ISSN: 2594-0252

AGRO PRODUCTIVIDAD

Water supply in artificial troughs:
a strategy to mitigate the impacts of climate change in the Maya forest

pág. 69

Año 17 • Volumen 17 • Número 4 • abril, 2024

Colegio de Postgraduados

Contenido

Año 17 • Volumen 17 • Número 4 • abril, 2024

AGRICULTURA — SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL
AGRO PRODUCTIVIDAD
Colegio de Postgraduados

Bases de datos de contenido científico

Directorios

Año 17, Volumen 17, Número 4, abril 2024, Agro productividad es una publicación mensual editada por el Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, Estado de México. CP 56264. Tel. 5959284427. www.colpos.mx. Editor responsable: Dr. Jorge Cadena Iñiguez. Reservas de Derechos al Uso Exclusivo No. 04-2017-031313492200-203. ISSN: 2594-0252, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, M.C. Valeria Abigail Martínez Sias. Fecha de última modificación, 6 de mayo de 2024.

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

Contacto de soporte
Soporte
5959284703
agroproductividadsoporte@gmail.com

Directrices para Autores/as

Naturaleza de los trabajos: Las contribuciones que se reciban para su eventual publicación deben ser resultados originales derivados de un trabajo académico de alto nivel sobre los tópicos presentados en la sección de temática y alcance de la revista.

Extensión y formato: Los artículos deberán estar escritos en procesador de textos, con una extensión de 15 cuartillas, tamaño carta con márgenes de 2.5 centímetros, Arial de 12 puntos, interlineado doble, sin espacio entre párrafos. Las páginas deberán estar foliadas desde la primera hasta la última en el margen inferior derecho. La extensión total incluye abordaje textual, bibliografía, gráficas, figuras, imágenes y todo material adicional. Debe evitarse el uso de sangría al inicio de los párrafos. Las secciones principales del artículo deberán escribirse en mayúsculas, negritas y alineadas a la izquierda. Los subtítulos de las secciones se escribirán con mayúsculas sólo la primera letra, negritas y alineadas a la izquierda.

Exclusividad: Los trabajos enviados a Agro Productividad deberán ser inéditos y sus autores se comprometen a no someterlos simultáneamente a la consideración de otras publicaciones; por lo que es necesario adjuntar este documento: Carta de originalidad.

Frecuencia de publicación: Cuando un autor ha publicado en la revista como autor principal o de correspondencia, deberá esperar tres números de ésta para publicar nuevamente como autor principal o de correspondencia.

Idiomas de publicación: Se recibirán textos en inglés con títulos, resúmenes y palabras clave en inglés.

ID Autores: El nombre de los autores se escribirán comenzando con el apellido o apellidos unidos por guion, sólo las iniciales del nombre, separados por comas, con un índice progresivo en su caso. Es indispensable que todos y cada uno de los autores proporcionen su número de identificador normalizado ORCID, para mayor información ingresar a (https://orcid.org).

Institución de adscripción: Es indispensable señalar la institución de adscripción y país de todos y cada uno de los autores, indicando exclusivamente la institución de primer nivel, sin recurrir al uso de siglas o acrónimos. Se sugiere recurrir al uso de la herramienta wyta (http://wayta.scielo.org/) de Scielo para evitar el uso incorrecto de nombres de instituciones.

Anonimato en la identidad de los autores: Los artículos no deberán incluir en ni en cuerpo del artículo, ni en las notas a pie de página ninguna información que revele su identidad, esto con el fin de asegurar una evaluación anónima por parte de los pares académicos que realizarán el dictamen. Si es preciso, dicha información podrá agregarse una vez que se acredite el proceso de revisión por pares.

Estructura de los artículos: Los artículos incluirán los siguientes elementos: Título, title, autores y adscripción, abstract, keywords, resumen, palabras clave, introducción, objetivos, materiales y métodos, resultados y discusión, conclusiones y literatura citada en formato APA.

Título: Debe ser breve y reflejar claramente el contenido, deberá estar escrito en español e inglés. Cuando se incluyan nombres científicos deben escribirse en itálicas. No deberá contener abreviaturas ni exceder de 20 palabras, se usará solo letras mayúsculas, en negritas, centrado y no llevará punto final.
Resumen y Abstract: Deberá integrarse un resumen en inglés y español (siguiendo ese orden), de máximo 250 palabras, donde se destaque obligatoriamente y en este orden: a) objetivo; b) diseño / metodología / aproximación; c) resultados; d) limitaciones / implicaciones; e) hallazgos/ conclusiones. El resumen no deberá incluir citas, referencias bibliográficas, gráficas ni figuras.
Palabras clave y Keywords: Se deberá incluir una lista de 3 a 5 palabras clave en español e inglés que permitan identificar el ámbito temático que aborda el artículo.
Introducción: Se asentará con claridad el estado actual del conocimiento sobre el tema investigado, su justificación e importancia, así como los objetivos del trabajo. No deberá ser mayor a dos cuartillas.
Materiales y Métodos: Se especificará cómo se llevó a cabo la investigación, incluyendo el tipo de investigación, diseño experimental (cuando se traten de investigaciones experimentales), equipos, substancias y materiales empleados, métodos, técnicas, procedimientos, así como el análisis estadístico de los datos obtenidos.
Resultados y Discusión: Puede presentarse en una sola sección. En caso de presentarse de forma separada, la discusión debe enfocarse a comentar los resultados (sin repetirlos), en términos de sus características mismas, su congruencia con la hipótesis planteada y sus semejanzas o diferencias con resultados de investigaciones similares previamente realizadas.
Conclusiones: Son la generalización de los resultados obtenidos; deben ser puntuales, claras y concisas, y no deben llevar discusión, haciendo hincapié en los aspectos nuevos e importantes de los resultados obtenidos y que establezcan los parámetros finales de lo observado en el estudio.
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.
Cuadros: Deben ser claros, simples y concisos. Se ubicarán inmediatamente después del primer párrafo en el que se mencionen o al inicio de la siguiente cuartilla. Los cuadros deben numerarse progresivamente, indicando después de la referencia numérica el título del mismo (Cuadro 1. Título), y se colocarán en la parte superior. Al pie del cuadro se incluirán las aclaraciones a las que se hace mención mediante un índice en el texto incluido en el cuadro. Se recomienda que los cuadros y ecuaciones se preparen con el editor de tablas y ecuaciones del procesador de textos.
Uso de siglas y acrónimos: Para el uso de acrónimos y siglas en el texto, la primera vez que se mencionen, se recomienda escribir el nombre completo al que corresponde y enseguida colocar la sigla entre paréntesis. Ejemplo: Petróleos Mexicanos (Pemex), después sólo Pemex.
Elementos gráficos: Corresponden a dibujos, gráficas, diagramas y fotografías. Deben ser claros, simples y concisos. Se ubicarán inmediatamente después del primer párrafo en el que se mencionen o al inicio de la siguiente cuartilla. Las figuras deben numerarse progresivamente, indicando después de la referencia numérica el título del mismo (Figura 1. Título), y se colocarán en la parte inferior. Las fotografías deben ser de preferencia a colores y con una resolución de 300 dpi en formato JPG, TIF o RAW. El autor deberá enviar 2 fotografías adicionales para ilustrar la página inicial de su contribución. Las gráficas o diagramas serán en formato de vectores (CDR, EPS, AI, WMF o XLS).
Unidades. Las unidades de pesos y medidas usadas serán las aceptadas en el Sistema Internacional.
Citas bibliográficas: deberán insertarse en el texto abriendo un paréntesis con el apellido del autor, el año de la publicación y la página, todo separado por comas. Ejemplo (Zheng *et al.*, 2017). El autor puede introducir dos distintos tipos de citas:

Citas directas de menos de 40 palabras: Cuando se transcriben textualmente menos de 40 palabras, la cita se coloca entre comillas y al final se añade entre paréntesis el autor, el año y la página. Ejemplo:

> Alineado al Plan Nacional de Desarrollo 2013-2018, (DOF, 2013), el Programa Sectorial de Desarrollo Agropecuario, Pesquero y Alimentario 2013-2018 establece "Construir un nuevo rostro del campo sustentado en un sector agroalimentario productivo, competitivo, rentable, sustentable y justo que garantice la seguridad alimentaria del país" (DOF, 2013).

Citas indirectas o paráfrasis: Cuando se interpretan o se comentan ideas que son tomadas de otro texto, o bien cuando se expresa el mismo contenido pero con diferente estructura sintáctica. En este caso se debe indicar el apellido del autor y el año de la referencia de donde se toman las ideas. Ejemplo:

> Los bajos rendimientos del cacao en México, de acuerdo con Avendaño *et al.* (2011) y Hernández-Gómez *et al.* (2015); se debe principalmente a la edad avanzada de las plantaciones.

Las referencias bibliográficas: al final del artículo deberán indicarse todas y cada una de las fuentes citadas en el cuerpo del texto (incluyendo notas, fuentes de los cuadros, gráficas, mapas, tablas, figuras etcétera). El autor(es) debe revisar cuidadosamente que no haya omisiones ni inconsistencias entre las obras citadas y la bibliografía. Se incluirá en la lista de referencias sólo las obras citadas en el cuerpo y notas del artículo. La bibliografía deberá presentarse estandarizada recurriendo a la norma APA, ordenarse alfabéticamente según los apellidos del autor.
De haber dos obras o más del mismo autor, éstas se listan de manera cronológica iniciando con la más antigua. Obras de un mismo autor y año de publicación se les agregará a, b, c… Por ejemplo:

Ogata N. (2003a).
Ogata N. (2003b).

Artículo de revista:
Wang, P., Zhang, Y., Zhao, L., Mo, B., & Luo, T. (2017). Effect of Gamma Rays on *Sophora davidii* and Detection of DNA Polymorphism through ISSR Marker [Research article]. https://doi.org/10.1155/2017/8576404

Libro:
Turner J. (1972). Freedom to build, dweller control of the housing process. New York: Macmillan.

Uso de gestores bibliográficos: Se dará prioridad a los artículos enviados con la bibliografía gestionada electrónicamente, y presentada con la norma APA. Los autores podrán recurrir al uso de cualquier gestor disponible en el mercado (Reference Manager, Crossref o Mendeley entre otros), o de código abierto tal como Refworks o Zotero.

Colegio de Postgraduados

The Mexican Beekeeping Agri-food System: A descriptive analysis

Álvarez-Sánchez, Geiner F.[1]; **Zaldívar-Cruz, Juan M.**[1]; **Sauri-Duch, Enrique**[2]; **Sol-Sanchez, Angel**[1]; **Moscoso-Ramírez, Pedro A.**[1]; **Sanchez-Chino, Xariss M.**[3]

[1] Colegio de Postgraduados Campus Tabasco, Ranchería Río Seco y Montaña, Cárdenas, Tabasco, Mexico, C.P. 86500.

[2] Instituto Tecnológico de Mérida, Mérida, Yucatán, Mexico, C.P. 97118. 3Colegio de la Frontera Sur - Unidad Villahermosa, Ranchería Guineo 2da. Sección, Villahermosa, Tabasco, Mexico, C.P. 86280.

* Correspondence: jmzaldivarc@gmail.com

ABSTRACT

Objective: To conduct a descriptive analysis of the historical and current situation of honey production in Mexico.

Design/Methodology/Approach: We used the Food Balance Sheet obtained from the FAOSTAT website and the statistics of livestock production in Mexico recovered from the website of the Servicio de Información Agrícola y Pesquera (SIAP). We chose ten variables: production, import, export, per capita consumption, number of hives, production volume, economic value, price paid to producers, yield, and revenue per unit. The analysis describes the variables in the last recorded year at a global and local level, as well as the changes and trends according to the available historical records.

Results: In 2019, Mexico held the ninth place in honey production with 64,000 t and had the capacity to export around 50 % of said production. Between 2006 and 2016, the number of hives increased by 6%, while production volume and yield decreased by 1.6% and 7.5%. In contrast, the economic value, the price paid to producers, and the revenue per unit increased by 14%, 5.7%, and 8.5%.

Study limitations/Implications: This type of study relies on records of statistical information systems, whose availability depends on the variable of interest.

Findings/Conclusions: In Mexico, beekeeping is practiced throughout the country, making it self-sufficient in terms of domestic honey consumption while allowing its participation in the international market. Therefore, the country has optimization opportunities, especially in the central and northern areas, that have the lowest number of hives and production volume levels.

Keywords: Beekeeping, Honey, Descriptive analysis.

Citation: Álvarez-Sánchez, G. F., Zaldívar-Cruz, J. M., Sauri-Duch, E., Sol-Sanchez, A., Moscoso-Ramírez, P. A., Sanchez-Chino, X. M. (2024). The Mexican Beekeeping Agri-food System: A descriptive analysis. *Agro Productividad*. https://doi.org/ 10.32854/agrop.v17i4.2553

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

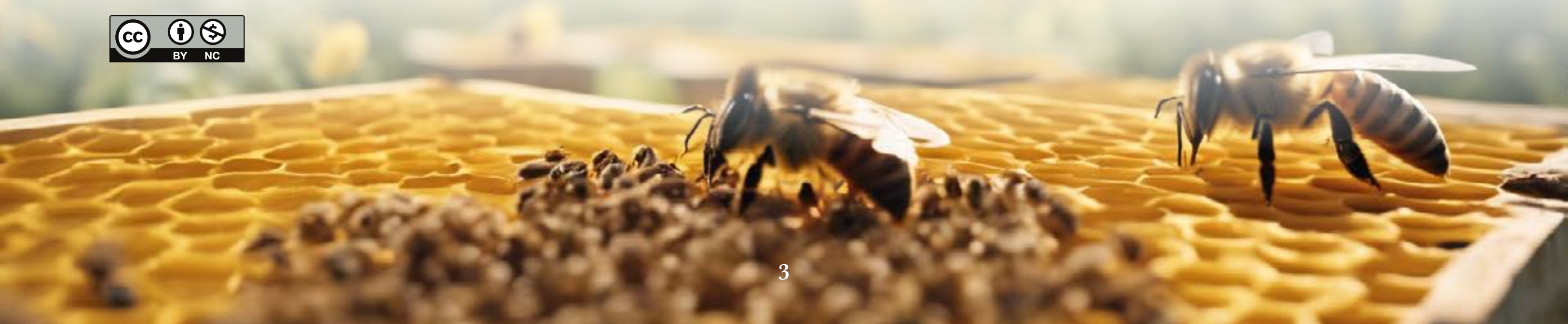
Received: March 23, 2023.
Accepted: January 15, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 3-10.

INTRODUCTION

Beekeeping has great relevance worldwide, providing not only environmental services of pollination —on which approximately 286 cultivated species depend (Castellanos-Potentiano *et al.*, 2016)— but also honey, which is the main beekeeping product marketed in 135 countries (FAO, 2021).

In Mexico, beekeeping has become socioeconomically salient since it represents a significant source of jobs and income in rural areas and an opportunity to attract foreign exchange (Magaña *et al.*, 2007). Honey production was recorded in the 32 states of the



country (31 states and Mexico City), which was recognized in 2019 as the ninth honey producer (with 64,000 t) and as the fifth exporter worldwide (with 32,000 t) (FAO, 2021).

Obtaining information on the performance of beekeeping production in Mexico is only possible thanks to statistical information systems (SIS). Using SIS and adequately interpreting agricultural statistics enable the making of public policies and optimal decisions that favor efficiency and better yields in the agriculture and livestock, agro-industrial, and forestry sectors (Chávez *et al.*, 2017).

In Mexico, several institutions are devoted to generating agricultural information and statistics, such as the Servicio de Información Agrícola y Pesquera (SIAP). However, despite their availability, these sources of information are seldom used in scientific research as a tool in the description of the beekeeping agri-food system (BAS).

Considering the above, the purpose of this study was to conduct a descriptive and referenced analysis of the historical and current situation of honey production in Mexico using statistical information from national and international institutions. Our goal is to obtain evidence that encourages the development of the beekeeping system.

MATERIALS AND METHODS

For this study, we analyzed three SIS: the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), the Sistema de Información Agroalimentaria y Pesquera (SIAP), and the Instituto Nacional de Estadística y Geografía (INEGI).

FAOSTAT offers worldwide statistical data on a wide range of topics. For our study, we selected the Food Balance Sheet (FBS). The SIAP is in charge of generating statistics and geographic information on agri-food matters in Mexico, offering production reports, monographic descriptions, statistics, and even satellite images and dynamic maps.

Food balance sheet (FBS)

Food Balance Sheets (FBS) are useful databases for assessing a country's or entity's food capacity concerning food availability.

We procured the FBS from the Food Balance Sheets section on the FAOSTAT website. From this source, we selected a total of five variables (production, import, export, national demand, and *per capita* consumption) recorded for a period of 29 years (1990-2019) (FAO, 2021).

Once downloaded, we integrated the data from the FBS into a spreadsheet and homogenized the fields and units of measurement. Finally, we generated a data table with 680,000 records. To conduct the exploratory data analysis, we used dynamic tables in Excel.

Performance of the beekeeping agri-food system (BAS)

We obtained national statistical data from 31 files on the SIAP website. Said files contain livestock production statistics from 1990 to 2020 (SIAP 2021). The data for livestock areas came from the 2016 Agricultural, Livestock, and Forestry Census on the INEGI website. We subsequently integrated the data into a spreadsheet to identify spelling errors, inconsistencies, and omissions. Finally, we generated a data table with 665,000 records.

Based on this last database, we identified a total of four variables of interest: Number of hives (NH), production volume (PV), economic value of production (EV), and average price paid to producers (PP). In addition, we generated two more variables: Yield (Y), by dividing PV by NH, and revenue per unit (RPU), which resulted from dividing EV by NH. Exploratory data analysis and calculations were performed using pivot tables in Excel.

The purpose of describing the BAS was to outline the most relevant aspects of the system. We divided our analysis into two levels. The first one comprised the statistical description of the variables of interest for 2016 (because 2016 was the last year providing records for number of hives, even though data for the rest of the variables are available until 2020). The second level of description considered the changes and trends of the variables of interest from 2006 to 2016. For our evaluation, the monetary values were deflated to the base year 2010 using the National Consumer Price Index (NCPI).

RESULTS AND DISCUSSION

BAS performance in a global context

In 2019, global honey production reached 2.4 million tons, with China contributing 457,000 t (24.7% of the world total), making this country the leading honey producer globally. Mexico contributed 64,000 t or 3.5% of the global production, which placed it as the ninth honey producer worldwide, third in the American continent, and second in Latin America, surpassed only by Argentina, that contributed 79,000 t of honey or 4.3% of the global total (Table 1).

The *per capita* consumption of honey worldwide for the same year was 0.43 kg. In Mexico, the *per capita* consumption reached only 140 g, three times less than the average in the leading producer countries (Table 1).

The apparent national demand (AND) amounted to 24,000 t and was supplied with 37.5% of the national production, which left 62.5% of the remaining production for export (Figure 1). These production and consumption values have had ups and downs between 1990 and 2018.

Table 1. Statistics of the main honey-producing countries for 2019 (FAO, 2021).

Principales países productores	Production (thousands of ton*)	Participation (%)	Consumption *per capita* (kg año^{-1})
China	457	24.7	0.22
Türkiye	108	5.8	1.17
Argentina	79	4.3	0.03
Iran	78	4.2	0.92
Ukraine	71	3.8	0.41
USA	70	3.8	0.64
India	67	3.6	0.01
Russia	65	3.5	0.41
Mexico	64	3.5	0.14
Ethiopia	50	2.7	0.43
Global	2427	100	0.43[1]

[1]=global average

Honey production in Mexico has remained mostly stable, ranging from 50,000 to 60,000 t. The lowest figure was recorded in 1995 and 1996, when only 49,000 t were obtained, while the maximum production occurred in 1991, reaching 66,000 t of honey (Figure 1).

During the same period, the AND —with its ups and downs— presented a slight increase of 4%, going from 23,000 t in 1990 to 24,000 t in 2018. In contrast, annual *per capita* consumption showed a decrease of 46% in the same period, going from 0.26 kg in 1990 to 0.14 kg in 2018, also with ups and downs throughout the period, with a maximum consumption of 0.36 kg person^{-1} year^{-1} in 2001 and a minimum consumption of 0.14 kg person^{-1} year^{-1} in 2015 (Figure 1).

Like production and consumption per capita, honey exports decreased from 44,000 t in 1990 to 40,000 t in 2018 —9% less for said period (Figure 1). Despite this decline, Mexico still holds an important place in the international market since its production volume is high enough to maintain its export vocation. Each year, the country places around 50% of its honey production in the international market (Chan-Chi *et al.*, 2018).

However, the international markets' strict quality and safety standards increase production costs, as they force producers to modify the traditional forms of hive management and solve health problems by acquiring superior materials. This, in turn, allows them to maintain their presence in said markets (Magaña *et al.*, 2016).

For their part, imports have been virtually unnecessary to satisfy national demand —1,000 tons of honey were imported in 2001 and 2002 only (Figure 1).

BAS performance from a local context

In 2020, beekeeping was practiced in all 32 states of the country and in 1,525 municipalities, with Jalisco, Yucatán, Chiapas, Campeche, Veracruz, and Oaxaca having the largest number of honey-producing municipalities (Table 2).

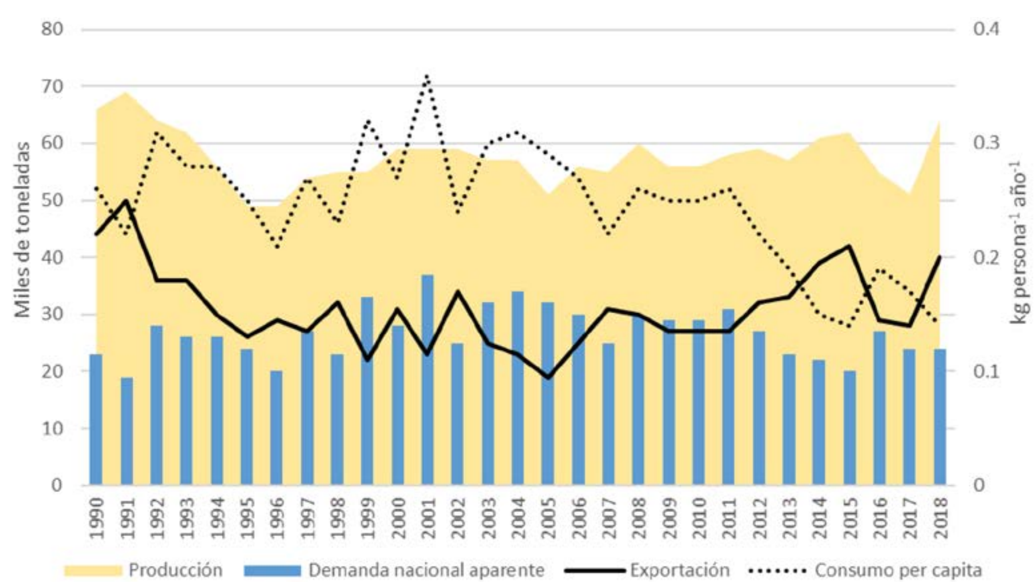


Figure 1. Food Balance Sheet (FBS) for Mexico, 1990-2018 (FAO, 2021).

In 2016, the NH nationwide reached 1,859,350 (Table 2). The states with the highest NH are in the south and southeast of the Mexican Republic, with Yucatán having the highest established NH: 250,073, that is 13.4% of the national total (Table 2). In contrast, most of the states with the lowest NH are in the north and northwest of the country (Figure 2a).

Table 2. BSA performance variables by state in Mexico for 2020 (SIAP, 2021).

Entidad	Municipios	NH[1]	PV (t)	EV (m$)	PP ($ kg^{-1})
Aguascalientes	11	17500	517.9	23.6	45.5*
Baja California	5	8672	297.9	14.7	48.7*
Baja California Sur	4	4680	201.7	9.6	47.6*
Campeche	13	205377*	5374.5*	165.5*	30.7
Chiapas	82	161822*	5434.5*	220.7*	41.6
Chihuahua	18	34061*	637.2	30.8	47.4*
Ciudad de México	7	9337	96.1	5.0	51.9*
Coahuila	19	17000*	222.3	11.3	51.2*
Colima	9	4000	494.3	22.9	46.0*
Durango	24	16884*	471.5	23.4	52.5*
Guanajuato	34	39523	654.1	32.2	49.5*
Guerrero	60	81194*	1963.3*	90.4*	47.7
Hidalgo	67	23454	1399.0*	59.6	42.7
Jalisco	109	120128*	6059.2*	283.2*	46.6*
México	73	40657*	876.1	44.2*	50.3*
Michoacán	113	67842*	2041.9*	105.9*	52.8*
Morelos	33	66180*	1954.2*	86.6*	43.4
Nayarit	16	11312	460.3	19.5	44.3
Nuevo León	8	4720	257.7	11.7	45.4*
Oaxaca	244	116860*	4592.3*	185.0*	40.9
Puebla	166	91951*	2449.6*	106.6*	43.7
Querétaro	15	2028	63.4	2.7	43.1
Quintana Roo	9	120188*	2132.8*	52.1*	23.2
San Luis Potosí	46	44202*	1145.5*	52.8*	47.8*
Sinaloa	19	19237	179.4	7.7	42.9
Sonora	28	19184	565.6	27.1	48.0*
Tabasco	17	10542	405.0	18.2	45.1*
Tamaulipas	24	22854	708.9	28.6	40.5
Tlaxcala	55	32003	454.0	19.7	43.3
Veracruz	122	138009*	4645.2*	202.8*	43.9
Yucatán	103	250073*	5528.6*	138.9*	25.0
Zacatecas	54	57876	1881.4*	83.8*	45.2*
Nacional	1525	1859350	54165.3	2187.0	
Promedio nacional		58104.7	1692.7	68.3	44.3

NH: number of hives; PV: production volume; EV: economic value of production; PP: average price paid to producers; t: tons, *: values over national mean; [1]: data for 2016.

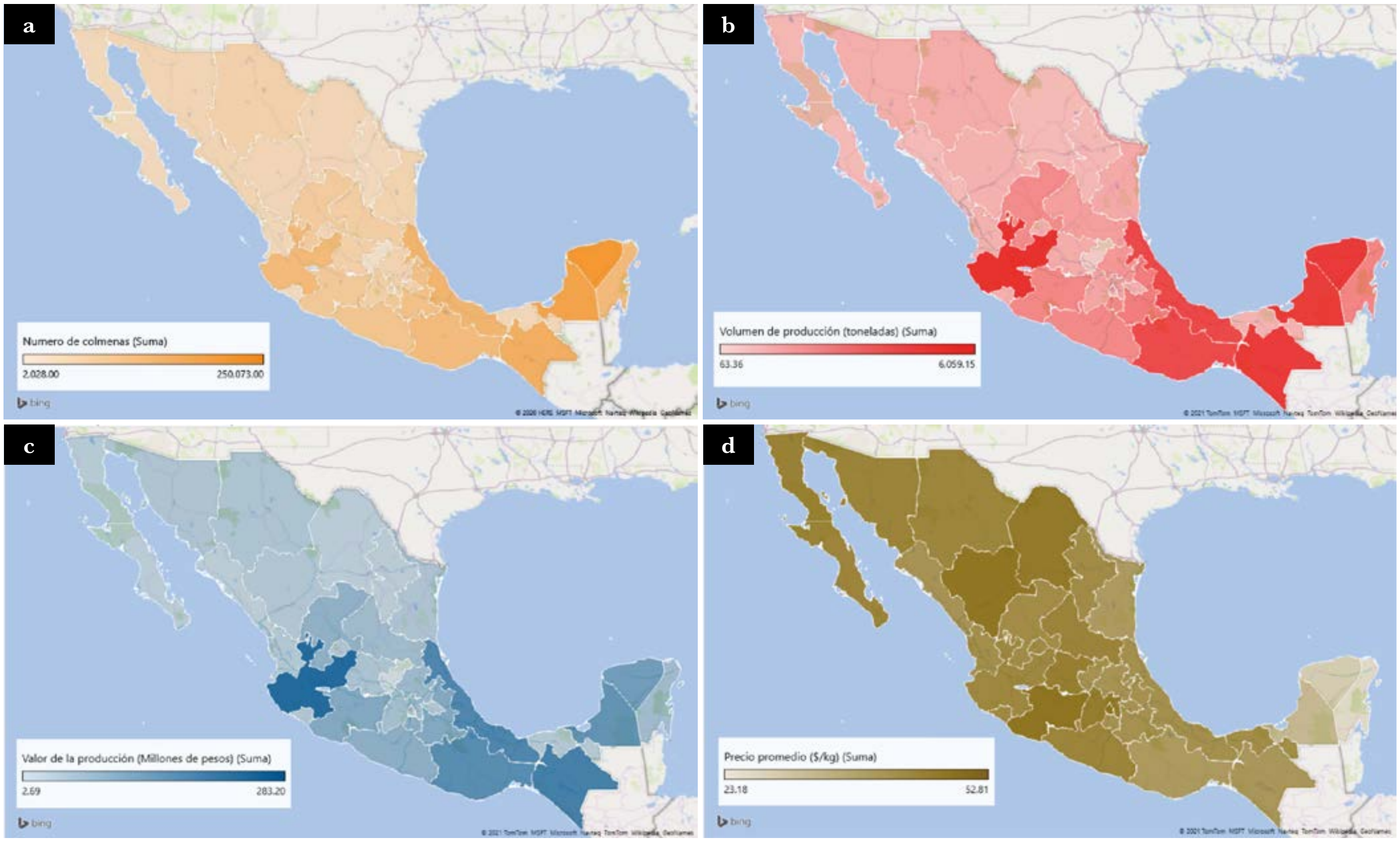


Figure 2. Geographic distribution of BAS performance variables in Mexico. a) NH for 2016, b) PV, c) EV, and d) PP for 2020.

In 2020, the total PV of honey was 54,165.3 t, which reached an EV of 2,187 million pesos. Jalisco had the highest PV with 6,059.2 t or 11.2% of the national production and an EV of 283.2 million pesos (Table 2). Again, the other states with high PV and EV are in the south and southeast of the Mexican Republic (Figures 2b and 2c).

The PP per kilogram of honey at the national level was estimated at \$44.3 kg^{-1} (Table 2). As for the geographical distribution, in Figure 2d we can observe that the best PPs converge in the north and center of the country, possibly due to the low production levels in this same area, where the states holding the last places in NH and PV are located. Michoacán has the maximum PP, estimated at \$52.8 kg^{-1} of honey (Figure 2d).

The recorded PPs for the country differ largely from the price paid per exported kilogram of honey, since the price paid to beekeepers is usually determined by the wholesale exporter or someone seeking to supply an international marketer. This depends both on the demand in the international market (import) and on the profit margin sought by commercial agents, which means that beekeepers have virtually no power to negotiate in most markets (Magaña *et al.*, 2016).

Changes and trends of BAS in Mexico, 2006-2016

The BAS has had a small increase in the number of productive hives at the national level, going from 1,747,033 in 2006 to 1,859,350 in 2016. This increase equals 6% of the initial value (Figure 3) and would also entail an increase in the PV of honey at the national level. However, the PV decreased: while in 2006, 58,121.3 t of honey were produced, in 2016 the figure was 57,202.4 t, a reduction of nearly 919 t or 1.6% of the initial PV (Figure 3). Even though the causes of this decrease in production are not clear, it may be attributed to diseases, pests, deficient use of agrochemicals, and even delays in the blooming of nectar-polliniferous plants due to climate change (Baena-Diaz *et al.*, 2022).

There was also a decrease in the average Y per hive: while in 2006, the yield reached 33.3 kg of honey per hive, in 2016, the yield was 30.8 kg —a decrease of 7.5% (Figure 3).

The analysis of the production EV for the BAS began with the deflation of prices through the NCPI based on 2010 data (Banxico, 2018). From 2006 to 2016, there was a notable upward trend in the production EV, which went from 1,728.5 million pesos in 2006 to 2,009.7 million pesos in 2016, that is, an increase of more than 281 million pesos, or 14% of the initial EV (Figure 3). This was the result of a 5.7% increase in the PP at the national level for the same period, which went from \$44.3 to \$47 kg^{-1}, generating an increase in the RPU from \$989.4 to \$1,080.9 per hive (Figure 3).

The slight decrease in the national beekeeping activity in terms of PV, NH, and Y is due to problems of a disparate nature that have affected productivity levels.

We can group the structural causes of these problems under two main points: 1) the Africanization of bees and the presence of the Varroa destructor mite, both of which constitute the main health problem of bee colonies since they considerably reduce the bee population and honey yield (Baena-Díaz *et al.*, 2022); and 2) the stagnation of Mexican honey competitiveness in the world market due to the lack of better handling and packaging technologies, as well as to the lack of interest in characterizing and differentiating the product, which could bring an added value (Campos-García *et al.*, 2018).

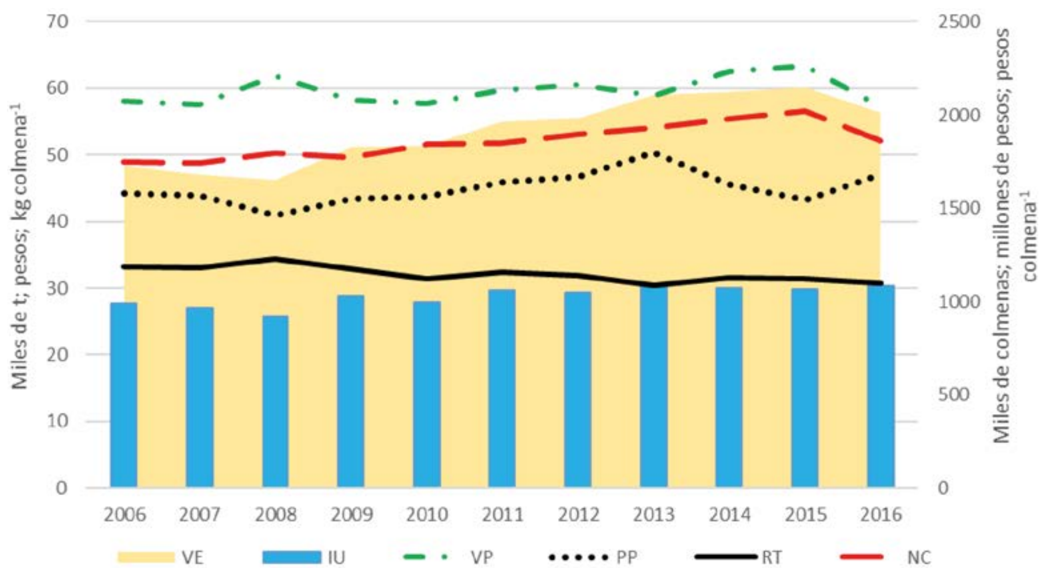


Figure 3. BAS performance in Mexico, 2006-2016: EV (millions of pesos), RPU (pesos $hive^{-1}$), PV (thousands of t), PP (pesos), Y (kg $hive^{-1}$), NH (thousands of hives).

CONCLUSIONS

Mexico has an outstanding beekeeping tradition: virtually all its states practice beekeeping. The country can satisfy the national demand for honey without resorting to imports due to its low *per capita* consumption (140 g person^{-1} year^{-1}). This enables the country to place nearly 50% of its production in the international market, meaning it holds a place among the top 10 countries with the highest volume of honey production.

Geographically, the south-southeast area of the country is home to the states with the best results in NH, PV, and EV. However, the states in the center and north of the country have the best PP per kilogram of honey.

Like any other agri-food system, the BAS has experienced ups and downs in performance variables over the years. Broadly, from 2006 to 2016, the average PV and Y per hive decreased by 1.6% and 7.5%. In contrast, the NH, EV, PP, and RPU showed 6%, 14%, 5.7%, and 8.5% increases. All this indicates that the BAS presents opportunities for management optimization, which would increase production levels and eventually contribute to the development of the municipalities and communities dedicated to beekeeping.

REFERENCES

Baena-Díaz, F., Chévez, E., Ruiz, de la M. F., y Porter-Bolland, L. (2022). *Apis mellifera* en México: producción de miel, flora melífera y aspectos de polinización. *Revisión. Revista Mexicana de Ciencias Pecuarias, 13*(2), 525-548. https://doi.org/10.22319/rmcp.v13i2.5960

Campos-García, M., Leyva-Molares, C., Ferráez-Puc, M., y Sanchez-Bolivar, Y. (2018). El mercado internacional de la miel de abeja y la competitividad de México. *Revista de Economía, 35*(90), 87-123.

Castellanos-Potenciano, B. P., Gallardo-López, F., Sol-Sanchez, A., Landeros-Sanchez, C., Diaz-Padilla, G., Sierra-Figueroa, P., Santibañez-Galarza, J. L. (2016). Impacto potencial del cambio climático en la apicultura. *Revista Iberoamericana de Bioeconomía y Cambio Climático. 2*(1): 1-19. doi: 10.5377/ribcc.v2i1.5673

Chan-Chi, J. R., Caamal-Cauich, I., Pat-Fernández, V. G., Martínez-Luis, D., & Pérez-Fernández, A. (2018). Social and economic characterization of bee honey production in the north of the state of Campeche, Mexico. *Textual*, 72, 103-124. doi: 10.5154/r.textual.2017.72.007

INEGI. (2017). Anuario estadístico y geográfico de Tabasco 2017. Instituto Nacional de Estadística y Geografía. México: INEGI. 440 p.

FAO. (2021). Hojas de balance alimentario. Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO). Recuperado el 10.12.2021, de http://www.fao.org/faostat/es/#data/FBS

Magaña, M. A., Aguilar, A., Lara, P., Sanginés, J. (2007). Caracterización socioeconómica de la actividad apícola en el estado de Yucatán, México. *Agronomía. 15*(2): 17-24.

Chávez, E. D., Arteaga, C. Y., García, Q. Y., y Zambrano, V. D. A. (2017). La contribución de la Estadística en la formación del profesional agropecuario, agroindustrial y forestal. *Revista Electrónica de Veterinaria. 18*(5): 1-9.

SIAP. (2021). Estadística de la producción pecuaria. Servicio de Información Agrícola y Pesquera. Recuperado el 22. 10. 2021, de http://infosiap.siap.gob.mx/gobmx/datosAbiertos_p.php

Magaña, M. A., Tavera, C. M. E., Salazar, B. L. L., Sanginés, G. J. R. (2016). Productividad de la apicultura en México y su impacto sobre la rentabilidad. *Revista mexicana de ciencias agrícolas. 7*(5): 1103-1115.

BANXICO. (2018). Índice Nacional de Precios al Consumidor. Banco de México. Recuperado el 09.10.2018, de http://www.banxico.org.mx/SieInternet/consultarDirectorioInternetAction.do?accion=consultarCuadro&idCuadro=CP154

Colegio de Postgraduados

Productive response of dual-purpose cows supplemented with chelate minerals and calcium salts

Ley-de Coss, Alejandro[1]; **Solís-Vázquez, Agustín**[1]; **Zambrano-Castillo, Beatriz**[1]; **Bran Reynerio, Adrián A.**[1]; **Montañez-Valdez, Oziel D.**[2]; **Guerra-Medina, Cándido E.**[3*]

[1] Universidad Autónoma de Chiapas, Cuerpo Académico Recursos Genéticos Tropicales, Facultad de Ciencias Agronómicas, Campus V, México.
[2] Universidad de Guadalajara, Grupo de Investigación en Nutrición Animal (GINA), Centro Universitario del Sur, 49000, Ciudad Guzmán, Jalisco, México.
[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Rosario Izapa, Tuxtla Chico, Chiapas, México.
* Correspondence: eguerranutricion@gmail.com

Citation: Ley-de Coss, A., Solís-Vázquez, A., Zambrano-Castillo, B., Bran Reynerio, A. A., Montañez-Valdez, O. D., Guerra-Medina, C. E., (2024). Productive response of dual purpose cows supplemented with chelate minerals and calcium salts. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2569

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: April 11, 2023.
Accepted: February 17, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 11-17.

ABSTRACT

Objective: To evaluate milk production, body condition and ethological behavior of grazing cows supplemented with chelated minerals and calcium salts, in dry tropical conditions.

Design/methodology/approach: Two treatments were evaluated, T1=Base feeding (grazing+4 kg of concentrated feed cow^{-1} day^{-1} (CF); T2=CF+300 g cow^{-1} day^{-1} of nucleus (chelated minerals+calcium salts) (N). Milk production data were evaluated during 32 days in four periods of seven days, the body condition at the beginning, 15 and 30 days after the evaluation, and the ethological behavior of the cows.

Results: In cows supplemented with nucelus, milk production increased 19.9% ($P \leq 0.05$); body condition improved 0.5 units, there was no difference in grazing time ($P > 0.05$) and rumination time was longer ($P \leq 0.05$).

Limitations on study/implications: The response to reproductive variables was not assessed in this study/ reproductive activity is likely to improve in response to improved body condition.

Findings/conclusions: By supplementing dual-purpose cows in the first third of lactation with chelated minerals and calcium salts, milk production increases, and body condition and rumination time improve.

Keywords: feeding, livestock farming, milk production, tropics.

INTRODUCTION

In Mexico, bovine livestock farming for milk production represents around 40% of the production units, and it is important due to the contribution of foods for human consumption such as milk, meat and dairy products (Loera and Banda, 2017). The dual-purpose (DP) system predominates in tropical regions, the animals used are racial crosses of

Bos indicus×*Bos taurus* because of their greater adaptation to conditions in the tropics, the producers obtain income from the sale of milk and weaned calves in the regional market (Orantes *et al*., 2010; Cortes *et al*., 2003). The milk production reported in the extensive system is 4.5 kg per cow day^{-1} and low profitability (Pech *et al*., 2007). Livestock feeding is based on extensive grazing, where the availability and amount of fodder is dependent on rainfall, and under these conditions the animals can present deficiencies in consumption of dry matter, protein, energy and microelements, which result in low productive and reproductive indices (Díaz, 2014; Spears, 2000). To correct nutritional deficiencies and improve productivity, it is necessary to complement the livestock with sources of nutrients which preferably are regionally available (Gómez *et al*., 2019). Lactating cows require higher consumption of energy and minerals, due to their functions in growth, gestation and lactation (Lipps and Bravo, 2016; Garmendina, 2005).

The aim of the study was to evaluate the effect of the supply of a nucleus based on chelated minerals and calcium salts to dual-purpose cows, on milk production, body condition, and ethological behavior.

MATERIALS AND METHODS

The study was carried out in the "San Isidro" Livestock Production Unit, municipality of Villaflores, Chiapas, located at 16° 14' 31.03" latitude North and 93° 13' 41.10" longitude West, at an altitude of 540 masl with an average temperature of 26 °C and mean annual precipitation of 813 mm; and in the Agriculture and Livestock Health Laboratory of the "San Ramón" CUTT of the Agronomic Sciences School Campus V of Universidad Autónoma de Chiapas.

In the study, 12 second-birth F1 cows (Swizz×Zebu) were used in the first stage of lactation; six cows per treatment with average weight of 470±5 kg of LW. Feeding of control cows was rotational grazing in African Star grass (*Cynodon nlemfuensis*; Vanderyst) pasturelands, and 4.0 kg of concentrated feed were allotted on mornings at the time of milking (Table 1); in T2, 300 g cow^{-1} day^{-1} were allotted in addition from the nucleus based on chelated minerals and calcium salts (Table 1).

Preparation of the nucleus based on chelated minerals and calcium salts

To obtain calcium salts, the process of saponification of African palm oil (*Elaeis guineensis* Jacq.) was carried out, which consisted in taking the oil to 80 °C to add the sodium hydroxide, and in a second step to add the calcium chloride to obtain calcium soap or calcium salt, and then mix with the chelated microminerals that were purchased from a commercial house[¥].

Management of cows and data collection

The cows had an adaptation period of eight days, followed by 32 days for data collection. Milking started at 6:00 hours, the livestock entered the waiting hall, and then went into individual pens, where they were given 4.0 kg of concentrated feed (Table 1). Milking was done manually, with pre-sealed management and disinfection of each the cow's nipples,

Table 1. Proportion of ingredients and chemical composition of concentrated feed and the nucleus.

Variable	**Balanced meal**	**Mineral base**
Ingredient	**Proportion (%)**	
Wheat bran	24.0	0.0
Ground corn	44.5	0.0
Palm kernel cake	16.0	0.0
Soybean cake	12.0	0.0
Urea	1.5	0.0
Micro mineral mix	2.0	0.0
Vehicle (inert material)	0.0	23.0
Macro mineral mix	0.0	45.0
Biuret	0.0	7.0
Chelated micromineral[¥]	0.0	1.0
Calcium salts	0.0	24.0
Chemical composition		
Dry matter	88.90	--
Crude protein	18.40	--
Ether extract	3.14	--
NDF	26.90	--
Calcium	0.7	--
Phosphorus	0.6	--
NLE (Mcal kg^{-1} dry matter)	1.6	--

DM: Dry matter; NLE: Net lactation energy; NDF: Neutral detergent fiber; ND: non-determined. [¥] for every 100 grams, it contains: Selenium, 0.03 g; Zinc, 11.0 g; Chromium, 0.040 g; Cobalt, 0.050 g; Copper, 3.0 g; Iodine 0.20 g; Manganese, 10.0 g; and Iron, 12.0 g.

and then the milking process began, which takes approximately 7 to 10 minutes per cow depending on production. After milking, the cows nursed the calves for 30 minutes, and they were transported to the star grass (*C. nlemfuensis*; Vanderyst) pastureland with availability of clean water, where they remained until the following day.

Evaluated variables

Milk production. At the end of milking, the milk produced by each cow was weighed with an electronic scale with capacity of 100 kg and accuracy of 100 g, with the aim of obtaining the productive data of cows in each treatment, taking daily data during 32 evaluation days.

Body condition. The scale of 1 to 5 from the NIRD (National Institute of Research in Dairying) was used to assess the body condition. The assessment was carried out on days 1, 15 and 30.

Ethological behavior. It was evaluated for 24 days on days 15 and 25 of the experimentation period, on all the cows from each treatment. It consisted in taking the time that each animal devoted to performing the activities of: grazing, milking, moving to pastureland (walking), moving to milking room, movingo to resting pastureland, standing rumination (rest), lying rumination (rest), water consumption, urination, and defecations. The data obtained were added and averaged to determine the time devoted to each activity.

Statistical design and analysis

The experimental design was completely random with two treatments and six repetitions each. The data of milk production, body condition, and ethological behavior were analyzed using the procedure of the general linear model (Proc GLM) of SAS (2011). The means were compared with Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Table 2 shows that milk production was higher in the cows with treatment 2 (with nucleus), during the four periods ($P \leq 0.05$).

The cows that consumed the nucleus (T2) produced 19.94% more milk than cows in the control group. The effect of calcium salts in milk production was reviewed by Moallem *et al.* (2007); they mention that fats protected from ruminal digestion increase milk production in Holstein multiparous cows, due to extraruminal effects and higher energy contribution. Duque *et al.* (2011) indicate that the cows in their first phase of lactation use most of the energy for milk production. The digestibility of palm oil calcium salts is 93 to 96%, they are a source of energy highly used by the ruminant at the level of small intestine, and it can be provided to cows with mean production of 4000 to 5000 liters, in amounts of 300 to 500 g cow^{-1} day^{-1}, during the first third of lactation (SAC Battilana Nutrición., 2019).

The use of chelated minerals in milking cows feed has shown favorable results in production and reproduction, as a result of their functions in the animal's metabolism, improving the assimilation of other nutrients, the functions of the immune system and of reproduction. When chelated minerals are supplied to milking cows, it has been observed that the number of somatic cells in milk decreases, and production and fertility increases (Gómez, 2020).

In studies developed by Gómez and Fernández (2020), Romero (2014), Méndez (2013), and Gagliostro *et al.* (2002), the authors observed increases in milk production when including calcium salts and chelated minerals in the diet. The different studies indicate

Table 2. Effect of the supply of chelated minerals and calcium salts on milk production in post-partum cows.

Treatments	Daily milk production (kg cow^{-1})			
	1*	2*	3*	4*
Without mineral base	9.0b	9.8b	9.9b	9.4b
With mineral base	11.3a	12.2a	11.6a	10.6a
SEM	0.89	0.27	0.42	0.19

*Period of eight days each; a, b: different letters in the same column indicate statistical difference according to Tukey's test ($p < 0.05$); SEM: standard error of the mean.

that during the first 90 days of lactation, it is the best stage to supplement with bypass fat (Romero, 2014).

Body condition

Table 3 presents the mean values obtained for body condition in the three periods analyzed. The body condition was similar at the start and at 15 days of supplementation ($P>0.05$), while at 30 days, it was higher ($P<0.05$) in the cows that consumed the nucleus of calcium salts and chelated minerals; in these animals, it was also seen that the body condition increased from 2.5 to 3.0 during the assessment period. Salgado *et al.* (2008) mention that when supplementing milking cows with calcium salts, more energy was contributed, which is used to recover their body reserves and to improve their productivity. In studies carried out by Castañeda *et al.* (2009), they observed that the body condition of lactating cows improved when they were supplemented with calcium salts. For their part, Tyagi *et al.* (2010) and Aguilar *et al.* (2009) reported that the body condition improved significantly in cows supplemented with calcium salts.

Ethological behavior

Table 4 shows the results obtained from the ethological behavior of each group. There was no difference in the time of grazing ($P>0.05$); the time devoted to standing grazing was

Table 3. Body condition of cows in the first third of lactation.

Treatment	Day 1 of suplementation	15 days of suplementation	30 days of suplementation
Without mineral base	2.50	2.55b	2.80
With mineral base	2.55	2.70a	3.30
SEM	0.057	0.053	0.315

a, b: different letters in the same column indicate statistical difference between treatments according to Tukey's test ($P<0.05$); SEM: standard error of the mean.

Table 4. Ethology of lactating cows with and without supplementation of calcium salts and chelated minerals.

Activity	Time (hours)		SEM
	Whitout mineral base	Whit mineral base	
Grazing	7.57	7.30	0.24
Time of milking	0.06	0.07	0.01
Walk to the pasture	0.10	0.10	0.05
Walk to the parlor	0.07	0.07	0.01
Walk to the pasture rest	0.10	0.10	0.01
Standing rumination	6.18a	4.05b	0.25
Lying rumination	9.58b	11.94a	0.54
Water consumtion	0.15	0.17	0.03
Urinations	0.08	0.09	0.01
Defecations	0.11	0.11	0.01

a, b: different letters in the same column indicate statistical difference between treatments according to Tukey's test ($P<0.05$); SEM: standard error of the mean.

higher in cows that did not consume nucleus ($P \leq 0.05$), while the time of lying rumination was higher in cows that consumed nucleus ($P \leq 0.05$). According to the results obtained, there is a difference of 2.36 more hours of rest in the group of cows that consumed nucleus. The milking cows must rest approximately 10 to 12 h per day. The time that cows devoted to rest is important; the rumination is optimized when a cow is lying down, it produces more saliva, and the risk of ruminal acidosis decreases. Likewise, the blood flow to the udder is higher in a lying cow (around 5 liters min^{-1}) than in a standing cow (around 3 liters min^{-1}); this improves the function of the udder and increases milk production (Temple *et al.*, 2016). In the same sense, Blanco (2019) mentions that the cows that produce most are the ones that rest most (in rest and rumination), because the udder's irrigation improves, there is more rumination time, more salivation, and there is more rest for the feet and relaxation of articulations.

CONCLUSIONS

In the dual-purpose system, milk production increased 19.94% by supplementing lactating cows in the first third of lactation with chelated minerals and calcium salts; during the assessment period, the body condition improved 0.5 units and the time devoted to rumination was 2.36 more hours.

REFERENCES

Aguilar, C., Ku-Vera, J., and Garnsworthy, P. (2009). Effects of bypass fat on energy balance, milk production and reproduction in grazing crossbred cows in the tropics. *Livestock Science* 121: 64-71.

Blanco, I. (2019). Una hora más de descanso de la vaca son dos litros de leche más por ordeño. Campo Galego. Disponible en: https://www.campogalego.es/una-hora-mas-de-descanso-de-la-vaca-son-dos-litros-de-leche-mas-por-ordeno/#comments [Fecha de consulta: 29 de agosto de 2021].

Castañeda, E., Pelton, S.H., Gilbert, R.O., and Butler, W.R. (2009). Effect of peripartum dietary energy supplementation of dairy cows on metabolites, liver function and reproductive variables. *Animal Reproduction Science* 112: 301-315.

Cortés, H. Aguilar, C. Vera, R. (2003). Sistemas bovinos doble propósito en el trópico bajo de Colombia, modelo de simulación. *Archivos de Zootecnia 52*(197): 25-34.

Díaz, A. (2014). Análisis de la situación y tendencia de la producción lechera bovina en el estado de Chiapas 2000-2012. Universidad Autónoma Agraria Antonio Narro. Saltillo, Coahuila, México. Recuperado de http://repositorio.uaaan.mx:8080/xmlui/bitstream/handle/123456789/294/63049%20%20%20DIAZ%20NU%C3%91EZ%2C%20%20ARTEMIO%20%20TESIS.pdf?sequence=1&isAllowed=y

Duque, M., Olivera, M., y Rosero, N. (2011). Metabolismo energético en vacas durante la lactancia temprana y el efecto de la suplementación con grasa protegida. *Revista Colombiana de Ciencias Pecuarias, 24*(1): 74-82. [Fecha de Consulta 20 de Julio de 2021]. ISSN: 0120-0690. Disponible en: https://www.redalyc.org/articulo.oa?id=295022380010

Garmendina, J. (2005). Suplementación estratégica de vacas de doble propósito alrededor del parto. IX seminario de pastos y forrajes 2005, 1(1) 126 pp. http://www.avpa.ula.ve/eventos/ix_seminario_pastosyforraje/Conferencias/C8-JulioGarmendia.pdf

Glagliostro, G. A., Vidaurreta, L. I., Schroeder, G.F., Rodríguez, A., y Gatti, P. (2002). Incrementando los valores basales de ácido linoleico conjugado (CLA) en la grasa butirosa de vacas lecheras en condiciones de pastoreo. *Revista Argentina de Producción Animal. 22*(1): 59-60. http://dx.doi.org/10.4067/S0717-75182009000200006

Gómez, C., Padilla, D., Fernández, M., y Hidalgo, V. (2019). Suplementación nutricional de vacunos en el trópico peruano. Lima, Perú: Universidad Nacional Agraria La Molina. *ResearchGate. 1*(1): 1 https://www.researchgate.net/publication/335541947.

Gómez, C., y Fernández, M. (2020). Minerales para mejorar producción de leche y fertilidad en vacas lecheras. Departamento de Nutrición, Universidad Nacional Agraria La Molina. Disponible en:

https://bmeditores.mx/ganaderia/minerales-para-mejorar-produccion-de-leche-y-fertilidad-en-vacas-lecheras/ [Fecha de consulta: 29 de agosto de 2021].

Lipps, E.M., y Bravo, S. (2016). Importancia de los minerales en la producción bovina. Sitio argentino de producción animal, 1-4. https://www.produccion-animal.com.ar/suplementacion_mineral/272-Importancia_de_los_minerales.pdf

Loera, J., y Banda, J. (2017). Industria lechera en México: parámetros de la producción de leche y abasto del mercado interno. *Revista de investigación Altoandinas, 19*(4), 422 pp. http://dx.doi.org/10.18271/ria.2017.317.

Méndez, M.T. (2013). Desempeño productivo y análisis económico de vacas lecheras primíparas suplementadas con grasa sobre pasante en una ración totalmente mezclada. Escuela Agrícola Panamericana Zamorano.

Moallem, U., Katz, M., Arieli, A., & Lehrer, H. (2007). Effects of peripartum propylene glycol or fats differing in fatty acid profiles on feed intake, production, and plasma metabolites in dairy cows. *J Dairy Sci. 90*(1). 3846-3856. doi:10.3168/jds.2007-0092

Orantes, M. A, Vilaboa, A. J., Ortega, J. E., Córdova, A. V. (2010). Comportamiento de los comercializadores de ganado bovino en la región centro del estado de Chiapas. *Revista Quehacer Científico 1*(9): 51-56.

Pech, M. V., Carvajal, H. M., y Montes, P. R. (2007). Impacto Económico de la mastitis subclínica en hatos bovinos de doble propósito de la zona centro del estado de Yucatán, México. *Tropical and Subtropical Agroecosystems 7*(2): 127-131.fecha de Consulta 14 de agosto de 2021]: https://www.redalyc.org/articulo.oa?id=93970207

SAC Battilana Nutrición. (2019). Uso de grasas protegidas como fuente de energía para vacas lecheras y cálculo de valores energéticos. Actualidad Ganadera. http://www.actualidadganadera.com/battilana/articulos/uso-de-grasas-protegidas-calculo-valores-energeticos.html

Salgado, R., Vergara, O., y Simanca, J. (2008). Relaciones entre peso, condición corporal y producción de leche en vacas del sistema doble propósito. *Rev MVZ Córdoba 13*(2): 1360-1364.

SAS. Statistical Analysis Software. 2011. SAS/STAT. Version 9.3 Edition. Cary (NC): SAS Institute Inc.

Spears, J. W. (2000). Micronutrients and immune function in cattle. *Proceedings of the Nutrition Society. 59*(4): 587-594.

Temple, D., Bargo, F., Mainau, E., Ipharraguerre, I., y Manteca, X. (2016). Conducta de descanso y eficiencia productiva de las vacas de leche - una visión práctica. FAWEC. 15 (1). https://www.fawec.org/media/com_lazypdf/pdf/Ficha_Tecnica_ FAWEC15 _n15_eficiencia_confort_es.pdf.

Tyagi, N., Thakur, S., and Shelke, S. (2010). Effect of bypass fat supplementation on productive and reproductive performance in crossbred cows. *Trop Anim Health Prod.* 42: 1749-1755. comments [Fecha de consulta: 29 de agosto de 2020]. Recuperado de: http://www.springerlink.com/content/5589422vqw8nl176/

Materials and methods for the microencapsulation of substances of food and agricultural interest

Ordóñez-García, Magali[1]; Morales-Ovando, Mario A.[2]; Villanueva-Romero, Laura G.[1]; García-Cano, Verónica G.[1]; Sánchez-Ortíz, Olalla[1]; Bustillos-Rodríguez, Juan C.[1*]

[1] Tecnológico Nacional de México, Instituto Tecnológico de Cd. Cuauhtémoc, Cuauhtémoc, Chihuahua, México, C.P. 31500.

[2] Universidad de Ciencias y Artes de Chiapas, Facultad Ciencias de la Nutrición y Alimentos, Sede Acapetahua, Acapetahua, Chiapas, México, C.P. 30580

* Correspondence: jbustillos@itcdcuauhtemoc.edu.mx

ABSTRACT

Microencapsulation involves trapping solid, liquid, or gas particles within an inert cover to protect them from the environment. This technique has numerous biotechnological applications in the food, agricultural, pharmaceutical, and other industries. Microcapsules are relevant for obtaining viable products and optimizing their efficacy, stability, safety, and ease of application. There is a wide range of coating materials and techniques used to microencapsulate various substances of interest. This article presents a review of encapsulating materials and microencapsulation methods used in the food and agricultural industries.

Keywords: Biotechnology, Cover, Microcapsule, Polymers, Protection.

Citation: Ordóñez-García, M., Morales-Ovando, M. A., Villanueva-Romero, L. G., García-Cano, V. G., Sánchez-Ortíz, O., & Bustillos-Rodríguez, J. C. (2024). Materials and methods for the microencapsulation of substances of food and agricultural interest. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2575

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: April 21, 2023.

Accepted: January 16, 2024.

Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 19-25.

INTRODUCTION

Microencapsulation is a method or technique that involves trapping solid particles, liquid droplets, or gases within an inert cover. Its aim is to protect substances from surrounding environments that inactivate them (Jyothi *et al.*, 2010; Nazzaro *et al.*, 2012; Solanki *et al.*, 2013). Microencapsulation originated in the late 1930s and has since had numerous applications in the food, agricultural, and pharmaceutical industries (Agnihotri *et al.*, 2012; Teixeira *et al.*, 2014). The microencapsulated substance can be called core material, substance of interest, active agent, or internal phase, while the substance that surrounds or covers it can be called coating, membrane, envelope, carrier material, wall material, external phase, or matrix. This coating forms a microcapsule (Figure 1), defined as a spherical particle with a size ranging from 1 to 1,000 μm (Singh *et al.*, 2010; Zuidam and Shimoni, 2010). Numerous materials have been reported as potential encapsulants. Overall, these materials maintain the integrity of the core, *i.e.*, they must form a barrier between the internal phase and its environment and be biodegradable. Polysaccharides are among the most used materials for food microencapsulation.

However, proteins and lipids are also appropriate for this application (Nedovic *et al.*, 2011; Ocampo-Salinas *et al.*, 2020). Aside from materials, different microencapsulation methods have been developed, *e.g.*, coacervation, solvent evaporation, thermal gelation, polymerization, spray drying, cold drying (lyophilization), extrusion, emulsification, and others. To decide which method is most appropriate, one must search for the most suitable formulation depending on the active ingredients (Jyothi *et al.*, 2010; Vemmer and Patel, 2013). Nevertheless, the efficiency of encapsulation —*i.e.*, the generation of microcapsules— will depend on many factors, such as the concentration of the wall material and the substance of interest, the rate of solvent removal, or the affinity between the internal phase and the coating (Jyothi *et al.*, 2010). Therefore, it is necessary to conduct research focused on studying the interaction and optimization of cover materials and substances of interest, as well as encapsulation methods, for their potentially better application in various industrial activities.

MATERIALS AND METHODS

Terms of interest were identified in the literature relevant to the topic. The terms were subsequently combined, and a search was conducted, both in English and Spanish, in the following scientific databases: Google Scholar, ScienceDirect, SciELO, Dialnet, MDPI, and ResearchGate. The keywords used were: “materials”, “methods”, “encapsulation”, “agriculture”, “food”, and “application”. After reviewing and analyzing summaries and keywords in the papers found, we were able to select the most significant ones to evaluate them. A selection of papers by section was conducted to determine the materials and methods used in the microencapsulation of substances of food and agricultural interest.

RESULTS AND DISCUSSION

The literature search suggests that a wide range of coating materials have been used to microencapsulate active ingredients in both the agricultural and food sectors. Most of these coating materials are biocompatible and have been approved as safe for humans (Escobar-Avello *et al.*, 2021). The selection of coating materials determines the physicochemical and functional properties of microcapsules. Among the most used materials we find a wide array of natural and synthetic polymers, including carbohydrates (starch, modified starches, dextrins, sucrose, cellulose, and chitosan); gums (gum arabic, alginate, xanthan, and carrageenan); lipids (wax, paraffin, monoglycerides and diglycerides, hydrogenated oils and fats); inorganic materials (calcium sulfate and silicates); and proteins (gluten, casein, gelatin, and albumin). These can be used alone or combined for microencapsulation (Agnihotri *et al.*, 2012; Dias *et al.*, 2017; Teixeira *et al.*, 2014). Coating materials must form a cohesive film or coating for the core material, be compatible and non-reactive with it, and provide the desired coating properties, such as strength, flexibility, impermeability, optical properties, and stability, to ensure the quality of the product (Bansode *et al.*, 2010; Ocampo-Salinas *et al.*, 2020).

Main applications of microencapsulation

Food

Microencapsulation in the food industry can offer numerous benefits by changing or improving the properties of active materials (Jafari *et al.*, 2008a). In addition to protecting sensitive food components, microencapsulation prevents nutritional loss, allows the use of sensitive ingredients and the incorporation of release or unusual mechanisms in the formulation, masks or preserves flavors and aromas, and transforms liquid ingredients into easy-to-handle solids (Desai and Park, 2005). The application of this technique in the food industry is broad. It is mainly used on different active compounds, such as aromas, vitamins, and antioxidants, which, in turn, are used in various powdered food products (soups, instant drinks, and sauces) and food supplements (Turchiuli *et al.*, 2005). When applying microencapsulation to food, it is essential to select the appropriate coating material, for which several requirements must be met, such as good emulsifying properties, good drying properties, non-hygroscopic character, mild flavor, non-reactivity, and low cost, in addition to being hypoallergenic, generally recognized as safe (GRAS), and affordable (Ocampo-Salinas *et al.*, 2020). Table 1 presents some examples of microencapsulated foods.

Table 1. Microencapsulated food products.

Active material (Core)	Coating material	Method used	Author (year)
Orange oil	Lactose and sodium caseinate	Emulsification and spray drying	Edris and Bergnstahl (2001)
Food flavors	Dextrin	Spray drying	Reineccius *et al.*, (2002)
Food flavors	Arabic gum, maltodextrin, modified starch and water-soluble soy protein	Spray drying	Soottitantawat *et al.*, (2003)
Food flavors	Maltodextrin	Spray drying	Shiga *et al.*, (2004)
Vegetal oil	Maltodextrin and Arabic gum	Emulsification, spray drying and agglomeration	Turchiuli *et al.*, (2005)
Vegetal oil	Maltodextrin and Arabic gum	Spray drying	Fuchs *et al.*, (2006)
Fish oil	Maltodextrin, modified starch and whey protein	Spray drying	Jafari *et al.*, (2008)
Fish oil	Chitosan, maltodextrin and whey protein	Ultrasonic atomization, emulsification and cold spray	Klaypradit and Huang (2008)
Food flavors	Arabic gum, modified starch, sodium caseinate, and whey and milk proteins	Spray drying	Charve and Reineccius (2009)
Caffeine	Sodium alginate	Emulsification	Cangueiro (2011)
Flaxseed oil	Maltodextrin, gum Arabic and modified starch	Spray drying	Carneiro *et al.*, (2013)
Oregano oil	Chitosan	Emulsification and ionic gelation	Hosseini *et al.*, (2013)
Vitamins (α-tocopherol and ascorbic acid)	Native and modified soy protein	Spray drying	Nesterenko *et al.*, (2014)
Vitamin E	Whey protein and Arabic gum	Emulsification	Ozturk *et al.*, (2014)
Rice bran oil	Tapioca starch and soy protein	Spray drying	Murali *et al.*, (2016)
Sunflower oil	Sodium alginate and calcium chloride	Emulsification	Martins *et al.*, (2017)
Caramel flavoring	Maltodextrin and medium chain triglycerides	Emulsification and Spray drying	Kim *et al.*, (2019)
Vanilla extract	Rice starch	Spray drying	Ocampo-Salinas *et al.*, (2020)
Grape juice	Hydroxypropyl-beta-cyclodextrin (HP-~CD) and maltodextrin	Spray drying	Escobar-Avello *et al.*, (2021)

Agricultural products

Agrochemical markets differ depending on the regulations of each country. However, the sectors involved (governments, production companies, and environmental groups) have set out initiatives for the use of more environmentally friendly products in crops so that food grown in the countryside does not contain agrochemical residues (insecticides, fungicides, herbicides, nematicides, and other chemical products), which has also bolstered the use of agricultural bio-input agents (John *et al.*, 2011; Przyklenk *et al.*, 2017). Microencapsulation can improve or confer several properties in biofertilizers and biopesticides (bacteria, fungi, viruses, and nematodes). This method stands out for its easy handling and application, providing protection to microorganisms against climatic factors, allowing a controlled release, and bestowing the biological agent with greater effectiveness in cultivation, in addition to facilitating long-term storage (Azaroual *et al.*, 2021; Patel *et al.*, 2005). These characteristics also reduce phytotoxicity and loss of soil nutrients. Moreover, microencapsulation reduces application costs by lowering the number of applications and doses (Tomaszewska and Jarosiewicz, 2006). Table 2 lists some examples of microencapsulated agricultural products.

Table 2. Microencapsulated agricultural products.

Active material (Core)	Coating material	Method used	Author (year)
Azospirillum brasilense	Sodium alginate and skim milk powder	Ionic gelation	Bashan *et al.*, (2002)
*Af*NPV	Corn flour, lignin and sugar	Spray drying	Tamez-Guerra *et al.*, (2002)
Bacillus thuringiensis (Berliner)	Gelatinized tapioca starch, sugar, milk powder, rice bran oil and polyvinyl alcohol	Spray drying	Teera-Arunsiri *et al.*, (2003)
Beauveria brongniartii (Saccardo)	Skimmed milk powder and polyvinylpyrrolidone	Spray drying	Horaczek and Viernstein (2004)
*Cp*GV	Lignin	Spray drying	Arthurs *et al.*, (2006)
NPK Fertilizer 6-20-30	Polysulfone	Spray drying	Tomaszewska and Jarosiewicz (2006)
Soluble granular fertilizer	Soluble starch and polyvinyl alcohol	Ionic gelation	Han *et al.*, (2009)
Beauveria bassiana (Balsamo) Vuillemin	Hydroxy-methyl-cellulose, chitosan, dextrin and skimmed milk powder	Spray drying	Liu and Liu (2009)
Trichoderma harzianum (Rifai)	Sucrose, molasses and glycerol	Spray drying	Jin and Custis (2011)
Heterorhabditis bacteriophora (Poniar)	Sodium alginate	Ionic gelation	Hiltpold *et al.*, (2012)
Pyrethrins	Lignin and calcium lignosulfonate	Spray drying and ionic gelation	Fernández-Pérez *et al.*, (2014)
Bacillus thuringiensis (Berliner)	Amaranth starch	Spray drying	Rodríguez-García *et al.*, (2015)
Metarhizium brunneum (Petch)	Sodium alginate, carboxymethyl cellulose, corn starch and potato starch	Ionic gelation	Przyklenk *et al.*, (2017)
Bacillus sp.	Maltodextrin, sodium alginate and phosphate	Spray drying	Azaroual *et al.*, (2021)

REFERENCES

Agnihotri, N., Mishra, R., Goda, C., y Arora, M. (2012). Microencapsulation–a novel approach in drug delivery: a review. *Indo Global Journal of Pharmaceutical Sciences, 2*(1), 1-20. https://doi.org/10.5958/2231-5659.2020.00009.0

Arthurs, S., Lacey, L., y Behle, R. (2006). Evaluation of spray-dried lignin-based formulations and adjuvants as solar protectants for the granulovirus of the codling moth, *Cydia pomonella* (L). *Journal of Invertebrate Pathology, 93*(2), 88-95. https://doi.org/10.1016/j.jip.2006.04.008

Azaroual, S. E., El Mernissi, N., Zeroual, Y., Bouizgarne, B., y Meftah Kadmiri, I. (2021). Effect of *Bacillus* spp. strains on wheat nutrient assimilation and bioformulation by new spray drying approach using natural phosphate powder. *Drying technology*, 1-15. https://doi.org/10.1080/07373937.2021.1950170

Bansode, S., Banarjee, S., Gaikwad, D., Jadhav, S., y Thorat, R. (2010). Microencapsulation: a review. *International Journal of Pharmaceutical Sciences Review and Research, 1*(2), 38-43.

Bashan, Y., Hernandez, J.-P., Leyva, L. A., y Bacilio, M. (2002). Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. *Biology and Fertility of Soils, 35*(5), 359-368. https://doi.org/10.1007/s00374-002-0481-5

Cangueiro, M. (2011). *In vitro* transdermal delivery of caffeine-loaded alginate particles. Paper presented at the I National Symposium on Nanoscience and Biomedical Nanotechnology, Lusophone University of Humanities and Technology, Lisboa, Portugal.

Carneiro, H. C., Tonon, R. V., Grosso, C. R., y Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering, 115*(4), 443-451. https://doi.org/10.1016/j.jfoodeng.2012.03.033

Charve, J. p., y Reineccius, G. A. (2009). Encapsulation performance of proteins and traditional materials for spray dried flavors. *Journal of Agricultural and Food Chemistry, 57*(6), 2486-2492. https://doi.org/10.1021/jf803365t

Desai, K., y Park, H. (2005). Recent developments in microencapsulation of food ingredients. *Drying Technology, 23*(7), 1361-1394. https://doi.org/10.1081/DRT-200063478

Dias, D. R., Botrel, D. A., Fernandes, R. V. D. B., y Borges, S. V. (2017). Encapsulation as a tool for bioprocessing of functional foods. *Current Opinion in Food Science*, 13, 31-37. https://doi.org/10.1016/j.cofs.2017.02.001

Edris, A., y Bergnstahl, B. (2001). Encapsulation of orange oil in a spray dried double emulsion. *Nahrung/Food, 45*(2), 133-137. https://doi.org/10.1002/1521-3803(20010401)45:2<133::AID-FOOD133>3.0.CO;2-C

Escobar-Avello, D., Avendaño-Godoy, J., Santos, J., Lozano-Castellón, J., Mardones, C., von Baer, D. (2021). Encapsulation of phenolic compounds from a grape cane pilot-plant extract in hydroxypropyl beta-cyclodextrin and maltodextrin by spray drying. *Antioxidants, 10*(7), 1130. https://doi.org/10.3390/antiox10071130

Fernández-Pérez, M., Flores-Céspedes, F., Daza-Fernández, I., Vidal-Peña, F., y Villafranca-Sánchez, M. (2014). Lignin and lignosulfonate-based formulations to protect pyrethrins against photodegradation and volatilization. *Industrial & Engineering Chemistry Research, 53*(35), 13557-13564. https://doi.org/10.1021/ie500186e

Fuchs, M., Turchiuli, C., Bohin, M., Cuvelier, M., Ordonnaud, C., Peyrat-Maillard, M., *et al.* (2006). Encapsulation of oil in powder using spray drying and fluidised bed agglomeration. *Journal of Food Engineering, 75*(1), 27-35. https://doi.org/10.1016/j.jfoodeng.2005.03.047

Han, X., Chen, S., y Hu, X. (2009). Controlled-release fertilizer encapsulated by starch/polyvinyl alcohol coating. *Desalination, 240*(1-3), 21-26. https://doi.org/10.1016/j.desal.2008.01.047

Hiltpold, I., Hibbard, B. E., French, B. W., y Turlings, T. C. (2012). Capsules containing entomopathogenic nematodes as a trojan horse approach to control the western corn rootworm. *Plant and Soil, 358*(1-2), 11-25. https://doi.org/10.1007/s11104-012-1253-0

Horaczek, A., y Viernstein, H. (2004). *Beauveria brongniartii* subjected to spray-drying in a composite carrier matrix system. *Journal of Microencapsulation, 21*(3), 317-330. https://doi.org/10.1080/02652040410001673892

Hosseini, S. F., Zandi, M., Rezaei, M., y Farahmandghavi, F. (2013). Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and *in vitro* release study. *Carbohydrate Polymers, 95*(1), 50-56. https://doi.org/10.1016/j.carbpol.2013.02.031

Jafari, S. M., Assadpoor, E., Bhandari, B., y He, Y. (2008). Nano-particle encapsulation of fish oil by spray drying. *Food Research International, 41*(2), 172-183. https://doi.org/10.1016/j.foodres.2007.11.002

Jin, X., y Custis, D. (2011). Microencapsulating aerial conidia of *Trichoderma harzianum* through spray drying at elevated temperatures. *Biological Control, 56*(2), 202-208. https://doi.org/10.1016/j.biocontrol.2010.11.008

John, R. P., Tyagi, R., Brar, S., Surampalli, R., y Prévost, D. (2011). Bio-encapsulation of microbial cells for targeted agricultural delivery. *Critical Reviews in Biotechnology, 31*(3), 211-226. https://doi.org/10.3109/07388551.2010.513327

Jyothi, N. V. N., Prasanna, P. M., Sakarkar, S. N., Prabha, K. S., Ramaiah, P. S., y Srawan, G. (2010). Microencapsulation techniques, factors influencing encapsulation efficiency. *Journal of Microencapsulation, 27*(3), 187-197. https://doi.org/10.3109/02652040903131301

Kim, G.-Y., Lee, J., Lim, S., Kang, H., Ahn, S.-I., Jhoo, J.-W., et al. (2019). Microencapsulation of caramel flavor and properties of ready-to-drink milk beverages supplemented with coffee containing these microcapsules. *Food Science of Animal Resources, 39*(5), 780. https://doi: 10.5851/kosfa.2019.e68

Klaypradit, W., y Huang, Y.-W. (2008). Fish oil encapsulation with chitosan using ultrasonic atomizer. *LWT-Food Science and Technology, 41*(6), 1133-1139. https://doi.org/10.1016/j.lwt.2007.06.014

Liu, C.-P., y Liu, S.-D. (2009). Low-temperature spray drying for the microencapsulation of the fungus *Beauveria bassiana*. *Drying Technology, 27*(6), 747-753. https://doi.org/10.1080/07373930902828005

Martins, E., Poncelet, D., y Renard, D. (2017). A novel method of oil encapsulation in core-shell alginate microcapsules by dispersion-inverse gelation technique. *Reactive and Functional Polymers*, 114, 49-57. https://doi.org/10.1016/j.reactfunctpolym.2017.03.006

Murali, S., Kar, A., Patel, A. S., Kumar, J., Mohapatra, D., y Dash, S. K. (2016). Encapsulation of rice bran oil in tapioca starch-soya protein isolate complex using spray drying. *Indian Journal of Agricultural Sciences, 86*(8), 984-991. http://krishi.icar.gov.in/jspui/handle/123456789/14672

Nazzaro, F., Orlando, P., Fratianni, F., y Coppola, R. (2012). Microencapsulation in food science and biotechnology. *Current Opinion in Biotechnology, 23*(2), 182-186. https://doi.org/10.1016/j.copbio.2011.10.001

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., y Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, 1, 1806-1815. https://doi.org/10.1016/j.profoo.2011.09.265

Nesterenko, A., Alric, I., Silvestre, F., y Durrieu, V. (2014). Comparative study of encapsulation of vitamins with native and modified soy protein. *Food Hydrocolloids*, 38, 172-179. https://doi.org/10.1016/j.foodhyd.2013.12.011

Ocamp-Salinas, I. O., Gómez-Aldapa, C. A., Castr-Rosas, J., Varga-León, E. A., Guzmán-Ortiz, F. A., Calcáneo-Martínez, N., *et al.* (2020). Development of wall material for the microencapsulation of natural vanilla extract by spray drying. *Cereal Chemistry, 97*(3), 555-565. https://doi.org/10.1002/cche.10269

Ozturk, B., Argin, S., Ozilgen, M., y McClements, D. J. (2014). Formation and stabilization of nanoemulsion-based vitamin E delivery systems using natural surfactants: Quillaja saponin and lecithin. *Journal of Food Engineering*, 142, 57-63. https://doi.org/10.1016/j.jfoodeng.2014.06.015

Patel, A., Slaats, B., Hallmann, J., Tilcher, R., Beitzen-Heineke, W., y Vorlop, K. (2005). Encapsulation and application of bacterial antagonists and a nematophagous fungus for biological pest control. *Gesunde Pflanzen, 57*(1), 30-33. https://doi.org/10.1007/s10343-004-0061-8

Przyklenk, M., Vemmer, M., Hanitzsch, M., y Patel, A. (2017). A bioencapsulation and drying method increases shelf life and efficacy of *Metarhizium brunneum* conidia. *Journal of Microencapsulation, 34*(5), 498-512. https://doi.org/10.1080/02652048.2017.1354941

Reineccius, T., Reineccius, G., y Peppard, T. (2002). Encapsulation of flavors using cyclodextrins: comparison of flavor retention in alpha, beta, and gamma types. *Journal of Food Science, 67*(9), 3271-3279. https://doi.org/10.1111/j.1365-2621.2002.tb09577.x

Rodríguez-García, A. P., Martínez, M. G., Barrera-Cortés, J., Ibarra, J. E., y Bustos, F. M. (2015). Bio-insecticide *Bacillus thuringiensis* spores encapsulated with amaranth derivatized starches: studies on the propagation "*in vitro*". *Bioprocess and Biosystems Engineering, 38*(2), 329-339. https://doi.org/10.1007/s00449-014-1273-7

Shiga, H., Yoshii, H., Ohe, H., Yasuda, M., Furuta, T., Kuwahara, H., *et al.* (2004). Encapsulation of shiitake (*Lenthinus edodes*) flavors by spray drying. *Bioscience, Biotechnology, and Biochemistry, 68*(1), 66-71. https://doi.org/10.1271/bbb.68.66

Singh, M., Hemant, K., Ram, M., y Shivakumar, H. (2010). Microencapsulation: A promising technique for controlled drug delivery. *Research in Pharmaceutical Sciences, 5*(2), 65. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3093624/

Solanki, H. K., Pawar, D. D., Shah, D. A., Prajapati, V. D., Jani, G. K., Mulla, A. M., *et al.* (2013). Development of microencapsulation delivery system for long-term preservation of probiotics as biotherapeutics agent. *BioMed Research International*, 2013. https://doi.org/10.1155/2013/620719

Soottitantawat, A., Yoshii, H., Furuta, T., Ohkawara, M., y Linko, P. (2003). Microencapsulation by spray drying: influence of emulsion size on the retention of volatile compounds. *Journal of Food Science, 68*(7), 2256-2262. https://doi.org/10.1111/j.1365-2621.2003.tb05756.x

Tamez-Guerra, P., McGuire, M. R., Behle, R. W., Shasha, B. S., y Pingel, R. L. (2002). Storage stability of *Anagrapha falcifera* nucleopolyhedrovirus in spray-dried formulations. *Journal of Invertebrate Pathology, 79*(1), 7-16. https://doi.org/10.1016/S0022-2011(02)00005-8

Teera-Arunsiri, A., Suphantharika, M., y Ketunuti, U. (2003). Preparation of spray-dried wettable powder formulations of *Bacillus thuringiensis*-based biopesticides. *Journal of Economic Entomology, 96*(2), 292-299. https://doi.org/10.1093/jee/96.2.292

Teixeira, P., Martins Fries, L. L., de Menezes, C. R., Tasch Holkem, A., Schwan, C. L., Francine Wigmann, É., et al. (2014). Microencapsulation: concepts, mechanisms, methods and some applications in food technology. *Ciencia Rural, 44*(7), 1304-1311. https://doi.org/10.1590/0103-8478cr20130971

Tomaszewska, M., y Jarosiewicz, A. (2006). Encapsulation of mineral fertilizer by polysulfone using a spraying method. *Desalination, 198*(1-3), 346-352. https://doi.org/10.1016/j.desal.2006.01.032

Turchiuli, C., Fuchs, M., Bohin, M., Cuvelier, M., Ordonnaud, C., Peyrat-Maillard, M., et al. (2005). Oil encapsulation by spray drying and fluidised bed agglomeration. *Innovative Food Science & Emerging Technologies, 6*(1), 29-35. https://doi.org/10.1016/j.ifset.2004.11.005

Vemmer, M., y Patel, A. V. (2013). Review of encapsulation methods suitable for microbial biological control agents. *Biological Control, 67*(3), 380-389. https://doi.org/10.1016/j.biocontrol.2013.09.003

Zuidam, N. J., y Shimoni, E. (2010). Overview of microencapsulates for use in food products or processes and methods to make them. In Encapsulation technologies for active food ingredients and food processing (pp. 3-29): Springer. https://doi.org/10.1007/978-1-4419-1008-0_2

Colegio de Postgraduados

Blood disorders caused by hypophosphatemia in dairy cows

Valdez-Arjona, Laura P.[1]; **Ramírez-Mella, Mónica**[2*]; **Ramírez-Bribiesca, J. Efrén**[1]; **Cruz-Tamayo, Alvar A.**[3]; **Sánchez-Torres Esqueda, M. Teresa**[1]; **Trejo-Téllez, Libia I.**[4]

[1] Colegio de Postgraduados, Campus Montecillo, Posgrado en Recursos Genéticos y Productividad-Ganadería, Texcoco, Estado de México, México, C. P. 56264.
[2] Colegio de Postgraduados, Campus Campeche, CONAHCYT-Posgrado en Bioprospección y Sustentabilidad Agrícola en el Trópico, Sihochac, Champotón, Campeche, México, C. P. 24450.
[3] Universidad Autónoma de Campeche, Facultad de Ciencias Agropecuarias, Escárcega, Campeche, México, C. P. 24350.
[4] Colegio de Postgraduados, Campus Montecillo, Posgrado en Edafología, Texcoco, Estado de México, México, C. P. 56264.
* Correspondence: monicara@colpos.mx

ABSTRACT
Objective: To describe the main blood disorders caused by hypophosphatemia (low P level in the blood) in dairy cows.
Design/Methodology/Approach: Publications about blood disorders caused by hypophosphatemia in dairy cows were analyzed.
Results: In addition to a decrease in milk production and several reproductive and metabolic disorders, hypophosphatemia can cause alterations in blood cells, mainly in erythrocytes, as a consequence of the decrease of the phosphorus (P) needed to generate adenosine triphosphate (ATP) and the alterations of the cell membrane phospholipids.
Study Limitations/Implications: Few studies have described how P affects different blood cells or their components.
Findings/Conclusions: Hypophosphatemia has been associated with structural and functional alterations in blood cells.

Keywords: Hypophosphatemia, dairy cows, phosphorus deficiency, blood cell abnormalities, haemolysis.

Citation: Valdez-Arjona, L. P., Ramírez-Mella, M., Ramírez-Bribiesca, J. E., Cruz-Tamayo, A. A., Sánchez-Torres-Esqueda, M. T., & Trejo-Téllez, L. I. (2024). Blood disorders caused by hypophosphatemia in dairy cows. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2578

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: April 26, 2023.
Accepted: February 28, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 27-33.

INTRODUCTION

Phosphorus (P) is one of the most important minerals for animal health. It fulfills many structural functions in the bones, the cell wall (Morse *et al.*, 1992; Zhang *et al.*, 2017), and in energy-dependent reactions, such as the development of high-energy phosphate ester bonds and the adenosine triphosphate (ATP) (Erickson and Kalscheur, 2020). The P in the cow's saliva influences the ruminal environment and acts as an enzyme cofactor, pH regulator, and medium osmolarity; in addition, it contributes to cellulose digestion and microbial synthesis (Ramírez-Pérez, 2005; Yosathai, 2014). The casein and the fat of the milk include P (Alvarez-Fuentes *et al.*, 2016); its concentration fluctuates between

0.7 and 1.2 g kg^{-1} (NRC, 2021). Therefore, a high milk production requires more P (Grünberg, 2008).

P deficiency in the blood of highly productive dairy cows is associated with reproductive problems and milk production decrease (Yosathai, 2014), as well as mastitis, metritis, and infertility (Soldá *et al.*, 2017). These problems take place during transitional periods (Chiwome *et al.*, 2017). In addition, Eisenberg *et al.* (2019) reported blood disorders caused by hypophosphatemia —including intravascular haemolysis of the red blood cells and cellular immunosuppression—, which have been related to metabolic disorders (Molefe and Mwanza, 2019). Consequently, this review aimed to describe the alterations in blood cells caused by hypophosphatemia in dairy cows.

MATERIALS AND METHODS

The data used in the study were gathered from Scopus, Google Scholar, and NCBI. The keywords used for the search (individually or combined) were: phosphorus, phosphorus deficiency, hypophosphatemia, blood cell disorder, erythrocytes, leucocytes, platelets, haemoglobin, haemolysis, and dairy cows.

Hypophosphatemia

Determining the phosphorus state and the requirements of the body of a cow is fundamental (Sharifi *et al.*, 2007), because inorganic phosphorus (IP) in the plasm is an indicator of its bioavailability in food (Montiel *et al.*, 2007). Recently, NRC (2021) reported that the normal P concentration in the plasm of dairy cows ranges from 1.3 to 2.6 mM (4 to 8 mg dL^{-1}). Therefore, hypophosphatemia takes place when the Pi plasm concentration is <2 mg dL^{-1} (Rahmati *et al.*, 2021). Hypophosphatemia decreases food consumption (Cohrs and Grünberg, 2018), mainly among cows during the transitional period (Ménard and Thompson, 2007; Ramírez-Nava, 2009). This disorder impacts up to 70% of the animals in production units (Kaczmarek *et al.*, 2021). Hypophosphatemia results from a sudden and growing loss of P through the milk (Albornoz *et al.*, 2016). P deficiency in the diet hinders the balance of such loss (Najarnezhad *et al.*, 2016). The clinical signs of low P concentration in the blood include: anorexia, paresis, and haemoglobinuria (Barrios *et al.*, 2010). This disorder is also associated with different pathologies such as Pica, Downer cow syndrome, rickets, osteomalacia (Eisenberg *et al.*, 2014; Soldá *et al.*, 2017; Molefe and Mwanza, 2019), displaced abomasum, abomasal volvulus, hepatic lipidosis, metritis, mastitis (Grünberg *et al.*, 2005; Grünberg, 2008; Ismail *et al.*, 2011; Zhang *et al.*, 2017; Macías *et al.*, 2018), retained placenta, silent heat, irregular estrus, inactive ovary, and delayed sexual maturity (Yosathai, 2014). In addition, it causes metabolic diseases such as milk fever, ketosis, and postpartum haemoglobinuria (Resum *et al.*, 2017). Hypophosphatemia has also been related to both functional and structural cellular alterations, including haemolysis of erythrocytes and cellular immunosuppression (Eisenberg *et al.*, 2019).

Blood disorders caused by hypophosphatemia

Blood disorders caused by hypophosphatemia have been reported in different animal species (pigs, rats, and cattle) and humans (Jubb *et al.*, 1990; Eisenberg *et al.*, 2014;

Najarnezhad *et al.*, 2016); erythrocytes are the most studied blood cells (Nozad *et al.*, 2012; Zhang *et al.*, 2017). Erythrocytes require ATP to control cell volume, keeping their shape and plasticity (Grünberg *et al.*, 2015). Intravascular haemolysis ocurrs when there is a low P concentration in the blood (0.4-1.5 mg dL^{-1}) of post-partum dairy cows. This disorder is caused by the decrease of the glyceraldehyde 3-phosphate dehydrogenase (G-3-PD) enzyme, which plays a key role in glycolysis and ATP formation (Macías *et al.*, 2018). Intravascular haemolysis causes the explosion of erythrocytes by capillary pressure (Brechbühl *et al.*, 2008), as a result of a deficient ATP intracellular concentration, which is fundamental to keep the integrity of the cell membrane (Abramowicz *et al.*, 2022). Erythrocyte haemolysis reduces the count of erythrocytes (RBC), haemoglobin (Hgb), hematocrit (Htc) (Kaczmarek *et al.*, 2021), and platelets (PLT) (Abramowicz *et al.*, 2022). In addition, Brechbühl *et al.* (2008) and Abramowicz *et al.* (2022) reported normocytic normochromic/hypochromic anemia, while Noro and Wittwer (2011) and Zhang *et al.* (2017) mentioned haemoglobinuria. Noro and Wittwer (2011) and Kaczmarek (2021) recorded polychromasia, reticulocytes, anisocytosis, macrocytosis, spherocytes, and Heinz bodies in the erythrocytes.

There is a relationship between P and cell protection, because the membranes are made up of phospholipids (Soldá *et al.*, 2017). Zhang *et al.* (2017) studied the morphofunctional state of erythrocytes of cows under a deficient P diet. They recorded a variation in the phospholipid content (particularly phosphatidylcholine and phosphatidylserine). In addition, they reported a decrease in the antioxidant activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the erythrocytes. The reduction of ATP erythrocyte synthesis is another source of weakness in the membrane. Ultimately, this phenomenon diminishes the cell defenses against oxidative stress (Ok *et al.*, 2009). Hypophosphatemia also causes alterations in immune cells. Eisenberg *et al.* (2014) reported a decrease in the concentration and survival of granulocytes and B lymphocytes in dairy cows. Langova *et al.* (2020) and Libera *et al.* (2021) suggested that, as a result of P deficiency, mastitis or laminitis in dairy cows can be related to a decrease in the phagocytic activity of the granulocytes. Other studies have reported the opposite effect: hypophosphatemia can increase leukocyte and neutrophil concentration, during a haemolytic condition (Brechbühl *et al.*, 2008; Noro and Wittwer, 2011). Table 1 shows the blood disorders and clinical signs recorded among dairy cows fed with a P-deficient diet.

Hypophosphatemia treatment and prevention

As a preventive measure, Noro and Wittwer (2011) recommend an appropriate P content in the diets, to meet the maintenance and milk production requirements, especially during the beginning of the lactation cycle. In addition, hematological analyses, such as hemogram (which considers the RBC, Htc, and Hgb parameters), are key to a timely prevention and detection of P deficiency (Abramowicz *et al.*, 2022). However, reverting the clinical signs of blood cell alterations (particularly in erythrocytes) is fundamental once hypophosphatemia is detected. Different treatments can be used to revert this situation, including intravenous therapies with phosphate salts, monosodium, disodium, and trisodium phosphates, and selenium, copper, manganese, and potassium (Cohrs and Grünberg, 2018; Rahmati *et*

Table 1. Blood disorders and clinical signs among dairy cows fed with a P-deficient diet.

Number of cows evaluated	Days in milk	P in diet (%)	Phosphatemia	Clinical signs	Blood alteration	Reference
72	28 days BC	1-TMR[1] -0.30% P 2-TMR[1] -0.31% P 3-TMR[1] -0.43% P	1-0.9 mmol L^{-1} 2-1.4 mmol L^{-1} 3-1.9 mmol L^{-1}	Haemoglobinuria Pica Syndrome ⇓ MP	Anemia Hypophosphatemia	(Stockdale *et al.*, 2005)
8	100 days	LP-TMR[2] 0.2% P DM AP-TMR[2] + NaH_2PO_4 0.36% P DM HP-TMR[2] + NaH_2PO_4 0.45% P DM	LP-0.07 mmol dL^{-1} AP-1.3 mmol dL^{-1} Hp-1.9 mmol dL^{-1}	No clinical signs	⇓ Gran y BL	(Eisenberg *et al.*, 2014)
10	100 and 200 days	LP-TMR[2] 0.2% P DM AP-TMR[2] + NaH_2PO_4 0.36% P DM HP-TMR[2] + NaH_2PO_4 0.42% P DM	LP-1.5 mg dL^{-1} AP-4.1 mg dL^{-1} HP-5.5 mg dL^{-1}	No clinical signs	Intravascular hemolysis Hypophosphatemia	(Grünberg *et al.*, 2015)
40	90 days	C-TMR[2] + NaH_2PO_4 0.35% P DM LP-TMR 0.03% P DM	C 2.02 mmol L^{-1} LP 0.48 mmol L^{-1}	Muscle weakness or recumbency Jaundice Anorexia ⇓ MP	⇓ RCB y MCH ⇑ BNP, AST y ALT ⇓ SOD y GSH-Px ⇓ PC ⇓ Na^+/K^+-ATPase and Mg^{2+}– ATPase Hyperacute hemolysis Macrocytic anemia	(Zhang *et al.*, 2017)
36	42 days BC and 28 days AC.	AP-TMR[3] NaH_2PO_4 0.44 % P DM LP-TMR[3] 0.20% P DM	AP 5.1 mmol L^{-1} LP 1.4-2.3 mmol L^{-1}	Haemoglobinuria Dystocia Anorexia ⇓ MP	Anemia Intravascular hemolysis.	(Grünberg *et al.*, 2019a)
36	42 days BC and 28 days AC.	AP –TMR[3] + NaH_2PO_4 0.28-0.44 % P of MS LP –TMR[3] 0.15-0.20% P DM	AP 1.7 mmol L^{-1} LP 0.5 mmol L^{-1}	Anorexia Haemoglobinuria Metritis Displaced abomasum	Intravascular hemolysis. Anemia Hepatic lipidosis Hypocalcemia Ketosis	(Grünberg *et al.*, 2019b)
18	42 days BC and 42 days AC.	AP -TMR[2] NaH_2PO_4 0.28-0.44 % P of DM LP –TMR[2] 0.15-0.20% P DM	AP 1.8 mmol L^{-1} LP 0.6 mmol L^{-1}	Hemoglobinuria Anemia	Intravascular hemolysis. ⇓ Gran y BL	(Eisenberg *et al.*, 2019)
30	42 days BC.	AP- TMR[4] 0.35%P DM LP- TMR 0.15% P DM	AP 1.7 mmol L^{-1} LP-1.4 mmol L^{-1}	Recumbency Metritis and endometritis Mastitis Limp Anorexia Haemoglobinuria	Hypophosphatemia Intravascular hemolysis Hypocalcemia	(Wächter *et al.*, 2022)

⇑, Increased; ⇓,decreased; C, control; AC, after calving; BC, before calving; BT, before treatment; AT, after treatment; P, phosphorus; LP, Low phosphorus; AP, adequate phosphorus; HP, high phosphorus; TMR, total mixed ration; DM, dry matter; RBC, red blood cell; MCH, mean corpuscular hemoglobin; Gran, granulocytes; BL, B lymphocytes; BNP, total bilirubin; AST, alanine aminotransferase; ALT, aspartate aminotransferase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; Na^+/K^+−ATPase, sodium-potassium pump; Mg−ATPase; magnesium pump; MP, milk production; NaH_2PO_4, sodium phosphate; TMR: [1] 1 -corn silage, barley grains, canola meal and hay. 2- TMR plus pellets (commercial concentrate), 3 -TMR plus soybean meal. [2] Corn silage, grass seed straw and beet pulp. [3] Corn silage, grass seed straw, beet pulp and soybean meal. [4] Corn silage, beet pulp, hay, straw and concentrate.

al., 2021). In addition, blood transfusions can immediately be carried out to balance the erythrocyte loss caused by haemolysis (Noro and Wittwer, 2011). Other treatments include the oral intake of calcium, magnesium, and iron solutions, mixed with phosphorus salts (Noro and Wittwer, 2011). These treatments can be supplied in the food or water (Rahmati *et al.*, 2021). Antioxidants (such as ascorbic acid) can also be used as a complement, to reduce the oxidative stress caused by the P deficiency in the cells (Soldá *et al.*, 2017).

CONCLUSIONS

Hypophosphatemia causes alterations in the erythrocytes and leucocytes of the blood cells of dairy cows. These alterations are both functional and structural. They impact the RBC, Htc, PLT, and leucocyte hematological parameters. Nevertheless, few studies have evaluated the disorder caused by hypophosphatemia in the different blood cells and their components in dairy cows.

REFERENCES

Abramowicz, B., Kurek, Ł., Lutnicki, K., Abramowicz, B., Biernacka, D., Sahinduran, S., & Urosevic, M. (2022). Changes in red blood cell characteristics over the course of subclinical phosphorus deficiency in dairy cows. *Journal of Elementology, 27*(1), 7-15. https://doi.org/10.5601/jelem.2022.27.1.2232

Albornoz, L., Albornoz, J., Morales, M., & Fidalgo Álvarez, L. (2016). Hipocalcemia puerperal bovina. Revisión. *Veterinaria, 52*(201), 29-39.

Alvarez-Fuentes, G., Appuhamy, J. A. D. R. N., & Kebreab, E. (2016). Prediction of phosphorus output in manure and milk by lactating dairy cows. *Journal of Dairy Science, 99*(1), 771–782. https://doi.org/10.3168/JDS.2015-10092

Barrios, M., Sandoval, E., Camacaro, O., & Borges, J. (2010). Importancia del fósforo en el complejo suelo-animal. *Mundo Pecuario, 6*(2), 151-156.

Brechbühl, M., Meylan, M., Kunz-Kirchhofer, C., Bodmer, M., Michel, A., & Kaufmann, T. (2008). Post-parturient haemoglobinuria in cows kept in the Swiss Alpine region. *Tierarztliche Praxis Ausgabe Grosstiere - Nutztiere, 36*(4), 236-240. https://doi.org/10.1055/s-0037-1621534

Chiwome, B., Kandiwa, E., Mushonga, B., Sajeni, S., & Habarugira, G. (2017). A study of the incidence of milk fever in Jersey and Holstein cows at a dairy farm in Beatrice, Zimbabwe. *Journal of the South African Veterinary Association, 88*(1), a1457. https://doi.org/10.4102/jsava.v88i0.1457

Cohrs, I., & Grünberg, W. (2018). Suitability of oral administration of monosodium phosphate, disodium phosphate, and magnesium phosphate for the rapid correction of hypophosphatemia in cattle. *Journal of Veterinary Internal Medicine, 32*(3), 1253-1258. https://doi.org/10.1111/JVIM.15094

Eisenberg, S., Ravesloot, L., Koets, A., & Grünberg, W. (2014). Influence of feeding a low-phosphorus diet on leucocyte function in dairy cows. *Journal of Dairy Science*, *97*, 5176-5184. https://doi.org/10.3168/jds.2014-8180

Eisenberg, S. W. F., Ravesloot, L., Koets, A. P., & Grünberg, W. (2019). Effect of dietary phosphorus deprivation on leukocyte function in transition cows. *Journal of Dairy Science, 102*(2), 1559-1570. https://doi.org/10.3168/jds.2018-15417

Erickson, P. S., & Kalscheur, K. F. (2020). Nutrition and feeding of dairy cattle. In F. Bazer; G. Cliff y G. Wu (Eds.), *Animal Agriculture Sustainability, Challenges and Innovations* (pp: 157-180). *Academic Press*. https://doi.org/10.1016/B978-0-12-817052-6.00009-4

Grünberg, W., Constable, P., Schröder, U., Staufenbiel, R., Morin, D., & Rohn, M. (2005). Phosphorus homeostasis in dairy cows with abomasal displacement or abomasal volvulus. *Journal of Veterinary Internal Medicine, 19*(6), 894-898. https://doi.org/10.1111/J.1939-1676.2005.TB02784.X

Grünberg, W. (2008). Phosphorus homeostasis in dairy cattle: some answers, more questions. In Proceedings of the 17th Annual *Tri-state Dairy Nutrition Conference*, Fort Wayne, Indiana, USA, 22-23 April, 2008 (pp: 29-35). Ohio State university.

Grünberg, W., Mol, J. A., & Teske, E. (2015). Red blood cell phosphate concentration and osmotic resistance during dietary phosphate depletion in dairy cows. *Journal of Veterinary Internal Medicine, 29*(1), 395-399. https://doi.org/10.1111/JVIM.12497

Grünberg, W., Scherpenisse, P., Cohrs, I., Golbeck, L., Dobbelaar, P., van den Brink, L. M., & Wijnberg, I. D. (2019a). Phosphorus content of muscle tissue and muscle function in dairy cows fed a phosphorus-deficient diet during the transition period. *Journal of Dairy Science, 102*(5), 4072-4093. https://doi.org/10.3168/jds.2018-15727

Grünberg, W., Witte, S., Cohrs, I., Golbeck, L., Brouwers, J. F., Müller, A. E., & Schmicke, M. (2019b). Liver phosphorus content and liver function in states of phosphorus deficiency in transition dairy cows. *PLoS ONE, 14*(7), e0219546. https://doi.org/10.1371/JOURNAL.PONE.0219546

Ismail, Z. A. B., Alzghoul, M. D. B., & Eljarah, A. (2011). Hematology, plasma biochemistry, and urinary excretion of glucose and minerals in dairy cows affected with parturient paresis. *Comparative Clinical Pathology, 20*(6), 631-634. https://doi.org/10.1007/S00580-010-1046-X

Jubb, T. F., Jerrett, I. V., Browning, J. W., & Thomas, K. W. (1990). Haemoglobinuria and hypophosphataemia in postparturient dairy cows without dietary deficiency of phosphorus. *Australian Veterinary Journal, 67*(3), 86-89. https://doi.org/10.1111/J.1751-0813.1990.TB07710.X

Kaczmarek, B. A. L. M. K. (2021). Effect of mineral deficiencies on the red blood cell parameters in cattle. *Medycyna Weterynaryjna – Veterinary Medicine-Science and Practice 77*(10), 480-483. https://doi.org/10.21521/mw.6570

Langova, L., Novotna, I., Nemcova, P., Machacek, M., Havlicek, Z., Zemanova, M., & Chrast, V. (2020). Impact of nutrients on the hoof health in cattle. *Animals, 10*(10), 1-22. https://doi.org/0.3390/ANI10101824

Libera, K., Konieczny, K., Witkowska, K., Żurek, K., Szumacher-Strabel, M., Cieslak, A., & Smulski, S. (2021). The Association between selected dietary minerals and mastitis in dairy cows—A Review. *Animals, 11*(8), 1-13. https://doi.org/10.3390/ANI11082330

Macías, A., Macíó, M., Sticotti, E., Fernández, J., Winter, M., & Magnano, G. (2018). Hemoglobinuria postparto asociada a hipofosfatemia en un tambo bovino en la Provincia de Córdoba. *FAV - UNRC Ab Intus, 1*(1), 88-93.

Ménard, L., & Thompson, A. (2007). Milk fever and alert downer cows: Does hypophosphatemia affect the treatment response? *The Canadian Veterinary Journal, 48*(5), 487-491.

Molefe, K., & Mwanza, M. (2019). Serum biochemistry in cows of different breeds presented with reproductive conditions. *The Onderstepoort Journal of Veterinary Research, 86*(1), 1-7. https://doi.org/10.4102/OJVR.V86I1.1742

Montiel, L., Tremblay, A., Girard, V., & Chorfi, Y. (2007). Preanalytical factors affecting blood inorganic phosphate concentration in dairy cows. *Veterinary Clinical Pathology, 36*(3), 278-280. https://doi.org/10.1111/J.1939-165X.2007.TB00224.X

Morse, D., Head, H. H., Wilcox, C. J., Van Horn, H. H., Hissem, C. D., & Harris, B. (1992). Effects of concentration of dietary phosphorus on amount and route of excretion. *Journal of Dairy Science, 75*(11), 3039-3049. https://doi.org/10.3168/jds.S0022-0302(92)78067-9

Najarnezhad, V., Jalilzadeh-Amin, G., Asri Rezaei, S., & Mohseni, M. (2016). Post parturient hemoglobinuria in a sheep flock. *Journal of the Hellenic Veterinary Medical Society, 67*(4), 265-268. https://doi.org/10.12681/jhvms.15649

Noro, M., & Wittwer Fernando, M. (2011). Hemoglobinuria posparto en vacas de tres rebaños lecheros de la región del Bío-Bío, Chile. *Revista MVZ Córdoba, 16*(3), 2785-2792. https://doi.org/10.21897/RMVZ.279

Nozad, S., Ramin, A.-G., Moghadam, G., Asri-Rezaei, S., Babapour, A., & Ramin, S. (2012). Relationship between blood urea, protein, creatinine, triglycerides and macro-mineral concentrations with the quality and quantity of milk in dairy Holstein cows. *Veterinary Research Forum, 3*(1), 55-59.

NRC. (2021) *Nutrient Requirements of Dairy Cattle*. 8 th revised edition. Whasington DC: National Academy Press. https://doi.org/10.17226/25806.

Ok, M., Guzelbektes, H., Sen, I., Coskun, A., & Ozturk, A. S. (2009). Post-parturient haemoglobinuria in three dairy cows. A case report. *Bulletin of the Veterinary Institute in Pulawy, 53*(3), 421-423.

Rahmati, S., Aziz, A., Tawfeeq, M. M., Zabuli, J., & Nazhat, S. A. (2021). Clinical Features of post-parturient hemoglobinuria in dairy cattle and buffaloes: A Review. *Open Journal of Veterinary Medicine, 11*(04), 143–155. https://doi.org/10.4236/ojvm.2021.114010

Ramírez-Nava, J. (2009). Composición mineral de la leche de vaca: los fosfatos. *Tecnología Láctea Latinoamericana. 57*(1), 47-53.

Ramírez-Pérez, A. M. F. (2005). Requerimiento de fósforo de los microorganismos ruminales: una revisión. *Interciencia, 30*(11), 664-670.

Resum, N. S., Kour, P., Singh, H., & Sharma, N. (2017). Post-Partum Hemoglobinuria (PPH) in Bovine. *Theriogenology Insight - An International Journal of Reproduction in All Animals, 7*(1), 51. https://doi.org/10.5958/2277-3371.2017.00016.x

Sharifi, K., Mohri, M., & Rakhshani, A. (2007). The relationship between blood indicators of phosphorus status in cattle. *Veterinary Clinical Pathology, 36*(4), 354-357. https://doi.org/10.1111/j.1939-165X.2007.tb00440.x

Soldá, N. M., Glombowsky, P., Campigotto, G., Bottari, N. B., Schetinger, M. R. C., Morsch, V. M., Favero, J. F., Baldissera, M. D., Schogor, A. L. B., Barreta, D., Machado, G., & da Silva, A. S. (2017). Injectable mineral supplementation to transition period dairy cows and its effects on animal health. *Comparative Clinical Pathology, 26*(2), 335-342. https://doi.org/10.1007/S00580-016-2378-Y

Stockdale, C.R., Moyes, T.E., & Dyson, R. (2005). Acute post-parturient haemoglobinuria in dairy cows and phosphorus status. *Australian Veterinary Journal, 83*(6), 362-366.

Wächter, S., Cohrs, I., Golbeck, L., Scheu, T., Eder, K., & Grünberg, W. (2022). Effects of restricted dietary phosphorus supply during the dry period on productivity and metabolism in dairy cows. *Journal of Dairy Science, 105*(5), 4370-4392. https://doi.org/10.3168/jds.2021-21246 4370-4392

Yosathai, R. (2014). Importance of minerals on reproduction in dairy cattle. *International Journal of Science, Environment and Technology 3*(6), 2051-2057.

Zhang, Z., Bi, M., Yang, J., Yao, H., Liu, Z. y Xu, S. (2017). Effect of phosphorus deficiency on erythrocytic morphology and function in cows. *Journal of Veterinary Science, 18*(3), 333. https://doi.org/10.4142/JVS.2017.18.3.333

Efficacy of chemical fungicides against the anthracnose disease caused by *Colletotrichum gloeosporioides* in *Carica papaya* fruits

Chan-Cupul, Wilberth; Ruelas-Jurado, Julio; Sánchez-Rangel, Juan Carlos; Buenrostro-Nava, Marco Tulio; Manzo-Sánchez, Gilberto*

Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima, Km. 40 Autopista Colima-Manzanillo, Tecomán, Colima, México. C.P. 28934. Phone/Fax: +52-3133229405 Ext. 52453.

* Correspondence: gmanzo@ucol.mx.

ABSTRACT

Objective: To evaluate the efficacy of chemical fungicides against *Colletotrichum gloeosporioides* in papaya fruits.

Design/methodology/approach: The effect of four chemical fungicides (A+F=azoxystrobin+fludioxinil, C+F=cypronil+fludioxinil, B+P=boscalid+pyraclostrobin and T=tiabendazole) at 250, 500, 750, and 1000 mg kg^{-1} were assessed on severity and the area under the disease progress curve (AUDPC) of *Colletotrichum gloeosporioides* inoculated on papaya fruits.

Results: At 12 days after inoculation (dai) A+F achieved a range of effectiveness between 63.0 (250 mg kg^{-1}) to 77.52 (1000 mg kg^{-1}); while the range of effectiveness for T was 12.8% (250 mg kg^{-1}) to 74% (1000 mg kg^{-1}). Both fungicides achieved the highest effectiveness at 1000 mg kg^{-1} that C+F (38.5%) and B+P (55.6%). The AUDPC achieved the same value at the four studied concentration in A+F, C+F, and B+P. Only 750 and 1000 mg kg^{-1} of T achieved the lowest AUDPC than 250 and 500 mg kg^{-1}.

Findings/conclusions: The fungicides A+F and T achieved adequate control of anthracnose in papaya fruits and the use of the diagrammatic logarithmic scale is easy to use to give a quick estimate of the disease, as well as being easy to reproduce.

Keywords: fungal disease, chemical control, postharvest, severity.

INTRODUCTION

Papaya fruit (*Carica papaya* L.) is the most commercially important species within the Caricaceae family; its production is destined to the national and international markets because its consumption promotes health benefits and due to its exotic nature in some countries (Mailafia *et al.*, 2017). It is a climacteric fruit, whose ripening occurs quickly after its harvest, therefore it is considered as a highly perishable fruit. *C. papaya* is a fruit with a short shelf life and very susceptible to postharvest handling and decay, which causes significant losses in quantity and quality of fruit when handled improperly (Suárez-Quiroz *et al.*, 2013). Main phytopathogenic fungi, which cause postharvest diseases in papaya fruits worldwide, are the genera: *Alternaria*, *Fusarium*, *Ascochyta*, *Rhizopus*, *Mycosphaerella*, *Phomopsis*, *Lasiodiplodia*, *Guignardia*, *Cladosporium*, *Penicillium* and *Colletotrichum* (Chávez-Quintal *et al.*, 2011; Rathod 2012).

Citation: Chan-Cupul, W., Ruelas-Jurado, J., Sánchez-Rangel, J. C., Buenrostro-Nava, M. T., & Manzo-Sánchez, G. (2024). Efficacy of chemical fungicides against the anthracnose disease caused by *Colletotrichum gloeosporioides* in Carica papaya fruits. *Agro Productividad*. https://doi.org/ 10.32854/agrop.v17i4.2583

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: August 12, 2023.
Accepted: March 28, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 35-45.

The anthracnose, which is caused by the micromycete *Colletotrichum gloeosporioides* Penz, is the most important fungal disease in *C. papaya*. It can manifest itself on leaves and petioles, but it is mainly a fruit disease (Molina-Chavez *et al.*, 2017). Symptoms on leaves show as gray to brown spots with darker margins and a yellow surrounding halo. The spots later enlarge and coalesce to form sizable necrotic areas. Small, light-colored spots appear first on the skin of fruits. As they mature, the spots grow considerably in size (up to 5 cm) and become round, dark brown lesions, often with a water-soaked or raised appearance. Pinkish to orange specks grow within the lesions in a concentric manner. Smaller, reddish-brown, sunken spots (up to 2 cm), referred to as "chocolate spots", are also visible. Fruits tend to fall off prematurely. These symptoms might develop after harvest, particularly if the fruits are refrigerated (Maeda and Nelson 2014).

Colletotrichum gloeosporioides has a greater impact on the shelf life or postharvest of papaya fruits (Zavala-León *et al.*, 2005). Losses ranging from 20 to 50% caused by anthracnose and other diseases have been reported; during the period of flowering and berthing of fruit, therefore fungicides of different mode of action must be applied to avoid latent infections and reduce the risk of resistance of the fungus (Ferreira-Demartelaere *et al.*, 2017).

Among the alternatives to control the anthracnose are the use of hot air, hydrothermal treatments (Ayón-Reyna *et al.*, 2017a; Ayón-Reyna *et al.*, 2017b), modified atmospheres, ultraviolet light, plant extracts (*Allium sativum*, *Azadirachta indica*, *Cinnamomun zeylanicum*, *Thymus vulgaris*, and *Ricinus communis*), biological control agents (*Trichoderma* sp. *Gliocladium* sp., *Streptomyces* sp., *Bacillus* spp., and *Coniothyrium* sp.) (Hewajulie and Wilson 2010) and some active ingredients (*a.i.*) of chemical fungicides such as trifloxystrobin, imazalil, benomyl, azoxistrobin and prochloraz (Subramanian *et al.*, 2006; Santamaría-Basulto, 2011).

However, constant use of chemical fungicides causes resistance in the plant phytopathogenic fungi including *C. gloeosporioides* (Rathod, 2012); hence, one strategy to avoid the fungicide resistance is the rotation of the active ingredients, as well as the exploration of the new ingredients available in the market, to find the most effective ones to the phytopathogenic fungi (Salvatore and Ritenour, 2007). In addition, to determine the mean effective concentration to *C. gloeosporioides* under postharvest conditions is needed to the integrated management of fruit diseases. The present study raised the use of some chemical active ingredients of fungicides available in the agricultural market, which poses registration for papaya exportation by the environmental protection agency (EPA) from U.S.A. This study aimed to evaluate the efficacy of chemical fungicides to postharvest control of *C. gloeosporioides* in papaya fruits and to calculate the median effective (EC_{50}) concentration of each molecule.

MATERIALS AND METHODS

Fungal source

A single strain of *C. gloeosporioides* was used for bioassays, this strain was isolated from fruits with anthracnose symptoms. The strain was TSWT, which belongs to the collection of phytopathogenic fungi of the Faculty of Biological and Agricultural Science of the Colima University. Strain was reactivated in potato dextrose agar supplemented with chloramphenicol (100 mg/L, Sigma-Aldrich, U.S.A.) under laboratory conditions (25±3.0 °C, 75±5.0% of relative humidity and 12:12 h light: darkness).

Diagrammatic logarithmic scale

Papaya cv. "Maradol" fruits (1.5 to 2.5 kg with a maturity stage 2) were used according to Santamaría-Basulto *et al.* (2009). Fruits were washed twice with a non-ionic detergent (Extran® 1.0%) and sodium hypochlorite (100 mg kg^{-1}), and then washed with distilled sterile water (Zavala-León and Cristobal-Alejo, 2012). Disinfected fruits were punctured with a sterile needle (0.8 mm) and inoculated with 15 μL of a conidia suspension of *C. gloeosporioides* (1.8×10^6 conidia/mL) using a micropipette. Inoculated fruits were incubated at laboratory conditions (25±3 °C, 80±50% of relative humidity and 12 h light: darkness) for 10 days to permit the fruit infection. After this period, fruits were photographed with a Kodak® Camera at 35 cm between the lent and the papaya fruit. Digital images were processed with the ImagenJ software (Rasband 2014) to measure the infected area (%) by anthracnose. The diagrammatic logarithmic scales with six classes were established to quantify the anthracnose severity caused by *C. gloeosporioides* in papaya fruits. Class 0 corresponded to healthy fruits, by other hand; the class 6 corresponded to an anthracnose percentage of 43.81% (Figure 1).

Chemical fungicides and doses-response bioassays

The fungicides Bankit® Gold (Syngenta), Switch® 62.5 WG (Syngenta), Cabrio® C (BASF) and Tecto® 60 (Syngenta) were used in this study. Each fungicide was evaluated

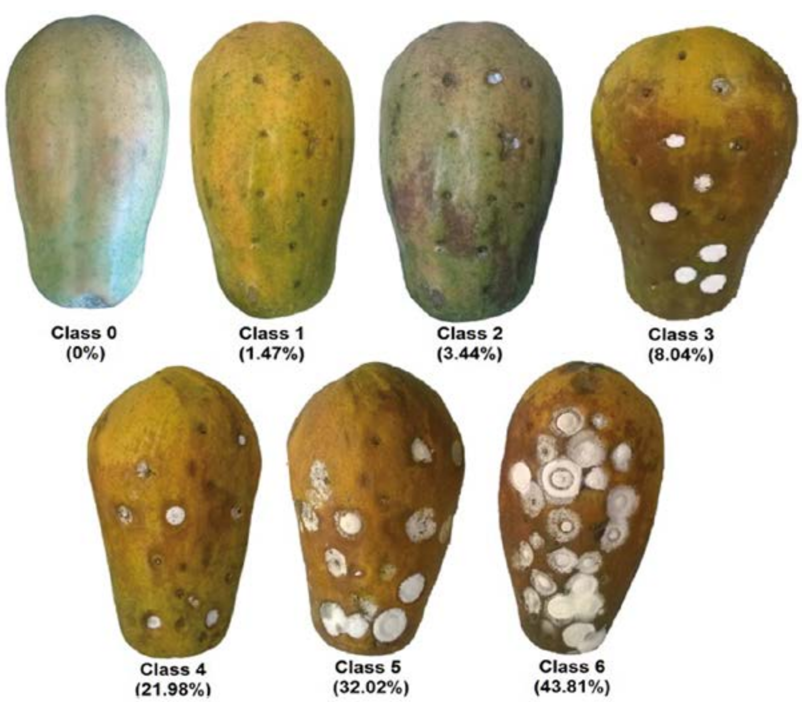


Figure 1. Diagrammatic logarithmic scale to evaluate the severity of anthracnose caused by *Colletotrichum gloeosporioides* in *Carica papaya* fruits.

using four concentrations (250, 500, 750, and 1000 mg kg^{-1}) through a dose-response bioassay. In addition, a control was established using distilled sterilized water. For bioassays, papaya fruits (maturity stage 2) were disinfected as was previously described, and then the fungicide treatments using four concentrations (Table 1) were diluted in distilled water plus 1 mL L^{-1} of a pH buffer (Buffer XS®, Mexico City). Fungicide concentration was applied by soaking the papaya fruits using a contained of 40 L of capacity with 20 L of the fungicides at the evaluated concentration. Fruits were soaked for three minutes and incubated at laboratory conditions. Fruits from the control were soaked only with distilled water with 1 mL/L of a pH buffer. Both the treated fruits with fungicides and the control were inoculated immediately with *C. gloeosporioides* as was mentioned previously (Zavala-León *et al.*, 2005; Santamaría-Basulto *et al.*, 2011). Treated fruits with fungicides and inoculated with *C. gloeosporioides* were incubated at laboratory conditions for 12 days. Response variables were evaluated as follow.

Response variables

Anthracnose severity by *C. gloeosporioides* on fruits was evaluated every four days using the diagrammatic scale, which was designed with six scales (Figure 1). The effectiveness of the fungicides concentrations was determined using the formula:

$$Effectiveness = (control - treatment) / control \times 100$$

The Area Under the Disease Progress Curve (AUDPC) was determined with the formula

Table 1. Chemical fungicides and concentrations.

Active ingredients	Chemical groups	Concentration (mg kg^{-1})	Key
azoxystrobin + fludioxonil	strobilurins fenilpirroles	250	A + F 250
		500	A + F 500
		750	A + F 750
		1000	A + F 1000
cyprodinil fludioxinil	anilinopirimidine fenilpirroles	250	C + F 250
		500	C + F 500
		750	C + F 750
		1000	C + F 1000
boscalid pyraclostrobin	anilidas strobilurins	250	B + P 250
		500	B + P 500
		750	B + P 750
		1000	B + P 1000
tiabendazole	benzimidazol	250	T 250
		500	T 500
		750	T 750
		1000	T 1000

$$AUDPC = \sum_{i=1}^{n}\left[\left(X_{i+1} + X_{i}\right)/2\right]\left[t_{i+1} - t_{i}\right]$$

Where, X_i is the damage ratio of the host tissue i^{th} per day, t_i is the time (days) after the onset disease i^{th} (days) and is the number of observations (Marin-Cortez *et al.*, 2019). The mean effective concentration (EC_{50}) was calculated through a Probit analysis using the fungicide concentration and the effectiveness percentage in the SAS software for windows.

Experimental design and data analysis

Independent experiments which involve the fungicides and concentrations were established through doses response bioassays. The studies concentrations and controls were established with the replicates, each of them with three treated fruits. The response variables: anthracnose severity and AUDPC was analyzed using an analysis of variance and a Tukey ($P \leq 0.05$) multiple comparison procedure. These analyses were performed using StatGraphics Plus for windows. The severity data of *C. gloeosporioides* on inoculated fruits of *C. papaya* did not show homogeneity of variance, therefore, they were transformed to $\sqrt{X+1}$ to develop the ANOVA.

RESULTS AND DISCUSSION

Diagrammatic logarithmic scale

The diagrammatic logarithmic scale generated to quantify the severity of *C. gloeosporioides* in *C. papaya* fruits has seven severity classes, where class 0 corresponded to fruits without damage, on the contrary, class 6 to fruits with the maximum percentage of damage or severity estimated with 43.81% (Figure 1).

Disease severity

Each fungicide was evaluated at four concentrations at the same time. At 4 dai of the concentrations of azoxystrobin+fludioxinil (A+F) achieved a significant difference ($F=25.96$, $P=0.00001$) between the concentrations of the fungicides and the control. However, no differences were found between the concentrations, the lowest severity values were found at 250 (0%), 750 (0%), and 1000 (0%) mg kg^{-1}. On the contrary, the highest severity was found in the control with 0.43% (Table 2). At the second evaluation (8 dai) the A+F concentrations, a significant difference ($F=13.78$, $P=0.0004$) was found for the fungicide concentrations and the control. However, no differences were found between the concentrations, the lowest value was obtained at 500 mg kg^{-1} with 1.01%. On the contrary, the highest severity was found in the control with 9.44% (Table 2). At 12 dai of A+F concentrations significant difference ($F=4.06$, $P=0.0330$) was achieved, the control together with concentrations 250, 750 and 1000 mg kg^{-1} showed the highest severity with 40.0, 14.8, 11.1 and 9.0%, respectively. By the other side, 500 of A+F registered the lowest severity (6.21%) (Table 2).

In the treatment boscalid+pyraclostrobin (B+P), at 4 dai, a significant difference ($F=5.60$, $P=0.125$) in severity was found, the control together with concentrations 750 and 1000 mg kg^{-1} showed the highest severity with 0.43, 0.13 and 0.13%, respectively.

While the concentrations 250 (0.0%) and 500 (0.07%) mg kg^{-1} showed the least severity (Table 2). At 8 dai, B+P treatment achieved a significant difference (F=4.82, P=0.199) in the severity, the control with the concentrations 250 and 1000 mg kg^{-1} showed the highest severity with 9.44, 3.50 and 2.38%, respectively. While the concentrations 500 and 750 mg kg^{-1} showed the lowest severity with 1.67 and 1.44%, respectively (Table 3). At 12 dai, the B+P concentrations did not achieve significant differences (F=25.96, P=0.00001). The lowest severity was found at 500 (10.2%) mg kg^{-1}. On the contrary, the highest severity was found in the control with 40.0% (Table 2).

In the cyprodinil+fludioxinil (C+F) evaluations at 4 (F=2.69, P=0.0931), 8 (F=2.79, P=0.0855) and 12 (F=0.86, P=0.5177) dai, did not achieved significant differences between the concentrations of the fungicides and the control (Table 2).

For the tiabendazole (T) treatment, at 4 dai significant difference was found (F=5.13, P=0.0165) in severity, the control together with concentrations 250 and 500 mg kg^{-1} showed the highest severity with 0.43, 0.25 and 0.31%, respectively. While the concentration of 1000 mg kg^{-1} showed the lowest severity with 0.03% (Table 2). At the second evaluation

Table 2. Anthracnose severity in papaya fruits under the application of fungicides at four concentrations.

Active ingredients	Concentrations (mg kg^{-1})	Days after inoculation		
		4	8	12
A+F	250	0.0 a	1.57 a	14.82 ab
A+F	500	0.05 a	1.01 a	6.21 a
A+F	750	0.0 a	2.07 a	11.10 ab
A+F	1000	0.0 a	1.16 a	9.0 ab
Control	0	0.43 b	9.44 b	40.05 b
B+P	250	0.00 a	3.50 ab	18.20
B+P	500	0.07 a	1.67 a	10.2
B+P	750	0.13 ab	1.44 a	15.5
B+P	1000	0.13 ab	2.38 ab	17.7
Control	0	0.43 b	9.44 b	40.0
C+F	250	0.10	3.91	20.82
C+F	500	0.10	2.81	20.88
C+F	750	0.13	3.28	19.78
C+F	1000	0.09	3.42	24.61
Control	0	0.43	9.44	40.05
T	250	0.25 ab	7.34 bc	34.91 b
T	500	0.31 ab	3.52 ab	26.67 ab
T	750	0.05 a	1.68 a	11.71 a
T	1000	0.03 a	1.30 a	10.36 a
Control	0	0.43 b	9.44 c	40.05 b

Means followed by different letter are significant different from each other according to Tukey test ($P \leq 0.05$). A+F=Azoxystrobin+Fludioxinil, C+F=Cypronil+Fludioxinil, B+P=Boscalid+Pyraclostrobin, and T=Tiabendazole.

(at 8 dai) of T concentrations, a significant difference (F=16.89, P=0.0002) in severity was found, the control together with the 250 mg kg^{-1} concentration showed the highest severity with 9.44 and 7.34%, respectively. While the concentration of 1000 mg kg^{-1} showed the lowest with 0.03% (Table 2). At 12 dai of T, a significant difference (F=7.63, P=0.0044) was found the control and the concentrations of 250 and 500 mg kg^{-1} showed the highest severity with 40.05, 34.91, and 26.67%, respectively. By the other side, the concentration of 1000 mg kg^{-1} showed the lowest severity with 10.36% (Table 2).

Effectiveness

The A+F treatments at 4 dai, achieved a significant difference (F=236.67, P=0.00001) was found between the concentrations of the fungicides and the control. However, no differences were found between the concentrations, the highest effectiveness values were found at 250 (100%), 750 (100%) and 1000 (100%) mg kg^{-1}. On the contrary, the lowest effectiveness was found in the control with 0.0% (Table 3).

At 8 dai, the concentrations of A+F showed a significant difference (F=104.16, P=0.00001). However, no differences were found between the concentrations; the highest effectiveness value was found at 500 mg kg^{-1} with 89.28%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3). At 12 dai of evaluation, A+F concentrations, a highly significant difference (F=32.58, P=0.00001) was found between the concentrations and the control. However, the highest effectiveness value was found at 500 mg kg^{-1} with 84.49%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3).

In the B+P treatment, significant difference (F=14.65, P=0.0003) was found at the 4 dai, the concentrations no differences were found, the highest effectiveness value was found at 250 mg kg^{-1} with 100%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3). At 8 dai of evaluation the B+P achieved significant difference (F=11.57, P=0.0009). However, no differences were found between the concentrations, the highest effectiveness value was found at 750 mg kg^{-1} with 84.7%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3). At 12 dai, the same behavior was found (F=12.75, P=0.0006), no differences were found between the concentrations, the highest effectiveness value was found at 500 mg kg^{-1} with 74.4%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 3).

For C+F treatment, at 4 dai significant difference (F=6.21, P=0.0089) was achieved. The same effectivity was found between the concentrations, the highest effectiveness value was found at 1000 mg kg^{-1} with 78.1%. On the contrary, the lowest effectiveness was found in the control with 0% (Table 8). At 8 (F=7.44, P=0.0048) and 12 (F=4.80, P=0.0203) dai significant difference were found between the concentrations and the control. However, between the concentrations no differences were found, the highest effectiveness value was found at 500 mg kg^{-1} with 70.2% and in 1000 mg kg^{-1} with 38.5% (12 dai). On the contrary, the lowest effectiveness was found in the control with 0% (Table 3).

In the T treatment, significant difference was found at 4 dai (F=7.29, P=0.0051), the control together with concentrations 250 and 500 mg kg^{-1} showed the lowest effectiveness with 0, 42.0, and 26.4%, respectively. While the concentration of 1000 mg kg^{-1} showed the highest effectiveness with 92.9%. At 8 dai, the control and the 250 mg kg^{-1} showed the lowest effectiveness with 0 and 22.2%, respectively (F=7.10, P=0.0056). While the concentration of 1000 mg kg^{-1} showed the highest effectiveness with 86.2% (Table 3). At 12 dai of T concentrations, the control and the 250 mg kg^{-1} application achieved the lowest effectiveness with 0.12.8% each one (F=27.43, P=0.00001). By the other side, the concentration of 1000 mg kg^{-1} showed the highest effectiveness with 74.1% (Table 3).

Area under the disease progress curve (AUDPC)

Figure 2 shows the AUDPC of fungicides on *C. gloeosporioides* in *C. papaya* fruits. For A+F a highly significant difference was found (F=8.71, P=0.0027) between the concentrations and the control. However, the four studied concentrations did no show significant differences. Concentration of 500 mg kg^{-1} achieved lowest value of ABCPE with 14.90. On the contrary, the highest area was found in control 30.15 (Figure 2). In B+P the four concentration reduced significantly (F=4.38, P=0.0265) the ABCPE. The lowest ABCPE was found at the concentration of 500 mg kg^{-1} with 17.11. On the contrary, the

Table 3. Effectiveness of chemical fungicides at four concentrations against anthracnose caused by *Colletotrichum gloeosporioides* in papaya fruits.

Active ingredients	Concentrations (mg kg^{-1})	Days after inoculation		
		4	8	12
A+F	250	100.0 b	83.3 b	63.0 b
A+F	500	83.3 b	89.3 b	84.5 b
A+F	750	100.0 b	79.1 b	72.3 b
A+F	1000	100.0 b	87.7 b	77.5 b
Control	0	0 a	0 a	0 a
B+P	250	100.0 b	62.9 b	54.3 b
B+P	500	82.8 b	82.2 b	74.4 b
B+P	750	69.3 b	84.7 b	61.0 b
B+P	1000	70.3 b	74.7 b	55.6 b
Control	0	0 a	0 a	0 a
C+F	250	77.1 b	58.5 b	48.2 b
C+F	500	76.1 b	70.2 b	47.8 b
C+F	750	68.8 b	65.2 b	50.6 b
C+F	1000	78.1 b	63.8 b	38.5 ab
Control	0	0 a	0	0
T	250	42.0 ab	22.2 b	12.8 b
T	500	26.4 ab	62.7 b	33.4 b
T	750	87.2 b	82.2 b	70.7 b
T	1000	92.9 b	86.2 b	74.1 b
Control	0	0 a	0 a	0 a

Means followed by different letter are significant different from each other according to Tukey test (P≤0.05). A+F=Azoxystrobin+Fludioxinil, C+F=Cypronil+Fludioxinil, B+P=Boscalid+Pyraclostrobin and T=Tiabendazole.

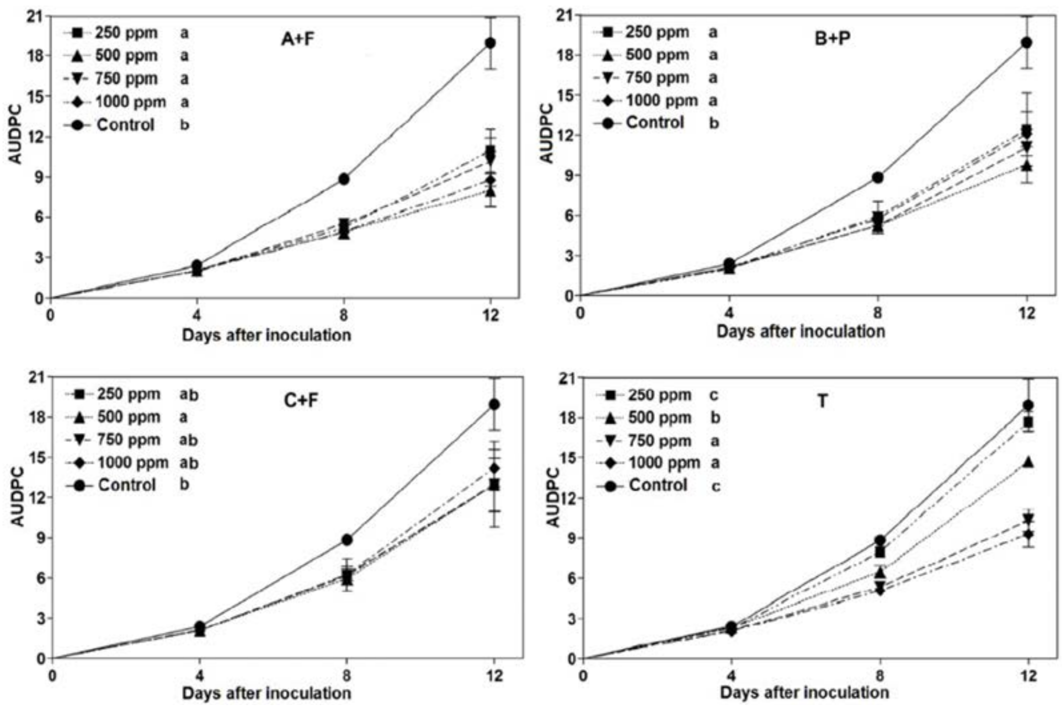


Figure 2. Area under the disease progress curve (AUDPC) of *Colletotrichum gloeosporioides* in *Carica papaya* fruits under different concentrations of chemical fungicides. A+F=Azoxystrobin+Fludioxinil, C+F=Cypronil+Fludioxinil, B+P=Boscalid+Pyraclostrobin and T=Tiabendazole. Means (± standard error, n=3) with different literals are significantly different from each other (Tukey test, P≤0.05).

highest area was found in control 30.15 (Figure 2). For C+F, only the 500 mg kg^{-1} (30.15) archived a low ABCPE regarding to the control (21.0) (Figure 2). Finally, in T highly the concentration of 750 and 1000 mg kg^{-1} (<27.889) achieved lower values of AUDPC in comparison to the control (30.15) (Figure 2).

DISCUSSION

Carica papaya fruits immersed in A+F at 500 mg kg^{-1} at 12 dai showed the least severity (6.21%) and the highest effectiveness (84.49%), these results were similar to the reported by Gutiérrez-Alonso and Gutiérrez-Alonso (2003), Gutiérrez-Alonso *et al.* (2004) and Zavala-León *et al.* (2005), in studies with *Psidium guajava*, *Mangifera indica* and *C. papaya* fruits, respectively, inoculated with *C. gloeosporioides*. A concentration of 500 mg kg^{-1} of A+F was ideal to obtain the highest effectiveness and lowest severity at that concentration.

In another study, López-Navarrete *et al.* (2011), reported that pyraclostrobin solution at 500 mg kg^{-1} in *C. papaya* fruits inoculated with *C. gloeosporioides*, a greater effectiveness is obtained regarding the control (without pyraclostrobin), a similar result was achieved at the present study using B+P at 500 mg kg^{-1} at 12 dai. On the contrary, the results obtained by Pérez-León *et al.* (2015), did no differ from the results obtained at present study, because when application of boscalid+pyraclostrobin immediately after anthracnose inoculation, these authors obtained 100% effectiveness on the disease caused in *Sansevieria trifasciata*.

The low efficacy of tiabendazole to inhibit the growth of *C. gloeosporioides* at 250 and 500 mg kg^{-1} were reported by Suárez-Quiroz *et al.* (2013), mentioning that tiabendazole was not able to inhibit fungal growth of papaya fruit disease such as *Fusarium crookwellense*, *F. oxysporum*, *C. gloeosporioides*, *Mucor hiemalis* and *M. circinel*. The tiabendazole has been reported with low effectivity against *C. gloeosporioides* in *Psidium guajava*. Despite these results, more investigation is needed to find the CL_{50} of the chemical molecules on postharvest diseases in papaya fruits.

CONCLUSION

In conclusion, the application of A+F at 500 mg kg^{-1} has the highest control of anthracnose caused by *C. gloeosporioides* in *C. papaya* fruits under laboratory conditions. In contrast, T at 250 mg kg^{-1} showed reduced sensitivity to anthracnose (*C. gloeosporioides*) with a difference in severity to the control (5.14%), therefore its use is not recommended at that concentration. Finally, the use of the diagrammatic logarithmic scale is easy to use to give a quick estimate of the disease, as well as being easy to reproduce.

REFERENCES

Ayón-Reyna, LE; González-Robles, A; Rendón-Maldonado, JG; Báez-Flores, ME; López-López, ME; Vega-García, M.O. 2017a. Application of hydrothermal-calcium chloride treatment to inhibit postharvest anthracnose development in papaya. *Postharvest Biol. Technol.* 124:85-90. https://doi.org/10.1016/j.postharvbio.2016.10.009

Ayón-Reyna, L.E., López-Valenzuela, J.A., Delgado-Vargas, F., López-López, M.E., Molina-Corral, F.J., Carillo-López, A., Vega-García, M.O. 2017b. Effect of the combination hot water – calcium chloride on the in vitro growth of *Colletotrichum gloeosporioides* and the postharvest quality of infected papaya. *Plant Pathol. J.* 33:572-581. https://doi.org/10.5423/PPJ.OA.01.2017.0004

Chávez-Quintal, P., González-Flores, T., Rodríguez-Buenfil, I., Gallegos-Tintoré, S. 2011. Antifungal activity in ethanolic extracts of *Carica papaya* L. cv. Maradol leaves and seeds. *Ind. J. Microbiol*, 51: 54-60. https://doi.org/10.1007/s12088-011-0086-5

Ferreira-Demartelaere, A.C., Cordeiro-Do, Nascimiento L., Camelo-Guimaraes, G.H., Araújo-Da Silva, J., Gi-De Luna, R. 2017. Elicitors on the control of anthracnose and postharvest quality in papaya fruits. *Pesq. Agrop. Trop.* 47:211-217. https://doi.org/10.1590/1983-40632016v4745093

Gutiérrez-Alonso, J.G., Gutiérrez-Alonso, O., Nieto-Angel, D., Téliz-Ortiz, D., Zavaleta-Mejía, E., Delgadillo-Sánchez, F. 2004. Manejo integrado de la antracnosis (*Colletotrichum gloeosporioides* (Penz.) Penz. y Sacc.) del mango (*Mangifera indica* L.) durante la postcosecha. *Rev. Mex. Fitopatol.* 22: 395-402.

Gutiérrez-Alonso, O., Gutiérrez-Alonso, J.G. 2003. Evaluación de resistencia a benomil, thiabendazole y azoxystrobin para el control de antracnosis (*Colletotrichum gloeosporioides* Penz.) en frutos de guayaba (*Psidium guajava* L.) en postcosecha. *Rev. Mex. Fitopatol.* 21: 228-232.

Hewajulie, I.G., Wijeratnam, S.W. 2010. Alternatives postharvest treatments to control anthracnose disease in papaya during storage. *Fresh Produce* 4:15-20.

López-Navarrete, M.C., Arevalo-Galarza, M.L.C., Nieto-Angel, D. 2011. Uso de fungicidas y tratamientos térmico postcosecha para el control de antracnosis en frutos de papaya Maradol (*Carica papaya*). *AgroProd* 4: 24-28.

Maeda, C., Nelson, S. 2014. Anthracnose of papaya in Hawaii. https://www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-103.pdf.

Mailafia, S., Okoh, G.R., Olabode, H.O.K., Osanupin, R. 2017. Isolation and identification of fungi with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Vet. World* 10: 393-397. https://doi.org/10.14202/vetworld.2017.393-397

Marín-Cortez, F.A., Chan-Cupul, W., Buenrostro-Nava, M.T., Hernández-Ortega, H.A., Manzo-Sánchez, G., Galindo-Velasco, E. 2019. Biological control of late rust disease [*Pucciniastrum americanum* (Farl.) Arthur] in raspberry (*Rubus idaeus* L.) using two biological products: *Bacillus subtilis* (Fungizard®) and *Larrea tridentata* botanic extracts (CleanCrop®) under screenhouse conditions. *Idesia* 37:125-133. http://dx.doi.org/10.4067/S0718-34292019005000504.

Molina-Chavez, A., Gómez-Alpízar, L., Umaña-Rojas, G. 2017. Identification of *Colletotrichum* species with anthracnose in papaya (*Carica papaya* L.) in Costa Rica. *Agron. Costarricense* 41:69-80. http://dx.doi.org/10.15517/rac.v41i1.29752.

Pérez-León, G., Castillo-Matamoros, R., Chavarría-Pérez, L., Brenes-Angulo, A., Gómez-Alpízar, L. 2015. Combate químico de la antracnosis de *Sansevieria trifasciata* var. *Hahnii* en un sistema de hojas separadas. *Agron. Mesoam*. 26: 305-313. https://doi.org/10.15517/am.v26i2.19323 Rathod, G. 2012. A review on biological control of post-harvest fungal diseases of fruits. Curr. Bot. 3:5-7.

Salvatore, J.J., Ritenour, M.A. 2007. Effectiveness of different fungicides applied preharvest at reducing postharvest decay of fresh Florida citrus. *Proc. Fla Stat. Hort. Soc*. 120: 281-284.

Santamaría-Basulto, F., Díaz-Plaza, R., Gutiérrez-Alonso, O., Santamaría-Fernández, J., Larqué-Saavedra, A. 2011. Control of two species of *Colletotrichum* causing anthracnose in Maradol papaya fruits. *Rev. Mex. Cienc. Agric.* 2: 631-643.

Santamaría-Basulto, F., Sauri-Duch, E., Espadas, F., Gil-Espadas, F., Díaz-Plaza, R., Larqué-Saavedra, A., Santamaría, J.M. 2009. Postharvest ripening and maturity indices for Maradol papaya. *Interciencia* 34: 583-588.

Suárez-Quiroz, M.L., Mendoza-Bautista, I., Monroy-Rivera, J.A., De La Cruz-Medina, J., Angulo-Guerrero, O., González-Ríos, O. 2013. Identification and antifungal susceptibility of phytopathogenic fungi in papaya cv. Maradol (*Carica papaya* L.). Rev. Iber. Tecn. Post. 14:115-124.

Zavala-León, M.J., Tun-Suarez, J.M., Cristóbal-Alejo, J., Ruiz-Sánchez, E., Gutiérrez-Alonso, O., Vázquez-Calderón, M., Méndez-González, R. 2005. Control postcosecha de la antracnosis en papaya y sensibilidad de *Colletotrichum gloeosporioides* (penz.) Sacc. a fungicidas organosintéticos. *Rev. Chap. Ser. Hort*. 11: 251-255.

Colegio de Postgraduados

Cardinal temperatures of populations developed from native maize (*Zea mays* L.) from central and southern, Tamaulipas, Mexico

García-Ramírez, Alejandro[1]; López-Santillán, José A.[1*]; Estrada-Drouaillet, Benigno[1]; Poot-Poot, Wilberth A.[1]; Varela-Fuentes, Sostenes E.[1]; Reséndiz-Ramírez, Zoila[2]; Mayek-Pérez, Netzahualcóyotl[3]

[1] Universidad Autónoma de Tamaulipas, Posgrado e Investigación de la Facultad de Ingeniería y Ciencias Cd. Victoria, Tamaulipas, México, C. P. 87000. Tel: 834-318-1721, Ext. 2146.
[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Río Bravo, Río Bravo, Tamaulipas, México, C. P. 88900.
[3] Universidad Autónoma de Tamaulipas, Unidad Académica Multidisciplinaria Reynosa Rodhe, Reynosa, Tamaulipas, México, C. P. 88779.
* Correspondence: jalopez@docentes.uat.edu.mx

ABSTRACT
Objective: To estimate the cardinal temperatures of native maize populations from Tamaulipas.
Design/Methodology/Approach: Ten genotypes developed from native germplasms were established in Güémez, Tamaulipas, on 12 planting dates (2019 and 2020). The cardinal temperatures (T_b, T_o, and T_u) of each of the cultivars were estimated through the decomposition of a quadratic model, using the days from sowing to tasseling and the average temperature of each sowing date from that period.
Results: From sowing to tasseling, the evaluated cultivars recorded 15.7-18.1 °C base temperatures (T_b), 28.3-30.1 °C optimum temperatures (T_o), and 32.3-34.4 °C threshold temperatures (T_u). The T_b and T_o values represent the high thermal requirement of the germplasm, while T_u stands for its resistance to high temperatures. The L3, L4, L5, and VHA cultivars stood out for their broader adaptation range (16.2 to 34.4 °C), while the L3, L4, and L6 cultivars have a higher resistance to high temperatures (average T_u: 34.5 °C).
Study Limitations/Implications: The cardinal temperatures determined in this study depended on the evaluated genotypes and the environment in which they developed.
Findings/Conclusions: The maize germplasm evaluated in this study was resistant to high temperatures. As a result of its adaptation to the conditions of central and southern Tamaulipas, this germplasm is a source of variation for the characteristics that provide resistance to the stress caused by high temperatures.

Keywords: *Zea mays*, native maize, tolerance, high temperature, genetic improvement.

Citation: García-Ramírez, A., López-Santillán, J. A., Estrada-Drouaillet, B., Poot-Poot, W. A., Varela-Fuentes, S. E., Reséndiz-Ramírez, Z. & Mayek-Pérez, N. (2024). Cardinal temperatures of populations developed from native maize (*Zea mays* L.) from central and southern, Tamaulipas, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2588

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: May 25, 2023.
Accepted: January 16, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 47-52.

INTRODUCTION

The wide agroecological diversity of Tamaulipas favors the genetic variability of native maize (Castro *et al.*, 2013; González-Martínez *et al.*, 2018), mainly in the center and south of the state. Many grain and forage production systems are established with these cultivars —mainly *Tuxpeño*, *Ratón*, *Olotillo*, and *Cónico* maize races (González-Martínez *et al.*, 2018). In addition, these races are known for their high adaptability to limiting agroclimatic conditions, such as high temperatures (Pecina-Martínez *et al.*, 2009). As a consequence of its geographical location, central and southern Tamaulipas reach temperatures >40 °C in summer (Castro *et al.*, 2013). Nevertheless, native maize developed under these conditions is a source of characteristics that provide resistance to this type of environmental stress (Castro-Nava *et al.*, 2011). Resistance to high temperatures is a feature with a high genotype-environment interaction (Naveed *et al.*, 2016). Consequently, determining the genetic effects that control the resistance to high temperatures in the available maize germplasm is fundamental to design effective evaluation and selection plans (Van Inghelandt *et al.*, 2019). Therefore, an accurate characterization of the cardinal temperatures (Yousaf *et al.*, 2018) of the Tamaulipas maize germplasm is required to select specific populations to be used as a base germplasm, in order to design an appropriate program for the improvement of the resistance to high temperatures (Chen *et al.*, 2012). This characterization must be based on the evaluation of germplasms with different temperature ranges —*i.e.*, the cardinal temperatures must be determined (Ruiz-Corral *et al.*, 2002; Aburto-Cansino *et al.*, 2018). In this regard, the temperature range of a given cultivar should be accurately determined. The temperature range includes a minimum or base temperature (T_b) and a maximum or threshold temperature (T_u). In addition, the optimal temperature (T_o) must be determined, based on the highest development rate (Hatfield and Prueger, 2015). When temperatures are lower or higher than the optimal temperature, they cause a decrease in the development rate of the plant; however, temperatures outside the range of the base and threshold temperatures cause a null development rate. This technique helps to determine the thermal growing periods for each cultivar, based on the thermal offer of each place (Arista-Cortes *et al.*, 2018).

MATERIALS AND METHODS

Phenological and temperature data were recorded in 12 sowing dates between 2019 and 2020 (Table 1). The data was collected in Güémez, Tamaulipas (23° 56’ 28” N, 99°

Table 1. Sowing dates and thermal characteristics of Güémez, Tamaulipas.

Sowing date	Season	Year	AT
August 01		2019	29.2
August 21	-	-	27.4
August 30	-	-	25.1
September 09	-	-	24.3
January 24	FW	2019-2020	23.0
February 07	-	-	24.1
February 22	-	-	25.7
March 27	-	-	28.4
July 22	SS	2020	27.5
August 04	-	-	27.7
August 17	-	-	27.0
August 31	-	-	25.5

Sp-Su (PV): Spring-Summer; A-W (OI): Autumn-Winter; Tm: Medium daily temperature.

06' 24" W, at 193 m.a.s.l.). This region has a semi-arid warm climate (Vargas *et al.*, 2007). Each sowing date was established under irrigation conditions and included 10 maize cultivars. The furrows were established 0.08 m apart from each other and the distance between plants was 0.25 m. Two maize seeds were sown per hole and thinning was carried out 14-21 days after the sowing. The ten maize cultivars included in this study came from a germplasm from the dry subtropics and are consequently adapted to central and southern Tamaulipas (Castro *et al.*, 2013).

The following variables were evaluated: tasseling (F) days and medium daily temperature (T_m). The former is the number of days from the sowing until 50% of the plants have released pollen. The latter was calculated as the mean between the maximum daily temperature and the minimum temperature of each sowing date. The 1/F was calculated to determine the development rates of the 12 environments, during the sowing-tasseling period. The data described a curvilinear shape and, consequently, the quadratic regression model used by Ruiz-Corral *et al.* (2002) was applied to calculate base and optimal temperatures, using the following formula:

$$1/F = \beta_0 + \beta_1 T_m \times \beta_2 T_m{}^2$$

Where T_m is the medium daily temperature of the *F* period (sowing-tasseling). Based on this equation, base temperature (T_b) was estimated using the following equation: .

$$T_b = \frac{\left(-\beta_1 + \beta_1^2 - 4\beta_0\beta_2\right)^{1/2}}{2\beta_2}$$

Optimal temperature (T_o) was calculated with the following equation:

$$T_o = \frac{-\beta_1}{2\beta_2}$$

Once T_b and T_o were calculated, the maximum threshold temperature (T_u) was determined with the following identity:

$$T_u = \frac{\ln\left(2C^{T_o} - C^{T_b}\right)}{\ln C}$$

where ln is the natural logarithm and *C* is equal to 1.15 (Ruiz-Corral *et al.*, 2002).

RESULTS AND DISCUSSION

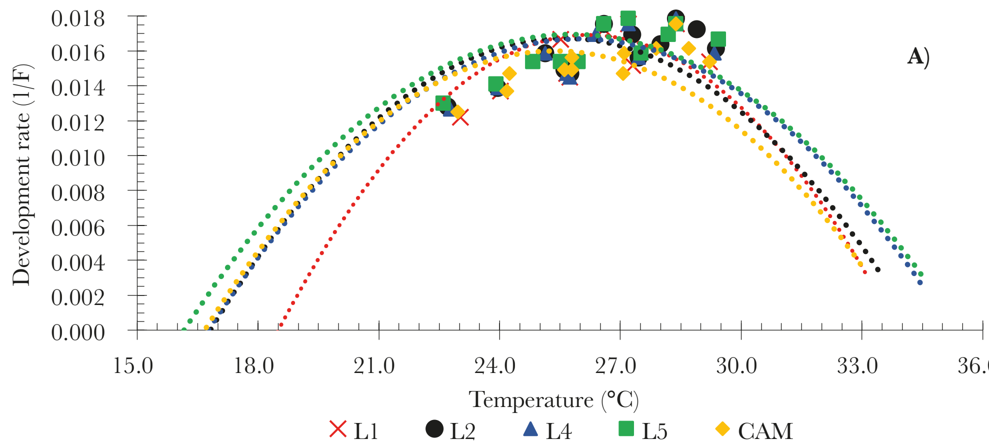
The quadratic model accurately represented the temperature-development rate relationship (R^2=0.88, Table 2). Figures 1A and 1B show the relationship between the maize development rate and the environmental temperature of the evaluated cultivars.

Table 2. Parameters of the quadratic regression and cardinal temperatures of the maize cultivars evaluated in Güémez, Tamaulipas.

Cultivar	β_0	β_1	β_2	Bt	Ot	Mtt	R^2
L1	−0.10535	0.00846	-1.46×10^{-4}	18.1	29.0	33.1	0.87
L2	−0.07200	0.00611	-1.05×10^{-4}	16.4	29.1	33.4	0.88
L3	−0.06314	0.00534	-8.86×10^{-5}	16.2	30.1	34.6	0.89
L4	−0.06614	0.00556	-9.25×10^{-5}	16.3	30.0	34.5	0.89
L5	−0.05935	0.00511	-8.48×10^{-5}	15.7	30.1	34.6	0.89
L6	−0.09536	0.00789	-1.39×10^{-4}	17.4	28.5	32.6	0.86
CAM	−0.06980	0.00598	-1.04×10^{-4}	16.3	28.8	33.1	0.87
VHA	−0.06771	0.00571	-9.63×10^{-5}	16.4	29.6	34.0	0.87
VCII	−0.10276	0.00844	-1.49×10^{-4}	17.8	28.3	32.3	0.86
CGMor18	−0.06677	0.00575	-9.98×10^{-5}	16.1	28.8	33.2	0.87

β_0, β_1, β_2: parameters of the quadratic regression; T_b: base temperature; T_o: optimal temperature; T_u: maximum threshold temperature; R^2: adjustment of the model; S-F: sowing to tasseling.

The interval of temperatures of the field experiments included the thermal levels of the suboptimal and optimal intervals for maize development (Valdez-Torres *et al.*, 2012). Based on these cardinal temperatures, the L3, L4, L5, and VHA cultivars recorded a 15.7-16.5 °C T_b, a 29.6-30.1 °C T_o, and a 34.0-34.6 °C T_u (Table 2), resulting in a 16.2-34.4 °C adaptation range (Figure 1A). Meanwhile, the L1, L6, and VCII cultivars obtained a 17.4-18.1 °C T_b, a 28.3-29.0 °C T_o, and a 32.3-33.1 °C T_u (Table 2), which results in a 17.8-32.7 °C adaptation range (Figure 1B). Finally, since the L2, CAM, and CGMor18 cultivars recorded a 16.1-16.4 °C T_b, a 28.8-29.1 °C T_o, and a 33.1-33.4 °C T_u (Table 2), a 16.3-33.2 °C adaptation range can be deduced (Figure 1B). Consequently, the L3, L4, L5, and VHA cultivars had a broader temperature adaptation range, as a result of their higher Tu (Figure 1A). These cultivars have a greater resistance to high temperatures, because they maintain the growing and development processes in temperatures >34 °C (Table 2, Figure 1A). The thermal requirements of the evaluated cultivars had a low variation, maintaining temperatures of 15.7-18.1, 28.3-30.1, and 32.3-34.6 °C, for T_b, T_o, and T_u, respectively (Table 2). The evaluated germplasm is native to a single region: central and southern Tamaulipas (Hernandez-Trejo *et al.*, 2022). These cultivars may have been developed in localities with a minimum mean temperature between 10 and 12 °C and a maximum mean temperature between 38 and 40 °C (Vargas *et al.*, 2007). The differentiation pattern of the cardinal temperatures between cultivars is the consequence of the origin or adaptation range of each cultivar (Arista-Cortes *et al.*, 2018), which explains the high values of the cardinal temperatures determined in this germplasm. Meanwhile, the levels of the cardinal temperatures were high, particularly compared with the findings of other research: 7-13°C T_b (Ruiz *et al.*, 1998; Steward *et al.*, 2018; Arista-Cortes *et al.*, 2018); 24.3-25 °C T_o (Ruiz *et al.*, 1998; Tojo *et al.*, 2005); and 28.8 °C T_u (Ruiz-Corral *et al.*, 2002).



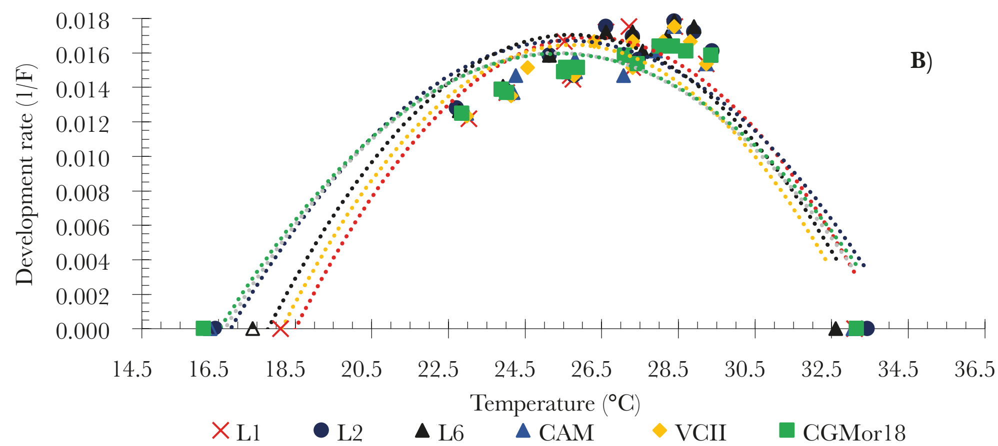


Figure 1. Relationship between temperature and development rate during the sowing-tasseling period of the following cultivars: A) L1, L2, L4, L5, and CAM and B) L3, L6, VHA, CGMor18, and VCII.

CONCLUSIONS

The variation of the cardinal temperatures between cultivars shows the need to estimate these parameters for each specific cultivar, in order to establish the adaptation range, according to the thermal offer of each place. The high threshold temperature of the cultivars proves that they are a source of variation for the selection of the characteristics that provide resistance to the stress caused by high temperatures.

REFERENCES

Aburto-Cansino, G. N., Ruiz-Corral, J. A., Sánchez, G. J. J. y González, E. D. R. (2018). Temperaturas cardinales de desarrollo del teocintle (*Zea* spp.). *Rev. Mex. de Cienc. Agríc*. 9:1269-1281.

Arista-Cortes, J., Quevedo, N. A., Zamora, M. B. P., Bauer M. R., Sonder K. y Lugo E. O. (2018). Temperaturas base y grados días desarrollo de 10 accesiones de maíz de México. *Rev. Mex. de Cienc. Agríc*. 9:1023-1033.

Castro N. S., López, S. J. A., Pecina, M. J. A., Mendoza C. M. C. y Reyes, M. C. A. (2013). Exploración de germoplasma nativo de maíz en el centro y sur de Tamaulipas, México. *Rev. Mex. de Cienc. Agríc*. 4:645-653.

Castro-Nava, S., Ramos-Ortíz, V. H., Reyes-Méndez, C. A., Briones-Encinia, F. and López-Santillán, J. A. (2011). Preliminary field screening of maize landrace germplasm from northeastern México under high temperatures. *Maydica* 56:76-82.

Chen, J., Xu, W., Velten, J. P., Xin, Z. and Stout, J. E. (2012). Characterization of maize inbred lines for drought and heat tolerance. *J. of S. and W. C. S*. 67:354-364. https://doi.org/10.2489/jswc.67.5.354

González-Martínez, J., Rocandio-Rodríguez, M., Chacón-Hernández, J. C., Vanoye-Eligio, V. y Moreno-Ramírez, Y. R. (2018). Distribución y diversidad de maíces nativos (*Zea mays* L.) En el altiplano de Tamaulipas, México. *Agro Productividad 11*(1):124-130.

Hatfield, J. L. and Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes* 10:4-10. https://doi.org/10.1016/j.wace.2015.08.001

Hernandez-Trejo, A., López-Santillán, J. A., Estrada-Drouaillet, B., Reséndiz-Ramírez, Z., Varela-Fuentes, S. E., Coronado-Blanco, J. M. and Malvar, R. A. (2022). Maize tolerance to *Spodoptera frugiperda* (J. E. Smith) leaf damage and insecticide application. *Agro Productividad* 15:23-31.

Naveed, M., Ahsan, M., Akram, H. M., Aslam, M. and Ahmed, N. (2016). Genetic effects conferring heat tolerance in a cross of tolerant × susceptible maize (*Zea mays* L.) genotypes. *Front. in Plant Sci.* 7:1-12. https://doi.org/10.3389/fpls.2016.00729

Pecina, M. J. A., Mendoza, C. M. C., López, S. J. A., Castillo, G. F. y Mendoza R. M. (2009). Respuesta morfológica y fenológica de maíces nativos de Tamaulipas a ambientes contrastantes de México. *Agrociencia 43*(7):681-694.

Ruiz, C. J. A., Sánchez, G. J. J. and Goodman, M. M. (1998). Base temperature and heat unit requirement of 49 mexican maize races. *Maydica* 43:277-282.

Ruiz-Corral, J. A., Flores-López, H. E., Ramírez-Díaz, J. L. y González-Eguiarte, D. R. (2002). Temperaturas cardinales y duración del ciclo de madurez del híbrido de maíz H-311 en condiciones de temporal. *Agrociencia* 36:569-577.

Steward, P. R., Dougilla, A. J., Thierfelderb, C., Pittelkowc, C. M., Stringera, L. C., Kudzalad, M. and Shackelford, G. E. (2018). The adaptive capacity of maize-based conservation agriculture systems to climate stress in tropical and subtropical environments: A meta-regression of yields. *Agric., Ecosyst. and Env*. 251:194-202. https://doi.org/10.1016/j.agee.2017.09.019

Tojo, C. M., Sentelhas, P. C. and Hoogenboom, G. (2005). Thermal time for phenological development of four maize hybrids grown off-season in a subtropical environment. *The J. of Agricultural Sci.* 143:169-182.

Valdez-Torres, J. B., Soto-Landeros, F., Osuna-Enciso, T. y Báez-Sañudo, M. A. (2012). Modelos de predicción fenológica para maíz blanco (*Zea mays* L.) y gusano cogollero (*Spodoptera frugiperda* J. E. Smith). *Agrociencia* 46:399-410.

Van Inghelandt, D., Frey, P. F., Ries, D. and Stich, B. (2019). QTL mapping and genome-wide prediction of heat tolerance in multiple connected populations of temperate maize. *Scientific Reports* 9:14418. https://doi.org/10.1038/s41598-019-50853-2

Vargas T. V., Hernández, R. M. E., Gutiérrez, L. J., Plácido, D. C. J. y Jiménez, C. A. 2007. Clasificación climática del estado de Tamaulipas, México. *CienciaUAT* 2:15-19.

Yousaf, M. I., Hussain, K., Hussain, S., Ghani, A., Arshad, M., Mumtaz, A. and Hameed, R. A. 2018. Characterization of indigenous and exotic maize hybrids for grain yield and quality traits under heat stress. *Intl. J. of Agric. and Biology* 20:333-337. https://doi.org/10.1038/s41598-019-50853-2

Evaluation of the effect of organic fertilizers on the morphophysiological parameters and antioxidant compounds of Swiss chard (*Beta vulgaris* L.).

Sánchez-Domínguez, Elisama[1]; Lara-Capistrán, Liliana[1*]; Aquino-Bolaños, Elia Nora[2]; Alba-Jiménez, Jimena Esther[3]

[1] Universidad Veracruzana, Facultad de Ciencias Agrícolas, *Campus* Xalapa, Circuito Universitario Gonzalo Aguirre Beltrán s/n, Zona Universitaria, Xalapa, Veracruz, México, C. P. 91090.

[2] Centro de Investigación y Desarrollo en Alimentos (CIDEA), Av. Dr. Rafael Sánchez Altamirano s/n, km 3.5 carretera Xalapa-Las Trancas, Col. Industrial, Las Animas, Xalapa, Veracruz, México, C. P. 91192.

[3] CONAHCyT-Centro de Investigación y Desarrollo en Alimentos (CIDEA). Av. Dr. Rafael Sánchez Altamirano s/n, km 3.5 carretera Xalapa-Las Trancas, Col. Industrial, Las Animas, Xalapa, Veracruz, México. C. P. 91192.

* Correspondence: llara_capistran@hotmail.com

Citation: Sánchez-Domínguez, E., Lara-Capistrán, L., Aquino-Bolaños, E. N., & Alba-Jiménez, J. E. (2024). Evaluation of the effect of organic fertilizers on the morphophysiological parameters and antioxidant compounds of Swiss chard (*Beta vulgaris* L.). *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2632

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: July 11, 2023.
Accepted: February 23, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 53-59.

ABSTRACT

Objective: To evaluate the effect of organic fertilizers on the morphophysiological parameters and antioxidant compounds of Swiss chard (*B. vulgaris* L.) var. 'Rainbow' grown under greenhouse conditions.

Design/Methodology/Approach: A completely randomized design was used with three treatments and 33 repetitions: T1 (poultry manure-based organic fertilizer (T1 PM)), T2 (coffe pulp-based vermicompost (T2 V)), and T3 (chemical fertilization (T3 F)). Plant height, stem diameter, number of leaves, leaf area, fresh biomass, dry biomass, chlorophyll A, chlorophyll B, total chlorophyll, carotenoids, total polyphenols, DPPH, and yield were evaluated. An analysis of variance and Tukey's test (α=0.05) were used.

Results: For most of the variables evaluated, the best results were obtained with the poultry manure-based organic fertilizer (T1 PM) treatment. The antioxidant compounds and the quality of Swiss chard were influenced by organic fertilizers, with 0.21 mg Eq. gallic acid g^{-1} fresh tissue (ft), total chlorophyll of 19.5 μg g^{-1} (ft), and a yield of 6,150 kg·m^2.

Study Limitations/Implications: The mineral content of this vegetable was not evaluated.

Findings/Conclusions: The use of poultry manure-based organic fertilizers can be an alternative nutritional management for Swiss chard cultivation.

Keywords: Vermicompost, total polyphenols, poultry manure, DPPH.

INTRODUCTION

Swiss chard is a green leafy vegetable with a thick stem from the Quenopodiaceae family. Very few varieties are cultivated, and they are classified according to their color, the size of their leaves and petioles, the thickness of the stem, and the quick recovery when their leaves are cut. According to Valero *et al.* (2018), based on their quality and flavor, the most well-known, cultivated, and appreciated varieties are the Blond de Lyon and the white-stem Bressane. The latter variety is valued for its fatty acids (omega 3), lutein, flavonoids, beta-carotene, and zeaxanthin, as well as its vitamins, fiber, folic acid, and mineral salts, including Ca, Na, K, Cu, Fe, and P (SADER, 2020). According to SIAP (2020), more than 12,000 tons of Swiss chard are produced every year in Mexico.

Although it can be grown all year round, as a monoculture, it has high fertilization requirements, especially regarding nitrogen. In addition, the excessive use of pesticides, from the beginning of plant growth to the end of cultivation (Ninfali *et al.*, 2017), requires finding ecological alternatives for its production, remarkably the addition of compost — an organic fertilizer formed by the microbial degradation of waste subjected to a natural decomposition process (Spaccini and Piccolo, 2020; Ro *et al.*, 2022). The application of this type of materials has become very important, as they increase the organic matter of agricultural soils, improving their fertility, structure, and water retention and preventing erosion and degradation. Likewise, Álvaro (2019), Mamun *et al.* (2020), and Yazid *et al.* (2020) mention that, in social and economic terms, these practices encourage the search and improvement of the systematization of agricultural production activities and practices. Regarding the effect that this kind of organic fertilizers has on horticultural crops, Libutti *et al.* (2020) mention that the growth variables and chlorophyll content of Swiss chard responded positively to the organic amendment, especially when the soil was treated with animal waste compost. Therefore, the objective of this research was to evaluate the effect of organic fertilizers on the morphophysiological parameters and antioxidant compounds of Swiss chard var. 'Rainbow' grown under greenhouse conditions.

MATERIALS AND METHODS

Location of the study area

The research was carried out in a tunnel greenhouse, with an average temperature of 35 °C and a relative humidity of 55%. Six-centimeter-high Swiss chard var. 'Rainbow' seedlings, with a pair of true leaves, were used for the experiment.

Experimental design and treatment description

A completely randomized design with three treatments was used: T1 (poultry manure-based organic fertilizer (T1 PM)), T2 (coffe pulp-based vermicompost (T2 V)), and T3 (chemical fertilization (T3 F)). Each treatment had 33 repetitions, resulting in a layout of 99 experimental units.

Crop establishment

The substrate consisted of a mixture of soil and *tepezil* in a 1:1 (v/v) ratio, disinfected with Full-Gro (6 L·1 L^{-1}). The substrate was used to fill 4-kg black polyethylene bags. Before transplanting, 273 g of vermicompost (V) and 273 g of poultry manure (PM) were applied, following the recommendations of SAGARPA (2014). The control treatment (F) was fertilized with a blue granular fertilizer (10 g·$plant^{-1}$). The poultry manure-based organic fertilizer had the following characteristics: 400-45% organic matter; 2-3% N, P, K; 6-8% Ca; 1-1.6% Mg; 0.4-1% Fe; 400-700 ppm Zn, 60-90 ppm Cu; 450-800 ppm Mn; 40-100 ppm B; and 24-28% organic carbon. The commercial vermicompost (TerraNova Lombricultores, Xalapa, Veracruz, Mexico) produced from coffee pulp (V) contained: 84% organic matter; pH 7.4; 0.108% organic P; 0.25% total P; 3.99% total N;

2.14% total K; 1.72% total C; 0.8% total Mg; 12:21 C:N ratio; 10.5% fulvic acids; and 15.1% humic acids.

Variables evaluated and statistical analysis

The following agronomic traits were evaluated at 45 days after transplanting (DAT): plant height (cm), with a Truper® flexometer; stem diameter (mm), with a Steren® graduated vernier; number of leaves (unit); leaf area (cm^2) in the Photoshop® software; fresh biomass (g), with a Truper® BASE-5EP digital scale; and dry biomass (g). The samples were dried in a Robert Shaw® oven at 75 °C for 7 days; each sample was then weighed on a Denver Instrument® APX 200 analytical balance. Variables related to compounds with antioxidant activity (chlorophyll A, chlorophyll B, total chlorophyll, and carotenoids ($\mu g\ g^{-1}$ tf)) were determined according to the 80% acetone extraction method, following Song *et al.* (2021), who used a UV-VIS® spectrophotometer (Shimadzu UV-1800), at 645, 663, and 440 nm wavelengths. Total polyphenols (mg- EAG g^{-1} tf) were determined with the method developed by Singleton and Rossi (1965); meanwhile, antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Brand-Williams *et al.* (1995). Finally, yield was measured in m^2. The assumptions of normality and homoscedasticity for the resulting data were verified following the criteria of Shapiro-Wilk (1965) and Hartley (1950). An analysis of variance (ANOVA) and Tukey's Least significant difference (LSD) test were performed, with a 5% significance level (α=0.05), using the Statistica software version 10.0 (StatSoft Inc., 2011) for Windows.

RESULTS AND DISCUSSION

Morphophysiological variables

The ANOVA showed significant differences for the variables evaluated (Tukey, P≤0.05). Table 1 shows that poultry manure-based fertilization was a better treatment than chemical fertilization (T3 F) regarding plant height, stem diameter, number of leaves, leaf area, fresh biomass, and dry biomass, with 6.28, 58.12, 77.44, 2.5, 109, and 111.47% increases, respectively. These results could be related to the contribution of macro and microelements (*e.g.*, a high content of nitrogen, phosphorus, and potassium) that the poultry manure-based fertilizer makes to the soil, as well as their influence on the improvement of soil characteristics (*e.g.*, the structure and texture of the substrate). Therefore, it enables a better absorption of nutrients and, consequently, a greater total biomass. These results match the findings of Cabaleiro *et al.* (2017), who applied different doses of dehydrated and granular broiler litter (BL) to lettuce crops, during the spring and winter cycles, and proved that fertilization with any dose of BL guarantees lettuce production, both regarding the fresh weight and number of commercial lettuces; these results are like those obtained with mineral fertilizers.

Chlorophyll content

According to the analysis of variance, the chlorophyll A content of Swiss chard leaves showed significant differences (P≤0.05) (Table 2). The T3 F with 12.1 μg-g^{-1} and the T1 PM with 11.9 μg-g^{-1} were statistically equal. Similarly, the best content of chlorophyll

Table 1. Morpho-physiological.

Variables evaluated	Mean Square Error	F	CV	T1 PM	T2 V	T3 F
Plant height (cm)	0.52	5.66	10.12	71±1.0a	55.20±4.87b	68.66±0.57a
Stem diameter (mm)	0.52	5.55	10.08	23.70±0.45a	22.20±0.43a	16.93±1.72b
Number of leaves	0.44	4.55	9.33	18.33±0.57a	10.33±1.52b	12.66±0.57b
Leaf area (cm^2)	0.33	4.23	8.55	56.45±0.62a	38.04±0.57c	44.62±0.99b
Total fresh biomass (g)	0.55	6.02	11.11	384.66±23.54a	186±8.0b	247±45.73b
Total dry biomass (g)	0.57	6.21	11.2	25.25±0.45a	11.94±0.96b	12.68±0.32b

Values with the same letters within columns are statistically equal (Tukey, P≤0.05). F: calculated, CV: coefficients of variation, and ±Standard deviation.

B and total chlorophyll were recorded by the T3 F (7.6 μg-g^{-1} and 19.8 μg-g^{-1}) and T1 PM (7.2 μg-g^{-1} and 19.5 μg-g^{-1}) treatments. Likewise, Moncayo-Luján *et al.* (2015) and Alvarado (2020) determined that the content of chlorophyll A, chloropyll B, and total chloropyll —in the crops of basil, lettuce, and radish, respectively— was influenced by chemical fertilization, obtaining a result similar to the treatment with organic fertilizers. This trend could be attributed to the availability of N (on which the chlorophyll content depends); this availability stimulates the photosynthetic process and therefore the synthesis of chlorophyll (Moncayo-Luján *et al.*, 2015).

Total polyphenols and antioxidant activity (DPPH)

The statistical analysis showed significant differences (P≤0.05) regarding the content of total polyphenols: the T3 F treatment had higher content (0.21 mg Eq. Gallic Acid-g^{-1} tf) than the other treatments (Figure 1a). This phenomenon may be related to the high N content, which at a given time can influence the presence of phenolic compounds. These results are similar to those reported by Vázquez-Vázquez *et al.* (2015), who reported that the chemical fertilization treatment showed a higher phenolic content than organic fertilizer treatments in a basil crop. Meanwhile, the antioxidant activity increased in the T1 PM treatment with 3.74 μmol E. Trolox-g^{-1} tf (Figure 1b). This figure may be related to the concentration of major elements (N, P, K) in this fertilizer, which matches the findings of Martínez *et al.* (2017), who mention that plant nutrition affects the antioxidant capacity and performance of plants.

Table 2. Chlorophyll content.

	Mean Square Error	F	CV	T1 PM	T2 V	T3 F
Chlorophyll A	2.64	11.97	14.33	11.9±0.57a	7.8±0.57b	12.1±0.57a
Chlorophyll B	0.51	5.86	10.27	7.2±0.25a	5.8±0.25b	7.6±0.25a
Chlorophyll T	5.45	10.26	12.54	19.5±0.87a	13.9±0.83b	19.8±0.85a

Values with the same letters within columns are statistically equal (Tukey, P≤0.05). F: calculated, CV: coefficients of variation, and ±Standard deviation.

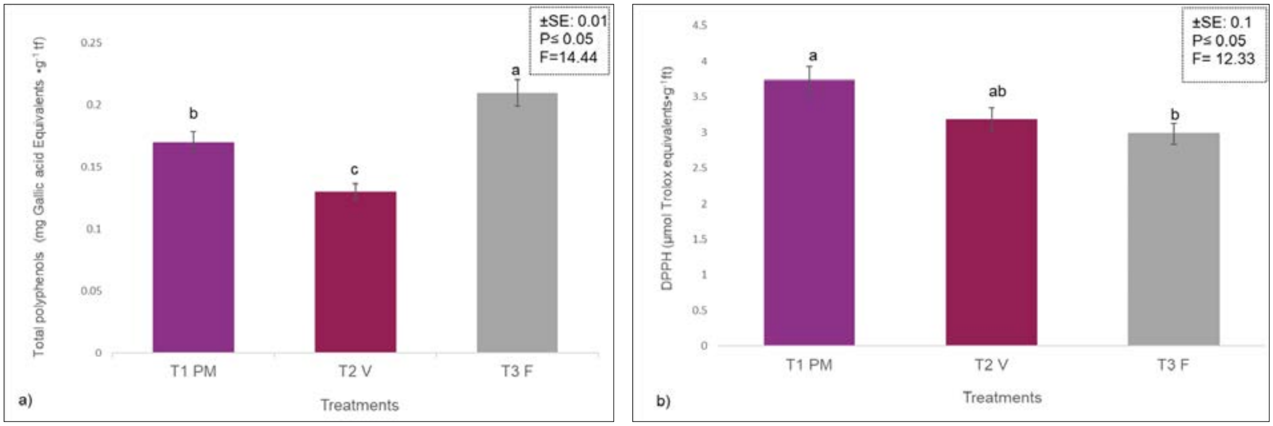


Figure 1. Polyphenol (mg Eq. Gallic acid • g^{-1} tf) a) and DPPH (μmol E. Trolox-g^{-1} tf) b) content in Swiss chard var. 'Rainbow' at 45 DAT. Columns with the same letter are statistically equal (Tukey, P≤0.05). The vertical lines on the bars represent the standard error (±).

Yield

The highest yield for the T1 PM treatment was quantified (6,150 kg-m^2) with an increase of 34.86% (Figure 2a and b), possibly as a consequence of the mineral content of the poultry manure-based fertilizer. These results were higher than those reported by Rodríguez *et al.* (2016) and by Huanca and Blanco (2019), who reported yields of 1,645 kg-m^2 and 2.32 kg-m^2, respectively.

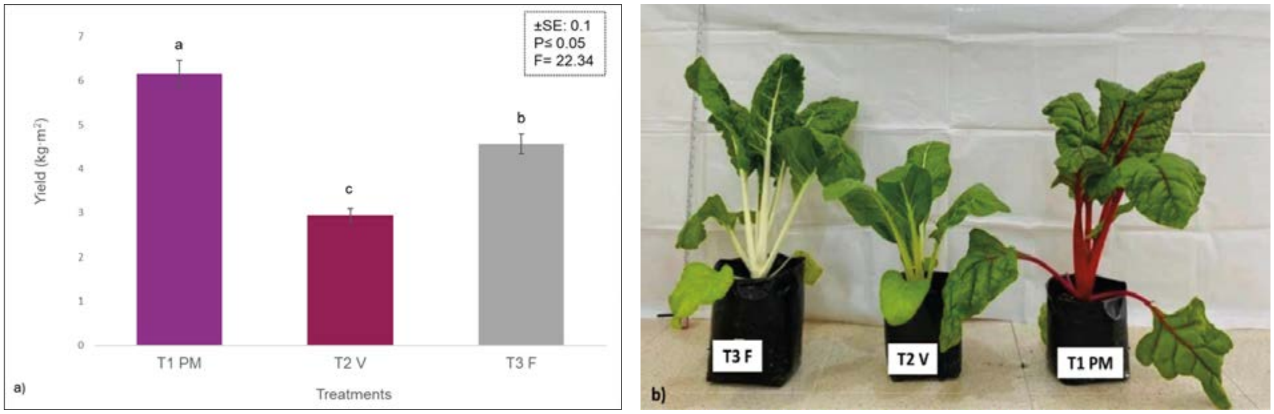


Figure 2. Yield (kg m^{2-1}) of Swiss chard var. 'Rainbow' a). Comparative effects b). Columns with the same letter are statistically equal (Tukey, P≤0.05). The vertical lines on the bars represent the standard error (±).

CONCLUSIONS

Fertilization with poultry manure-based organic fertilizer could be a viable nutritional management alternative, with a positive effect on Swiss chard var. 'Rainbow' grown under greenhouse conditions, increasing its morphophysiological variables and some antioxidant compounds.

ACKNOWLEDGEMENTS

The authors would like to thank the authorities of the Facultad de Ciencias Agrícolas, for the financial support provided, and to the Centro de investigación y Desarrollo en Alimentos (CIDEA-UV), for the analysis of antioxidant compounds.

REFERENCES

Alvarado, J, A. A. 2020. Efecto de la incorporación de diferentes abonos orgánicos en el desarrollo y contenido en lípidos, azúcares y clorofilas en cultivos de hortalizas. Tesis de Licenciatura. Universitat Politècnica de Catalunya. Barcelona. 31 de Enero del 2020.

Álvaro, G.J. 2019. Ventajas y desventajas del compostaje. Obtenido en la Red Mundial el 4 de octubre de 2022. Disponible en https://www.fertibox.net/single-post/ventajas-compost

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity using the DPPH free radical method. Lebensmittel Wissenchaft und Technologie Food Science and Technology, 28

Cabaleiro, F. A; López-Mosquera, E; Sainz, M. J; & Seoane-Labandeira, S. 2017. Estiércol de pollo peletizado: Potencial fertilizante inmediato y residual en cultivo de lechuga. *Recursos Rurais*. (13). 55-62. DOI: https://doi.org/10.15304/rr.id5059

Hartley, H. (1950). 'The Maximum F-Ratio as a Short-Cut Test for Heterogeneity of Variance', *Biometrika* 37, 308-312.

Huanca A, O; y Blanco V. M. W. 2019. Efecto de la aplicación de abonos orgánicos en el rendimiento del cultivo de beterraga (*Beta vulgaris* L.) en la Estación Experimental de Patacamaya. *Apthapi. 5*(3). 1704. Disponible en URL https://apthapi.umsa.bo/index.php/ATP/article/view/34/34

Libutti, A; Trotta, V; y Rivelli, A.R. 2020. Biochar, vermicompost, and compost as soil organic amendments: Influence on growth parameters, nitrate and chlorophyll content of swiss chard (*Beta vulgaris* L. var. Cycla). *Agronomy. 10*(3). DOI: https://doi.org/10.3390/agronomy10030346

Mamun, A.A; Haya, N; Malarvizhi, C.A.N; y Zainol, N.R.B. 2020. Economic and environmental sustainability through green composting: A study among low-income households. *Sustainability*. 12. Article ID 6488. DOI: 10.3390/su12166488

Martínez, J. D. L., Salas-Pérez, L., Soto, R. V., García, V. J. B., Rangel, P. P., & Seañez, A. R. R. 2017. Efecto del potasio en el contenido fenólico y capacidad antioxidante de *Ocimum basilicum* L. *Revista Mexicana de Ciencias Agrícolas, 8*(1), 133-145.doi.org/https://doi.org/10.29312/remexca.v8i1.77

Moncayo-Luján, M. D. R; Álvarez-Reyna, V. D. P; González Cervantes, G; Salas Pérez, L; & Chávez Simental, J. A. 2015. Producción orgánica de albahaca en invernadero en Comarca Lagunera. *Terra Latinoamericana. 33*(1). 69-77. https://www.terralatinoamericana.org.mx/index.php/terra/article/view/46

Ninfali, P; Antonini, E; Frati, A; y Scarpa, E. S. 2017. C-glycosyl flavonoids from *Beta vulgaris* Cicla and betalains from *Beta vulgaris* rubra: Antioxidant, anticancer and antiinflammatory activities-A review. *Phytotherapy Research. 31*(6): 871-884. DOI: 10.1002/ptr.5819

Ro, S., Long, V., Sor, R., Pheap, S., Nget, R. y William, J. 2022. Alternative feed sources for vermicompost production. *Environment and Natural Resources Journal. 20*(4): 393-399. DOI: 10.32526/ennrj/20/202200009

Rodríguez, G.Y., Alemán P.D.R, Domínguez B.J., Soria R.S., Hernández R.H., Salazar G.Ch., Jara A.M del R. 2016. Efecto de dos abonos orgánicos (compost y biol) sobre el desarrollo morfológico de *Beta vulgaris* L. var. cicla bajo condiciones de invernadero. *Revista Amazónica Ciencia y Tecnología 5*(2), 104-117.

SAGARPA (Secretaría de Agricultura Ganadería, Desarrollo Rural Y Pesca y Alimentación). 2014. Cultivo de acelga. Programa Integral de Desarrollo Rural 2014 Componente de Agricultura Familiar Periurbana y de Traspatio. Carta tecnológica no. 1.

SADER (Secretaría de Agricultura y Desarrollo Rural). 2020. Acelga, una hortaliza muy nutritive. Disponible en https://www.gob.mx/agricultura/articulos/acelga-una-hortaliza-muy-nutritiva

Shapiro, S.S.; Wilk, M.B. (1965). An analysis of variance test for normality: complete samples. *Biometrika*, 52: 591-611.

SIAP (Secretaría de Información Agroalimentaria y Pesquera). 2020. Acelga, una hortaliza muy nutritiva. Disponible en https://www.gob.mx/agricultura/articulos/acelga-una-hortaliza-muy-nutritiva.

Singleton, V.L. y Rossi J. A. 1965. Colorimetry of total phenolics with phosphomolmolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture 16*(3):144-158

Song D., Gao D., Sun H., Qiao L., Zhao R., Tang W. & Li M. 2021. Chlorophyll content estimation based on cascade spectral optimizations of interval and wavelength characteristics. *Computers and Electronics in Agriculture*, 189: Art. 106413. DOI: 10.1016/j.compag.2021.106413

Spaccini, R; y Piccolo, A. 2020. Amendments with humified compost effectively sequester organic carbon in agricultural soils. *Land Degradation & Development. 31*(10): 1206-1216. DOI: https://doi.org/10.1002/ldr.3524

StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10.0. USA: SAS Institute Inc.

Valero G. T; Rodríguez A. P; Ruiz M. E; Ávila T. J.M. y Varela M. G. La alimentación española; características nutricionales de los principales alimentos de nuestra dieta. 2ª. ed. Fundación Española de la Nutrición/Ministerio de Agricultura, Pesca y Alimentación, Madrid, España. 2018. 654 p.

Vázquez-Vázquez, C; Ojeda-Mijares, G. I; Fortis-Hernández, M; Preciado-Rangel, P; & Antonio-González, J. 2015. Sustratos orgánicos en la producción de albahaca (*Ocimum basilicum* L.) y su calidad Fitoquímica. *Revista mexicana de ciencias agrícolas. 6*(8). 1833-1844. DOI: https://doi.org/10.29312/remexca.v6i8.499

Yazid, M; Pusfasari, W; y Wilayana, E. 2020. Social, economic and ecological benefits and farmers' perception of agricultural waste processing in Banyuasin Regency. *IOP Conference Series: Earth and Environmental Science 473 012020*. DOI: 10.1088/1755-1315/473/1/012020

Colegio de Postgraduados

Modification of the composition of thyme (*Thymus vulgaris*) essential oil based on the quality of the light

Morales-Becerril, Carlos de J.[1]**; Colinas-León, Maria T.**[1*]**; Soto-Hernández, Ramón M.**[2]**; Martínez-Damián, Ma. T.**[1]**; Méndoza-Castelán, Guillermo**[1]

[1] Universidad Autónoma Chapingo, Chapingo, Texcoco, Estado de México, México. C. P. 56227.
[2] Colegio de Posgraduados-Área de Botánica. Km. 36.5 carretera México-Texcoco, Montecillo, Texcoco Estado de México, México. C. P. 56264.
* Correspondence: email: lozcol@gmail.com

ABSTRACT

Objective: To identify the changes in the concentration of the main components of thyme (*Thymus vulgaris*) essential oil in response to five different LED colors.

Design/Methodology/Approach: A completely randomized experimental design was used. The design included five treatments (white light; blue light; red light; 75% blue light and 25% red light; and 75% red light and 75% blue light) and 10 repetitions, at a 25 μmol m^{-2} s^{-1} luminous intensity, during a 16 h photoperiod. The thyme plants were sown in a pot with a substrate made up of 50% peat, 48% perlite, and 2% vermicompost. Each plant was an experimental unit. The plants were placed in light isolation chambers and subjected to the treatment for 35 days.

Results: The concentration of the main molecules in the essential oil recorded considerable changes between treatments: the concentration of thymol (its main component) increased in the white light treatments, as well as in the red light (75%) and blue light (75%) treatments. In addition, the composition of the essential oil resulting from these treatments is different to the composition reported in the references.

Study Limitations/Implications: The light intensity used in this experiment was lower than the light intensity required for plant growth; however, it was enough to produce changes in the secondary metabolism.

Findings/Conclusions: The changes in the quality of the light modify the composition of the thyme essential oil. Even at a low light intensity (25 μmol m^{-2} s^{-1}), the changes in the spectrum composition under which the plants grow influence the composition of the essential oil.

Keywords: *Thymus vulgaris*, LEDs, secondary metabolism, terpenoids.

Citation: Morales-Becerril, C. de J., Colinas-León, M. T., Soto-Hernández, R. M., Martínez-Damián, Ma. T., & Méndoza-Castelán, G. (2024). Modification of the composition of thyme (*Thymus vulgaris*) essential oil based on the quality of the light. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2644

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: July 17, 2023.
Accepted: March 18, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 61-67.

INTRODUCTION

Thyme (*Thymus vulgaris*) is a popular plant of the Lamiaceae family; it is native to the Mediterranean and its global distribution is the result of its multiple uses (Hosseinzadeh *et al.*, 2015). Thyme produces up to 1.2% (fresh weight) of essential oil, which is made up of thymol (40-50%), p-cymene (15-20%), y-terpinene (3-15%), carvacrol (3-4%), and δ-cadinene (1-3%) (Soković *et al.*, 2009; Nikolić *et al.*, 2014). Thyme essential oil has antioxidant (Kulisic *et al.*, 2005; Nikolić *et al.*, 2014), antibiotic (Basch *et al.*, 2004; Nikolić *et*

al., 2014), antiviral (Nolkemper *et al.*, 2006), antitumor (Nikolić *et al.*, 2014), and antitussive (Knols *et al.*, 1994) properties. Marchese *et al.* (2016) and Martínez-Pabón and Ortega-Cuadros (2020) agree that these properties are the result of the high content of thymol in the essential oil. Consequently, many researches seek to change its composition, using different chemotypes of thyme and favoring thymol concentration.

Many researches have aimed to prove that changes in the quality of light can influence the growth, development, and secondary metabolism of plants. Currently, this type of researches uses light-emitting diodes (LED) to modify the light environment in which plants develop. LEDs have multiple advantages compared with the light sources used in the past, including: long life, low heat emission, light intensity adjustment, high energy conversion efficiency, and a specific wavelength (Gupta and Agarwal, 2017). As a result of the requirements of the plants, most of the experiments are carried out using at least 80 μmol m^{-2} s^{-1} light intensity.

Nishioka *et al.* (2008) used a red LED (150 μmol m^{-2} s^{-1}, 16 h photoperiod) to carry out a treatment with corn mint (*Mentha arvensis* L.), recording higher menthol, menthone, and limonene contents than the experiments carried out using blue and green LEDs. Nishimura *et al.* (2009) experimented with *Perilla frutescens* (L.) Britt. var. Acuta using red light, red-blue, and red-green wavelength treatments, and obtained plants with lower perillaldehyde and limonene concentrations. These two elements are the main components of that essential oil.

Noguchi and Amaki (2016) conducted an experiment using Mexican mint (*Plectranthus amboinicus*) treated with red light (100 μmol m^{-2} s^{-1},16 h photoperiod). This treatment promoted a higher concentration of α-pinene, β-pinene, and limonene compounds, recording a lower retention time in the column, during the gas chromatography. Meanwhile, green light promoted the concentration of borneol and bornyl acetate compounds, recording an intermediate retention time in the column. Finally, blue light promoted an accumulation of β-farnesene, germancrene D, and elemene compounds, recording the maximum retention time in the column. The authors pointed out that Mexican mint is very sensitive to changes in the quality of the light. Therefore, they concluded that blue light promotes the biosynthetic metabolic pathway of the sesquiterpenoids.

These examples prove that modifying the light environment in which plants grow can promote phytochemical modifications. This situation opens the door to further researches aimed to improve the quality of essential oils of anthropocentric importance, using technologies such as high- and low-intensity LEDs. Consequently, the objective of this research is to describe the changes in the composition of the essential oil of thyme plants, treated with different qualities of low-intensity LEDs.

MATERIALS AND METHODS

The experiment was established from April to July 2021, in a greenhouse of the Posgrado en Horticultura of the Autonomous University of Chapingo. The thyme (*Thymus vulgaris*) seedlings used in the experiments were grown from seeds (Vita®).

The seeds were sown in a substrate made up of 70% peat and 30% perlite; they were grown under greenhouse conditions and watered with running water. Fourteen days after germination, the seedlings were planted in 4-inch-wide pots, with a 50% peat, 48% perlite, and 2% vermicompost substrate. Fertilization was carried out once a week, using a dose of 1,000 mg L^{-1} of the Ultrasol® multipurpose fertilizer (NO_3^- : 9%, NH_4^+ : 9%, P_2O_5: 18%, K_2O: 18%, MgO: 1%, EDTA-Fe: 0.04%, EDTA-Mn: 0.02%, EDTA-Zn: 0.02%, B: 0.01%, EDTA-Cu: 0.01%, Mo: 0.01%). Watering was carried out every two days, using running water. The plants were kept under natural light conditions for 28 days and were subjected to two trims (at 10 and 24 days), in order to promote branching and homogenization of the aerial part of the plant. Subsequently, the plants were subjected to completely controlled light conditions, remaining under these conditions until the experiment was completed.

Each thyme plant was an experimental unit. Five treatments with ten repetitions each were established. The experimental design was completely random. The treatments were: monochromatic blue (B) light; monochromatic red (R) light; a combination of 75% blue light and 25% red light (75B:25R); and 75% red light and 75% blue light (75R:25B); and white light (W).

RGB 5050 (Weluvfit®) LED lightning tapes were used as source of blue and red lights and their combinations. They had 30 modules per meter (5 m) and were installed on 15×40 cm wooden plates. As source of white light, a 3528 (Tunix®) LED lightning tape was used, with 60 modules per meter. The height of the plates was adjusted to influence the plants. The 25 μmol m^{-2} s^{-1} light intensity was measured with a QMSW-SS Apogee® radiometer. The photoperiod lasted for 16 hours, from 6:00 am to 10:00 pm. The treatments were established in 80 cm long×40 cm wide×80 cm tall boxes, with a white interior. Air circulation was provided with a 4-inch fan (12 v), placed lengthways in the back side of the chamber. The fan was switched on every hour: 10 minutes, from 6:00 to 11:00 am and 06:00 and 10:00 pm and 15 minutes, from 12:00 to 05:00 pm. The temperature inside the greenhouse was kept under 28 °C, using fans controlled by a temperature sensor. Inside the boxes, the plants were rotated on a daily basis and the treatments were changed to another box, every four days, in order to reduce the experimental error.

The aerial part of the plants was harvested 35 days after the beginning of the LED treatments. In order to extract the essential oil, the thyme was crushed and 7.4 ml of ethyl acetate (Sigma®) were added per gram of plant material. The mixture was allowed to rest for 30 m and, afterwards, it was filtered.

The extracts were taken to 10 mL with ethyl acetate; subsequently, they were dried with anhydrous sodium sulfate and filtered. In order to analyze the oils, an Agilent Technologies 5973Network Mass Selective Detector (with a HP 5973 coupled mass detector) and a column with an internal diameter of HP5/30 m×0.225 mm were used. The temperature of the oven was initially kept at 60 °C for 1 minute; afterwards, it was linearly increased to 260 °C, at a 7 °C min^{-1} speed. This final temperature was kept for 1 minute. The helium constant flux was 1 mL min^{-1} and the gas had a 99.999% purity. The temperature of the splitless injector was 260 °C. The ionization energy was 70 eV. The temperatures of the ion source and the quadruple were 250 and 150 °C, respectively. The interphase temperature was 280 °C. The injection volume was 1 μL. The compounds were identified comparing the mass spectra of the peaks of interest and the NIST 2011 library.

The statistical analysis was carried out with an analysis of variance, while the following variables were subjected to a Tukey's mean comparison test ($p \leq 0.05$): concentration of the five main molecules of the essential oil of each treatment and concentration of each main molecule in the different treatments. The SAS® 9.0 statistical software was used.

RESULTS AND DISCUSSION

The main compounds of the essential oil obtained in this research were: thymol (38-52%), β-terpinene (14-23%), sulphureous acid, dodecyl 2-propyl ester (6-20%), perillene (4-7%), and thymol acetate (3-8%). Except for thymol, the content and concentration of the other molecules contrast with the data reported in the references, because several authors agree that the thyme essential oil is made up of: thymol (40-50%), p-cimene (15-20%), γ-terpinene (3-15%), carvacrol (3-4%), β-cariophyllene (2-4%), and δ-cadinene (1-3%) (Soković *et al.*, 2009; Nikolić *et al.*, 2014). The practically inexistent match between those results and the results of this study can be the consequence of the different light intensity used in the researches. The plants of those studies were grown outdoor or in greenhouses (high light intensity), while the plants of this research were subjected to a low light intensity (25 μmol m^{-2} s^{-1}). These differences strongly influence the composition of the essential oil (Noguchi and Amaki, 2016; Milenković *et al.*, 2021).

Thymol recorded the highest concentration in all the treatments. In addition, all the following molecules were identified in all the treatments: the β-terpinene and perillene cyclical monoterpenoids (Figure 1), which are products of the same biosynthetic pathway than thymol (Tholl, 2015). Only the red light treatment and the two dichromatic treatments reported the presence of γ-terpinene di-epoxide (a precursor of thymol) (Thompson *et al.*, 2003).

The effect of the quality of the light on the concentration of the identified molecules is remarkable (Table 1). The thymol concentration was higher in the white light and 75R:25B treatments and lower in the rest of the treatments. These results contrast with the findings of Tohini *et al.* (2020), who reported a higher increase of thymol concentration in thyme plants subjected to a blue light treatment. Ahmadi *et al.* (2021) carried out an experiment with Melissa officinalis (300 μmol m^{-2} s^{-1}, 16 h photoperiod) and determined that the quality of the light interacts with the genotype in the case of the thymol synthesis. In one of the studied genotypes, the maximum amount of thymol was recorded with the blue light treatment, while the highest accumulation was recorded by another treatment with the use of white light. The authors concluded that red light promoted the accumulation of monoterpenes in both genotypes, while blue light promoted the accumulation of sesquiterpenes. Meanwhile, the effects of white light were intermediate for both colors. Such differences can be the result of the unequal light intensity used in both experiments. Tohini *et al.* (2020) used a 300 μmol m^{-2} s^{-1} intensity, while the intensity used in this study was 12 times lower. For their part, Rios-Estepa *et al.* (2008) reported that changes in light intensity can influence some of the steps of the biosynthetic path of the different molecules that make up the essential oil.

Consequently, further research is need to evaluate the effects of light intensity, through different spectrum compositions, on the secondary metabolism of different species.

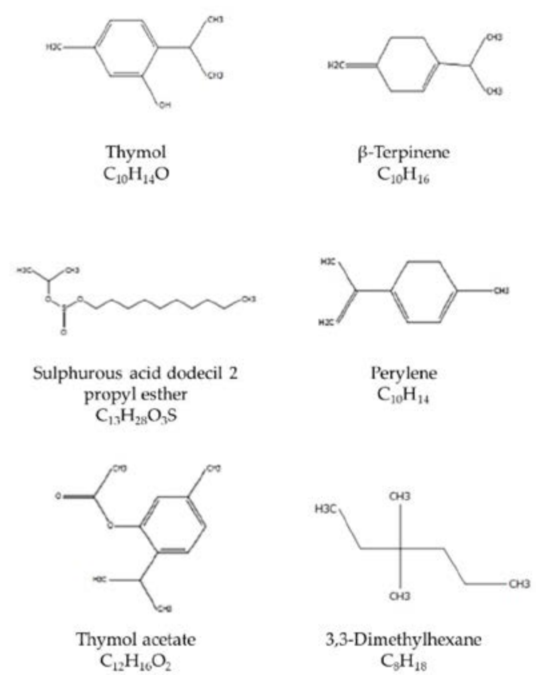


Figure 1. Structures of the molecules with higher concentration in the essential oil of thyme plants, grown under LEDs with different colors.

Table 1. Relative concentration of the main molecules found in the essential oil of thyme, grown with different LED colors.

<	**R.T.*** **(min.)**	**Relative content (%)**				
Molecule		**Blanco**	**Rojo**	**75R:25A**	**75A:25R**	**Azul**
Thymol	11.61	51.08 a**	43.98 b	52.26 a	38.56 b	41.42 b
β-Terpinene	7.01	21.55 a	23.13 a	21.13 a	16.44 b	14.42 b
Sulphurous acid dodecil 2 propyl esther,	29.42	14.72 b	13.9 b	6.4 c	17.75 a	18.24 a
Perylene	6.36	5.07	5.03	4.74	6.81	6.52
Thymol acetate	12.81	3.57 b	7.87 a	4.93 b	4.37 b	5.01 b
4-acetoxy-3-methoxystyrene	11.81	1.81	1.52	1.67	1.43	1.44
Hexadecane	29.18	-	-	-	-	14.28
3, 3-Dimethyilhexane	29.21	-	-	6.64 b	13.89 a	-
γ-Terpinene diepoxide	7.84	-	0.94 b	0.83 b	1.45 a	-

*RT (T.R): Retention time. **Values followed by similar letters in each row are not statistically different ($p \leq 0.05$). Relative content was calculated based on the areas of the curves obtained from the gas chromatography.

Most of the molecules found in the experiment recorded a low retention time in the column and only three of them reached a high value (>15 min). The blue and 75B:25R treatments recorded a higher ($p \leq 0.05$) relative concentration of sulfurous acid and dodecyl 2-propyl ester (Table 1). Hexadecane was only found in the blue monochromatic treatment; meanwhile, the highest 3, 3-dimethylhexane values were recorded when the blue light ratio was higher. However, it was not identified in the monochromatic blue treatment. The results suggest that, under a monochromatic or a high ratio of blue light, the plants accumulate molecules of high retention time in the column. This effect has already been reported by Noguchi and Amaki (2016), who carried out experiments with *Plectranthus amboinicus*. In addition, these authors reported that red light promotes the synthesis of compounds with a low retention time in the column. These data match the findings of this research, but only in the case of β-terpinene and perillene. This response is related to the gene expression codified by different enzymes that take part in the biosynthetic path of terpenoids, which are positively regulated by blue and red lights (Fu *et al.*, 2015).

This study proves that modifying the composition of thyme essential oil is possible, if the plants are subjected to different spectra compositions, even at a low light intensity and for a relatively short period.

CONCLUSIONS

The changes in the quality of the light modify the composition of the thyme essential oil. Even at a low light intensity (25 μmol m^{-2} s^{-1}), the changes in the spectrum composition under which the plants grow influence the composition of the essential oil.

REFERENCES

Ahmadi, T., Shabani, L. & Sabzalian, R. (2021). LED light sources improved the essential oil components and antioxidant activity of two genotypes of lemon balm (*Melissa officinalis* L.). *Botanical Studies, 62*(1), 1-13. https://doi.org/10.1186/s40529-021-00316-7

Basch, E., Ulbricht, C., Hammerness, P., Bevins, A. & Sollars, D. (2004). Thyme (*Thymus vulgaris* L.), thymol. *Journal of herbal pharmacotherapy 4*(1): 49-67. https://doi.org/10.1080/J157v04n01_07

Fu, X., Chen, Y., Mei, X., Katsuno, T., Kobayashi, E., Dong, F., Watanabe, N. & Yang, Z. (2015). Regulation of formation of volatile compounds of tea (*Camellia sinensis*) leaves by single light wavelength. *Scientific reports 5*(1): 1-11. https://doi.org/10.1038/srep16858

Gupta, S. & Agarwal, A. (2017). Artificial lighting system for plant growth and development: Chronological advancement, working principles, and comparative assessment. In Light emitting diodes for agriculture (pp. 1-25). Springer, Singapore. https://doi.org/10.1007/978-981-10-5807-3_1

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. & Armand, R. (2015). The application of medicinal plants in traditional and modern medicine: a review of *Thymus vulgaris*. *International Journal of Clinical Medicine 6*(09): 635. https://doi.org/10.4236/ijcm.2015.69084

Knols, G., Stal, P. & Van Ree, J. (1994). Productive coughing complaints: Sirupus Thymi or Bromhexine? A double-blind randomized study. *Huisarts en Wetenschap* 37: 392-394.

Kulisic, T., Radonic, A. & Milos, M. (2005). Antioxidant properties of thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) essential oils. *Italian journal of food science 17*(3): 315.

Marchese, A., Orhan, I., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S., ... & Nabavi, S. (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food chemistry* 210: 402-414. https://doi.org/10.1016/j.foodchem.2016.04.111

Martínez-Pabón, M. & Ortega-Cuadros, M. (2020). Timol, mentol y eucaliptol como agentes para el control microbiológico en cavidad bucal: una revisión exploratoria. *Revista Colombiana de Ciencias Químico Farmacéuticas 49*(1). https://doi.org/10.15446/rcciquifa.v49n1.87006

Milenković, L., Ilić, Z., Šunić, L., Tmušić, N., Stanojević, L., Stanojević, J. & Cvetković, D. (2021). Modification of light intensity influence essential oils content, composition and antioxidant activity of thyme, marjoram and oregano. *Saudi Journal of Biological Sciences*. https://doi.org/10.1016/j.sjbs.2021.07.018

Nikolić, M., Glamočlija, J., Ferreira, I., Calhelha, R., Fernandes, Â., Marković, T., & Soković, M. (2014). Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. *Industrial Crops and Products* 52: 183-190. https://doi.org/10.1016/j.indcrop.2013.10.006

Nishimura, T., Ohyama, K., Goto, E. & Inagaki, N. (2009). Concentrations of perillaldehyde, limonene, and anthocyanin of Perilla plants as affected by light quality under controlled environments. *Scientia Horticulturae 122*(1): 134-137. https://doi.org/10.1016/j.scienta.2009.03.010

Nishioka, N., Nishimura, T., Ohyama, K., Sumino, M., Malayeri, S. H., Goto, E., & Morota, T. (2008). Light quality affected growth and contents of essential oil components of Japanese mint plants. In International Workshop on Greenhouse Environmental Control and Crop Production in Semi-Arid Regions 797: 431-436. https://doi.org/10.17660/ActaHortic.2008.797.62

Noguchi, A. & Amaki, W. (2016). Effects of light quality on the growth and essential oil production in Mexican mint. In VIII International Symposium on Light in Horticulture 1134 (pp. 239-244). https://doi.org/10.17660/ActaHortic.2016.1134.32

Nolkemper, S., Reichling, J., Stintzing, F., Carle, R. & Schnitzler, P. (2006). Antiviral effect of aqueous extracts from species of the Lamiaceae family against Herpes simplex virus type 1 and type 2 *in vitro*. *Planta medica 72*(15): 1378-1382. https://doi.org/10.1055/s-2006-951719

Rios-Estepa, R., Turner, G., Lee, M., Croteau, R. & Lange, B. (2008). A systems biology approach identifies the biochemical mechanisms regulating monoterpenoid essential oil composition in peppermint. *Proceedings of the National Academy of Sciences, 105*(8), 2818-2823. https://doi.org/10.1073/pnas.0712314105

Soković, M., Vukojević, J., Marin, P., Brkić, D., Vajs, V. & Van Griensven, L. (2009). Chemical composition of essential oils of thymus and mentha species and their antifungal activities. *Molecules 14*(1): 238-249. https://doi.org/10.3390/molecules14010238

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. *Biotechnology of isoprenoids* 63-106. https://doi.org/10.1007/10_2014_295

Thompson, J., Chalchat, J., Michet, A., Linhart, Y. & Ehlers, B. (2003). Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *Journal of chemical ecology 29*(4): 859-880. https://doi.org/10.1023/A:1022927615442

Tohidi, B., Rahimmalek, M., Arzani, A. & Sabzalian, M. (2020). Thymol, carvacrol, and antioxidant accumulation in Thymus species in response to different light spectra emitted by light- emitting diodes. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2019.125521

Water supply in artificial troughs: a strategy to mitigate the impacts of climate change in the Maya forest

Contreras-Moreno, Fernando M.[1,2*]; Jesús-Espinosa, Daniel[3]; Cruz-Romo, Lizardo[4]; Sánchez-Pinzón, Khiavett G.[3]; Méndez-Tun, José M.[3,5]; Pérez-Méndez, Florentino[6]; Tamay-Yah, Luis[6]; Gutiérrez-Cortés, Ernesto[6]; Duque-Moreno, Víctor D.[3]; Díaz-Ruiz, Yulisenia[3,7]; Servín-Camacho, Dulce Y.[3,7]; Méndez-Saint Martin, Gabriela[2]; David Simá-Pantí[8]; Pedro Bautista-Ramírez[3]

[1] World Wildlife Fund Inc.-México, Xpujil, Calakmul, Campeche, México, C. P. 24640.
[2] Universidad Tecnológica de Calakmul, Xpujil, Calakmul, Campeche, México, C. P. 24640.
[3] Grupo de Monitoreo Socioambiental, Balancán, Tabasco; México, C. P. 86930.
[4] Espacios Naturales y Desarrollo Sustentable A.C. Tlalpan, Ciudad de México, México, C.P. 14090
[5] Universidad Autónoma de Campeche, San Francisco de Campeche, Campeche, México, C. P. 24039.
[6] Reserva de la Biosfera de Calakmul, Xpujil, Calakmul, Campeche, México, C. P. 24640.
[7] Universidad para el Bienestar Benito Juárez García-Calakmul, Xpujil, Calakmul, Campeche, México, C. P. 24640.
[8] Santuario Islas de la Bahía de Chamela, Punta Perúla, La Huerta, Jalisco, C. P. 48850.
* Correspondence: fernandom28@hotmail.com

Citation: Contreras-Moreno, F. M., Jesús-Espinosa, D., Cruz-Romo, L., Sánchez-Pinzón, K. G., Méndez-Tun, J. M., Pérez-Méndez, F., Tamay-Yah, L., Gutiérrez-Cortés, E., Duque-Moreno, V. D., Díaz-Ruiz, Y., Servín-Camacho, D. Y., & Méndez-Saint, M. G. (2024). Water supply in artificial troughs: a strategy to mitigate the impacts of climate change in the Maya forest. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2647

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: July 21, 2023.
Accepted: March 25, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 69-77.

ABSTRACT

Objective: To verify the functionality of drinking troughs based on fauna record.
Design/Methodology/Approach: Most of the approximately 70 artificial drinking troughs were installed inside the core zones of the Calakmul Biosphere Reserve (CBR). The remaining troughs were installed in communities and left under the protection and supervision of beekeepers. A camera-trap station was associated with each of the drinking troughs installed.
Results: Ninety-one wildlife species that drink water from the artificial troughs have been identified, including 30 mammals, 53 birds, 5 reptiles, and three amphibians. Jaguars, tapirs, and other endangered species are some of the most frequent visitors to these sites, especially during the dry season.
Study Limitations/Implications: The use of artificial water troughs is an adequate alternative to guarantee water availability in the Calakmul region. The high diversity of species that constantly visit the drinking troughs during the dry season makes evident the functionality of the water supply strategy with artificial drinking troughs.
Findings/Conclusions: The drinking troughs can be a tool for various objectives in the region. It arose from the need to respond to emergency climatological events (*i.e.*, droughts), but it has been adapted to the regional needs and other activities. It has been considered a successful management strategy in the face of climate change.

Keywords: Drinking troughs, camera-trapping, mammals, drought, climate change.

INTRODUCTION

Climate change is characterized by long-term changes in temperature and climate patterns (Kardol *et al.*, 2010). The hydrological cycle has been one of the main axes of study, given the possible increase in flood and drought risks (Chou *et al.*, 2013).

Water availability is a habitat-specific resource that can influence the spatial distribution of wildlife (Rich *et al.*, 2019), especially in sites where precipitation has been compressed. However, some animals meet their water demand with their food (Nagy and Gruchacz, 1994). Although most species depend on surface water (Moro-Ríos *et al.*, 2008), they adjust their behavior in the face of scarcity (Hofmann *et al.*, 2015).

In places where water has been identified as a limiting resource, rainfall can have a major impact, since it influences many of the animals' movements (Bello *et al.*, 2001).

Artificial water sources (*i.e.*, artificial ponds, dams, reservoirs, and drinking troughs) have historically been used to counteract the impacts of prolonged droughts in Mexico (Villarreal, 2006). This measure contributes to the enrichment of the habitat and the maintenance of wildlife populations (Bello *et al.* 2001). In particular, the implementation of drinking troughs for wildlife has been reported as a successful strategy to mitigate the consequences of the lack of water in times of drought, mainly in places where water is the limiting resource (Mandujano-Rodríguez and Hernández, 2019; Borges-Zapata *et al.*, 2020).

The use of these artificial water sources is part of various strategies, some of which aim to maintain wildlife populations within sites with better conditions, such as protected natural areas (PNA). These strategies would prevent the death of animals when they explore new sites (Borges-Zapata *et al.*, 2020). Filling drinking troughs with water is conceived as a measure that can contribute to the short-term maintenance of wildlife populations, since it allows animals to have access to water during the dry season (Borges-Zapata *et al.*, 2020).

Mardero *et al.* (2018) have documented increasingly extreme temperatures in the Yucatan Peninsula in recent years, with more severe and longer temperature events, which force wildlife to resort to water as a thermo-regulation factor. The Maya Forest, particularly the Calakmul region (in southeastern Mexico), lacks fast-flowing rivers or extensive surface water bodies (García-Gil *et al.*, 2002). The mismatch in precipitation patterns that has been recorded in recent years (Mardero *et al.*, 2018) has prevented the aguadas, natural water reservoirs found in the region (Reyna-Hurtado *et al.*, 2022), from capturing enough water to remain full during the dry season.

Given the imminent need for surface water in the Maya Forest that will be the consequence of global climate change, the Calakmul Biosphere Reserve, in collaboration with non-governmental organizations (NGOs), has established a water supply strategy for wild fauna, through a network of drinking troughs. Discussing that experience is the focus of this article.

MATERIALS AND METHODS

The Calakmul Biosphere Reserve (CBR) is located within the Yucatan Peninsula, to the southeast of the state of Campeche. It is part of the Greater Calakmul Region, which includes the Maya Biosphere Reserve in Guatemala and the Río Bravo Dos Milpas conservation area in Belize. It has an area of 723,185.12 ha (Reyna-Hurtado *et al.*, 2022).

The CBR has a warm and subhumid climate (Aw), with an average annual temperature of 24.6 °C. The maximum height is found on the Champerico hill (390 m.a.s.l.), while the minimum height varies from 100 to 150 m. The dominant vegetation types are medium sub-evergreen forests, medium sub-deciduous forests, and low sub-deciduous forests (Martínez and Galindo, 2002; Martínez-Kú *et al.*, 2008).

Installation of drinking troughs

As part of the efforts of the CBR, in collaboration with the GEF project for species at risk and WWF Mexico, to counteract the impacts of climate change in the region, approximately 70 artificial drinking troughs were installed in a water supply network. Most of them were located within the core zones of the PNA, while the rest were established in communities under the protection and supervision of beekeepers. The drinking troughs that were set up in the CBR consisted of 300 L black Rotoplas® plastic structures (Figure 3). The troughs were distributed along the CBR access road (Figure 1), with a minimum distance of 2 km between stations (troughs). At the beginning of the dry season (Figure 4), water was usually supplied twice a month (every 15 days); however, as the dry season marched on and became harsher, sometimes water had to be supplied every 7 days.

Data from camera traps

To verify if the strategy was functional for the purposes of the CBR, the biological monitoring protocol in drinking troughs was created in 2018, based mainly on the recording of wildlife with camera traps (Borges-Zapata *et al.*, 2020; Contreras-Moreno *et al.*, 2020).

RESULTS AND DISCUSSION

Currently, 91 wildlife species that drink water from the troughs have been identified: 30 mammals, 53 birds, 5 reptiles, and 3 amphibians (Table 1). Twenty-nine of these species are classified in one of the risk categories established by the NOM-059-SEMARNAT-2010

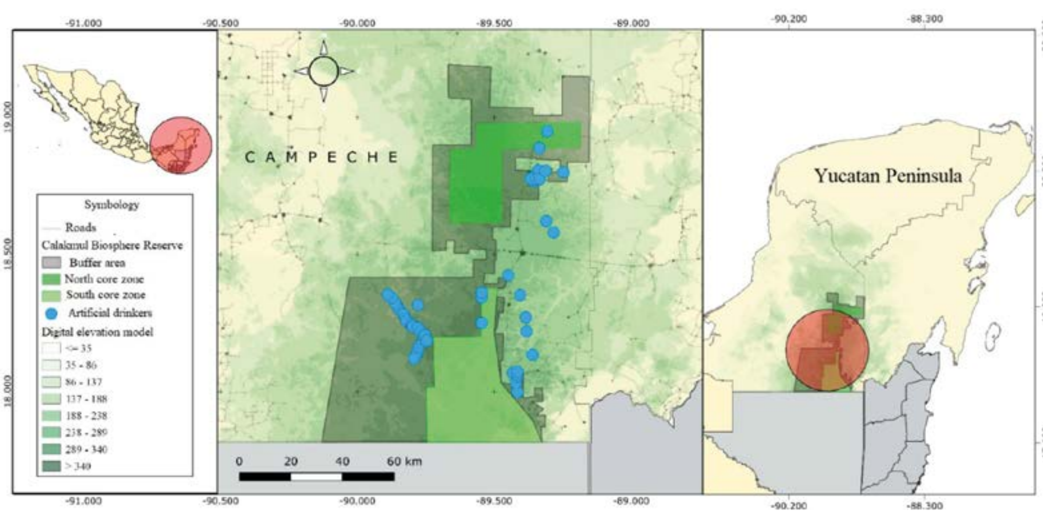


Figure 1. The position of the drinking troughs in the Calakmul Biosphere Reserve and neighboring ejidos in southeastern Mexico are shown in blue circles.

Table 1. Species recorded in drinking troughs.

Group	Family	Taxonomy	Risk category	
			NOM-059	UICN
Anfibios	Hylidae	*Dendropsophus microcephalus*	-	LC
		Scinax staufferi	-	LC
		Smilisca baudinii	-	LC
Reptiles	Anolidae	*Anolis sagrei*	-	LC
	Colubridae	*Drymobius margaritiferus*	-	LC
	Kinosternidae	*Kinosternon creaseri*	-	LC
	Phrynosomatidae	*Sceloporus chrysostictus*	-	LC
	Teiidae	*Holcosus gaigeae*	-	LC
Aves	Accipitridae	*Buteo plagiatus*	-	LC
		Buteogallus anthracinus	Pr	LC
		Chondrohierax uncinatus	Pr	LC
		Leptodon cayanensis	Pr	LC
		Rupornis magnirostris	-	LC
		Spizaetus ornatus	P	NT
	Aramidae	*Aramus guarauna*	A	LC
	Cardinalidae	*Cyanocompsa cyanoides*	-	LC
		Cyanocompsa parellina	-	LC
		Cyanoloxia cyanoides	-	LC
		Passerina ciris	Pr	LC
		Piranga rubra	-	LC
	Cathartidae	*Cathartes aura*	-	LC
	Columbidae	*Claravis pretiosa*	-	LC
		Geotrygon montana	-	LC
		Patagioenas flavirostris	-	LC
		Patagioenas nigrirostris	Pr	LC
		Patagioenas speciosa	Pr	LC
	Corvidae	*Psilorhinus morio*	-	LC
		Cyanocorax yucatanicus	-	LC
	Cracidae	*Crax rubra*	A	VU
		Ortalis vetula	-	LC
		Penelope purpurascens	A	LC
	Cuculidae	*Piaya cayana*	-	LC
	Falconidae	*Micrastur ruficollis*	Pr	LC
		Micrastur semitorquatus	Pr	LC
	Furnariidae	*Dendrocincla homochroa*	-	LC
	Icteridae	*Quiscalus mexicanus*	-	LC
	Mimidae	*Dumetella carolinensis*	-	LC
		Melanoptila glabrirostris	Pr	NT
	Momotidae	*Eumomota superciliosa*	-	LC
	Parulidae	*Geothlypis formosa*	-	LC
		Mniotilta varia	-	LC
		Setophaga citrina	-	LC
		Setophaga ruticilla	-	LC
	Phasianidae	*Meleagris ocellata*	A	NT
	Picidae	*Dryocopus lineatus*	-	LC
	Rallidae	*Aramides albiventris*	-	LC

Table 1. Continues...

Group	Family	Taxonomy	Risk category	
			NOM-059	UICN
Aves	Ramphastidae	*Ramphastos sulfuratus*	A	LC
		Pteroglossus torquatus	Pr	LC
	Strigidae	*Glaucidium brasilianum*	-	LC
		Strix virgata	-	LC
	Thraupidae	*Thraupis episcopus*	-	LC
	Tinamidae	*Tinamus mayor*	A	NT
		Crypturellus cinnamomeus	Pr	LC
	Trochilidae	*Amazilia yucatanensis*	-	LC
	Turdidae	*Catharus ustulatus*	-	LC
		Hylocichla mustelina	-	LC
		Turdus grayi	-	LC
	Tyrannidae	*Pachyramphus aglaiae*	-	LC
		Pitangus sulphuratus	-	LC
		Pyrocephalus rubinus	-	LC
Mamíferos	Atelidae	*Ateles geoffroyii*	P	EN
	Canidae	*Canis latrans*	-	LC
		Urocyon cinereoargenteus	-	LC
	Cervidae	*Mazama pandora*	-	VU
		Mazama temama	-	DD
		Odocoileus virginianus	-	LC
	Cuniculidae	*Cuniculus paca*	-	LC
	Dasypodidae	*Dasypus novemcinctus*	-	LC
	Dasyproctidae	*Dasyprocta punctata*	-	LC
	Didelphidae	*Didelphis marsupialis*	-	LC
		Didelphis virginiana	-	LC
		Philander opossum	A	LC
		Tlacuatzin canescens	-	LC
	Felidae	*Herpailurus yagouaroundi*	A	LC
		Leopardus pardalis	P	LC
		Leopardus wiedii	P	NT
		Panthera onca	P	NT
		Puma concolor	-	LC
	Mephitidae	*Conepatus semistriatus*	-	LC
		Spilogale angustifrons	-	LC
	Mustelidae	*Eira barbara*	P	LC
	Myrmecophagidae	*Tamandua mexicana*	P	LC
	Phyllostomidae	*Desmodus rotundus*	-	LC
	Procyonidae	*Nasua narica*	-	LC
		Procyon lotor	-	LC
	Sciuridae	*Sciurus deppei*	-	LC
		Sciurus yucatanensis	-	LC
	Sigmodontinae	*Sigmodon toltecus*	-	LC
	Tapiridae	*Tapirus bairdii*	P	EN
	Tayassuidae	*Pecari tajacu*	-	LC
		Tayassu pecari	P	VU

official Mexican standard: 11 as subject to special protection (Pr), 8 as threatened (A), and 9 in danger of extinction (P).

The mammals that frequently visit the drinking troughs include both predators (*i.e.*, jaguars and pumas; Figure 2a and 2b) and herbivores (Figure 2c and 2d). Females with young have been likewise observed. Photographs taken in the troughs help to identify the species and their body state, and intra and interspecies interactions, as well as little-known behaviors (Figures 3a-3d).

The presence of key species that constantly use drinking troughs in the Maya Forest region makes it clear that this strategy is ecologically functional for the conservation of high-priority species in terrestrial ecosystems. Therefore, this method partially addresses one of the multiple threats that these species face.

Birds also constantly use the troughs. The most striking specimes belong to the regional birds of prey (*e.g.*, ornate hawk-eagles), tropical forest species (*e.g.*, toucans), and endemic species (*e.g.*, ocellated turkeys) (Figure 4 a,b,c, d).

The number of drinking troughs installed in the CBR was doubled in 2019, as a response to the peak of an intense and prolonged drought recorded in the Calakmul region. In that same year, a high number of sightings of tapirs (*Tapirus bairdii*) in poor physical condition and with dehydration symptoms was registered near or within human settlements (Pérez-Flores *et al.*, 2021). However, it is likely that the impacts of 2019 were not only caused by the lack of water, but also by the increase in the number of fires in the area (Contreras-Moreno, personal communication).

Figure 2. Jaguars (2a) and pumas (2b) are species of great importance for the Calakmul Biosphere Reserve, along with all herbivores such as tapirs (2c *Tapirus bairdii*), and red brocket deer (2d *Mazama temama*). All these species benefit from the water supply provided by the drinking troughs.

Figure 3. The photographic record helps to identify the intra and inter-species relationships of the animals that drink from the troughs. Figure 3a shows a coati (*Nasua narica*) and a great curassow (*Crax rubra*), while figure 3b shows a pair of Yucatan brown brockets (*Mazama pandora*). Species that rarely come down to the ground, such as the Geoffroy's spider monkey (*Ateles geoffroyi*), or curious carnivores such as the tayra (*Eira barbara*), have likewise been photographed (Figures 3c and 3d).

Figure 4. The jungle birds of the Calakmul region (*e.g.*, ornate hawk-eagles (4a) and eagles (4b)) also use artificial drinking troughs, not only for drinking but also as a bathtub. Likewise, tropical birds, such as the ocellated turkey (4c) and the toucan (4d), benefit from the throughs.

The impacts of climate change have likely increased the probability of negative interactions between some wildlife species and people (Abrahms *et al.* 2023). Therefore, supplying water to wildlife through drinking troughs could minimize these potential conflicts.

CONCLUSIONS

The use of drinking troughs is a good alternative to guarantee the availability of water in the Calakmul region. The high diversity of species that constantly visit the drinking troughs in the Maya Forest during the dry season makes evident the functionality of the water supply strategy using artificial drinking troughs. Drinking troughs provide individuals from diverse populations with access to water and enable their interaction. Therefore, throughs could be considered spaces for socialization, as shown by the photos of their interactions. The drinking troughs strategy helps to reduce conflict between various wildlife species and people.

ACKNOWLEDGMENTS

The authors would like to thank project 00092169: "Strengthening Management of the PA System to Better Conserve Endangered Species and Their Habitats". The project was implemented by the United Nations Development Program (UNDP), executed by the Comisión Nacional de Áreas Naturales Protegidas (CONANP), and funded by the Global Environment Facility (GEF). The authors would also like to thank the personnel of the Calakmul Biosphere Reserve (CBR), who were always eager to support the monitoring project. We would also like to thank the Word Wildlife Fund Inc. (WWF-Mexico) for the funding granted through the "Monitoring water bodies in the CBR" project, part of the "Saving the jaguar: an ambassador for the Americas" project. Finally, we would like to thank to community monitors of the ejidos of Calakmul, who were always eager to support the monitoring project and the community surveys.

REFERENCES

Abrahms, B., Carter, N. H., & Clark-Wolf, T. J., Gaynor, K. N., Johansson, E., McInturff, A., Nisi, A. C., Rafiq, K., & West, L. (2023). Climate change as a global amplifier of human–wildlife conflict. *Nature Climate Change* 13, 224-234. https://doi.org/10.1038/s41558-023-01608-5

Bello, J., Gallina, S., Equihua, M., Mandujano, S., & Delfín, C. (2001). Activity areas and distance to water sources by White-tailed deer in northeastern Mexico. *Vida Silvestre Neotropical, 10*(1-2), 30-37.

Borges-Zapata, J. Y., Contreras-Moreno, F. M., Serrano-MacGregor, I., Sima-Pantí, D. E., Coutiño-Cal, C., Zúñiga-Morales, J. A., Duque-Moreno, V., Hernandez-Pérez, E., & López-Chan, J. A. (2020). Uso de bebederos artificiales por el sereque centroamericano (*Dasyprocta punctata*) en la reserva de la biosfera de Calakmul, México. *Agro Productividad, 13*(1).

Chou, C., Chiang, J., Lan, C. W. Lan, C., Chung, C., Liao, L., & Lee, C. (2013). Increase in the range between wet and dry season precipitation. *Nature Geoscience* 6, 263-267 (2013). https://doi.org/10.1038/ngeo1744

García-Gil, G., Palacio, J., & Ortiz, M. (2002) Reconocimiento geomorfológico e hidrográfico de la Reserva de la Biosfera Calakmul, México", en *Investigaciones Geográficas* 48:7-23.

Kardol, P., Campany, C., Souza, L., Norby, R., Weltzin, J., & Classen, A. (2010). Climate change impacts on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. *Global Change Biology* 16, 2676-2687. https://doi.org/10.1111/j.1365-2486.2010.02162.x

Mandujano-Rodriguez, S., & Carlos, H. (2019). Uso de bebederos artificiales por venado cola blanca en una UMA extensiva en la reserva de la biosfera Tehuacan-Cuicatlan, México. *Agroproductividad* 12, 37-42. https://doi.org/10.32854/agrop.v0i0.1406

Mardero, S., Schmook, B., Christman, Z., Metcalfe, S. E., & De la Barreda-Bautista, B. (2020). Recent disruptions in the timing and intensity of precipitation in Calakmul, Mexico. *Theoretical and Applied Climatology*, 140, 129-144. https://doi.org/10.1007/s00704-019-03068-4

Martinez, E., & Galindo-Leal, C. G. (2022). La vegetacion de Calakmul, Campeche, México: clasificacion, descripcion y distribucion. *Boletin de la Sociedad Botanica de Mexico*, (71), 7-32.

Martínez-Kú, D. H., Escalona-Segura, G., & Vargas-Contreras, J. A. (2008). Importancia de las aguadas para los mamíferos de talla mediana y grande en Calakmul, Campeche, México. Avances en el estudio de los mamiferos II. *Asociacion Mexicana de mastozoologia AC México*, 449-468.

Moro-Rios, R. F. (2008). Obtençao de água por um grupo de Alouattaclamitans (Primates: Atelidae), em floresta com araucária: variaçoes sazonais, sexo-etárias e circadianas. *Rev Bras Zool*, 25, 558-562.

Pérez-Flores, J., Mardero, S., López-Cen, A., & Contreras-Moreno, F. M. (2021). Human-wildlife conflicts and drought in the greater Calakmul Region, Mexico: implications for tapir conservation. *Neotropical Biology and Conservation* 16, 539-563. doi: 10.3897/neotropical.16.e71032

Reyna-Hurtado, R., García-Anleu, R., García-Vetorazzi, M., Sanchez-Pinzón, K., Slater, K. Barão-Nobrega, J., Contreras, F., Méndez-Saint Martin, G., Sima-Panti, D., Martínez, W., Cal, R., & Ponce, G. (2022). Aguadas de la Selva Maya: Santuarios de vida silvestre que unen esfuerzos de conservación internacional. *Ciencia Nicolaita* 84. https://doi.org/10.35830/cn.vi84.610

Rich, L. N., Beissinger, S. R., Brashares, J. S., & Furnas, B. J. (2019). Artificial water catchments influence wildlife distribution in the Mojave Desert. *The Journal of Wildlife Management, 83*(4), 855-865. https://doi.org/10.1002/jwmg.21654

Villarreal J. 2006. Venado Cola Blanca: Manejo y Aprovechamiento Cinegético. 2ª Ed. Unión Ganadera Regional de Nuevo León. México. 410 pp.

Colegio de Postgraduados

Levels of fat for potential consumption of juvenile *Ambystoma mexicanum* (Shaw & Nodder, 1798) axolotls: Lipid levels

Manjarrez-Alcívar, Itzel[1,2]; **Martínez-Cárdenas, Leonardo**[3*]; **Vega-Villasante, Fernando**[2]; **Badillo-Zapata, Daniel**[2,4,*]; **Montoya-Martínez, Cynthia E.**[2,5]; **López-Félix, Erick F.**[6]

[1] Universidad Autónoma de Nayarit, Maestría en Ciencias Biológico-Agropecuarias, México.
[2] Universidad de Guadalajara, Laboratorio de Calidad de Agua y Acuicultura Experimental. Centro Universitario de la Costa, México.
[3] Universidad Autónoma de Nayarit, Secretaría de Investigación y Posgrado, Ciudad de la Cultura Amado Nervo s/n. Tepic, Nayarit. México. C.P. 63190.
[4] Investigadoras e Investigadores por México, CONAHCyT, México.
[5] Centro de Investigaciones Biológicas del Noroeste S.C. México.
[6] PIMVS AXOS Criadero de Ajolotes. Juan de la Barrera no. 69, Lomas de la Cruz, C.P. 63037, Tepic, Nayarit, México.
* Correspondence: leonardo_martinez@uan.edu.mx; danielbad00@gmail.com

Citation: Manjarrez-Alcívar, I., Martínez-Cárdenas, L., Vega-Villasante, F., Badillo-Zapata, D., Montoya-Martínez, C. E., & López-Félix, E.F. (2024). Levels of fat for potential consumption of juvenile *Ambystoma mexicanum* (Shaw & Nodder, 1798) axolotls: Lipid levels. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2649

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: July 21, 2023.
Accepted: March 15, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 79-85.

ABSTRACT

Objective: The present study focused on the effect of different lipid levels on growth (weight and length) and survival in juvenile *Ambystoma mexicanum*.

Design/methodology/approach: Four diets with the same 45% protein level and different lipid levels: 6, 8, 10 and 12% were tested for a period of 81 days. For the preparation of the diets, two key ingredients were used, such as fishmeal and fish oil, these as protein base and lipid source. The experiment consisted of placing six organisms per experimental unit in tubs with 40 L of water for a period of 81 days; period during which four biometrics were performed, the organisms were fed every 48 hours to the weight of their biomass. The digestibility of the diets, initial and final height, initial and final weight, weight gained, weight gained per day, specific growth rate, survival, Fulton index and protein efficiency rate were recorded.

Results: At the end of the experiment, significant differences ($p>0.05$) were observed in the growth and survival of the axolotls in the diets of 8, 10 and 12% lipids.

Limitations on study/implications: A wider range of lipid levels could not be tested, due to the number of individuals available for the experiment.

Findings/conclusions: According to the data obtained in this study, it is recommended to include a level of 45% protein and 8% lipids in the diets of juvenile *Ambystoma mexicanum*, for their better development and nutrition.

Keywords: nutrition, lipid, feeding, axolotl, amphibian, caudata.

INTRODUCTION

Today aquaculture is the fastest growing activity worldwide in terms of the production of aquatic organisms (Garzón *et al.*, 2019) and within this activity is the aquarium hobby

Image by Juan Carlos Palau Díaz at Pixabay that is based on the hobby of keeping in ornamental aquariums or ponds whether they are fish, crustaceans, mollusks, echinoderms, amphibians and reptiles (Wabnitz *et al.*, 2003; Lango-Reynoso *et al.*, 2012). To meet the needs of these farming systems, it is important to recognize the needs of the species for their development (Sicuro, 2021). The foregoing includes their digestive physiology, life stage, feeding, as well as the particle size of the food, texture, palatability, buoyancy or sinking among others. The clear objective of the industry dedicated to the manufacture of artificial food is to formulate mixtures in adequate quantities and using quality ingredients that correspond to the nutritional profiles of the species (Garzón *et al.*, 2019). The correct formulation of the food supplied to an ornamental organism improves the quality of the water and reduces excessive maintenance costs (Velasco-Santamaría, 2011). Thus, fulfilling the basic requirements for both survival and growth.

The main source for obtaining energy for an animal comes from the macronutrients consumed in its diet, among which lipids and carbohydrates stand out, taking into account that proteins are the main source of muscle growth (Evers *et al.*, 2019; Huaspa Kana, 2019). Lipids are one of the most studied nutrients in fish and crustaceans since their metabolic function is based on energy distribution in the body and cell structure. It has been determined that aquatic organisms fed low-lipid diets have slow growth, low survival, and unfavorable physical-metabolic changes (Khalili & Sampels, 2018). Lipids are the most efficient macronutrients for a good energy intake, maximizing their storage (Tocher, 2003). The knowledge of the food and nutritional requirements for each organism that is used in the aquarium market, allows to position a healthy product with high economic potential (Velasco-Santamaría, 2011). Therefore, the objective of this work was to evaluate the optimal requirement of lipid content in diets supplied to juvenile *Ambystoma mexicanum* (Shaw & Nodder, 1798) axolotls and the effect on their growth and survival.

Most studies related to the nutrition of organisms are focused on mammals and birds and in the case of aquaculture, the most popular species with high commercial value. There are few studies that address breeding methods and the development of experimental diets that improve the growth of amphibians. For the particular case of the genus *Ambystoma*, there are no studies that address the development of inert diets for maintenance in captivity and some are limited to giving general recommendations (McWilliams, 2008) or establish eating habits in free life or in captivity, based on organisms typical of their habitat (zooplankton) (Chaparro-Herrera *et al.*, 2011). The possibility of having a specific diet for this species, considered in danger of extinction, is of priority importance to develop adequate management plans.

MATERIALS AND METHODS

Axolotl obtention

This study was carried out in the Laboratory of Water Quality and Experimental Aquaculture (LACUIC) belonging to the University of Guadalajara, located in Puerto Vallarta, Jalisco. A total of 72 *A. mexicanum* specimens (with an average weight of 16.4±3.2 g and an average length of 11.8±1.6 cm) were obtained by donation from the Axos-PIMVS production center located in the city of Tepic, Nayarit, Mexico. The juveniles were transferred in individual bags and placed in Styrofoam boxes with ice to maintain a constant temperature (18.0 °C) during their transfer to LACUIC. Once in the laboratory, they were placed in plastic containers with a capacity of 500 mL per individual for a quarantine process, (40 days) while they were given a prophylactic treatment with the product Azoo Diesase Treatment® at a concentration of 1 mL per 10 L. During the quarantine they were supplied with silver cup El Pedregal food every 48 hours (55% protein and 12% lipids). Throughout the trial they were provided with a controlled environment at a stable temperature of 18.0±1.0°C. All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors

Preparation of diets

The elaboration of the diets was based on the results obtained by Manjarrez-Alcívar *et al.*, (2022). The ingredients used to prepare the four isoproteic diets (Table 1) were weighed on an analytical scale (Nimbo NBL®; d=0.0001 g) and mixed in a food processor (Kitchen Aid®) until a homogeneous mixture was achieved, which was processed in an extruder (Kitchen Aid®) to remove excess water and form a food into 3.0 mm diameter pellets, which were allowed to dry in an oven (NOVATECH®) at 65 °C for a period of approximately 24 hours. To determine that the diets met the theoretical amounts of protein and lipids necessary to develop the experiment, proximal analyzes were performed according to the protocols established by the A.O.A.C (1995). The determination of the nitrogen content was carried out by the micro Kjeldahl method and a factor of 6.25 was used for the calculation of total protein. Total lipid analyzes were performed using the Soxhlet method using hexane as carrier solvent. The ash content was determined by gravimetric difference with the calcination method in a Thermolyne muffle at 550 °C for a period of 8 h. The determination of the extract free of nitrogen (ELN) was calculated by the difference of the dry matter, with the formula

$$ELN = 100 - (\%\ crude\ protein + \%\ total\ lipids + \%\ ash)$$

Experimental design

After the quarantine period (40 days), the axolotls were randomly placed in plastic tubs, with a capacity of 80 liters, with a working volume of 25 L. Four diets with the same 45% protein level and different lipid levels: 6, 8, 10 and 12% were tested for a period of 81 days (Four treatments in triplicate). Six organisms were placed per tub (one axolotl for every 4.5 L of water) and they were fed every 48 hours at 4% of their initial biomass, after this time, the uneaten food was removed from the tubs with a net and the tub was completely cleaned to feed again. During the 81 day trial four measurements were performed in which the size and weight of the axolotls were recorded.

Response variables

The response variables were: final weight, final length, total weight gain, weight gained, weight gained per day, specific growth rate, and survival. Survival was calculated using the formula:

$$SE(ti) = SP(ti) \times SP(t2) \times SP(t1)$$

where $SP(ti)$=mean population survival in the interval $(ti-1,\ ti)$, The specific growth rate (SGR) was calculated with the formula

$$(SGR\ \%\ increase\ in\ weight\ per\ day) = \left[\left(\ln Wf - \ln Wi\right) / t\right] \times 100$$

where Wf=final weight (g), Wi=initial weight (g), and t=time (days) and the

$$size\ heterogeneity = CV\ Wf\ /\ CV\ Wi$$

was calculated; where Wf=final weight, Wi=initial weight, and CV=coefficient of variation.

Statistical analysis

Shapiro Wilk normality and homoscedasticity tests were performed for each of the measured variables. A one-way analysis of variance (ANOVA) was carried out to compare among the treatments the average of: survival, initial and final length (cm), initial and final wet weight (g), weight gain and length gain, coefficient of variation (g), size heterogeneity (fish body weight g), and final biomass. In all cases a significance level of $P<0.05$ was used. Tukey's post-hoc test was used to identify differences between treatment means using the statistical program SPSS version 17.0.

RESULTS AND DISCUSSION

Table 1 shows the proximal composition of the diets made with different lipid levels and a protein level, in which the data obtained in the determination of these are similar to those necessary to carry out the experiment. Total, protein levels were 45.0±0.5% and lipid levels presented a maximum difference of 0±0.4%.

Figure 1 shows the growth of juvenile axolotl *A. mexicanum*. The final weight obtained from the juveniles for each of the diets per axolotl was: P45/L6 with 35.7±0.8 g, P45/L8 35.8±4.9 g, P45/L10 with 37.1±0.8 g and P45/L12 with 37.4±2.8 g.

According to what was obtained (Table 2) in the initial weight and initial length, total weight gain, weight gained, weight gained per day and specific growth rate, the axolotls did not present statistical differences between the treatments except in survival, where was observed a decreased survival on the P45/L6 diet ($P<0.05$).

Despite the fact that in this experiment a trend of greater biomass was observed in the juveniles of the 12% lipid treatment, when performing the statistical analysis of the evaluated variables there were no significant differences in: weight, length, total weight gain, weight gained, weight gained per day and specific growth rate (Table 2). This suggests that *A. mexicanum* has an adaptability to lipid intake, coinciding with Garzón *et al.* (2019) where it was reported for other ornamental fish species such as: *Poecilia latipinna*, *Trichogaster trichoptrus* and *Pterophyllum scalare* that the nutritional requirements necessary for their best

Table 1. Formulation and proximal composition in the experimental diets of juvenile Axolotls *A. mexicanum* with a protein level of 45.0% and different lipid levels: 6, 8, 10 and 12% during a period of 81 days.

Ingredients	P45/L6	P45/L8	P45/L10	P45/L12
Fish meal	59.1	59.1	59.1	59.1
Cornmeal	16.0	16.0	16.0	16.0
Fish oil	5.3	4.9	4.3	3.6
Cornstarch	33.8	26.8	19.9	12.9
Gelatin	5.0	5.0	5.0	5.0
Vitamins and minerals	3.0	3.0	3.0	3.0
Vitamin C	0.5	0.5	0.5	0.5
Sodium benzoate	0.2	0.2	0.2	0.2
Alpha tocopherol	0.01	0.01	0.01	0.01
Total (g)	100.0	100.0	100.0	100.0
Proximal composition				
Crude protein (%)	45.5±0.8	45.0±0.1	45.01±0.2	45.2±0.5
Total lipids (%)	6.5±0.2	8.5±0.1	10.2±0.1	12.2±0.4
Ashes (%)	11.3±0.1	11.6±0.5	11.1±0.4	11.3±0.2
Nitrogen free extract	36.5	34.7	33.6	31.1
Kcal consumables	4.701	4.815	4.901	4.998

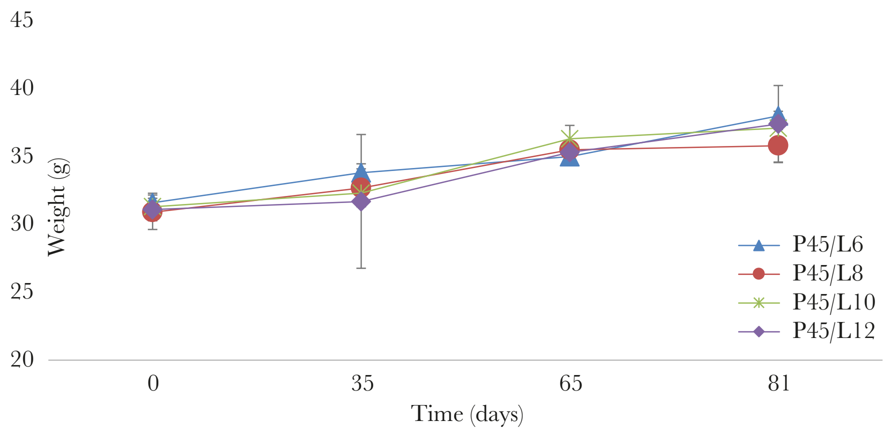


Figure 1. Weight gain in juvenile axolotls *A. mexicanum* fed with a 45% protein level and different lipid levels: 6, 8, 10 and 12% during a period of 81 days.

performance are in a range of 40 to 50% protein and 6 to 8% lipids. In other cold-water fish such as salmon, it was estimated that a high lipid intake between 15 and 20% contributes to a 13% decrease in the amount of protein in diets, from 48% to 35% without altering their growth, obtaining the same protein efficiency and better energy utilization, attributing this to its energy density (Akoh, 2017).

Regarding survival, this was greater than 80% in all treatments. The treatments of 8 and 12% of lipids obtained 100%, this means that the animals were kept in adequate conditions, the above coincides with what was reported by: Bolasina and Fenucci (2007) for the fish *Urophycis brasiliensis* where three different diets with 42% protein and different

Table 2. Effect of four diets with a protein level of 45% and different lipid levels 6, 8, 10 and 12% on: weight, length, survival, weight gained per day, weight gained per day, Fulton's K and rate. growth factor (SGR) and feed conversion factor (FCA) in juvenile axolotls *A. mexicanum* cultured for 81 days.

Response variables	Treatments			
	P45/L6	**P45/L8**	**P45/L10**	**P45/L12**
Survival (%)	83.3±2.8 [b]	100±0.0 [a]	94.4±9.6 [a]	100±0.0 [a]
Initial weight (g)	31.6±0.1 [a]	30.9±0.7 [a]	31.3±0.3 [a]	31.1±0.3 [a]
Final weight (g)	35.7±0.8 [a]	35.8±4.9 [a]	37.1±0.8 [a]	37.4±2.8 [a]
Initial length (cm)	15.4±0.0 [a]	15.4±0.4 [a]	14.7±0.9 [a]	15.3±0.1 [a]
Final length (cm)	16.4±0.1 [a]	16.3±0.6 [a]	16.2±0.2 [a]	16.4±0.6 [a]
Total weight gain (%)	20.5 [a]	15.6 [a]	18.3 [a]	20.33 [a]
Weight gained (g)	6.5±0.9 [a]	4.9±4.5 [a]	5.7±0. 5 [a]	6.3±3.1 [a]
Weight gained per day (g/day)	0.1±0.0 [a]	0.1±0.0 [a]	0.1±0.1 [a]	0.1±0.0 [a]
Specific growth rate	0.1±0.1 [a]	0.1±0.0 [a]	0.1±0.0 [a]	0.1±0.0 [a]
Fulton's K	0.80±0.04 [a]	0.82±0.02 [a]	0.87±0.04 [a]	0.84±0.01 [a]
Food convertion factor (%)	2.7±0.3 [a]	2.8±0.5 [a]	2.4±0.5 [a]	2.7±0.7 [a]

levels of crude lipids 5, 8 and 11% lipids for 40 days where the best survival was recorded in the 8% lipid diet. Toledo *et al*., (2014) reported a survival greater than 98% in diets with 8, 10 and 12% lipids in *Calyptocephalella gayi*.

CONCLUSIONS

According to the results obtained in this study, it is recommended to include a level of 45% protein and 8% lipids in the diets of juvenile *Ambystoma mexicanum*. These results contribute to the formulation of specific diets for the optimal development and nutrition of the species, as well as to improve management and cultivation plans.

ACKNOWLEDGEMENTS

We thank Dr. Jackob Parker and Dr. Candido Silva-Peña for their kind as-sistance on the improvement of earlier manuscript drafts.

REFERENCES

Akoh, C. C. (2017). Food lipids: chemistry, nutrition, and biotechnology. CRC press.

A.O.A.C. (1995) Official Methods of Analysis 16 th edn. Association of Official Analytical Chemists. Arlington, VA, USA 684p

Bolasina, S. N., & Fenucci, J. L. (2007). Effects of dietary lipid level on growth, survival and body composition of Brazilian codling (*Urophycis brasiliensis* Kaup, 1858). *Revista de Biología Marina y Oceanografía. 42*(1): 23-27.

Chaparro-Herrera, D.J., Nandini, S., Sarma S.S.S. & Zambrano, L. (2011). Feeding behaviour of larval *Ambystoma mexicanum*. *Amphibia Reptilia* 32, 509-517.

Evers, H. G., Pinnegar, J. K., & Taylor, M. I. (2019). Where are they all from? –sources and sustainability in the ornamental freshwater fish trade. *Journal of Fish Biology, 94*(6), 909-916.,

Garzón, J. S. V., & Gutiérrez-Espinosa, M. C. (2019). Aspectos nutricionales de peces ornamentales de agua dulce. *Revista politécnica. 15*(30): 82-93.

Huaspa Kana, V. N. (2019). Evaluación de diferentes Niveles de Lípidos totales en la dieta sobre el Crecimiento y Supervivencia de los Juveniles de Corvina (*Cilus Gilberti*) en el Centro de Acuicultura Morro Sama del distrito de Sama las Yaras, provincia de Tacna, región Tacna.

Khalili Tilami, S., & Sampels, S. (2018). Nutritional value of fish: lipids, proteins, vitamins, and minerals. *Reviews in Fisheries Science & Aquaculture, 26*(2), 243-253.

Lango Reynoso, F., Castañeda-Chávez, M., Zamora-Castro, J. E., Hernández-Zárate, G., Ramírez-Barragán, M. A., & Solís-Morán, E. (2012). La acuariofilia de especies ornamentales marinas: un mercado de retos y oportunidades. *Latin american journal of aquatic research, 40*(1): 12-21.

Manjarrez-Alcívar, I., Vega-Villasante, F., Montoya-Martínez, C. E., López-Félix, E. F., Badillo-Zapata, D., & Martínez-Cárdenas, L. (2022). New findings in the searching of an optimal diet for the axolotl *Ambystoma mexicanum*: protein levels. *Agroproductividad, 15*(6).

Sicuro, B. (2021). The Real Meaning of Ornamental Fish Feeds in Modern Society: The Last Frontier of Pet Nutrition?. In Sustainable Aquafeeds (pp. 113-120). CRC Press.

Tocher, D.R. (2003). Metabolismo y funciones de lípidos y ácidos grasos en peces teleósteos. *Revisiones en ciencia pesquera. 11*(2): 107-184.

Toledo, P. H., Suazo, R., & Viana, M. T. (2014). Formulated diets for giant Chilean frog *Calyptocephalella gayi* tadpoles. *Ciencia e investigación agraria: revista latinoamericana de ciencias de la agricultura. 41*(1): 13-20.

Wabnitz, C., Taylor, M., Green, E., & Razak, T. (2003). From ocean to aquarium. UNEP World Conservation Monitoring Centre, Cambridge, 64 pp.

Colegio de Postgraduados

Factors affecting the profitability of wheat production in the states of Guanajuato and Nuevo Leon, Mexico

Ayala-Garay, Alma V.[1*]; Espitia-Rangel, Eduardo[1]; Almaguer-Vargas Gustavo[2]; Buendía-Ayala, Blanca L.[3]; Marín-Vázquez, Esmeralda[3]

[1] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP). Campo experimental Valle de México. Carretera los Reyes-Texcoco km 13.5, Coatlinchán Texcoco, Estado de México. C. P. 56250.
[2] Universidad Autónoma Chapingo (UACh). Departamento de Fitotecnia. Carretera México-Texcoco km 38.5, Chapingo, México. C.P. 56230.
[3] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP). Campo experimental Valle de México. Carretera los Reyes-Texcoco km 13.5, Coatlinchán Texcoco, Estado de México. C. P. 56250.
* Correspondence: ayala.alma@inifap.gob.mx

ABSTRACT
The objective of this research was the analysis of the main factors involved in the profitability of bread wheat in the states of Guanajuato and Nuevo León, Mexico. To obtain the information, semi-structured interviews were applied to key informants who indicated the context of the crop in their region. In order to estimate profitability, production performance and costs production were determined. The production cost information, for the two Mexican states, was obtained through the producer panels methodology in 2019. Producers were asked about the costs expended in land preparation, cultivation inputs, harvest and others such as water, electricity and financial rights. In addition, wheat yields and the price received per ton sold at the market. It was noticed that Nuevo León and Guanajuato have an agro-industrial infrastructure that demands Mexican wheat. In each state, the profitability of the crop depends on a number of factors such as the planting system and the production technology used; but in all the places studied, bread wheat had positive profitability. However, there are high production costs and a continuous deterioration of the real prices of the product. It is necessary to develop soft wheat varieties that adapt to rainfall conditions for agricultural regions where wheat cultivation is under those conditions. Although Mexico is self-sufficient in the production of flour, each year it is necessary to import an additional volume of bread-making wheat since Mexican industry is deficient facing the high demand for bread production.

Keywords: yield, production costs, profitability.

Citation: Ayala-Garay, A. V., Espitia-Rangel, E., Almaguer-Vargas G., Buendía-Ayala, B. L., & Marín-Vázquez, E. (2024). Factors affecting the profitability of wheat production in the states of Guanajuato and Nuevo Leon, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2666

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: August 26, 2023.
Accepted: March 05, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 87-94.

INTRODUCTION

During 2020, the national production of grain wheat reached three million tons. Among the main producing states, Sonora topped the list with 49% of production, followed by Guanajuato with 12%, Baja California with 10%, while Nuevo León, Tlaxcala and Chihuahua together contributed 8% of national production (SIAP, 2023). In Mexico, the milling industry acquires the entire harvest of bread-making wheat and around 720 thousand tons of crystalline wheat, for the production of semolina that is used in the manufacture of pasta (CANIMOLT, 2016).

Wheat production in Mexico shows a downward trend, in 2008 the production was 4 million 214 thousand tons, in contrast to the 2 million 943 thousand obtained in 2020; which represented 70% of what was produced in 2008. This trend tends to be accentuated, especially with bread-making varieties. This is attributable to the scenario of falling international prices and competition for the area available for planting with crops such as barley and oilseeds (CANIMOLT, 2016). Also, to the reduction in profitability, as producers have high production costs and a continuous deterioration in the real prices of the product.

Between 1980 and 2015, the input price index for wheat production grew at an average annual rate of 30.44%, while that of the producer price index decreased by 2.1% and yields barely grew 0.4%, implying a reduction in profitability. Despite the loss of profitability, coupled with the increase in import volumes and the deregulation of the market, there are no studies that accurately investigate the main problems involved in reducing the profitability and competitiveness of wheat cultivation. The objective of this research was to analyze the factors involved in the profitability of wheat in the states of Guanajuato and Nuevo Leon, Mexico.

MATERIALS AND METHODS

The study was conducted in the municipalities Penjamo and Valle de Santiago, Guanajuato; and in the municipalities Los Ramones, Allende, and Valle de Salinas, in Nuevo León. The information was obtained by applying a semi-structured interview, directed to key informants. The first interview was with eight actors, from research institutions, such as INIFAP, the State Delegation of the Ministry of Agriculture and Rural Development (SADER) and the Secretariat of Rural Development in Guanajuato (SDR). A second interview was conducted with 40 farmers in the two states, from which data were obtained to identify problems, causes and solutions in the production and sale of wheat.

For the analysis of crop profitability and to identify actions to make a competitive production chain (Lundy *et al.*, 2004), production costs were obtained with the methodology of producer panels (Agroprospecta, 2010; Ireta-Paredes *et al.*, 2015, Ireta-Paredes *et al.*, 2018) with groups of producers, with similar characteristics of technological level and surface area under cultivation. Two producer panels were held in each locality, with small and large producers, each one was made up of four to six producers who were invited directly, the requirement was that they had grown wheat in 2019. After obtaining production costs, the complement to the profitability analysis was yield evaluation (Swenson and Haugen, 2013). Then, the following algebraic expressions, based on economic theory, were used to determine profitability (Krugman and Wells, 2006; Samuelson and Nordhaus, 2009).

Total cost was determined through this function

$$CT = PxX$$

where CT=total cost; Px=input or activity cost x and X=activity or input.

And the total income per hectare was obtained by multiplying the yield of the crop in tons (Megagrams, Mg) times its market price.

$$IT = PyY$$

where IT=total gain (MXN \$ ha^{-1}), Py=sales price (MXN \$) at the market per Megagram of the crop (MXN \$ Mg^{-1}); Y=crop yield in Megagrams per hectare (Mg ha^{-1}).

Finally, profitability was calculated:

$$Profitability = IT - TC$$

RESULTS AND DISCUSSION

Profitability analysis

The producing areas of Valle de Santiago and Penjamo, in Guanajuato recorded an average yield of 7.5 Mg ha^{-1} and an average profit of MXN $ 8201 per ha; in contrast, the municipalities of Allende, Los Ramones and Valle de Salinas in Nuevo Leon presented an average yield of 4 Mg ha^{-1} with average profit of MXN $ 2305 per ha. In Nuevo Leon, the municipality of Valle de Salinas has the highest investment compared to the rest of the production units evaluated, which is attributable to the high costs of irrigation and land leasing.

In Table 1, it can be seen that the municipality of Penjamo had the highest profitability with $10 344 per ha, while in Valle de Santiago, it was MXN $6059 pesos per hectare cultivated with soft wheat. The variation in profitability between Penjamo and Valle de Santiago, Guanajuato can be attributed to the fact that in the municipality of Penjamo the sale of wheat is carried out by the producer organization to which they belong, so they achieve consolidated sales and obtain a better price per ton.

In the case of Valle de Santiago, producers resort to contract farming with intermediaries for different mills, which is done individually and therefore their bargaining power is lower, their only advantage is that they will be able to place their harvest at a sale price previously agreed in the contract. If the guarantee prices in force since the 2019 agricultural cycle are considered, the profitability is almost the same in both municipalities. However, that of Valle de Santiago is still lower due to higher production costs. Despite those, Valle de Santiago reaches a profitability per hectare greater than MXN $ 10 000.

The Mexican state of Guanajuato, where irrigated agriculture predominates, has the highest profits per hectare compared to wheat-producing regions in Nuevo Leon, where wheat crop is irrigated and rain-fed. Los Ramones municipality, in the Mexican state of Nuevo Leon, is the one with the highest profit obtained per hectare (MXN $3720 ha^{-1}), when real rural average price MXN $4300 Mg^{-1} is considered (without adding the warranty price). In this municipality, wheat is produced under irrigation system and mechanized labor (Table 1). Despite yield and income per hectare are higher in Valle de Salinas, their high production costs and a minor price paid to the producer, both affect profitability per ha. When profitability is calculated with the warranty price, income rises;

Table 1. Comparison of production costs and incomes per hectare of wheat in the states of Guanajuato and Nuevo Leon, Mexico with 2019 prices.

Concept	**Guanajuato**		**Nuevo León**		
Costs (MXN $ ha^{-1})	**Penjamo**	**Valle de Santiago**	**Los Ramones**	**Allende**	**Valle de Salinas**
Type of irrigation	Irrigated	Irrigated	Irrigated	Rainfed	Irrigated
Land preparation	6462	5917	5180	1500	5900
Agricultural labor	2000	1425	1500	1000	1425
Seeds	1740	1800	1740	1050	1740
Fertilizer	4249	4137	-	-	4500
Fungicide	340	233	400	401	400
Herbicides	1625	1313	520	120	1300
Irrigation	1240	2116	1200	-	3240
Harvest	2250	2250	940	700	1410
Production cost	19 906	19 191	11 480	4771	19 915
Land leasing	5000	7000	2000	2000	2000
Production cost plus land leasing (MXN $ ha^{-1})	24 906	26 191	13 480	6771	21 915
Income					
Yield (Mg ha^{-1})	7.5	7.5	4	2	6
Price paid to producer (MXN $ Mg^{-1})	4700	4300	4300	3700	4080
Income (MXN $ ha^{-1})	35 250	32 250	17 200	7400	24 480
Profitability (MXN $ ha^{-1})	10 344	6059	3720	629	2565
Profit per Megagram (MXN $ Mg^{-1})	1379	808	930	315	428
Profitability (MXN $ ha^{-1}) (with warranty prices set at MXN $ 5790 per Mg)	18 519	17 234	9680	4809	12 825

Source: elaborated by the authors with field data obtained in 2019.

the municipality with the highest profitability is then Valle de Salinas (MXN $ 12 825 ha^{-1}), because it also has greater production per hectare.

Factors Affecting Profitability in Wheat

These are government subsidies, credits, and other governmental grants in the wheat production process. The government grant received was from Pro Agroproductivo, which granted $1300 per hectare of wheat cultivated until 2018. Starting in the 2019 agricultural year, Mexican Federal Government implemented the Basic Food Products Warranty Price Program, which included bread-making wheat that was paid with a warranty price of $5790.00 MXN per Megagram, with a purchase limit of 100 tons per producer. The agency in charge of the operation of the Program, the Mexican Food Security Agency (SEGALMEX), paid the producer with the difference between the actual price obtained for the delivery of their crop to the industry and the established warranty price.

With SEGALMEX operating rules for the 2019-2020 Autumn-Winter cycle, the warranty price remained at MXN $ 5790.00 per ton of bread-making wheat with the limit of up to 100 tons per producer. The productivity incentive was added for tons 101 to 300, which received 50% of the support, and the competitiveness incentive for crystalline wheat, intended for the domestic milling industry, from 1 to 50 tons, with a support of 40% of the grant defined as the warranty price of bread wheat. This has been favorable for these producers, however, it should be applied in its entirety to all producers in Mexico.

Producers tend to resort to financing and in Guanajuato loans are higher than MXN $100 000 with an interest rate of 1 to 13%, compared to Nuevo León, where producers request support for amounts higher than MXN $500 000 with a 3 to 13% interest rate. Most of the loans are granted by financial institutions, or by the companies with which they sign contract farming, as it is the case of DC-TRIMA, in the municipality of Valle de Santiago, Guanajuato.

Exports and imports

The production of bread-making wheat is insufficient to meet the demand of the national milling industry (SAGARPA, 2017). As for crystalline wheat, the domestic market for human consumption has surpluses, which are destined for export as a market of opportunity and to the livestock sector as a market that consumes surpluses (CANIMOLT, 2016). The main destination country for soft wheat and crystalline wheat is the United States, which accounted for 91% of Mexican exports (2009 to 2019) of soft wheat and 80% of crystalline wheat (SIAVI, 2019).

Imports in 2019 showed a decrease of 27.54% compared to those in 2018 (SIAVI, 2019). In the period 2009–2019, imports of soft wheat accounted for 97.1% of total imports, of which 61.5% came from the United States of America. In turn, Russia, Lebanon, Italy, and the United Kingdom have become the main suppliers of crystalline wheat (SIAVI, 2019).

Wheat prices in México

Figure 1 shows the dynamics of the real rural average price (RRAP) paid to the producer per type of wheat; which has an Average Annual Growth Rate (AAGR or TMAC) of -1.73; composed of -1.05 for soft wheat and -1.98 for crystalline wheat (Figure 1).

When analyzing the wheat Producer Price Index (wPPI) for the period 2008-2021, an upward trend is observed in the prices of inputs used to grow wheat, whose AAGR showed a 4.12% increase, the fertilizer price index FPI grew at a 3.16% rate, and the wheat Rural Average Price Index decreased by 1.73% (Figure 2).

When analyzing the average rural price and the producer price index for the period from 2008 to 2022 and comparing the AAGRs, a loss of profitability for the producer is observed. Since the price of inputs shows a higher growth rate than that of the AAGR of the real price paid to the producer at the national scale.

Wheat market

The soft wheat market in Guanajuato has two main uses, the pasta making industry and the baking industry. According to the information obtained in the field, "Molinera de México", which is part of the TRIMEX Group, acquires wheat for the industrial consumption of PepsiCo Mexico, while "Harinera Irapuato" acquires wheat for "Grupo

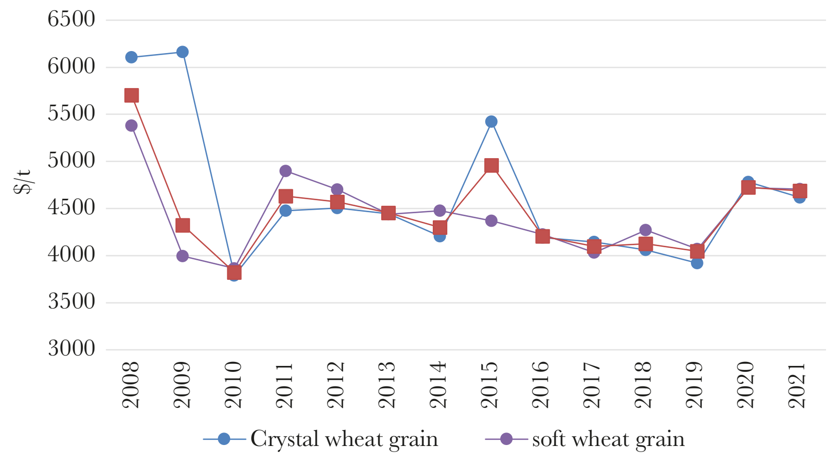


Figure 1. Real Rural Average Price (RRAP) paid per Megagram (ton) of wheat to the producer for the period 2008-2021.
Source: elaborated by the authors based on data from INEGI (2023) and SIAP (2023). RRAP-Real rural average price for wheat grain.

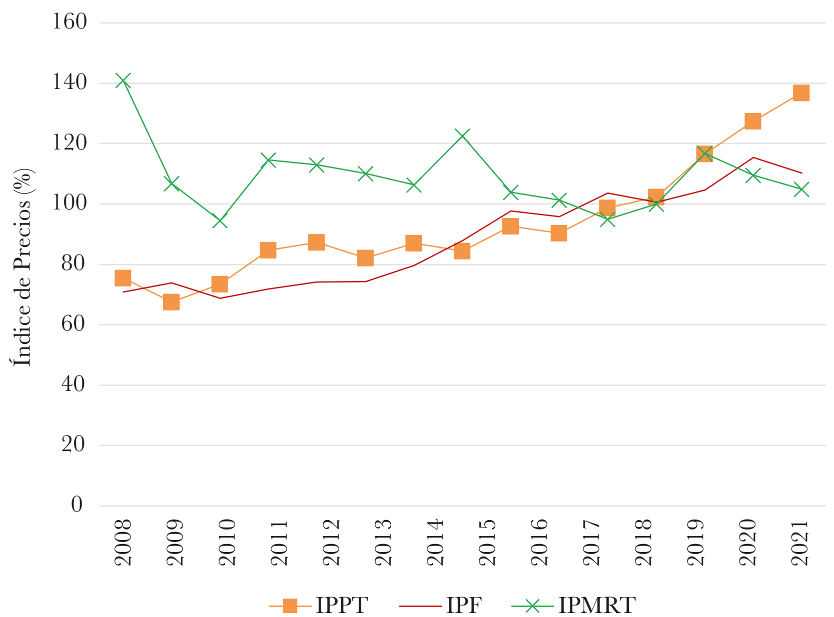


Figure 2. Price indices for the production and sale of wheat in Mexico (2008-2022).
Source: elaborated by the authors. wPPI - Producer Price Index for wheat; FPI - Fertilizers Price Index based on INEGI (2023) and wRAPI - Rural Average Price Index for wheat based on SIAP (2023).

BIMBO". In the case of crystalline wheat, it is acquired by the "Los Pirineos" flour mill, located in the municipality of Salamanca, Guanajuato, for the company "Grupo La Moderna", which makes pasta, and also by "Grupo MINSA", which transports the wheat to Jalisco. The "San Blas" flour mill belongs to "La Italiana" Industrial Group, which makes the past brand "Italpasta", and mill consumption is also durum wheat, which are processed in the municipalities of Salamanca, where "La Italiana" produces flour, and Irapuato, where it elaborates pasta.

Another market for wheat is to sell it to bakery industries such as PepsiCo which pays on time, although the disadvantage is that this Company does not invest in storage facilities and asks to use the facilities that belong to the producer organizations. Another purchasing scheme of the bakery companies was that of "Grupo Bimbo", which according to the percentage of protein that was present in the wheat grain, paid an economic stimulus over the price to the producer. More than 12% protein was required to reach the best stimulus over the price of wheat.

In Nuevo León, mills that produce wheat flour or semolina (or both) for pasta-making are the first buyers of wheat. They are followed by manufacturers of feed for cattle fattening (SAGARPA, 2017). Nuevo León is an eminent consumer of wheat, since what it exports are wheat products, mainly flour in its different presentations and qualities. Once this Mexican state satisfies its export target, it buys grain from other states or from USA and Canada to meet its domestic needs.

CONCLUSIONS

Nuevo León and Guanajuato have an agro-industrial infrastructure that demands Mexican wheat, both from the center of the country and abroad. In each state, the profitability of the crop depends on a number of factors, such as the planting system and the production technology used. But in all the places studied, wheat had positive returns; despite the fact that there are high production costs and a continuous deterioration of the real prices of the product.

When considering the warranty prices, the profitability of the grain increases three-fold in Valle de Santiago, and five-fold in Valle de Salinas. Although Mexico is self-sufficient in the production of flours, in the North of the country producers prefer to import wheat flour, then process it to export pastries, cookies, and bread destined for USA and Canadian markets. Overall, Mexico is self-sufficient in the production of wheat flour, but each year it must import an additional volume of bread-making wheat, because it is deficient in bread production, due to the degree of national consumption.

REFERENCES

Agroprospecta. 2010. Red mexicana de investigación en política agroalimentaria. Reporte de Unidades Representativas de Producción Agrícola, Panorama Económico 2008-2018. Reporte 2010-01. Universidad Autónoma Chapingo. 208 p.

CANIMOLT (Cámara Nacional de la Industria Molinera de Trigo). 2016. Reporte estadístico 2015 con datos de 2016. https://issuu.com/canimolt/docs/reporte-estadi-stico-2015-2016.

INEGI (Instituto Nacional de Estadistica y Geografía). 2023. Indice Nacional de Precios al Productor. Banco de Información Económica (BIE), https://www.inegi.org.mx/temas/inpp/ (Recuperado: 06/07/2023).

Ireta-Paredes, A. R., Altamirano-Cárdenas, J. R., Ayala-Garay, A. V., Covarrubias-Gutiérrez, I. 2015. Análisis macroeconómico y microeconómico de la competitividad del arroz en México. *Agricultura, Sociedad y Desarrollo* 12: 499-514.

Ireta-Paredes, A. R., Pérez-Hernández, P., Bautista-Ortega, J., y Rosas-Herrera, E. L. 2018. Análisis de la red de valor calabaza chihua (*Cucurbita argyrosperma* Huber) en Campeche, México. *Agrociencia* 52: 151-167.

Krugman, P. y Wells, R. 2006. Introducción a la Economía: microeconomía. Reverte. Barcelona, España. 537 p.

Lundy, M., Gottret, M. V., Cifuentes, W., Ostertag, C.F. y Best, R. 2004. Diseño de estrategias para aumentar la competitividad de cadenas productivas con productores de pequeña escala. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia. 85 p.

SADER (Secretaría de Agricultura y Desarrollo Rural). 2023. Sistema de Información Agroalimentaria de Consulta (SIACON). México.

SAGARPA (Secretaría de Agricultura, Ganadería, Recursos naturales, Pesca y Alimentación). 2017. Plan Nacional Trigo 2017-2030. SADER– Agricultura. https://www.gob.mx/cms/uploads/attachment/file/256434/B_sico-Trigo_Cristalino_y_Harinero.pdf

Samuelson, PA., y Nordhaus, WD. 2009. Economía. McGraw-Hill. 19ª Edición. España. 744 p.

SIAP (Servicio de Informacion Agroalimentaria y Pesquera). 2023. Estadisticas de Trigo grano. Anuario Estadístico de la Producción Agrícola (Sistema de consulta en línea; por cultivo, ciclo y entidad federativa). https://nube.siap.gob.mx/cierreagricola/

SIAVI (Sistema de Información Arancelaria vía internet). 2019. SIAVI 5.0, http://www.economia-snci.gob.mx/ (Sitio con datos disponibles actualizados solo hasta noviembre 2021).

Swenson, A., y Haugen, R. 2013. Projected crop budgets. North West, ND, USA. http://www.ag.ndsu.edu/pubs/agecon/ecguides/nw2013.pdf

Colegio de Postgraduados

Food losses from farm to retail operations: agricultural produces supply chain of Baja Peninsula, México

Martínez-Camacho, Rubí A.[1]; Yahia-Kazuz, Elhadi[3]; Rivas-García, Tomás[2]; Castellanos-Cervantes, Thelma R.[1]; Voegele, Ralf T.[4]; Beltrán-Morales Luis F.[1].; Ascencio-Valle, Felipe[1*]

[1] Instituto Politécnico Nacional Centro de Investigaciones Biológicas del Noroeste, SC #125. La Paz, C.P. 23096, B.C.S., México.
[2] CONAHCyT- Universidad Autónoma de Chapingo, Carr. Federal Méxco-Texcoco km 38.5 C.P. 56235, Texcoco, México.
[3] Universidad Autónoma de Querétaro, Facultad de Ciencias Naturales, Avenida de las Ciencias S/N C.P. 76230 Juriquilla, Querétaro, México.
[4] University of Hohenheim, Faculty of Agricultural Sciences, Institute of Phytomedicine, Department of Phytopathology, Otto-Sander-Str. 5, 70599 Stuttgart, Germany
* Correspondence: ascencio@cibnor.mx

Citation: Martínez-Camacho, R. A., Yahia-Kazuz, E., Rivas-García, T., Castellanos-Cervantes, T. R., Voegele, R. T., Beltrán-Morales L. F., &Ascencio-Valle, F. (2024). Food losses from farm to retail operations: agricultural produces supply chain of Baja Peninsula, México. *Agro Productividad*. https://doi.org/ 10.32854/agrop.v17i4.2669

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: August 31, 2023.
Accepted: March 14, 2024.
Published on-line: May 06, 2024.

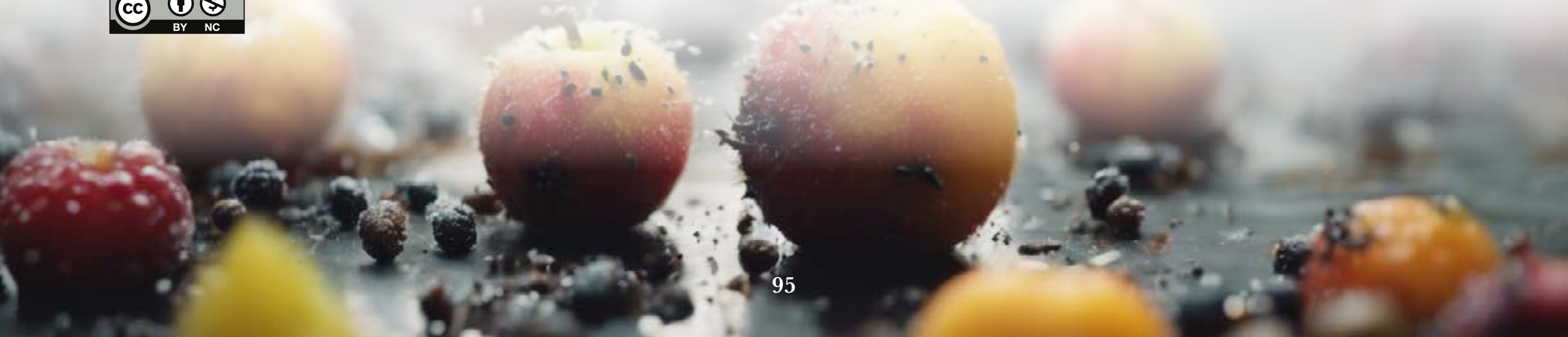
Agro Productividad, 17(4). April. 2024. pp: 95-103.

ABSTRACT
Objective: To evaluate food losses (FL) volumes generated by farms in Baja California Peninsula, México, of five agricultural commodities.
Design/methodology/approach: Baja California Sur (BCS) state was the study area. Information was gathered from a total of 380 sampled chain actors in asparagus, mango, strawberry, orange and tomato by survey and personal interviews. Tobit technique was applied to identify factors that influence FL percentage.
Results: Data shows about 11.8% of asparagus is lost during harvesting and distribution, as well as 8.5% of strawberry, 26% of mango, 17.8% of oranges and 3.5% of tomatoes, representing 29.9% loss rate of marketed yield.
Limitations on study/implications: This study did not classify commodities in the last steps of the supply chain. The five commodities used in the current study correspond to the more important agricultural produces in BCS, but given changing market, harvesting time and produce availability did not consider the waste of the supply chain.
Findings/conclusions: Commodity, type of transportation and distribution, education, and human resources has been identified as influence factors in the volume of FL. This exploratory study fills the void in information in terms of its geographic scope and food group number, and farm owners willing to manage food losses for the purpose of obtaining bioactive compound.

Keywords: Food loss, agrifood, desert agriculture, food security, retail.

INTRODUCTION

Food losses and waste (FLW) has been recognized as a major global, environmental and societal challenge. The United Nations (UN) through their Sustainable Development Goals in 2030 Agenda has been clear about it setting two specific goals, "2 Zero hunger" and "12



responsible consumption and production" (ONU, 2015). Besides, FLW has a significant impact in food related companies decreasing economic growth and viability. One-third of all food produced globally is lost or wasted, which means that annually 1.3 billion tons of probably perfect human consume food is discarded along food supply (FAO, 2013). FLW implies that in addition to throwing away food, greenhouse gases are released in food production, nutrient soil is wasted, but also when wasted food is sent to landfill (Qin and Horvarth, 2022). The issue of FLW is one of the greatest challenges of the new century. It is important to document that food loss does not mean only a boost in food production, if not; it is something even more complex. FLW reduction/disappear includes the adaptation of production habits and the implementation of new technologies and strategies that allow food production system to be sustainable and environmentally friendly even in pandemic times (Saboori *et al.*, 2022).

Although the terms 'food loss' and 'food waste' are often used interchangeably, there is an important difference between these two concepts (Affognon *et al.*, 2015). Food loss describes the situations whereby edible food leaves the food supply chain because of unintentional events, such as bad growing, size, shape, no special color or flavor, no temperature control in distribution or packing, among others. Quantification of FLW may go beyond the FLW definition scope and accounting for production-level factors during FLW measurement may be important to FLW management strategies. Using post-harvest losses implies that the timing of when FLW is considered occurs after harvest has been completed. However, post-harvest loss may in fact account for losses that occur during harvest (Affognon *et al.*, 2015). Different supply chain stages where loss occurs, and associated reasons for loss, respond differently to distinct types of policy incentives and measurements (HLPE, 2017).

The Causes and feedback process of FLW along supply chain may be different that the stage where FLW occurs even in different countries or different territories (HLPE, 2014). For example, although the amount of FLW is similar in developed and developing countries, 670 and 630 million tons (Mts), they are different on per capita factor (HLPE, 2017). More than 160kg/capita of food is thrown away annually from agricultural production to retail, while the actual amount changes from region to region (Amicarelli and Box, 2020). The highest loss occurs in Latin America with 200kg/capita, while the lowest loss occurs in South and Southeast Asia (100 kg/capita) (Amicarelli and Box, 2020). Several recent studies have used self-report or direct measurement methods to quantify region —and supply chain— specific FLW of perishable foods, finding losses concentrated at the producer level (Benites-Zapata *et al.*, 2021; Pereira and Filimonau, 2022; Ronen, 2023).

Our study assesses the extent, stages, and determinants of FLW along five perishable vegetable species (commodities) supply chains in Baja California Sur, México, from farm to retail. Unique to this study, we use data collected at the harvest and distribution levels to compare FLW. Using detailed data on production, harvest, and postharvest context, we examine the associated determinant of FLW at the farmer stages.

MATERIALS AND METHODS

This study was conducted in Baja California Sur State, in five municipalities (Figure 1). Food losses of every commodity were assessed in Los Cabos, La Paz, Comondú, Loreto

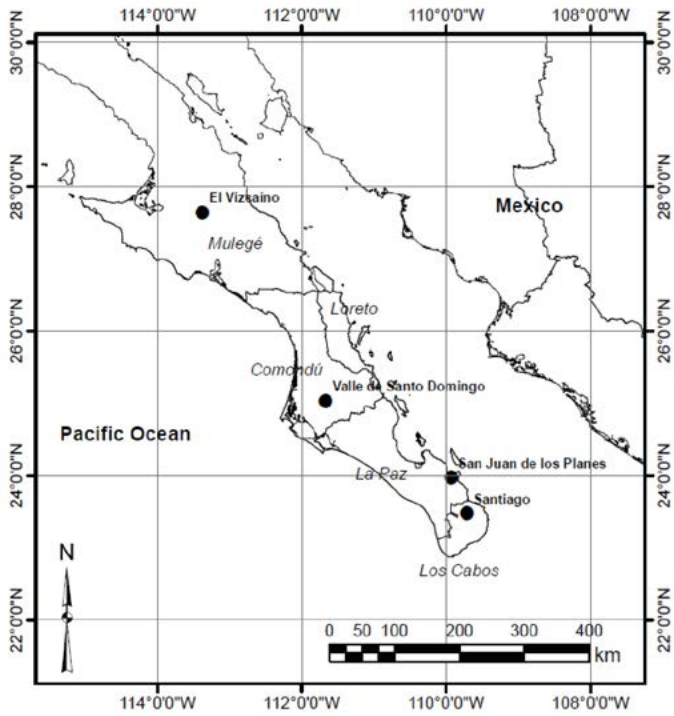


Figure 1. Map of Baja California Sur indicating the municipalities and localities included in the study.

and Mulege, across supply chains from farm to retail stages. Data were collected using surveys and pile-sort group discussions from August 2021 to February 2022.

Descriptive statistics and study variables

Participants in the survey were the owners of the farms used in the study. Direct variables included location, crop, cropland size, season, production quantity (ton), losses due to diseases, technical difficulties, packing method, packing location, type of distribution and channel, and quantities of losses due to distribution (Table 1).

Food losses (FL) were calculated as percentage of total production (TP) in each farm minus marketed production delivered (DP) to a distributor, as described below:

$$MY = \left(\left(TP - DP\right)\right) / TP * 100 \quad (1)$$

Selection of the estimation method

Food losses data in percentage causes that independent variable food loss volume was truncated. Data truncation nature requires the selection of a suitable estimation approach. Data were submitted to Tobit modeling technique providing three equations linking location, season, and commodity with the percentage of food loss. Therefore, each equation modeling the percentage of food loss in a given category is independently estimated. This approach is further supported by the variability of technical difficulties across season and commodity characteristics of different farmlands and often unpredictability of specific solutions that solve these problems. The Tobit equation is represented by three algebraic

Table 1. Descriptive statistic of demographic and socioeconomic characteristics.

Variable name	Units	Mean[a]	Standard deviation	Min	Max
Farm size[b]	Size category	2.86	0.34	1	4
Number of employees[c]	Employee category	3.84	0.35	1	5
Farm location[d]	Municipality category	4.27	0.15	1	5
Farm owner education	1=university degree; 0=otherwise	0.62	0.11	0	1
Employee technical training	1=Basically training or more; 0=no training	0.43	0.17	0	1
Gender	1=man; 0=woman	0.71	0.50	0	1

[a] In case of binary variables the reported figure is share.
[b] Size categories are: 1=100 ha or less; 2=101-300 ha; 3=301-500 ha; 4=500 ha or more.
[c] Employee categories are: 1=100 or less; 2=101-300; 3=301-500; 4=501-800; 5=801 or more.
[d] Municipality categories are: 1=La Paz; 2=Los Cabos; 3=Loreto; 4=Comondú; 5=Mulegé.

sentences where N is the number of observations; Y_i is the observed dependent variable and Y_i^* is the unobserved variable; β is parameters vector, and ε_i is the error for equation; this error must be normally distributed with the mean in cero and variance σ^2. Tobit equation is represented as:

$$\begin{gathered} Y*im = X_i' \ \beta_m + \varepsilon_i, i = 1,2,\ldots,N \text{ and} \\ Y_i = Y_i^* \text{ if } Y_i^* > 0 \\ Y_i = 0 \text{ if } Y_I^* \leq 0 \end{gathered} \tag{2}$$

Independently, a binary dependent variable equation was specified to model the decision to agree to use food losses for the regional obtaining of bioactive compounds. The yes/no nature of the decision suggested the use of the probit technique to identify the factors that encourage or impede such a choice. The variables used in the specified empirical model are commonly used in consumer studies and surveys such as including socio-economic and demographic characteristics of the respondent and their farmland. In this case the variables are, among others, crop, season, production (ton), income by commodity production, farmland size, number of employees, farmer education and harvest technique. These variables capture factors identified in earlier studies as associated in other desert regions as well as agricultural farmlands to food losses. Crop and season are associated with water scarcity and land fertility as well as culture region (Gao *et al.*, 2022). Farmer education is negatively correlates with loss food, the better educated owner and farmer would be expected to lose less food if that is their concern. Smaller farmland tends to consume more water, having more technical difficulties and lose more food. The current study adds a square measure of the farmland implying that the empirically tested effect on the volume of food loss can change as farmland size diminish. Similarly, number of employees squared of a respondent can capture changes in food losses.

RESULTS AND DISCUSSION

Factors influencing food losses in commodities

The identified factors influencing the amount of food losses vary across commodities. Consequently, it appears that reduction of food losses requires a different approach if the goal is to alter harvesting and distribution behavior. Food losses are remarkably different within the five commodities, *i.e.*, asparagus, strawberry, mango, orange, and tomato (Table 2). It appears that asparagus food losses are positively associated with packing conditions after harvest and harvest time from farm to the primary packing location. These factors were affected by the non-controlled transportation temperature (Table 3). Strawberry and tomato food losses were affected by 8 out of 16 explanatory variables (Table 3). The high quantity of tomato losses reported here and in earlier studies is possibly associated with the packing and distribution (Abera *et al.*, 2020; Chaboud and Moustier, 2021). Strawberry and tomato seasons are directly affected by hot weather in the farm, which causes technical difficulties for transportation (Sasaki *et al.*, 2021b). Moreover, tomato trade characteristics have a limited window especially if it must be transported long distances. Packing is affected by the fruit characteristics like color, size, shape, and optimal conditions to distribution, as strawberry and tomato trade has very rigorously policies associated to national and international markets. Packaging systems play an essential role in the logistic chain for protecting, labelling, and stacking of valuable or fragile perishable commodities (Sasaki *et al.*, 2021a). Our results were positively affected by this variable showing high food losses in packaging step. Farm owner education has significant impact to mitigate food losses all along harvest and distribution stages (Table 3).

Oranges and mangos had the highest food losses estimates, related to different variables (Table 3). One of them is hot weather in the farm (Table 4) causing high food losses (Tang *et al.*, 2020; Liu *et al.*, 2022). Refrigerated distribution of perishable food is required, but almost no cooling practices are done at any stage of the chain in the studied municipalities. Some producers transport their produce to the local market covered with available materials to reduce exposure to the sun. The lack of optimum temperature management positively influences the food losses volume in the distribution stage in all the commodities assessed. There was a significative difference among the level of transportation and its influence on food losses. In 2007 clarified that during transportation the commodities should be

Table 2. Food losses estimation of commodities at harvest and distribution step.

Commodity	Supply chain step losses		Food losses Ton (%)
	Harvesting Ton (%)	Distribution Ton (%)	
Asparagus	2,638 (9.5%)**	659 (2.3%)	3,297 (11.8%)
Strawberry	378 (5.6%)*	201 (3%)	579 (8.5%)
Mango	1,540 (18%)**	673 (8%)	2,213 (26%)
Orange	6451 (12.8%)**	2,481 (5%)	8,932 (17.8%)
Tomato	2,223 (2.5%)**	794 (1%)	3,017 (3.5%)

Results are the mean in the municipalities. *, ** indicate significant differences ($p<0.05$) and highly significative difference ($p<0.01$), respectively, according to ANOVA, followed by a Tukey post hoc test ($p<0.05$) between supply chain step losses by commodity.

Table 3. Tobit model estimation results of the percentage of losses from asparagus, strawberry, orange, mango and tomato by farmland sector BCS, México.

Variable name / Parameter	Asparagus	Strawberry	Mango	Orange	Tomato
	t-value				
Intercept	−0.65	−1.12	−0.72	3.40***	−1.7
Farm size	1.53*	0.9	1.53**	0.53**	0.7
Farm size squared	−0.56	−0.56	−0.61	−0.03	−0.33
Number of employees	20.34	9.8123	12.34	11.09*	6.5612
Number of e. squared	−0.65	−0.35	−0.12	−0.08	−0.15
Packing in farm	1.32**	1.28*	0.35**	−1.12**	1.17*
Packing outfarm	1.91*	0.81***	0.73**	1.05**	0.91***
Farm location	0.23	0.03***	1.4***	0.5***	0.01***
Local distribution	−0.04	−0.12	−0.17	−0.07	−0.14
National distribution	1.17	2.34***	2.01	1.17	1.34***
International distribution	0.61	3.71***	1.63	0.8	4.56***
Farm owner education	1.32*	0.31	0.13	0.04	0.43
Gender	1.78*	0.72*	0.23	1.62*	0.61**
Employee technical training	0.11	0.23	1.4**	0.3**	0.15
Transportation temperature control	−1.77*	−5.89**	−2.55*	−3.77*	−7.89**
No transportation temperature control	1.95*	1.37*	3.47**	3.8**	2.15*
Sorting for bioactive compounds	0.16	0.27	0.4567	0.23	0.78

*Significant at 0.10; ** Significant at 0.05; *** Significant at 0.01.

packaged and stacked to avoid excessive movement (Rehman *et al.*, 2007). A commonly explanatory variables is the kind of cultivation, harvesting method, according to collected data analysis these commodities have harvesting time associated to farm size, collecting type and employee technical training (Table 3), among other factors that increase food losses in the farm. It was reported that food losses volume was significantly influenced by farmland size, use of water and these factors influence the emissions (Karthikeyan *et al.*, 2020; Qin and Horvarth, 2022). However, the latter also found a negative correlation between the crop size and food losses is that is using a high level of sensor technology to irrigate.

The ordered probit results suggested that farm owners are interested in sorting food losses to produce bioactive compounds and this decision was positively influenced by socio-economic and demographic factors (Table 4). Those that are more likely to sort food losses for the obtaining bioactive compounds have a technical training in agriculture or university degree. The results are consistent with expectations that education favors higher environmental awareness and encourages behavior consistent with environmentalism (Arslan, 2012). Respondents participating in the survey recognized they can convert food losses into a useful resource. The positive effect on farm owner's decision to separate food

Table 4. Ordered probit estimation results of the willingness to separate food loss for the purpose to obtain bioactive compounds.

Variable name /Parameter	Estimated coefficient	Standard error	t-value
Intercept	−0.6789	1.1302	−0.65
Farm size	0.7892	0.0067	2.31**
Farm size squared	−0.0023	0.0567	2.09*
Number of employees	0.3781	0.0124	1.20*
Number of e. squared	−0.7821	0.2301	−1.18*
Packing in farm	−0.6283	0.3201	−3.45***
Packing outfarm	−0.5674	0.1569	−0.62**
Farm location	0.1173	0.2583	0.34
Local distribution	−0.0105	0.1123	−0.63
National distribution	−0.6573	0.5743	−1.11*
International distribution	−0.2006	0.1893	−0.98**
Farm owner education	1.6743	0.1673	6.43***
Employee technical training	−0.5042	0.0981	−0.28*
Gender	−0.7823	0.0011	−4.85***

*Significant at 0.10; ** Significant at 0.05; *** Significant at 0.01.

losses seems to capture the attitudes of individuals who have human resources available in the farm and therefore have more flexibility of schedule.

Food loss estimation

There was a significant difference between losses during harvesting and distribution, accounting for 2.5%-18% and 1-8%, respectively (Table 2). This represents food loss between 3.5% and 26% in the commodities across the supply chain. The crops with the lowest losses were tomato and strawberry with losses of 2.5% and 5.6% during harvesting, respectively. Mango presented an interesting case. More than 3.5% of the total mango produced was left in the field, with an estimated loss of 3,017 ton over the whole season of mango produced in total. During harvesting, ripe mango fruit fall off the tree, crashed on the ground, becoming inedible (Zhang *et al.*, 2019). Losses of mangos pointed out the important need for proper food loss definition. In the actual loss definition ripe mango not intended to be harvested and therefore not intended for human consumption is considered a loss (FAO, 2019).

Results showed that losses vary significatively ($p<0.05$) between crops during the harvesting and distribution stages. The loss volume of asparagus during distribution was highly significatively different ($p<0.01$) with respect to the losses in the distribution with the differences between 2,638 ton to 659 ton approximately. The same case has been shown for strawberry, mango, orange, and tomato (Table 2).

In Baja California Sur desert, geographical location plays an import role in the commodities distribution, associated with weather and the conditions of storage, both in marketability and in food loss (Widener, 2018). Recent studies show that geographical proximity is beneficial for food supply chains, especially with regard to transport time, due to the short farm-to-market transport time, decreasing the probability of losses (Widener, 2018; FAO, 2019; Zhang *et al.*, 2019).

In México there are no geographical limited studies about food losses. Our study pointed up causes of small and large farm food losses generation in a desert region with critical weather conditions. Commodities assessed showed a different losses behavior which has crucial impact to future treatments and facilities to use all nutrients and compounds of food losses.

CONCLUSIONS

Characteristics of agricultural desert system in BCS state peninsula achieved to estimation the amount of food losses of five commodities. Food losses share in both categories decrease as the farm size increased, strongly suggesting that the smaller farms may be a primary source of food losses. Rural farm owners of desert in BCS peninsula with asparagus, strawberry, mango, orange and tomato commodities, with large farms and a high employee number are likely to sort their food losses for the purpose of obtaining bioactive compound. The willingness to undertake sorting is essential in using food losses in BCS state peninsula.

REFERENCES

Abera, G., Ibrahim, A. M., Forsido, S. F., & Kuyu, C. G. 2020. Assessment on post-harvest losses of tomato (*Lycopersicon esculentem* Mill.) in selected districts of East Shewa Zone of Ethiopia using a commodity system analysis methodology. *Heliyon*. 6. https://doi.org/10.1016/j.heliyon.2020.e03749

Affognon, H., Mutungi, C., Sanginga, P., & Borgemeister, C. 2015. Unpacking postharvest losses in Sub-Saharan Africa: a Meta-analysis. *World dev*. 66: 49–68. https://doi.org/10.1016/j.worlddev.2014.08.002

Amicarelli, V., & Box, C. 2020. Food waste measurement toward a fair, healthy and environmental-friendly food system: a critical review. *Br. Food j.* 123 https://doi.org/10.1108/BFJ-07-2020-0658

Arslan, S. 2012. The Influence of Environment Education on Critical Thinking and Environmental Attitude. *Procedia - social and behavioral sciences*. 55:902–909. https://doi.org/10.1016/j.sbspro.2012.09.579

Benites-Zapata, V., Urrunaga-Pastor, D., Solorzano-Vargas, M., Herrera-Añazco, P., Uyen-Cateriano, A., Bendezu-Quispe, G., Toro-Huamanchumo, C., & Hernandez, A. 2021. Prevalence and factors associated with food insecurity in Latin America and the Caribbean during the first wave of the COVID-19 pandemic. *Heliyon*. 7. https://doi.org/10.1016/j.heliyon.2021.e08091

Chaboud, G., & Moustier, P. 2021. The role of diverse distribution channels in reducing food loss and waste: The case of the Cali tomato supply chain in Colombia. *Food policy*. 98. https://doi.org/10.1016/j.foodpol.2020.101881

FAO. Food wastage footprint. Impacts on natural resources. 2013. https://www.fao.org/3/i3347e/i3347e.pdf (Access date: junio 2023)

FAO. The State of Food and Agriculture 2019. 2019. https://www.fao.org/3/ca6030en/ca6030en.pdf

Gao, M., Wu, Z., Guo, X., & Yan, D. 2022. Emergy evaluation of positive and negative benefits of agricultural water use based on energy analysis of water cycle. *Ecological indicators*. 139. https://doi.org/10.1016/j.ecolind.2022.108914

HLPE. Food Losses and Waste in the Context of Sustainable Food Systems, A Report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security (Rome). 2014.

HLPE. Nutrition and Food Systems, A Report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security (Rome). 2017. https://doi.org/10.1016/j.jafr.2022.100431

Karthikeyan, L., Chawla, I., & Mishra, A. K. 2020. A review of remote sensing applications in agriculture for food security: Crop growth and yield, irrigation, and crop losses. *Journal of hydrology*. 586. https://doi.org/10.1016/j.jhydrol.2020.124905

Liu, X., Zhang, Y., Leng, X., Yang, Q., Chen, H., Wnag, X., & Cui, N. 2022. Exploring the optimisation of mulching and irrigation management practices for mango production in a dry hot enviroment based

on the entropy weight method. *Scientia horticulturae*. 291. https://doi.org/https://doi.org/10.1016/j.scienta.2021.110564
Pereira, R., Galo, N., & Filimonau, V. 2022. Food loss and waste from farm to gate in Brazilian soybean production. *Journal of agriculture and food research*. 10.
Qin, Y., & Horvath, A. 2022. What contributes more to life-cycle greenhouse gas emissions of farm produce: Production, transportation, packaging, or food loss? *Resources, conservation and recycling*. 176. https://doi.org/10.1016/j.resconrec.2021.105945
Rehman, M. U., Khan, N., & Jan, I. 2007. Post harvest losses in tomato crop: a case of peshawar valley. *Sarhad Journal of Agriculture*. 23.
Ronen, K. 2023. Quantification of food waste in retail operations: A fruit and vegetable wastage case in Paraguay. *Enviromental challenges*. 10. https://doi.org/10.1016/j.envc.2022.100665
Saboori, B., Radmehr, R., Zhang, Y., & Zekri, S. 2022. A new face of food security: A global perspective of the COVID-19 pandemic. *Progress in disaster science*. 16. https://doi.org/10.1016/j.pdisas.2022.100252
Sasaki, Y., Orikasa, T., Nakamura, N., Hayashi, K., Yasaka, Y., Makino, N., Shobatake, K., Koide, S., & Shiina, T. 2021b. Optimal packaging for strawberry transportation: Evaluation and modeling of the relationship between food loss reduction and enviromental impact. *Journal of food engineering*. 314. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2021.110767
Sasaki, Y., Orikasa, T., Nakamura, N., Hayashi, K., Yasaka, Y., Makino, N., Shobatake, K., Koide, S., & Shiina, T. 2021a. Dataset for life cycle assessment of strawberry-package supply chain with considering food loss during transportation. *Data in brief*. 39. https://doi.org/10.1016/j.dib.2021.107473
Tang, Y., Ma, X., Li, M., & Wang, Y. 2020. The effect of temperature and light on strawberry production in a solar greenhouse. *Solar energy*. 195:318–328. https://doi.org/10.1016/j.solener.2019.11.070
United Nations. Transforming Our World: The 2030 Agenda for Sustainable Development. 2015. https://sdgs.un.org/goals (Access date: Julio 2023)
Widener, M. J. 2018. Spatial access to food: Retiring the food desert metaphor. *Physiology and Behavior*, 193: 257–260. https://doi.org/10.1016/j.physbeh.2018.02.032
Zhang, D., Wang, C., & Li, X. lin. 2019. Yield gap and production constraints of mango (*Mangifera indica*) cropping systems in Tianyang County, China. *Journal of integrative agriculture*, 18: 1726–1736. https://doi.org/10.1016/S2095-3119(18)62099-4

Colegio de Postgraduados

Geographic distribution prediction of an invading species in Mexico: the case of the monk parakeet (*Myiopsitta monachus*)

Salazar-Borunda, Manuel A.[1]; Valdez-Abdo, Rebeca[1]; Pereda-Solís, Martín E.[1*]; Martínez-Guerrero, José H.[1]; Saucedo-Rentería, Andrés[1]; Peredo-Rivera, Ernesto[2]

[1] Universidad Juárez del Estado de Durango. Durango, Durango, México. C. P. 34000.
[2] Colegio de Postgraduados Campus San Luis Potosí. Salinas de Hidalgo, San Luis Potosí, México. C.P. 78620.
* Correspondence: mepereda@ujed.mx

ABSTRACT
Objective: To determine the potential distribution of the monk parakeet (*Myiopsitta monachus*) in Mexico.
Design/methodology/approach: The study generated the model with confirmed presences of the species, the MaxEnt algorithm, and bioclimatic and elevation information. The evaluation, calibration and selection were carried out with the kuenm package in R. The model generated was projected to the geographic space of Mexico.
Results: The model estimated the most favorable areas for the species in Mexico, based on the similarity of the climate and elevation conditions of the sites with its natural distribution. The most favorable sites for the species are distributed in the central-southern regions of the country. Variables influencing its distribution are derived from temperature, precipitation and elevation.
Limitations on study/implications: The model can contribute to the planning of management and monitoring strategies that mitigate the invasion of this species.
Findings/conclusions: The areas in Mexico where there is a high risk of invasion by the monk parakeet were identified.

Keywords: Exotic species, invasive species, invasion risk, biodiversity loss, MaxEnt.

Citation: Salazar–Borunda, M. A., Valdez–Abdo, R., Pereda–Solís, M. E., Martínez–Guerrero, J. H., Saucedo–Rentería, A., & Peredo–Rivera, E. (2024). Geographic distribution prediction of an invading species in Mexico: the case of the monk parakeet (*Myiopsitta monachus*). *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2678

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: September 11, 2023.
Accepted: March 18, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 105-112.

INTRODUCTION

Most of the invading species in Mexico have been introduced due to human activities related to trade (CONABIO, 2010). In the case of birds, their traffic has experienced a constant increase (Ribeiro *et al.*, 2019) and their liberation or escape into other environments represents a significant risk. This is because their propagation can cause economic losses, damage to biodiversity, and harmful effects for native species (CONABIO, 2010).

Although not all the exotic species manage to establish self-sustainable populations in the places where they are introduced, the risk increases significantly when the climate conditions are similar to those of their places of origin (Liu *et al.*, 2020). Therefore, the

spatial identification of sites that are more favorable for their propagation can be essential in the management of their local populations, in addition to promoting an early reaction against unwanted initial colonies.

In Mexico, out of the 348 species recognized as invaders, at least 16 bird species have been established and reproduced successfully (DOF, 2016). The monk parakeet (*Myiopsitta monachus*, Boddaert 1783), originally from South America (Salgado-Miranda *et al*., 2016), was introduced as an ornamental bird since 1970 and has become one of the invading parrots with highest distribution in Mexico, confirming its presence in different states of the south (Pablo, 2009), center (Pineda and Malagamba, 2011), and north (Guerrero-Cárdenas *et al*., 2012) of the country.

Although the monk parakeet is widening its distribution and can be abundant locally, the species has received scarce attention in Mexico. In addition, considering the importance of increasing its study, due to the damage that it generates in its environment (Hernández-Brito *et al*., 2020; Castro *et al*., 2022; Briceño *et al*., 2023), the objective of this study was to model the potential distribution of the monk parakeet in Mexico, with the aim of determining the favorable zones for the species and estimating its possible expansion. An approach of such broad scale can help to elucidate some of the factors responsible for the increase in population and the distribution area of this potentially harmful species.

MATERIALS AND METHODS

Study area and records of presence

The study area corresponds to the Mexican Republic, which covers a continental surface of 1 959 248 km^2 and includes the totality of the physiographic provinces (INEGI, 2021). The coordinates of presence of the monk parakeet were obtained from the Global Biodiversity Information Facility platform (GBIF, 2021; 239 594 points) and field records (30 points). The spatial correlation between coordinates decreased considering a buffer circular area of 1.22 ha between each record, in function of the home range of this species (Senar *et al*., 2021). The points that were outside this threshold were eliminated and the coordinates duplicated with spThin (Aiello-Lammens *et al*., 2015) and *remove.duplicates* (Bivand *et al*., 2013; R Core Team, 2021) packages, respectively.

Bioclimatic variables and calibration area

Bioclimatic variables generated from the interpolation of data from monthly meteorological stations were used, considering the average values covering from 1970 to 2000 (WorlClim, version 2.1; Fick and Hijmans, 2017), with a spatial resolution of 30 seconds of arc ($\sim$1 km^2). From the set of variables downloaded (n=19), the average temperatures of the most humid (BIO 8) and driest (BIO 9) trimester were excluded, as well as the amount of rainfall in the warmest (BIO 18) and coldest (BIO 19) trimester, due to the uncertainty that is generated when combining information about temperature and precipitation in the same raster (Escobar *et al*., 2014). The variables were selected based on their coefficient of correlation (Pearson, $r \geq 0.8$) and biological importance (Avery *et al*., 2012). The delimitation of the calibration area was fundamental in the modelling of niches and distribution of species, as well as in the generation of the model

(Soberón *et al*., 2017). This site (Figure 1) was defined as the original distribution area of the monk parakeet and it was generated using the centroid of the presences and the minimum convex polygon.

Execution and evaluation of the model

The calibration, construction and evaluation of the model were carried out using the kuenm package (Cobos *et al*., 2019), within the R Core Team environment (2021). The adjustment of the model was determined through the partial ROC analysis (operative curves characteristic of the receptor), the omission rate and the complexity of the model (Akaike information criterion). The exit format adopted was the logistic, where the maps represented values of environmental suitability in a range of 0 to 1, for the monk parakeet. The type ASCII file generated by the software was projected to the geographic space of the study area through QGIS (version 3.16; QGIS, 2021).

RESULTS AND DISCUSSION

Presence data and distribution model

The coordinates of presence were refined and reduced to 10862 single ones and spatially non-correlated. In the study, 1433 models and 85 sets of environmental variables were evaluated. The model selected was constructed with refined records (7603 for training and 3259 for execution) and the set of bioclimatic variables: isothermality (BIO 3), maximum temperature of the warmest month (BIO 5), annual temperature interval (BIO 7), mean temperature of the coldest quarter (BIO 11), annual precipitation (BIO 12), precipitation of the driest month (BIO 14), precipitation of the driest quarter (BIO 17), and elevation. The final model presented a satisfactory adjustment, with AUC rates of 0.96, omission rates of 0.01%, and partial ROC value of 0.

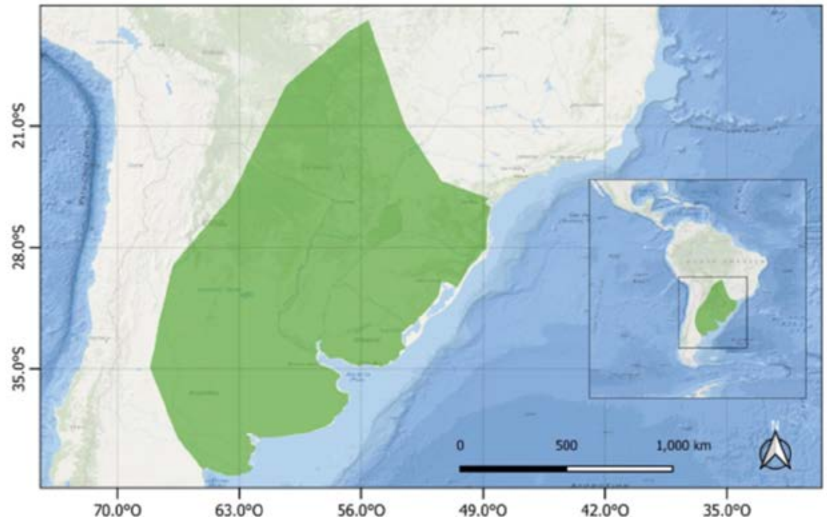


Figure 1. Calibration area (original distribution) used for the potential distribution model of the monk parakeet (*Myiopsitta monachus*).
Source: Prepared by the authors.

Bioclimatic variables

The bioclimatic variables selected through the Jack-knife process allowed identifying the climate spaces where the species groups together. These sites are characterized by having a partially fresh climate, whose temperature fluctuates between 3 and 14 °C in the coldest month and between 15 and 20 °C in the warmest month (Table 1).

Potential distribution of the monk parakeet in Mexico

The model projected zones of high climate suitability in the entire territory, especially highlighting regions in the center and southwest of Mexico (Figure 2).

These areas include the states of Jalisco, Michoacán, Puebla, Guanajuato, Guerrero and Oaxaca in areas where the species has not been recorded.

Table 1. Percentage contribution of the bioclimatic variables to the potential distribution model of the monk parakeet (*Myiopsitta monachus*) in Mexico.

Bioclimatic variables	Contribution (%)
Mean Temperature of Coldest Quarter (BIO 11)	61.2
Elevation	25.7
Precipitation of Driest Quarter (BIO 17)	4.7
Isothermality (BIO 3)	3.9
Maximum Temperature of Warmest Month (BIO 5)	1.8
Temperature Annual Range (BIO 7)	1.4
Precipitation of Driest Month (BIO 14)	1.2
Annual Precipitation (BIO 12)	0.1

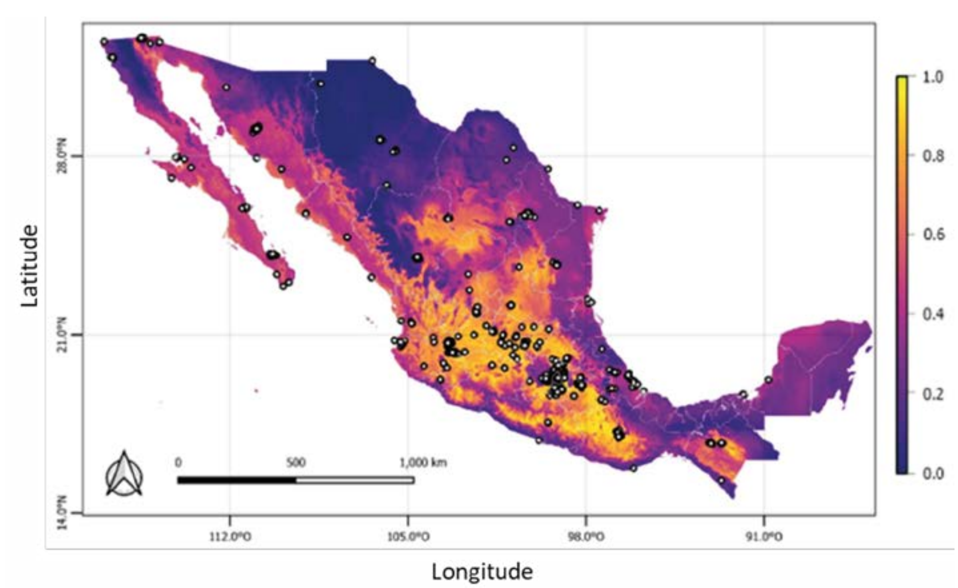


Figura 2. Potential distribution model for the monk parakeet (*Myiopsitta monachus*) in Mexico. The map indicates the continuous logistic estimation with lighter areas (values close to 1) that contain greater bioclimatic suitability. The circles indicate known presences of *M. monachus*.

DISCUSSION

The invasion anticipated by the model

The distribution of invading species can be modelled using environmental information from their region of origin (Thuiller, 2005). This study has been built to estimate the distribution of the monk parakeet in Mexico. It was developed with a limited set of bioclimatic and elevation variables that could influence its possible invasion. The predicted areas extend on most of the territory, especially in the zones of warm and temperate climates of the central portion of the country.

One of the most desirable characteristics of this model is that it classifies as favorable areas some sites where the species is absent. This could be explained by taking into account that the monk parakeet has invaded Mexico for some years (CONABIO, 2015) and that the colonization process is probably not over. Although the speed of dispersion of this species is low and there is no evidence that it conducts long-distance movements (>10 km, Borray-Escalante *et al.*, 2023), it is likely that the settlement of the sites estimated as favorable by the model is slow. Especially considering that these sites have continuity with those that have presence recorded and with favorable or intermediate favorable characteristics (>0.6). In these sites, the populations could be well established and act as sources from which monk parakeets disperse toward nearby favorable areas. However, the dispersion of the species observed during recent years has been fast and extensive (Da Silva *et al.*, 2010; Dawson *et al.*, 2021; Borray-Escalante *et al.*, 2023). This phenomenon has been attributed to this species being a broadly traded parrot, whose success in colonization and establishment increases notably with the rate of escape (Duncan *et al.*, 2003), which is why the model generated could be modified by including this factor.

As an exotic species, the demographic growth and of dispersion ends around 30 years after its phase of establishment (Shigesada and Kawasaki, 2002) and perhaps this invading species needs to surpass some other factors and biological interactions before occupying its potential distribution. However, if the establishment of the monk parakeet in Mexico was in an expansion phase (CONABIO, 2015), it would probably imply that demographic growth will continue in the zones with potential distribution, especially considering that its reproductive capacity increases in invaded areas (Senar *et al.*, 2019).

Need for monitoring

Once the exotic and invading species are established, their control or eradication is costly and difficult to carry out (Beever *et al.*, 2019). The monk parakeet can be considered exotic and invader in Mexico and its establishment has taken place successfully, since its presence is reported in most of the country (Zuria *et al.*, 2017).

Without adequate management, the expansion and demographic growth and of dispersion of this species could continue due to its adaptability to urban environments (Dickinson *et al.*, 2023), because its diet is nearly omnivorous of anthropogenic origin (Mazzoni *et al.*, 2021), and due to its high reproductive capacity (Senar *et al.*, 2019). This phenomenon has been documented in other countries (Hernández-Brito *et al.*, 2022) and at the same time, it has been accompanied by other potential damages in agricultural areas (Castro *et al.*, 2022), since it competes over nesting sites with native birds (Hernández-Brito *et al.*, 2020) and it can transmit zoonotic diseases (Briceño *et al.*, 2023); therefore, without a doubt they are factors that should be taken into consideration while monitoring the zones with potential for distribution.

CONCLUSIONS

The areas in Mexico where there is a high risk of invasion by the monk parakeet have been identified. Although it is clear that some variables not included in the model, such as climate change, the influence of human activities, or the biotic relationships with native fauna, can influence the dispersion of this species, the map generated can contribute to the planning of management and monitoring strategies. Likewise, the estimation can be considered as a first step toward a future expansion and invasion in Mexico, which justifies a close tracking of the populations of this species, especially in the areas detected as favorable by the model.

ACKNOWLEDGEMENTS

The authors wish to thank the School of Veterinary Medicine and Animal Science at the Universidad Juárez del Estado de Durango and Colegio de Postgraduados, San Luis Potosí Campus; and the reviewers who substantially improved this manuscript.

REFERENCES

Aiello-Lammens, M., Boria, R., Radosavljevic, A., Vilela, B. & Anderson, R. (2015). spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. *Natural Sciences, 38*(5), 541-545. https://doi.org/10.1111/ecog.01132

Avery, M. L., Tillman, E. A., Keacher, K. L., Arnett, J. E., & Lundy, K. J. (2012). Biology of invasive monk parakeets in South Florida. *The Wilson Journal of Ornithology, 124*(3), 581-588. https://doi.org/10.1676/11-188.1

Beever, E. A., Simberloff, D., Crowley, S. L., Al-Chokhachy, R., Jackson, H. A., & Petersen, S. L. (2019). Social-ecological mismatches create conservation challenges in introduced species management. *Frontiers in Ecology and the Environment, 17*(2), 117-125. https://doi.org/10.1002/fee.2000

Bivand, R., Pebesma, E., & Gomez-Rubio, V. (2013). Applied spatial data analysis with R. New York: Springer. Pp. 237-268.

Borray-Escalante, N. A., Baucells, J., Carrillo-Ortiz, J., Hatchwell, B. J., & Senar, J. C. (2023). Long-distance dispersal of monk parakeets. *Animal biodiversity and conservation, 46*(1), 71-78. http://hdl.handle.net/2072/531519

Briceño, C., Marcone, D., Larraechea, M., Hidalgo, H., Fredes, F., Ramírez-Toloza, G., & Cabrera, G. (2023). Zoonotic Cryptosporidium meleagridis in urban invasive monk parakeets. *Zoonoses and Public Health*. https://doi.org/10.1111/zph.13067

Castro, J., Sáez, C., & Molina-Morales, M. (2022). The monk parakeet (*Myiopsitta monachus*) as a potential pest for agriculture in the Mediterranean basin. *Biological Invasions, 24*(4), 895-903. https://doi.org/10.1007/s10530-021-02702-5

Cobos, M., Peterson, A., Barve, N., & Osorio-Olvera, L. (2019). kuenm: An R package for detailed development of ecological niche models using Maxent. *PeerJ* 7:e6281. https://doi.org/10.7717/peerj.6281

Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). (2010). Estrategia nacional sobre especies invasoras en México, prevención, control y erradicación. México, CONABIO. http://www.conabio.gob.mx/institucion/Doc/Estrategia_Invasoras_Mex.pdf

Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). (2015). Diagnóstico de la invasión de cotorra argentina (*Myiopsitta monachus*) en las áreas prioritarias. México, CONABIO. http://www.conabio.gob.mx/institucion/proyectos/resultados/InfLI047.pdf

Da Silva, A. G., Eberhard, J. R., Wright, T. F., Avery, M. L., & Russello, M. A. (2010). Genetic evidence for high propagule pressure and long-distance dispersal in monk parakeet (*Myiopsitta monachus*) invasive populations. *Molecular Ecology, 19*(16), 3336-3350. https://doi.org/10.1111/j.1365-294X.2010.04749.x

Dawson Pell, F. S., Senar, J. C., Franks, D. W., & Hatchwell, B. J. (2021). Fine-scale genetic structure reflects limited and coordinated dispersal in the colonial monk parakeet, *Myiopsitta monachus*. *Molecular ecology, 30*(6), 1531-1544. https://doi.org/10.1111/mec.15818

Diario Oficial de la Federación (DOF) (2016). Listado de especies exóticas. Secretaría de Medio Ambiente y Recursos Naturales. http://www.dof.gob.mx/nota_detalle.php?codigo=5464456&fecha=07/12

Dickinson, E., Young, M. W., Tanis, D., & Granatosky, M. C. (2023). Patterns and Factors Influencing Parrot (Order: Psittaciformes) Success in Establishing Thriving Naturalized Populations within the Contiguous United States. *Animals, 13*(13), 2101. https://doi.org/10.3390/ani13132101

Duncan, R. P., Blackburn, T. M., & Sol, D. (2003). The ecology of bird introductions. *Annual Review of Ecology, Evolution, and Systematics, 34*(1), 71-98. https://doi.org/10.1146/annurev.ecolsys.34.011802.132353

Escobar, L. E., Lira-Noriega, A., Medina-Vogel, G., & Peterson, A. T. (2014). Potential for spread of the white-nose fungus (*Pseudogymnoascus destructans*) in the Americas: use of Maxent and NicheA to assure strict model transference. *Geospatial health, 9*(1), 221-229. https://doi.org/10.4081/gh.2014.19

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology, 37*(12), 4302-4315. https://doi.org/10.1002/joc.5086

Global Biodiversity Information Facility (GBIF). (2021). *Myiopsitta monachus* (Boddaert, 1783) GBIF Backbone Taxonomy. Checklist dataset. https://doi.org/10.15468/39omei accessed via GBIF.org

Guerrero-Cárdenas, I., Galina-Tessaro, P., Caraveo-Patiño, J., Tovar-Zamora, I., Cruz-Andrés, O. y Álvarez-Cárdenas, S. (2012). Primer registro de la cotorra argentina (*Myiopsitta monachus*) en Baja California Sur, México. *Huitzil, 13*(2), 156-161. https://www.scielo.org.mx/pdf/huitzil/v13n2/v13n2a10.pdf

Hernández-Brito, D., Blanco, G., Tella, J. L., & Carrete, M. (2020). A protective nesting association with native species counteracts biotic resistance for the spread of an invasive parakeet from urban into rural habitats. *Frontiers in Zoology, 17*(13), 1-13. https://doi.org/10.1186/s12983-020-00360-2

Hernández-Brito, D., Carrete, M., & Tella, J. L. (2022). Annual censuses and citizen science data show rapid population increases and range expansion of invasive rose-ringed and monk parakeets in Seville, Spain. *Animals, 12*(6), 677. https://doi.org/10.3390/ani12060677

Instituto Nacional de Estadística y Geografía (INEGI). 2021. Catálogo Único de Claves de Áreas Geoestadísticas Estatales, Municipales y Localidades. México. https://www.inegi.org.mx/contenidos/app/ageeml/Ayuda/Ayuda_Gral_Cat_Unico.pdf

Liu, C., Wolter, C., Xian, W., & Jeschke, J. M. (2020). Most invasive species largely conserve their climatic niche. *Proceedings of the National Academy of Sciences, 117*(38), 23643-23651. https://doi.org/10.1073/pnas.2004289117

Mazzoni, D., Pascual, J., Montalvo, T., González-Solís, J., & Senar, J. C. (2021). The diet of Monk Parakeet nestlings (*Myiopsitta monachus*) in an urban area: a study using stable isotopes. *Bird study, 68*(4), 455-461. https://doi.org/10.1080/00063657.2022.2113856

Pablo, R. (2009). Primer registro del perico argentino (*Myiopsitta monachus*) en Oaxaca, México. *Huitzil, 10*(2), 48-51. https://www.scielo.org.mx/pdf/huitzil/v10n2/v10n2a2.pdf

Pineda, R. y Malgamba, A. (2011). Nuevos registros de aves exóticas en la ciudad de Querétaro, México. *Huitzil, 12*(2), 22-27. https://doi.org/10.28947/hrmo.2011.12.2.127

QGIS Development Team. (2021). QGIS Geographic Information System. Open-Source Geospatial Foundation. http://qgis.osgeo.org

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Ribeiro, J., Reino, L., Schindler, S., Strubbe, D., Vall-llosera, M., Araújo, M. B., ... & Nuno, A. (2019). Trends in legal and illegal trade of wild birds: A global assessment based on expert knowledge. *Biodiversity and conservation*, 3343-3369. https://doi.org/10.1007/s10531-019-01825-5

Salgado-Miranda, S., Medina, J., Sánchez-Jasso, J. M., y Soriano-Vargas, E. (2016). Registro altitudinal más alto en México para la cotorra argentina (*Myiopsitta monachus*). *Huitzil, 17*(1),155-159. https://doi.org/10.28947/hrmo.2016.17.1.238

Senar, J. C., Carrillo-Ortiz, J. G., Ortega-Segalerva, A., Dawson Pell, F. S. E., Pascual, J., Arroyo, L., ... & Hatchwell, B. J. (2019). The reproductive capacity of Monk Parakeets *Myiopsitta monachus* is higher in their invasive range. *Bird Study, 66*(1), 136-140. https://doi.org/10.1080/00063657.2019.1585749

Senar, J. C., Moyà, A., Pujol, J., Tomas, X., & Hatchwell, B. J. (2021). Sex and Age Effects on Monk Parakeet Home-Range Variation in the Urban Habitat. *Diversity, 13*(12), 648. https://doi.org/10.3390/d13120648

Shigesada, N., & Kawasaki, K. (2002). Invasion and the range expansion of species: effects of long-distance dispersal. *Dispersal ecology*, 350-373.

Soberón, J., Osorio-Olvera, l. y Peterson, T. (2017). Diferencias conceptuales entre modelación de nichos y modelación de áreas de distribución. *Revista Mexicana de Biodiversidad, 88*(2), 437-441. https://doi.org/10.1016/j.rmb.2017.03.011

Thuiller, W., Richardson, D. M., Pyšek, P., Midgley, G. F., Hughes, G. O., & Rouget, M. (2005). Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Global change biology, 11*(12), 2234-2250. https://doi.org/10.1111/j.1365-2486.2005.001018.x

Zuria, I., Castellanos, I., Valencia-Herverth, R. y Carbó-Ramírez, P. (2017). Primeros registros de la cotorra argentina (*Myiopsitta monachus*) en el estado de Hidalgo, México. *Huitzil, 18*(1), 33-37. https://doi.org/10.28947/hrmo.2017.18.1.261

Bibliometric analysis of wastewater treatment with microalgae in the period 1985-2023

Santillán-Ángeles, Abel[1]; **Quevedo-Nolasco, Abel**[1*]; **Macedo-Cruz, Antonia**[1]; **Rico-Sánchez, Axel Eduardo**[1]; **Zamora-Morales, Bertha Patricia**[2]

[1] Colegio de Postgraduados, Programa de Hidrociencias. Km. 36.5 carretera México-Texcoco Montecillo, Texcoco, Estado de México. MÉXICO. C.P. 56230.

[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Progreso No. 5, Barrio de Santa Catarina, Alcaldía Coyoacán, México D.F., C.P. 04010.

* Correspondence: abcdqn@gmail.com, anolasco@colpos

ABSTRACT

Objective. To indicate the condition of the wastewater treatment with microalgae (WTwM) research.

Methodology. The words treatment, wastewater, and microalgae, were entered as keywords in a search under "article title" in SCOPUS. The documents found were saved and exported as a file with a .bb extension. From R Studio, the BIBLIOMETRIX interface was linked to R Statistics. The interface was opened from MOZILLA to import the .bb file.

Results. There is a significant increase in the number of papers published since 2013, up to 68 articles in 2022. The authors with the highest number of contributions on the subject are Ivet Ferrer and Joan García. The countries most involved in this issue are China, Spain, India and Brazil; China is the one with the highest number of publications. The WTwM studies in 2023 are concerned with investigating biomass accumulation and nutrient removal as a way to make sustainable use of the process.

Conclusions. WTwM is a research topic that is studying and disseminating knowledge since the 80's. The author who stands out the most is Ivet Ferrer. China has little collaboration with scientists from other countries. The most recent WTwM studies address issues related to biodiesel production and biogas production. The topics to be addressed in future research will be related to the study of temperature, osmotic capacity, pH and O_2 levels.

Keywords: biological wastewater treatment, biomass, biodiesel, biogas.

Citation: Santillán-Ángeles, A., Quevedo-Nolasco, A., Macedo-Cruz, A., Rico-Sánchez, A. E., Zamora-Morales, B. P., (2024). Bibliometric analysis of wastewater treatment with microalgae in the period 1985-2023. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2682

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: September 14, 2023.
Accepted: March 23, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 113-125.

INTRODUCTION

Literature analysis is a way of identifying the development and growth of knowledge. And it is an alternative to evaluate scientific activity through the documents published on a particular topic. The importance of carrying out an exhaustive, representative and up-to-date bibliographic analysis lies in the assessment and selection of the information in a scientific database, through the appropriate use of bibliometric indicators, as a tool that measures the impact of the information.

At the international scale, SCOPUS and Web of Science (WoS) propose some indicators to determine the quality of scientific information (González-Sanabria, 2019). However, a bibliometric analysis is capable of analyzing the evolution, the degree of progress, as well as the scientific imperfection of a particular subject, as well as allowing to determine the rate of productivity, dispersion and scientific rigor with which knowledge is developed.

On this basis and considering that the scarcity of fresh water is currently a cause for concern; although various actions are carried out for the treatment of wastewater, most of these require large amounts of fossil energy, produce greenhouse gases and there is little use of the products obtained, it becomes necessary to search alternatives which would lead the scope of research efforts. In this regard, Atif *et al.* (2023) pointed out that the use of microalgae is a sustainable method for the production of biomass from wastewater (W). For this reason, the objective of this study was to indicate the current state of research on wastewater treatment with microalgae (WTwM); through bibliographic indicators that allow us to know the growth and development of the subject, when biological processes are studied as a sustainable alternative response to the increase in volumes of wastewater (W) worldwide.

MATERIALS AND METHODS

The keywords "treatment, wastewater, microalgae" were entered for a search in the SCOPUS database. This search only considered the words in "article title". It was sorted by document type ("DOCTYPE"), publication stage ("PUBSTAGE"), font type ("SRTYPE") and language ("LANGUAGE"). Documents found were saved in the "My Scopus" folder, which was exported as a .bb extension file using the "BibTex" tab of SCOPUS.

At R Studio with the "bibliometrix" library active, it was requested to run the "biblioshiny" command to link the BIBLIOMETRIX interface with R Statistics. The BIBLIOMETRIX interface was opened at MOZILLA, where a tab was opened in which the file with a .bb extension was imported using "DATA"-"Load Data".

RESULTS AND DISCUSSION

Results generated from the search in SCOPUS showed 504 records that considered the words "treatment, wastewater, microalgae" in their title. Of these, 329 documents were selected filtered by document type (DOCTYPE), publication phase (PUBSTAGE), font type (SRTYPE) and language (LANGUAGE) which are shown in Table 1.

Main information analysed by BIBLIOMETRIX

As a result of the analysis, in the foreground BIBLIOMETRIX summarizes the main information obtained (Table 2).

Table 1. Results of the search of documents in the SCOPUS database, which in their title include the words "treatment, wastewater, with microalgae".

Data base	Search	Records	Documents
Scopus	TITLE (treatment AND wastewater AND with AND microalgae) AND PUBYEAR > 1987 AND PUBYEAR < 2023 AND (LIMIT-TO (SRCTYPE , "j")) AND (LIMIT-TO (PUBSTAGE , "final")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))	503	329

* "DOCTYPE": type of document; "ar": article; "PUBSTAGE": publication phase; "final": final; "SRCTYPE": font type; "j": journal; "LANGUAGE": language; "English": English.

Table 2. Main information of the documents found in BIBLIOMETRIX.

Main information	Results
Artícle	329
Journals	109
Autors	1,305
Average documents per year	4.77
Average citation per document	34.73

Annual scientific production

The analysis showed that of the total number of articles identified and related to the topic since 1985 to 2012, less than one document (0.75) was published on average per year (Figure 1). From 2013 onwards, the number of documents shows a positive trend, as the publication of articles related to WTwM increased, from seven documents in that year to 68 in 2022 for a total of 293 articles. In 2017, the trend dropped, as only 19 papers were published, two fewer articles than in 2016.

Average number of citations per article

In this regard, Figure 2 shows a positive trend and two negative trends. The positive trend goes from six average citations in 1985 to 84 citations in 1996. On the other hand, the first negative trend went from 161 citations in 2001 to 12.67 in 2009; while the second negative trend is observed from 2011 to 2022, when it dropped from 105.33 to 8.35 average citations, respectively. The maximum average number of citations per document was observed in 2001, with a value of 161; while the average minimum was three in 1998.

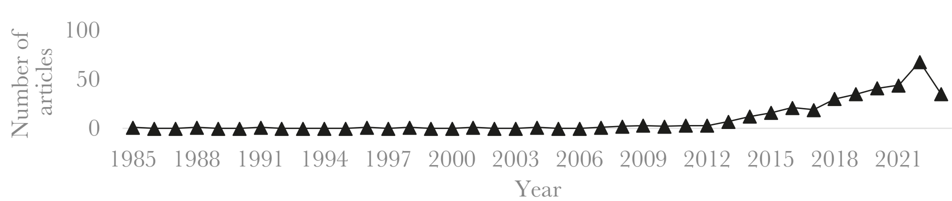


Figure 1. Behavior of scientific production in the period 1985-2023.

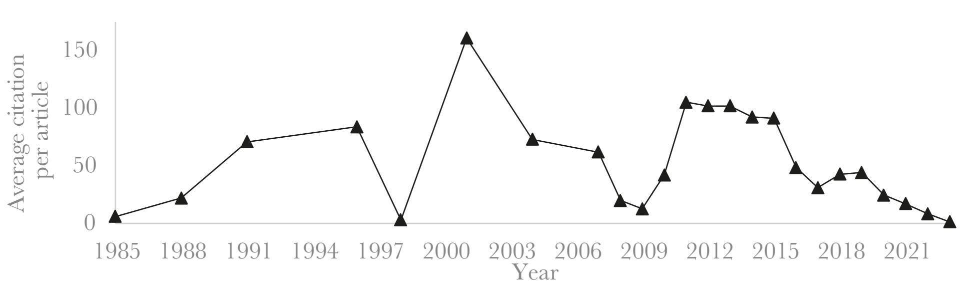


Figure 2. Behavior of the average number of citations per article in the period 1985-2023.

Most cited documents

The ten most cited documents are shown in Figure 3; those published by the authors Víctor Matamoros, Jyoti Prakassh Mayty, Sunja Cho, Annelies Beuckels and Esther Posadas stand out with more than 200 citations.

The paper "Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study" by Matamoros *et al.* (2015), published in Journal of Hazardous Materials, is the most cited with 292 citations. It is followed by the article "Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review" by Maity *et al.* (2014), published in Energy, which is cited 278 times.

The authors Cho *et al.* (2011) published the paper "Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production" in Bioresource Technology, which is cited 239 times. On the other hand, Beuckels *et al.* (2015) and Posadas *et al.* (2015) with their articles "Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment" and "Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways"; the first one published in Water Research and the second in Chemical Engineering Journal, these have 235 and 212 citations, respectively.

The most relevant Journal in 2023

Of the 109 journals analyzed, Figure 4 shows that Bioresource Technology, Algal Research, Journal of Environmental Management, and Water Science and Technology are those with the highest number of documents related to the topic of WTwM in 2023, with 52, 21, 13 and 11 respectively. The journals Environmental Science and Pollution Research, Journal of Water Process Engineering, Science of the Total Environment and Water Research have 10 published papers each.

Chemosphere has eight publications related to WTwM. In addition, Biochemical Engineering Journal and Journal of Environmental Chemical Engineering have seven published papers each.

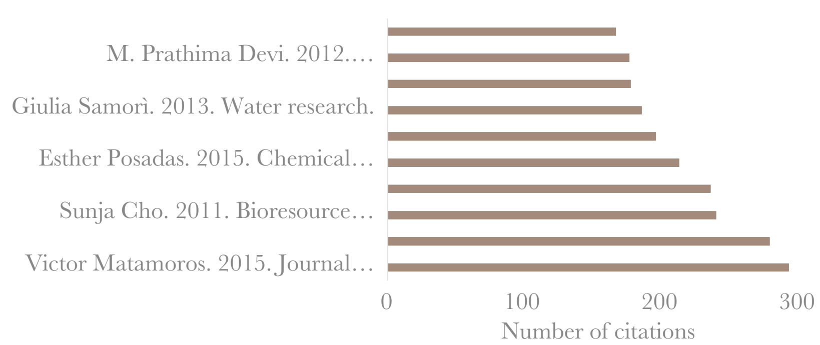


Figure 3. Number of citations included per article, which are related to Wastewater treatment with microalgae (WTwM).

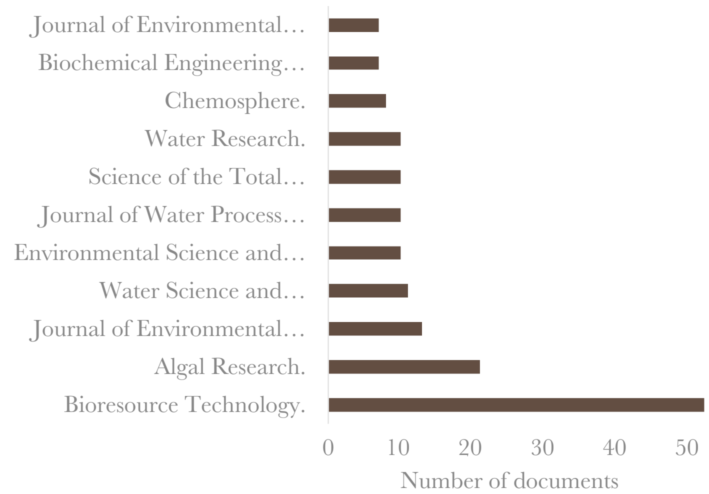


Figure 4. Behavior of journals that publish articles related to WTwM in 2023.

Journal production over time

Regarding the frequency of publication, Figure 5 shows that the journal Water Research has frequently published documents related to WTwM since 1988, with a cumulative number of 130 articles. While Bioresouce Technology, despite started publising in 1996, it has 334 articles on the subject to date.

On the other hand, of the journals with a recent incursion into the publication of related articles about wastewater treatment with microalgae (WTwM), Science of the Total Environment stands out, which began to be published in 2018 with a cumulative number of 29 papers. Environmental Science and Pollution Research, and Journal of Water Process Engineering have been publishing since 2019 with a cumulative number of articles 35 and 33 respectively. In addition, Journal of Environmental Management, publising since 2016, has 35; Algal Research with 109 published papers since 2016, and Water Science and Technology, publising since 2009, has a cumulative number of 75 articles.

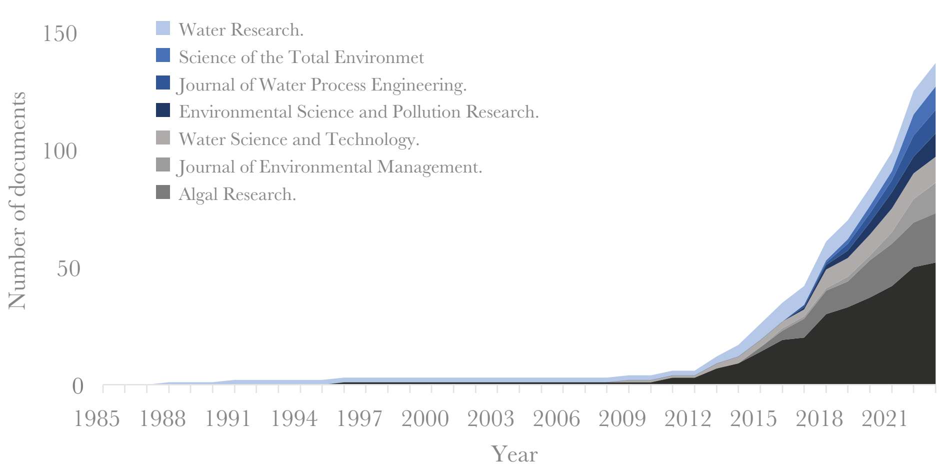


Figure 5. Trend in the production of articles per journal in the period 1985–2023.

Most relevant authors

Regarding this, Figure 6 shows that out of 1305 authors, those who have worked the most on the subject are Ivet Ferrey and Joan García, each with 10 articles published. They are followed by Liu Y., with nine publications (which initial includes either Yang Liu, Yu Liu, Yuhuan Liu, Yanbo Liu or Yuanqi Liu, as it was observed in the review of articles). Afterwards, Zhang Y., with eight published papers (the initial aggregates Yulei Zhang, Yaping Zhang, Yakun Zhang, Yifeng Zhang; Yalei Zhang, Yangguo Zhang, as it was observed in the review of those articles).

It is also noted that Uggetti Enrica published seven papers. Marianna Garfí, Raúl Muñoz and Li X (initial includes either Xiang Li, Xuyang Li, Xiangxing Li, Xunzhou Li, Xin Li or Xue Li; as it was observed in the review of articles) is a group of authors who participated in the publication of six papers each.

In another group, with five published documents each, we found mentioned Germán Buitrón, Chen X (several authors), Raquel Gutiérrez, Li Z (several authors) and Fabiana Passos.

Authors' production over time

Figure 7 shows that the authors Ivet Ferrer, Joan García and Raúl Muñoz have been publishing almost consistently since 2014; however, Ferrer did not publish in 2019 and 2021, García did not publish from 2019 to 2021, neither did Muñoz in 2017, 2020 and 2021.

Enrica Uggetti behaves similarly to the previous mentioned authors; however, this author started publishing in 2015 and did not publish from 2019 to 2021. In turn, Raquel Gutiérrez only published from 2015 to 2017. On the other hand, the authors Mariana Garfí and German Buitrón, who have been publishing since 2017 and 2016 respectively, are authors who also have recently published.

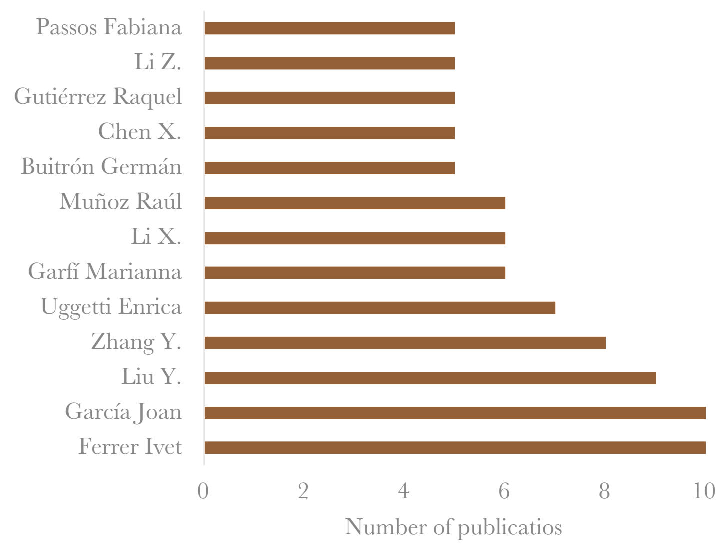


Figure 6. Behavior of the trend on most relevant authors.

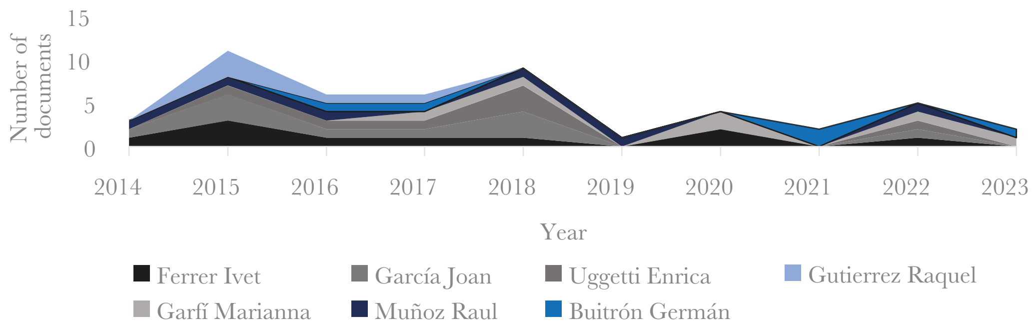


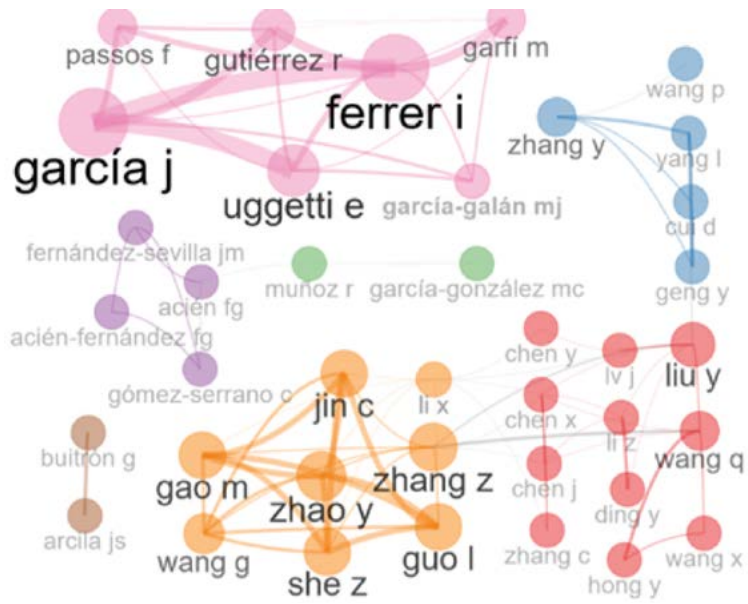
Figure 7. Trend in the production of articles per author in the period 2014-2023.

Collaborative network among authors

The analysis shows that there are seven networks of researchers who have worked with WTwM, of these, there are two groups that collaborate with each other; while two networks do not collaborate with any other network (Figure 8).

In the first group of networks with mutual collaboration (purple and green), seven authors participate in the first one, and Francisco Gabriel Ascíen stands out, who is the nexus with Raúl Muños of the second network (green) with only two authors. In the second group there are three collaborative networks, Zhang Z (orange) is Liu Y's nexus (red), who in turn is Geng Y's nexus (blue). Authors in these networks are eight in the first one (orange), 12 in the second, and five in the third (blue).

The first collaboration network without any nexus to other (pink) is made up of seven authors, including Joan Garcia, Ivet Ferrer and Enrica Uggetti. In the second no-nexus network (brown) only Germán Buitrón and Juan S. Arcila participate.



Source: BIBLIOMETRIX with the SCOPUS database.
Figure 8. Behavior of collaboration networks among authors.

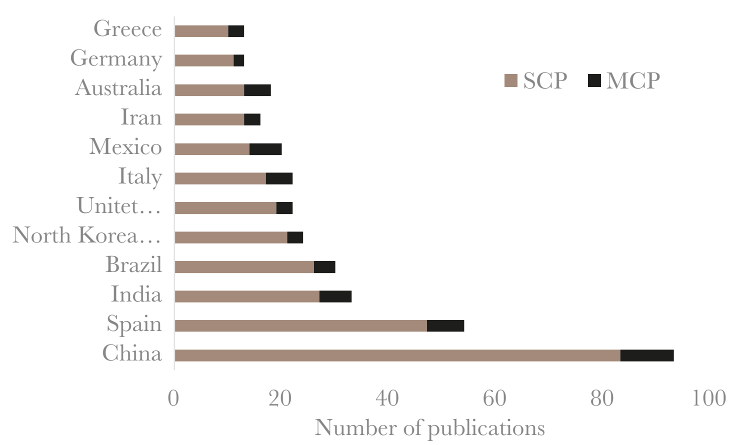
Production per country

The analysis showed that China is the country with the highest number of documents published regarding WTwM (Figure 9). Of these, 83 were published independently and 10 were published in collaboration with other countries. On the other hand, Spain published 47 articles independently and seven in collaboration, India 27 independently and six in collaboration, Brazil 24 independently and four were collaborative.

The list also considers South Korea, the United States, Italy, Mexico, Iran, Australia, Germany, and Greece with 21, 19, 17, 14, 13, 13, 11 and 10 documents published independently (3, 3, 5, 6, 3, 5, 2 and 3 in collaboration), respectively. Therefore, one outstanding point is that most countries publish in collaboration with other countries.

Keyword cloud

The bibliometric analysis shows in Figure 10 a cloud of keywords among which the word "biomass" stands out with a frequency of occurrence of 340. Other important words



* MCP: multiple country publications; SCP: single country publications.
Figure 9. Number of publications per country, with and without collaboration.

Source: BIBLIOMETRIX with the SCOPUS database.
Figure 10. Keyword cloud.

are "nitrogen" and "phosphorus" with frequencies of 200 and 119. Also, words related to species and genus of microalgae appear in the cloud, these are *Chlorela vulgaris* and *Scenedesmus* with frequencies of 100 and 57, respectively.

Reference content analysis

Biomass accumulation, through the removal of nutrients present in W through the use of microalgae, was the objective of researchers such as Fan *et al.* (2023), Paulenco *et al.* (2023), Liu *et al.* (2023), Zhuang *et al.* (2023), Zuo *et al.* (2023), Li *et al.* (2023), Qv *et al.* (2023) and Muthuraman *et al.* (2023). The microalgae species used were *Chlorella* sp., *Nannochloris* sp., Hybrid, Chlorella FACHB-30, Chlorella FACHB-5, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Coelastrella* sp.; mostly pre-grown in BG11 medium at 25 °C and light: dark periods of 12:12 h by using microbial consortium, Li *et al.* (2023) and Liu *et al.* (2023) obtained biomass values of 6.22 and 1.79 mg L^{-1}. In turn, Paulenco *et al.* (2023) and Fan *et al.* (2023) obtained values of 1600 and 375 mg L^{-1} without using the consortium.

Several authors studied the behavior of microalgae to remove W nutrients, through the use of microalgae strains in a simple and combined way. Nandini *et al.* (2023), Paulo de Sousa *et al.* (2023), Wang *et al.* (2023), and Krasaesueb *et al.* (2023) used *Chlorella vulgaris*, *Scenedesmus* sp., Chlorococcus AY122332.1, and Synechocystis ΔSphU (mutant cyanobacteria), respectively, as simple strains. On the other hand, Gu *et al.* (2023) did not specify the strain of microalgae used. The NH_4^+ removal was 100%, 94%, 91%, 45%, and 98%, respectively; while that of PO_4^{-3} varied from 71 to 97%.

Nutrient removal, by microbial consortium and the combination of strains from *Chlorella vulgaris*, *Scenedesmus* sp., *Chlorococcum* sp., and *Oscilatoria* sp., was performed by Nagabalaji *et al.* (2023) and removed 86.6% of NH_4^+. Soroosh *et al.* (2023) also experimented with a consortium and the combination of strains from *Chlorella* sp., *Desmodesmus* sp., and *Tribonema* sp., those authors obtained a removal of NH_4^+ and PO_4^{-3} of 88 and 98%.

The production of biodiesel through the biomass accumulated in microalgae by the removal of nutrients present in W was the objective established by Zhao *et al.* (2023), El-Sheekh *et al.* (2023), Vasistha *et al.* (2023) and Khalaji *et al.* (2023). El-Sheekh *et al.* (2023) managed to accumulate 98.2% fatty acids with the microalgae *Chlamydomonas reinhardtii* from a biomass of 48.62 mg L^{-1}, which originated from the removal of 69.2 and 7% of NH_4^+ y PO_4^{-3}, respectively in W. On the other hand, Khalaji *et al.* (2023) with an inoculum of 13 million mL^{-1} cells of *Chlorella vulgaris* in a 25% dilution of W from the dairy industry, observed the accumulation of 23.3% of palmitic acid.

In turn, Satheesh *et al.* (2023) experimented with the production of biohydrogen from the culture of *Chlorella pyrenoidosa*, *Scenedesmus obliquus* and *Chlorella sorokiniana*; *Scenedesmus obliquus* was able to produce 45.5 mL of H_2 g^{-1} VS, from the accumulation of 25.34% lipids in 710 mg L^{-1} of biomass. While Oliveira *et al.* (2023) produced 8175.7 N mL^{-1} Gsv^{-1} with a Chlorophycea-class microalgae.

In the analysis, the average number of articles published per year (4.77) shows little importance in WTwM research. From 1985 to 2012, the average annual publication was only 0.75, where the maximum number of articles published per year was three in 2009. In addition, in 15 years of this period, no publication was reported. In this way, the analysis shows that WTwM research started growing from 2013 onwards. In this regard, in 2007 the United Nations updated Millennium Development Goal number Seven, which established as number 7c, "for 2015, to reduce by-half the percentage of people who did not have sustainable access to safe drinking water and basic sanitation services in 1990". WTwM is currently investigated as a way to reduce greenhouse gas emissions intending to limit global warming by producing sustainable biofuels (CEPAL, 2015; Paddock, 2019).

The average number of citations per article in the analysis indicates that, in the first years, the number of citations increases as the years go by, reaching a maximum in 2001. In turn, there has been a decrease in the average number of citations from 2001 to date. However, the average number of citations an article has over time is 52.82. In this sense, Price (1973) pointed out that the number of queries made about a work is a measure that indicates its scientific importance. On the other hand, Cañedo (1999) mentioned that citations are a way of evaluating scientific publications, but dependent on the stage in which the research topic is in relation to time.

Of the first 13 researchers, Ivet Ferrer Martí, a researcher in the Environmental Engineering and Microbiology Group of the Polytechnic University of Catalonia in Barcelona, Spain, leads the research line on anaerobic digestion and biogas production in the GEMMA Research Group. This author's research focuses on the recovery of waste streams to obtain bioproducts and bioenergy, within the framework of the circular bioeconomy. That author specializes in the fields of anaerobic digestion and algae biotechnology (UPC, 2023a).

From the same research centre, Joan García is the second author who stands out in this analysis.This scientist studies the development and improvement of environmental biotechnology in the treatment of W, mimics natural decomposition processes, and maximizes eco-efficiency. And is focused on the recovery of bioproducts and bioenergy in the context of the circular bioeconomy, nature-based solutions and water reuse (UPC, 2023b).

On his part, the scientist Raúl Muños is another of the expert co-authors in WTwM. This researcher is Professor in the Department of Chemical Engineering and Environmental Technologies at the University of Valladolid in Spain. His main field of research is biological gas treatment and wastewater treatment with algae and bacteria in photobioreactors (ResearchGate, 2023).

In the 329 papers analyzed, it was observed that the number of authors involved in co-authorship, which exceeds five individuals on average per paper, demonstrates that the research is carried out as a team. However, authors such as Sofia Chaudry, Hai-Ming Jiang, and Hee-Jeong Choi[†], independently published their respective papers. In this sense, Wuchty *et al.*, (2007) mentioned that in the last five decades, the number of studies carried out by research teams is greater than those carried out by individual authors; this is, when the topics are related to science and engineering. However, the complexity of the study is the element that justifies the number of people involved (Repiso, 2020).

The analysis showed that most of the countries involved in WTwM research collaborate with other countries; and that China is the country with the largest number of documents related to the subject. Spain is the country with the most relevant authors in the research and generation of knowledge in the WTwM. In this regard, Van Raan (1998) discussed the epistemic advantage of an international collaborative work that also provides them with influence and impact.

Regarding the most important keywords, "biomass" is directly related to the cultivation of microalgae in wastewater, and corresponds to the assimilation of nutrients, minerals and molecules (lipids, carbohydrates, polymers and pigments) by these (Viera *et al.*, 2023). In this regard, Atif *et al.* (2023) pointed out that microalgae use various biochemical processes to remove chemicals and nutrients such as nitrogen and phosphorus from the W to produce biomass.

El-Sheekh *et al.* (2023) pointed out that microalgae bioengineering should be aimed at increasing lipid production and fatty acid accumulation, in order to meet energy demand through the use of microalgae as feedstock in the production of third-generation biofuels. On the other hand, Atif *et al.* (2023) mentioned that future research would be directed to the study of parameters such as temperature, biomass production, osmotic capacity, pH and O_2 levels in the efficiency of WTwM to determine optimal culture conditions on a large scale and within an industrial environment.

CONCLUSIONS

The analysis indicates that WTwM is a research topic that has been studying and disseminating knowledge since the 80's; however, this has been generated intermittently to date. Among the authors who stand out the most in the publication of scientific articles on WTwM is Ivet Ferrer, who deals with the recovery of waste streams to obtain bioproducts and bioenergy, and specializes in the fields of anaerobic digestion and algae biotechnology.

China is the country with the highest number of WTwM publications; however, this country, as well as Spain, India, Brazil, among others, publish individually and their collaboration with groups of scientists from other countries is limited. The most recent WTwM studies address issues related to biomass accumulation, nutrient removal, biodiesel production and biogas production, as a way to make sustainable use of the process.

The topics to be addressed in future research on the efficiency of WTwM will be related to the study of parameters such as temperature, biomass production, osmotic capacity, pH and O_2 levels. The availability of scientific documents documenting processes associated with WTwM is limited, which evidences large gaps in knowledge regarding this subject. Regardless, the importance of this issue in relation to water supply facing climate vulnerability makes it relevant to be addressed.

REFERENCES

Atif A.; Zunera, K.; Allam, A. A.; Jamaan, A. S. 2023. Wastewater treatment by using microalgae: Insights into fate, transport, and associated challenges. *Chemosphere*, 338, 139501. https://doi.org/10.1016/j.chemosphere.2023.139501

Beuckels, A.; Smolders, E.; Muylaert, K. 2015. Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water Research*, 77: 98-106. https://doi.org/10.1016/j.watres.2015.03.018

Cañedo A, R. 1999. Los análisis de citas en la evaluación de los trabajos científicos y las publicaciones seriadas. *ACIMED, 7*(1): 30-39. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1024-94351999000100004

CEPAL (Comisión Económica para América Latina y el Caribe). 2015. Objetivos de desarrollo del milenio. https://www.cepal.org/es/temas/objetivos-de-desarrollo-del-milenio-odm/objetivos-desarrollo-milenio

Cho, S.; Luong, T. T.; Lee, D.; Oh, You-Kwan; Lee, T. 2011. Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. *Bioresource Technology, 102*(18): 8639-8645. https://doi.org/10.1016/j.biortech.2011.03.037

El-Sheekh, M. M.; Galal, H. R.; Mousa, A. SH. H.; Farghl, A. A. M. 2023. Coupling wastewater treatment, biomass, lipids, and biodiesel production of some green microalgae. *Environmental Science and Pollution Research*, 30: 35492–35504. https://doi.org/10.1007/s11356-023-25628-y

Fan, J.; Feng, S.; Tang, Q.; Guo, S.; Cai, Z. 2023. Using steel slag as Ca_2^+ supplement to trigger microalgae growth and wastewater treatment. *Biochemical Engineering Journal*, 197, 108982. https://doi.org/10.1016/j.bej.2023.108982

González-Sanabria, J. S.; Díaz-Peñuela; J.S.; Castro-Romero; A. 2019. Análisis de los Indicadores de Citación de las Revistas Científicas Colombianas en el Área de Ingeniería. *Información Tecnologica. 30*(2). http://dx.doi.org/10.4067/S0718-07642019000200293

Gu, Z.; Zhang, Q.; Sun, G.; Lu, J.; Liu, Y.; Huang, Z.; Xu, S.; Xiong, J.; Liu, Y. 2023. Pretreatment of Biogas Slurry by Modified Biochars to Promote High-Value Treatment of Wastewater by Microalgae. *Sustainability, 15*(4), 3153. https://doi.org/10.3390/su15043153

Li, X.; Liu, J.; Tian, J.; Pan, Z.; Chen, Y.; Ming, F.; Wang, R.; Wang, L.; Zhou, H.; Li, J.; Tan, Z. 2023. Co-cultivation of microalgae-activated sludge for municipal wastewater treatment: Exploring the performance, microbial co-occurrence patterns, microbiota dynamics and function during the startup stage. *Bioresource Technology*, 374, 128733. https://doi.org/10.1016/j.biortech.2023.128733

Liu, Y.; Zhang, G.; Li, W.; Ding, Y.; You, H.; Zhu, J.; Leng, H.; Xu, C.; Xing, X.; Xu, J.; Li, Z. 2023. The characteristic evolution and formation mechanism of hybrid microalgae biofilm and its application in mariculture wastewater treatment. *Journal of Environmental Chemical Engineering, Volume 11*(3), 109645. https://doi.org/10.1016/j.jece.2023.109645

Mahtab, K.; Seyed, A. H.; Rasoul, G.; Nasser, A.; Hasan, R.; Michael, K.; Eleni, K. 2023. Treatment of dairy wastewater by microalgae *Chlorella vulgaris* for biofuels production. *Biomass Conversion and Biorefinery*, 13: 3259–3265. https://doi.org/10.1007/s13399-021-01287-2

Maity, J. P.; Bundschuh, J.; Chen, Chien-Yen; Bhattacharya, P. 2014. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. *Energy*, 78: 104-113. https://doi.org/10.1016/j.energy.2014.04.003

Matamoros, V.; Gutiérrez, R.; Ferrer, I.; García, J.; Bayona, J. M. 2015. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. *Journal of Hazardous Materials*, 288: 34-42. https://doi.org/10.1016/j.jhazmat.2015.02.002

Muthuraman, R. M.; Murugappan, A.; Soundharajan, B. 2023. Highly effective removal of presence of toxic metal concentrations in the wastewater using microalgae and pre-treatment processing. *Applied Nanoscience*, 13: 475–481. https://doi.org/10.1007/s13204-021-01795-7

Nagabalaji, V.; Maharaja, P.; Nishanthi, R.; Sathish, G.; Suthanthararajan, R.; Srinivasan, S. V. 2023. Effect of co-culturing bacteria and microalgae and influence of inoculum ratio during the biological treatment of tannery wastewater. *Journal of Environmental Management*, 341, 118008. https://doi.org/10.1016/j.jenvman.2023.118008

Nandini, M.; Namrata, J.; Robin, C. 2023. Impact of Microalgae in Domestic Wastewater Treatment: A Lab-Scale Experimental Study. *Pollution, 9*(1): 211-221. https://doi.org/10.22059/poll.2022.344705.1513

Nattawut, K.; Jarungwit, B.; Cherdsak, M.; Wanthanee, K. 2023. Highly effective reduction of phosphate and harmful bacterial community in shrimp wastewater using short-term biological treatment with immobilized engineering microalgae. *Journal of Environmental Management*, 325, Part A, 116452. https://doi.org/10.1016/j.jenvman.2022.116452

Oliveira S., E.; Mendes F., N.; Vargas H., E.; Konrad, O.; Lutterbeck, C. A.; Machado, Ê. L.; Ribeiro R., L. 2023. Energy recovery by anaerobic digestion of algal biomass from integrated microalgae/constructed wetland wastewater treatment. *Environmental Science and Pollution Research*, 30: 13317–13326. https://doi.org/10.1007/s11356-022-23019-3

Paddock, M. 2019. Microalgae Wastewater Treatment: A Brief History. Preprints, 2019120377. https://doi.org/10.20944/preprints201912.0377.v1

Paulenco, A.; Vintila, A. C. N.; Vlaicu, A.; Ciltea-Udrescu, M.; Galan, A. M. 2023. *Nannochloris* sp. Microalgae Strain for Treatment of Dairy Wastewaters. *Microorganisms, 11*(6), 1469. https://doi.org/10.3390/microorganisms11061469

Paulo de Sousa O., A.; Assemany, P.; Covell, L.; Perlini T., G.; Calijuri, M. L. 2023. Microalgae-based wastewater treatment for micropollutant removal in swine effluent: High-rate algal ponds performance under different zinc concentrations. *Algal Research*, 69, 102930. https://doi.org/10.1016/j.algal.2022.102930

Posadas, E.; Morales, M.; Gomez, C.; Acién, F. G.; Muñoz, R. 2015. Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. *Chemical Engineering Journal*, 265: 239-248. https://doi.org/10.1016/j.cej.2014.12.059

Price, D. J. de S. 1973. Hacia una ciencia de la ciencia. Ariel. Barcelona, España. 128 p.

Qv, M.; Dai, D.; Liu, D.; Wu, Q.; Tang, C.; Li, S.; Zhu, L. 2023. Towards advanced nutrient removal by microalgae-bacteria symbiosis system for wastewater treatment. *Bioresource Technology*, 370, 128574. https://doi.org/10.1016/j.biortech.2022.128574

Repiso, R. 2020. La autoría: ¿Cuántos firman, quiénes y en qué orden? https://doi.org/10.3916/escuela-de-autores-121

ResearchGate. 2023. https://www.researchgate.net/profile/Raul-Munoz-17

Satheesh, S.; Pugazhendi, A.; Al-Mur, B. A.; Balasubramani, R. 2023. Biohydrogen production coupled with wastewater treatment using selected microalgae. *Chemosphere*, 334, 138932. https://doi.org/10.1016/j.chemosphere.2023.138932

Soroosh, H.; Otterpohl, R.; Hanelt, D. 2023. Influence of supplementary carbon on reducing the hydraulic retention time in microalgae-bacteria (MaB) treatment of municipal wastewater. *Journal of Water Process Engineering*, 51, 103447. https://doi.org/10.1016/j.jwpe.2022.103447

UPC (Universidad Politécnica de Cataluña, Barcelonatech). 2023a. https://gemma.upc.edu/en/staff/faculty/ivette-ferrer

UPC. (Universidad Politécnica de Cataluña, Barcelonatech). 2023b. https://gemma.upc.edu/en/staff/faculty/joan-garcia

Van R., A. F. J. 1998. The influence of international collaboration on the impact of research results. Some simple mathematical considerations concerning the role of self-citations. *Scientometrics, 42*(3), 423-428. https://doi.org/10.1007/BF02458380

Vasistha, S.; Balakrishnan, D.; Manivannan, A.; Rai, M. P. 2023. Microalgae on distillery wastewater treatment for improved biodiesel production and cellulose nanofiber synthesis: A sustainable biorefinery approach. *Chemosphere*, 315, 137666. https://doi.org/10.1016/j.chemosphere.2022.137666

Vieira C., J. A.; Zaparoli, M.; Aguiar C., A. P.; Barcelos C., B.; da Silva V., B.; Greque de M., M.; Botelho M., J. 2023. Biochar production from microalgae: a new sustainable approach to wastewater treatment based on a circular economy. *Enzyme and Microbial Technology*, 169, 110281. https://doi.org/10.1016/j.enzmictec.2023.110281

Wang, H.; Liu, Z.; Cui, D.; Liu, Y.; Yang, L.; Chen, H.; Qiu, G.; Geng, Y.; Xiong, Z.; Shao, P.; Xubiao L. 2023. A pilot scale study on the treatment of rare earth tailings (REEs) wastewater with low C/N ratio using microalgae photobioreactor. *Journal of Environmental Management*, 328, 116973. https://doi.org/10.1016/j.jenvman.2022.116973

Wuchty, E.; Jones, B. F.; Uzzi, B. 2007. The Increasing Dominance of Teams in Production of Knowledge. *Science*, 316 (5827), 1036-1039. https://www.science.org/doi/10.1126/science.1136099

Zhao, Q.; Han, F.; You, Z.; Huang, Y.; She, X. 2023. Evaluation of the relationship of wastewater treatment and biodiesel production by microalgae cultivated in the photobioreactor. *Fuel*, 350, 128750. https://doi.org/10.1016/j.fuel.2023.128750

Zhuang, Y.; Su, Q.; Wang, H.; Wu, C.; Tong, S.; Zhang, J.; Qiao, H. 2023. Strain Screening and Conditions Optimization in Microalgae-Based Monosodium Glutamate Wastewater (MSGW) Treatment. *Water, 15*(9): 1663. https://doi.org/10.3390/w15091663

Zuo, W.; Chen, Z.; Zhang, J.; Zhan, W.; Yang, H.; Li, L.; Zhu, W.; Mao, Y. 2023. The microalgae-based wastewater treatment system coupled with Cerium: A potential way for energy saving and microalgae boost. *Environmental Science and Pollution Research*, 30: 60920–60931. https://doi.org/10.1007/s11356-023-26639-5

AGRO
PRODUCTIVIDAD

Colegio de Postgraduados

Drip-tape irrigation depth: water use efficiency, yield and forage quality in maize

Ortiz-Diaz, Sergio A.[1]; **Reyes-González Arturo**[2]; **Fortis-Hernández Manuel**[1]; **Rocha-Santillano Jessica J.**[1]; **Ayala Garay, Alma V.**[3,4]; **Preciado-Rangel Pablo**[1*]

[1] Tecnológico Nacional de México-Campus Instituto Tecnológico de Torreón. Carretera Torreón-San Pedro km 7.5, ejido Ana, Torreón, Coahuila, México. CP. 27170.
[2] Instituto Nacional de Investigaciones Forestales, Agríciolas y Pecuarias, Campo Experimental La Laguna. Blvd. José Santos Valdés núm. 1200 Pte., Matamoros, Coahuila, México. CP. 27440.
[3] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental Valle de México, Chapingo, Texcoco, Estado de México, México, C.P. 56230.
[4] Instituto Tecnológico Superior de Acatlán de Osorio. Del Maestro, Unidad Tecnológica, Acatlán de Osorio, Puebla, México, C.P. 74949.
* Correspondence: ppreciador@yahoo.com.mx

ABSTRACT

Objective: to evaluate the effect of the drip-tape irrigation depth on the efficiency of water use, yield, nutritional quality and profitability of forage maize, a study was established by installing drip-tape at a depths 0.05, 0.15 and 0.30 meters.

Design/Methodology/Approach: a randomized block experimental design was used. Treatments evaluated consisted of the installation of drip-tape at three depths 0.05, 0.15 and 0.30 m; each treatment in three replicates. The experimental unit was a 15 m^2 surface (comprising four 5m-long furrows, with a 0.76 m separation between furrows).

Results: results showed that with the drip-tape installed at a depth of 0.15 m, the highest biomass production and water use efficiency were obtained, without modifying the bromatological quality of the forage. However, the best benefit-cost ratio corresponded to the drip-tape installed at 0.3 m, recovering $1.27 for each MXN peso invested in crop production.

Limitations/Implications of the study: water scarcity in arid and semi-arid regions is a global problem, so it is necessary to use irrigation methods that make water use more efficient without reducing crop yield.

Findings/conclusions: the installation of the drip-tape at a depth of 0.15 m is recommended, due to the improvement in yield and water use efficiency without affecting nutritional quality of the forage or profitability of maize crop.

Keywords: *Zea mays* L., water potential, water use efficiency, profitability.

Citation: Ortiz-Diaz, S. A., Reyes-González, A., Fortis-Hernández, M., Rocha-Santillano, J. J., Ayala Garay, A. V., & Preciado-Rangel P. (2024). Drip-tape irrigation depth: water use efficiency, yield and forage quality in maize. *Agro Productividad*. https://doi.org/ 10.32854/agrop.v17i4.2682

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: September 15, 2023.
Accepted: March 16, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 127-135.

INTRODUCTION

The Comarca Lagunera, located between the states of Coahuila and Durango (Mexico) is the main basin destined to dairy production in the country, which demands large amounts of fodder per year, such as maize (*Zea mays* L.), one of the forages with the highest production and largest cultivated area in the region (50 thousand ha). This crop is established in the spring and summer cycles, and is one of the main sources of feed for dairy cattle (SADER, 2021). To produce the large amount of fodder that livestock demands, a high volume of irrigation water is required; because water scarcity, high temperatures, and long periods of drought affect crop development (Moore *et al.*, 2021).

Water scarcity for agricultural use forces the search for irrigation techniques that increase water productivity, as well as crop yields and profitability. Subsurface drip irrigation (SDI) is an irrigation technique that increases crop yields and improves irrigation water use efficiency. Unlike other systems, drip-tape can be installed at different depths of the soil, providing moisture directly to the root zone of the plant and minimizing water losses through direct evaporation, runoff, and percolation (Sandhu *et al.*, 2019).

SDI helps to minimise the negative effects that climatic conditions can have on plant physiology during the production cycle. In addition, different fertilisation doses can be injected in instalments, thus improving nutrient absorption, increasing the production and nutritional quality of forages (Moore *et al.*, 2021). Therefore, this study aimed to evaluate the effect of the drip-tape irrigation depth on water use efficiency, yield, nutritional quality and profitability of forage maize.

MATERIALS AND METHODS

Location of the experiment

The research was established at "La Laguna" (CELALA) Experimental Facilities under the National Institute of Forestry, Agriculture and Livestock Research (INIFAP, in Mexico), located at the geographical coordinates of 25° 32' N and 103° 14' W, at an altitude of 1150 m. The climatic conditions correspond to a semi-warm dry climate (Bwh) with low atmospheric humidity, average annual temperature of 22.6 °C; while the average rainfall is 215.5 mm and the average annual evaporation is 2000 mm. The soil has a clay loam texture, alkaline pH, non-saline, low in organic matter content, low in phosphorus and available nitrogen. Land preparation consisted of subsoil plow after fallow, harrowing, leveling and subsurface laying of the drip-tape.

The vegetative material used was the hybrid 20W41 (Syngenta®), an intermediate-cycle variety that is resistant to lodging and has good plant and ear health. Sowing was carried out manually on July 28, 2021 with a plant spacing of 0.12 m and 0.76 m between rows for a population density of 105 thousand plants per ha. The fertilization dose used was 200-100-00 (N-P_2O_5-K_2O); $CO(NH_2)_2$ and $(NH_4)_2SO_4$ were used as nitrogenous sources; and $NH_4H_2PO_4$ as a source of phosphorus. All the phosphorus and half of the nitrogen with $CO(NH_2)_2$ were applied at the time of planting and the rest of the nitrogen with $(NH_4)_2SO_4$ was injected every 15 days according to the phenology of the crop by means of a Venturi injector (Zavala-Borrego *et al.*, 2022).

For the SDI, a Ro-DRIP 8(mil) irrigation drip-tape (Rivulis Irrigation Inc., San Diego, CA, USA) was used, with a nominal wall thickness of 0.2 mm, nominal diameter of 16 mm, emitters at 0.2 m separation an a flow rate of 0.5 L h^{-1} per emitter. The SDI operated at a pressure range of 8 PSI with irrigation frequency every third day. The irrigation time was the same for all three depths throughout the cycle, but not for germination. An atmometer (ETgage A model, ETgage Company Loveland, Colorado, USA) located 20 m from the experimental site was used to measure the reference ET, where daily readings were taken and multiplied by the Kc to estimate the ETc. To calculate the Kc, the equation

$$Kc = 1.1705 * NDVI + 0.0535$$

was used for fodder maize with subsurface drip irrigation (Reyes-González *et al.*, 2019a).

Experimental design and treatments

A randomized block experimental design was used, the treatments evaluated consisted of the installation of the drip-tape at three depths 0.05, 0.15 and 0.30 m, each treatment was replicated three times. The experimental unit with a surface of 15 m^2 (four 5m-long furrows with a 0.76 m separation between rows).

Variables evaluated

Plant Height

Plant height was taken at five plants at random from each experimental unit at harvest time (105 days after sowing, DAS). It was measured from the base of the stem to the spike with a measuring tape.

Water Potential

To quantify the water potential, the Scholander pressure pump was used. Two samplings were taken per treatment and repeated between 12:00 and 14:00 h. Each week, the second leaf was selected from the upper part of the crop, taking two leaves per treatment and replicate which were covered with moistened cloth to avoid moisture loss before measurement.

Fresh and Dried Forage Biomass

Crop was harvested at 105 DAS when the maturation of the grain presented an advance of 1/3 of the milk line. For the production of green fodder, a line of three meters was taken as the useful plot per each experimental unit. After weighing, a sample of 500 g was taken and dried in a forced air convection oven (UF 260 Plus, Memmert, Germany) at a temperature of 65 °C until a constant weight was reached, to determine the dry matter (DM) production. With the production of green forage and the percentage of DM, the dry forage yield was estimated (DY).

Water Use Efficiency

Water use efficiency (WUE) was obtained by dividing the harvested dry forage yield (DY, kg) by the total volume of water used (m^3) in each treatment.

Bromatological quality

Bromatological quality was assessed by analysing 200 g-samples of dry forage from each treatment and replicate previously ground and identified. Nutritional content was determined by near-infrared reflectance spectroscopy –NIRS (Valenciaga and Simões, 2006). The parameters evaluated were crude protein (CP), neutral organic matter detergent fiber (NDF_{om}), net lactation energy (NLE), starch, lignin and digestibility of neutral detergent fiber at 30 h of incubation ($NDFD\text{-}_{30\ h}$).

The determination of crude protein (CP) was quantified using the microKjeldhal method (AOAC, 2005); Fat content using the Soxhlet method using a Goldfish extractor (Labconco, USA). The percentages of acid- and neutral detergent fibers (ADF and NDF) were quantified with the detergent fractionation method and subsequent filtration (Van Soest *et al.*, 1978).

Profitability

An estimation of the calculation of the profitability corresponding to the production cycle of the forage corn crop was made by means of the benefit/cost ratio (B/C). In each treatment, the total production costs of the crop were considered, including irrigation water costs and the income from the sale of green fodder obtained per hectare (Megagrams, Mg ha^{-1}). For the income estimation, we used the market price in the growing cycle in which the experiment was established.

Statistical Analysis

To establish whether there were significant differences between the variables evaluated, an analysis of variance was performed using the GLM procedure of SAS© v. 9.3 (SAS Institute Inc., Cary, NC, USA); when statistical differences were detected ($p \leq 0.05$), the Tukey's mean difference test was applied ($p \leq 0.05$).

RESULTS AND DISCUSSION

Leaf Water Potential (Ψh)

The drip-tape buried at 0.30 m provided the best water condition for the crop. While for the treatment with the drip-tape at 0.05 m depth, water potential was −1.4 MPa, the most negative value observed at 82 DAS (Figure 1), which resulted in lower plant height and biomass production. The lower water content with the drip-tape buried at 0.05 m caused water stress in the plant, which was quantified in the leaf, generating inadequate vegetative growth and lower biomass production (May-Lara *et al.*, 2011).

Reyes *et al.* (2019a) reported similar values in the evaluation of the water potential in forage sorghum, obtaining values in a range of −1.0 MPa in treatments with drip-tape compared to treatments with flood irrigation, with average values of −1.5 MPa

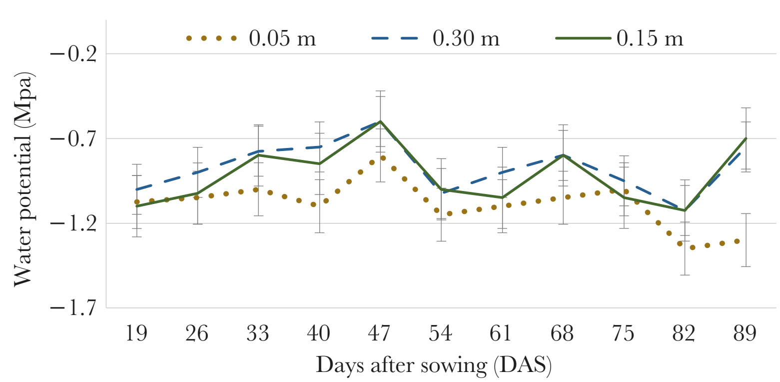


Figure 1. Water Potential values at different drip-tape irrigation depths in forage maize.

during the crop production cycle. More negative values than those found in our study were reported by Zavala-Borrego *et al.* (2022) in the evaluation of SDI with three levels of evapotranspiration and a control with gravity irrigation in the production of forage maize. Those authors found values that varied from −0.6 to −1.92 MPa, during one crop cycle in the Comarca Lagunera.

Plant Height

The height of the plant was not affected ($p \leq 0.05$) by the different depths of the irrigation drip-tape (Table 1). However, results show a trend to higher plant height with the irrigation tape buried at a depth of 0.15 m, with average plant height of 2.20 m. In relation to the drip-tape at a depth of 0.05 m, which obtained a lower height (2.08 m). This was due to greater exposure of the irrigation water to the surface, which increases direct evaporation and decreases water availability in the root zone (Reyes *et al.*, 2019).

On the other hand, Duan *et al.* (2007) mentioned that water stress causes changes in plant structure, which are reflected in a decrease in the growth rate during the crop cycle. Reyes-González *et al.* (2019b) reported that low levels of soil moisture negatively affect plant height. Similar results were reported by Sánchez-Hernández *et al.* (2013) who obtained forage maize heights of 2.44 m using subsurface drip irrigation.

Fresh and Dried Forage Biomass

The different depths of the drip-tape caused differences in the yield of green forage, the highest yields were obtained with the irrigation tape at depths of 0.15 and 0.30 m with 57.93 and 59.39 Mg ha^{-1}, respectively. The treatment with the lowest yield was 0.05 m with 52.20 Mg ha^{-1} (Table 1). Reta-Sánchez *et al.* (2007) found that the increase in biomass is due to the higher rate of leaf area that develops in the early stages of cultivation. Bame *et al.* (2014) pointed out that in the subsurface drip irrigation system, maize production increases due to the higher plant height and the weight of ears per plant.

The results obtained in this study are similar to those reported by Ortiz-Diaz *et al.* (2022), who evaluated irrigation tape depths in forage maize production and reported higher yields with irrigation tape at 0.30 m depth. Douh and Boujelben (2011) evaluated irrigation tape depths in maize cultivation and found that between 20 and 35 cm deep there is a better water distribution and content, therefore, greater use by the plant, which produced high yields. Dry matter production was not affected by the different depths of

Table 1. Plant height (PH), green forage (GF), dry forage yield (DY), net irrigation depth (NID), and water use efficiency (WUE) in forage maize.

Depth (m)	PH (m)	GF (t ha^{-1})	DF (t ha^{-1})	Irrigation (cm)	WUE (kg FS m^{-3})
0.05	2.08 a*	52.20 b	22.37 a	44.0 c	5.08 a
0.15	2.20 a	57.93 a	23.77 a	48.7 b	4.88 a
0.30	2.18 a	59.39 a	23.98 a	54.8 a	4.37 b

PH=Plant height; GF=Green fodder; DF=Dry forage; WUE=Efficient use water. * Different letters indicate significant difference (Tukey; $p \leq 0.05$).

the drip-tape ($p \leq 0.05$). However, as the depth of the irrigation tape increases, higher yields were obtained (Table 1).

These yields are higher than those reported by Gutiérrez-Guzmán *et al.* (2022), who obtained lower dry forage yields in forage corn production with two irrigation systems and three levels of applied evaporation, using irrigation tape installed at a depth of 0.36 m. Similar results were reported by Lamm and Trooien, (2006), where maize production was not affected by the depths of the placement of the irrigation tape, however, a slight trend in increasing yield was observed where the irrigation tape was used at greater depths. This can be explained because favorable conditions of humidity are created, which result in greater biomass production and nutritional quality (Sánchez-Hernández *et al.*, 2013).

Net irrigation depth

The accumulated net irrigation depth (NID) that was used during the production cycle in the evaluated treatments, including precipitation (5.28 cm), is shown in Table 1. The NID applied for the treatment with irrigation tape depth at 0.30 m was 54.8 cm, followed by the treatments with depths at 0.15 and 0.05 m with NID of 48.7 and 44.0 cm, respectively. Those differences can be explained by the fact that, at sowing, the treatment with a 0.05 m drip-tape used less water (2 cm), in relation to the depths of 0.15 and 0.30 m (6.7 and 12.8 cm), probably due to the proximity of the irrigation tape to the soil surface that favored seed germination. Similar values of net irrigation depths were applied by Reyes-González *et al.* (2023) who used the subsurface drip irrigation system at 0.3 m depth with accumulated net irrigation depths of 43.2, 51.8 and 54.2 cm; results of different levels of evapotranspiration in the production of maize for fodder.

Water Use Efficiency (WUE)

The SDI installed at different depths affected the WUE, the highest efficiency corresponded to the treatment with the irrigation tape at a depth of 0.05 m (Table 1), with a WUE of 5.08 kg of DY m^{-3}, followed by the treatment of 0.15 m (4.88 kg of DY m^{-3}), with a very similar saving in irrigation water. However, the treatment with the drip-tape at 0.15 m generated higher yields. The treatment with the drip-tape irrigation depth at 0.30 m was the one that showed the lowest WUE, because it used more irrigation water (54.8 cm). Zavala-Borrego (2022) pointed out that the highest WUE is obtained where less net irrigation depth is used.

The WUE results obtained in this study were superior to those reported by Ortiz-Diaz *et al.* (2022), who recorded average values of 3.42 kg FS m^{-3} in forage maize with irrigation tape depths similar to those established in our study. Those authors reported higher WUE values at depths of 0.05 m; in contrast to Solano *et al.* (2021), who obtained higher WUE in treatments with irrigation tape depths betwen 0.20 and 0.30 m, compared to the depth of 0.10 m. Variations in WUE results can be generated by the variation of climatological conditions in each region, cultural practices, and the irrigation system used in crop production (Reyes-González *et al.*, 2023).

Nutritional Quality

The nutritional quality of the forage was not affected by the different depths of the drip-tape irrigation (Table 2). The highest CP content was found in the treatment with irrigation tape depth at 0.05 m (7.86%), followed by treatments at 0.15 and 0.3 m depth (7.15% and 7.48%). Average crude protein values can range from 7.5 to 8.6% (Silva *et al.*, 2015).

The treatment with irrigation tape depth at 0.05 m showed the best percentages of ADF and NDF (24.47 and 39.35), lower than the treatments with depths at 0.15 and 0.30 m (Table 2). These variables represent the fibrous part of the forages, which is related to feed consumption by livestock. The higher the percentage, the lower the digestibility and acceptance by cattle (Pinos *et al.*, 2002).

Similar results to those in this study (Table 2) were found by Zaragoza-Esparza *et al.* (2019), lower than those obtained by Gutiérrez-Guzmán *et al.* (2022), who reported higher percentages of ADF and NDF ranging from 30.4 to 36.8, and 51.27 to 60.53%, respectively, in forage maize production in the Lagunera Region.

Regarding the yield of starch and lignin (Table 2), very similar percentages were obtained among treatments. Values ranged from 4.71 to 4.77% and from 30.63 to 32.26%, respectively; the treatment with the drip-tape at 0.30 m depth was the one with the lowest percentage in starch and lignin, therefore the one with the highest digestibility. These results coincide with those found by Granados-Niño *et al.* (2022), who obtained percentages of 29.91% (starch) and 4.99% (lignin) during the summer cycle, in the production and nutritional quality of forage maize in the Comarca Lagunera.

Regarding net lactation energy (NLE), the highest values were found in the treatment with drip-tape irrigation depth at 0.05 m (1.59 Mcal kg^{-1}), while in the treatments of 0.15 and 0.30 m depth, lower values were obtained (1.55 Mcal kg^{-1}). This is due to the fact that they obtained greater fiber production, therefore, the energy value was negatively associated with those concentrations, which in turn had an impact on the production of NLE (Gutiérrez-Guzmán *et al.*, 2022). The NLE results of this study are superior to those reported by Yescas *et al.* (2015), who obtained concentrations of 1.36 and 1.08 Mcal kg^{-1} of NLE in their quality evaluation of forage maize at different levels of subsurface drip irrigation.

Profitability

Table 3 shows the total production costs based on the forage maize technology package for the Comarca Lagunera, and the income obtained afther the fodder sale from each

Table 2. Crude protein (CP), acid detergent fiber (ADF), lignin, starch, neutral detergent fiber (NDF), and net lactation energy (NLE), in samples of forage maize.

Depth (m)	CP (%)	ADF (%)	Lignin (%)	Starch (%)	NDF (%)	NEL (Mcal kg^{-1})
0.05	7.86 a*	24.47 a	4.74 a	32.26 a	39.35 a	1.59 a
0.15	7.15 a	25.56 a	4.77 a	30.63 a	41.41 a	1.55 a
0.30	7.48 a	25.69 a	4.71 a	31.01a	41.82 b	1.55 a

CP=Crude protein; ADF=Acid detergent fiber; NDF=Neutral detergent fiber; LNE=Net energy for lactation. * Different letters indicate significant difference (Tukey; $p \leq 0.05$).

Table 3. Benefit-cost (B/C) ratios in forage maize production.

Treatment	Sale price ($ kg^{-1})	Yield (kg ha^{-1})	Total income ($)	Production cost ($)	B/C ($)
0.05 m	1.25	52,200	65,250.0	31,754	2.05
0.15 m	1.25	57,930	72,412.5	32,136	2.25
0.30 m	1.25	59,390	74,237.5	32,736	2.27

Source: Data based on the Statistics Yearbook of Agricultural Production, crop cycle 2021. SADER Delegación Comarca Lagunera, Ciudad Lerdo, Durango, Mexico.

of the treatments evaluated. The estimated B/C ratio in the most profitable treatments was 2.25 and 2.27 for the treatments with drip-tape buried at 0.15 and 0.30 m depth, respectively. These values indicate that for each Mexican peso invested in the production of forage maize with subsurface drip irrigation, 2.25 and 2.27 $ MXN were obtained from sales; in regard to the depth at 0.05 m, a lower B/C ratio was obtained (2.05).

Regarding the economic productivity of water use, the cost of gravity irrigation water in the Comarca Lagunera in the current production cycle was $0.91 m^{-3} (MXN). The production of green forage per m^3 of water used indicated that in the case of the 0.15 m depth treatment, the yield was 13.16 kg GF m^{-3}, this is, higher than those produced at 0.05 and 0.30 m depth treatments (9.5 and 12.19 kg GF m^{-3}), which indicates that for each m^3 of water invested in the production of maize for fodder with drip-tape irrigation at 0.15 m depth, the producer obtained $15.23 (MXN) of gross profit with the price per kg of GF set at $1.25 in the production cycle.

CONCLUSIONS

The subsurface drip irrigation system with the drip-tape installed at different depths affects yield and water use efficiency, without affecting forage quality. It is recommended to install the drip-tape at a depth of 0.15 m, due to the improvement in yield and water use efficiency without affecting the nutritional quality of the forage or the crop profitability.

REFERENCES

AOAC. (2005). Official Methods of Analysis. AOAC International. Gaithersburg, MD, EEUU, 18(Ed.), 179.

Bame, I. B., Hughes, J. C., Titshall, L. W., & Buckley, C. A. (2014). The effect of irrigation with anaerobic baffled reactor effluent on nutrient availability, soil properties and maize growth. *Agricultural Water Management*, 134, 50-59. https://doi.org/10.1016/j.agwat.2013.11.011

Douh, B., & Boujelben, A. (2011). Improving water use efficiency for a sustainable productivity of agricultural systems with using subsurface drip irrigation for maize (*Zea mays* L.). *Science and Technology*, 1, 881–888.

Granados-Niño, J. A., Sánchez-Duarte, J. I., Ochoa-Martínez, E., Rodríguez-Hernández, K., Reta-Sánchez, D. G., & López-Calderón, M. J. (2022). Efecto del ciclo de producción sobre el potencial de rendimiento y calidad nutricional del maíz forrajero en la Comarca Lagunera. *Revista Mexicana de Ciencias Agrícolas, 13*(28), 207-217. https://doi.org/10.29312/remexca.v13i28.3276.

Gutiérrez-Guzmán, U. N., Ríos-Vega, M. E., Núñez-Hernández, G., Esquivel-Romo, A., Vázquez-Navarro, J. M., & Anaya-Salgado, A. (2022). Producción de maíz forrajero con dos sistemas de riego y tres niveles de la evaporación aplicada. *Revista Mexicana de Ciencias Agrícolas, 13*(SPE28), 263-273. https://doi.org/10.29312/remexca.v13i28.3281

Lamm, F. R., & Trooien, T. P. (2006). Effect of dripline depth on field corn production in kansas. *Irrigation Assn. Int'l. Irrigation Technical Conf, 23*(1), 18-20.

May-Lara, C., Pérez-Gutiérrez, A., Ruiz-Sánchez, E., Ic-Caamal, A. E., & García-Ramírez, A. (2011). Efecto de niveles de humedad en el crecimiento y potencial hídrico de *Capsicum chinense* Jacq. Y su relación con el desarrollo de *Bemisia tabaci* (Genn.). *Tropical and Subtropical Agroecosystems, 14*(3), 1039-1045.

Montemayor, T. J. A., Gómez, M. O. Á., Olague, R. C. R., Fortis, H. M. F., Salazar, S. E., & Aldaco, N. R. (2006). Efecto de tres profundidades de cinta de riego por goteo en la eficiencia de uso de agua y en el rendimiento de maíz forrajero. *Revista Mexicana de Ciencias Pecuarias, 44*(3), 359-364.

Moore, C. E., Meacham, H. K., Lemonnier, P., Slattery, R. A., Benjamin, C., Bernacchi, C. J., Lawson, T., & Cavanagh, A. P. (2021). The effect of increasing temperature on crop photosynthesis: From enzymes to ecosystems. *Journal of Experimental Botany, 72*(8), 2822–2844. https://doi.org/10.1093/jxb/erab090

Ortiz-Diaz, S. A., Reyes-González, A., Fortis Hernández, M., Santana, O. I., Zermeño González, H., & Preciado-Rangel, P. (2022). Profundidad de la cinta de riego y estiércol solarizado en la producción y calidad de maíz forrajero. *Revista Mexicana de Ciencias Agrícolas, 13*(SPE28), 275-286. https://doi.org/10.29312/remexca.v13i28.3282

Pinos, R J. M., Gonzalez, S. S., Mendoza, G. D., Barcena, R., Cobos, M. A., Hernandez, A., Ortega, M. E. (2002). Effect of exogenous fibrolytic enzyme on ruminal fermentation and digestibility of alfalfa and rye–grass hay fed to lambs. *Journal of Animal Science, 80*(11), 3016-3020. https://doi.org/10.2527/2002.80113016x

Reta-Sánchez DG, Cueto-Wong JA, Gaytan-Mascorro A, Santamaria-Cesar J (2007) Rendimiento y extraccióndе nitrógeno, fosforo y potasio de maíz forrajero en surcos estrechos. *Agricultura Técnica en México*, 33,145-151

Reyes, G. A., Reta Sánchez, D. G., Sánchez Duarte, J. I., Martínez, E. O., Hernández, K. R., & Preciado, R. P. (2019a). Estimación de la evapotranspiración de maíz forrajero apoyada con sensores remotos y mediciones *in situ*. *Terra Latinoamericana, 37*(3), 279–290. https://doi.org/10.28940/terra.v37i3.485

Reyes-Gonzalez, A., Kjaersgaard, J., Trooien, T., Reta-Sanchez, D.G., Sanchez-Duarte, J.I., Preciado-Rangel, P., & Fortis-Hernandez, M. (2019b). Comparison of leaf area index, surface temperature, and actual evapotranspiration estimated using the METRIC model and *in situ* measurements. *Sensors*, 19, 1857. https://doi.org/10.3390/s19081857

Reyes-González, Arturo., Reta-Sánchez, D. G., Sánchez-Duarte, J. I , Preciado-Rangel, P., Rodríguez-Moreno, V. M., y Ruiz-Alvarez, O. (2023). Uso del atmómetro y coeficiente de cultivo en la programación del riego en maíz forrajero. *Ecosistemas y Recursos Agropecuarios, 10*(1), 6. https://doi.org/10.19136/era.a10n1.3160

Sánchez-Hernández, M. A., Aguilar-Martínez, C.U., Valenzuela-Jiménez, N., Joaquín-Torres, B. M., Sánchez-Hernández, C., Jiménez-Rojas, M. C., & Villanueva-Verduzco, C. (2013). Rendimiento en forraje de maíces del trópico húmedo de México en respuesta a densidades de siembra. *Revista Mexicana de Ciencias Pecuarias*, 4, 271-288.

Sandhu, O. S., Gupta, R. K., Thind, H. S., Jat, M. L., Sidhu, H. S., & Yadvinder-Singh. (2019). Drip irrigation and nitrogen management for improving crop yields, nitrogen use efficiency and water productivity of maize-wheat system on permanent beds in north-west India. *Agricultural Water Management, 219*(March), 19–26. https://doi.org/10.1016/j.agwat.2019.03.040

Silva, M. J. da S., Cabreira, J. C., Poppi, E. C., Tres, T. T., & Osmari, M. P. (2015). Production technology and quality of corn silage for feeding dairy cattle in Southern Brazil. *Revista Brasileira de Zootecnia, 44*(9), 303–313. https://doi.org/10.1590/S1806-92902015000900001

Solano, J. L. C., Urdaneta, A. B. S., De Ortega, C. B. C., Vásquez, E. R., & Alcalá, J. O. (2021). Impacto del riego por goteo subsuperficial en la eficiencia de uso del agua en maíz (*Zea mays* L.). *Revista Científica Agroecosistemas, 9*(1), 49-57.

Valenciaga, D. y Simoes, D. O. (2006). La espectroscopia de reflectancia en el infrarojo cercano (NIRS) y sus potencialidades para la evaluación de forrajes. *Revista Cubana de Ciencia Agrícola, 40*(3), 259-267.

Van Soest, P. J., Mertens, D. R., & Deinum, B. (1978). Preharvest factors influencing quality of conserved forage. *Journal of Animal Science, 47*(3), 712-720. https://doi.org/10.2527/jas1978.473712x

Yescas, C. P., Segura, C. M. A., Martínez, L. C., Álvarez, R. V. P., Montemayor, A, T. J., Orozco, V. J. A., & Frías, R. J. E. (2015). Rendimiento y calidad de maíz forrajero (*Zea mays* L.) con diferentes niveles de riego por goteo subsuperficial y densidad de plantas. *Phyton, 9457*(2), 272–279.

Zaragoza-Esparza, J., Tadeo-Robledo, M., Espinosa-Calderón, A., López-López, C., García-Espinosa, J. C., Zamudio-González, B., & Rosado-Núñez, F. (2019). Rendimiento y calidad de forraje de híbridos de maíz en Valles Altos de México. *Revista Mexicana de Ciencias Agrícolas, 10*(1), 101-111. https://doi.org/10.29312/remexca.v10i1.1403

Zavala-Borrego, F., Reyes-González, A., Álvarez-Reyna, V. D. P., Cano-Ríos, P., & Rodríguez-Moreno, V. M. (2022). Efecto de la tasa de evapotranspiración en área foliar, potencial hídrico y rendimiento de maíz forrajero. *Revista Mexicana de Ciencias Agrícolas, 13*(3), 407-420. https://doi.org/10.29312/remexca.v13i3.2294

Colegio de Postgraduados

In vitro gas production and digestibility of oat and triticale forage mixtures ensiled with fibrolytic enzymes and inoculants

González Reyes, Mónica[1]; López-Rodríguez, Fredy[1]; Gayosso-Barragán, Odilón[2]; Miranda-Romero, Luis Alberto[3]; Tirado-González, Deli Nazmín[4*]; Tirado-Estrada, Gustavo[4*]

[1] Tecnológico Nacional de México/Tecnológico El Llano Aguascalientes, División de Estudios de Posgrado e Investigación, , Carretera Aguascalientes-S.L.P. Km. 18, El Llano, Aguascalientes, México, C. P. 20330.

[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)/Centro Nacional de Investigación Disciplinaria Agricultura Familiar (CENID AF), Carretera Ojuelos-Lagos de Moreno Km 8.5, Ojuelos, Jalisco, México, C. P. 47540.

[3] Universidad Autónoma Chapingo, Posgrado en Producción Animal, Departamento de Zootecnia, Carretera Federal México-Texcoco Km 38.5, Texcoco, Estado de México, México, C. P. 56230.

[4] Tecnológico Nacional de México/Tecnológico El Llano Aguascalientes, Departamento de Ingenierías, Carretera Aguascalientes-S.L.P. Km. 18, El Llano, Aguascalientes. México. C.P. 20330.

* Correspondence: deli.tg@llano.tecnm.mx; gustavo.te@llano.tecnm.mx

ABSTRACT

Objective: To assess the effects of adding fibrolytic enzymes (FE) or lactic acid bacteria (LAB) inoculants to 40 d silages with oat and triticale (O:T) mixtures on the ratio and composition of neutral detergent fiber (NDF) and its subsequent *in vitro* gas production (GP) and *in vitro* dry matter digestibility (IVDMD) at 24 h.

Design/Methodology/Approach: Silages elaborated with two O:T ratios (60:40 and 80:20) treated with low (LD), medium (MD), and high (HD) doses of FE (0.75, 1, and 1.25 g/kg forage in wet basis (WB), respectively), and LAB (0.188, 0.25, and 0.31 g/kg WB, respectively). In both cases (FE and LAB), the control had a value of 0. Subsequently, pH, NDF, acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose (HEM), cellulose (CEL), dry matter (DM), crude protein (CP), GP parameters, and IVDMD were assessed. GP parameters included maximum velocity (Vmax), fractional rate (S), and lag. Experiments were planned in complete randomized designs (CRD), including factorial and split-plot arrangements. Variance analysis (ANOVA) models included fixed (doses, additives, and FR) and random (place/moment of sampling) effects.

Results: LAB improved the IVDMD at 24 h of 60:40 and 80:20 O:T silages. FE did not reduce the NDF of 60:40 silages, but LD and MD increased the HEM and CP, and reduced the ADF, ADL, and CEL; these results are correlated (r) with the improvement of pH pattern, GP, and IVDMD.

Study Limitations/Implications: The differences in the NDF of FR mixtures could affect the effectiveness of FE and LAB.

Findings/Conclusions: Although FE and LAB did not reduce the NDF, they changed the ratios of ADF, ADL, HEM, CEL, and CP of silages, potentially improving the GP and IVDMD.

Keywords: Oats and triticale, ensiling, fibrolytic enzymes, lactic acid bacteria, gas production, ruminal degradability.

Citation: González-Reyes, M., López-Rodríguez, F., Gayosso-Barragán, O., Miranda-Romero, L. A. Tirado-González, D. J., & Tirado-Estrada, G. (2024). *In vitro* gas production and digestibility of oat and triticale forage mixtures ensiled with fibrolytic enzymes and inoculants. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2711

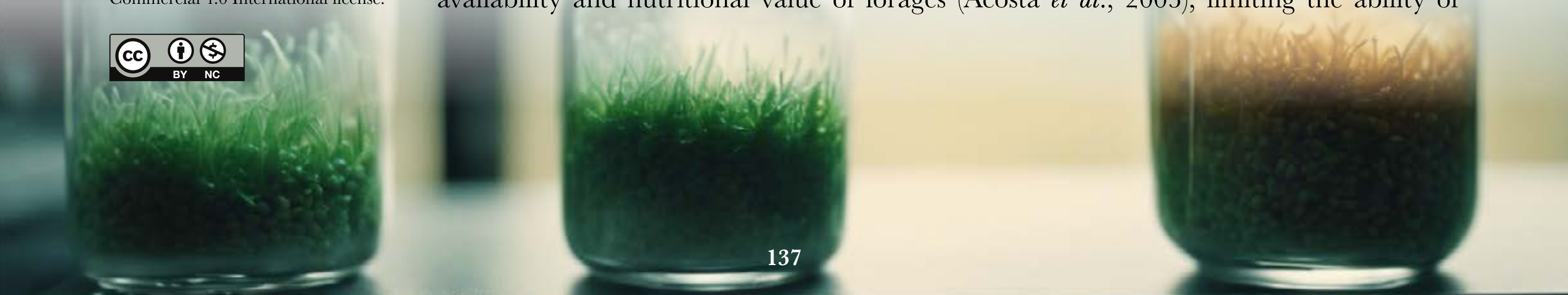
Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: October 24, 2023.
Accepted: March 18, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 137-149.

INTRODUCTION

In semi-arid regions, the high frequency of droughts has a negative effect on the availability and nutritional value of forages (Acosta *et al.*, 2003), limiting the ability of



producers and ranchers from mainly extensive and family systems to maintain the production of bovine milk and sheep, goat, and bovine meat. Therefore, maximizing the ratio of fibrous forages in the diet of ruminants, without reducing the quality and quantity of the products (Tirado-González *et al.*, 2018; Tirado-Estrada *et al.*, 2020), can help to reduce production costs (Oba and Allen, 1999, 2000a, b; Tirado-Estrada *et al.*, 2015), improve animal health (Saleem *et al.*, 2012; Petri *et al.*, 2013), and diminish the environmental impact of deforestation, consequently maintaining intensive grain production (McGinn *et al.*, 2004; Knapp *et al.*, 2014; Mora de Alba *et al.*, 2018).

The ensiling process contributes to the conservation and modification of the degradability of the cell walls of the forages, improving the nutritional characteristics of the forage through the formation of lactic acid and alcohols and the increase in crude protein (CP) (Tiwari *et al.*, 2008; Ajila *et al.*, 2015; Chen *et al.*, 2016). Meanwhile, the use of lactic acid bacteria (LAB) inoculants can improve the carbohydrate fermentation period, while the volatile fatty acid (VFA) profile and the formation of lactic acid increase the aerobic stability and quality of silages (Tabacco *et al.*, 2011; Skládanka *et al.*, 2012; Guo *et al.*, 2013; Schroeder, 2013). Fibrolytic enzymes (FE) can help to break the bonds of the cellulose and hemicellulose components (Arriola *et al.*, 2017; Kholif *et al.*, 2017; Tirado-González *et al.*, 2015, 2018), favoring the fermentation process through the increase of the sugars available for the microorganisms (Gado *et al.*, 2013; Salem *et al.*, 2015; Kholif *et al.*, 2017). However, the efficient action of LAB and FE depends on the interaction of various factors, such as: type and ratios of forages (Dehghani *et al.*, 2012), LAB strain or FE mixture (Dean *et al.*, 2005; Lynch *et al.*, 2012), dose (Del Valle *et al.*, 2019), and ensiling time (Lynch *et al.*, 2012). This phenomenon is caused by the high specificity of the enzymatic components, which record small differences in their neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) composition (Tirado-González *et al.*, 2016).

The objective of this work is to assess the effect of the use of EF and LAB in silages with oat and triticale mixtures on the contents and compositions of NDF and their relationship with GP and IVDMD.

MATERIALS AND METHODS

Experiment location and biological material

Representative samples of 60:40 and 80:20 ratios of oat (*Avena sativa* L.) and triticale (*Triticosecale* Wittmack. Ex. A. Camus) were taken in two locations in the central-northern region of Mexico:

1) El Llano, Aguascalientes (21° 55’ 00- N, 101° 58’ 00- W; 2,021 m.a.s.l.). The climate is semi-arid (BS1kw, Köppen), with an average temperature of 17.4 °C and an average annual precipitation of 540 mm. The soil mainly consists of planosol (66%) and phaeozem (23%).
2) Lagos de Moreno, Jalisco (21° 21- 23- N, 101° 55- 45- W; 1,942 m.a.s.l.). The climate is subtropical (Csa, Köppen), with an average temperature of 18.4 °C and an average annual precipitation of 670 mm. The soil is mainly composed of lithosol, planosol, and phaeozem (31%, 29% and 25%, respectively).

The samples were taken 120 hours after the cultivation began: 10 samples (20 kg of complete plants/sample) were selected at random in each of the blocks (DM=33.1±2.7%).

Preparation of microsilages

The microsilages and chemical analyzes were carried out at TecNM/ITEL (21° 55' N, 101° 58' W; 1,840 m.a.s.l.), in El Llano, Aguascalientes (average annual temperature of 17 °C, average annual precipitation of 455 mm).

The additives applied to the microsilages were an FE preparation —with different ratios of cellulases and xylanases (Fibrozyme, Alltech Inc., Nicholasville, KY, USA)— and a LAB inoculant —with *Lactobacillus plantarum* and *Pediococcus pentosaceus* (EnziBiolac, Enzimas y Productos Químicos, S. A. de C. V., SAGARPA Reg. A-9912-001, Mexico). Samples of 1±0.1 kg (WB) of the 60:40 and 80:20 O:T mixtures collected in the two locations (DM=30±2.5%) were chopped into 2 cm particles and treated with: 1) 0 (control), 0.75 (LD), 1 (MD), and 1.25 g (LD) doses of FE/kg WB; and 2) 0 (control), 0.188 (LD), 0.25 (MD), and 0.31 g (HD) doses of LAB/kg WB.

The FE and LAB mixtures were dissolved in distilled water and sprayed uniformly on the forage. The treatments were placed in 5.08 cm wide×30 cm long polyvinyl chloride (PVC) pipes (with reinforced PVC end caps installed at both ends of the pipe). The treatments were compacted with a metal piston (to eliminate as much oxygen as possible inside the microsilages) and stored in a closed room with an average temperature of 20 °C. They were then allowed to ferment for 40 days.

Chemical analysis

At the time of ensiling and every 0, 10, 20, 30, and 40 d after the start of the ensiling process, 300 g WB/microsilage fractions were sampled from different parts of each microsilage. These samples were placed in a Felisa® AR-290 forced air oven at 60 °C, until a constant weight (initial DM; 0 d) was reached. The dried samples were ground with a 1 mm sieve.

In the chemical analysis, the pH of the samples was measured using an Orion Star™ A2110 pH-meter (Thermo Scientific). The initial and final DM, crude protein (CP), and ash (ASH) were determined using the 10.136, 990.03 and 942.05 methods, respectively (AOAC, 2005). The NDF, ADF, and ADL of the samples collected from the microsilages at 40 d were determined (Van Soest *et al.*, 1991). For this purpose, the samples were adapted for the reagents of the F57 filters (2016, Ankom Technol Technology, Macedon, NY, USA) and an ANKOM^{200} Fiber Analyzer (ANKOM Technology,). Hemicellulose (HEM) and cellulose (CEL) were calculated by difference (HEM=NDF−ADF and CEL=ADF−ADL−ASH). Each sample was analyzed in duplicate.

***In vitro* fermentation**

The gas production of the silage samples taken at 40 d was analyzed using the gas production technique published by Menke and Steingass (1998). Ruminal fluid was obtained from two cannulated Dorper sheep (live weight: 60±5 kg) fed with a 76:24 forage:concentrate ratio (forage: barley (*Hordeum vulgare*) straw; concentrate: mixture of corn and soybean grain). The chemical composition of the forage was: 58% NDF, 10% CP, and 44.1% crude fiber (CF). Meanwhile, the chemical composition of the concentrate was: 22.4% NDF, 16% CP, and 5.4% CF.

The ruminal fluid was filtered with an 8-layer gauze and mixed with the mineral solutions reported by Cobos and Yokoyama (1995). Once the ruminal inoculum was prepared, it was placed in amber bottles with 0.5 g of DM and 90 mL of inoculum from the samples taken from the microsilages at 0 and 40 d (each sample was analyzed in duplicate). Incubation was carried out in a water bath (39 °C) under conditions of continuous flow of carbon dioxide (CO_2). The excess CO_2 from each bottle was extracted with the 63100 analog manometer (Metron®). The pressure of the fermentation gas (0 to 1 kg/cm^2) was measured with the manometer at 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, and 72 h of incubation and it was converted to mL.

At the end of the incubation period, the residue from each bottle was filtered through a previously weighed filter paper (Whatman® qualitative filter paper, Grade 4: 1004-110, pore size: 20-25 μm); the filter papers with residue were dried at 65 °C for 48 h and subsequently weighed. After 24 h, the fermentation of half of the flasks was stopped and the IVDMD was determined as follows:

$$IVDMD24 = 100 - \left[\left(initialDM - finalDM\right) / \left(initialDM * 100\right)\right]$$

The maximum volume, fractional rate, and lag phase (Vmax, S, and Lag) parameters of gas production were optimized in Equation (1) of Schofield *et al.* (1994).

$$Vo = \frac{V\max}{1 + e^{2-4S(t-Lag)}} \qquad (1)$$

Where: Vo=accumulated GP volume; $Vmax$=maximum volume of GP; S=fractional rate; Lag=lag phase.

Statistical analysis

Data analysis was performed with the SAS [9.2] statistical software (Statistical Analysis System). An analysis of variance (ANOVA) took into consideration the DCA, with factorial and split-plot arrangements with 4 repetitions per treatment, as well as 2 sub-repetitions for the fixed and random effects of Models 1 and 2. The significances, coefficients of determination (R^2), and coefficients of variation (CV) were obtained using the General Linear Procedure (Proc GLM); the LsMeans instruction was used for the adjusted means; and the standard errors (SE) were determined with the Mixed Models Procedure (Proc Mixed). The DMS were calculated using the SE values (P=0.05).

Model 1

$$Y = \mu + Rep(Loc)_{ij} + Tra_k + T_l + (Tra * T)_{kl} + \varepsilon_{ijkl}$$

Where: Y=pH; μ is the overall mean; $Rep\ (Loc)_{ij}$ is the random effect of the i-th repetition within the j-th sowing location; Tra_k is the effect of the k-th treatment; T_l is the effect of the l-th time; $(Tra*T)_{kl}$ is the interaction between fixed factors; ε_{ijkl} is the random error.

Model 2

$$Y = \mu + Subrep\left(Rep\right)_{ij} + D_k + A_l + PAT_m + \left(D * A\right)_{kl} + \left(D * PAT\right)_{km} + \left(A * PAT\right)_{lm} + \left(D * A * PAT\right)_{ijklm} + \varepsilon_{ijklm}$$

Where: Y=*initial DM*, *final DM*, *Vmax*, *S*, *Lag*, *pH*, *NDF*, *ADF*, *ADL*, *CP*, *ASH*, and *IVDMD24*; $Subrep\ (Rep)_{ij}$ is the random effect of the i-th subrepetition within the j-th locality or analysis time; D_k is the effect of the k-th dose; A_l is the effect of the l-th additive; PAT_m is the effect of the m-th ratio of O:T; $(D*A)_{kl}$, $(D*PAT)_{km}$, $(A*PAT)_{lm}$, and $(D*A*PAT)_{ijklm}$ are the interactions of the fixed factors; ε_{ijklm} is the random error.

Correlation analysis. Simple Pearson correlation analyzes were performed to analyze the relationship between the Vmax, S, Lag, NDF, ADF, ADL, CP, ASH, and IVDMD24 variables.

RESULTS AND DISCUSSION

Changes in chemical composition and pH

Chemical composition. Table 1 shows the chemical composition of the silages. Overall, the use of LD and MD of FE and LAB in the 60:40 O:T silages did not affect the ratio of NDF. However, the HD of both additives increased the ratio of NDF in the 60:40 O:T silages. The LD of FE reduced the ratio of NDF ($P<0.0009$) in 80:20 O:T silages. Furthermore, the ratio of ADF did not diminish in any of the FE and LAB treatments in both types of silage; even treatments with HD of LAB showed the highest ADF values compared to the control ($P<0.02$). Although the additives did not consistently reduce NDF and ADF, their inclusion did affect the ratios of HEM and CEL: the 60:40 O:T silages had more HEM and less CEL than the 80:20 silages ($P<0.008$), while silages with FE had a lower CEL content than those treated with LAB ($P<0.01$). The ratios of ADL and ASH were lower in 60:40 O:T silages than in 80:20 silages ($P<0.0002$); likewise, ASH were higher in silages treated with FE ($P<0.03$).

The 60:40 O:T silages had better CP content than 80:20 silages ($P<0.0001$). In the 60:40 O:T silages, the use of FE did not improve the CP in relation to the Control. In the 80:20 O:T silages, the use of LD, MD, and HD of FE increased the CP. Similarly, LD, MD, and HD of LAB improved the CP ratios of the 60:40 and 80:20 O:T silages ($P<0.05$).

Higher initial and final DM were observed in the 60:40 O:T silages than in the 80:20 silages ($P<0.0001$), as well as in those treated with FE with regard to those treated with LAB ($P<0.01$).

Table 1. Nutritional quality of silages of oat and triticale (O:T) forage mixtures (60:40 and 80:20 ratios), supplemented with various doses of fibrolytic enzymes (FE) and lactic acid bacteria (LAB).

Silages		NDF (%)	HEM (%)	ADF (%)	CEL (%)	ADL (%)	CP (%)	Ashes (%)	PreE-DM (%)	PostE-DM (%)
Oats: triticale 60:40%										
EFE	Control	61.38c	28.28b	33.51de	19.25cd	4.15c	7.25c	9.69cd	36.88cd	41.88cd
	LD	61.48c	28.15ab	33.34e	19.32cd	4.17c	7.80bc	9.86bc	39.65a	44.81a
	MD	62.28c	28.78ab	33.5de	20.18cd	4.27c	7.76bc	9.04d	38.15b	43.16ab
	HD	64.61a	29.23a	35.38c	21.70bc	4.53bc	7.37c	9.15d	38.59ab	43.71b
ALB	Control	62.69bc	28.37ab	34.20d	20.36cd	4.23c	6.92d	9.17d	37.13c	42.13c
	LD	62.29c	29.24a	34.05d	20.85bc	4.26c	9.06a	8.94d	37.11c	42.08cd
	MD	62.54bc	27.95b	34.59cd	21.54bc	4.31c	8.06b	8.74d	37.73bc	42.89bc
	HD	63.68ab	28.39ab	35.29c	21.55bc	4.29c	7.35c	9.46cd	35.83d	40.98d
Oats: triticale 80:20%										
EFE	Control	63.01ab	28.86ab	35.16cd	19.38cd	4.49bc	5.09h	9.28cd	28.43f	33.53f
	LD	61.89c	27.29bc	34.6cd	19.33d	4.57b	6.38e	10.69ab	28.28f	33.44f
	MD	63.27b	27.45bc	35.81ab	20.49c	4.57b	5.92f	10.75a	29.48ef	34.87e
	HD	62.66bc	27.30c	34.36d	20.53c	4.65b	5.80f	10.18b	28.26f	33.36f
ALB	Control	63.74a	28.13b	35.61c	20.56c	4.62b	4.83h	9.27cd	29.14ef	34.24ef
	LD	63.71ab	27.25c	36.46b	22.05ab	4.75ab	5.47g	9.66c	27.68f	32.58fg
	MD	64.41a	26.88c	37.53a	23.23a	5.02a	5.44gh	9.28cd	28.22f	33.16g
	HD	64.25a	27.07c	37.17a	21.85b	4.89ab	5.41gh	9.44cd	29.05ef	34.01ef
P-values										
	Doses	0.006	0.84	<0.0001	0.006	0.12	0.0004	0.36	0.56	0.89
	Additive	0.004	0.01	<0.0001	0.004	0.24	0.04	0.003	0.03	0.05
	OT	0.37	<0.0001	<0.0001	0.008	0.0002	<.0001	<0.0001	<0.0001	<0.0001
	D*A	0.009	0.03	0.17	0.06	0.83	0.003	0.23	<0.0001	<0.0001
	D*OT	0.11	0.26	0.05	0.02	0.67	0.21	0.22	<0.0001	<0.0001
	A*OT	0.10	0.17	0.02	0.99	0.11	0.002	0.10	0.13	0.01
	D*A*OT	0.47	0.38	0.66	0.93	0.26	0.05	0.40	0.004	0.05
	R^2	0.65	0.59	0.8	0.57	0.44	0.87	0.48	0.98	0.97
	V.C. (%)	1.77	3.20	2.19	4.91	11.77	7.92	6.00	2.99	4.01
	LSD (0.05) =	1.05	1.04	0.72	1.20	0.34	0.46	0.50	0.95	1.01
	S.E.	0.62	0.63	0.42	0.73	0.20	0.27	0.29	0.583	0.571

Different letters represent statistical media differences; FE, fibrolytic enzymes; LAB, lactic acid bacteria; LD, low dose; MD, medium dose; HD, high dose; Initial DM, initial dry matter (0 d, prior to the ensiling process); Final DM, final dry matter (DM of the silage at 40 d); NDF, neutral detergent fiber; HEM, hemicellulose; ADF, acid detergent fiber; CEL, cellulose; ADL, acid detergent lignin; CP, crude protein; P-values, probability values; ASH, ashes; D, doses; A, additive (FE or LAB); ROT, oat:triticale ratio; R^2, determination coefficient; V.C., variation coefficient; S.E., standard error; LSD, least significant difference ($P<0.05$).

pH modifications during the ensiling process. Figure 1 shows the pH changes of the treatments over the course of the ensiling period. The pH of the 60:40 O:T silages decreased from 6.1 to 4.5 (average) in the first 10 d. The use of FE had no significant effect on the reduction of pH, but there were significant effects resulting from the use of LD and MD of LAB ($P<0.05$). On the one hand, the use of HD of LAB increased pH (on day 20 after the fermentation began) with regard to the LD and MD of LAB ($P<0.0001$). On the other hand, in the 80:20 O:T silages, the pH was reduced from 6.1 to 4.2 in the first 30 d. However, in average, the pH increased after 30 d of fermentation ($P<0.01$) in all treatments.

In vitro fermentation of DM

Table 2 shows the Vmax, S, and Lag phase of GP of the treatments assessed in vitro, as well as the IVDMD24. Overall, differences in Vmax and S were observed in the two types of silage ($P<0.0001$). In 80:20 O:T silages, the use of FE and LAB did not increase Vmax and S with regard to the control, but, in the 60:40 O:T silages, the use of LD and MD of FE and LAB resulted in higher Vmax and S than in the control treatments ($P<0.003$). Similarly, the MD of FE, and the LD, MD, and HD of LAB reduced the Lag phase time of the 60:40 O:T silages (5.73 h *vs*. 6.55 h, MD of FE *vs*. Control; 5.92 h *vs*. 6.97 h, average LD, MD, and HD of LAB *vs*. Control), but did not affect the Lag time of the 80:20 O:T silages ($P<0.03$). The use of LD of FE and MD of LAB increased the IVDMD24 of the

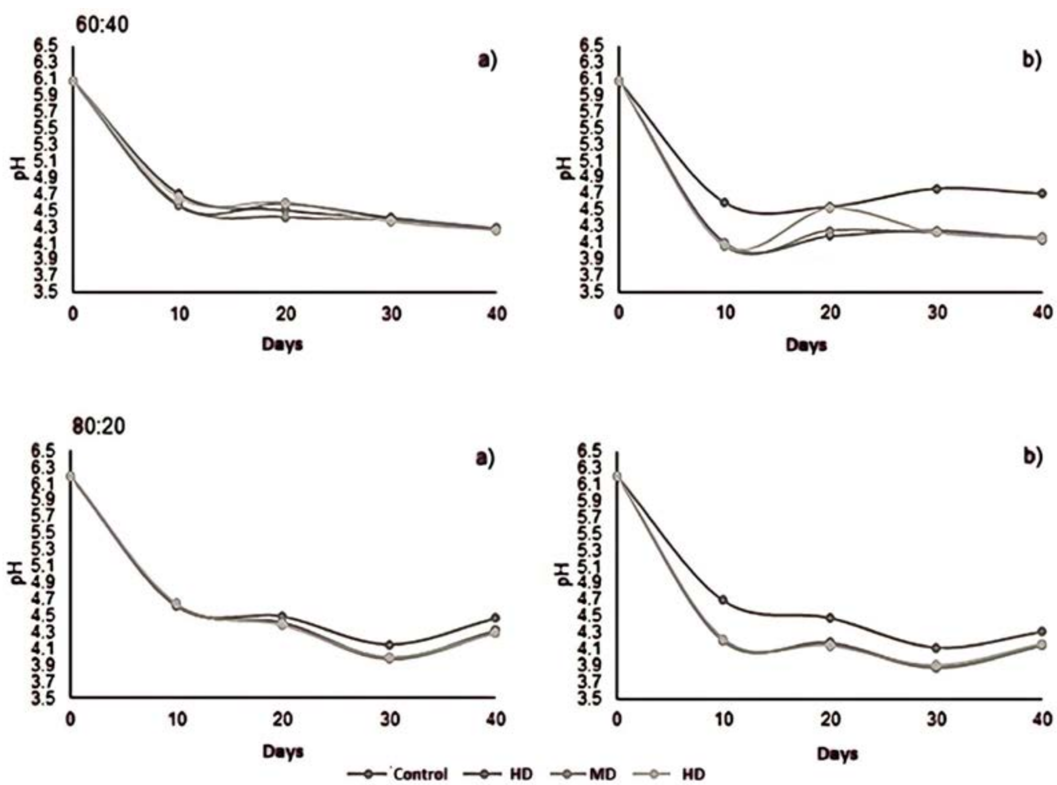


Figure 1. Effects on pH during the ensiling process of 60:40 and 80:20 oat:triticale silages supplemented with additives: a) fibrolytic enzymes (FE) and b) lactic acid bacteria (LAB).

Table 2. *In vitro* gas production (GP) and *in vitro* dry matter digestibility (IVDMD) at 24 h of silages with 60:40 and 80:20 oat:triticale (O:T) ratios supplemented with different doses of fibrolytic enzymes (FE) and lactic acid bacteria (LAB).

Silages		Vmax mL/g	S mL gas/h	Lag h	IVDMD %
Oats: triticale 60:40%					
EFE	Control	302.53cd	0.032b	6.55b	66.41b
	LD	314.24bc	0.033a	6.52b	67.70a
	MD	333.28ab	0.032b	5.73c	65.21c
	HD	322.75b	0.031c	6.60b	65.81bc
ALB	Control	308.33c	0.032b	6.97ab	65. 68bc
	LD	330.55ab	0.033a	6.02c	65.21c
	MD	331.58b	0.032b	6.01c	66.27ab
	HD	346.18a	0.033a	5.73c	62.66d
Oats: triticale 80:20%					
EFE	Control	283.83de	0.032b	6.50ab	60.95e
	LD	295.58cd	0.031c	6.61b	62.14de
	MD	294.28d	0.031c	6.79ab	59.96ef
	HD	302.01cd	0.031c	6.52b	59.95ef
ALB	Control	288.96de	0.032b	7.18a	59.76ef
	LD	300.79cd	0.031c	6.97ab	60.62ef
	MD	292.93de	0.031c	6.73ab	59.71f
	HD	277.10e	0.030d	7.01ab	59.95ef
P-values					
	Doses	0.25	0.05	0.42	0.0002
	Additives	0.05	0.84	0.83	<0.0001
	OT	<0.0001	<0.0001	<0.0001	<0.0001
	D*A	0.11	0.002	0.78	<0.0001
	D*OT	0.24	0.28	0.64	0.25
	A*OT	0.003	0.003	0.0005	0.0002
	D*A*OT	0.10	0.11	0.03	<0.0001
	R^2	0.80	0.79	0.94	0.91
	C.V. (%)	6.67	4.27	9.67	3.14
	LSD (0.05)=	17.00	0.001	0.52	1.20
	S.E.	10.33	0.0007	0.31	0.72

FE, fibrolytic enzyme; LAB, lactic acid bacteria; LD, low dose; MD, medium dose; HD, high dose; Vmax, maximum volume of GP; S, fractional gas production rate; Lag, lag phase; IVDMD, in vitro dry matter digestibility at 24 h; P-values, probability values; D, doses; A, additive (FE or LAB); ROT, oat: triticale ratio; R^2, determination coefficient; V.C., variation coefficient; S.E., standard error; LSD, last significant difference ($P<0.05$).

60:40 O:T silages (67.70% *vs.* 66.41%, LD of FE *vs.* Control; 66.27% *vs.* 65.68%, MD of LAB *vs.* Control) ($P<0.0001$); however, using FE and LAB did not have a positive effect on the IVDMD of 80:20 O:T silages.

Pearson correlations

The IVDMD24 did not correlate with NDF (Table 3); however, it showed a positive correlation with the HEM and CP ratio, but negative correlation with ADF, CEL, and ADL ($P<0.0001$). Consequently, the IVDMD24 had a positive correlation with Vmax and S and a negative correlation with the Lag phase ($P<0.01$).

FE has been used to improve the digestibility of forages intended for ruminant feed. These products can combine different ratios of forms and isoforms of cellulases and xylanases, depending on the extraction time of the FE and the substrate from which it was obtained (Tirado-González *et al.*, 2015, 2016, 2018; Carrillo-Díaz *et al.*, 2022). Using FE can have positive effects on the production and kinetics of GP and IVDMD (Phakachoed *et al.*, 2013; Salem *et al.*, 2015; Li *et al.*, 2018).

The variation in the oat and triticale ratios in this work affected the action of the FE and LAB added to the silages, confirming the high specificity of the enzymes extracted from fungi and the potential synergisms and negative interactions between enzymes and bacteria reported in previous research (Tirado-González *et al.*, 2016). Furthermore, the inconsistent fermentation and in vitro degradability results arising from the use of enzymes between silages with different O:T ratios are explained by the interaction between the types of cell walls with the exogenous enzyme preparations and the activity of bacteria and endogenous FE (Beauchemin *et al.*, 2003; Tirado-González *et al.*, 2015). The components and structures of the cell walls of forages depend on their type, maturity, and stress (Jung and Casler, 2006a, b).

Therefore, this research shows how the optimal activity of commercial FE depends more on changes in the structure and composition of NDF and its interaction with endogenous and exogenous bacteria than on the total ratio of NDF (Tirado-González *et al.*, 2018; 2021). Likewise, changes in the ratios of ADF, HEM, CEL, and ADL can improve the activity of FE. The relationships between cellulases and xylanases in commercial FEs depend on the fungus from which they were extracted, the type of substrate, and the extraction time. In addition, their action, combined with or independent from LAB, will be reflected in the pH —indicating greater production of acids during the fermentation of the silage (Skládanka *et al.*, 2012; Mora de Alba *et al.*, 2018; Li *et al.*, 2018)—, in the chemical composition of the silages at the end of fermentation (Gado *et al.*, 2013; Kholif *et al.*, 2017), in the subsequent fermentation (Tirado-Estrada *et al.*, 2015), and in the rumen degradability of NDF (Carrillo-Díaz *et al.*, 2022; Khan *et al.*, 2015).

This study shows how LD and MD of FE improved IVDMD24 in 60:40 O:T silages. However, their addition did not have positive effects in 80:20 O:T silages, partially showing the effects of interactions between the ratio and composition of NDF and the exogenous and endogenous enzymes and bacteria. This phenomenon is relevant because NDF degradability has previously been related to DM intake, as well as to the potential production and quality of ruminant milk and meat (Oba and Allen, 1999, 2000a, b; Arriola *et al.*, 2017; Tirado-González *et al.*, 2018, 2020). Likewise, changes in the productive behavior of ruminants are caused by changes in rumen kinetics —which depend on the type of successive populations of highly specific microorganisms that

Table 3. Pearson correlations among the following variables: composition, gas production (GP), and in vitro dry matter digestibility (IVDMD) at 24 h.

	S	**Lag**	**IVDMD**	**PreE-DM**	**Ash**	**NDF**	**HEM**	**ADF**	**CEL**	**ADL**	**pH**	**CP**	**PostE-DM**
Vmax	0.32**	−0.74***	0.66***	0.68***	−0.43***	−0.03	0.46***	−0.41***	−0.03	−0.44**	0.03	0.56***	0.68***
S		−0.50***	0.64***	0.43**	−0.09	−0.23§	0.24§	−0.45**	−0.30*	−0.31*	−0.17	0.61***	0.43**
Lag			−0.76***	−0.60***	0.21§	0.16	−0.38**	0.50***	0.22§	0.46***	−0.08	−0.62***	−0.60***
IVDMD				0.74***	−0.18	−0.21	0.48***	−0.62***	−0.37**	−0.47***	0.03	0.76***	0.74***
PreE−DM					−0.40**	−0.13	0.57***	−0.61***	−0.25*	−0.46***	0.09	0.77***	0.87***
Ash						0.06	−0.12	0.17	−0.40**	0.15	0.11	−0.33§	−0.40§
NDF							0.50***	0.70***	0.64***	0.22	0.01	−0.22	−0.13
HEM								−0.27*	−0.05	−0.38**	0.15	0.52***	0.57***
ADF									0.75***	0.56***	−0.12	−0.68***	−0.61***
CEL										0.07	−0.11	−0.36**	−0.25*
ADL											−0.16	−0.45***	−0.46***
pH												−0.14	0.11
CP													0.77***

*, **, ***, statistical significances at $P<0.001$, $P<0.01$, and $P<0.05$, respectively; Vmax, maximum volume of GP; S, fractional rate; Lag, lag phase; Initial DM, initial dry matter (0 d, prior to the ensiling prwocess); ASH, ashes; NDF, neutral detergent fiber; HEM, hemicellulose; ADF, acid detergent fiber; CEL, cellulose; ADL, acid detergent lignin; CP, crude protein; IVDMD24, *in vitro* dry matter digestibility at 24 h; Final DM, final dry matter (DM of the silage at 40 d).

degrade the components of the NDF and non-fibrous carbohydrates of the diets, and on their efficient use to provide net production energy (Knapp *et al.*, 2014).

The use of LAB can contribute to the stability and quality of silages by promoting an increase in the lactic acid:acetic acid ratio (Guo *et al.*, 2013; Chen *et al.*, 2016; Mora de Alba *et al.*, 2018) and by limiting the growth of yeast in the silage (Tabacco *et al.*, 2011).

In this study, LD and MD of LAB improved the quality of the silages, based on the CP contents of the 60:40 O:T silages; in addition, they improved the IVDMD24, Vmax, S, and Lag of the 80:20 O:T silages. Lynch *et al.* (2012) and other authors have found that some strains and/or doses of Lactobacillus may not improve or that they may even worsen the quality of silage, as a consequence of the reduction in the concentration of lactic acid that can be used as a substrate by some lactic acid bacteria.

Finally, this study proves the relationship between IVDMD24 and GP during *in vitro* fermentation with ruminal fluid. This relation is deeply connected with the changes in the ratios of hemicellulose and cellulose caused by the use of additives during the ensiling process. However, they are not always related to the total NDF content, which may not even be affected by the use of FE or LAB.

CONCLUSIONS

This work shows the degree of specificity with which enzyme preparations and lactic acid bacteria act on the different types of cell walls of different oat and triticale ratios in a silage. The contents of cell walls (NDF) and CP, *in vitro* degradability and fermentation were better in mixtures containing a lower ratio of oat. Although the additives did not consistently reduce NDF and ADF, their inclusion did affect the hemicellulose and cellulose ratios: the 60:40 O:T silages had more hemicellulose and less cellulose, ADL, and ASH than 80:20 silages, while silages with FE had a lower cellulose content than those treated with LAB. Although the use of additives does not directly improve the ratio of NDF during the ensiling process, it can increase the ratios of hemicellulose and reduce the ratios of cellulose. Hemicellulose and cellulose could be highly correlated with the fermentation and degradability of forages in the rumen.

REFERENCES

AOAC. (2005). Official Methods of Analysis of AOAC International (OMA). AOAC International, Gaithersburg, MD.

ANKOM Technol. (2016). Method 6, 20/01/16: Neutral Detergent Fiber in Feeds Filter Bag Technique (For A200, A200I). Ankom Technology, Macedon, N NY. Disponible en: https://www.ankom.com/sites/default/files/document-files/Method 13 NDF Method A2000 RevE 4 10 15.pdf.

Ajila, C. M., Sarma, S. J., Brar, S. K., Godbout, S., Cote, M., Guay, F., Verma, M., Valéro, J. R. (2015). Fermented apple pomace as a feed additive to enhance growth performance of growing pigs and its effects on emissions. *Agriculture* 5:313-329.

Arriola, K.G., Oliveira, A.S., Ma, Z.X., Jean, I.J., Giurcanu, M.C., Adesogan A.T. (2017). A meta-analysis on the effect of dietary application of exogenous fibrolytic enzymes on the performance of dairy cows. *Journal of Dairy Science 100*(6):4513-4527.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z. (2003). Use of the exogenous fibrolytic enzyme to improve feed utilization by ruminants. *Jorunal of Animal Science* 81: E37-E47.

Carrillo-Díaz, M.I., Miranda-Romero, L.A., Chávez-Aguilar, G., Zepeda-Batista, J.L., González-Reyes, M., García-Casillas, A.C., Tirado-González, D.N., Tirado-Estrada, G. (2022). Improvement of ruminal neutral detergent fiber degradability by obtaining and using exogenous fibrolytic enzymes from White-Rot Fungi. *Animals* 12: 843. https://doi.org/10.3390/ani12070843

Chen, L., Yuan, X., Li, J., Wang, S., Dong Z., Shao T. (2016). Effect of lactic acid bacteria and propionic acid on conservation characteristics, aerobic stability and *in vitro* gas production kinetics and digestibility of whole-crop corn based total mixed ration silage. *Journal of Integrative Agriculture 15*(7):1592-1600.

Cobos, M.A., Yokoyama, M.T. (1995). Clostridium paraputrificum var ruminantum: colonization and degradation of shrimp carapaces in vitro observed by scanning electron microscopy. In: Wallas, R.J., Lahlou-Kassi, A. (Eds.), Rumen Ecology Research Planning, Proceedings of a Workshop. International Livestok Research Institute, Addis Abeba, Ethiopia, pp. 151-161.

Del Valle, T.A., Antonio, G., Zenatti, T.F., Campana, M., Zilio, E.M., Ghizzi, L.G., Gandra, J.R., Osório, J.A.C., Morais, J.P.G. (2019). Effects of xylanase on the fermentation profile and chemical composition of sugarcane silage. *The Journal of Agricultural Science 156*(9):1123-1129.

Gado, H.M., Salem, A.Z.M., Camacho, L.M., Elghandour, M.M.Y., Salazar M.C. (2013). Influence of exogenous enzymes on *in vitro* ruminal degradation of ensiled straw with DDGS. *Animal Nutrition and Feed Technology* 13:569-574.

Guo, X.S., Undersander, D.J., Combs, D.K. (2013). Effect of Lactobacillus inoculants and forage dry matter on the fermentation and aerobic stability of ensiled mixed-crop tall fescue and meadow fescue. *Journal of Dairy Science 96*(3):1735-1744.

Jung, H.G., Casler, M.D. (2006a). Maize stem tissues: cell wall concentration and composition during development. *Crop Science* 46:1793-1800.

Jung, H.G., Casler, M.D. (2006b). Maize stem tissues: impact of development on cell wall degradability. *Crop Science* 46:1801-1809.

Khan, N.A., Yu, P., Ali, M., Cone, J.W., Hendricks W.H. (2015). Nutritive value of maize silage in relation to dairy cow performance and milk quality. *Journal of the Science of Food and Agriculture 95*(2):238-252.

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P., Tricarico J.M. (2014). Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emmisions. *Journal of Dairy Science* 97:3231-3261.

Kholif, A.E., Elghandour, M.M.Y., Rodríguez, G.B., Olafadehan, O.A., Salem, A.Z.M. (2017). Anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktail as a cleaner and sustainable biological product. *Journal of Cleaner Production* 142:2649-2655.

Li, J., Yuan, X., Dong, Z., Mugabe, W., Shao, T. (2018). The effects of fibrolytic enzymes, cellulolytic fungi and bacteria on the fermentation characteristics, structural carbohydrates degradation, and enzymatic conversion yields of *Pennisetum sinese* silage. *Bioresource Technology* 264:123-130.

Lynch, J.P., Kiely, P.O., Waters, S.M., Doyle, E.M. (2012). Conservation characteristics of corn ears and stover ensiled with addition of *Lactobacillus plantarum* MTD-1, *Lactobacillus plantarum* 30114, or *Lactobacillus buchneri* 11A44. *Journal of Dairy Science* 95:2070-2080.

McGinn, S.M., Beauchemin, K.A., Coates, T., Colombatto D. (2004). Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. *Journal of Animal Science* 82:3346-3356.

Menke, K.E., Steingass H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research Development* 27:7-55.

Mora de Alba, M.E., Tirado-González, D.N., Quezada-Tristán, T., Guevara-Lara, F., Jáuregui-Rincón, J., Larios-González, R., Tirado-Estrada, G. (2018). Calidad nutricional del bagazo de manzana ensilado con fuentes nitrogenadas orgánicas e inorgánicas. *Revista Mexicana de Ciencias Agrícolas 9*(1):229-235.

Oba, M., Allen, M. (1999). Evaluation of the importance of the digestibility of NDF from forage: effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science* 82:589-596.

Oba, M., Allen, M. (2000a). Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 1. Feeding behavior and nutrient utilization. *Journal of Dairy Science* 83:1333-1341.

Oba, M., Allen, M. (2000b). Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 2. Digestibility and microbial efficiency. *Journal of Dairy Science* 83:1350-1358.

Phakachoed, N., Suksombat, W., Colombatto, D., Beauchemin, K.A. (2013). Use of fibrolytic enzymes additives to enhance *in vitro* ruminal fermentation of corn silage. *Livestock Science* 157: 100-112.

Petri, R.M., Schwaniger, T., Penner, G.B., Beauchemin, K.A., Forster, R.J., McKinnon, J.J., McAllister, T.A. (2013). Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well during and after an acidotic challenge. *PlosOne 8*(12):e83424.

Salem, A.Z.M., G. Buendía-Rodríguez, G., Elghandour, M.M.M., Berasain, M.A.M., Jiménez, F.J.P., Pliego, A.B., Chagoyan, J.C.V., Cerrillo, M.A., Rodríguez, M.A. (2015). Effects of cellulase and xylanase enzymes mixed with increasing doses of *Salix babylonica* extract on *in vitro* rumen gas production kinetics of a mixture of corn silage with concentrate. *Journal of Integrative Agriculture 14*(1):131-139.

Saleem, F., Ametaj, B.N., Bouatra, S., Mandal R., Zebeli, Q., Dunn, S.M., Wishart, D.S. (2012). A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. *Journal of Dairy Science* 95: 6606-6623.

Schofield, P., Pitt, R.E., Pell, A.N. (1994). Kinetics of fiber digestion from *in vitro* gas production. *Journal of Animal Science* 72:2980-2991.

Shroeder, J.W. (2013). Silage fermentation and preservation. NDSU Extension Service: AS1254.

Skládanka, J., Mikyska, F., Dolezal, P., Seda, J., Havlícek, Z., Mikel, O., Hosková, S. (2012). Effect of the technology of the additional sowing of drought-resistant clover-grass mixture and silage additives on fermentation process quality and nutritive value of baled grass silages. *African Journal of Agriculture Research 7*(2): 325-333.

Tabacco, E., Piano. S., Revello-Chion, A., and Borreani, G. (2011). Effect of *Lactobacillus buchneri* LN4627 and *Lactobacillus buchenri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. *Journal of Dairy Science* 94: 5589-5598.

Tawari, S. P., Narag, M. P., Dubey, M. (2008). Effect of feeding apple pomace on milk yield and milk composition in crossbred (Red Sindhi × Jersey) cow. *Livestock Research for Rural Development 4*(29): 293-297.

Tirado-Estrada, G., Mejía-Haro, I., Cruz-Vázquez, C.R., Mendoza-Martínez, G.D., Tirado-González, D.N. (2015). Degradación in situ y patrones de fermentación del rastrojo de maíz (*Zea mays* L.) tratado con enzimas exógenas en vacas Holstein. *Interciencia 40*(10: 716-724.

Tirado-Estrada, G., Tirado-González, D.N., Medina-Cuéllar, S.E., Miranda-Romero, L.A., González-Reyes, M., Sánchez-Olmos, L.A., Castillo-Zúñiga, I. (2020). Global effects of maximizing the forage in production and quality of bovine milk and meat. A meta-analysis. *Interciencia 45*(10):461-468.

Tirado-González, D.N., Tirado-Estrada, G., Miranda-Romero, L.A. (2015). Sobre el efecto de enzimas fúngicas en la alimentación de rumiantes. *Interciencia 40*(11):758-766.

Tirado-González, D.N., Jauregui-Rincóon J., Tirado-Estrada G., Martínez-Hernández, P.A., Guevara-Lara, F., Miranda-Romero L.A. (2016). Production of cellulases and xylanases by white-rot fungi cultured in corn stover media for ruminant feed applications. *Animal Feed Science and Technology* 221:147-156.

Tirado-González, D.N., Miranda-Romero, L.A., Ruiz-Flores, A., Medina-Cuéllar, S.E., Ramírez-Valverde, R., Tirado-Estrada, G. (2018). Meta-analysis: effects of exogenous fibrolytic enzymes in ruminants. *Journal of Applied Animal Research 46*(1):771-783.

Tirado-González, D.N., Tirado-Estrada, G., Miranda-Romero, L.A., Ramírez-Valverde, R., Medina-Cuéllar, S.E., Salem, A.Z.M. (2021). Effects of addition of exogenous fibrolytic enzymes on digestibility and milk and meat production- A Systematic Review. *Ann. Anim. Sci. 21*(4): 1159-1192.

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: 3583-3592.

Knowledge management for small-scale agricultural producers: a thematic proposal for strengthening rural economic units

Muñoz-González, Arturo Erik[1]; Vivanco-Vargas, Martin[1]; Bravo-Vinaja, Ángel[2]; Méndez-Gallegos S. de Jesús[2]; Vasco-Leal, José F.[1*]

[1] Universidad Autónoma de Querétaro, Facultad de Contaduría y Administración. Querétaro, Querétaro. México. C. P. 76010.

[2] Colegio de Postgraduados Campus San Luis Potosí. Posgrado de Innovación en Manejo de Recursos Naturales. Salinas de Hidalgo, San Luis Potosí. México. C.P. 78622.

* Correspondence: jose.vasco@uaq.mx

ABSTRACT

Objective: To establish a thematic proposal for the management of knowledge of small-scale agricultural producers (peasants) and the strengthening of rural economic units in the state of Querétaro, to energize the social fabric and improve the quality of life of the rural population.

Design/methodology/approach: A bibliometric study of the scientific production on peasant knowledge in Mexico was carried out, as well as an observational analysis focused on specific and general problems. According to the information obtained and the problems detected, three areas for knowledge management were established: technical-productive, managerial and entrepreneurial.

Results: In the technical-productive area, training in good agricultural practices should be addressed, along with technological innovation and the generation of added value. In the management area, issues related with strategic management with a broad entrepreneurial vision that could help create strategies for agribusiness development. Concerning entrepreneurship, the internal and external factors of the environment stand out, which allow awakening their interest, encouraging leadership and direction for business development, in addition to promoting associativity in farming regions. The implementation of the topics proposed in the research will strengthen and boost small-scale agricultural production in the state of Querétaro.

Limitations on study/implications: This study can serve as a reference for small-scale agricultural producers (peasant).

Findings/conclusions: There is a great opportunity through knowledge management to increase the capacities, knowledge and skills of small-scale agricultural producers (peasants) in the state of Querétaro regarding technical-productive, managerial and entrepreneurial themes, which will generate economic, social and environmental impacts for the benefit of this rural sector.

Keywords: small-scale farming, peasants, training, human capital, rural development.

Citation: Muñoz-González, A. E., Vivanco-Vargas, M., Bravo-Vinaja, Á., Méndez-Gallegos S. de J., Vasco-Leal, J. F. (2024). Knowledge management for small-scale agricultural producers: a thematic proposal for strengthening rural economic units. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2714

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: October 26, 2023.
Accepted: January 26, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 151-166.

INTRODUCTION

In Mexico, agricultural production is considered the main activity in the farming sector and the one of highest economic relevance in relation to the livestock production, aquaculture and fishing sectors; likewise, it offers multiple social and environmental benefits (SADER, 2018). According to the National Agriculture and Livestock Production

Survey (INEGI and SADER, 2019), from the agricultural production of grains in Mexico, 0.5% is destined to sowing seed, 4.3% is devoted to family consumption, 7.8% is used as fodder for the livestock, and the remaining 87.4% to sale. From the latter, 53.1% is traded with intermediaries, 25.1% in direct deals with the final consumer, 11.5% are negotiated with storerooms, warehouses or stockpiling centers, 3.8% established under contract, 1.2% is destined to packinghouses or industrial use, 0.9% is sent to central markets, 0.3% is traded with shopping centers or supermarkets, and 4.7% with other types of buyers. These figures exhibit an excessive participation of intermediaries, weak negotiations, and low integration to the markets, resulting in important monetary losses for the rural economic units (REUs).

According to the diagnosis of the rural and fishing sector in Mexico carried out in the year 2012 by the Ministry of Rural Development (*Secretaría de Desarrollo Rural*, SADER) and the Food and Agriculture Organization of the United Nations (FAO) (SADER and FAO, 2014), REUs can be classified into six strata: (S1) Subsistence family-based without link to the market, (S2) Subsistence family-based linked to the market, (S3) In transition, (S4) Entrepreneurial with fragile profitability, (S5) Pushing entrepreneurial, and (S6) Dynamic entrepreneurial. For the state of Querétaro, it was reported that, 46.6% of the REUs belong to S1, 35.1% to S2, 5.8% to S3, 6.9% to S4, 4.9% to S5, and 0.7% to S6 (FAO and SAGARPA, 2013).

Strata segmentation in the REUs in Querétaro could be related, primarily, to the small-scale farmers not having the managerial and productive capacities and abilities for entrepreneurial management. This is associated with the limited access to knowledge adoption, finding with this organizations that do not generate utilities and which, in contrast, show low productive levels and high production costs. This low positioning in the market is generated because they do not comply with the demanding characteristics of quality and a certain degree of differentiation, which has provoked disinterest and abandonment of primary activities and has derived into high rates of migration of the workforce and apathy from young people in rural zones.

Knowledge management provides the opportunity of transcending in the REUs, with the interest of having the ability to generate and adopt new knowledge, share it among members of the sector, and materialize it into technological innovations, goods, services and systems. Therefore, this would also allow being more productive and efficient to obtain competitive advantage through continuous innovation (Carson, 2018; Alavi and Leidner, 2002; Nonaka and Takeuchi, 1999). In addition, it would allow developing the knowledge, abilities and appropriate attitudes in the agricultural producers for agribusiness, making it easier to attain better results in the agro-industrial area, as well as improving the generational shift and economic dynamics (Toillier *et al.*, 2020; Ikuemonisan *et al.*, 2022).

Presently, the agro-entrepreneurial sector is found in a context of globalization, of high technological innovation and growing demand, so a greater emphasis is required on each task of its management, in addition to being in a continuous process of adaptation and permanence in time, in order to respond to the expectations and political, economic and social changes (Arteaga-Coello *et al.*, 2016). In this sense, Spielman and Birner (2008) suggested a series of points for the creation and implementation of an innovation system for agriculture, highlighting that agricultural education should be directed toward the development of human capital, which will be reflected in the increase of yields, the generation of added value, the capacity for innovation, and the interest for entrepreneurship, among other processes. For its part, the Mexican Agrifood Innovation System has the objective of creating policies, executing projects, managing innovation, transferring knowledge and technology for the agrifood sector to incorporate science, technology and innovation within its activities as a motor for productivity, competitiveness and sustainability (Deschamps-Solorzano and Escamilla-Caamal, 2010). It should be highlighted that the promotion of agricultural innovation normally requires the support of the State, as has happened in diverse countries of the world as a way of encouraging producers to investigate, experiment and then implement cutting edge practices with the aim of improving their productivity, decreasing their agro-environmental impact, and facing market challenges (Wesseler *et al.*, 2017; Akkaya *et al.*, 2021).

Taking into consideration that public and private institutions that work on agricultural development in Mexico conduct an important role in knowledge and technology generation, they will be able to respond to the needs and quandaries identified based on the applied research. These should be linked in the regions with specialists, researchers and extension workers with the aim of contributing to the economic and social welfare of this economic sector. Because of this, the study suggests the objective of establishing a thematic proposal for knowledge management of small-scale agricultural producers (peasants) and the strengthening of rural economic units in the state of Querétaro, in order to make more dynamic the social fabric and improve the quality of life of the rural population.

MATERIALS AND METHODS

Part of the documental analysis carried out was the identification of research themes, with a bibliometric analysis of the scientific production on peasant knowledge in Mexico published in "mainstream" journals (Salager-Zeyer 2015) during the 1991-2023 period; for this purpose, a search was done of documental information in the Science Citation Index Expanded SCIE and Social Sciences Citation Index (SSCI) databases through the search expression:

$$TS = \left(\left(Farmer*\ \ OR\ peasant*\right)\ AND\ Knowledge\right)$$

The resulting bibliographic records were refined by the types of documents: scientific article, review article, and anticipated access, and then by country, selecting the articles published by authors with institutions located in Mexico.

A total of 271 bibliographic records were obtained, which were reviewed to select those that treated the subject effectively, and with that, the final database was made up by 181 documents, distributed into 172 research articles, nine review articles, and one of anticipated access, which was also classified as scientific article. The resulting records were exported to a file in text format compatible with the VosViewer software (van Eck and Waltman (2010), with the aim of performing the mapping of keywords of the authors and keywords assigned by the Science Web (Keyword Plus) of documents recovered through the joint-words analysis, thus identifying clusters of the research themes, as well as identifying the research trends. The thematic research maps were carried out through the option of co-occurrence analysis (Tijssen and Van Raan 1994), selecting at least three repetitions of keywords or phrases (normalized and translated into Spanish) contained in the bibliographic records. To normalize the clusters, the LingLog option was selected, instead of the association one that appears by assignment in VosViewer.

For the second phase, the next step was the observational analysis according to the experience/knowledge focused on suggesting and limiting the study problem, taking into account the causes that provoke the economic and social deterioration of small-scale agricultural producers (peasants) in the state of Querétaro. Then, based on the diagnosis conducted and the experience/knowledge obtained in the field, the reach and perspective of the study was assessed. Finally, stemming from bringing together the information, a thematic proposal was designed to train small-scale farmers (peasants) and strengthen the rural economic units in the state of Querétaro.

RESULTS AND DISCUSSION

Research themes about peasant knowledge in Mexico

In the density map of keywords, six clusters of keywords are identified, which represent the research themes about peasant knowledge in Mexico (Figure 1). Cluster 1 (red) groups the research on traditional peasant, ethnobotanical, ecological knowledge, about ecologic restoration, agroforestry systems, forests and rainforests, ecological reserves, protected areas, wild flora, medicinal plants, and the Maya; examples of these are the studies by: Suárez *et al.* (2012), Beltrán-Rodríguez *et al.* (2014), Orantes-García *et al.* (2018), Parraguez-Vergara *et al.* (2018), Falkowski *et al.* (2019), Flores-Silva *et al.* (2021), and Heinze *et al.* (2022). Cluster 2 (green) groups the themes of ecosystem services, biodiversity conservation, sustainability, environment, agrosilvipasture systems, socioecological systems, agricultural landscapes, intensive agriculture, food sovereignty, coffee and Chiapas; in this cluster, the studies that stand out are: Valencia *et al.* (2015), Barton *et al.* (2016), García-Barrios *et al.* (2017), Castillo *et al.* (2021), Rendon-Sandoval *et al.* (2021), and Contreras-Medina *et al.* (2022). Cluster 3 (dark blue) deals with themes of local and indigenous knowledge, perception of farmers, and traditional agriculture, among which some included are soils, crops, irrigation, foods, health, pests and diseases, and safety in pesticide handling; some studies that represent this group are: Reséndiz-Paz *et al.* (2013), Bautista *et al.* (2019), Torres-Guerrero *et al.* (2019), Sánchez-Gervasio *et al.* (2021), and Trejo *et al.* (2022). Cluster 4 (yellow) groups the themes of climate change, and their adaptation to it, vulnerability, agroecology, small-scale farmers, family gardens, rainfed agriculture, rotating agriculture, sheep, tropical agriculture, local varieties, the *milpa*, and Maya knowledge. Some examples of studies on this are: Benz *et al.* (2007), Jiménez-Ferrer *et al.* (2007), Aguilar-Stoen *et al.* (2009), Charcas S. *et al.* (2010), Castellanos *et al.* (2013), Bermeo *et al.* (2014), Camacho-Villa *et al.* (2021), and Martínez-Herrera *et al.* (2021). Cluster 5 (purple) groups the themes of technology adoption, innovations, rural development, conservation agriculture, peasant agriculture, dual-purpose livestock, and Michoacán. As examples of studies in these themes, there are: Flores López *et al.* (2020), Contreras-Medina *et al.* (2020), Lastiri-Hernández *et al.* (2021), Subercaseaux *et al.* (2021), Villarroel-Molina *et al.* (2022), and Barragán-Ocaña and del-Valle-Rivera (2016). Cluster 6 (light blue) groups peasant knowledge about genetic resources, particularly corn, food security, and the participation of peasants in agricultural research projects; among the articles that deal with these themes, there are: Zavala *et al.* (2005), Benz *et al.* (2007), Rodríguez *et al.* (2007), Bermeo *et al.* (2014), Berget *et al.* (2015), and Hernández-Ramos *et al.* (2020).

Research trends

The mapping techniques in science, using the co-occurrence analysis, allow visualizing the research trends, from the point of view that the most current themes are the trend. The word networks shown in Figure 2 show the most recent peasant knowledge themes, identified in yellow, such as technology adoption, conservation agriculture, intensive agriculture, ecologic intensification, women's participation, alternative control of diseases and pests, irrigation implementation, adaptation to climate change, ecology, and food sovereignty, among others.

Taking into account the results obtained from the maps of research themes on peasant knowledge in Mexico (Figure 1) and research trends (Figure 2), the need is identified to specifically analyze the impact of the technical-productive, managerial, and entrepreneurial management of small-scale agricultural producers (peasants) in the state of Querétaro.

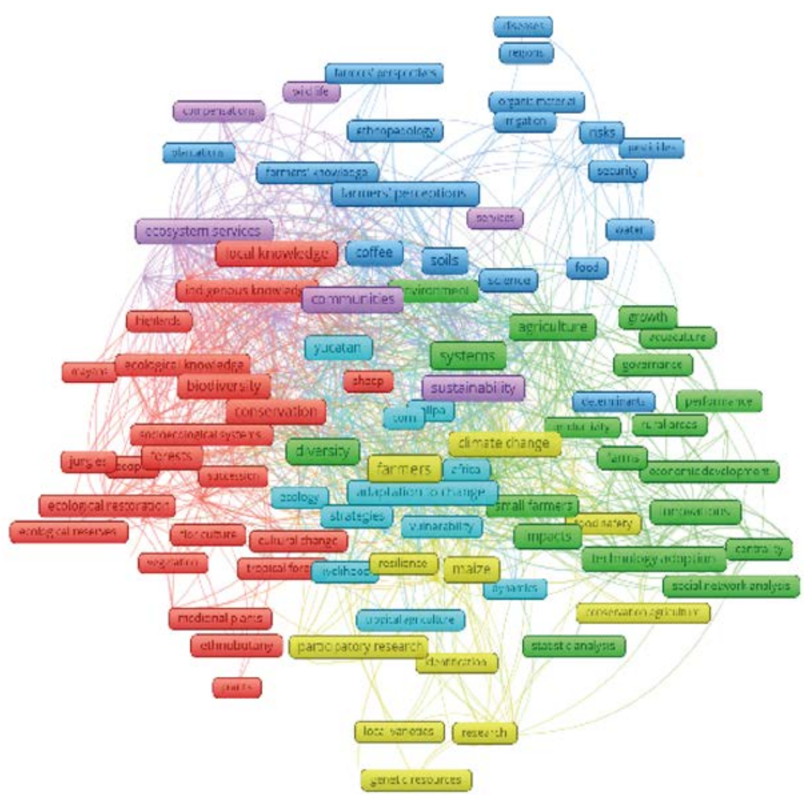


Figure 1. Research themes on peasant knowledge in Mexico in mainstream journals (1991-2023). Source: Prepared by authors.

Figure 2. Map of research trends on peasant knowledge in Mexico in mainstream journals (1991-2023). Source: Prepared by the authors.

Current situation of the technical-productive, managerial and entrepreneurial management of small-scale agricultural producers (peasants) in the state of Querétaro

Presently, the country is immersed in economic uncertainty, resulting from the pandemic, political and macroeconomic changes, among others, having as a result unemployment, economic backwardness, impoverishing of the rural population, etc. This makes it even more important to mobilize and activate strategies for initiatives by the public and private sectors for the strengthening of rural economic units (REUs), which could allow generating employment in their communities and seeking the rootedness of the rural population.

Next, a series of problems identified in the field are listed (Table 1), through the different projects carried out with small-scale agricultural producers (peasants) in the state of Querétaro and which match the problems presented in the National Agriculture and Livestock Production Survey (INEGI and SADER, 2019), with the aim of addressing the causes and generating productive, economic, social results, among others, to forge sustainable entrepreneurial growth and consolidation.

Thematic proposal for continuous training of small-scale agricultural producers (peasants)

Table 1 presents the main problems identified in agricultural production of the rural economic units. According to each problem identified, it is necessary to establish the knowledge management that allows addressing, resolving or minimizing these problems. In general, training will be directed toward the objective of developing capacities, abilities,

Table 1. Problems identified in the rural economic units.

<table>
<tr><th>Thematic</th><th>Specific problems</th><th>General problems</th></tr>
<tr><td rowspan="3">Technical-productive</td><td>✓ Deficiency in good agricultural practices</td><td rowspan="9">✓ Low educational level
✓ Low income
✓ Low investment capacity for continuous training
✓ Lack of diagnostics that take into account specific needs in terms of knowledge, culture, etc.
✓ Demotivation due to lack of positive results
✓ Poverty and low quality of life
✓ Fear of failure
✓ Conformity with the results obtained
✓ Weaknesses in the methodological approach to knowledge and technology transfer by the extension sector
✓ Resistance to change
✓ Insufficient infrastructure and resources
✓ Generational change with no interest in the rural sector
✓ Migration to domestic and international urban areas
✓ Lack of market knowledge
✓ Low level of profits
✓ Marginalization and social inequality
✓ Among others</td></tr>
<tr><td>✓ Low use of technological innovations</td></tr>
<tr><td>✓ Lack of capacity for transformation and generation of added value</td></tr>
<tr><td rowspan="5">Management</td><td>✓ Lack of strategic management</td></tr>
<tr><td>✓ Lack of entrepreneurial vision</td></tr>
<tr><td>✓ Low market integration</td></tr>
<tr><td>✓ Lack of interest in business development</td></tr>
<tr><td>✓ Lack of leadership and direction</td></tr>
<tr><td>Entrepreneurship</td><td>✓ Low level of associativity in agricultural production regions</td></tr>
</table>

and competences in technical-productive, managerial, and entrepreneurial themes in agricultural producers.

Learning-teaching should be conducted according to the specific needs identified by region, productive chain, socioeconomic characteristics, and level of investment, among other aspects, through courses and workshops; also, through the establishment of demonstrative platforms and to explain good agricultural practices in an applied manner. Likewise, attendance to agricultural exhibitions and fairs should be encouraged, for farmers to be able to interact and exchange knowledge and experiences about agricultural production in their plots. Next, each theme will be addressed from the different perspectives of knowledge management.

Technical-productive

It is important to prioritize for the implementation of good technical-productive management to allow the strengthening of REUs. This management has been connected with the prior use of good agricultural practices by the producer, which greatly influences the success of their implementation. Garrido-Rubiano *et al.* (2017) report that producers with a better use of good agricultural practices present a greater interest for acquiring technical knowledge. Likewise, the Food and Agriculture Organization of the United Nations (FAO), suggests the need to incorporate innovations in agricultural production to increase productivity and profitability, in order to allow improving the quality of life of the rural population (FAO, 2014). From this the importance of proposing themes that allow managing the technical-productive knowledge of small-scale agricultural producers (Table 2).

Table 2. Technical-productive themes.

Good agricultural practices	✓ Adoption of technological packages for agronomic management, nutrition, harvesting, postharvest handling, and packaging.
	✓ Good agricultural practices and good management practices
	✓ Establishment of the agricultural plot
	✓ Definition of the size of the agricultural parcel
	✓ Procurement of supplies
	✓ Soil preparation and application of cultural tillage
	✓ Establishment of the agricultural crop
	✓ Cultural practices for crop maintenance
	✓ Estimated crop yield per planted area
	✓ Organic agriculture
	✓ Pest/disease management and prevention
	✓ Use of residues and harvest surpluses
Technological innovations	✓ Efficient use of agricultural irrigation and water-saving techniques
	✓ Use of renewable energies
	✓ Use of rainwater harvesting and recirculation systems
	✓ Use of highly productive vegetative material
	✓ Using big data for decision-making in agriculture
	✓ Use of technologies for the sustainable management of the rural economic unit.
Value added	✓ Alternatives for the generation of added value
	✓ Importance of physical, chemical, nutritional, and microbiological quality.
	✓ Quality specifications (size, color, texture, appearance, odor)
	✓ Safety in the production, storage, and distribution chain
	✓ Cooling in the storage and distribution chain
	✓ Agro-ecological products
	✓ Strategies for packaging, packing and wrapping
	✓ Labeling, branding, and corporate image
	✓ Certifications and regulations

Source: Prepared by the authors.

Development of managerial capacities that potentiate the economy of agricultural producers

The training strategy proposal in agribusiness themes can help in the management of rural economic units, through the development of the managerial knowledge, capacities and abilities of farmers, in order to tend toward the development of a fair, profitable farmland and which result in economic benefits of the rural zone. Avendaño-Ruiz *et al.* (2017), in a study on technological innovations in the vegetable sector in northwestern Mexico, make evident the importance in productive units of connecting good agricultural practices, integrated management of pests, and adoption of international standards of safety, innocuousness and food quality with administrative, marketing and commercial elements, to gain access to specialized markets such as that of exports. For their part, Mendoza-Velázquez and Pastrana-López (2021) highlight the importance of knowledge in themes of financing, agriculture and livestock insurance and contingency funds that could cover the potential economic losses of agricultural production in presence of price fluctuation, climatological problems, among other contingencies; also, generating willingness in important themes such as investment in the improvement of lands, modernization and establishment of infrastructure, which if it is not taken into account leads to a high risk for agricultural production. Considering the aforementioned, this study suggests taking into account the following managerial themes in the knowledge management process that would allow growth in the REUs (Table 3).

Table 3. Managerial themes.

Strategic management	✓ Planning, organization, direction and control
	✓ Project management: Suppliers, risks, uncertainty
	✓ Organizational development and human resources
	✓ Business vision
	✓ Strategic planning
	✓ Development of administrative systems
	✓ Organization: Legal figures for regularization
	✓ Management of financial operations
	✓ Accounting management (budget, investment, cost and profit)
	✓ Management of the tax regime for the primary sector
	✓ Digital media management for business positioning
Agribusiness market management	✓ Contract farming and crop insurance
	✓ Market trends
	✓ Market studies for new products and by-products
	✓ Identification of value and market networks
	✓ Marketing channels
	✓ Web positioning (social networks, official website)
	✓ Marketing schemes (producer - final consumer)
	✓ Market: Opportunities, sales, production, type of product, prices, etc.
	✓ Pricing
	✓ Consumer segmentation and profiling
	✓ Product differentiation
Agribusiness	✓ Corporate Social Responsibility
	✓ Current status of local, regional, national and international production
	✓ Dynamics in recent years (volume, area sown, economic value, etc.)
	✓ Import and export statistics (quantity and economic value)
	✓ Identification of the main key players in the value chain
	✓ Integration into the production chain
	✓ Generation of products from the transformation of raw materials
	✓ Classification by standards, certifications
	✓ Access to national and international markets through digital marketplaces

Source: Prepared by authors.

Training theme for training of leading agricultural producers with entrepreneurial vision

In the study proposed by the Economic Commission for Latin America and the Caribbean (ECLAC), the Food and Agriculture Organization of the United Nations (FAO) and the Inter-American Institute for Cooperation on Agriculture (IICA) (CEPAL, FAO and IICA, 2021), there was agreement in the importance of taking into account entrepreneurship and cooperativism as agents of change, in order to ease social rootedness, resource sustainability, as well as agriculture and livestock production and trade, territory connectivity, and the necessary infrastructure to improve the efficiency of productive and commercial processes, which will strengthen the social economy. Meanwhile, Jurado Paz (2022) manifested that there is a disparity on training in entrepreneurial education themes in rural environments, where they rarely receive training in themes of solidary economy, cooperativism, inclusive businesses, sustainable rural development, which could contribute to the sustainable economic, social and cultural growth of the territories. According to the information gathered, it will be of great importance to incorporate entrepreneurship in the education of small-scale agricultural producers, in order to generate through knowledge management a change in mentality, create a business profile, and implement the entrepreneurial culture to make agricultural production attractive (Table 4).

Impacts to be obtained from training of small-scale agricultural producers and strengthening of rural economic units

The implementation strategies of technical-productive, managerial and entrepreneurial capacities in agricultural producers could favor knowledge management, thus generating economic, social and environmental impacts (Figure 3).

Next, the possible economic, social and environmental impacts are presented, which could provide knowledge management to small-scale agricultural producers through a thematic proposal for the strengthening of rural economic units.

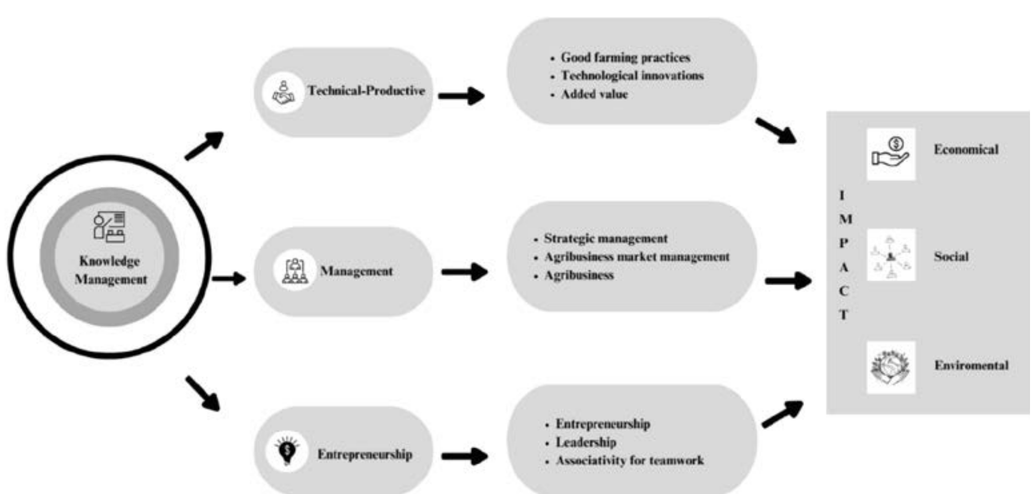


Figure 3. Plan for knowledge management of small-scale agricultural producers (peasants).
Source: Prepared by authors.

Table 4. Entrepreneurial themes.

Entrepreneurship	✓ Agro-entrepreneurship and formulation of productive projects
	✓ Investment mechanisms and access to public and private financing
	✓ Strategic networks for business generation
	✓ Entrepreneurial culture
	✓ Characteristics and profile of the entrepreneur
	✓ Change management
	✓ Entrepreneurial ecosystem
	✓ Failure vs Success
Leadership	✓ Address
	✓ Assertive communication
	✓ Negotiation
	✓ Business Vision
	✓ Collaboration
	✓ Teamwork
	✓ Resilience
	✓ Continuous improvement
Partnership for teamwork	✓ Network development for teamwork
	✓ Actors and roles in a strategic network
	✓ Building trust and creating a sense of belonging
	✓ Organization and operation of a strategic network
	✓ Motivation, responsibility, and mutual commitment
	✓ Establishment of common objectives and decision-making for community well-being.
	✓ Creation of a sense of belonging

Source: Prepared by the authors.

CONCLUSIONS

There is a great opportunity through knowledge management to elevate the capacities, knowledge and abilities of small-scale agricultural producers (peasants) in the state of Querétaro in technical-productive, managerial and entrepreneurial themes, which will generate economic, social and environmental impacts in benefit of this rural sector. Likewise, the articulation of the agricultural sector should be encouraged, in its different levels and links, with educational institutions (universities, technological institutes, centers for agricultural and livestock technological education, among others), research centers, and institutions related to the sector in the region. Strategies for transference of knowledge and technology could be established with these, to address the basic needs of training and to foster the creation of strategic projects and the generation of added value, which could transform rural economic units into productive and competitive units, to allow generating better living conditions, more sources of employment, and an adequate regional economic development.

Table 5. Economic, social and environmental impacts that the project's implementation could offer.

Economic	Social	Environmental
✓ Promote the strengthening of small agricultural producers in the state of Querétaro. ✓ It will contribute to rural development. ✓ Organize producers into formal groups that can have negotiating capacity. ✓ It will increase GDP participation through the dynamics of the economic circuits related to the agricultural sector in the state of Querétaro. ✓ It will promote associativity for more efficient commercialization. ✓ It will provide agricultural producers with tools that will enable them to create strategic alliances and formally organize and/or strengthen themselves as productive associations to market their products competitively. ✓ Strengthen rural economic units to reduce costs and gain competitiveness. ✓ You will have access to information on prices, technological alternatives, services, and credits to producers.	✓ Promote the creation of new sources of employment within the area of influence, improving the social and economic environment of the region where the productive projects are established. ✓ It will allow the integration of new trained producers who will be influenced by the results of this project, diversifying their productive offer. ✓ It will encourage regional and national production of food products for self-consumption and will help the food sovereignty of the population of Querétaro and Mexico. ✓ Promote the associativity of agricultural producers by developing negotiation, administrative, and liaison skills with different actors (public, private, and civil society sectors). ✓ Provide cooperation for social and community development in the state of Querétaro. ✓ It will favor the creation of productive projects that help improve the quality of life of its population and the growth of rural communities. ✓ It will encourage the rural population's interest in entrepreneurship, reducing the migration phenomenon. ✓ It will offer socioeconomic importance due to the number of jobs it will generate and benefit producers, suppliers, transporters, agricultural and industrial laborers, and consumers, which is why the business consolidation of this economic sector is of great importance. ✓ It will strengthen self-employment in the regions where agricultural production plots and agroindustries are located, thanks to the strengthening of rural economic units. ✓ It will contribute to improving the quality of life of producers, employees, and workers through access to well-paid jobs with good conditions. ✓ Increase the level of education through the training of agricultural producers.	✓ Use natural resources in a sustainable manner in agricultural crops with rational use of water, soil, and environment to minimize the damage caused by the misuse of natural resources. ✓ Preserve ecosystems by employing measures to reduce the impacts generated by productive activities. ✓ It will achieve the sustainable development of the Mexican Bajío Region by promoting training and environmental transformation processes that lead to the sustained and sustainable improvement of the rural environment. ✓ It will use new agronomic technologies for production optimization. ✓ Perform maintenance and remediation for the sustainable use of soils.

Source: Prepared by the authors.

ACKNOWLEDGEMENTS

The authors wish to express their most sincere gratitude to the FONDEC-UAQ, for funding for the project "Strategic connection and transference of knowledge and technology to the agricultural sector in the municipality of San Juan del Río, Querétaro, Mexico", of which this study is part.

REFERENCES

Aguilar-Stoen, M., Moe, S. R., & Lucia Camargo-Ricalde, S. (2009). Home Gardens Sustain Crop Diversity and Improve Farm Resilience in Candelaria Loxicha, Oaxaca, Mexico. *HUMAN ECOLOGY, 37*(1), 55–77. https://doi.org/10.1007/s10745-008-9197-y

Akkaya, D., Bimpikis, K., & Lee, H. (2021). Government interventions to promote agricultural innovation. *MANUFACTURING & SERVICE OPERATIONS MANAGEMENT, 23*(2), 437-452. https://doi.org/10.1287/msom.2019.0834

Alavi, M., & Leidner, D. E. (2002). Sistemas de gestión del conocimiento: cuestiones, retos y beneficios. In Sistemas de gestión del conocimiento: teoría y práctica (pp. 17-40). Thomson-Paraninfo.

Arteaga-Coello, H. S., Intriago-Manzaba, D. M., & Mendoza-García, K. A. (2016). La ciencia de la administración de empresas. *DOMINIO DE LAS CIENCIAS, 2*(4), 421-431. http://dx.doi.org/10.23857/dc.v2i4.265

Avendaño-Ruiz, B. D., Hernández-Alcantar, M. L., & Martínez-Carrasco-Pleite F. (2017). Innovaciones tecnológicas en el sector hortícola del noroeste de México: rapidez de adopción y análisis de redes de difusión. *CORPOICA CIENCIA Y TECNOLOGÍA AGROPECUARIA, 18*(3), 495-511 https://doi.org/10.21930/rcta.vol18_num3_art:740

Barton, D., Benjamin, T., Cerdan, C., DeClerck, F., Madsen, A., Rusch, G., Salazar, A., Sanchez, D., & Villanueva, C. (2016). Assessing ecosystem services from multifunctional trees in pastures using Bayesian belief networks. *ECOSYSTEM SERVICES*, 18, 165–174. https://doi.org/10.1016/j.ecoser.2016.03.002

Bautista, F., Barajas, A., & Alcalá-de Jesús, M. (2019). Peasant knowledge about the soils of the Zicuiran-Infiernillo Biosphere Reserve. *REVISTA CHAPINGO SERIE CIENCIAS FORESTALES Y DEL AMBIENTE, 25*(3), 369–381. https://doi.org/10.5154/r.rchscfa.2018.02.019

Barragán-Ocaña, A., & del-Valle-Rivera, M. C. (2016). Rural development and environmental protection through the use of biofertilizers in agriculture: An alternative for underdeveloped countries? *TECHNOLOGY IN SOCIETY*, 46, 90–99. https://doi.org/10.1016/j.techsoc.2016.06.001

Beltrán-Rodríguez, L., Ortiz-Sánchez, A., Mariano, N. A., Maldonado-Almanza, B., & Reyes-García, V. (2014). Factors affecting ethnobotanical knowledge in a mestizo community of the Sierra de Huautla Biosphere Reserve, Mexico. *JOURNAL OF ETHNOBIOLOGY AND ETHNOMEDICINE*, 10, 14. https://doi.org/10.1186/1746-4269-10-14

Benz, B., Perales, H., & Brush, S. (2007). Tzeltal and Tzotzil farmer knowledge and maize diversity in Chiapas, Mexico. *CURRENT ANTHROPOLOGY, 48*(2), 289–300. https://doi.org/10.1086/512986

Berget, C., Duran, E., & Bray, D. B. (2015). Participatory Restoration of Degraded Agricultural Areas Invaded by Bracken Fern (*Pteridium aquilinum*) and Conservation in the Chinantla Region, Oaxaca, Mexico. *HUMAN ECOLOGY, 43*(4), 547–558. https://doi.org/10.1007/s10745-015-9762-0

Bermeo, A., Couturier, S., & Galeana Pizana, M. (2014). Conservation of traditional smallholder cultivation systems in indigenous territories: Mapping land availability for milpa cultivation in the Huasteca Poblana, Mexico. *APPLIED GEOGRAPHY*, 53, 299–310. https://doi.org/10.1016/j.apgeog.2014.06.003

Castellanos, E. J., Tucker, C., Eakin, H., Morales, H., Barrera, J. F., & Diaz, R. (2013). Assessing the adaptation strategies of farmers facing multiple stressors: Lessons from the Coffee and Global Changes project in Mesoamerica. *ENVIRONMENTAL SCIENCE & POLICY*, 26, 19–28. https://doi.org/10.1016/j.envsci.2012.07.003

Carson, K. I. (2018). Agricultural training and the labour productivity challenge. *International Journal of Agricultural Management, 6*(1029-2019-929), 131-133. 10.22004/ag.econ.287297

Camacho-Villa, T. C., Martínez-Cruz, T. E., Ramirez-Lopez, A., Hoil-Tzuc, M., & Teran-Contreras, S. (2021). Mayan Traditional Knowledge on Weather Forecasting: Who Contributes to Whom in Coping With Climate Change? *FRONTIERS IN SUSTAINABLE FOOD SYSTEMS*, 5, 618453. https://doi.org/10.3389/fsufs.2021.618453

Castillo, X., Etchevers, J., Aguirre, A., & Hidalgo, C. (2021). Peasant management of horti-floristic system: case study. *AGROCIENCIA, 55*(2), 159–176. https://doi.org/10.47163/agrociencia.v55i2.2393

CEPAL, FAO e IICA (2021). Perspectivas de la Agricultura y del Desarrollo Rural en las Américas: una mirada hacia América Latina y el Caribe 2021-2022 /. – San José, C.R.: IICA. https://repositorio.cepal.org/bitstream/handle/11362/47208/1/CEPAL-FAO21-22_es.pdf

Charcas S, H., Aguirre R, J. R., Antonio Reyes-Agüero, J., & Martin Duran-García, H. (2010). Runoff agriculture in the highlands of San Luis Potosi state, Mexico. *INTERCIENCIA, 35*(10), 716–722.

Contreras-Medina, D. I., Medina-Cuellar, S., & Rodríguez-García, J. (2022). Roadmapping 5.0 Technologies in Agriculture: A Technological Proposal for Developing the Coffee Plant Centered on Indigenous Producers' Requirements from Mexico, via Knowledge Management. *PLANTS-BASEL, 11*(11). https://doi.org/10.3390/plants11111502

Contreras-Medina, D. I., Miguel Contreras-Medina, L., Pardo-Núñez, J., Alberto Olvera-Vargas, L., & Mario Rodríguez-Peralta, C. (2020). Roadmapping as a Driver for Knowledge Creation: A Proposal for Improving Sustainable Practices in the Coffee Supply Chain from Chiapas, Mexico, Using Emerging Technologies. *SUSTAINABILITY, 12*(14), 5817. https://doi.org/10.3390/su12145817

Deschamps-Solorzano, L., & Escamilla-Caamal, G. (2010). Hacia la consolidación de un sistema mexicano de innovación agroalimentaria. Instituto Interamericano de Cooperación para la Agricultura (IICA). https://repositorio.iica.int/handle/11324/19598

de Lourdes Maldonado-Mendez, M., Luis Romo-Lozano, J., & Ismael Monterroso-Rivas, A. (2022). Determinant Indicators for Assessing the Adaptive Capacity of Agricultural Producers to Climate Change. *ATMOSPHERE, 13*(7), 1114. https://doi.org/10.3390/atmos13071114

Falkowski, T., Chankin, A., Diemont, S., & Pedian, R. (2019). More than just corn and calories: A comprehensive assessment of the yield and nutritional content of a traditional Lacandon Maya milpa. *FOOD SECURITY, 11*(2), 389–404. https://doi.org/10.1007/s12571-019-00901-6

FAO (2014). El estado mundial de la agricultura y la alimentación. Roma, Italia: FAO.

FAO y SAGARPA. (2013). Propuestas de políticas públicas para el desarrollo del sector rural y pesquero (SRP) en México", México. https://www.agricultura.gob.mx/sites/default/files/sagarpa/documento/2019/01/28/1608/01022019-informe-final-propuesta-de-politicas-publicas-para-el-desarrollo-del-sector-rural-y.pdf

Flores López, J. G., Ochoa Jiménez, S., & Jacobo Hernández, C. A. (2020). Knowledge Management and Innovation in Agricultural Organizations: An Empirical Study in the Rural Sector of Northwest Mexico. *CUADERNOS DE DESARROLLO RURAL, 17*(86). https://doi.org/10.11144/Javeriana.cdr17.kmia

Flores-Silva, A., Cuevas-Guzman, R., Baptista, G., Olvera-Vargas, M., & Mariaca-Mendez, R. (2021). Dynamic Edible Plant Theoretical Knowledge in a Changing Western Mexican Rural Community. *JOURNAL OF ETHNOBIOLOGY, 41*(4), 465–480. https://doi.org/10.2993/0278-0771-41.4.465

García-Barrios, L., Cruz-Morales, J., Vandermeer, J., & Perfecto, I. (2017). The Azteca Chess experience: Learning how to share concepts of ecological complexity with small coffee farmers. *ECOLOGY AND SOCIETY, 22*(2). https://doi.org/10.5751/ES-09184-220237

Garrido-Rubiano, MF., Martínez-Medrano, JC., Martínez-Bautista, H., Granados-Carvajal, RE., & Rendón-Medel R. (2017). Pequeños productores de maíz en el Caribe colombiano: estudio de sus atributos y prácticas agrícolas. *Corpoica Ciencia y Tecnología Agropecuaria. 18*(1):7-23 http://dx.doi.org/10.21930/rcta.vol18_num1_art:556

Heinze, A., Bongers, F., Marcial, N., Barrios, L., & Kuyper, T. (2022). Farm diversity and fine scales matter in the assessment of ecosystem services and land use scenarios. *AGRICULTURAL SYSTEMS*, 196. https://doi.org/10.1016/j.agsy.2021.103329

Hernández-Ramos, M. A., Guevara-Hernández, F., Luis Basterrechea-Bermejo, J., Coutino-Estrada, B., La O-Arias, M. A., & Pinto-Ruiz, R. (2020). Diversity and conservation of local maize from La Frailesca, Chiapas, Mexico. *REVISTA FITOTECNIA MEXICANA, 43*(4), 471–479.

Ikuemonisan, E. S., Abass, A. B., Feleke, S., & Ajibefun, I. (2022). Influence of Agricultural Degree Programme environment on career in agribusiness among college students in Nigeria. *Journal of Agriculture and Food Research*, 7, 100256. https://doi.org/10.1016/j.jafr.2021.100256

INEGI y SADER. (2019). Encuesta Nacional Agropecuaria. https://www.inegi.org.mx/contenidos/programas/ena/2019/doc/rrdp_ena2019.pdf

Jiménez-Ferrer, G., Pérez-López, H., Soto-Pinto, L., Nahed-Toral, J., Hernández-López, L., & Carmona, J. (2007). Livestock, nutritive value and local knowledge of fodder trees in fragment landscapes in Chiapas, Mexico. *INTERCIENCIA, 32*(4), 274–280.

Jurado-Paz, I. (2022). Emprendimiento rural como estrategia de desarrollo territorial: una revisión documental. *Económicas CUC, 43*(1), 257–280. https://doi.org/10.17981/econcuc.43.1.2022.Org.7

Lastiri-Hernández, M. A., Álvarez-Bernal, D., Moncayo-Estrada, R., Cruz-Cárdenas, G., & Silva Garcia, J. T. (2021). Adoption of phytodesalination as a sustainable agricultural practice for improving the productivity of saline soils. *ENVIRONMENT DEVELOPMENT AND SUSTAINABILITY, 23*(6), 8798–8814. https://doi.org/10.1007/s10668-020-00995-5

Martinez-Herrera, G., Trejo, I., Moreno-Calles, A., Fernanda de Alba-Navarro, M., & Martinez-Balleste, A. (2021). Knowing the Clouds through the Land: Perceptions of Changes in Climate through Agricultural Practices in Two Nahua Indigenous Communities. *JOURNAL OF ETHNOBIOLOGY, 41*(3), 349–367. https://doi.org/10.2993/0278-0771-41.3.849

Mendoza-Velázquez, A., Pastrana-López, C. (2021). La cobertura de riesgos agrícolas en México: una propuesta de fondo contingente para los estados. *Agricultura, Sociedad y Desarrollo, 18*(2), 279-304. Nonaka, I., Takeuchi, H. (1999). La organización creadora de conocimiento. Cómo las compañías japonesas crean la dinámica de la innovación. Oxford University Press, México.

Orantes-García, C., Moreno-Moreno, R., Caballero-Roque, A., & Farrera-Sarmiento, O. (2018). Useful plants in traditional medicine of peasant and indigenous communities of Selva Zoque, Chiapas, Mexico. *BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMATICAS, 17*(5), 503–521.

Parraguez-Vergara, E., Contreras, B., Clavijo, N., Villegas, V., Paucar, N., & Ther, F. (2018). Does indigenous and campesino traditional agriculture have anything tocontribute to food sovereignty in Latin America? Evidence from Chile, Peru, Ecuador, Colombia, Guatemala and Mexico. *INTERNATIONAL JOURNAL OF AGRICULTURAL SUSTAINABILITY, 16*(4–5), 326–341. https://doi.org/10.1080/14735903.2018.1489361

Rendon-Sandoval, F., Casas, A., Sinco-Ramos, P., Garcia-Frapolli, E., & Moreno-Calles, A. (2021). Peasants' Motivations to Maintain Vegetation of Tropical Dry Forests in Traditional Agroforestry Systems from Cuicatlan, Oaxaca, Mexico. *FRONTIERS IN ENVIRONMENTAL SCIENCE*, 9. https://doi.org/10.3389/fenvs.2021.682207

Reséndiz-Paz, M., Gutiérrez-Castorena, M., Gutierrez-Castorena, E., Ortiz-Solorio, C., Cajuste-Bontempts, L., & Sánchez-Gúzman, P. (2013). Local soil knowledge and management of Anthrosols: A case study in Teoloyucan, Mexico. *GEODERMA*, 193, 41–51. https://doi.org/10.1016/j.geoderma.2012.09.004

Rodríguez, J., Pena Olvera, B. V., Muñoz, A. G., Martínez Corona, B., Manzo, F., & Salazar Liendo, L. (2007). *In situ* recovery of "poblano" pepper in Puebla, Mexico. *REVISTA FITOTECNIA MEXICANA, 30*(1), 25–32.

SADER, (2018). ¿Cómo beneficia la agricultura a las familias mexicanas? https://www.gob.mx/agricultura/es/articulos/como-beneficia-la-agricultura-a-las-familias-mexicanas#:~:text=As%C3%AD%20pues%2C%20la%20agricultura%20beneficia,de%20cultura%20y%20tradici%C3%B3n%20gastron%C3%B3mica.

SADER y FAO. (2014). Diagnóstico del sector rural y pesquero de México 2012. https://www.agricultura.gob.mx/sites/default/files/sagarpa/document/2019/01/28/1608/01022019-1-diagnostico-del-sector-rural-y-pesquero.pdf

Salager-Zeyer F. 2015. Peripheral scholarly journals: From locality to globality. *Iberica* 30: 15–36. https://www.redalyc.org/pdf/2870/287042542002.pdf

Sánchez-Gervacio, B., Legorreta-Soberanis, J., Bedolla-Solano, R., Rosas-Acevedo, J., Valencia-Quintana, R., Juarez-López, A., & Paredes-Solís, S. (2021). Impact of a Non-Formal Environmental Education Program on safe handling of pesticides among Mexican subsistence farmers: A participatory pilot study. *HUMAN AND ECOLOGICAL RISK ASSESSMENT, 27*(6), 1636–1654. https://doi.org/10.1080/10807039.2020.1868285

Spielman, D. J., & Birner, R. (2008). How innovative is your agriculture?: Using innovation indicators and benchmarks to strengthen national agricultural innovation systems. Washington, DC, USA: World Bank. https://documents1.worldbank.org/curated/en/696461468316131075/pdf/448700NWP0Box327419B01PUBLIC10ARD0no1041.pdf

Suarez, A., Williams-Linera, G., Trejo, C., Valdez-Hernández, J., Cetina-Álcala, V., & Vibrans, H. (2012). Local knowledge helps select species for forest restoration in a tropical dry forest of central Veracruz, Mexico. *AGROFORESTRY SYSTEMS, 85*(1), 35–55. https://doi.org/10.1007/s10457-011-9437-9

Subercaseaux, D., Moreno-Calles, A. I., Astier, M., & de Jesus Hernandez L., J. (2021). Emerging Agro-Rural Complexities in Occident Mexico: Approach from Sustainability Science and Transdisciplinarity. *SUSTAINABILITY, 13*(6), 3257. https://doi.org/10.3390/su13063257

Tijssen RJW, Van Raan AFJ. 1994. Mapping changes in science and technology: Bibliometric cooccurrence analysis of the R&D literature. *Evaluation Review 18*(1): 98–115. https://doi.org/10.1177/0193841X9401800110

Toillier, A., Guillonnet, R., Bucciarelli, M., & Hawkins, R. (2021). Developing capacities for agricultural innovation systems: lessons from implementing a common framework in eight countries. Rome, FAO and Paris, Agrinatura. https://doi.org/10.4060/cb1251en

Torres-Guerrero, C., Gutiérrez-Castorena, M., Ortiz-Solorio, C., Herrera, J., Gutiérrez-Castorena, E., & Etchevers, J. (2019). Rate of root decomposition of maize at plots and regions using local land knowledge and technical analysis of soils. *AGROCIENCIA, 53*(5), 661–680.

Trejo, L., Velazquez, M., Vallejo, M., & Montoya, A. (2022). Differentiating Knowledge of Agave Landraces, Uses, and Management in Nanacamilpa, Tlaxcala. *JOURNAL OF ETHNOBIOLOGY, 42*(1), 31–50. https://doi.org/10.2993/0278-0771-42.1.31

Valencia, V., West, P., Sterling, E., Garcia-Barrios, L., & Naeem, S. (2015). The use of farmers' knowledge in coffee agroforestry management: Implications for the conservation of tree biodiversity. *ECOSPHERE, 6*(7). https://doi.org/10.1890/ES14-00428.1

van Eck NJ, Waltman L. 2010. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 84: 523–538. https://doi.org/10.1007/s11192-009-0146-3

Villarroel-Molina, O., De-Pablos-Heredero, C., Barba, C., Rangel, J., & García, A. (2022). Does Gender Impact Technology Adoption in Dual-Purpose Cattle in Mexico? *ANIMALS, 12*(22), 3194. https://doi.org/10.3390/ani12223194

Wesseler, J., Smart, R. D., Thomson, J., & Zilberman, D. (2017). Foregone benefits of important food crop improvements in Sub-Saharan Africa. *PLoS One, 12*(7), e0181353. https://doi.org/10.1371/journal.pone.0181353

Colegio de Postgraduados

The use of native yeasts to improve the organoleptic characteristics and yield of artisanal mezcal

Carrasco-López, Amairany[1]; Romero-Ramírez, Yanet[1]; López-Barrera, José A.[2]; Moreno-Terrazas-Casildo, Rubén D.[3]; Ramírez-Peralta, Arturo[4]; Patricio-Hernández, Alberto[1]; Forero-Forero, Angela V.[5]; Toribio-Jiménez, Jeiry[1*]

1 Universidad Autónoma de Guerrero, Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Facultad de Ciencias Químico-Biológicas, Av. Lázaro Cárdenas s/n, Ciudad Universitaria, Apdo. Postal 39070, Chilpancingo, Guerrero, México.
2 Universidad de Guadalajara, Laboratorio de Fermentaciones y Biocatálisis, Marcelino García Barragán No. 1421, Guadalajara, Jalisco, México, C. P. 44430.
3 Universidad Iberoamericana, Departamento de Ingeniería y Ciencias Químicas, Prolongación Paseo de la Reforma 880, Ciudad de México, México.
4 Universidad Autónoma de Guerrero, Laboratorio de Patometabolismo, Facultad de Ciencias Químico-Biológicas, Av. Lázaro Cárdenas s/n, Ciudad Universitaria, Apdo. Postal 39070, Chilpancingo, Guerrero, México.
5 Universidad Nacional Autónoma de México Facultad de Ciencias, Av. Universidad 3000, Circuito Exterior S/N, Delegación Coyoacán, Ciudad Universitaria, Ciudad de México. México, C. P. 04510.
* Correspondence: jeiryjimenez2014@gmail.com

Citation: Carrasco-López, A., Romero-Ramírez, Y., López-Barrera, J. A., Moreno-Terrazas-Casildo, R. D., Ramírez-Peralta, A., Patricio-Hernández, A., Forero-Forero, A., V., & Toribio-Jiménez, J. (2024). The use of native yeasts to improve the organoleptic characteristics and yield of artisanal mezcal. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2755

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: November 24, 2023.
Accepted: February 15, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 167-176.

ABSTRACT
Objective: To assess the use of native yeasts on the yield and on the organoleptic characteristics of artisanal mezcal in Guerrero, Mexico.
Design/Methodology/Approach: Native yeasts from fermentation vats containing *Agave angustifolia* and *A. cupreata* were subjected to molecular isolation and identification in three regions of the state of Guerrero. A consortium of yeasts was designed and used to produce artisanal mezcal, using *A. cupreata* as a substrate. The experimental mezcal (with yeasts) and the control (without yeasts, only producer lore) were compared. Finally, the physicochemical and sensory characteristics of the final product were assessed at the Concours Mondial de Bruxelles 2020.
Results: *S. cerevisiae* A5GM, *T. delbrueckii* A6GM, *S. cerevisiae* A3GM, and *K. marxianus* A2GM were identified and used in a consortium that produced 46% more yield (8.3 kg L^{-1} compared to 10.5 kg L^{-1}). The mezcal produced with this consortium obtained the Grand Gold medal based on its sensory characteristics.
Study Limitations/Implications: To work with the producer in both systems with and without yeast.
Findings/Conclusions: The consortium improved the production yield by 26%, along with the sensory characteristics; therefore, the use of native yeasts is feasible and recommended for the improvement of processes in the production of artisanal mezcal from Guerrero.

Keywords: agave, artisanal mezcal, native yeasts, yields, sensory characteristics.

INTRODUCTION

Artisanal mezcal in Mexico is generally produced on a small scale in different states that are protected by the Denomination of Origin Mezcal (DOM), through the spontaneous fermentation of agave must (Torres-Velázquez *et al.*, 2022; Domínguez-Coronado *et al.*, 2021). However, this type of fermentation results in productions with varying organoleptic characteristics, which generates different yields per production (Aldrete-Tapia *et al.*, 2020).

The spontaneous fermentation of mezcal is carried out by microorganisms from the agave or the environment of the production system (Pérez-Hernández *et al.*, 2022). These specialized native microorganisms participate throughout the process and their different fermentative capacities can be modified by pH, type of substrate, and temperature (Alcázar-Valle *et al.*, 2019, Núñez-Guerrero *et al.*, 2019). Therefore, *mezcaleros* (mezcal producers) consider that the yields and organoleptic characteristics of the production of artisanal mezcal must be standardized and increased, using native yeasts from the agave must through starter cultures and defined production methodologies (Aldrete-Tapia *et al.*, 2020). The most important conventional yeasts are *S. cerevisiae* —which has a high tolerance to ethanol production— and non-*Saccharomyces* yeasts —due to their influence on the organoleptic composition of the drink, given their perfect adaptation to the environment and because they frequently are more numerous than *S. cerevisiae* (Aldrete-Tapia *et al.*, 2018, Nuñez-Guerrero *et al.*, 2019). The presence of non-*Saccharomyces* native yeasts in the must leads to a competition for nutrients (Alvarez-Ainza *et al.*, 2021). The diversity of native microbiota during fermentation has been described in different regions that produce agave distillates. The prevailing species was *S. cerevisiae*, followed by *Kluyveromyces marxianus*, *Zygosaccharomyces rouxii*, *Torulaspora delbrueckii*, *Pichia membranifaciens*, and other species (Aldrete-Tapia *et al.*, 2018; Pérez-Hernández *et al.*, 2022; Núñez-Guerrero *et al.*, 2019; Kirchmayr *et al.*, 2017; Alvarez-Ainza *et al.*, 2021; Vera-Guzmán *et al.*, 2018). *T. delbrueckii* produces a lower concentration of acetic acid than *S. cerevisiae*, which benefits alcoholic beverages, since a >0.8 g/L concentration could generate a vinegar odor (Ogawa *et al.*, 2022). Likewise, *K. marxianus* and *S. cerevisiae* can be found at the end of the fermentation stage, with similar levels of resistance to ethanol in the fermentation medium (Martínez-Estrada *et al.*, 2019). In addition, the microbiota in the most of the agave can be affected or favored by various factors such as: species, maturity, geographical location of the fields, factories, climatic conditions, and the artisanal production experience of each mezcalero (Lappa *et al.*, 2020; Ruiz-Teran *et al.*, 2019). The objective of this study was to assess the potential use of native yeasts in fermentation processes to increase the yield (kg) of agave per liter and the sensory quality of artisanal mezcal.

MATERIALS AND METHODS

Selection of native yeasts from agave must

Must was obtained from various fermentation stages in five mezcal factories of the following two municipalities: Eduardo Neri and Mochitlán. Tlanipatla (TI) in Eduardo Neri is located at −99.451667 longitude (dd) and +17.808056 latitude (dd). Mochitlán (Mo) in Mochitlán is located at −99.369167 longitude (dd) and +17.471389 latitude (dd).

To isolate and identify the yeasts most frequently found in the must, it was subjected to serial dilutions until a 1:1,000 ratio was reached; they were inoculated on yeast extract peptone dextrose agar (YPD) (Becton Dickinson, USA) and incubated at 30 °C for 48 h. Colonies were expressed in CFU/mL (Mambuscay *et al.*, 2013).

Amplification of the ITSI1-5.8S-ITSI2 region of the 5.8S rRNA gene

The total DNA of the yeast was extracted according to Querol *et al.* (1992). Subsequently, two variable regions that flank the 5.8S ribosomal RNA (rRNA) gene were amplified, using:

- 1 μL of DNA plus 24 μL of PCR master mix for a final volume of 25 μL: 50 mM ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) (White *et al.*, 1990; Suárez *et al.*, 2007),
- 2 μL of dNTP, 1X Taq Buffer with $(NH_4)_2SO_4$ (750 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20),
- 3 μL of $MgCl_2$, and
- 0.5 μL of Taq DNA Polymerase (Thermo Scientific, USA).

The amplification was carried out in a GeneAmp® PCR 2400 thermal cycler (Applied Biosystems) following the initial denaturation: 94 °C for 5 min, 94 °C for 1 min (30 cycles), 55 °C for 1 min, and 72 °C for 2 min, with a final extension of 72 °C for 10 min. PCR outputs were analyzed by electrophoresis in 1% agarose gel, stained with ethidium bromide, and visualized and recorded with a GVM30UV transilluminator (Syngene, UK). Finally, the size of the bands was analyzed using a Generuler 1Kb molecular weight marker (Fermentas, USA). To obtain the sequences, the PCR outputs were sent to the Institute of Biotechnology of UNAM. Once the sequences were obtained, they were analyzed with the Chromas 2.6 software (Technelysium Pty. Ltd.). The results were input into the database of Blast program, in order to search for homology with reported yeast sequences. Finally, the identified species were subjected to a phylogenetic analysis in the Mega 7.1 software (Mega Ltd), using the neighbor-joining method based on the distance matrix specified by Jukes and Cantor (1969).

Fermentation stage

Obtaining raw materials

Four tons of cooked *Agave cupreata*, with 8 to 10 years of maturation, were used. They were harvested at the El Calvario community, municipality of Chilpancingo, Guerrero. Agave was cooked in a ground oven for 6 days. Once the agave hearts were cooked, they were fragmented into ≈15 cm pieces and the bagasse were obtained using a 13 Hp hammer mill (CIATEJ, 2014).

Pre-inoculum preparation

A single consortium of yeasts (A5GM, A6GM, A3GM, and A2GM) was formed and cultured for 48 h at 30 °C in a Sabouraud Dextrose Agar (SDA) growth medium (MCD-

Lab). Two pre-inocula were prepared using 20 L of *A. cupreata* juice with bagasse, in clean and new 20 L containers (one container per pre-inocula). They were adjusted to 6 °Bx with an AT-HHR2N optical refractometer (Twilight) (CIATEJ, 2014).

Inoculum preparation

The final volume of the inoculum for each experimental vat was 50 L of agave juice with consortium adjusted to 6 °Bx. The control vats were not inoculated. The containers with the inoculum were incubated for 12 h prior to the preparation of the vats (CIATEJ, 2014).

Fermentation vats

The control and the experimental samples were established in 1,000 L wooden fermentation vats with the following preparation quantities: 650 kg of cooked agave and 350 L of water in triplicate, adjusted to 12 °Brix following the conditions of the CIATEJ (2014). During the fermentation process, °Brix were measured every 12 h, with an AT-HHR2N optical refractometer (Twilight). The entire process was based on the *mezcalero* lore (characteristic smell of the must) and °Brix measurements (CIATEJ, 2014).

Distillation and product refinement processes

The distillation in each fermentation vat (with and without yeasts) was carried out when the °Brix reached 2.0. First, a 600 L stainless steel still was used and the distillate (ordinary) was received after it began to boil. Subsequently, at the end of the first distillation, the equipment was washed before a second distillation (refining) started. This stage was done over a low heat to avoid smoking or burning the product. The heads, hearts, and tails were separated. Finally, the mezcal was adjusted to 48° ABV (CIATEJ, 2014). During distillation, alcohol content was measured using a PCE-ALK handheld refractometer (PCE Instruments) (CIATEJ, 2014).

Mezcal quality analysis

One L of the control and one of experimental mezcal were sent to the Centro de Innovación y Desarrollo Agroalimentario de Michoacán (CIDAM) (accredited by the EMA, the Mexican accreditation agency), where the following analyses were according to the NOM-070-SCFI-2016 (Bebidas alcohólicas-Mezcal-Especificaciones) Official Mexican Standard: alcohol volume at 20 °C, dry extract, higher alcohols, methanol, furfural, aldehydes, and esters.

Sensory analysis

The sensory analysis was based on the participation of the control and experimental mezcals in the Spirits Selection by Concours Mondial de Bruxelles. The drinks were assessed by a panel of internationally renowned experts, with a total of 103 international judges of 28 nationalities. The number of samples tasted per session is deliberately restricted to 35; the samples were assessed anonymously, hiding the shape of the bottle and the name of the drink from the testers. The appearance, aroma, and flavor of the drink were assessed.

Three types of medals are awarded in this competition: Grand Gold Medal, Gold, and Silver Medals.

RESULTS AND DISCUSSION

Five manufacturing factories were visited in Tlanipatla (Eduardo Neri) and Mochitlán, Guerrero. A letter code (A to E) was assigned to each factory in the order of the sampling. Additionally, a number was assigned according to the vat from which the sample was taken; the said number included day of fermentation, place, and raw material data. Once the samples were sent to the research laboratory, the colony forming units were determined for each sample on YPD agar after 48 hours of incubation. The number of microorganisms decreased as the days of fermentation passed —*i.e.*, the samples corresponding from days 1 to 3 recorded more microorganisms (36×10^4 CFU/mL of yeasts per factory) than the samples from days 4 to 12 ($2\text{-}3\times10^3$ CFU/mL of yeasts per factory).

Based on the macro and microscopic morphology, close to 20 different yeast phenotypes were identified, with approximately 80 strains in the different factories. The 1% carbohydrate fermentation (glucose, fructose, maltose, and others) was carried out and eight representative profiles were obtained and selected for molecular identification (Table 1).

Some researchers report that, in the first days of fermentation, the most proliferate genera are non-conventional yeasts. After the first days, a supposed intolerance to ethanol and/or a nutritional limitation cause a drastic reduction in these genera (Kunkee, 1984); this phenomenon allows the growth of other species which have greater tolerance to ethanol —such as *Saccharomyces*, which is considered the main species responsible for alcoholic fermentations (Ribéreau 1985, Aldrete-Tapia *et al.*, 2018).

Only eight strains were selected from the two mezcal producing regions. They were subjected to a molecular identification and the analysis of the resulting sequences identified only four genera (Table 2): *Torulaspora delbrueckii*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, and *Pichia kluyveri*.

Table 1. Colony forming units during the fermentation process.

Agave angustifolia				***Agave cupreata***			
Sample		**CFU/mL**	**Time (h)**	**Sample**		**CFU/mL**	**Time (h)**
	A1	65.5×10^4	24		B1	29×10^4	24
	A2	14.5×10^4	72		B2	33×10^4	48
	A3	11.5×10^4	144	TL	B3	30.5×10^4	72
	D1	4.5×10^4	24		C1	10.5×10^4	168
AX	D2	12×10^4	96		C2	7×10^4	192
	E1	5.5×10^4	72		C3	3.5×10^4	216
	E2	13.5×10^4	96	MO	C4	3×10^4	240
					C5	8×10^4	264
					C6	6×10^4	96

CFU/mL results on average, (h): fermentation time at sample collection. Sampling location: AX: Axaxacualco, TL: Tlanipatla, -MO: Mochitlán.

Table 2. Molecular identificación of yeasts.

Isolation	5.8S rDNA	Maximum identified
A1GM	*Torulaspora delbrueckii*	99%
A2GM	*Kluyveromyces marxianus*	98%
A3GM	*Saccharomyces cerevisiae*	99%
A4GM	*Pichia kluyveri*	98%
A5GM	*Saccharomyces cerevisiae*	99%
A6GM	*Torulaspora delbrueckii*	98%
A7GM	*Torulaspora delbrueckii*	99%
A9GM	*Torulaspora delbrueckii*	99%

The following yeasts were used to form the consortium for the inoculation of fermentation vats: *S. cerevisiae* A5GM, *T. delbrueckii* A6GM, *S. cerevisiae* A3GM, and *K. marxianus* A2GM. During the fermentation process, the sugar consumption was observed and measured in °Brix. The initial (control and experimental) fermentation vats were adjusted to 12 °Brix. The consortium was formed according to the characteristics attributed to each species. *K. marxianus* has a higher growth rate and lower production of ethyl acetate and acetaldehyde in *A. cupreata*; it can also produce the same or more ethanol than *S. cerevisiae* (Alcázar-Valle *et al.*, 2019; Martínez-Estrada *et al.*, 2019). *T. delbrueckii* has been more studied in wines and it is known to increase the concentration of the thiol 4MMP (4-mercapto-4-methylpentan-2-one), which is related to floral and herbaceous aromas (notes of boxwood, cassis, or broom flower) (AGROVIN, 2020; Ogawa *et al.*, 2022). *S. cerevisiae* is the largest producer of esters and it is the yeast with the greatest resistance to alcohol levels; likewise, it is considered the largest producer of ethanol (Alcázar-Valle *et al.*, 2019; Pretorius, 2000; Gabriel *et al.*, 2012). Between 6 and 7 days, the fermentation process reached 2 °Brix in both conditions, with a temperature between 28 to 33 °C. Pérez-Hernández *et al.*, (2022) reported similar results, finding that the optimal fermentation condition is an optimized temperature of 32.5 °C, which favors the production of biomass.

Once the refinement was completed, the alcohol levels were adjusted to obtain the mezcal at 48% ABV. Finally, 123.5 L±0.7 and 156 L±1 of mezcal were obtained for the control and the experiment, respectively.

Once the total L of mezcal produced in both cases was determined, the calculations were made considering the amount of raw material and distillation —*i.e.*, the amount of previously cooked and weighed agave, related to the liters of total ethanol obtained—, in order to observe the yield (L) of mezcal kg^{-1} of agave used. On the one hand, for every 10.5 kg L^{-1} of agave in the control vats, one liter of mezcal was obtained; on the other hand, for every 8 kg L^{-1} of agave in the experimental vats, one liter of mezcal was obtained. In conclusion, the use of native yeasts results in a 26% increase in yield. The quality analysis was based on the NOM-070-SCFI-2016 (Bebidas alcohólicas-Mezcal-Especificaciones) Mexican official standard. Table 2 shows the parameters of both mezcals within the range established by the said standard. The physicochemical analysis and its results allowed the research team to proceed with the sensory and consumer acceptance analyses. For this purpose, the mezcal was entered into the Spirits Selection by Concours Mondial de Bruxelles, a competition where tasting judges assess drinks from different parts of the world. The 103 judges awarded this experimental mezcal the Grand Gold medal (Table 3).

According to the quality analysis (Table 4), the experimental mezcal showed values within the NOM-070-SCFI-2016 Mexican official standard; the concentrations of higher alcohols were better in the experimental unit (191.63 mg/100 mL) which is related to the sensory descriptors. The production of higher alcohols depends on the species of agave and yeast used (Vera *et al.*, 2009). For example, fermentation the juice with bagasse of *A. cupreata* generates a high production. Meanwhile, the three species used in the consortium can produce high concentrations of 1-propanols.

Meanwhile, only *T. delbrueckii* did not record a good isobutanol and amyl alcohol production, unlike *S. cerevisiae* and *K. marxianus* —the former of which provided the highest concentration. Regarding the aldehydes, low values were obtained in both mezcals (Alcázar-Valle, 2019). The medium was suitable for the growth and development of the yeasts, given the imbalance in the medium (*e.g.*, a lack of nutrients) resulting from a high concentration of aldehydes. This situation affects the activity of the yeasts.

Table 3. Mezcal quality analysis according to the NOM-070-SCFI-2016 (Alcoholic beverages-mezcal-specifications) .

Essay	Mezcal			Applied standard
	Control	Experimental		
Alcohol volumen at 20 °C	46.70	40.55	% alcohol/Vol. A 20 °C	NMX-V-013-NORMEX-2013
Dry extract	0.06	0.07	G L^{-1}	NMX-V-017-NORMEX-2014
Higher alcohols	19.61	191.63	mg 100^{-1} mL AA	NMX-V-005-NORMEX-2013
Methanol	218.53	164.05	mg 100^{-1} mL AA	NMX-V-005-NORMEX-2013
Furfural	3.70	2.94	mg 100^{-1} mL AA	NMX-V-004-NORMEX-2013
Aldehydes	<4.61	5.55	mg 100^{-1} mL AA	NMX-V-005-NORMEX-2013
Esters	15.97	45.58	mg 100^{-1} mL AA	NMX-V-005-NORMEX-2013

note: results obtained in the distillation process, reported as means and with standard deviation (S) in each of the tests.

Table 4. Tasting notes obtained in the competition Spirits Selection by Concours Mondial de Bruxelles.

Mezcal	Qualification	Tasting Note
Control	66/100	**Appearance**: Transparent **Nose**: Dried and exotic fruit with a citrus and smoky touch on the back. **Palate**: Spicy tone continues with interesting sweetness on the palate. Tasty but luxurious alcohol tone that adds an intense spicy character. **Overall**: lack of elegance and grace, robust style with a warm but moderate length. Sound product with a discreet finish.
Experimental	Gran Gold Medal	**Appearance**: Clear and bright, without faults. **Nose**: A nice palette of fruity agave, harmonious florality, spicy and nutty clove and well-integrated smoky impressions caress the nose. **Paladar**: On the palate, a round, full, and soft body with characterful, earthy aromas is accompanied by a powerful, complex spiciness that makes you want to drink more. **Overall**: A lovely mezcal that feels like a relaxed stroll in a Winter Christmas market! ¡Bravo!

Meanwhile, the production of esters takes place in the final phase of fermentation. They provide fruity notes to alcoholic beverages and are related to the species of yeast and agave used. Ogawa (2022) has recently shown that *T. delbrueckii* may be related to ester production. The methanol values can indicate a correct cooking process, since they are produced by the degradation of the pectins found in the agave heart. Species of yeast with the pectin-methyl-esterase enzyme have been found in tequila, which allows them to hydrolyze pectins in fermentation and generate methanol; therefore, the presence of yeasts with this enzymatic capacity can be taken into consideration (Gallardo Valdez *et al.*, 2020). Furfural involves a procedure similar to that of methanol, except that its production is based on the degradation of carbohydrates; in the case of mezcal, they are generated during cooking and therefore also function as an indicator that verifies the use of agave as a raw material (Gallardo Valdez *et al.*, 2020). This phenomenon could be related to the results obtained, since relatively higher values for both compounds are observed in the control mezcal than in the experimental one (CIATEJ, 2014). Compared with the results obtained in the distillation, the tails obtained were low —if the standard deviation is taken into account. In terms of yield, a 46% increase was recorded in the experimental mezcal —higher than the result obtained by CIATEJ (2014), which studied the application of yeast in three different factories. In the said study, the B factory has a higher yield (11 kg L^{-1}) and the yeasts found (*S. cerevisiae*, *T. delbrueckii*, *Kazachstania exigua*, and *K. marxianus*) were similar to the consortium used in this study. In the case of the tasting descriptors, the aroma characteristics were better in the experimental mezcal (a more diverse palette of flavors) than in the control. In conclusion, these sensory descriptors seem to be provided by the consortium of yeasts used.

CONCLUSIONS

The consortium of native yeasts used achieved a 46% increase in yield (liters of mezcal) and improved the organoleptic and physicochemical characteristics of the product, leading to the award of the Grand Gold Medal in Brussels 2020. Therefore, the use of native yeasts is a good biotechnological proposal for the agave-mezcal production chain.

ACKNOWLEDGMENTS

The authors would like to thank Gerardo Huerta Beristain (ScD) and María de los Ángeles Garibay-Marcelo (BSc in Chemical Bacteriology and Parasitology), for their technical support. Likewise, they would also like to thank Mr. José Calixto García, producer of artisanal mezcal from the El Calvario community. Finally, they would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the scholarship (No. 732517) granted for the Master in Biosciences program.

REFERENCES

AGROVIN (2019) La Semana Vitivinícola - Noticias del vino. Empleo De Torulaspora delbrueckii Cepa NSTD Para Incremento Sensorial De Vinos.

Alcázar-Valle M, Gschaedler A, Gutiérrez-Pulido H, Arana-Sánchez A, Arellano-Plaza M (2019) Fermentative capabilities of native yeast strains grown on juices from different Agave species used for tequila and mezcal production. *Brazilian Journal of Microbiology*. 50: 379-388.

Álvarez JM, Campo E, San-Juan F, Coque JJ, Ferreira V, Hernández P (2012) Sensory and chemical characterization of the aroma of Prieto Picudo rosé wines: The differential role of autochthonous yeast strains on aroma profiles. *Food Chemistry* 133: 284-292.

Álvarez ML, Zamora QKA, Acedo FE (2009) Perspectivas para el uso de levaduras nativas durante la elaboración de bacanora. *Revista Latinoamericana de Microbiología*, 5: 58-63.

Belda, I., Navascués, E., Alonso, A. Marquina, D. y Santos, A. (2014). Microbiología del proceso de vinificación: selección de levaduras Saccharomyces cerevisiae autóctonas con óptimas propiedades enológicas. *REDUCA (Biología) Serie Microbiología, 7*(1): 1-14. Disponible en: http://revistareduca.es/index.php/biologia/article/view/1593/1614

CIATEJ (2014) Manual para la estandarización de los procesos de producción del mezcal Guerrerense. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco.

Corona-González RI, Ramos-Ibarra JR, Gutiérrez-González P, Pelayo-Ortiz C, Guatemala-Morales GM, & Arriola-Guevara E. (2013) El uso de la metodología de superficie de respuesta para evaluar las condiciones de fermentación en la producción de tepache. *Revista Mexicana de Ingeniería Química* 12:19-28.

De León-Rodríguez, A., Escalante-Minakata, P., Barba de la Rosa, A.P., y Blaschek, H.P. (2008). Optimization of fermentation conditions to produce the mezcal from *Agave salmiana* using response surface methodology. *Chemical Engineering and Processing: Process Intensification, 47*(1): 76-82. Disponible en: https://doi.org/10.1016/j.cep.2007.08.010

Instituto Mexicano de la Propiedad Intelectual (2018). Emite IMPI nuevas modificaciones a la Declaración General de Protección a la Denominación de Origen Mezcal. Disponible en: https://dof.gob.mx/nota_detalle.php?codigo=5534192&fecha=08/08/2018

Días, A., Verruma, M.R., Correa, L.A., Mendes, M.T., Tieko, R., et al., (2012). Effect of the spontaneous fermentation and the ageing on the chemo-sensory quality of Brazilian organic cachaca. *Ciencia Rural, Santa Maria, 42*(5): 918-925. Disponible en: https://doi.org/10.1590/S0103-84782012000500026

Escalante-Minakata, P., Blaschek, H. P., Barba de la Rosa, A. P., Santos, L., y De León-Rodríguez, A. (2008). Identification of yeast and bacteria involved in the mezcal fermentation of *Agave salmiana*. *Letters in Applied Microbiology, 46*(6): 626–630. https://doi.org/10.1111/j.1472-765X.2008.02359.x

García, M., Esteve-Zarzoso, B., y Arroyo, T. (2016). Non-Saccharomyces yeasts: Biotechnological role for wine production. En A. Morata & I. Loira (Eds.), Grape and Wine Biotechnology. InTech. http://www.intechopen.com/books/grape-and-wine-biotechnology/non-saccharomyces-yeasts-biotechnological-role-for-wine-production. 10.5772/64957.

González, J.C., Pérez, E., Damián, R.M. y Chávez, M.C. (2012). Aislamiento, Caracterización molecular y fermentativa de una Levadura usada en la producción de etanol durante la Elaboración de mezcal. *Revista Mexicana de Ingeniería Química. 11*(3): 389-400.

González-Pérez, D., García-Ruiz, E., y Alcalde, M. (2012). *Saccharomyces cerevisiae* in directed evolution: An efficient tool to improve enzymes: An efficient tool to improve enzymes. *Bioengineered Bugs. 3*(3): 172–177. https://doi.org/10.4161/bbug.19544

Hernández, L. Y. y Salas, J. (2015). Mezcal cupreata, fuente de admiración. *Revista de la Academia Mexicana de Ciencias. 66*(3), 40-47.

Jukes TH, Carton CR (1969) Evolution of protein molecules. In: Munro HN (ed.) Mammalian protein metabolism. Academic Press. New York. 21-132. http://dx.doi.org/10.1016/B978-1-4832-3211-9.50009-7

Kirchmayr, M. R., Segura-García, L. E., Lappe-Oliveras, P., Moreno-Terrazas, R., y de la Rosa y Gschaedler-Mathis A. (2017). Impact of environmental conditions and process modifications on microbial diversity, fermentation efficiency and chemical profile during the fermentation of Mezcal in Oaxaca. LWT - *Food Science and Technology*, 79: 160–169. https://doi.org/10.1016/j.lwt.2016.12.052

Kunkee D. (1984). Selection and modification of yeasts and lactic acid bacteria for wine fermentation. *Food Microbiology*, 1: 315-332.

López, W. A., Ramírez, M., Mambuscay, L. A. y Osorio, E. (2010). Diversidad de levaduras asociadas a chichas tradicionales de Colombia. *Revista Colombiana de Biotecnología, 12*(2):176-186.

Mambuscay M., L. A., López A., W. A., Cuervo M., R. A., Argote V., F. E., y Osorio C., E. (2013). Identificación de las levaduras nativas presentes en zumos de piña, mora y uva. *Biotecnoloía en el Sector Agropecuario y Agroindustrial, 11*(SPE), 136–144.

Norma Oficial Mexicana NOM-070-SCFI-2016, Bebidas alcohólicas-Mezcal-Especificaciones. Disponible en: http://www.dof.gob.mx/normasOficiales/6437/seeco11_C/seeco11_C.html

Oviedo, L., Lara, C. y Mizger, M. (2009) Levaduras autóctonas con capacidad fermentativa en la producción de etanol a partir de pulpa de excedentes de plátano Musa (AAB Simmonds) en el departamento de Córdoba, Colombia. *Revista Colombiana de Biotecnologia, 11*(1): 40-47.

Páez-Lerma, J. B., Arias-García, A., Rutiaga-Quiñones, O. M., Barrio, E., y Soto-Cruz, N. O. (2013). Yeasts isolated from the alcoholic fermentation of *Agave duranguensis* during mezcal production. *Food biotechnology, 27*(4): 342–356. https://doi.org/10.1080/08905436.2013.840788

Padilla, B., Gil, JV y Manzanares, P. (2016). Pasado y futuro de las levaduras no Saccharomyces: de microorganismos de deterioro a herramientas biotecnológicas para mejorar la complejidad del aroma del vino. *Fronteras en microbiología*, 7:411.

Pérez, E., Chávez, M. y Gonzáles, J. C. (2016). Revisión del agave y el mezcal. *Revista Colombiana de Biotecnología, 18*(1): 148-164.

Pérez, E., González, J. C., Chávez, M. C. y Cortés, C. (2013). Fermentative characterization of producer's ethanol yeast from Agave cupreata juice in mezcal elaboration. *Revista Mexicana de Ingeniería Química, 12*(3): 451-461.

Pretorius, I. S. (2000) Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast*, 16: 675-729.

Querol, A., Barrio, E., y Ramón, D. (1992). A comparative study of different methods of yeast strain characterization. *Systematic and Applied Microbiology, 15*(3): 439–446. https://doi.org/10.1016/s0723-2020(11)80219-5

Ribérau-Gayon, P. (1985). New developmets in wine microbiology. *Revista Americana de Enología y Viticultura, 36*(1): 1-10.

Ruiz-Terán, F., Martínez-Zepeda, P. N., Geyer-de la Merced, S.Y., Nolasco-Cancino, H., y Santiago-Urbina, J.A. (2018). Mezcal: indigenous *Saccharomyces cerevisiae* strains and their potential as starter cultures. *Food Science and Biotechnology*, 28, 459–467 (collection 2019).

Suárez, B., Pando, R., Fernández, N., Querol, A. y Rodríguez, R. (2007). Yeast species associated with the spontaneus fermentation of cider. *Food microbiology*, 24: 25-31

Tataridis, P., Kannellis, A., Logothetis, S. y Nerantzis, E. (2013). Use of non-Saccharomyces Torulaspora delbrueckii yeast strains in winemaking and brewing. *Matica Srpska Journal for Natural Sciences, 124*(2): 415-426.

Vázquez, H.J. y Dacosta, O. (2007). Fermentación alcohólica: Una opción para la producción de energía renovable a partir de desechos agrícolas. *Ingeniería, Investigación y Tecnología, 8*(4): 249-259.

Vera G. A. M., Santiago G., Patricia A., y López, M. G. (2009). Compuestos volátiles aromáticos generados durante la elaboración de mezcal de *Agave angustifolia* y *Agave potatorum*. *Revista Fitotecnia Mexicana, 32*(4): 273-279.

Verdugo Valdez, A., Segura García, L., Kirchmayr, M., Ramírez Rodríguez, P., González Esquinca, A., Coria, R., & Gschaedler Mathis, A. (2011). Yeast communities associated with artisanal mezcal fermentations from *Agave salmiana*. *Antonie van Leeuwenhoek, 100*(4): 497–506. https://doi.org/10.1007/s10482-011-9605-y

White, T. J., Bruns, T., Lee, E. y Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. En: PCR protocols: a guide to methods and applications. Innis et ál. (ed.). Academic Press, 315-322

Wittmann, C., Hans, M., y Bluemke, W. (2002). Metabolic physiology of aroma producing *Kluyveromyces marxianus*. *Yeast (Chichester, England), 19*(15): 1351–1363. https://doi.org/10.1002/yea.920

Comparative analysis of the chemical quality of fishmeal produced on the Northwest coast of Mexico

Lizárraga-Hernández, Francisco D.[1,2]; **Soberanes-Yepiz, Maritza L.**[3]; **Cortés-Jacinto, Edilmar**[3*]; **Rangel-Dávalos, Carlos**[1]; **Cienfuegos-Martínez, Kathia**[3]

[1] Universidad Autónoma de Baja California Sur - Departamento de Ciencias Marinas y Costeras, Carretera al Sur Km 5.5, Colonia El Mezquitito, C.P. 23080, La Paz, Baja California Sur, México.
[2] University of the Littoral Opal Coast – Institute of Marine and Littoral Sciences, Quai Masset – Bassin Napoléon, 62200 Boulogne-sur-Mer, France
[3] Centro de Investigaciones Biológicas del Noroeste SC- Programa de Acuacultura, Avenida Instituto Politécnico Nacional 195, C.P. 23096, La Paz, Baja California Sur, México.
* Correspondence: ecortes04@cibnor.mx

ABSTRACT

Objective: To evaluate the physico-chemical quality of fishmeal produced by four companies in different states of the Republic (Baja California Sur, Jalisco, and Sinaloa).

Design/methodology/approach: The analyzed fishmeals were from six batches, sardine meal from California pilchard and Pacific thread herring (*S. sagax* and *O. libertate*), and skipjack tuna and (*K. pelamis*) processed by different Mexican companies. Proximal chemical analysis was carried out at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR).

Results: The fishmeal's quality parameters analyzed in this study showed similar values to those reported in the literature. The variations observed in their proximate chemical composition allow them to be classified according to the results of the analyses.

Limitations on study/implications: Considering that four of the six flours were produced from the same raw material, *S. sagax*, the high variability in their physico-chemical quality parameters indicates a lack of standardization in both production methods and quality controls among the producing companies.

Findings/conclusions: *K. pelamis* by-products can produce meals of equal or better physico-chemical quality than those produced from *S. sagax*. The development of official regulations establishing quality standards to fishmeal production at national level is desirable for competitiveness.

Keywords: fishmeal, proximate composition, California pilchard, Skipjack tuna.

Citation: Lizárraga-Hernández, F. D., Soberanes-Yepiz, M. L., Cortés-Jacinto, E., Rangel-Dávalos, C., Cienfuegos-Martínez, K. (2024). Comparative analysis of the chemical quality of fishmeal produced on the Northwest coast of Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i4.2870

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: November 29, 2023.
Accepted: April 02, 2024.
Published on-line: May 06, 2024.

Agro Productividad, 17(4). April. 2024. pp: 177-184.

INTRODUCTION

Fishmeal has been one of the most important agri-food products due to its extensive use in livestock, poultry, and aquaculture (Naylor *et al*., 2009; Kaiser *et al*., 2022). Therefore, the latter, these ingredients have been used in feeds for carnivorous fish and crustaceans due to their high protein content, good digestibility, palatability, and optimal balance between amino acids and lipids (Yen-Ortega *et al*., 2021; Zlaugotne *et al*., 2022). However, there have been efforts to replace fishmeal with alternative protein sources for more than two decades to reduce pressure on small pelagic fish stocks (Gaxiola and Cuzón, 2014; FAO, 2022).

Although alternative animal protein sources, such as soybean or insect meals (Martínez-Córdova *et al.* 2013), have allowed the gradual reduction of fishmeal in aquaculture diets, full substitution of traditional ingredients has not been possible (Hua *et al.*, 2019). Alternative meals often have lower digestibility, anti-nutritional factors, or poor palatability (Zlaugotne *et al.*, 2022). Consequently, it has been recommended that the quality of fishmeal be controlled and optimized as much as possible to ensure good diet performance (Cozzolino *et al.*, 2005; Boyd *et al.*, 2022).

Mexico is the world's fourteenth largest producer of fishmeal, which is mainly made from clupeids, whose fishing and processing are concentrated in the states of Sinaloa, Sonora, Baja California, and Baja California Sur (CONAPESCA, 2021; FAO, 2022). Despite this, there is a lack of standards defining the parameters for the quality control of fishmeal, with NOM-242-SSA1-2009 being the only official document establishing a limit of 100 mg/kg of histamine (SSA, 2009). Consequently, product quality in the national market tends to be heterogeneous between factories due to the non-standardisation of production processes (Hernández-Cerón, 2020).

The present study aimed to evaluate the proximal chemical quality of fishmeal collected in 6 different processing plants distributed in the Northwest region of Mexico to establish a baseline on its parameters that will allow an eventual qualitative standardization.

MATERIALS AND METHODS

The present study was conducted at the Proximal Chemical Analysis laboratory of the Centro de Investigaciones Biológicas del Noroeste, S. C. (CIBNOR), La Paz, Baja California Sur (24° 08' 10.03" LN and 110° 25' 35.31" LO), and at the Food Science and Technology Laboratory of the Universidad Autónoma de Baja California Sur (UABCS), campus La Paz (24° 6'-3.14"-LN, 110° 18'-54.44" LO).

A completely randomized experimental design was used with one factor under study (fishmeal). Table 1 shows the six treatments considered, each with three replicates per variable.

Table 1. Species, producing company, and origin of fishmeal.

Treatment	Company and location	Fish species
Fishmeal 1	Bahía Magdalena Harinera S.A de C.V., Puerto San Carlos, B.C.S.	California pilchard (*Sardinops sagax*)
Fishmeal 2	Maz Industrial S.A. de C.V., Mazatlán, Sin.	California pilchard (*Sardinops sagax*)
Fishmeal 3	Proteínas Marinas y Agropecuarias S.A. de C.V., Guadalajara, Jal.	Skipjack tuna (*Katsuwonus pelamis*)
Fishmeal 4	Proteínas Marinas y Agropecuarias S.A. de C.V., Guadalajara, Jal.	California pilchard (*Sardinops sagax*)
Fishmeal 5	Sardinera del Real S. de R.L. de C.V., Puerto San Carlos, B.C.S.	California pilchard (*Sardinops sagax*)
Fishmeal 6	Sardinera del Real S. de R.L. de C.V., Puerto San Carlos, B.C.S.	Pacific thread herring (*Opisthonema libertate*)

The proximate chemical composition was analysed according to AOAC (2005) methods: constant weight for moisture; charring in the furnace for ash; Soxhlet method for ether extract (EE, crude fat); acid and alkaline hydrolysis for crude fiber (CF); Kjeldahl method for crude protein (CP); and subtraction for free nitrogen extract (FNE). An analytical balance (Mettler Toledo®, CDMX, Mexico) was used for all gravimetry.

Statistical analyses were performed with Statistica® v. 10.0 for Windows (StatSoft®, 2011). Data were checked for normality (Kolmogorov-Smirnov, $p>0.20$) and homoscedasticity (Levene, $p=0.05$). Analysis of variance (ANOVA) and multiple comparisons of means were performed. For all variables, means were considered significantly different when $p \leq 0.05$.

RESULTS AND DISCUSSION

In general, the quality parameters of the fishmeals analyzed in the present study showed similar values to those previously reported in the literature (Cabello *et al.*, 2013; Quijije *et al.* 2019). However, the variations observed in their proximate chemical composition make it necessary to classify them according to the results of the analyses.

Fishmeals' moisture was statistically different between all treatments, except for fishmeals 1 and 5 ($F=928.2$, $p<0.000$). Figure 1 shows that fishmeal 4 obtained the highest parameter value (7.61±0.10%), which is within the range suggested by De Koning (2002) of 5 to 10% to avoid the development of microorganisms. Except for fishmeal 6 (4.18%±0.03), all of them complied with the standard. The low percentage of the latter suggests overheating during drying (Hilmarsdottir *et al.*, 2020). Also, its nutritional quality is compromised as this generates a reaction between lysine and histamine that produces gizzerosine, an irritant toxin in the digestive tract of crustaceans, fish, and chickens (Cruz-Suarez *et al.*, 1999; Takakuwa *et al.*, 2021).

Ash percentages ranged from 13 to 25% (see Figure 2), with fishmeal 1 having the lowest value (13.06±0.03%) and fishmeal 2 the highest (24.87±0.09%). Statistically, all treatments showed significant differences ($F=7153.3$, $p<0.000$). This is consistent

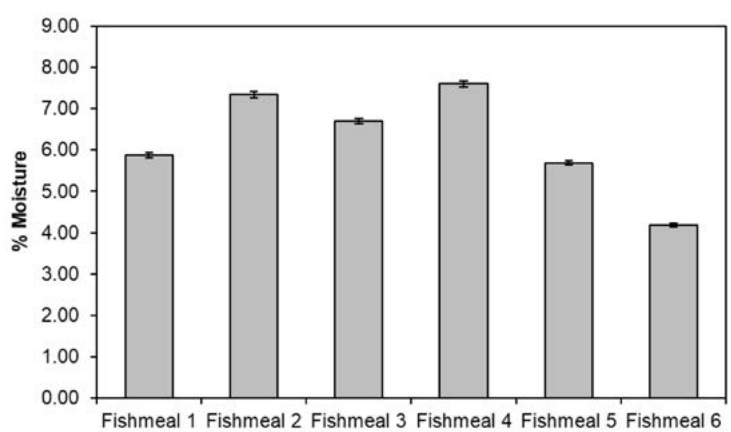


Figure 1. Mean percentages of the moisture content in fishmeal ($p<0.001$). Vertical bars show ± standard error.

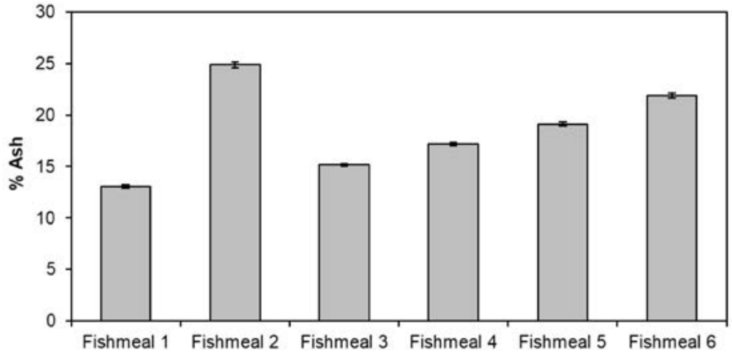


Figure 2. Mean percentages of ash content in fishmeal ($p<0.001$). Vertical bars show ± standard error.

with previous studies, as fishmeals usually contain between 10 to 25% ash since the bones of the raw material are not discarded entirely (Rossi and Davis, 2014; Quijije *et al.*, 2019). In the case of fishmeal 2, it exceeds the maximum of 20% ash recommended by New and Wijkström (2002), thus its nutritional quality is diminished as excess ash reduces digestibility. In contrast, in fishmeal 3, the low value of the parameter may be a result of its raw material, skipjack tuna, which, due to its size, is minced before being introduced into the wet pressing machine, allowing a greater bone removal (Kim *et al.* 2019).

The EE values ranged from 4 to 9.5% as shown in Figure 3, and showed statistical differences between all treatments, apart from fishmeals 2 and 4 ($F=8235.2$, $p\leq0.000$). This is consistent with previous reports mentioning that fishmeal normally contains between 6 to 10% fat (Mih and Lacherai, 2020). Fishmeal 1 obtained the highest EE value (9.41±0.05%), which exceeds the maximum optimum of 8% recommended by FAO (1986), and therefore falls into category B (medium quality). This is usual for sardine meals, which in many cases contain up to 20% lipids (Chaula *et al.*, 2019; Mih and Lacherai, 2020). In contrast, the low EE level observed in meal 6 (4.14±0.02%) indicates overheating that combusted its fats (Hilmarsdottir *et al.*, 2020; Takakuwa *et al.*, 2021). Likewise, fishmeal 3

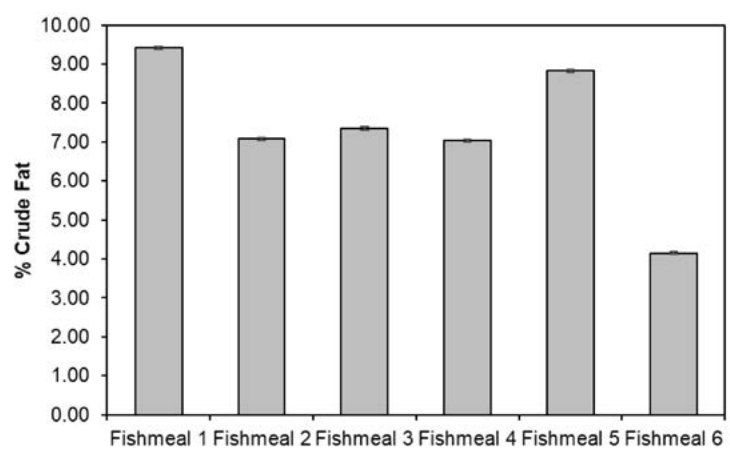


Figure 3. Mean percentages of EE in fishmeal ($p<0.001$). Vertical bars show ± standard error.

presents an EE (7.34±0.03%) consistent with previous studies on tuna meals (Hernández *et al.*, 2014; Souza *et al.*, 2017).

The CF content presented values below 0.20% in all the fishmeals (Figure 4). Significant differences were also found ($F=61.00$, $p \leq 0.0001$): fishmeals 2, 3, and 5 were the same, as well as fishmeals 4, and 6. Only fishmeal 1 was different from all the others. These products regularly have low FC in the form of celluloses and non-digestible carbohydrates (Villarreal-Cavazos *et al.*, 2019; Rawski *et al.*, 2020). Therefore, good fishmeal should not contain more than 1% FC as a high percentage can lead to digestibility problems (Morales *et al.*, 1999; Arriaga-Hernández *et al.*, 2021).

The CP levels of the tested flours ranged from 53 to 62%, with statistical differences between three groups ($F=11.90$, $p \leq 0.00025$). Fishmeals 1, 2, and 6 were equal (see Figure 5); meal 4 showed no differences between them and fishmeal 5. Fishmeal 5

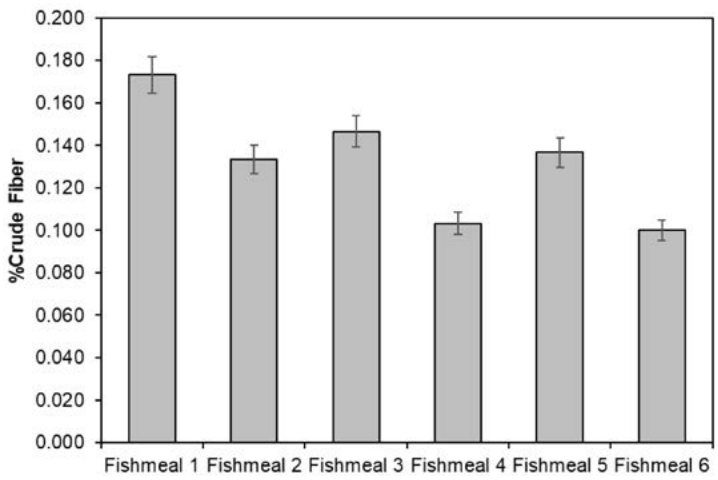


Figure 4. Percentage averages of CF in fishmeal ($p<0.001$). Vertical bars show ± standard error.

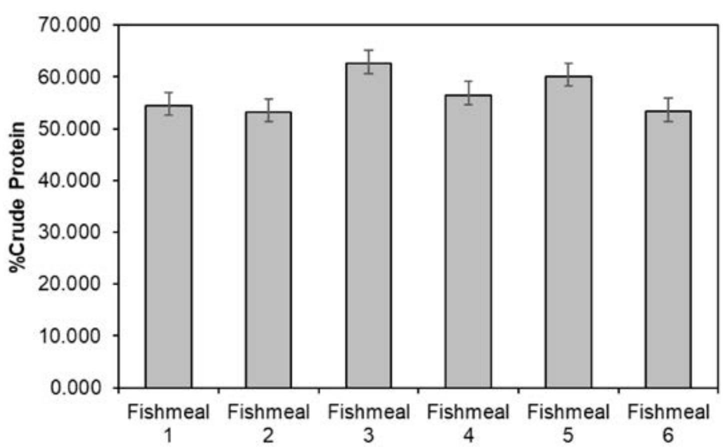


Figure 5. Percentage mean CP in fishmeal. Different letters differ statistically ($p<0.001$). Vertical bars show ± standard error.

(60.05±0.80) was equal to meal 3, which obtained the highest value (62.55%±1.26). In contrast, fishmeal 2 showed the lowest CP content (53.15±1.17%). Regularly, the protein level of fishmeal ranges from 50 to 72% depending on the species processed and the production process, which is consistent with these results (Arriaga-Hernández *et al.*, 2016; FAO, 2022). Fishmeal 3 can be classified as category A (superior quality), which is common for tuna meals (Souza *et al.*, 2017; Kim *et al.*, 2019; Li *et al.*, 2023). Likewise, fishmeal 5 is in category A, although its protein content is relatively low considering that the best Monterey sardine meals have up to 70% CP (Hernández *et al.*, 2014; Arriaga-Hernández *et al.*, 2021). All other fishmeals are considered category B as they do not reach 60% CP, which may be a consequence of protein denaturation during manufacture (meal 6), high inclusion of bones (fishmeal 2), or poor nutritional status of the fish at the time of capture (De Koning, 2002; Cabello *et al.*, 2013; Chaula *et al.*, 2019).

The FNE presented percentages between 6 and 17% (Figure 6), with differences between treatments ($F=16.53$, $p \leq 0.00005$). Fishmeal 1 obtained the highest value of

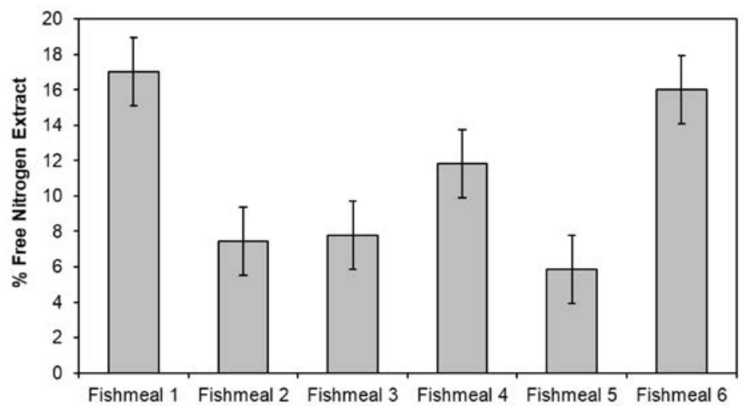


Figure 6. Mean percentages of the FNE in fishmeal. Different letters differ statistically ($p<0.001$).

the parameter (17.03%±2.74%), being equal to fishmeals 4, and 6 (11.55±2.92% and 16.39±1.96%). Fishmeal 5 showed the lowest percentage (6.18±0.08%) and had no significant differences with fishmeal 2, and 5. Fishmeal 4 was statistically equal for both groups. Generally, the FNE percentage of fishmeal's ranges from 3 to 12%, which is consistent with part of the results (Hernández *et al.*, 2014). However, higher than 10% is discouraged for the formulation of feeds for carnivorous fish and crustaceans as they do not metabolise carbohydrates efficiently (Ween *et al.*, 2017; Yen-Ortega *et al.*, 2021). Consequently, fishmeals 2, 3, and 5 would provide the optimal carbohydrate intake without detracting from other parameters (CP or EE), while fishmeals 1, 4, and 6 would have a lower nutritional quality.

CONCLUSIONS

Fishmeal produced on the northwest coast of Mexico has considerable variation in its proximate chemical composition due to the lack of standardised fabrication processes and quality control norms. In particular, the differences observed between the California pilchard meals confirm the information. Likewise, fishmeals 3, and 5 are the most recommendable for inclusion in aquaculture diets as they comply with the recommended quality intervals, while the use of fishmeal 6 is not recommended due to its possible overheating. A focused evaluation of tuna-derived meals is also recommended to assess their variability between production plants.

ACKNOWLEDGEMENTS

We thank the Consejo Nacional de Humanidades, Ciencia y Tecnología for funding the project (FC 2016 /2930) from which this work originated. We also extend our gratitude to the companies that participated in this study by providing us with the fishmeal samples.

REFERENCES

Arriaga-Hernández, D., Hernández, C., Martínez-Montaño, E., Ibarra-Castro, L., Lizárraga-Velázquez, E., Leyva-López, N., & Chávez-Sánchez, M. C. (2021). Fish meal replacement by soybean products in aquaculture feeds for white snook, *Centropomus viridis*: Effect on growth, diet digestibility, and digestive capacity. *Aquaculture, 530*(1), 735823. https://doi.org/10.1016/j.aquaculture.2020.735823

AOAC (2005). Official Methods of Analysis. Association of Official Agricultural Chemists. Gaithersburg, USA. 771 pp.

Boyd, C. E., McNevin, A. A., & Davis, R. P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food Security 14*(3), 805-827. https://doi.org/10.1007/s12571-021-01246-9

Cabello, A., García, A., Figuera, B., Higuera, Y., & Vallenilla, O. (2013). Physicochemical quality of Venezuelan fishmeal. *Saber 25*(4), 414-422.

Chaula, D., Laswai, H., Chove, B., Dalsgaard, A., Mdegela, R., & Hyldig, G. (2019). Fatty acid profiles and lipid oxidation status of sun dried, deep fried, and smoked sardine (*Rastrineobola argentea*) from Lake Victoria, Tanzania. *Journal of Aquatic Food Product Technology 28*(2), 165-176. https://doi.org/10.1080/10498850.2019.1570992. https://doi.org/10.1080/10498850.2019.1570992

Cho, J. H., & Kim, I. H. (2011). Fish meal - nutritive value. *Journal of Animal Physiology and Animal Nutrition 95*(6), 685-692.

CONAPESCA. (2021). Anuario Estadístico de Acuacultura y Pesca 2021. Comisión Nacional de Pesca y Acuacultura. Mazatlán, Mexico. 292 pp.

Cozzolino, D., Chree, A., Scaife, J. R., & Murray, I. (2005). Usefulness of near-infrared reflectance (Nir) spectroscopy and chemometrics to discriminate fishmeal batches made with different fish species. *Journal of Agricultural and Food Chemistry 53*(11), 4459-4463.

Cruz-Suárez, L.E., Ricque, D., Pike, I.H. & Cuzon, G. (1999). Determination of some factors affecting the nutritional and biotoxicological value of fish meal for use in feed for fish culture and to establish quality control standards. *International Fishmeal and Oil Manufacturers Association 3*(3): 1-54.

De Koning, A. (2002). Quantitative quality tests for fish meal. II. An investigation of the quality of South African fish meals and the validity of a number of chemical quality indices. *International Journal of Food Properties 5*(3), 495-507. https://doi.org/10.1081/JFP-120015487

Gaxiola, G. & Cuzon, G. (2014). El uso de ingredientes alternativos a las harinas de pescado. En LM. Martínez-Córdova, M. Martínez-Porchas & E. Cortés-Jacinto (Eds.), Alimentos y estrategias de alimentación para una acuacultura sustentable (pp. 67-98). Universidad de Sonora.

FAO. (2022). The State of World Fisheries and Aquaculture 2022: Towards the Blue Transformation. Food and Agriculture Organisation of the United Nations. Rome, Italy. 288 pp. https://www.fao.org/3/cc0461es/cc0461es.pdf

Hua, K., Cobcroft, J. M., Cole, A., Condon, K., Jerry, D. R., Mangott, A., Praeger, C., Vucko, M. J., Zeng, C., Zenger, K., & Strugnell, J. M. (2019). The Future of Aquatic Protein: Implications for Protein Sources in Aquaculture Diets. *One Earth 1*(3), 316-329. https://doi.org/10.1016/j.oneear.2019.10.018

Hernández, C., Hardy, R. W., Contreras-Rojas, D., López-Molina, B., González-Rodríguez, B., & Domínguez-Jiménez, P. (2014). Evaluation of tuna by-product meal as a protein source in feeds for juvenile spotted rose snapper *Lutjanus guttatus*. *Aquaculture Nutrition 20*(6), 574-582. https://doi.org/10.1111/anu.12110

Hernández-Cerón, A,J. (2020). Efecto de la inclusión de harina de pescado artesanal en la alimentación de ovinos de pelo en finalización, sobre las características de la canal y calidad de la carne [Tesis de maestría, Universidad Autónoma de Nayarit]. Repositorio Institucional Aramara – Universidad Autónoma de Nayarit.

Kaiser, F., Harbach, H., & Schulz, C. (2022). Rapeseed proteins as fishmeal alternatives: A review. *Reviews in Aquaculture 14*(4), 1887-1911. https://doi.org/10.1111/raq.12678

Kim, K., Park, Y., Je, H., Seong, M., Damusaru, J. H., Kim, S., Jung, J., & Bai, S. C. (2019). Tuna byproducts as a fishmeal in tilapia aquaculture. *Ecotoxicology and Environmental Safety 172*(1), 364-372. https://doi.org/10.1016/j.ecoenv.2019.01.107.

Li, R., & Cho, S. H. (2023). Substitution Impact of Tuna By-Product Meal for Fish Meal in the Diets of Rockfish (*Sebastes schlegeli*) on Growth and Feed Availability. *Animals, 13*(22), 3586. https://doi.org/10.3390/ani13223586

Martínez-Córdova, L. R., Campaña Torres, A., Villarreal-Colmenares, Martínez-Porchas, M., M. Ezquerra Brauer & Cortés-Jacinto, E. 2013. Evaluation of partial and total replacement of formulated feed by live insects, *Trichocorixa* sp. (Heteroptera: Corixidae) on the productive and nutritional response, and post-harvest quality of shrimp, *Litopenaeus vannamei* (Boone 1931). *Aquaculture Nutrition*. 19, 218-226

Mih, H., & Lacherai, A. (2020). Nutritional Properties of Fish Meal Produced from Fresh By-Products of Sardina pilchardus. *Journal of Fisheries and Environment 44*(2), 16-23.

Morales, A., Cardenete, G., Sanz, A., & de la Higuera, M. (1999). Re-evaluation of crude fibre and acid-insoluble ash as inert markers, alternative to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). *Aquaculture 179*(1-4), 71-79.

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., Forster, I., Gatlin, D. M., Goldburg, R. J., Hua, K., & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences 106*(36), 15103-15110. https://doi.org/10.1073/pnas.0905235106

New, M.B. & Wijkström, U.N. (2002). The use of fish meal and fish oil in aquafeeds: Further thoughts on the fish meal trap. *Food and Agriculture Organisation*. Rome, Italy. 71 pp.

Quijije-Mero, R., Villareal-De la Torre, D., & Chinga-Alcívar, B. (2019). Evaluación bromatológica de la harina de pescado procesada en la fábrica TADEL S.A. *Revista De Ciencias Del Mar y Acuicultura YAKU. 2*(3), 16-25

Rawski, M., Mazurkiewicz, J., Kierończyk, B., & Józefiak, D. (2020). Black Soldier Fly Full-Fat Larvae Meal as an Alternative to Fish Meal and Fish Oil in Siberian Sturgeon Nutrition: The Effects on Physical Properties of the Feed, Animal Growth Performance, and Feed Acceptance and Utilization. *Animals, 10*(11), 2119. https://doi.org/10.3390/ani10112119

Rossi, W., & Davis, D. A. (2014). Meat and Bone Meal as an Alternative for Fish Meal in Soybean Meal-Based Diets for Florida Pompano *Trachinotus carolinus* L. *Journal of the World Aquaculture Society, 45*(6), 613-624. https://doi.org/10.1111/jwas.12155

Souza, M. L. R. R. de, Yoshida, G. M., Campelo, D. A. V., Moura, L. B., Xavier, T. O., & Goes, E. S. dos R. (2017). Formulation of fish waste meal for human nutrition. *Acta Scientiarum. Technology 39*(5), 525-531. https://doi.org/10.4025/actascitechnol.v39i5.29723

Takakuwa, F., Hayashi, S., Yamada, S., Biswas, A., & Tanaka, H. (2022). Effect of additional heating of fish meal on *in vitro* protein digestibility and growth performance of white trevally (*Pseudocaranx dentex*) juveniles. *Aquaculture Research 53*(4), 1254-1267. https://doi.org/10.1111/are.15659

SSA. (2009). Norma Oficial Mexicana NOM-242-SSA1-2009, Productos y servicios-Productos de la pesca frescos, refrigerados, congelados y procesados-Especificaciones sanitarias y métodos de prueba. Secretaría de Salud - Diario Oficial de la Federación. Mexico City, Mexico. 128 pp.

Villarreal-Cavazos, D. A., Ricque-Marie, D., Nieto-López, M., Tapia-Salazar, M., Lemme, A., Gamboa-Delgado, J., & Cruz-Suárez, L. E. (2019). Apparent digestibility of amino acids in feedstuffs used in diets for the Pacific white shrimp, *Penaeus vannamei*. *Ciencias Marinas 45*(3). https://doi.org/10.7773/cm.v45i3.3007

Ween, O., Stangeland, J. K., Fylling, T. S., & Aas, G. H. (2017). Nutritional and functional properties of fishmeal produced from fresh by-products of cod (*Gadus morhua* L.) and saithe (*Pollachius virens*). *Heliyon 3*(7)

Yen-Ortega, E. E., Correa-Reyes, J. G., & Hernández-Rodríguez, M. (2021). Growth, thermal preference and critical thermal maximum for *Totoaba macdonaldi*: Effect of acclimation temperature and inclusion of soybean meal in the diet. *Latin American Journal of Aquatic Research 49*(2), 258-271. https://doi.org/10.3856/vol49-issue2-fulltext-2563

Zlaugotne, B., Pubule, J., & Blumberga, D. (2022). Advantages and disadvantages of using more sustainable ingredients in fish feed. *Heliyon 8*(9), 1-6. https://doi.org/10.1016/j.heliyon.2022.e10527