplasm. *Cell Biology International*, *38*(2), 211-218. <https://doi.org/10.1002/cbin.10191>
- Pickett H. (2011). Shave and punch biopsy for skin lesions. *American Family Physician*, *84*(9), 995-1002.
- Saikhun, J., Pavasuthipaisit, K., Jaruanuwat, M., & Kitiyanant, Y. (2002). Xenonuclear transplantation of buffalo (*Bubalus bubalis*) fetal and adult somatic cell nuclei into bovine (*Bos indicus*) oocyte cytoplasm and their subsequent development. *Theriogenology*, *57*(7), 1829-1837. [https://doi.org/10.1016/s0093-691x\(02\)00667-2](https://doi.org/10.1016/s0093-691x(02)00667-2)
- Saini, M., Selokar, N. L., Palta, P., Chauhan, M. S., Manik, R. S., & Singla, S. K. (2018). An update: reproductive handmade cloning of water buffalo (*Bubalus bubalis*). *Animal Reproduction Science*. <https://doi.org/10.1016/j.anireprosci.2018.08>
- Santos, M. D. C. B., Aquino, L. V. C., Nascimento, M. B., Silva, M. B., Rodrigues, L. L. V., Praxedes, É. A., & Pereira, A. F. (2021). Evaluation of different skin regions derived from a postmortem jaguar, *Panthera onca* (Linnaeus, 1758), after vitrification for development of cryobanks from captive animals. *Zoo Biology*, *40*(4), 280-287. doi:10.1002/zoo.21605
- Seaby, R., Alexander, B., King, W., & Mastromonaco, G. (2013). *In vitro* development of bison embryos using interspecies somatic cell nuclear transfer. *Reproduction in Domestic Animals*, *48*(6), 881-887. <https://doi.org/10.1111/rda.12180>
- Selokar, N. L., George, A., Saha, A. P., Sharma, R., Muzaffer, M., Shah, R. A., & Singla, S. K. (2011). Production of interspecies handmade cloned embryos by nuclear transfer of cattle, goat and rat fibroblasts to buffalo (*Bubalus bubalis*) oocytes. *Animal Reproduction Science*, *123*(3-4), 279-282. doi:10.1016/j.anireprosci.2011.01.008
- Seluanov, A., Vaidya, A., & Gorbunova, V. (2010). Establishing primary adult fibroblast cultures from rodents. *Journal of Visualized Experiments*, (44), 2033. <https://doi.org/10.3791/2033>
- Srirattana, K., Kaneda, M., & Parnpai, R. (2022). Strategies to improve the efficiency of somatic cell nuclear transfer. *International Journal of Molecular Sciences*, *23*(4), 1969. <https://doi.org/10.3390/ijms23041969>
- Takashima, A. (1998). Establishment of fibroblast cultures. *Current Protocols in Cell Biology*, *2*(1), 1-12. <https://doi.org/10.1002/0471143030.cb0201s00>
- Tarnowski, B. I., Spinale, F. G., & Nicholson, J. H. (1991). DAPI as a useful stain for nuclear quantitation. *Biotechnic & Histochemistry: Official Publication of the Biological Stain Commission*, *66*(6), 297-302.
- Tecirlioglu, R. T., Guo, J., & Trounson, A. O. (2006). Interspecies somatic cell nuclear transfer and preliminary data for horse-cow/mouse iSCNT. *Stem Cell Reviews*, *2*(4), 277-287. <https://doi.org/10.1007/BF02698054>
- Tovar, H., Navarrete, F., Rodríguez, L., Skewes, O., & Castro, F. O. (2008). Cold storage of biopsies from wild endangered native Chilean species in field conditions and subsequent isolation of primary culture cell lines. *In vitro Cellular & Developmental Biology - Animal*, *44*(8/9), 309-320. <http://www.jstor.org/stable/40205903>
- Vajta, G., Lewis, I. M., Hyttel, P., Thouas, G. A., & Trounson, A. O. (2001). Somatic cell cloning without micromanipulators. *Cloning*, *3*(2), 89-95. <https://doi.org/10.1089/15204550152475590>
- Vazquez-Avendaño, J. R., Cortez-Romero, C., Ambriz-García, D. A., Rodríguez-Suástegui, J. L., Hernández-Pichardo, J. E., & Navarro-Maldonado, M. D. C. (2024). The influence of oviductal and uterine fluid supplementation on the *in vitro* development and quality of cloned sheep embryos. *Animals*, *14*(19), 2894. <https://doi.org/10.3390/ani14192894>
- Verma, G., Arora, J., Sethi, R., Mukhopadhyay, C., & Verma, R. (2015). Handmade cloning: recent advances, potential and pitfalls. *Journal of Animal Science and Biotechnology* *6*:43 <https://doi.org/10.1186/s40104-015-0043-y>
- Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., & Campbell, K. H. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature*, *385*(6619), 810-813. <https://doi.org/10.1038/385810a0>
- Yadav, B. R., Katiyar, P. K., Chauhan, M. S., & Madan, M. L. (1997). Chromosome configuration during *in vitro* maturation of goat, sheep and buffalo oocytes. *Theriogenology*, *47*(4), 943-951. [https://doi.org/10.1016/s0093-691x\(97\)00050-2](https://doi.org/10.1016/s0093-691x(97)00050-2)
- Yelisetti, U. M., Komjeti, S., Katari, V. C., Sisinthy, S., & Brahmasani, S. R. (2016). Interspecies nuclear

transfer using fibroblasts from leopard, tiger, and lion ear piece collected postmortem as donor cells and rabbit oocytes as recipients. *In vitro* cellular & developmental biology. *Animal*, 52(6), 632-645. <https://doi.org/10.1007/s11626-016-0014-4>

Yu, M., Muteti, C., Ogugo, M., Ritchie, W. A., Raper, J., & Kemp, S. (2016). Cloning of the african indigenous cattle breed kenyan Boran. *Animal Genetics*, 47(4), 510-511. <https://doi:10.1111/age.1244>



Evaluation of Grass Weed Control in Sugarcane Using Phosphite Applied as Phosphorous Acid

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ABSTRACT

Objective: To evaluate the effect of Phi application on the control of a grass weed population composed predominantly of stargrass (*Cynodon nlemfuensis* and *C. plectostachyus*) and native grasses (*Axonopus* spp., *Cynodon* spp., and *Paspalum* spp.) in sugarcane cultivation.

Design/methodology/approach: Three 2×2 m plots were established to evaluate foliar spraying with 0, 400, and 800 mM Phi. The trial was conducted in a one-year-old ratoon sugarcane field. Toxicity level, damaged leaf area, and percentage of weed population controlled were assessed through image analysis using ImageJ software. Additionally, antioxidant activity and total chlorophyll concentration in leaf tissue were measured. Data were processed using RStudio version 1.2.5033 (analysis of variance and Tukey's test).

Results: Phi exhibited moderate toxicity at 400 mM and high toxicity at 800 mM. The predominant undesired vegetation included stargrass species (*Cynodon nlemfuensis* and *C. plectostachyus*) and native grasses of the genera *Axonopus*, *Cynodon*, and *Paspalum*. Despite clear foliar damage at both Phi concentrations, most weeds recovered within two to three weeks after application, indicating an efficient physiological strategy to counteract Phi-induced stress. This represents a challenge for the use of Phi as an herbicidal agent. Damaged leaf area reached 20.38% at 400 mM and nearly 51% at 800 mM Phi. Antioxidant activity increased with higher Phi concentrations, whereas total chlorophyll content declined as Phi dosage increased.

Limitations on study/implications: This study focused solely on the effects of phosphorous acid-derived Phi on grass weeds in one-year-old sugarcane. Further research is needed to assess its impact throughout the crop cycle and on a broader range of grass weed species.

Findings/conclusions: Foliar applications of 400 and 800 mM Phi caused moderate and severe phytotoxic effects, respectively, but were not sufficient to effectively control Poaceae weed populations in sugarcane.

Keywords: Weeds, undesired vegetation, sugarcane agroecosystems, phosphite, alternative herbicides.

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INTRODUCTION

Phosphorous acid (PhA) and its conjugate base, phosphite (Phi), can be used as an alternative to glyphosate for controlling undesired vegetation, since its application at high concentrations can induce phytotoxicity and plant death (Li *et al.*, 2025).



In Mexico, sugarcane (*Saccharum* spp. hybrids) is the second most important agroindustrial crop economically, following maize (*Zea mays* L.), with estimated values of 4.4 and 5.4 billion USD, respectively (Statista, 2025). Currently, sugarcane is cultivated on approximately 800,000 hectares across 15 states and 290 municipalities, generating around 500,000 direct jobs and approximately 2 million indirect jobs (PRONAC, 2020; CONADESUCA, 2024).

Although this crop is highly efficient at capturing solar energy and converting it into biomass and sugar, its productivity relies heavily on the intensive use of fertilizers and herbicides (Patel *et al.*, 2024). Considering this, a growing trend is the development of sustainable alternatives for weed management in agriculture to reduce or replace the use of glyphosate (Gandhi *et al.*, 2021; Turek *et al.*, 2022). Among these alternatives, phosphite is gaining attention as an ion with herbicidal potential against numerous weed species in strategic crops worldwide (Achary *et al.*, 2017; Trejo-Téllez *et al.*, 2024; Li *et al.*, 2025).

Phosphorus (P) is an essential element for higher plants, primarily absorbed as inorganic phosphate (Pi), in the form of H_2PO_4^- and HPO_4^{2-} anions (Puga *et al.*, 2024). In the plasma membrane of plant cells, Pi uptake is facilitated by specific transporter proteins (PHT, Phosphate Transporter Family), which are also capable of absorbing other inorganic phosphorus forms such as the phosphite anions (Phi: H_2PO_3^- and HPO_3^{2-}).

Phi is an isostere of Pi, differing by the replacement of one oxygen (O) atom bonded to phosphorus with a hydrogen (H) atom. Despite this structural similarity, Phi cannot substitute Pi as a source of phosphorus once inside the plant cell and can even attenuate phosphate starvation responses (PSR), leading to phytotoxic effects if not properly managed (Achary *et al.*, 2017; Trejo-Téllez *et al.*, 2024). This occurs because Phi triggers a false sufficiency signal for Pi, thereby suppressing the molecular pathways involved in phosphate deficiency responses (McDonald *et al.*, 2001).

The deprotonation of phosphorous acid (PhA; H_3PO_3) and phosphoric acid (PiA; H_3PO_4) generates the Phi and Pi anions, respectively. In both cases, protons are sequentially released, first forming H_2PO_3^- and H_2PO_4^- , followed by HPO_3^{2-} and HPO_4^{2-} . However, the loss of a third proton is only feasible for the HPO_4^{2-} anion, since in HPO_3^{2-} the tetrahedral structure is formed by a hydrogen atom directly bonded to phosphorus instead of a fourth oxygen atom, which is present in HPO_4^{2-} (Trejo-Téllez *et al.*, 2024).

Although subtle, these structural differences have important physiological consequences. In practice, the simple substitution of an oxygen atom with a hydrogen atom significantly alters plant responses to the application of either anion or their parent acids. Pi serves as the main readily available source of phosphorus for plants, whereas Phi cannot be metabolized within plant cells, as plant genomes naturally lack genes encoding for the enzyme phosphite dehydrogenase (PTDH or PtxD), which is required to oxidize Phi into Pi and integrate it into metabolic processes (Trejo-Téllez and Gómez-Merino, 2018). As a result, Phi accumulates in the cells, triggering dose-dependent hormetic responses that may be beneficial, neutral, or harmful, depending on Pi availability, Phi concentration, plant genotype, and other factors (Gómez-Merino *et al.*, 2022).

This study evaluated the effect of three concentrations of phosphorous acid—resulting in Phi anions through deprotonation—on the control of grass weeds in sugarcane

cultivation. The concentrations tested were 0, 400, and 800 mM Phi, applied to the foliage, with the aim of assessing their herbicidal potential in this crop.

MATERIALS AND METHODS

Experimental site, plant material, and treatments

The study was conducted in sugarcane (*Saccharum* spp. hybrids) fields located at the experimental facilities of the Colegio de Postgraduados, Córdoba Campus, in Amatlán de los Reyes, Veracruz, Mexico (18° 51' 20" N; 96° 51' 37" W; 654 m a.s.l.).

Three treatments corresponding to foliar applications of phosphite (Phi), supplied in the form of phosphorous acid (H_3PO_3), were evaluated at concentrations of 0, 400, and 800 mM. Each treatment was applied to 2×2 m plots, with three replicates per treatment.

To ensure that the observed effects were solely attributable to Phi, no additional weed control methods—manual, mechanical, or chemical—were employed.

Measured variables

Photographs of damaged foliage were taken, and the affected leaf area was quantified using the ImageJ software (Rasband, 2018). Based on this information, the percentage of damaged leaf area, the phytotoxicity level of Phi, and the proportion of controlled vegetation were determined.

Phytotoxicity was evaluated according to the general guidelines of the EPPO PP 1/135 (4) protocol, *Phytotoxicity assessment* (EPPO, 2014), using the percentage of affected leaf area as a quantitative indicator. For comparative purposes, phytotoxicity levels were classified as follows: none or very low (0-5%), slight (>5-20%), moderate (>20-40%), high or severe (>40-70%), and very high or total damage (>70-100%). This classification allowed for a standardized interpretation of the visual effects of phosphite treatments on grass weeds.

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical method, following the procedure described by Silva *et al.* (2024).

The total chlorophyll concentration was determined according to the protocol described by Jiménez-Lao *et al.* (2021).

Experimental design and statistical analysis

A completely randomized design was used, consisting of three treatments and three replicates. Data were processed using the statistical software RStudio version 1.2.5033 (RStudio Team, 2016). An analysis of variance (ANOVA) was performed to determine statistically significant differences at a 95% confidence level ($P \leq 0.05$), followed by Tukey's test for mean comparisons.

RESULTS AND DISCUSSION

Phytotoxicity level, affected leaf area, and weed population control

Foliar application of Phi exhibited a moderately toxic effect at a concentration of 400 mM and a highly toxic effect at 800 mM, as shown in Table 1.

The predominant species of unwanted vegetation in the assessed population were *Cynodon nlemfuensis* and *C. plectostachyus* (commonly known as star grasses), along with native grasses from the genera *Axonopus*, *Cynodon*, and *Paspalum*.




In a study conducted by Herrera-Solano *et al.* (2023) on Poaceae weeds in sugarcane (*Saccharum* spp.) fields in the central region of Veracruz, Mexico, various species were identified, including *Brachiaria utica*, *Panicum purpurascens*, *Brachiaria fasciculata*, *Chloris virgata*, *Cynodon dactylon*, *Cynodon plectostachyus*, *Digitaria ciliaris*, *Panicum maximum*, *Rhynchelytrum repens*, *Rottboelia exaltata*, and *Sorghum halepense*.

Despite the evident foliar tissue damage caused by the application of 400 and 800 mM Phi, most grasses exhibited a noticeable recovery within two to three weeks post-treatment. This rapid recovery suggests that these species possess highly efficient physiological mechanisms to counteract the imbalances induced by Phi, posing a significant challenge for their control using herbicidal formulations based on this anion.

In the grass weed population, leaf area was differentially affected depending on the Phi concentration applied. At 400 mM Phi, approximately 20.38% of the foliage exhibited visible symptoms of burn injury, whereas at 800 mM Phi, this value significantly increased to nearly 51% (Table 2).

The application of 400 mM Phi caused phytotoxicity and mortality in approximately 6% of the weed population, while the 800 mM Phi treatment increased this value to 10.2%. Despite these initial effects, as previously noted, most plants exhibited full recovery approximately one month after application, indicating a remarkable resilience capacity of these species to Phi-induced stress (Table 2).

Table 1. Phytotoxicity level of phosphite (Phi) on grass weeds in sugarcane (*Saccharum* spp.) crops in experimental fields in Amatlán de los Reyes, Veracruz, Mexico.

Phi (mM)	0	400	800
Toxicity level in grass weed population	None	Moderate	High or severe
Visual evidence of toxicity level			

Evidence of toxicity documented 48 hours after treatment application.

Table 2. Affected leaf area and controlled weed population in response to the application of phosphite (Phi) in grass weeds in sugarcane (*Saccharum* spp.) cultivation under field conditions in Amatlán de los Reyes, Veracruz, Mexico.

Phi (mM)	Affected leaf area (%)	Controlled population (%)
0	0.00 c	0.00 c
400	20.38 b	5.79 b
800	50.67 a	10.23 a

Data recorded 48 hours after treatment application. Means followed by different letters within each variable are statistically different (Tukey; $P \leq 0.05$).

The stoloniferous grass species evaluated in this study exhibited notable resistance to systemic herbicides, primarily because their leaves absorb only a fraction of the applied compound, which is not efficiently translocated to the roots and stolons (UNL, 2011; Beck *et al.*, 2013). For instance, in German grass (*Echinochloa polystachya*), the application of the herbicides cyhalofop and penoxsulam resulted in only 37% stolon control, while quinclorac achieved just a 12% reduction (Bottoms *et al.*, 2011). In the case of Bermuda grass (*Cynodon dactylon*), stolons that come into contact with the pre-emergent herbicide oxadiazon absorb only minimal amounts of the compound, preserving the root system almost intact, which facilitates rapid regrowth after treatment (Beck *et al.*, 2013).

Antioxidant activity and chlorophyll content

The antioxidant activity recorded in the grass weeds showed a positive correlation with the applied Phi concentration, with significant differences observed among treatments (Table 3). Plants treated with 800 mM Phi exhibited the highest antioxidant activity, with a 65% increase compared to the control. Similarly, the application of 400 mM Phi resulted in a 52% increase in antioxidant activity relative to untreated plants.

In contrast, the total chlorophyll concentration decreased significantly with increasing Phi concentration. The highest mean value was recorded in the control group (3.51 mg g⁻¹ fresh weight), whereas treatments with 400 and 800 mM Phi resulted in reductions to 2.19 and 1.23 mg g⁻¹ fresh weight, respectively. These reductions correspond to approximately 37.6% and 62.3% relative to the value observed in plants not treated with Phi (Table 3).

Herbicides can induce oxidative stress in plants, thereby activating defense mechanisms such as increased antioxidant activity (Caverzan *et al.*, 2019). This response is associated with the generation of reactive oxygen species (ROS), which accumulate as a consequence of herbicide-induced oxidative damage. In this context, plants with greater herbicide tolerance often exhibit enhanced antioxidant activity as an adaptive strategy (Ecciza *et al.*, 2023). In the present study, total antioxidant activity increased significantly with rising Phi concentrations, suggesting that this compound acted as a stressor that triggered antioxidant responses in grass weed species.

Herbicides such as iodosulfuron-methyl, clodinafop-propargyl, and 2,4-D can reduce chlorophyll and carotenoid contents while simultaneously inducing oxidative stress by

Table 3. Total antioxidant activity and total chlorophyll concentration in grass weeds in sugarcane (*Saccharum* spp.) in response to foliar application of phosphite (Phi) under field conditions in Amatlán de los Reyes, Veracruz, Mexico.

Phi (mM)	Total antioxidant activity ($\mu\text{g g}^{-1}$ FWB)	Total chlorophylls (mg g^{-1} FWB)
0	448.92 c	3.51 a
400	683.34 b	2.19 b
800	745.12 a	1.23 c

Data recorded 48 h after treatment application. Means followed by different letters within each column are significantly different (Tukey; $P \leq 0.05$). FWB: Fresh weight biomass.

increasing hydrogen peroxide (H_2O_2) levels and lipid peroxidation, both in wheat (*Triticum aestivum*) and perennial ryegrass (*Lolium perenne*) (Tarouco *et al.*, 2024). In agreement with these findings, the present study also recorded a significant reduction in total chlorophyll concentrations in response to Phi applications, reinforcing the compound's potential phytotoxic effect on leaf tissue.

Plants activate their antioxidant defense system in response to oxidative stress, which is divided into two components: enzymatic and non-enzymatic. The enzymatic system is composed, as the name implies, of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), among others. The non-enzymatic system includes compounds such as chlorophylls, carotenoids, glutathione, and polyphenols (Rao *et al.*, 2025).

Hasan *et al.* (2022) evaluated the efficacy of WeedLock, a broad-spectrum plant-based bioherbicide (*a.i.* EGX-101™, a natural derivative from *Solanum habrochaites*), on ageratum (*Ageratum conyzoides*), goosegrass (*Eleusine indica*), red amaranth (*Amaranthus gangeticus*), and maize. The herbicide caused significant reductions in chlorophyll content and disrupted photosynthetic processes in all treated species. The greatest inhibition of photosynthesis was recorded in *A. conyzoides*, with a 74.88% decrease observed 24 hours after application. In all evaluated species, malondialdehyde (MDA) levels increased by more than 100%, accompanied by elevated proline concentrations —both physiological indicators of oxidative stress. Plants exhibited chlorosis and signs of wilting as early as one hour after treatment application.

In several species of the genus *Urochloa*, increasing doses of glyphosate significantly reduced the quantum efficiency of photosystem II and the electron transport rate, with more pronounced effects under shaded conditions (de Oliveira *et al.*, 2024).

In the present study, foliar application of 400 and 800 mM Phi caused severe damage to the leaf tissue of grass weeds. Nevertheless, most plants were able to recover within two to three weeks after treatment, suggesting that these species possess highly efficient physiological mechanisms to counteract the imbalance induced by Phi.

On the other hand, the sugarcane crop did not exhibit any damage from Phi applications at any of the tested concentrations. It is important to emphasize that the plants were one year old and in the ratoon cycle. Further studies are needed to evaluate the effects of Phi during the early developmental stages of the crop, particularly from the time of planting.

CONCLUSIONS

Although the application of Phi at 400 and 800 mM caused visible damage to the foliar tissue of grass weed populations in sugarcane fields, these weed species exhibited a remarkable recovery capacity, fully reestablishing approximately one month after treatment. Phi application induced oxidative stress in the weeds, as evidenced by a significant increase in antioxidant capacity and a reduction in total chlorophyll concentration. These results indicate that Phi triggers defense mechanisms in weeds but fails to achieve effective control of the grass weed populations tested at the evaluated doses. Therefore, under the conditions and concentrations tested, Phi does not represent a viable herbicidal alternative for managing grass weeds in sugarcane cultivation.

Notably, Phi did not cause phytotoxicity in sugarcane plants when applied at an advanced developmental stage (ratoon crop, one year old), close to harvest. Further studies are recommended to assess its effects during earlier stages of the crop cycle, in order to more clearly establish its safety and potential implications during critical growth phases.

REFERENCES

- Achary, V. M. M., Ram, B., Manna, M., Datta, D., Bhatt, A., Reddy, M. K., & Agrawal, P. K. (2017). Phosphite: A novel P fertilizer for weed management and pathogen control. *Plant Biotechnology Journal* 15(12): 1493-1508. doi: 10.1111/pbi.12803
- Beck, L. L., Cooper, T., Hephner, A. J., Straw, C. M., & Henry, G. M. (2013). Effect of preemergence herbicides on the recovery of bermudagrass from spring dead spot. *Applied Turfgrass Science* 10(1): 1-7. doi: 10.1094/ATS-2013-0328-01-RS.
- Bottoms, S. L., Webster, E. P., Hensley, J. B., & Blouin, D. C. (2011). Effects of herbicides on growth and vegetative reproduction of creeping rivergrass. *Weed Technology* 25(2): 262-267. doi: 10.1614/WT-D-10-00113.1
- Caverzan, A., Piasecki, C., Chavarria, G., Stewart, C. N. J., & Vargas, L. (2019). Defenses against ROS in crops and weeds: The effects of interference and herbicides. *International Journal of Molecular Sciences* 20(5): 1086. doi: 10.3390/ijms20051086
- CONADESUCA (Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar). 2024. Cuenta Pública 2024 del Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar. Ciudad de México. https://www.cuentapublica.hacienda.gob.mx/work/models/CP/2024/tomo/VII/MAT_Print.8AFU.01.INTRO.pdf
- de Oliveira, V. A. V., Donato, L. M. S., Ruas, M. A. O., de Oliveira J. Â. M., de Souza, R. F., & Santos, L. D. T. (2024). The light intensity in the cultivation environment and the impact of glyphosate on plants of the *Urochloa* genus. *Journal Environmental Science and Health B, Part B* 59(8): 540-549. doi: 10.1080/03601234.2024.2381934
- Eceiza, M. V., Barco-Antoñanzas, M., Gil-Monreal, M., Huybrechts, M., Zabalza, A., Cuypers, A., & Royuela, M. (2023) Role of oxidative stress in the physiology of sensitive and resistant *Amaranthus palmeri* populations treated with herbicides inhibiting acetolactate synthase. *Frontiers in Plant Science* 13: 1040456. doi: 10.3389/fpls.2022.1040456
- EPPO (European and Mediterranean Plant Protection Organization). (2014). PP 1/135 (4) Phytotoxicity assessment. *EPPO Bull* 44(3): 265-273. doi: 10.1111/epp.12134
- Gómez-Merino, F. C., Gómez-Trejo, L. F., Ruvalcaba-Ramírez, R., & Trejo-Téllez, L. I. (2022). Application of phosphite as a biostimulant in agriculture”, In *New and Future Developments in Microbial Biotechnology and Bioengineering* (Elsevier, Amsterdam), pp. 135-153. doi: 10.1016/B978-0-323-85581-5.00002-1
- Gandhi, K., Khan, S., Patrikar, M., Markad, A., Kumar, N., Choudhari, A., Sagar, P., & Indurkar, S. (2021). Exposure risk and environmental impacts of glyphosate: Highlights on the toxicity of herbicide co-formulants. *Environmental Challenges* 4: 100149. doi: 10.1016/j.envc.2021.100149
- Hasan, M., Mokhtar, A. S., Mahmud, K., Berahim, Z., Rosli, A. M., Hamdan, H., Motmainna, M., & Ahmad-Hamdani, M. S. (2022). Physiological and biochemical responses of selected weed and crop species to the plant-based bioherbicide WeedLock. *Scientific Reports*, 12, 19602. doi: 10.1038/s41598-022-24144-2
- Herrera-Solano, A., Verdejo-Lara, R. A., Real-Garrido, C. J., Hernández-Gastelú, A., & Castillo-Morán, A. (2023). Caracterización de las principales malezas (Poaceae) de la caña de azúcar en la zona de influencia del Ingenio La Providencia, S. A. de C. V., Veracruz. *Revista Biológico Agropecuaria Tuxpan* 11(2): 1-5. doi: 10.47808/revistabioagro.v11i2.508
- Jiménez-Lao, R., García-Caparros, P., Pérez-Saiz, M., Llanderal, A., & Lao, M. T. (2021). Monitoring optical tool to determine the chlorophyll concentration in ornamental plants. *Agronomy* 11: 2197. doi: 10.3390/agronomy11112197
- Li, Z., Kong, X., Zhang, Z., Tang, F., Wang, M., Zhao, Y., & Shi, F. (2025). The functional mechanisms of phosphite and its applications in crop plants. *Frontiers in Plant Science* 16: 1538596. doi: 10.3389/fpls.2025.1538596
- McDonald, A. E., Grant, B. R., & Plaxton, W. C. (2001). Phosphite (phosphorous acid): Its relevance in the environment and agriculture and influence on plant phosphate starvation response. *Journal of Plant Nutrition* 24(10): 1505-1519. doi: 10.1081/PLN-100106017

- Patel, T., Dudhat, M. S., Patel, D., & Thanki, J. D. (2024). Comparative evaluation of agronomical, mechanical and chemical management of weeds and their impact on sugarcane productivity. *Indian Journal of Weed Science* 56(2): 167-175. doi: 10.5958/0974-8164.2024.00027.X
- PRONAC (Programa Nacional de la Agroindustria de la Caña de Azúcar). (2020). PRONAC 2021-2024 (p. 84). Secretaría de Agricultura y Desarrollo Rural - Comisión-Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar (CONADESUCA). Ciudad de México. https://www.gob.mx/cms/uploads/attachment/file/709503/PROGRAMA_PRONAC_2021-2024vf_web.pdf
- Puga, M. I., Poza-Carrión, C., Martínez-Hevia, I., Pérez-Liens, L., & Paz-Ares, J. (2024). Recent advances in research on phosphate starvation signaling in plants. *Journal of Plant Research* 137(3): 315-330. doi: 10.1007/s10265-024-01545-0
- Rao, M. J., Duan, M., Zhou, C., Jiao, J., Cheng, P., Yang, L., Wei, W., Shen, Q., Ji, P., Yang, Y., Conteh, O., Yan, D., Yuan, H., Rauf, A., Ai, J., & Zheng, B. (2025). Antioxidant defense system in plants: reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. *Horticulturae* 11(5): 477. doi: 10.3390/horticulturae11050477
- Rasband, W.S. (2018). ImageJ. US National Institutes of Health, Bethesda, Maryland, USA. <https://imagej.net/ij/>
- RStudio_Team. (2016). RStudio: Integrated Development for R.; RStudio, Inc.: Boston, MA, USA. Retrieved from <http://www.rstudio.com/>
- Silva, F., Veiga, F., Cardoso, C., Dias, F., Cerqueira, F., Medeiros, R., & Paiva-Santos, A. C. (2024). A rapid and simplified DPPH assay for analysis of antioxidant interactions in binary combinations. *Microchemical Journal* 202: 110801. doi: 10.1016/j.microc.2024.110801
- Statista. (2025). Industry revenue of “Cane sugar manufacturing” in Mexico from 2012 to 2024. Retrieved from <https://www.statista.com/forecasts/409877/cane-sugar-manufacturing-revenue-in-mexico>
- Tarouco, C. P., Ulguim, A. R., Nohatto, M. A., Manica-Berto, R., de Avila, L. A., Senseman, S. A., & Agostinetti, D. (2024). Antioxidant detoxification system of wheat and ryegrass plants subjected to various herbicides. *Ciência Rural* 54(7): e20230132. doi: 10.1590/0103-8478cr20230132
- Trejo-Téllez, L. I., & Gómez-Merino, F. C. (2018). Phosphite as an inductor of adaptive responses to stress and stimulator of better plant performance”, In *Biotic and Abiotic Stress Tolerance in Plants* (Springer, Singapore), pp. 203-238. doi: 10.1007/978-981-10-9029-5_8
- Trejo-Téllez, L. I., Carbajal-Vázquez, V. H., Lavín-Castañeda J., & Gómez-Merino, F. C. (2024). Phosphite as a sustainable and versatile alternative for biostimulation, biocontrol, and weed management in modern agriculture. *Processes* 12(12): 2764. doi: 10.3390/pr12122764
- Turek, M., Biczak, R., Pawłowska, B., Różycka-Sokołowska, E., Owsianik, K., Marciniak, B., & Balczewski, P. (2022). The need to change the approach to the safe use of herbicides by developing chiral and environmentally friendly formulations: A series of enantioselective (R)- and (S)-phenylethylammonium chloroacetates. *Green Chemistry* 24(4): 1693-1703. doi: 10.1039/D1GC03970A
- UNL (University of Nebraska-Lincoln). (2011). Broadleaf weed control in home lawns. Turfgrass Science Program. Lincoln, NE, USA. Retrieved from <https://turf.unl.edu/sites/unl.edu/ianr.agronomy-horticulture.turf/files/media/file/Broadleaf-weed-control-home-lawns2011b.pdf>

Use of pine extract (*Pinus patula*) for weed control in agave cultivation (*Agave tequilana* Weber var. Azul)

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ABSTRACT

Objective: To evaluate the pine extract with two solvents (water and ethanol), as a pre-emergence weed control in agave crop in the town of San Miguel el Alto, Jalisco.

Design/methodology/approach: The experimental design was in randomized blocks, with six treatments and four replicates, using two synthetic herbicides and two pine extracts (aqueous and ethanolic), during the rainfed period from July to September 2023. Plots were 30 m² with 10 agaves. Applications were made pre-emergence and twice more per month at a concentration of 25%. The variables recorded for weeds were number of specimens, species and visual coverage; for the crop, number of detached leaves, plant height, leaf width and length. The Shannon index, % control, EC and pH were obtained per treatment.

Results: The pine extract with both solvents showed phytotoxicity (chlorosis and stunting), and similar control to that observed with synthetic herbicides was not observed. The type of weed leaf determines the efficacy of the herbicide used, as it does for the extracts. Although the desired significant differences between treatments were not found, changes in weed populations were observed, suggesting that the extracts should be further evaluated.



Limitations on study/implications: N/A

Findings/conclusions:

- First report on the phytotoxic effect of *Pinus patula* extract.
- Pine extract with both solvents causes phytotoxic symptoms (stunting and chlorosis).
- The ideal application rate must be determined.
- *Digitaria ciliaris*, *Paspalum lividum* and *Chloris virgata* are reported as species tolerant or resistant to glyphosate and glufosinate ammonium.

Keywords: Allelopathy, plant extracts, *Pinus patula*, weed control.

INTRODUCTION

Weeds are a problem in crops, since they decrease the yield when they do not receive timely attention, causing important losses; they are one of the biotic factors with greatest impact in agriculture. The use of synthetic agrochemicals is the main tool for producers, even if they cause damage to the environment and to the population's health (Scavo and Mauromicale, 2020). In this regard, glyphosate residues have been detected in foods such as soy and maize, and these residues reach the end consumer causing harm to health, the environment and wildlife (CONACYT, 2020; Suppa *et al.*, 2020; Ordoñez-Beltrán *et al.*, 2019; Serpa *et al.*, 2015). In Jalisco, agave cultivation has designation of origin, so it is one of the most important crops, in addition to recently having gained terrain, with 133,763.52 ha planted by the end of 2023 (SIAP, 2024). This crop does not have selective herbicides and herbicides evaluated in other crops, to which agave turns out to be tolerant, are used. An herbicide of common use in this crop is glufosinate ammonium, which is efficient although quite residual; for example, when agave is harvested and maize is to be planted, it cannot be established because of the toxicity of the herbicide applied. Then, the producer must seek options that allow planting maize. An efficient control method is manual, but it must be applied two to three times during the rainy season, which is costly; the optimal option would be integrated control (Dominschek *et al.*, 2021). Derived from this, there is a requirement to find less costly strategies by observing the behavior of nature. Plant extracts have been reported since 1970 with inhibitory activity on plant germination and growth (Belz, 2007; Cruz-Ortiz and Flores-Méndez, 2021). These are options for weed control, since their metabolites (natural chemical substances) could alter the cell structure and cause biodegradation or the inhibition of germination. In the market, "organic" products for weed control have been found, and a constant factor is pine. Therefore, the objective of this study is to evaluate the pine extract with two solvents (water and ethanol), as pre-emergence control on weeds in the agave crop in the locality of San Miguel el Alto, Jalisco. In addition, there are reports in the literature that pine essence (*Pinus patula* L.) can inhibit the germination of *Sinapis arvensis* L., *Lolium rigidum* (Gaudin) Weiss ex Nyman, *Phalaris canariensis* L. and *Trifolium campestre* Schreb (Amri *et al.*, 2011), and 93% of inhibition in weed germination was obtained with the aqueous extract of *Pinus caribbean* Morelet, without reporting the species (Jiménez Ferrer *et al.*, 2006). On the other hand, the pine extract had significant results in the germinative inhibition of *Arthraxon quartinianus* (A. Rich.) Nash (Portuguez-García *et al.*, 2020).

MATERIALS AND METHODS

The study was conducted on a plot located in San Miguel el Alto, which is on the limits with the municipality of Arandas in the state of Jalisco, Mexico, belonging to the zone called Altos de Jalisco; it is located on geographical coordinates 20.897021674240175 latitude, 102.4581126284207 longitude. The climate is semi-warm, semi-humid (34.1%), and temperate sub-humid (65.9%). The mean annual temperature is 17.3 °C and the mean annual precipitation is 919 mm, with the month of July being the one with most rainfall. The predominant soil is Luvisol (34.6%) and presents reddish soils that accumulate a large amount of clay; the ones used for agriculture have moderate yields and are quite susceptible to erosion. Agriculture (61.7%) is the dominant land use in the municipality (INEGI, 2001, 2018). The experiment was established in the rainfed season (July-October 2023) and had a completely random block arrangement, where the treatments were randomized via a raffle.

Treatments

The experiment was established in a newly transplanted, one month old, agave plantation (*Agave tequilana* f.a.c. Weber), with planting density of 3,333 plants/ha. An experimental plot of 720 m² was established with six treatments and four replicates, T1: Clean control (kept clean with hoe and shovel), T2: Weedy control (without treatment), T3: Glufosinate ammonium, T4: Glyphosate, T5: Aqueous pine extract, T6: Ethanolic pine extract; this results in a total of 24 quadrants of 30 m² each (3×10 m). For the design of completely randomized blocks (James *et al.*, 2002), the plot was divided into lines of 10 agaves per treatment, leaving two agaves without any application, between treatments.

Preparation of plant extracts (ethanolic and aqueous)

The plant material (plant) that was used for this experiment was chosen because of its availability in the region and its allelopathic qualities reported for the genus and/or species (Marín-Velásquez and Barrutia-Barreto, 2020), in addition to the prior mention about its use in commercial “organic” products. Specimens of *Pinus patula* were found in the region and fresh needles were collected that were transported to the laboratory for their processing. Two types of extracts were elaborated, aqueous and ethanolic. For the elaboration of the extracts, 200 g of fresh needles were used for every liter of solution, aqueous or ethanolic; for the case of the latter, a solution of denatured ethanol at 70% was used, and for the aqueous, the ethanol was replaced for potable water, with proportion of 1:100 p/v. The needles were diced to facilitate grinding, for which a classic Oster[®] blender was used; the plant was placed and ethanolic solution was added, grinding was done for 10 seconds in three repetitions, and then the entire content of grinding was placed in a wide-mouth and amber-color recipient; it was left to rest for 15 days and shaken twice per day for 5 min. At the end of the resting period, the extract obtained was filtered with a kitchen strainer to remove the plant residues, then with a tulle fabric sieve to remove the large particles of plant tissue from the extract, and finally with number 4 Whatman[®] filter paper and a funnel to remove any small plant particle that could clog up the nozzle of the spraying pumps. The filtered extract was placed in duly labeled amber color containers.

Preparation of herbicide extracts

From the extracts obtained, a dose of 25% from each extract was used and 5.7 liters per extract were prepared to be applied on the agave plot and each of the four repetitions; to determine the amount of extract necessary, the spraying bag was previously calibrated.

Preparation of synthetic herbicides

The synthetic herbicides used were glyphosate (chemical control) (3 L ha^{-1}) and glufosinate ammonium (1.5 L ha^{-1}); the latter is the chemical regional control (which received that name because it is the one the producer uses). The doses are those recommended on the label. Glyphosate was applied with a bell to avoid damaging the agave, since it is a non-selective herbicide. For the extract, 50 L ha^{-1} were used, and its concentration was 25%. The treatments were applied with a spraying pump (SWEDISH HUSKY POWER® Electrical Fumigator with Tank Capacity of 20 L), at a pressure between 30 and 40 pounds, measuring with a manometer adapted to the pump and TeeJet® 8004 nozzle.

Variables evaluated and statistical analysis

The applications were carried out 30 days after the crop establishment; meanwhile, the extracts were applied three times, one per month for three months; and for the evaluation of the effect of treatment on weeds, the first measurement was carried out 15 days after application (DAA) and the next two, with one month separation between them. The number of plants per treatment/repetition was recorded, and the visual coverage (%) which represents the effect of the herbicides. The percentage of control was calculated, as well as the diversity of species present; for this purpose, the quadrant method was used, with a quadrant of $50 \times 50 \text{ cm}$, thrown twice, and whatever was inside the quadrant was recorded (species and frequency of appearance) (Monroy-Reyes *et al.*, 2005), with which the Shannon index was obtained. The electrical conductivity and pH of a sample made up of soil per treatment after its application was also recorded. To evaluate the effect of weeds and treatments applied on the crop, a sample of 5 plants (agave) was recorded, per repetition and treatment. The initial number of leaves, the number of leaves detached, and those totally detached from the shoot were recorded as detached leaves. The plant height, width of the last leaf detached, and height of this same leaf were recorded; to avoid confusion, the tip of the last leaf detached was cut (Monroy-Reyes *et al.*, 2005; Pimienta-Barrios *et al.*, 2005), and plant toxicity observations were also made on the crop. These measurements were carried out once per month, for four months. In the data analysis, a logarithmic (variable number of weeds) and square root (variables of percentage of control and cover) data transformation was conducted; in the end, the assumptions were not fulfilled, and a repeated measurements analysis was used with Kruskal Wallis (Kay *et al.*, 2021). Then, a contrast comparison test was carried out with $\alpha=0.05$ (Elkin *et al.*, 2021).

RESULTS AND DISCUSSION

The treatments with extracts were applied in pre-emergence and the glufosinate and glyphosate once the weeds had emerged (post-emergence), so the first measurement of

the number of weeds was recorded at 15 DAA. Results were grouped per type of leaf in the weeds: broad leaf refers to the dicotyledons, and narrow leaf mostly to the grasses, although Cyperasea were also grouped here. The results from the statistical analysis (Kruskal Wallis) for the variable “number of weeds” between treatments shows significant differences ($p\text{-value}=1.4244^{e-07}$) (Figure 1).

A significant interaction was also found between treatment *type of leaf ($p\text{-value}=5.9778^{e-10}$), meaning that the effect from the treatment depends on the type of leaf (broad and narrow). These effects have been mentioned by the producers, because the synthetic chemical herbicides cannot manage to control all the species cataloged as weed in a plot. Because of the type of plant metabolism and the synthetic chemical used, some species are affected more than others and the extracts functioned equally, affecting the broad-leaf or narrow-leaf species ($p\text{-value}=1.5524^{e-07}$) in different ways. Therefore, different herbicides are used depending on the crop and the type and species of weeds present (Herrera-Murillo and Picado-Arroyo, 2024). Grasses are mostly difficult to control, and commercial herbicides take time to have an impact and do not completely eliminate them; this can be observed since this group (grasses/narrow leaf) was higher in number of individuals in all the treatments and their decrease is observed with time, also with the extracts, except more slowly. The aqueous and ethanolic pine extracts do not compete with the synthetic herbicides with the dose applied. The ethanolic extract affects more the emergence of broad-leaf weeds, the same as glyphosate and glufosinate ammonium (Figure 1). Regarding the effect through the three measurements (months), a significant difference was also found ($p\text{-value}=0.014799$), which was expected, because the effect of the synthetic

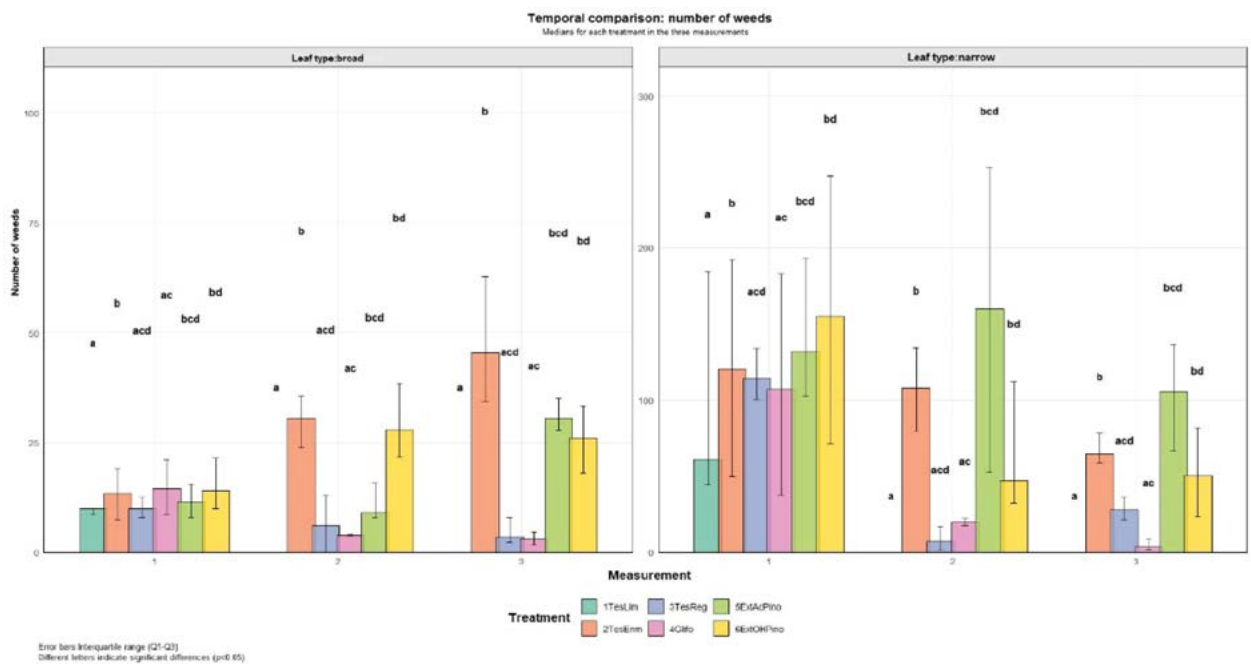


Figure 1. Means comparison of the number of weeds present in the agave crop through treatment and type of leaf, in the three measurements 1, measurement 1, 2, measurement 2 and 3, measurement 3. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlifo: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHlPino: Alcoholic pine extract.

chemical herbicides and of the extracts is not immediate, and this is corroborated through the measurements.

Regarding Dunn’s test (Table 1), significant differences are found when the treatments are compared, so that they can be grouped into weedy control, with higher number of weeds, clean control with lower number of weeds, glyphosate with a small number of weeds, and treatments with glufosinate ammonium (regional control) and the two extracts (aqueous and ethanolic) that had an intermediate level of weeds.

This indicates that there are strong temporal effects which were observed in symptomatology recorded, such as chlorosis and stunting. However, the extracts present effectiveness which is generally variable in time (Figure 2), since they reduce the number of weeds at the end of the study. This is important because most commercial organic herbicides contain pine extract, although only effects of chlorosis, stunting and possible

Table 1. Significant values in Dunn’s Test for Treatment.

Comparison	P_value
Clean control-Weedy control	7.050 ^{e-07***}
Weedy control - Glufosinate	3.72 ^{e-02*}
Weedy control - Glif	4.99 ^{e-03**}
Clean control - ExtAcPino	3.92 ^{e-05***}
Clean control - ExtOHPino	2.32 ^{e05***}
Glif-ExtOHPino	4.89 ^{e-02*}

1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlifo: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHPino: Alcoholic pine extract.

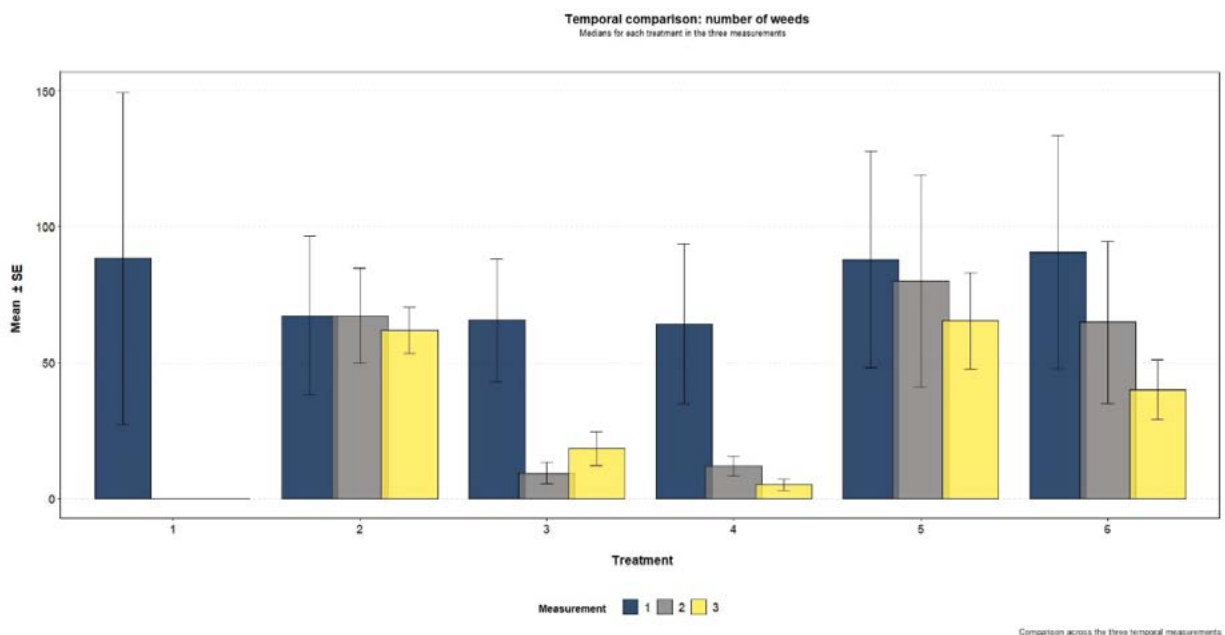


Figure 2. Number of weeds accumulated and encompassed without considering the type of leaf, during the three months recorded. 1 Clean control, 2 Weedy control, 3 Glufosinate ammonium, 4 Glyphosate, 5 Aqueous pine extract, 6 Alcoholic pine extract.

reduction of emergence are suggested, in this study, by reducing the number of plants with both solvents in different degrees (Treatments 5 and 6, Figure 2). Something else that can be seen is the effect on the reduction of weeds with Treatments 3 and 4, which are glufosinate ammonium and glyphosate, respectively. Although they do not completely eliminate the weeds, this is because of the presence of seasonal rains, since weeds continue to emerge when it is still raining, because glyphosate is a semi-systemic herbicide just like glufosinate ammonium, and its effect was not enough to eliminate the weeds.

The visual coverage expressed in percentage of soil covered (area) represents the vertical projection of the aerial part of the weeds and reflects the capacity for competition better than the number of weeds present, at the same time that it represents the control exerted by herbicides; it is a fast visual evaluation: with more coverage, less control, and vice versa. Regarding this variable (Figure 3), the Kruskal Wallis statistical analysis shows $p\text{-value}=1.08346^{e-11}$, indicating significant differences between treatments, although a statistically equal effect with both pine extracts. Herrera-Murillo and Picado-Arrollo (2024) mention that some herbicides do not cause chlorosis, but a reduction in coverage is observed which agrees with what was observed in the third measurement for the aqueous extract in narrow-leaf plants compared to the second and third measurements.

To visualize this variable better, a correlation was conducted between the coverage and the number of weeds or species present (Figure 4). It shows that, in general, there is a moderate positive correlation, since each treatment must be taken into account to analyze punctually; for example, in the regional control (glufosinate ammonium), the coverage is

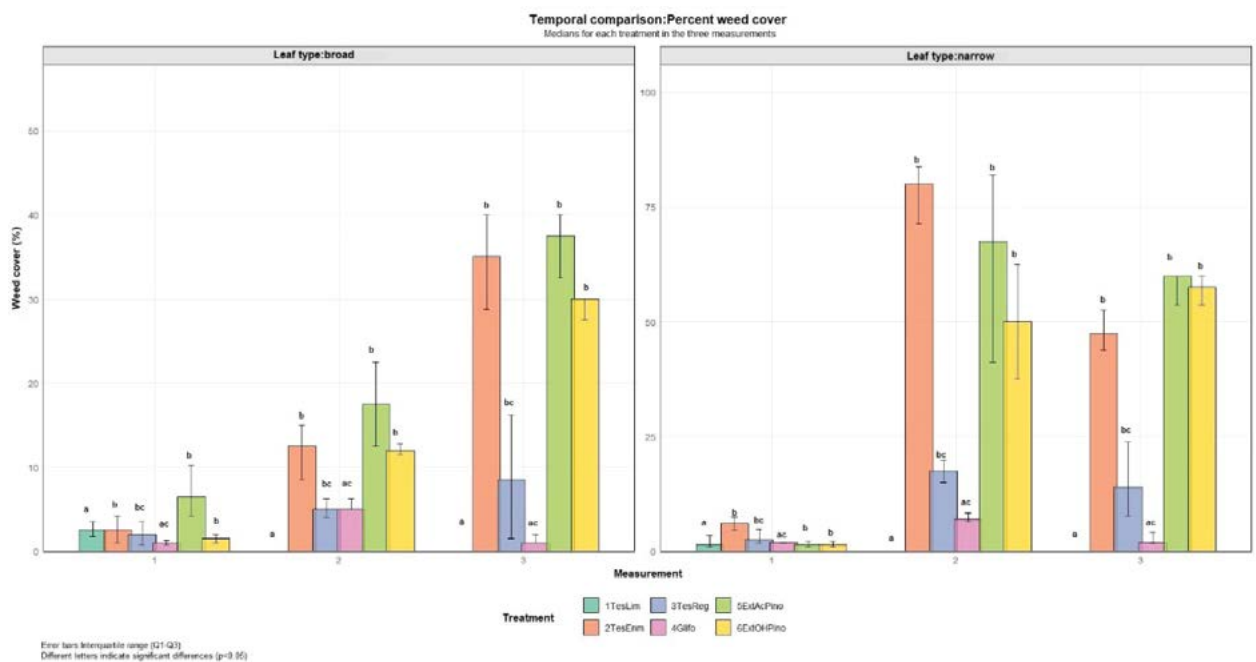


Figure 3. Means comparison of visual coverage of weeds (%) (control) present in the agave crop per treatment and type of leaf, in the three measurements 1, measurement 1, 2, measurement 2 and 3, measurement 3. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlf: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHPino: Alcoholic pine extract.

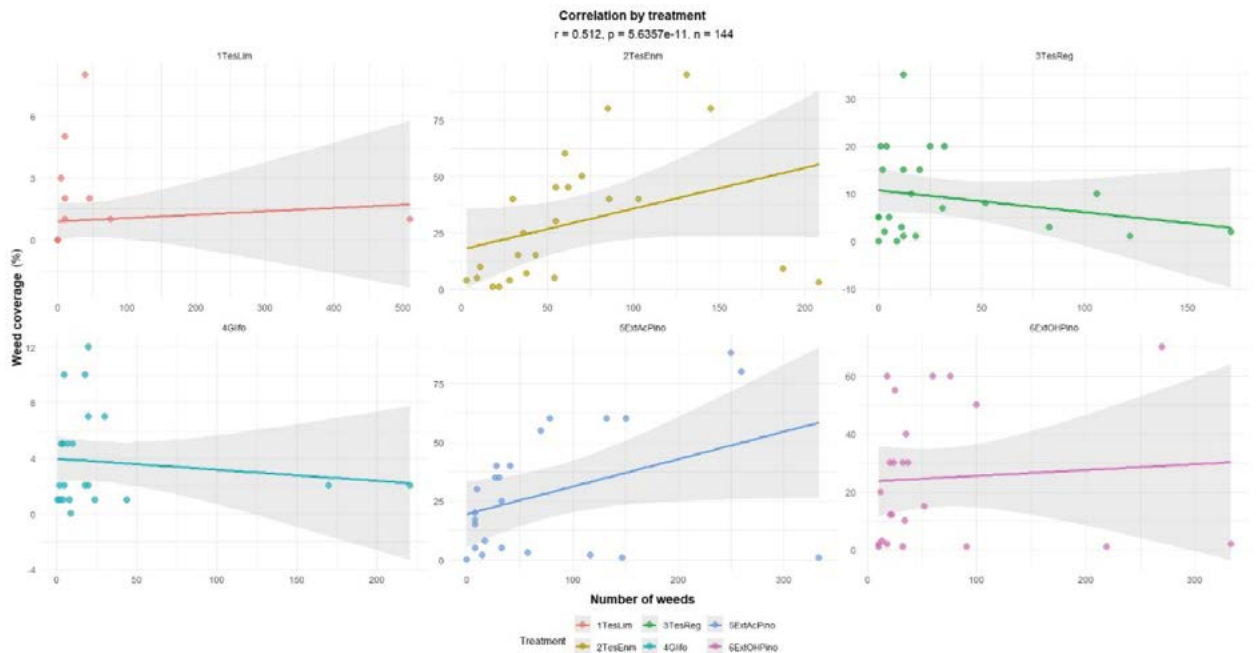


Figure 4. Correlation between number of weeds and coverage. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlif: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHpino: Alcoholic pine extract.

generally low and when it comes to the number of weeds present in the case of broad leaf, it ranges between 3 and 10 individuals, while for the narrow leaf the number fluctuates between 110 individuals in the first measurement and 20 in the last. Regarding the behavior of ethanolic and aqueous extracts, the trend was positively moderate but with variable points, so the effectiveness is qualified as weak for both, with their particularities, which will be discussed when addressing the species present in each treatment. This is probably because of the type of extraction performed; by using only water, certain metabolites can be extracted (acids, amines, ketones, aldehydes, tannins, lignans, stilbenes, and sugars), which will probably not be extracted with the alcohol solvent used (terpenes, tannins, phenols, siloxanes, alkanes) (Amri *et al.*, 2011).

However, Alam *et al.* (2022) mention that in extracts of needles from *Pinus roxburghii* Sargent, elaborated with alcohol and aqueous solvent, the same metabolites were obtained, except more concentrated with the organic solvent (ethanol). When it comes to plant extracts, their action can be due to the compounds that the bibliography refers are found in *Pinus patula*, such as alpha-pinene, limonene, among others (Amri *et al.*, 2011; Iñíguez-Guillén *et al.*, 2007; Portuguese-García *et al.*, 2020). However, it is necessary to work on elaborating more concentrated extracts. For the chemical-synthetic treatments, the values are shown for glyphosate in the base of the figure compared to the regional control (glufosinate ammonium), so glyphosate is qualified as efficient when presenting a lower number of weeds and less coverage. Regarding both extracts, the percentage of coverage was similar, that is, without significant difference between them (Figure 3).

According to the European scale (EWRS), the levels of activity decrease as the effectiveness of the herbicide increases, so that, with less visual coverage, the effect is

greater. And in this sense, glufosinate ammonium has a classification of 3 and glyphosate of 2, the aqueous pine extract 8, and the ethanolic extract 7.

No impacts were seen on the agave crop and symptoms were observed in the weeds such as chlorosis, stunting, and in some cases necrosis; this is the first report of phytotoxic or allelopathic action of *Pinus patula* elaborated in a proportion of 1:100 (p-v) applied at 25%. Regarding the species that were present in the different treatments, the Shannon index was calculated, where the low diversity there is in broad-leaf weeds (Figure 5) and narrow-leaf weeds (Figure 6) is shown; clear differences are seen between the diversity present per treatment, and the value of 1 is not even reached, which is the lowest value mentioned for this index; the herbicide that decreases diversity the most is glyphosate.

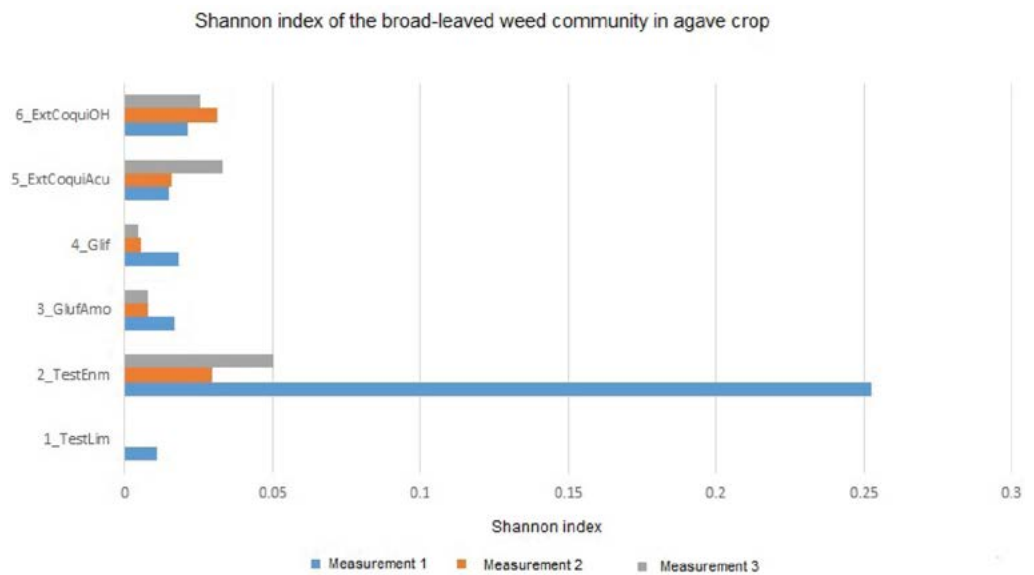


Figure 5. Shannon index in the treatments applied with broad-leaf weeds.

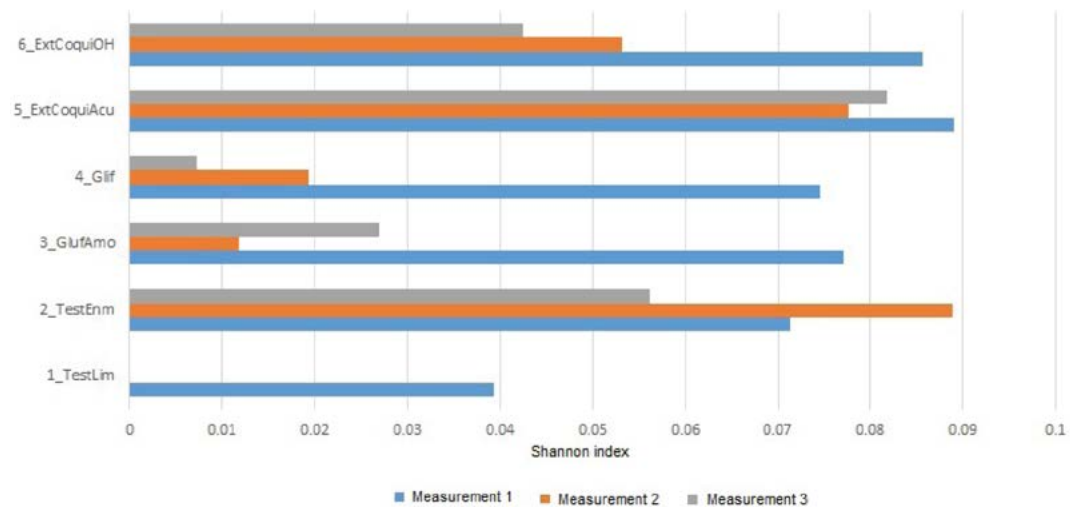


Figure 6. Shannon index in the treatments applied with narrow-leaf weeds.

All the treatments reduce the diversity in both types of leaf, including the weedy control; this is because of the environmental conditions and the life cycle of some weeds that appear and disappear according to climate conditions, and this treatment has the highest values in diversity.

Although the areas sampled are relatively small to estimate this index, this gives us an idea of the decrease in diversity that is caused by using herbicides; in many cases they are species with medicinal or allelopathic properties that could well be used. For example, in the species of broad-leaf weeds that were identified because they were not controlled (Figure 7), without this indicating resistance, *Salvia tilifolia* Vahlque was found, which is reported as forage, edible and medicinal (Vibrans, 2009c). *Mentha rotundifolia* L. was also identified, which has medicinal properties and is considered an aromatic plant similar to another *Salvia* species (Vibrans, 2009b). One species that is tolerant or resistant to herbicides applied is *Anoda cristata* L., which has been seen in the region that tolerates their application and was not the exception in this study; in addition, it is medicinal, forage, edible, and melliferous (Vibrans, 2009a).

When it comes to the presence of weeds per species, treatment and measurement (Figure 7), it was seen that there was a decrease in the number of species in the third measurement, just as with glyphosate, but more gradual or slower with the extracts. The grasses that were identified because they could not be controlled completely (Figure 7), agree with what was reported by Pimienta *et al.* (2005); some species present resistance to both glyphosate and glufosinate ammonium, and they were *Digitaria ciliaris* (Ketz.) Koeler, *Paspalum lividum* Trin. Ex Schtdl. and *Chloris virgate* Sw, although further studies must be done due to the

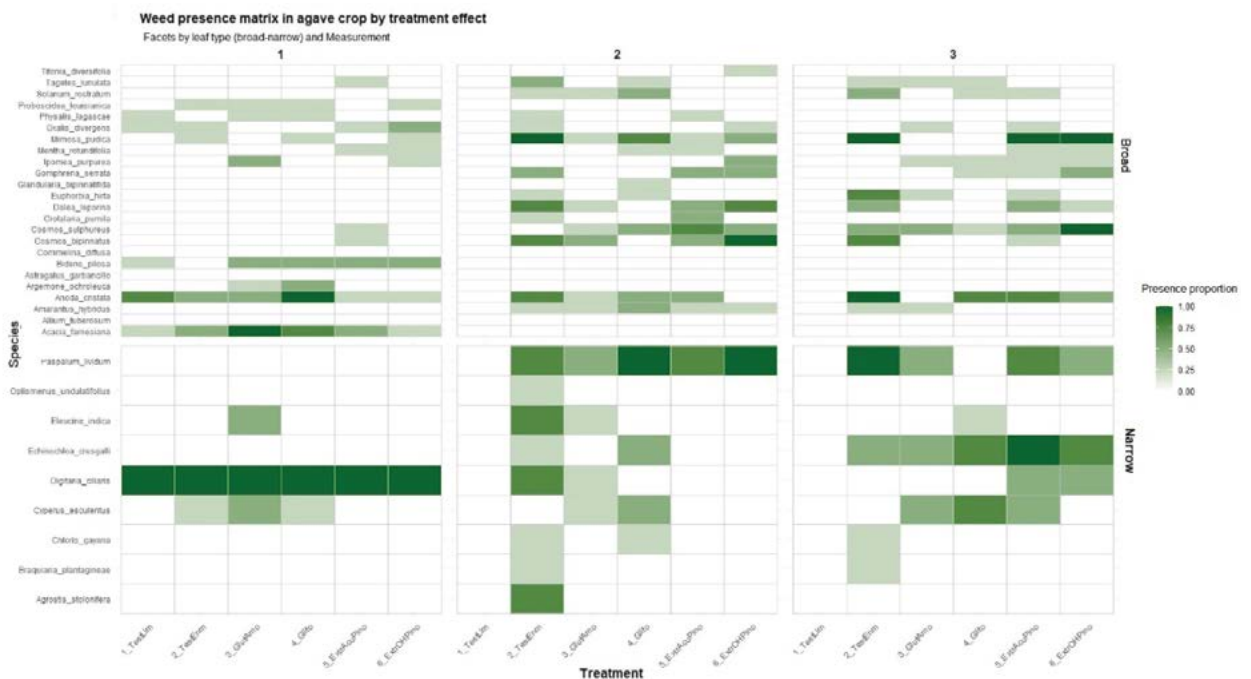


Figure 7. Presence of species per Measurement (1, 2, 3) and Treatment. Trat 1: Clean control, Trat 2: Weedy control, Trat 3: Glufosinate ammonium, Trat 4: Glyphosate, Trat 5: Aqueous pine extract, Trat 6: Alcoholic pine extract.

existence of ecotypes. Glufosinate ammonium is used to control weeds that are resistant to glyphosate (Takano and Dayan, 2020), and in this study species with resistance to both herbicides applied were found, such as those mentioned before. The total species in the aqueous pine extract were 31, and from these only eight could be controlled (*Proboscidea lousitanica* (Mill.) Thell., *Argemone ochroleuca* Swet, *Eleusine indica* L., *Chloris gayana* Kunt and *Agrostis stolonifera* L., among others) and there were 25 species reported for the ethanolic pine extract, of which 15 could be controlled (*Physalis lagascae* Roem. & Schult, *Argemone ochroleuca*, *Cyperus esculentum* L. and *Eleusine indica*, among others); regarding the 33 species reported in the treatment with glyphosate, nine could be controlled (*Oxalis divergens* Benth. & Lindl., *Cosmos bipinnatus* Cav., *Dalea leporine* (Ait.) Bullock and *Crotalaria pumila* Ort.).

Regarding plant herbicides, it is reported that when *Pinus caribean* Morelet var. *caribaea*. needles decompose, they form a compound called pycnogenol that can inhibit the germination of some plants considered weeds, since this compound inhibits the activity of the indole-acetic-oxidase acid, preventing seeds from germinating (Jiménez Ferrer *et al.*, 2006). For *Pinus patula*, ethanolic extract components reported include 62.4% of monoterpene hydrocarbons, α -pinene (35.2%), and β -phellandrene (19.5%) (Amri *et al.*, 2011). However, as a second study, it is necessary to evaluate the compounds of the extract. In a study, CO₂ was used to apply herbicides with pine oil and ensure the entry of the organic herbicide to the vascular system of the weed; it was reported that the pine oil had a good performance for weed control (James *et al.*, 2002).

Analysis of the agave plant

Concerning the statistical analysis of the parameters measured in the agave plant, the results showed significant differences between treatments (Table 2, leaf width, 4a, detached leaves, 4b, leaf length, 4c, plant height), which indicate that the treatments applied exert a different effect on the growth variables of the agave plant found.

Table 2. Analysis of variance for measurements repeated with non-parametric analyses of the variable leaf width.

	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	5.7250	0.1391
Treatment	Withn	5	472	3.3291	0.00576**
4a. Repeated measures analysis of variance with non-parametric analysis for the detached agave leaves variable					
	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	0.21875	0.68601
Treatment	Withn	5	472	2.64343	0.02267*
4b. Repeated measures analysis of variance with non-parametric analysis for the leaf's length variable					
	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	4.2061	0.1767
Treatment	Withn	5	472	2.5963	0.024857*
4c. Repeated measures analysis of variance with non-parametric analysis for the plant height variable					
	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	4.8333	0.1589
Treatment	Withn	5	472	2.4689	0.0318*

The four parameters measured indicate significant differences in all the parameters, although when the post hoc analysis is conducted (Figure 8), differences are only found in the variable detached leaves. It is seen that the treatment with glyphosate has greater leaf detachment, and this is explained because glyphosate is the only treatment that was not applied on the plant, because of its well-known effects, so there was no toxic effect on the crop; glyphosate has been used in other crops and it affects the seedlings due to the movement of this product in the soil and its contact with roots (Tofiño Rivera *et al.*, 2020). In addition, in this study, when it comes to plant growth, no impact was observed with the variables measured; this is because it is applied on the roads and with a hood, taking care that the glyphosate does not touch the plants. So its effect on its physiology is non-existent in the time of observation, although it was also observed that it does not affect the roots; this could be because of the low electrical conductivity recorded in dS/m (0.1) which was obtained with the application of this herbicide. The pH found was 6.03, or due to the low amount of organic matter present in the plot soils; something to remember is that the soils are clayey Luvisols, and another aspect to mention is that the pH interferes in the water quality and at the same time in the efficiency of the herbicide, where if the water is more alkaline, the efficiency decreases (García Ruiz and Sánchez Ortiz, 2005).

For the aqueous pine extract, these values were EC (0.7) and pH (6.43), and this treatment was the one that resulted in the lowest number of leaves detached, which could be due to a higher concentration of salts, given the EC recorded and this could cause a slight osmotic stress in the plant, in addition to the pH closer to 7 that can help to achieve better nutrient absorption; the compounds obtained are also water-soluble and possibly more persistent. When it comes to the ethanolic extract, with EC (0.8) and pH (6.7), in this

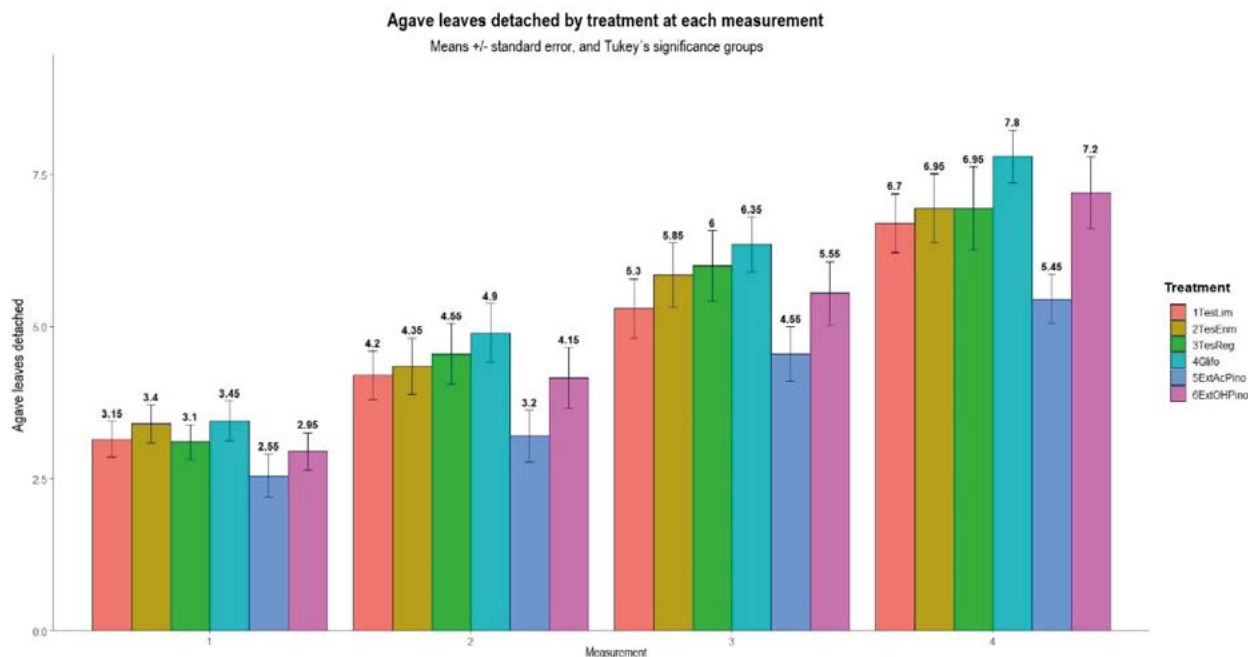


Figure 8. Means comparison of leaves detached in four months of records. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGliF: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHPino: Alcoholic pine extract.

case the compounds that were obtained are more volatile, so they exert their ephemeral allelopathic effect. The compounds that are obtained with the different solvents depend on their affinity with the polarity of the solvent; ethanol is catalogued by Adam *et al.* (2022) as one of the best solvents to extract compounds from the pine needles. Regarding the regional control, which was glufosinate ammonium, the EC was 0.16 and the pH 6.37; for the clean control where the weeds were only removed manually, the EC was 0.13 and the pH 6.3; the weedy control showed EC of 0.11 and pH of 6.67. The variations caused by the application of the treatments are slight and a study should be conducted considering the persistence and the identification of the metabolites extracted. However, this is a first approximation to the activity of *Pinus patula* needle extract in the field. This variation in pH could affect the soil microbiota, not only the variation but also the application of glyphosate, since the shikimate pathway that this herbicide impacts is in the metabolism of microorganisms, so they are also affected (González Ortega *et al.*, 2022), and this aspect should be studied further.

Regarding the costs that each treatment represents (Table 3), the extracts have a low cost, and what increases the cost is the workday that must be paid for any application. And given the total cost and the efficacy of glyphosate, it continues to be first place in use.

Table 3. Cost of treatments applied.

Treatments	Cost of supplies/ Daily labor cost	Total
1-Clean control	\$0.00/\$500	\$500.00
2- Weedy control	\$0.00/\$0.00	\$0.00
3- Glufosinate-ammonium	\$797.00/\$500	\$1,297
4- Glyphosate	\$350.00/\$500	\$850.00
5- Aqueous pine extract	\$5.00/\$500	\$505.00
6- Alcoholic pine extract	\$45.00/\$500	\$545.00

CONCLUSIONS

This is the first report of phytotoxic activity for *Pinus patula*. The pine extract with both solvents, in the dose used in this study, showed phytotoxic symptoms in weeds such as stunting, chlorosis, and to a lesser extent, necrosis, as well as a decrease in emergence. The aqueous and ethanolic pine extracts cause changes in number of species and coverage of weeds. The extracts from pine needles with both solvents and in the dose used do not match the effect of a synthetic herbicide. The variation in their performance in the last month of record, even in grasses, suggests that they are extracts that should still be studied. The species *Digitalia ciliaris*, *Paspalum lividum* and *Chloris virgata* are reported as not being able to be controlled with glyphosate or glufosinate ammonium. The type of leaf (broad or narrow) is determinant in the efficacy of herbicides.

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REFERENCES

- Alam, T., Jilani, G., Chaudhry, A. N., Ahmad, M. S., Aziz, R., & Ahmad, R. (2022). Terpenes and phenolics in alcoholic extracts of pine needles exhibit biocontrol of weeds (*Melilotus albus* and *Asphodelus tenuifolius*) and insect-pest (*Plutella xylostella*). *Journal of King Saud University - Science*, *34*(4), 101913. <https://doi.org/10.1016/J.JKSUS.2022.101913>
- Amri, I., Lamia, H., Gargouri, S., Hanana, M., Mahfoudhi, M., Fezzani, T., Ezzeddine, F., & Jamoussi, B. (2011). Chemical composition and biological activities of essential oils of *Pinus patula*. *Natural Product Communications*, *6*(10), 1531-1536. <https://doi.org/10.1177/1934578x1100601031>
- Belz, R. G. (2007). Allelopathy in crop/weed interactions - An update. In *Pest Management Science* (Vol. 63, Issue 4). <https://doi.org/10.1002/ps.1320>
- CONACYT. (2020). Expediente científico sobre el glifosato y los cultivos GM.
- Cruz-Ortiz, L., & Flores-Méndez, M. (2021). Avances en el desarrollo de nuevos herbicidas biológicos a partir de extractos vegetales fitotóxicos aplicados *in vitro*. *Informador Técnico*, *86*(1). <https://doi.org/10.23850/22565035.3648>
- Dominschek, R., Barroso, A. A. M., Lang, C. R., de Moraes, A., Sulc, R. M., & Schuster, M. Z. (2021). Crop rotations with temporary grassland shifts weed patterns and allows herbicide-free management without crop yield loss. *Journal of Cleaner Production*, *306*. <https://doi.org/10.1016/j.jclepro.2021.127140>
- Elkin, L. A., Kay, M., Higgins, J. J., & Wobbrock, J. O. (2021). An Aligned Rank Transform Procedure for Multifactor Contrast Tests. *ACM DL Digital Library*, 2102, 1-14. <http://arxiv.org/abs/2102.11824>
- García Ruiz, I., & Sánchez Ortiz, M. (2005). Influencia del pH del agua sobre la efectividad de varios herbicidas utilizados en Caña de azúcar. *Fitosanidad*, *9*(3), 37-40. https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0188-49992022000100113
- González Ortega, E., Fuentes Ponce, M. H., González Ortega, E., & Fuentes Ponce, M. H. (2022). DINÁMICA DEL GLIFOSATO EN EL SUELO Y SUS EFECTOS EN LA MICROBIOTA. *Revista Internacional de Contaminación Ambiental*, *38*, 127-144. <https://doi.org/10.20937/RICA.54197>
- Herrera-Murillo, F., & Picado-Arroyo, G. (2024). Selectividad de herbicidas en camote (*Ipomoea batatas* (L.) Lam.) variedad Criollo. *Agronomía Mesoamericana*, *35*, 1–13. <https://doi.org/10.15517/am.2024.54654>
- INEGI. (2001). Síntesis de Información geográfica del estado de México.
- INEGI. (2018). Comunicado de Prensa NÚM. 304 Comunicación Social Producción, Los Principales Cultivos del País son Caña de Azúcar, Maíz Grano Blanco, Maíz Grano Amarillo, Jitomate, Naranja, Trigo Grano y Plátano: Encuesta Nacional Agropecuaria (ENA) 2017. https://www.inegi.org.mx/contenidos/saladeprensa/boletines/2018/ena/ena2018_07.pdf
- Iñíguez-Guillén, M. R., Esqueda-Reyes, C. H., Escoto García, T., Guillermo Ochoa, H., Rodríguez Rivas, A., & Contreras Quiñones, J. H. (2007). Chemical characterization of essential oils and wood extracts from two pine species of La Primavera forest. *Revista Mexicana de Ciencias Forestales*, *6*(28), 42-57.
- James, T. K., Rahman, A., Trollove, M., & Frith, H. (2002). Efficacy of a certified organic herbicide based on pine essence. *New Zealand Plant Protection*, *55*, 207-212.
- Jiménez Ferrer, L., Valdés Zayas, D., & Álvarez Puentes, R. (2006). Efecto alelopático de *Pinus caribea* en la germinación de arvenses en casas de cultivo protegido. *Centro Agrícola*, *33*(4), 1-5.
- Kay, M., Elkin, L., Higgins, J., & Wobbrock, J. (2021). ARTool: Aligned rank transform for nonparametric factorial ANOVAs. R package version 0.11.1. <https://Github.Com/Mjskay/ARTool>.
- Marín-Velásquez, T., & Barrutia-Barreto, I. (2020). Extracto hidroalcohólico de cáscara de *Citrus sinensis* y aceite de *Pinus patula*, una alternativa de biorremediación. *Journal of the Selva Andina Research Society*, *11*(2), 103-113.
- Monroy-Reyes, B., Pimienta-Barrios, E., Hurtado de la Peña, S. A., & García-Galindo, J. (2005). Efecto competitivo de la maleza sobre el crecimiento y la respuesta fisiológica de agave (*Agave tequilana* Weber var. azul) en la región de Tequila, Jalisco, México. *Scientia-CUCBA*, *7*(2), 113-130.
- Ordoñez-Beltrán, V., Frías-Moreno, M. N., Parra-Acosta, H., & Martínez-Tapia, M. E. (2019). Estudio sobre el uso de plaguicidas y su posible relación con daños a la salud. *Revista Toxicología*, *36*(2), 148-162.
- Pimienta-Barrios, E., Zañudo-Hernández, J., Nobel, P. S., & García-Galindo, J. (2005). Respuesta fisiológica a factores ambientales del agave azul (*Agave tequilana* Weber). *Scientia-CUCBA*, *7*(2), 85-97.

- Portuguez-García, M. P., Agüero-Alvarado, R., & González-Lutz, M. I. (2020). Preemergence activity in the greenhouse on *Arthraxon quartinianus*, of an extract from *Pinus* sp. *Agronomía Mesoamericana*, 31(3), 773-779. <https://doi.org/10.15517/AM.V31I3.39361>
- Serpa, I., Calvo, K., Torres-Monserrat, V., Carbonaro, M., Martínez, M. Á., Rodríguez, M., Peralta, E., & Coscarelli, E. (2015). Efectos del glifosato sobre el endometrio de ratas hembra. *Reproducción*, 30, 14-25.
- Suppa, A., Kvist, J., Li, X., Dhandapani, V., Almulla, H., Tian, A. Y., Kissane, S., Zhou, J., Perotti, A., Mangelson, H., Langford, K., Rossi, V., Brown, J. B., & Orsini, L. (2020). Roundup causes embryonic development failure and alters metabolic pathways and gut microbiota functionality in non-target species. *Microbiome*, 8(1). <https://doi.org/10.1186/s40168-020-00943-5>
- Takano, H. K., & Dayan, F. E. (2020). Glufosinate-ammonium: a review of the current state of knowledge. In *Pest Management Science* (Vol. 76, Issue 12, pp. 3911–3925). John Wiley and Sons Ltd. <https://doi.org/10.1002/ps.5965>
- Tofiño Rivera, A. P., Carbono Murgas, R. E., Melo Ríos, A. E., & Merini, L. J. (2020). Effect of glyphosate on microbiota, soil quality and biofortified bean crop in Codazzi, department of Cesar, Colombia. *Revista Argentina de Microbiología*, 52(1), 61-71. <https://doi.org/10.1016/j.ram.2019.01.006>
- Vibrans, H. (2009a, April 15). *Anoda cristata* - ficha informativa. Malezas de México, CONABIO. <http://www.conabio.gob.mx/malezasdemexico/malvaceae/anoda-cristata/fichas/ficha.htm>
- Vibrans, H. (2009b, April 15). *Mentha × rotundifolia* - ficha informativa. Malezas de México, CONABIO. <http://www.conabio.gob.mx/malezasdemexico/lamiaceae/mentha-rotundifolia/fichas/ficha.htm>
- Vibrans, H. (2009c, April 15). *Salvia tiliifolia* - ficha informativa. Malezas de México, CONABIO. <http://www.conabio.gob.mx/malezasdemexico/lamiaceae/salvia-tiliifolia/fichas/ficha.htm>