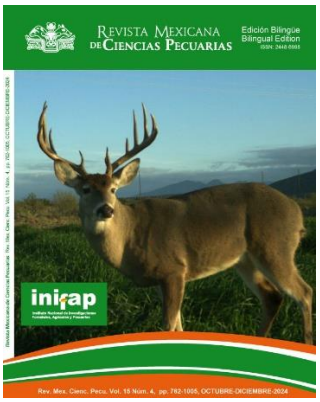
Revista Mexicana de Ciencias Pecuarias

Edición Bilingüe
Bilingual Edition
ISSN: 2448-6698

inifap
Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Revista Mexicana de Ciencias Pecuarias Rev. Mex. Cienc. Pecu. Vol. 15 Núm. 4, pp. 762-1005, OCTUBRE-DICIEMBRE-2024

Rev. Mex. Cienc. Pecu. Vol. 15 Núm. 4, pp. 762-1005, OCTUBRE-DICIEMBRE-2024



Venado cola blanca texano en parcela de triticale en el Rancho San Juan. Monclova, Coahuila.
Autor: **Eloy Alejandro Lozano Cavazos.**

REVISTA MEXICANA DE CIENCIAS PECUARIAS Volumen 15 Numero 4, Octubre-Diciembre 2024. Es una publicación trimestral de acceso abierto, revisada por pares y arbitrada, editada por el Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Avenida Progreso No. 5, Barrio de Santa Catarina, Alcaldía Coyoacán, C.P. 04010, Cuidad de México, https://www.gob.mx/inifap.
Distribuida por el Centro de Investigación Regional Sureste, Calle 6 No. 398 X 13, Avenida Correa Racho, Col. Díaz Ordaz, Mérida Yucatán, C.P. 97130.
Editor responsable: Arturo García Fraustro Reservas de Derechos al Uso Exclusivo número 04-2022-033116571100-102, ISSN: 2448-6698, otorgados por el Instituto Nacional del Derecho de Autor (INDAUTOR).
Responsable de la última actualización de este número: Arturo García Fraustro, Campo Experimental Mocochá, Km. 25 Antigua Carretera Mérida–Motul, Mocochá, Yuc. C.P. 97454. http://cienciaspecuarias. inifap.gob.mx, la presente publicación tuvo su última actualización en noviembre de 2024.

DIRECTORIO

Indizada en el "Journal Citation Report" Science Edition del ISI . Inscrita en el Sistema de Clasificación de Revistas Científicas y Tecnológicas de CONACyT; en EBSCO Host y la Red de Revistas Científicas de América Latina y el Caribe, España y Portugal (RedALyC) (www.redalyc.org); en la Red Iberoamericana de Revistas Científicas de Veterinaria de Libre Acceso (www.veterinaria.org/revistas/ revivec); en los Índices SCOPUS y EMBASE de Elsevier (www.elsevier. com).

REVISTA MEXICANA DE CIENCIAS PECUARIAS

La Revista Mexicana de Ciencias Pecuarias es un órgano de difusión científica y técnica de acceso abierto, revisada por pares y arbitrada. Su objetivo es dar a conocer los resultados de las investigaciones realizadas por cualquier institución científica, relacionadas particularmente con las distintas disciplinas de la Medicina Veterinaria y la Zootecnia. Además de trabajos de las disciplinas indicadas en su Comité Editorial, se aceptan también para su evaluación y posible publicación, trabajos de otras disciplinas, siempre y cuando estén relacionados con la investigación pecuaria.

Se publican en la revista tres categorías de trabajos: Artículos Científicos, Notas de Investigación y Revisiones Bibliográficas (consultar las Notas al autor); la responsabilidad de cada trabajo recae exclusivamente en los autores, los cuales, por la naturaleza misma de los experimentos pueden verse obligados a referirse en algunos casos a los nombres comerciales de ciertos productos, ello sin embargo, no implica preferencia por los productos citados o ignorancia respecto a los omitidos, ni tampoco significa en modo alguno respaldo publicitario hacia los productos mencionados.

Todas las contribuciones serán cuidadosamente evaluadas por árbitros, considerando su calidad y relevancia académica. Queda entendido que el someter un manuscrito implica que la investigación descrita es única e inédita. La publicación de Rev. Mex. Cienc. Pecu. es trimestral en formato bilingüe Español e Inglés. El costo total por publicar es de $ 7,280.00 más IVA por manuscrito ya editado.

Se publica en formato digital en acceso abierto, por lo que se autoriza la reproducción total o parcial del contenido de los artículos si se cita la fuente.

El envío de los trabajos de debe realizar directamente en el sitio oficial de la revista. Correspondencia adicional deberá dirigirse al Editor Adjunto a la siguiente dirección: Calle 36 No. 215 x 67 y 69 Colonia Montes de Amé, C.P. 97115 Mérida, Yucatán, México. Tel/Fax +52 (999) 941-5030. Correo electrónico (C-ele): rodriguez_oscar@prodigy.net.mx.

La correspondencia relativa a suscripciones, asuntos de intercambio o distribución de números impresos anteriores, deberá dirigirse al Editor en Jefe de la Revista Mexicana de Ciencias Pecuarias, Campo Experimental Mocochá, Km. 25 Antigua Carretera Mérida–Motul, Mocochá, Yuc. C.P. 97454; garcia.arturo@inifap.gob.mx o arias.alfonso@inifap.gob.mx.

Inscrita en la base de datos de EBSCO Host y la Red de Revistas Científicas de América Latina y el Caribe, España y Portugal (RedALyC) (www.redalyc.org), en la Red Iberoamericana de Revistas Científicas de Veterinaria de Libre Acceso (www.veterinaria.org/revistas/ revivec), indizada en el "Journal Citation Report" Science Edition del ISI (http://thomsonreuters. com/) y en los Índices SCOPUS y EMBASE de Elsevier (www.elsevier.com)

VISITE NUESTRA PÁGINA EN INTERNET
Artículos completos desde 1963 a la fecha y Notas al autor en: http://cienciaspecuarias.inifap.gob.mx

Revista Mexicana de Ciencias Pecuarias is an open access peer-reviewed and refereed scientific and technical journal, which publishes results of research carried out in any scientific or academic institution, especially related to different areas of veterinary medicine and animal production. Papers on disciplines different from those shown in Editorial Committee can be accepted, if related to livestock research.

The journal publishes three types of papers: Research Articles, Technical Notes and Review Articles (please consult Instructions for authors). Authors are responsible for the content of each manuscript, which, owing to the nature of the experiments described, may contain references, in some cases, to commercial names of certain products, which however, does not denote preference for those products in particular or of a lack of knowledge of any other which are not mentioned, nor does it signify in any way an advertisement or an endorsement of the referred products.

All contributions will be carefully refereed for academic relevance and quality. Submission of an article is understood to imply that the research described is unique and unpublished. Rev. Mex. Cien. Pecu. is published quarterly in original lenguage Spanish or English. Total fee charges are US $ 425.00 per article in both printed languages.

Part of, or whole articles published in this Journal may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying or otherwise, provided the source is properly acknowledged.

Manuscripts should be submitted directly in the official web site. Additional information may be mailed to Associate Editor, Revista Mexicana de Ciencias Pecuarias, Calle 36 No. 215 x 67 y 69 Colonia Montes de Amé, C.P. 97115 Mérida, Yucatán, México. Tel/Fax +52 (999) 941-5030. E-mail: rodriguez_oscar@prodigy.net.mx.

For subscriptions, exchange or distribution of previous printed issues, please contact: Editor-in-Chief of Revista Mexicana de Ciencias Pecuarias, Campo Experimental Mocochá, Km. 25 Antigua Carretera Mérida–Motul, Mocochá, Yuc. C.P. 97454; garcia.arturo@inifap.gob.mx or arias.alfonso@inifap.gob.mx.

Registered in the EBSCO Host database. The Latin American and the Caribbean Spain and Portugal Scientific Journals Network (RedALyC) (www.redalyc.org). The Iberoamerican Network of free access Veterinary Scientific Journals (www.veterinaria.org/ revistas/ revivec). Thomson Reuter´s "Journal Citation Report" Science Edition (http://thomsonreuters.com/). Elsevier´s SCOPUS and EMBASE (www.elsevier.com) and the Essential Electronic Agricultural Library (www.teeal.org)

.

VISIT OUR SITE IN THE INTERNET
Full articles from year 1963 to date and Instructions for authors can be accessed via the site http://cienciaspecuarias.inifap.gob.mx

REVISTA MEXICANA DE CIENCIAS PECUARIAS

REV. MEX. CIENC. PECU. VOL. 15 No. 4 OCTUBRE-DICIEMBRE-2024

CONTENIDO
Contents

ARTÍCULOS
Articles

Pág.

Momento de la suplementación sobre carga animal y producción de leche por hectárea de vacas Holstein en pastoreo

The effects of supplementation timing on stocking rate and milk production per hectare in grazing Holstein dairy cows

Citlali Anais Castro Jaime, Rodolfo Ramírez Valverde, Juan Andrés Burgueño Ferreira, Jacinto Efrén Ramírez Bribiesca, Luis Alberto Miranda Romero, Ricardo Daniel Améndola Massiotti 762

Respuesta productiva, características de la canal y calidad de la carne de ovinos alimentados con niveles crecientes de frutos secos triturados de *Acacia farnesiana*

Productive response, carcass traits, and meat quality of sheep fed with increasing levels of crushed dry fruits of *Acacia farnesiana*

Miguel Ángel Zarza-Albarrán, Agustín Olmedo-Juárez, Pedro Mendoza- de Gives, Jaime Ancelmo-Mondragón, Javier Arece-García, Francisca Aviles-Nova, Benito Albarrán-Portillo, Rolando Rojo-Rubio 778

Modelo de predicción para la extensión de la vida productiva en registros censurados de ganado Holstein de México

Prediction model for productive life extension in censored records of Holstein cattle from Mexico

Sandra Giovanna Núñez-Soto, Adriana García-Ruiz, Hugo Oswaldo Toledo Alvarado, Felipe de Jesús Ruiz-López 800

Evaluación antihelmíntica *in vitro* de curcumina contra huevos y larvas de aislados de *Haemonchus contortus* con distinta susceptibilidad a ivermectina

In vitro anthelmintic evaluation of curcumin against the eggs and larvae of three *Haemonchus contortus* isolates with different susceptibility to ivermectin

Lisandro Alberto Encalada-Mena, Raquel López-Arellano, María Eugenia López-Arellano, María Gabriela Mancilla-Montelongo, Carlos Alfredo Sandoval-Castro, Agustín Olmedo-Juárez, Juan Felipe de Jesús Torres-Acosta 811

Effect of spent coffee grounds aqueous extract as an antioxidant in raw pork patties during refrigerated storage

Efecto del extracto acuoso de granos de café gastado como antioxidante en hamburguesas de cerdo crudas durante el almacenamiento refrigerado

Juan Luis Murillo Hernández, Rey David Vargas Sánchez, Brisa del Mar Torres Martínez, Nelson Huerta Leidenz, Gastón Ramón Torrescano Urrutia, Armida Sánchez Escalante 833

Prevalence and diversity of zoonotic intestinal parasites in household dogs in urban areas of the Colombian Caribbean

Prevalencia y diversidad de parásitos intestinales zoonóticos en perros domésticos en un área urbana en el Caribe colombiano

Luz Adriana Sarmiento-Rubiano, Yina García Toscano, Julieannie Paola Ruiz, Lucena Delgado Soraca, Alfonso Bettin Martínez, Jimmy Becerra Enriquez 848

Transmisión espacial y vertical de los precios de leche del mercado internacional a mercados regionales y nacional de México

Spatial and vertical transmission of milk prices from the international market to Mexico's regional and national markets

Benigna González Ortiz, José Luis Jaramillo Villanueva, Samuel Vargas López, Ángel Bustamante González, Juan de Dios Guerrero Rodríguez, Vinicio Horacio Santoyo Cortés 861

Competitividad y ventaja comparativa de ganado bovino de carne en la Sierra Norte de Puebla, México

Competitiveness and comparative advantage in beef cattle production in the Sierra Norte of Puebla, Mexico

Lissete Abigail Rojas-Juárez, José Luis Jaramillo-Villanueva, Samuel Vargas-López, Juan Cabas-Monje 879

Dinámica estacional de pasto banderita [*Bouteloua curtipendula* (Mich.) Torr.] en Chihuahua, México: enfoque geoestadístico

Seasonal dynamics of sideoats grama [*Bouteloua curtipendula* (Mich.) Torr.] in Chihuahua, Mexico: a geostatistical approach

Ireyli Zuluamy Iracheta-Lara, Carlos Raúl Morales-Nieto, Gerardo Méndez-Zamora, Jaime Neftalí Márquez-Godoy, Federico Villarreal-Guerrero 898

Comportamiento productivo de una asociación Mombaza-Kudzú en diferentes épocas del año

Productive behavior of a Mombasa-Kudzu association at different times of the year

Eduardo Daniel Bolaños-Aguilar, Javier Francisco Enríquez-Quiroz, Abraham Fragoso-Islas, Roberto Omar Castañeda-Arriola, Maribel Montero-Lagunes, Julio César Vinay-Vadillo 913

REVISIONES DE LITERATURA

Reviews

Características de las curvas de lactación en ovejas y factores que influyen en su variación: Revisión

Characteristics of lactation curves in ewes and factors influencing their variation: A review

Gabriela Castillo-Hernández, Ana Erika Ochoa-Alfaro, Manuel Antonio Ochoa-Cordero, Jorge Alonso Maldonado Jáquez, Glafiro Torres-Hernández 930

NOTAS DE INVESTIGACIÓN

Technical notes

Capacidad de carga del venado cola blanca (*Odocoileus virginianus texanus*) en el noreste de México

Carrying capacity of white-tailed deer (*Odocoileus virginianus texanus*) in northeast Mexico

Fernando Isaac Gastelum Mendoza, Fernando Noel González Saldívar, Eloy Alejandro Lozano Cavazos, José Isidro Uvalle Sauceda, Guillermo Romero Figueroa, Enrique de Jesús Ruíz Mondragón, César Martín Cantú Ayala 951

The management of irrigated elephant grass intercropped with legumes in the semi-arid region

Manejo del pasto elefante de riego intercalado con leguminosas en la región semiárida

Jéssica Daisy do Vale Bezerra, João Virgínio Emerenciano Neto, Tamiris da Cruz da Silva, Patrick Bezerra Fernandes, Stela Antas Urbano, Felipe Pontes Teixeira das Chagas, Gelson dos Santos Difante, Marcone Geraldo Costa, Antonio Leandro Chaves Gurgel, Francisco Israel Lopes Sousa, Rodrigo da Silva Santos, Ana Beatriz Graciano da Costa 963

Evaluación *in vitro* de una fuente de nitrato protegido para rumiantes: efecto sobre la degradación de la materia seca y producción de metano

In vitro evaluation of a protected ruminant nitrate source: effect on dry matter degradation and methane production

María Elizabeth Rendón-Correa, Sandra Lucia Posada-Ochoa, Jaime Ricardo Rosero-Noguera……979

Color, humedad y contenido polínico de mieles del ecosistema manglar de la costa de Tabasco, México

Color, moisture, and pollen content of honeys from the mangrove ecosystem of the coast of Tabasco, Mexico

Hugo Alvarado-Díaz, Juan Manuel Zaldívar-Cruz, Ángel Sol-Sánchez, Said Cadena-Villegas, Jorge Ismael Tucuch Haas, Rocío Guadalupe Acosta-Pech 991

Actualización: septiembre, 2024

NOTAS AL AUTOR

La **Revista Mexicana de Ciencias Pecuarias** se edita completa en dos idiomas (español e inglés) y publica tres categorías de trabajos: Artículos científicos, Notas de investigación y Revisiones bibliográficas.

Los autores interesados en publicar en esta revista deberán ajustarse a los lineamientos que más adelante se indican, los cuales, en términos generales, están de acuerdo con los elaborados por el Comité Internacional de Editores de Revistas Médicas (CIERM) Bol Oficina Sanit Panam 1989;107:422-437.

1. Sólo se aceptarán trabajos inéditos. No se admitirán si están basados en pruebas de rutina, ni datos experimentales sin estudio estadístico cuando éste sea indispensable. Tampoco se aceptarán trabajos que previamente hayan sido publicados condensados o in extenso en Memorias o Simposio de Reuniones o Congresos (a excepción de Resúmenes).

2. Todos los trabajos estarán sujetos a revisión de un Comité Científico Editorial, conformado por Pares de la Disciplina en cuestión, quienes desconocerán el nombre e Institución de los autores proponentes. El Editor notificará al autor la fecha de recepción de su trabajo.

3. El manuscrito deberá someterse a través del portal de la Revista en la dirección electrónica: http://cienciaspecuarias.inifap.gob.mx, consultando el "**Instructivo para envío de artículos** en la página dela Revista Mexicana de Ciencias Pecuarias". Para su elaboración se utilizará el procesador de Microsoft Word, con letra **Times New Roman a 12 puntos**, a doble espacio. Necesariamente el autor que postule el manuscrito en la plataforma deberá fungir como autor para correspondencia, con quien únicamente el Comité Editorial tendrá comunicación con él. Asimismo, se deberán llenar los formatos de postulación, carta de originalidad y no duplicidad disponibles en el propio sitio oficial de larevista.

4. Por ser una revista con arbitraje, y para facilitar el trabajo de los revisores, todos los renglones de cada página deben estar numerados de manera continua a lo largo de todo el documento; asimismo cada página debe estar numerada, inclusive cuadros, ilustraciones y gráficas.

5. Los artículos tendrán una extensión máxima de 20 cuartillas a doble espacio, sin incluir páginas de Título, y cuadros o figuras (los cuales no deberán exceder de ocho y ser incluidos en el texto). Las Notas de investigación tendrán una extensión máxima de 15 cuartillas y 6 cuadros o figuras. Las Revisiones bibliográficas una extensión máxima de 30 cuartillas y 5 cuadros.

6. Los manuscritos de las tres categorías de trabajos que se publican en la **Rev. Mex. Cienc. Pecu**. deberán contener los componentes que a continuación se indican, empezando cada uno de ellos en página aparte.

   Página del título
   Resumen en español
   Resumen en inglés
   Texto
   Agradecimientos y conflicto de interés
   Literatura citada

7. **Página del Título**. Solamente debe contener el título del trabajo, que debe ser conciso pero informativo; así como el título traducido al idioma inglés. En el manuscrito no se debe incluir información como nombres de autores, departamentos, instituciones, direcciones de correspondencia, etc., ya que estos datos tendrán que ser registrados durante el proceso de captura de la solicitud en la plataforma del OJS (revisar el Instrucctivo para envío de artículos en la dirección: http://ciencias pecuarias.inifap.gob.mx.

8. **Resumen en español**. En la segunda página se debe incluir un resumen que no pase de 250 palabras. En él se indicarán los propósitos del estudio o investigación; los procedimientos básicos y la metodología empleada; los resultados más importantes encontrados, y de ser posible, su significación estadística y las conclusiones principales. A continuación del resumen, en punto y aparte, agregue debidamente rotuladas, de 3 a 8 palabras o frases cortas clave que ayuden a los indizadores a clasificar el trabajo, las cuales se publicarán junto con el resumen.

9. **Resumen en inglés**. Anotar el título del trabajo en inglés y a continuación redactar el "abstract" con las mismas instrucciones que se señalaron para el resumen en español. Al final en punto y aparte, se deberán escribir las correspondientes palabras clave ("keywords").

10. **Texto**. Las tres categorías de trabajos que se publican en la **Rev. Mex. Cienc. Pecu**. consisten en lo siguiente:

a) Artículos científicos. Deben ser informes de trabajos originales derivados de resultados parciales o finales de investigaciones. El texto del Artículo científico se divide en secciones que llevan estos encabezamientos:
Introducción
Material y Métodos
Resultados
Discusión
Conclusiones e implicaciones
Literatura citada

En los artículos largos puede ser necesario agregar subtítulos dentro de estas divisiones a fin de hacer más claro el contenido, tanto en Material y métodos como en las secciones de Resultados y de Discusión, las cuales también pueden presentarse como una sola sección.

b) Notas de investigación. Consisten en modificaciones a técnicas, informes de casos clínicos de interés especial, preliminares de trabajos o investigaciones limitadas, descripción de nuevas variedades de pastos; así como resultados de investigación que a juicio de los editores deban así ser publicados. El texto contendrá la misma información del método experimental señalado en el inciso a), pero su redacción será corrida del principio al final del trabajo; esto no quiere decir que sólo se supriman los subtítulos, sino que se redacte en forma continua y coherente.

c) Revisiones bibliográficas. Consisten en el tratamiento y exposición de un tema o tópico de relevante actualidad e importancia; su finalidad es la de resumir, analizar y discutir, así como poner a disposición del lector información ya publicada sobre un tema específico. El texto se divide en: Introducción, y las secciones que correspondan al desarrollo del tema en cuestión.

11. **Agradecimientos y conflicto de interés**. Siempre que corresponda, se deben especificar las colaboraciones que necesitan ser reconocidas, tales como a) la ayuda técnica recibida; b) el agradecimiento por el apoyo financiero y material, especificando la índole del mismo; c) las relaciones financieras que pudieran suscitar un conflicto de intereses. Las personas que colaboraron pueden ser citadas por su nombre, añadiendo su función o tipo de colaboración; por ejemplo: "asesor científico", "revisión crítica de la propuesta para el estudio", "recolección de datos", etc. Siempre que corresponda, los autores deberán mencionar si existe algún conflicto de interés.

12. **Literatura citada**. Numere las referencias consecutivamente en el orden en que se mencionan por primera vez en el texto. Las referencias en el texto, en los cuadros y en las ilustraciones se deben identificar mediante **números arábigos** entre paréntesis, sin señalar el año de la referencia. Evite hasta donde sea posible, el tener que mencionar en el texto el nombre de los autores de las referencias. Procure abstenerse de utilizar los resúmenes como referencias; las "observaciones inéditas" y las "comunicaciones personales" no deben usarse como referencias, aunque pueden insertarse en el texto (entre paréntesis).

Reglas básicas para la Literatura citada

Nombre de los autores, con mayúsculas sólo las iniciales, empezando por el apellido paterno, luego iniciales del materno y nombre(s). En caso de apellidos compuestos se debe poner un guión entre ambos, ejemplo: Elías-Calles E. Entre las iniciales de un autor no se debe poner ningún signo de puntuación, ni separación; después de cada autor sólo se debe poner una coma, después del último autor se debe poner un punto.

El título del trabajo se debe escribir completo (en su idioma original) luego el título abreviado de la revista donde se publicó, sin ningún signo de puntuación; inmediatamente después el año de la publicación, luego el número del volumen, seguido del número (entre paréntesis) de la revista y finalmente el número de páginas (esto en caso de artículo ordinario de revista).

Puede incluir en la lista de referencias, los artículos aceptados, aunque todavía no se publiquen; indique la revista y agregue "en prensa" (entre corchetes).

En el caso de libros de un solo autor (o más de uno, pero todos responsables del contenido total del libro), después del o los nombres, se debe indicar el título del libro, el número de la edición, el país, la casa editorial y el año.

Cuando se trate del capítulo de un libro de varios autores, se debe poner el nombre del autor del capítulo, luego el título del capítulo, después el nombre de los editores y el título del libro, seguido del país, la casa editorial, año y las páginas que abarca el capítulo.

En el caso de tesis, se debe indicar el nombre del autor, el título del trabajo, luego entre corchetes el grado (licenciatura, maestría, doctorado), luego el nombre de la ciudad, estado y en su caso país, seguidamente el nombre de la Universidad (no el de la escuela), y finalmente el año.

Emplee el estilo de los ejemplos que aparecen a continuación:

Revistas

Artículo ordinario, con volumen y número. (Incluya el nombre de todos los autores cuando sean seis o menos; si son siete o más, anote sólo el nombre de los seis primeros y agregue "et al.").

I) Basurto GR, Garza FJD. Efecto de la inclusión de grasa o proteína de escape ruminal en el comportamiento de toretes Brahman en engorda. Téc Pecu Méx 1998;36(1):35-48.

Sólo número sin indicar volumen.

II) Stephano HA, Gay GM, Ramírez TC. Encephalomielitis, reproductive failure and corneal opacity (blue eye) in pigs associated with a paramyxovirus infection. Vet Rec 1988;(122):6-10.

III) Chupin D, Schuh H. Survey of present status of the use of artificial insemination in developing countries. World Anim Rev 1993;(74-75):26-35.

No se indica el autor.

IV) Cancer in South Africa [editorial]. S Afr Med J 1994;84:15.

Suplemento de revista.

V) Hall JB, Staigmiller RB, Short RE, Bellows RA, Bartlett SE. Body composition at puberty in beef heifers as influenced by nutrition and breed [abstract]. J Anim Sci 1998;71(Suppl 1):205.

Organización, como autor.

VI) The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;(164):282-284.

En proceso de publicación.

VII) Scifres CJ, Kothmann MM. Differential grazing use of herbicide treated area by cattle. J Range Manage [in press] 2000.

Libros y otras monografías

Autor total.

VIII) Steel RGD, Torrie JH. Principles and procedures of statistics: A biometrical approach. 2nd ed. New York, USA: McGraw-Hill Book Co.; 1980.

Autor de capítulo.

IX) Roberts SJ. Equine abortion. In: Faulkner LLC editor. Abortion diseases of cattle. 1rst ed. Springfield, Illinois, USA: Thomas Books; 1968:158-179.

Memorias de reuniones.

X) Loeza LR, Angeles MAA, Cisneros GF. Alimentación de cerdos. En: Zúñiga GJL, Cruz BJA editores. Tercera reunión anual del centro de investigaciones forestales y agropecuarias del estado de Veracruz. Veracruz. 1990:51-56.

XI) Olea PR, Cuarón IJA, Ruiz LFJ, Villagómez AE. Concentración de insulina plasmática en cerdas alimentadas con melaza en la dieta durante la inducción de estro lactacional [resumen]. Reunión nacional de investigación pecuaria. Querétaro, Qro. 1998:13.

XII) Cunningham EP. Genetic diversity in domestic animals: strategies for conservation and development. In: Miller RH et al. editors. Proc XX VI eltsville Symposium: Biotechnology's role in genetic improvement of farm animals. USDA. 996:13.

Tesis.

XIII) Alvarez MJA. Inmunidad humoral en la anaplasmosis y babesiosis bovinas en becerros mantenidos en una zona endémica [tesis maestría]. México, DF: Universidad Nacional Autónoma de México; 1989.

XIV) Cairns RB. Infrared spectroscopic studies of solid oxigen [doctoral thesis]. Berkeley, California, USA: University of California; 1965.

Organización como autor.

XV) NRC. National Research Council. The nutrient requirements of beef cattle. 6th ed. Washington, DC, USA: National Academy Press; 1984.

XVI) SAGAR. Secretaría de Agricultura, Ganadería y Desarrollo Rural. Curso de actualización técnica para la aprobación de médicos veterinarios zootecnistas responsables de establecimientos destinados al sacrificio de animales. México. 1996.

XVII) AOAC. Official methods of analysis. 15th ed. Arlington, VA, USA: Association of Official Analytical Chemists. 1990.

XVIII) SAS. SAS/STAT User's Guide (Release 6.03). Cary NC, USA: SAS Inst. Inc. 1988.

XIX) SAS. SAS User´s Guide: Statistics (version 5 ed.). Cary NC, USA: SAS Inst. Inc. 1985.

Publicaciones electrónicas

XX) Jun Y, Ellis M. Effect of group size and feeder type on growth performance and feeding patterns in growing pigs. J Anim Sci 2001;79:803-813. http://jas.fass.org/cgi/reprint/79/4/803.pdf. Accessed Jul 30, 2003.

XXI) Villalobos GC, González VE, Ortega SJA. Técnicas para estimar la degradación de proteína y materia orgánica en el rumen y su importancia en rumiantes en pastoreo. Téc Pecu Méx 2000;38(2): 119-134. http://www.tecnicapecuaria.org/trabajos/20021217 5725.pdf. Consultado 30 Ago, 2003.

XXII) Sanh MV, Wiktorsson H, Ly LV. Effect of feeding level on milk production, body weight change, feed conversion and postpartum oestrus of crossbred lactating cows in tropical conditions. Livest Prod Sci 2002;27(2-3):331-338. http://www.sciencedirect. com/science/journal/03016226. Accessed Sep 12, 2003.

13. **Cuadros**, **Gráficas** e **Ilustraciones**. Es preferible que sean pocos, concisos, contando con los datos necesarios para que sean autosuficientes, que se entiendan por sí mismos sin necesidad de leer el texto. Para las notas al pie se deberán utilizar los símbolos convencionales.

14 **Versión final**. Es el documento en el cual los autores ya integraron las correcciones y modificaciones indicadas por el Comité Revisor. Se les enviará a los autores un instructivo que contendrá los puntos esenciales para su correcta elaboración. Las fotografías e imágenes deberán estar en formato jpg (o compatible) con al menos 300 dpi de resolución. Tanto las fotografías, imágenes, gráficas, cuadros o tablas deberán incluirse en el mismo archivo del texto. Los cuadros no deberán contener ninguna línea vertical, y las horizontales solamente las que delimitan los encabezados de columna, y la línea al final del cuadro.

15. Una vez recibida la versión final, ésta se mandará para su traducción al idioma inglés o español, según corresponda. Si los autores lo consideran conveniente podrán enviar su manuscrito final en ambos idiomas.

16. **Tesis**. Se publicarán como Artículo o Nota de Investigación, siempre y cuando se ajusten a las normas de esta revista.

17. Los trabajos no aceptados para su publicación se regresarán al autor, con un anexo en el que se explicarán los motivos por los que se rechaza o las modificaciones que deberán hacerse para ser reevaluados.

18.

Abreviaturas de uso frecuente:

cal caloría (s)
cm centímetro (s)
°C grado centígrado (s)
DL_{50} dosis letal 50%
g gramo (s)
ha hectárea (s)
h hora (s)
i.m. intramuscular (mente)
i.v. intravenosa (mente)
J joule (s)
kg kilogramo (s)
km kilómetro (s)
L litro (s)
log logaritmo decimal
Mcal megacaloría (s)
MJ megajoule (s)
m metro (s)
msnm metros sobre el nivel del mar
µg microgramo (s)
µl microlitro (s)
µm micrómetro (s)(micra(s))
mg miligramo (s)
ml mililitro (s)
mm milímetro(s)
min minuto (s)
ng nanogramo (s)
P probabilidad (estadística)
p página
PC proteína cruda
PCR reacción en cadena de la polimerasa
pp páginas
ppm partes por millón
% por ciento (con número)
rpm revoluciones por minuto
seg segundo (s)
t tonelada (s)
TND total de nutrientes digestibles
UA unidad animal
UI unidades internacionales
vs versus
xg gravedades

Cualquier otra abreviatura se pondrá entre paréntesis inmediatamente después de la(s) palabra(s) completa(s).

19. Los nombres científicos y otras locuciones latinas se deben escribir en cursivas.

inifap
Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

REVISTA MEXICANA DE CIENCIAS PECUARIAS

Updated: September, 2024

INSTRUCTIONS FOR AUTHORS

Revista Mexicana de Ciencias Pecuarias is published in its entirety in two languages (Spanish and English) and publishes three categories of papers: Scientific Articles, Research Notes and Bibliographic Reviews.

Authors interested in publishing in this journal must comply with the guidelines indicated below, which, in general terms, are in accordance with those developed by the International Committee of Medical Journal Editors (ICMJE) Bol Oficina Sanit Panam 1989;107:422-437.

1. Only unpublished works will be accepted. They will not be admitted if they are based on routine tests, or experimental data without statistical study when this is indispensable. Nor will works that have been previously published condensed or *in extenso* in Proceedings or Symposium of Meetings or Congresses (with the exception of Abstracts) be accepted.

2. All papers will be subject to review by a Scientific Editorial Committee, made up of Peers of the Discipline in question, who will not know the name and Institution of the proposing authors. The Editor will notify the author of the date of receipt of their work.

3. The manuscript must be submitted through the portal of the Journal at the electronic address: http://cienciaspecuarias.inifap.gob.mx, consulting "**Instructions for submitting articles** on the web page of Revista Mexicana de Ciencias Pecuarias". For its preparation, the Microsoft Word processor will be used, with **Times New Roman font at 12 points**, double-spaced. The author who submits the manuscript on the platform, must only serve as corresponding author, Editorial Committee will have only communication with him. Likewise, the forms of submission, letter of originality and non-duplicity, which are available on the official website of the journal, must be completed.

4. Because it is a peer-reviewed journal, and to facilitate the work of the reviewers, all the lines of each page must be numbered continuously throughout the document; each page must also be numbered, including tables, illustrations, and graphs.

5. The articles will have a maximum length of 20 double-spaced pages, not including Title pages, and tables or figures (which must not exceed eight and must be included in the text). The Research Notes will have a maximum length of 15 pages and 6 tables or figures. Bibliographic Reviews a maximum length of 30 pages and 5 tables.

6. The manuscripts of the three categories of papers that are published in **Rev. Mex. Cienc. Pecu.** must contain the components indicated below, each of them starting on a separate page.

Title page
Abstract in Spanish
Abstract in English
Text
Acknowledgments and conflict of interest
Literature cited

7. **Title Page.** It must only contain the title of the work, which should be concise but informative, as well as the title translated into Spanish. The manuscript should not include information such as names of authors, departments, institutions, correspondence addresses, etc., since these data will have to be registered during the process of capturing the application on the OJS platform (look at the Instructions for submitting articles at the address: http://ciencias pecuarias.inifap.gob.mx.

8. **Abstract in Spanish.** On the second page, an abstract that does not exceed 250 words must be included. It will indicate the purposes of the study or research; the basic procedures and methodology used; the most important results found, and if possible, their statistical significance and main conclusions. After the abstract, and on a separate line, add, properly labeled, 3 to 8 key words or short phrases that help indexers classify the work, which will be published along with the abstract.

9. **Abstract in English.** Write down the title of the work in English and then write the "abstract" with the same instructions that were indicated for the abstract in Spanish. At the end, and on a separate line, the corresponding keywords must be written.

10. **Text.** The three categories of papers that are published in **Rev. Mex. Cienc. Pecu.** consist of the following:

a) Scientific Articles. They must be reports of original works derived from partial or final results of studies. The text of the Scientific Article is divided into sections that have these headings:

Introduction
Material y methods
Results
Discussion
Conclusions and implications
Literature cited

In long articles, it may be necessary to add subheadings within these divisions in order to clarify the content, both in Material and methods and in the Results and Discussion sections, which can also be presented as a single section.

b) Research Notes. They consist of modifications to techniques, reports of clinical cases of special interest, preliminary work or limited research, description of new grass varieties, as well as research results that, in the opinion of the editors, should be published. The text will contain the same information as the experimental method referred to in subparagraph (a) but will be worded continuously from the beginning to the end of the work; this does not mean that subheadings are only deleted, but that it is written in a continuous and consistent manner.

c) Bibliographic Reviews. They consist of the treatment and presentation of a topical and important theme or issue; they purpose is to summarize, analyze and discuss, as well as to make available to the reader information already published on a specific theme. The text is divided into: Introduction, and the sections that correspond to the development of the topic in question.

11. **Acknowledgements and conflict of interest.** Where appropriate, collaborations that need to be recognized must be specified, such as a) technical assistance received; b) appreciation for financial and material support, specifying the nature of the support; c) financial relationships likely to give rise to a conflict of interest. People who collaborated can be cited by name, adding their function or type of collaboration; for example: "scientific advisor", "critical review of the proposal for the study", "data collection", etc. Where appropriate, authors should mention if there is any conflict of interest.

12. **Literature cited.** Number references consecutively in the order in which they are first mentioned in the text. References in the text, tables, and illustrations must be identified by **arabic numerals** in parentheses, without indicating the year of reference. Avoid, as far as possible, having to mention in the text the name of the authors of the references. Try not to use abstracts as references; "unpublished observations" and "personal communications" must not be used as references, although they may be inserted in the text (in parentheses).

Basic rules for Literature cited

Name of the authors, with capital letters only the initials, starting with the paternal surname, then initials of the maternal one and name (s). In case of compound surnames, a hyphen must be put between them, example: Elías-Calles E. Between the initials of an author, no punctuation mark, or separation should be placed; after each author, only a comma must be placed; after the last author, a period must be placed.

The title of the paper must be written in full (in its original language) then the abbreviated title of the journal where it was published, without any punctuation mark; immediately after, the year of publication, then the volume number, followed by the number (in parentheses) of the journal and finally the number of pages (this in the case of an ordinary journal article).

The list of references may include accepted articles, even if they are not yet published; indicate the journal and add "in press" (in square brackets).

In the case of books by a single author (or more than one, but all responsible for the total content of the book), after the name or names, the title of the book, the edition number, the country, the publishing house, and the year must be indicated.

When it comes to the chapter of a book by several authors, the name of the author of the chapter must be indicated, then the title of the chapter, then the name of the editors and the title of the book, followed by the country, the publishing house, year, and the pages covered by the chapter.

In the case of a thesis, the following must be indicated: name of the author, the title of the work, then the degree (bachelor's, master's, doctorate) in brackets, then the name of the city, state and, where appropriate, country, followed by the name of the University (not the school), and finally the year.

Use the style of the following examples:

Journals

Ordinary article, with volume and number. (Include the name of all authors when they are six or less; if there are seven or more, write down only the name of the first six and add "et al.").

I) Basurto GR, Garza FJD. Efecto de la inclusión de grasa o proteína de escape ruminal en el comportamiento de toretes Brahman en engorda. Téc Pecu Méx 1998;36(1):35-48.

Only the number without indicating the volume.

II) Stephano HA, Gay GM, Ramírez TC. Encephalomielitis, reproductive failure and corneal opacity (blue eye) in pigs associated with a paramyxovirus infection. Vet Rec 1988;(122):6-10.

III) Chupin D, Schuh H. Survey of present status of the use of artificial insemination in developing countries. World Anim Rev 1993;(74-75):26-35.

The author is not indicated.

IV) Cancer in South Africa [editorial]. S Afr Med J 1994;84:15.

Journal supplement.

V) Hall JB, Staigmiller RB, Short RE, Bellows RA, Bartlett SE. Body composition at puberty in beef heifers as influenced by nutrition and breed [abstract]. J Anim Sci 1998;71(Suppl 1):205.

Organization as author.

VI) The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;(164):282-284.

In the process of publication.

VII) Scifres CJ, Kothmann MM. Differential grazing use of herbicide treated area by cattle. J Range Manage [in press] 2000.

Books and monographs

Total author.

VIII) Steel RGD, Torrie JH. Principles and procedures of statistics: A biometrical approach. 2nd ed. New York, USA: McGraw-Hill Book Co.; 1980.

Chapter author.

IX) Roberts SJ. Equine abortion. In: Faulkner LLC editor. Abortion diseases of cattle. 1rst ed. Springfield, Illinois, USA: Thomas Books; 1968:158-179.

Meeting proceedings.

X) Loeza LR, Angeles MAA, Cisneros GF. Alimentación de cerdos. En: Zúñiga GJL, Cruz BJA editores. Tercera reunión anual del centro de investigaciones forestales y agropecuarias del estado de Veracruz. Veracruz. 1990:51-56.

XI) Olea PR, Cuarón IJA, Ruiz LFJ, Villagómez AE. Concentración de insulina plasmática en cerdas alimentadas con melaza en la dieta durante la inducción de estro lactacional [resumen]. Reunión nacional de investigación pecuaria. Querétaro, Qro. 1998:13.

XII) Cunningham EP. Genetic diversity in domestic animals: strategies for conservation and development. In: Miller RH et al. editors. Proc XX VI eltsville Symposium: Biotechnology's role in genetic improvement of farm animals. USDA. 996:13.

Thesis.

XIII) Alvarez MJA. Inmunidad humoral en la anaplasmosis y babesiosis bovinas en becerros mantenidos en una zona endémica [tesis maestría]. México, DF: Universidad Nacional Autónoma de México; 1989.

XIV) Cairns RB. Infrared spectroscopic studies of solid oxigen [doctoral thesis]. Berkeley, California, USA: University of California; 1965.

Organization as author.

XV) NRC. National Research Council. The nutrient requirements of beef cattle. 6th ed. Washington, DC, USA: National Academy Press; 1984.

XVI) SAGAR. Secretaría de Agricultura, Ganadería y Desarrollo Rural. Curso de actualización técnica para la aprobación de médicos veterinarios zootecnistas responsables de establecimientos destinados al sacrificio de animales. México. 1996.

XVII) AOAC. Official methods of analysis. 15th ed. Arlington, VA, USA: Association of Official Analytical Chemists. 1990.

XVIII) SAS. SAS/STAT User's Guide (Release 6.03). Cary NC, USA: SAS Inst. Inc. 1988.

XIX) SAS. SAS User´s Guide: Statistics (version 5 ed.). Cary NC, USA: SAS Inst. Inc. 1985.

Electronic publications

XX) Jun Y, Ellis M. Effect of group size and feeder type on growth performance and feeding patterns in growing pigs. J Anim Sci 2001;79:803-813. http://jas.fass.org/cgi/reprint/79/4/803.pdf. Accessed Jul 30, 2003.

XXI) Villalobos GC, González VE, Ortega SJA. Técnicas para estimar la degradación de proteína y materia orgánica en el rumen y su importancia en rumiantes en pastoreo. Téc Pecu Méx 2000;38(2): 119-134. http://www.tecnicapecuaria.org/trabajos/20021217 5725.pdf. Consultado 30 Ago, 2003.

XXII) Sanh MV, Wiktorsson H, Ly LV. Effect of feeding level on milk production, body weight change, feed conversion and postpartum oestrus of crossbred lactating cows in tropical conditions. Livest Prod Sci 2002;27(2-3):331-338. http://www.sciencedirect. com/science/journal/03016226. Accessed Sep 12, 2003.

13. **Tables, Graphs, and Illustrations.** It is preferable that they be few, concise, with the necessary data to be self-sufficient, that they be understood alone without having to read the text. For footnotes, conventional symbols should be used.

14 **Final version.** It is the document in which the authors have already integrated the corrections and modifications indicated by the Review Committee. Instructions that will contain the essential points for its correct preparation will be sent to the authors. Photographs and images must be in jpg format (or compatible) with at least 300 dpi resolution. Photographs, images, graphs, charts, or tables must be included in the same text file. Tables should not contain any vertical lines, and horizontal lines should contain only those delimiting column headings, and the line at the end of the table.

15. Once the final version is received, it will be sent for translation into English or Spanish, as appropriate. If the authors consider it convenient, they may send their final manuscript in both languages.

16. **Thesis**. They will be published as an Article or Research Note, as long as they conform to the standards of this journal.

17. Works not accepted for publication will be returned to the author, with an annex explaining the reasons why it is rejected or the modifications that must be made to be reevaluated.

18.

Frequently used abbreviations:

cal	calorie (s)
cm	centimeter (s)
°C	degree centigrade (s)
LD_{50}	lethal dose 50%
g	gram (s)
ha	hectare (s)
h	hour (s)
i.m.	intramuscular (-ly)
i.v.	intravenous (-ly)
J	joule (s)
kg	kilogram (s)
km	kilometer (s)
L	liter (s)
log	decimal logarithm
Mcal	megacalorie (s)
MJ	megajoule (s)
m	meter (s)
masl	meters above sea level
µg	microgram (s)
µL	microliter (s)
µm	micrometer (s)(micra(s))
mg	milligram (s)
mL	milliliter (s)
mm	millimeter (s)
min	minute (s)
ng	nanogram (s)
P	(statistical) probability
p	page
CP	crude protein
PCR	polymerase chain reaction
pp	pages
ppm	parts per million
%	per cent (with number)
rpm	revolutions per minute
sec	second (s)
t	tonne (s)
TDN	total digestible nutrients
AU	animal unit
IU	international units
vs	versus
xg	gravities

Any other abbreviation should be placed in parentheses immediately after the full word(s).

19. Scientific names and other Latin phrases must be written in italics

https://doi.org/10.22319/rmcp.v15i4.6680

Article

The effects of supplementation timing on stocking rate and milk production per hectare in grazing Holstein dairy cows

Citlali Anais Castro Jaime [a]

Rodolfo Ramírez Valverde [a]

Juan Andrés Burgueño Ferreira [b]

Jacinto Efrén Ramírez Bribiesca [c]

Luis Alberto Miranda Romero [a]

Ricardo Daniel Améndola Massiotti [a*]

[a] Universidad Autónoma Chapingo. Programa de Posgrado en Producción Animal, km 38.5 Carretera México-Texcoco, 56230 Texcoco, Estado de México, México.

[b] Centro Internacional de Mejoramiento de Maíz y Trigo. El Batán, Texcoco, Estado de México, México.

[c] Colegio de Postgraduados. Campus Montecillo. Rama de Ganadería, Texcoco, Estado de México, México.

[*] Corresponding author: r_amendola@yahoo.com

Abstract:

Concentrate supplementation can affect multiple parameters in grazing dairy systems. In mixed pastures (*Medicago sativa* L. and *Dactylis glomerata* L.) grazed by New Zealand Holstein cows, a study was done of the effects of concentrate supplementation timing on individual production, stocking rate and milk production per hectare. Two experiments were done, one in winter and another in spring-summer. Experimental design was 3x3 crossover with treatments defined by concentrate (5.0 kg DM cow^{-1} d^{-1}) supplement administration times: after morning milking (AM), after afternoon milking (PM), and equally divided

between both milkings (AM-PM). The experimental units were batches of six (winter) or five cows (spring-summer), which received the treatments, and their respective grazing areas. The rotational grazing management criterion was 8 cm residual forage height in all treatments, which allowed estimation of the effects of the treatments on stocking rate. Stocking rate did not differ ($P>0.05$) between treatments. Milk production per cow in the AM treatments was an average of 10.2 % higher than the other two treatments, both in winter (8.6 %, $P=0.0002$) and spring-summer (11.7 %, $P<0.0001$). The increase in milk production per hectare (9 %) was due to individual response and not to differences in stocking rate. Use of a uniform residual forage height was a simple way of estimating the response in stocking rate and thus milk production per hectare.

Key words: Concentrate, Individual production, Grazing Management, Crossed design.

Received: 07/05/2024

Accepted: 05/08/2024

Introduction

In research models, improvements in management of grazing dairy production systems have driven increases in milk production per hectare[1]. Two of the main interrelated factors responsible for this increased productivity are stocking rate[2] and use of concentrate supplements[3]. Though already thoroughly studied[4], use of concentrate supplements continues to receive broad attention[5,6,7].

Concentrate supplementation is commonly used in grazing systems. It increases energy intake, which helps to optimize animal nutritional status and body condition[8], as well as individual milk production[9]. Supplementation can lead to changes in milk composition in terms of nutraceutical feed[10]. Animals consuming supplements generally reduce forage intake in the pasture, allowing greater forage utilization efficiency through increased stocking rates (SR). This in turn raises milk production per unit area, and improves milk composition[11]. In grazing systems, SR is vital to calculating system efficiency[12]. Increasing forage utilization efficiency leads to greater milk production per hectare, a principal goal in maximizing profitability per grazed area[13].

Rises in milk production per hectare in response to the higher SRs allowed by supplementation[14], are more affected by changes in SR than by changes in individual

production[15]. Responses to supplementation can also be influenced by the timing of concentrate administration because this variable influences pasture forage substitution[16], fiber digestion, and other ruminal fermentation variables[17]. Since ruminal environment, forage composition, and cattle feeding behavior follow circadian cycles, the effects caused by supplementation timing are attributed to changes in the diurnal routine[17].

Cows graze more intensively before sunset, regardless of the supplement provided in their diet[16]. Afternoon or evening grazing is therefore longer and more important for forage intake, due to the effect of circadian rhythms in photosynthesis, and accumulation of dry matter (DM), carbohydrates, and fatty acids, which facilitates forage particle decomposition during the initial phases of digestion[18]. Considering this, it was hypothesized that morning supplementation would result in greater individual milk production than afternoon supplementation or morning/afternoon supplementation (the most common practice). The study objective was to evaluate the impact of concentrate supplementation timing in a dairy grazing system as a tool to increase individual productive performance, SR, and consequently milk productivity per hectare.

Material and methods

Study location

In 2022, two grazing experiments were done at the Grazing Module of the Universidad Autónoma Chapingo, Texcoco, Estado de Mexico (19º 29' N, 98º 54' W; 2,240 m asl). The first ran from February 4 to March 26 (winter), and the second from June 6 to July 26 (spring-summer). Regional climate is temperate subhumid with summer rains, 636 mm average annual precipitation, and 15.2 ºC average annual temperature[19].

The experimental units were batches of six (winter) or five (spring-summer) lactating New Zealand Holstein cows in 17-d periods, and their respective grazing areas. The batches were homogenized based on initial live weight, number of births, days lactation and milk production during the two weeks prior to batch creation.

Pastures and grazing management

Ten pastures in a 4.5 ha total area were used. Forage was alfalfa (*Medicago sativa L.*) associated with orchard grass (*Dactylis glomerata L.*) of between two and three years age. Grazing was intensive and rotational, with an average of five days grazing followed by 42 days' rest in winter and 40 days' rest in spring-summer. Each pasture was divided into three equal sections corresponding to the three experimental treatments. A residual forage height (RFH) of 8 cm was maintained in the three treatments by controlling access to specific pasture areas for each batch of cows using a mobile electric fence. Forage was measured with a descending disc. Controlling RFH was essential because SR was a response variable.

Experimental design and treatments

The experimental design was 3 x 3 crossed[20], using three treatments corresponding to three post-milking supplementation timings: divided between morning and afternoon (AM-PM), morning (AM) and, afternoon (PM). A twenty-day, pre-experimental adaptation period was implemented during which the batches were formed, and the animals accustomed to grazing management practices and concentrate composition. Each experiment lasted 51 d, divided into three, seventeen-day periods. Each period was divided into two phases: a) a twelve-day adaptation to treatment concentrate level, and b) a five-day response variable data-recording phase. Based on previous results from the same site[15], a 5.0 kg DM cow^{-1} d^{-1} concentrate supplementation level was administered. Concentrate composition was calculated considering previously reported average forage composition[15], and prepared in the Grazing Module. The formulation was based on rolled corn, ground sorghum, gluten meal, bypass fat, molasses and minerals; average concentrate chemical composition in both experiments was 16.65 % crude protein (CP), 20.43 % neutral detergent fiber (NDF), and 4.98 % acid detergent fiber (ADF).

Measurement and experimental procedure

Pasture access was controlled using mobile electric fencing. Following a previously reported pasture management method[15,21,22], every day each batch of cows was initially given access to a 12 × 4 m area. Residual forage height (RFH) was measured frequently in 12 m-wide strips. When RFH reached 8 cm, an additional 2 m-wide area was opened, and RFH

measurement continued. This required that during a single day a batch of cows be moved several times within the same pasture unit.

Each pasture was divided into thirds of equal width, ensuring that grazing progress was similar between the batches of cows. Grazed area was measured before (night progress) and after (day progress) the daytime grazing period. The daily area grazed per experimental unit was used to calculate SR (Equation 1).

The concentrate supplement was administered in individual feeders in a separate pen, after each milking, and at the times corresponding to each treatment. When grazing in the pastures, the animals had free access to water in mobile containers located at one end of the grazing area (Table 1).

Table 1: Average times of dairy cow management activities in a grazing system with concentrate supplementation at different times of day

Activity	Time
Morning milking	06:00-07:00
Morning period in supplementation pen	06:30-07:30
Grazing (daytime)	08:00-15:00
Afternoon milking	15:00-16:00
Afternoon period in supplementation pen	15:30-16:30
Grazing (nighttime)	17:00-06:00 (+1 day)

Samples of offered forage (OF) and residual forage (RF) were collected from each experimental unit. Samples were collected by mowing five strips averaging 0.50 x 5 m to an 8 cm height using a mower (Truper®, Mexico)[23]. Residual forage samples were paired with the corresponding OF samples. All samples were dried in a circulating air oven at 50 °C to constant weight.

Composition of consumed forage was estimated based on samples collected by experimental unit. Each sample consisted of ten subsamples collected using the simulated grazing technique[24]; this was modified in that samples were collected at 8 cm above ground level. For measurement of nutritional composition, the forage samples and the concentrate were first dried at 55 °C to constant weight, then ground to 1 mm in a mill (Thomas model 4 Wiley®, USA). Following AOAC methods[25], CP was measured using the Micro-Kjeldahl method; NDF and ADF were estimated using a fiber analyzer (ANKOM 200, Ankom Technology, USA); and acid-insoluble ash (AIA) was also determined.

During the five-day measurement phase, milk production was recorded per cow using Alfa Laval® automatic meters during the morning and afternoon milkings. Milk composition was quantified individually using samples taken with an Alfa Laval® automatic sampler and plastic vials. Fat, protein and total solids in milk samples were measured with a milk analyzer (MilkoScan®, Foss, Denmark). Milk production per hectare was estimated using individual milk production and grazing cycle SR per pasture. Stocking rate (SR) was estimated using the weighted mean of the instantaneous stock density for occupied and rest periods, with Equation 1.

$$SR = ISD * OP/(OP + RP) \quad \text{(Equation 1)}$$

Where: SR= stocking rate [cows^{-1} ha^{-1} (grazing cycle)]; ISD= instantaneous stock density (cows^{-1} ha^{-1}); OP= occupied period (days); RP= rest period (days).

Cow live weight (LW) was measured using an electronic scale (TruTest®, New Zealand; 1 kg accuracy, 1,000 kg capacity) after morning milking, for 2 d at the beginning and two days at the end of each experimental period. After each weighing, body condition (BC) was estimated by two trained observers using a 1-to-5 scale[26]. Changes in LW and BC were calculated as the difference between the measurements taken at the beginning and end of each period, for each variable.

Statistical analysis

The statistical model includes the effects of period, batch and treatment:

$$Yijk = \mu + Period_i + Batch_j + Treatment_k + Eijk \quad \text{(Equation 2)}$$

Where: Yijk is the average value of LW, BC, SR, individual production, and fat, protein and total solids content across cows and measurement days. Period, batch and treatment were fixed effects. The experimental error was the interaction between these three factors, and it was assumed that this interaction was not of biological or practical importance. Due to differences in climate, forage growth and, in some cases, lactation stage, analyses were run within each experimental period. Response variable analysis was done using the GLM procedure in the SAS package[27]. The means were compared between treatments with LSMEANS using the Tukey-Kramer test.

Results and discussion

Residual forage height (RFH) did not differ between treatments ($P>0.05$), and averaged 8.4 cm in both experiments. This allowed forage utilization to remain at the same efficiency in all treatments. It also served as the basis for estimating the effect of concentrate administration timing on SR, and thus on milk production per hectare. Assigned areas averaged 210 m^2 per day per batch in winter and 233 m^2 in spring; no differences were observed between treatments.

No differences ($P>0.05$) between treatments were observed for OF, either in winter (average = 2,183 kg DM ha^{-1}) or in spring-summer (average= 2,630 kg DM ha^{-1})(Table 2). The lower figure in winter is to be expected since environmental conditions in winter such as low temperatures, frost, and low solar radiation and shorter photoperiod, result in a lower rate of forage accumulation[28,29]. In a study of an alfalfa-orchard grass association, decreased growth rates in winter were caused by low temperatures (<10 °C)[30]. In contrast, forage accumulation is greater in spring-summer because the forage growth season begins in late April and ends in mid-October. One study reported an accumulation 2,333 kg DM ha^{-1} in spring-summer[31], which is comparable to the 2,630 kg DM ha^{-1} in the present results.

Neither were differences observed in RF ($P>0.05$), which is due to the 8 cm residual height criterion used here. Using this criterion, grazing efficiency was 76 % in winter and 80 % in spring-summer. Average grazing efficiency was 78 %, similar to the 75.3 % reported for smaller pastures.

Table 2: Offered and residual forage (kg DM ha^{-1}) above 8 cm height, in pastures grazed by cows administered concentrate supplementation at three different timings, in winter and spring-summer experiments

Experiment	Parameter	Treatment	Mean	SE
Winter	OF	AM-PM	2140	34
		AM	2201	
		PM	2206	
	RF	AM-PM	495	14
		AM	527	
		PM	528	
Spring-summer	OF	AM-PM	2624	35
		AM	2672	
		PM	2655	
	RF	AM-PM	512	10
		AM	506	
		PM	517	

OF = Offered forage, RF = Residual forage, AM-PM, AM, PM = concentrate supplement administration timing, SE= standard error; $^{\epsilon}$ (P<0.05) = significance level.

The OF nutritional composition results showed decreases in NDF, ADF and CP between morning and afternoon measurements (Table 3). In the winter experiment, NDF decreased by 7.5 %, ADF by 3.8 % and CP by 7.7 %. In the spring-summer experiment, NDF decreased by 9.6 %, ADF by 8.1 % and CP by 10.4 %.

Table 3: Nutritional composition (% DM) of forage offered to grazing dairy cows administered a concentrate supplement at different timings during the day

	Winter			Spring-summer		
	Forage		SE	Forage		SE
Component	AM	PM		AM	PM	
DM	$22.3^{b\beta}$	25.5^{a}	0.41	$21.6^{y\mu}$	24.8^{x}	0.44
NDF	39.4^{a}	37.9^{b}	0.33	49.5^{x}	45.5^{y}	0.87
ADF	29.2^{a}	27.0^{b}	0.33	34.4^{x}	31.1^{y}	0.72
Ash	9.7	9.4	0.20	9.9	9.4	0.17
CP	19.5^{a}	18.0^{b}	0.26	20.3^{x}	18.2^{y}	0.11

AM, PM= sample time; DM= dry matter; NDF= neutral detergent fiber; ADF= acid detergent fiber; CP= crude protein; SE= standard error; (P<0.05) = significance level. $^{\beta\,\mu}$ Different letter superscripts in the same row and season indicate significant difference (winter= ^{ab}, spring-summer= ^{xy}) (P<0.05).

The decreases observed in NDF, ADF and CP between the morning and evening OF samples (Table 3) may be due to dilution of these components caused by diurnal fluctuations in soluble carbohydrates concentration as a product of plant photosynthetic activity[32]. These levels are similar to those reported previously. For example, in *Lolium perenne*, between 08:00 and 19:00 h, non-structural carbohydrates (not studied here) were found to increase by 30 % while NDF decreased 8.7 % and CP by 6.1 %, the latter in response to the rise in non-structural carbohydrates[32]. This coincides with reported higher ADF and CP contents in alfalfa forage harvested in the morning than in that harvested in the afternoon[33]. Fluctuations in photosynthetic product concentrations exhibit higher diurnal fluctuations in the leaves than in the stems and pseudostems[18].

Changes in LW were minor over the experimental period, the highest being 45 g cow^{-1} d^{-1} in the winter/AM-PM treatment (Table 4). This is greater (P<0.05) than in the winter/AM and winter/PM treatments. In contrast, the spring-summer/AM-PM treatment exhibited greater (P<0.05) LW gain than in the spring-summer/PM treatment but did not differ (P>0.05) from LW in the spring-summer/AM treatment. Changes in LW caused by supplementation are a function of lactation period; during the first third of lactation, supplementation can reduce weight loss, while later in lactation it can lead to increased weight gain[21]. No changes in body condition were observed between treatments (P<0.05).

Table 4: Changes in live weight and body condition in grazing dairy cows administered concentrate supplements at different times

Experiment	Parameter	Treatment			Mean	SE
		AM-PM	AM	PM		
Winter	Change LW	$2.3^{a\dagger}$	1.8^{b}	1.8^{b}	2	0.17
	Change BC	0.01^{a}	0.003^{a}	-0.006^{b}	0.002	0.005
Spring-Summer	Change LW	$1.9^{x\epsilon}$	1.7^{xy}	1.2^{y}	1.6	0.20
	Change BC	-0.004	0.008	-0.004	-0.00002	0.004

LW= live weight (kg); BC= body condition (units); AM-PM, AM, PM= concentrate supplementation timing; SE= standard error; $^{\dagger\epsilon}$ Different letter superscripts in the same row indicate significant difference (winter= ^{ab}, spring-summer= ^{xy}) (P<0.05).

In both experiments, milk production in the AM treatment was higher than in the other two treatments (Table 5): 6.1 % in winter (P=0.0002) and 8.5 % in spring-summer (P<0.0001). The overall average increase in milk production (both experiments) was 7.3 %.

Table 5: Milk production and composition in grazing dairy cows administered concentrate supplements at different times

Experiment	Parameter	Treatment			Mean	SE
		AM-PM	AM	PM		
Winter	Milk production, L cow^{-1} d^{-1}	21.7^{b†}	23.8^{a}	21.8^{b}	22.5	0.24
	Protein, %	3.5	3.6	3.4	3.5	0.02
	Fat, %	3.9	4.0	3.9	3.9	0.03
	Total solids, %	13.3	13.5	13.2	13.3	0.04
Spring-summer	Milk production, L cow^{-1} d^{-1}	20.3$^{y\epsilon}$	22.6^{x}	19.6^{y}	20.8	0.29
	Protein, %	3.6	3.5	3.5	3.6	0.03
	Fat, %	4.0	3.9	3.9	4.0	0.03
	Total solids, %	13.5	13.2	13.3	13.3	0.07

AM-PM, AM, PM= concentrate supplementation timing; SE= standard error; $^{\dagger\,\epsilon}$ Different letter superscripts in the same row indicate significant difference (winter= ^{ab} [P=0.0002], spring-summer= ^{xy} [P<0.0001]).

In a similar study (same study site, cow type, pastures, concentrate supplement level and grazing management criteria)[15], a winter/AM-PM treatment had 21.4 L cow^{-1} d^{-1} production, slightly higher than the 20.3 L cow^{-1} d^{-1} in the same treatment in the present study. However, the present spring-summer/AM treatment produced 23.8 L cow^{-1} d^{-1}, 10 % higher than in the previous study. This coincides with a similar study in which morning administration of concentrate increased (P<0.07) individual milk production by 0.5 L cow^{-1} d^{-1} compared to other timings[16]. In another study, milk production was 2.1 L cow^{-1} d^{-1} higher (P<0.001) when grazing dairy cows were administered concentrate in the afternoon than in the morning[34].

Milk protein, fat and total solids contents did not differ (P>0.05) between treatments. In the literature, milk composition results vary widely. For instance, one study found that milk protein content was higher (P<0.05) with evening supplementation than with morning supplementation or none at all[35]. This may have been caused by a restricted daily forage allowance, since a reduction in diet forage proportion in dairy cows increases milk production volume and protein concentration[35]. Another study reported that milk fat content was lower when supplements were administered only in the morning[36]. Finally, a third study found no differences in milk production, milk solids production or fat and protein concentration and

amount were observed with treatments with and without corn silage supplementation, and different grazing time allowances[37].

Concentrate administration timing had no effect (P<0.5) on SR in either experiment (Table 6); average SR in winter was 6.7 cows ha^{-1} and in spring-summer it was 5.1 cows ha^{-1}. Stocking rate is normally described as the number of cows per surface unit and time (SR_{Annual} = cows^{-1} ha^{-1} year^{-1}, or SR_{Daily} = cows^{-1} ha^{-1} d^{-1})[15]; however, in the present study it was quantified as $SR_{Grazing\ cycle}$.

Table 6: Stocking rate (cows ha^{-1} / grazing cycle) in grazing dairy cows administered concentrate supplements at different times

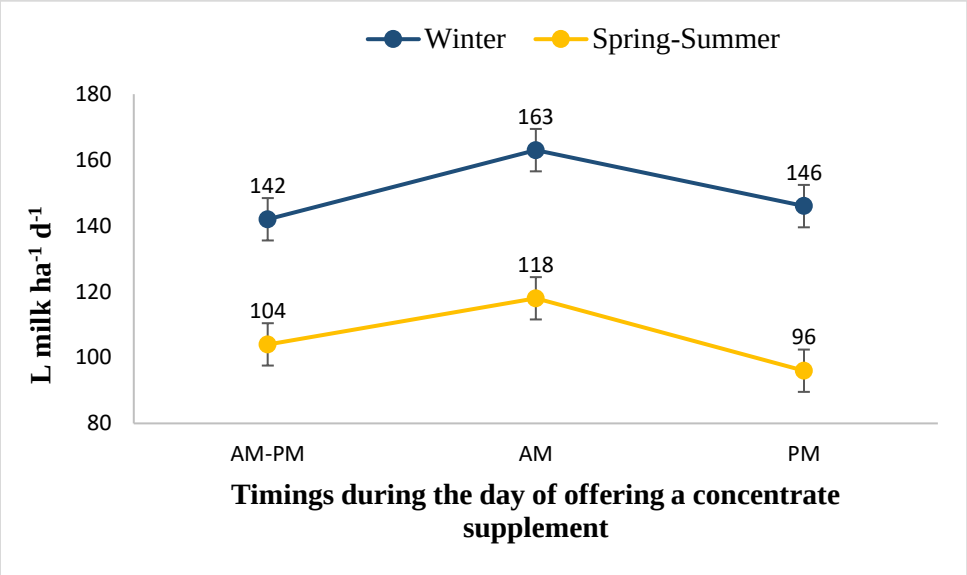
Experiment	AM-PM	SE	AM	SE	PM	SE
Winter	6.5	0.39	6.9	0.05	6.7	0.29
Spring-summer	5.1	0.38	5.2	0.23	4.9	0.22

AM-PM, AM, PM = concentrate supplementation timing; SE = standard error; (P<0.5).

The substitution effect caused by supplementation can allow increases in SR and consequently milk production per hectare[22]. At higher supplementation levels, the substitution effect reduces forage consumption in the pasture and, as a result, forage utilization efficiency[21]. If SR did not respond to changes in supplementation timing, no differences occurred in the substitution effect. This may be due to the fact that total supplementation level did not differ between the three treatments[38]. Treatments can cause changes in circadian rhythms in grazing activity and thus in forage intake[39]; only this type of effect could have caused differences in the substitution effect in the present study, but none were detected.

Milk production per hectare (Figure 1) was the result of individual production per SR. In the AM treatment of both experiments, this parameter exceeded the average of the other two treatments by 8.4 % during winter and 11.3 % during spring-summer. These differences were similar between experiments and originated in differences in individual production, since SR did not differ between treatments.

Figure 1. Milk production per hectare in grazing dairy cows administered concentrate supplements at different times



Offering concentrate once daily in the morning improved system productivity. This approach took advantage of changes in forage composition throughout the day such that, as mentioned elsewhere[40], maximum use of forage occurs in the afternoon, when its nutritional value is highest[35]. The improved milk production in the AM treatments was due to increased individual production, without changes in SR. This contradicts previous reports indicating that increases in milk production per hectare when SR varies respond to increases in SR rather than improvements in individual production[2,15,21].

The advantage of quantifying responses to supplementation in terms of milk production per hectare instead of milk production per cow is that this approach includes this technology's impact on the production system (production unit)[14,15], allowing more accurate estimation of its effect on system economic performance.

Conclusions and implications

Use of concentrate supplementation timing helped in attaining uniform forage utilization efficiency in the pasture, and consequently estimating stocking rate and milk production per hectare. Providing concentrate supplementation only after the morning milking increased individual milk production an average of 10.2 % and improved milk production per hectare by 9.9 %. This strategy raised production system efficiency without increasing inputs because it was based on circadian changes in forage composition and forage intake behavior.

Acknowledgements and conflicts of interests

The CONAHCYT supported the Ph.D. studies of the first author. The authors declare no conflicts of interest.

Literature cited:

1. McCarthy B, Delaby L, Pierce KM, Journot F, Horan B. Meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems. Animal 2011;5(5):784-794

2. Macdonald KA, Penno JW, Lancaster JAS, Roche JR. Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. J Dairy Sci 2008;91(5):2151-2163.

3. Bargo F, Muller LD, Delahoy JE, Cassidy TW. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. J Dairy Sci 2002;85(7):1777-1792.

4. Leaver JD. Effects of supplements on herbage intake and performance. In: Frame J. editor. Grazing. Occasional Symposium No. 19. British Grassland Society. 1985:79-88.

5. John AJ, Garcia SC, Kerrisk KL, Freeman MJ, Islam MR, Clark EF. The effect of temporal variation in feed quality and quantity on the diurnal feeding behavior of dairy cows. Animal 2019;13(11):2519-2526.

6. Bica GS, Pinheiro LC, Teixeira DL, De Sousa KT, Hötzel MJ. Time of grain supplementation and social dominance modify feeding behavior of heifers in rotational grazing systems. Front Vet Sci 2020;1-7.

7. Cahill L, Patton D, Reilly B, Pierce KM, Horan B. Grazing season length and stocking rate affect milk production and supplementary feed requirements of spring-calving dairy cows on marginal soils. J Dairy Sci 2023;106(2):1051-1064.

8. Stockdale CR. Effects of body condition score at calving and feeding various types of concentrate supplements to grazing dairy cows on early lactation performance. Livestock Sci 2008;116(1-3):191-202.

9. Albarrán M, Balocchi OA, Noro M, Wittwer F, Pulido RG. Effect of the type of silage on milk yield, intake and rumen metabolism of dairy cows grazing swards with low herbage mass. Anim Sci J 2016;87(7):878-884.

10. Ramírez-Mella M, Hernández-Mendo O, Ramírez-Bribiesca EJ, Améndola-Massiotti RD, Crosby-Galván MM, Burgueño-Ferreira JA. Effect of vitamin E on milk composition of grazing dairy cows supplemented with microencapsulated conjugated linoleic acid. Trop Animal Healt Prod 2013;45:1783-1788.

11. Bargo F. Suplementación en pastoreo: conclusiones sobre las últimas experiencias en el mundo. Facultad de Agronomía, Universidad de Buenos Aires 2003;1:1-21 https://www.agro.uba.ar/sites/default/files/catedras/bargo.pdf. Consultado 30 Nov, 2023.

12. Fales SL, Muller LD, Ford SA, O'sullivan M, Hoover RJ, Holden LA, *et al*. Stocking rate affects production and profitability in a rotationally grazed pasture system. J Prod Agr 1995;8(1):88-96.

13. Dillon PAT, Hennessy T, Shalloo L, Thorne F, Horan B. Future outlook for the Irish dairy industry: a study of international competitiveness, influence of international trade reform and requirement for change. Inter J Dairy Tec 2008;61(1):16-29.

14. Baudracco J, Lopez-Villalobos N, Holmes CW, Macdonald KA. Effects of stocking rate, supplementation, genotype and their interactions on grazing dairy systems: A review. N Z J Agr Res 2010;53(2):109-133.

15. Castro JC. Suplementación con concentrado sobre producción de leche individual y por hectárea de vacas Holstein en pastoreo [Tesis Maestría]. Texcoco, Estado de México; Universidad Autónoma Chapingo, 2020.

16. Sheahan AJ, Gibbs SJ, Roche JR. Timing of supplementation alters grazing behavior and milk production response in dairy cows. J Dairy Sci 2013;96(1):477-483.

17. Gekara OJ, Prigge EC, Bryan WB, Nestor EL, Seidel G. Influence of sward height, daily timing of concentrate supplementation, and restricted time for grazing on forage utilization by lactating beef cows. J Animal Sci 2005;83(6):1435-1444.

18. Gregorini P. Diurnal grazing pattern: its physiological basis and strategic management. Animal Prod Sci 2012;52(7):416-430.

19. García E. Modificaciones al Sistema de Clasificación Climática de Köpen. 5ta ed. México, DF: Instituto de Geografía-UNAM; 2004.

20. Herrera Haro JG, García Artiga C. Bioestadística en ciencias veterinarias: Procedimientos de análisis de datos con SAS. Universidad Complutense de Madrid: Madrid: área Ciencias de la Salud; 2010.

21. Amendola RD. A dairy system based on forages and grazing in temperate Mexico [PhD Thesis]. The Netherlands. Wageningen Universitiet, 2002.

22. Ramírez-Mella M, Hernández-Mendo O, Améndola-Massiotti RD, Ramírez-Bribiesca EJ, Mendoza-Martínez GD, Burgueño-Ferreira JA. Productive response of grazing dairy cows to fresh chopped maize supplementation under a small farming system in the Mexican Highlands. Trop Animal Health Prod 2010;(42):1377-1383.

23. Jiménez-Rosales JD, Améndola-Massiotti RD, Burgueño-Ferreira JA, Ramírez-Valverde R, Topete-Pelayo P, Huerta-Bravo M. Herbage intake of dairy cows in mixed sequential grazing with breeding ewes as followers. Trop Animal Health Prod 2018;(50):531-536.

24. Bonnet O, Hagenah N, Hebbelmann L, Meuret M, Shrader AM. Is hand plucking an accurate method of estimating bite mass and instantaneous intake of grazing herbivores? Range Eco Manag 2011;64(4):366-374.

25. AOAC. Official Methods of Analysis. 18th ed. Washington, DC; USA Association of Official Agricultural Chemists. 2005.

26. Garcia A, Hippen A. Feeding dairy cows for body condition score. Dairy Sci 2008;137.

27. SAS. SAS/STAT:9.3 User's Guide (3rd ed.) Cary, NC, USA: SAS Inst. Inc. 2010.

28. Esparza JZ, Hernández-Garay A, Pérez JP, Herrera JG, Haro FOG, Hernández PAM, *et al.* Análisis de crecimiento estacional de una pradera asociada alfalfa-pasto ovillo. Tec Pecu Mex 2009;47(2):173-188.

29. Cullen BR, Eckard RJ, Callow MN, Johnson IR, Chapman DF, Rawnsley RP, *et al*. Simulating pasture growth rates in Australian and New Zealand grazing systems. Aust J Agr Res 2008;59(8):761-768.

30. Álvarez FG, López OR. Rendimiento agronómico, digestibilidad *(in vitro*) y contenido de proteína cruda de nueve variedades de alfalfa (*Medicago sativa* L.) en Nazareno, Etla, Oaxaca [Tesis Profesional]. Texcoco, Estado de México. Universidad Autónoma Chapingo, 1992.

31. Lemus V, Guevara A, García JA, Gaspar D, García JG, Pacheco D. Producción de leche de vacas en pastoreo de alfalfa (*Medicago sativa*) en el altiplano mexicano. Rev Mex Cien Pecu 2020;11(1):1-18.

32. Delagarde R, Peyraud JL, Delaby L, Faverdin P. Vertical distribution of biomass, chemical composition and pepsin—cellulase digestibility in a perennial ryegrass sward: Interaction with month of year, regrowth age and time of day. Animal Feed Sci Tec 2000;84(1-2):49-68.

33. Putnam D, Mueller S, Marcum D, Frate C, Lamb C, Canevari M, *et al*. Diurnal changes in alfalfa forage quality. In: Proc, 28th Alfalfa Symposium. California, NV. 1998:31-39.

34. Trevaskis LM, Fulkerson WJ, Nandra KS. Effect of time of feeding carbohydrate supplements and pasture on production of dairy cows. Live Prod Sci 2004;85(2-3):275-285.

35. Jenkins TC, McGuire MA. Major advances in nutrition: impact on milk composition. J Dairy Sci 2006;89(4):1302-1310.

36. Abrahamse PA, Tamminga S, Dijkstra J. Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. J Agr Sci 2009;147(6):721-730.

37. Al-Marashdeh O, Gregorini P, Edwards GR. Effect of time of maize silage supplementation on herbage intake, milk production, and nitrogen excretion of grazing dairy cows. J Dairy Sci 2016;99(9):7123-7132.

38. Bargo F, Muller LD, Kolver ES, Delahoy JE. Invited review: Production and digestion of supplemented dairy cows on pasture. J Dairy Sci 2003;86(1):1-42.

39. Gregorini P. Estado interno. Estímulos que motivan el consumo y ciertas conductas ingestivas de rumiantes en pastoreo. Producción Animal en Pastoreo. Buenos Aires, Ediciones INTA 2011;513-65.

40. Soca P, González H, Manterola H, Bruni M, Mattiauda D, Chilibroste P, Gregorini P. Effect of restricting time at pasture and concentrate supplementation on herbage intake, grazing behaviour and performance of lactating dairy cows. Liv Sci 2014;(170):35-42.

https://doi.org/10.22319/rmcp.v15i4.6338

Article

Productive response, carcass traits, and meat quality of sheep fed with increasing levels of crushed dry fruits of *Acacia farnesiana*

Miguel Ángel Zarza-Albarrán [a]

Agustín Olmedo-Juárez [b*]

Pedro Mendoza- de Gives [b]

Jaime Ancelmo-Mondragón [a]

Javier Arece-García [c]

Francisca Aviles-Nova [a]

Benito Albarrán-Portillo [a]

Rolando Rojo-Rubio [a*]

[a] Universidad Autónoma del Estado de México, Centro Universitario UAEM-Temascaltepec, km 67.5. Carretera Federal Toluca-Tejupilco, Temascaltepec, 51300, Estado de México. México.

[b] Instituto Nacional de Investigaciones Agrícolas, Forestales y Pecuarias. Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad. Morelos, México.

[c] Estación Experimental de Pastos y Forrajes Indio Hatuey, Universidad de Matanzas, Central España Republicana, Matanzas, Cuba.

*Corresponding author: olmedo.agustin@inifap.gob.mx; aolmedoj@gmail.com

Abstract:

The present research evaluated the productive response, carcass traits, and meat quality of sheep fed with increasing levels of crushed dry fruits of *Acacia farnesiana* (CDFAf). Thirty-two sheep (20 ± 2.5 kg and age 70 ± 15 d) were used. Four levels of CDFAf (T0=0.0, T1=1.5, T2=3.0, and T3=4.5 %) were evaluated. The stages of growing (21 d) and finishing (49 d) were assessed. Initial and final live weight (ILW and FLW), dry matter intake (DMI), daily and total weight gain (DWG and TWG), and feed efficiency

(FE) were measured. On d 70, the animals were slaughtered to determine carcass traits (CaT), carcass morphometry (CaM), primal cut weight (PrCW), viscera weights (ViW), and meat quality parameters (MeQ). The addition of CDFAf did not affect the DMI; it positively affected DWG and TWG in the growing stage (P<0.05). No differences (P>0.05) were found in the productive variables during the finishing stage. The PrCWs differed (P<0.05), with T1 and T3 registering the highest weights in the loin and neck, respectively. The MeQ shows significant differences in the shear force and water retention capacity at 24 and 72 h. Better tenderness in the meat was observed in T1, and a greater loss of water and greater shear force was observed in T3. It is concluded that CDFAf improves weight gain and yield of primal cuts.

Keywords: Sheep, Safety, Meat quality, Huisache.

Received: 13/10/2022

Accepted: 28/06/2024

Introduction

Currently, intensive sheep production systems are affected by the increase in the cost of inputs used in feeding, such as energy ingredients (corn and sorghum) and protein ingredients, such as soybeans and rapeseed[(1)], a situation that has an impact on the production costs of small-scale sheep systems[(2)]. Given this situation, it is necessary to incorporate nutritional strategies that reduce the use of external inputs, such as the use of local forage resources from trees[(3)] and leguminous shrubs, which have nutraceutical properties (protein and bioactive compounds) that, at low concentrations (50 g/kg of DM) in the diet, could improve the productive response of the animals. Forage plants that have a high concentration of condensed tannins, flavonoids, saponins, organosulfur compounds, and essential oils have the ability to favorably modify rumen fermentation by reducing the oxidation of amino acids[(4)], antimicrobial action on some intestinal microorganisms, improving intestinal health and consequently the absorption of nutrients, increasing propionic acid production[(5)], increasing food palatability, and stimulating intake by decreasing lipid oxidation[(6)]. In addition, these plants can continue to produce biomass under conditions of low soil moisture content[(5)]. *Acacia farnesiana* is a shrubby legume distributed in tropical and subtropical climates of Mexico, and one of its best adaptive agronomic benefits is that it is one of the first plants to appear in soils once they have been degraded by anthropogenic activities, giving rise to ecological succession to plants that are more demanding in nutrients[(7)]. This plant species represents a source of nutrients mainly of protein origin (up to 20 % of CP)[(8)] and high digestibility of organic matter[(7)]; its fruits are rich in secondary metabolites (condensed tannins, flavonoids, and polyphenolic compounds), chemical compounds that benefit animal health by improving its productive performance and meat quality[(8-13)]. Likewise, it has been reported that some

secondary metabolites present in *A. farnesiana*, such as flavonoids and tannins, contain antimicrobial, anti-inflammatory, antioxidant, and anthelmintic properties[11-16]. There is evidence that adding inclusion levels of up to 12 % of *A. farnesiana* dry fruits in diets (dry basis) for sheep does not affect production parameters[7]. For this reason, the present research aimed to evaluate the inclusion of increasing levels based on the amount of an organic fraction (EtOAc-F) present in crushed dry fruits of *A. farnesiana* in sheep feed during the growing and finishing stages in pen on the production parameters, carcass traits, primal cuts, carcass quality, and changes in viscera weight.

Material and methods

Experimental site

The study was conducted out at the Metabolic Unit of the UAEM-Temascaltepec University Center, located at 19º 2′ 40″ N and -100º 2′ 42″ W, at 1,800 m asl, in Temascaltepec de González, State of Mexico, Mexico. With rains in summer and an average annual temperature of 18 °C[17].

Plant material

Ripe fruits of *A. farnesiana* were collected in seven different localities (7 shrubs per site) in the municipality of Tejupilco (latitude 18°90′ 58″ N and longitude -100°15′27″ W) in the southwestern area of the State of Mexico, Mexico, during the spring. The fruits were collected between 0600 and 0700 h and transferred to the Animal Nutrition Laboratory of the UAEM-Temascaltepec University Center, where they were dried in the shade until they reached a constant weight and then ground in a hammermill (New Holland, 2315) to a particle size of 5 mm. This research group[14,15] previously reported the anthelmintic activity and identification of the main secondary metabolites of the plant material used in the present study.

Animals and feed

Thirty-two (32) crossbred male sheep (Katahdin x Charollais; LW 20 ± 2.5 kg and age 70 ± 15 d) were used; upon arrival at the Metabolic Unit of the UAEM-Temascaltepec University Center, they were weighed to group them according to their weight from highest to lowest and form eight homogeneous blocks of four animals each. Each animal was housed in an individual pen (0.8 x 1 m), which was equipped with a feeder and drinker. In each block, treatments were randomly assigned. After this, the sheep received intramuscularly one milliliter of ADE vitamin complex (Vigantol ®), equivalent to 250,000 IU of vitamin A, 37,500 IU of vitamin D3, and 25 mg of vitamin E, and 2.5 ml of 8-way bacterin (BOBACT 8 ®) for the prevention of clostridial diseases and pneumonia.

All animals received experimental diets (Table 1) for the growing stage (15 % CP and 2.9 Mcal/kg) and another for the finishing stage (14 % CP and 3.0 Mcal/kg), according to their nutritional requirements[18]. Both diets underwent proximate chemical analysis[19] and fiber fractionation[20] (Table 2). The diet was administered at three frequencies: 0700, 1300, and 1900 h, under the following proportions: 30, 30, and 40 %. All animals were fed throughout the experiment, considering their voluntary consumption, and they received clean and fresh water at will.

Table 1: Experimental diets for growing and finishing sheep added with different levels of crushed dry fruits of *Acacia farnesiana*

Ingredients (%)	Growing				Finishing			
	¥Control	T1	T2	T3	¥Control	T1	T2	T3
Rolled corn	37.8	37.1	36.3	35.3	50.0	50.0	50.0	50.0
Soybean meal	9.0	9.0	9.0	9.0	7.0	7.0	7.0	7.0
Rapeseed meal	6.0	6.0	6.0	6.0	7.5	7.5	7.5	7.5
Whole sorghum	9.0	9.0	9.0	9.0	9.3	8.5	7.5	6.5
CDFAf[Ψ]	0.0	1.5	3.0	4.5	0.0	1.5	3.0	4.5
Molasses	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Urea	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Alfalfa hay	22.5	21.7	21	20.5	10.0	9.3	8.8	8.3
Corn stover	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Mineral premix	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Calcium carbonate					0.5	0.5	0.5	0.5
Common salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

[¥] Treatments: Control: 0.0, T1: 1.5, T2: 3.0, and T3: 4.5 as % inclusion (BD) of the diet, [Ψ]CDFAf, crushed dry fruits of *Acacia farnesiana*.

Table 2: Chemical composition (%) of experimental diets and crushed dry fruits of *Acacia farnesiana*

Nutrient (%)	Growing				Finishing				*A. farnesiana*
	¥Control	T1	T2	T3	¥Control	T1	T2	T3	
DM	91.89	91.99	91.95	91.84	91.65	91.39	91.64	91.03	87.54
CP	15.17	15.45	15.35	15.06	14.32	14.04	14.12	14.08	12.52
EE	3.81	3.85	3.38	3.96	4.06	4.36	4.14	4.04	3.27
NDF	25.08	29.31	33.06	34.62	19.12	20.08	20.46	22.37	38.80
ADF	21.25	24.86	26.67	28.76	16.31	17.43	16.87	18.46	34.22
OM	89.80	90.20	90.05	90.20	91.10	91.40	91.10	90.60	91.30
Minerals	10.20	9.80	9.95	9.80	8.90	8.60	8.90	9.40	8.70

[¥] Treatments: Control: 0.0, T1: 1.5, T2: 3.0, and T3: 4.5 as % inclusion (BD) of the diet; DM= dry matter, CP= crude protein, EE= ethereal extract, NDF= neutral detergent fiber, ADF= acid detergent fiber; OM= organic matter.

Experimental test

The feeding test lasted 80 d, of which 10 were for adaptation to the pen and diets, and there were two experimental periods: growing stage for 21 d and finishing stage for 49 d of feeding. The treatments were different levels of crushed dry fruits of *A. farnesiana* (CDFAf): Control: 0, T1: 1.5, T2: 3.0, and T3: 4.5 %, of the basal diet (BD) both in the growing stage and the finishing stage. For the inclusion levels of CDFAf, they were based considering the bioactive compounds of an organic fraction (EtOAc-F), using the same batch of pods as those of the present study. The specific chemical compounds within this fraction were: gallic acid, ethyl gallate, naringin, and naringenin[15]. The yield of EtOAc-F was 3.75 %, which was equivalent to 562, 1,125 and 1,687 mg of EtOAc-F in T1, T2, and T3, respectively.

Evaluation of the productive response

After the 10 d of adaptation to the individual pens and feeding, the animals were weighed for three consecutive days (with prior fasting) to know the initial live weight (ILW), then they were weighed on d 21 (growing phase) and d 70 (finishing period). Dry matter intake, total weight gain, daily weight gain, feed conversion, and feed efficiency were recorded throughout the experimental phase.

Post mortem variables

On d 70 of the experimental period, the animals were transferred to a private slaughterhouse in the municipality of Capulhuac, State of Mexico, to be slaughtered according to NOM-033-SAG/ZOO-2014 and Colomer-Rocher *et al*[21]. The live weight (LW) of the animals when leaving the farm and arriving at the slaughterhouse was recorded to estimate the farm yield (farm yield, %= (LW arrival at the slaughterhouse, kg/LW exit of the farm, kg)*100. Twelve (12) hours after arrival at the slaughterhouse, the LW was previously recorded to determine the commercial yield (%)= (hot carcass, kg/LW at slaughter, kg)*100.

Viscera and byproducts

Once the animal was slaughtered, the weights of blood, skin, head and legs, red viscera (heart, liver, lungs, and trachea) and empty green viscera (rumen, reticulum, omasum and abomasum, and large and small intestines) were recorded. The weight of some organs of the reproductive system (testicles and penis) and the weight of the total internal fat of the thoracic and abdominal cavities were also recorded.

Carcass traits and quality

At 45 min *post mortem*, the weight of the hot carcass (portable digital scale, Rhino), pH and temperature (Hanna potentiometer) of the *Longissimus thoracis* muscle between the 12th and 13th ribs were recorded[22]. The carcass was then taken to the cold chamber (4 °C) and at 24 h, the weight of the cold carcass, pH, and temperature were recorded. The color of the *Pectoralis profundus* muscle and the color of the superficial fat of the *Gluteus medius* muscle were measured; this variable was evaluated by the L* (lightness), a* (reddish), and b* (yellowish) system[23] with a Minolta colorimeter (Chroma Metro CR-200, Minolta Camara C., Osaka, Japan)[22]. GR grades were also measured, which indicate the total depth of tissue (mm) between the carcass surface and the rib, over the 12th rib region and at a point 11 cm from the midline; this indicator estimates subcutaneous fat: little or no fat cover (GR 0 to 4 mm), moderate fat cover (GR to 9 mm), abundant fat cover (GR 10 to 15 mm), excessive fat cover (GR >15 mm)[24]. Between the 12th and 13th rib, the area of *Longissimus thoracis* muscle was determined; the area under the rib was measured in the 12th rib by using a plastic grid or by tracing the eye on acetate paper and then using a grid (GRID-USDA) to determine the area in centimeters[25].

Morphometric characteristics and primal carcass cuts

Cañeque *et al*[22] and Colomer-Rocher *et al*[21] methodologies were used to measure carcass length, rump perimeter, leg length and circumference (tape measure), and the greatest and smallest width of the thorax (metric compass). The entire carcass was divided to record weight (Torrey digital scale with an accuracy of 0.05 g) of commercial parts: legs, neck, shoulder, rack, ribs, and loin[26].

Water loss, shear force, and meat color

For the meat quality analysis, 350 g of meat was taken from the *Longissimus thoracis* muscle from the 6th to 3rd rib of the cold carcass. The sample was deposited in a cooler to be transported to the meat quality laboratory of the UAEM-Temascaltepec University Center, which was used to determine the variables: drip water loss, shear force, and meat color (24, 48, and 72 h). Honikel's[27] technique was used to determine drip water loss. Two fat-free samples of 50 g each with a thickness of 1.5 cm were taken. Each sample was hooked and placed in an airtight bag, so that the meat was suspended inside the bag. In this way, all samples were hung inside a refrigerator at 4 °C. Weights were recorded at 24, 48, and 72 h later (analytical scale, Ohaus ± 0.05 g). Drip water loss was calculated using the following formula:

$$Drip\ water\ loss\ (\%) = \frac{Sample\ final\ weight\ (g)}{Sample\ initial\ weight\ (g)}\ X\ 100$$

In meat shear force, Bratzler's[28] methodology was used. Samples 4 cm long x 4 cm wide and 2.5 cm thick; these samples were previously vacuum packed and refrigerated at 4 °C for 3 d in order to reach 80 % softening. After that period, the samples were unpacked and placed in a plastic bag, sealed, and cooked in a double boiler (70-75 °C) for an hour and a half; at the end, the internal temperature was recorded, they were left to cool (30 min in clean water), and the shear force (kg) parallel to the muscle fibers was determined with the help of a texture meter (TAXT2, Stable Microsystems Corp, NY, USA) equipped with WarnerBrazler shear blades at a speed of 50 mm/min.

Meat color was determined with a Minolta CR-20 colorimeter, Konica Minolta, Osaka, Japan, using the CIE methodology[29]. It measures the color with the Hunter system: high values of L* are associated with pale colors: 0 (black), 100 (white); a*, high values determine a higher intensity of red: a*>0 (red), a*<0 (green); b*, high values are associated with a more yellowish tone of the meat: b*>0 (yellow), b*<0 (blue). These measurements were made during the 24 h *post mortem* using a sample of 4 x 4 cm with a thickness of 2.5 cm. The same sample was refrigerated at 4 °C for the determinations at 48 and 72 h. Readings were taken at three sample sites free of excess intramuscular fat and blood spots.

Experimental design and analysis of results

The results obtained were subjected to an analysis of variance using the GLM procedure of SAS[30] under a randomized complete block design, taking the animals' initial live weight (ILW) as a blocking factor, which was used as a covariate in the statistical analyses. The comparison of means between treatments was determined with Tukey's test; significant differences were declared when $P \leq 0.05$ and trends when $0.05 < P \leq 0.1$.

Results

Productive response

Dry matter intake was not affected ($P \geq 0.05$) by the addition of the different levels of crushed dry fruits of *A. farnesiana* (CDFAf) in both evaluation periods. During the growing period of the animals, CDFAf increased ($P \leq 0.05$) DWG, TWG, and FLW, while FE tended ($P=0.1$) to improve (Table 3). During the finishing stage, no significant differences or trends ($P>0.1$) were found between treatments.

Table 3: Productive behavior of sheep during the growing and finishing stages receiving different levels of crushed dry fruits of *Acacia farnesiana*

Stage/Variable		Treatments				SEM	*P*-value
		Control	T1	T2	T3		
Growing	ILW, kg	22.87	22.94	22.79	22.76		
	FLW, kg	28.36^{b}	29.95^{ab}	29.83^{ab}	30.43^{a}	0.44	0.02
	DMI, kg/d	1.50	1.53	1.49	1.62	0.17	0.45
	DWG, kg/d	0.26^{b}	0.33^{ab}	0.33^{ab}	0.36^{a}	0.05	0.02
	TWG, kg	5.52^{b}	7.10^{ab}	6.99^{ab}	7.59^{a}	1.25	0.02
	FE, kg	0.17^{b}	0.22^{a}	0.22^{a}	0.22^{a}	0.04	0.10
Finishing	ILW, kg	28.05	29.28	29.65	30.91		
	FLW, kg	45.69	45.70	46.88	46.23	2.79	0.81
	DMI, kg/d	1.52	1.50	1.50	1.63	0.17	0.52
	DWG, kg/d	0.33	0.33	0.35	0.34	0.05	0.82
	TWG, kg	16.29	16.28	17.45	16.82	2.78	0.82
	FE, kg	0.21	0.21	0.23	0.21	0.30	0.06

¥ Treatments: Control: 0.0, T1: 1.5, T2: 3.0, and T3: 4.5 as % inclusion (BD) of the diet, ILW= initial live weight, FLW= final live weight, DMI= dry matter intake, DWG= daily weight gain, TWG= total weight gain, FE= feed efficiency. SEM= standard error of the mean.

[ab] Different literal in the same row indicates differences ($P \leq 0.05$).

Carcass traits, primal cuts, and viscera weight

Carcass traits (Table 4) and morphometry (Table 5) were not affected ($P > 0.05$) by the addition of CDFAf. In the commercial primal cuts (Table 6), T3 tended to improve ($P = 0.09$) neck weight. The addition of CDFAf at the 1.5 % level improved ($P \leq 0.01$) the weight of the loin. No significant differences ($P > 0.05$) were found in the non-meat components between the treatments evaluated.

Table 4: Carcass traits of sheep finished in pens added with different levels of crushed dry fruits of *Acacia farnesiana*

Variable	Treatments				SEM	*P*-value
	¥Control	T1	T2	T3		
FY, %	46.63	48.35	48.37	48.50	2.03	0.24
CY, %	50.93	52.70	52.87	52.81	1.95	0.17
L* carcass	38.72	39.22	40.17	41.52	2.90	0.26
a* carcass	10.45	12.43	10.42	10.20	2.03	0.13
b* carcass	8.15	9.94	6.02	7.24	2.90	0.09
L* fat	70.92	69.62	69.62	70.65	3.05	0.75
a* fat	1.72	1.68	2.18	2.20	0.82	0.45
b* fat	10.39	10.67	10.97	11.03	1.25	0.73
pH45	6.61	6.62	6.60	6.49	0.17	0.44
pH24	5.83	5.66	5.65	5.80	0.21	0.26
T°45	28.34	29.18	28.82	29.60	1.48	0.39
T°24	1.72	2.43	2.06	2.31	1.03	0.54
Back fat, mm	2.43	2.79	3.26	2.92	0.88	0.34
GR Grades	10.94	12.06	12.75	12.48	2.89	0.62
RiEA, cm^2	21.9	23.7	23.0	22.7	2.71	0.64

¥ Treatments: Control: 0.0, T1: 1.5, T2: 3.0, T3: 4.5 as % inclusion (BD) of the diet, FY= farm yield, CY= commercial yield, RiEA= rib eye area.

Table 5: Morphometry of carcasses of sheep finished in pens added with different levels of crushed dry fruits of *Acacia farnesiana*

Variables	Treatments				SEM	*P*-value
	¥Control	T1	T2	T3		
HCW, kg	21.75	23.11	23.29	23.29	1.65	0.22
CCW, kg	21.19	22.44	22.65	22.61	1.67	0.27
CL, cm	66.27	65.07	66.52	66.69	2.01	0.39
LL, cm	35.56	34.58	36.23	34.89	1.81	0.30
LD, cm	41.14	41.49	43.33	42.33	1.97	0.16
RP, cm	61.62	62.18	63.18	59.49	5.12	0.54
RW, cm	21.34	22.05	21.85	22.00	1.04	0.52
LWT, cm	23.47	24.45	23.59	24.51	1.73	0.50
SWT, cm	19.23	19.67	19.54	19.53	0.91	0.80

¥ Treatments: Control: 0.0, T1: 1.5, T2: 3.0, and T3: 4.5 as % inclusion (BD) of the diet. SEM= standard error of the mean. HCW= hot carcass weight, CCW= cold carcass weight, CL= carcass length, LL= leg length, LD= leg diameter, RP= rump perimeter, RW= rump width, LWT= largest width of the thorax, and SWT= smallest width of the thorax.

Table 6: Weight of primal cuts (kg) of sheep added with different levels of crushed dry fruits of *Acacia farnesiana*

Variable (kg)	Treatments				SEM	*P*-value
	¥Control	T1	T2	T3		
Legs	6.74	7.09	6.65	7.18	1 .14	0.75
Neck	1.01 [b]	0.98 [b]	1.02[b]	1.3[a]	0.18	0.09
Shoulder	6.07	6.44	7.01	6.91	1.24	0.43
Rack	1.98	2.11	2.12	2.12	0.33	0.82
Ribs	3.51	3.34	3.29	3.26	0.50	0.75
Loin	1.88[b]	2.32[a]	2.17[ab]	2.16[ab]	0.25	0.01

¥ Treatments: Control: 0.0, T1: 1.5, T2: 3.0, T3: 4.5 as % inclusion (BD) of the diet. SEM= standard error of the mean.

[ab] Different literal in the same row indicates statistical differences ($P \leq 0.05$).

Meat quality

In the meat quality variables (Table 7), differences ($P<0.05$) were observed on drip water loss at 24 and 72 h, with T1 having the lowest water loss with 6.76 %, while T2 presented the highest runoff with 8.99 % at 72 h. The shear force also showed significant differences ($P<0.05$), where the control group obtained a lower shear force compared to the other treatments.

Table 7: Quality parameters of meat from sheep finished in pens added with different levels of crushed dry fruits of *Acacia farnesiana*

Variable/Treat	Hour	Treatments				SEM	*P*-value
		¥Control	T1	T2	T3		
L* meat		29.45	28.93	28.29	28.00	2.42	0.64
a* meat	H0	8.45	8.03	8.29	7.68	1.20	0.60
b* meat		9.04	8.70	8.80	8.36	1.19	0.72
L* meat		33.22	32.69	32.6	31.134	2.87	0.51
a* meat	H24	8.35	9.06	9.13	8.80	1.59	0.75
b* meat		10.90	11.10	10.20	9.80	2.38	0.67
L* meat		32.83	32.96	32.45	32.15	2.35	0.90
a* meat	H48	9.61	10.26	10.67	10.31	1.27	0.42
b* meat		11.27	11.59	12.17	11.71	1.54	0.71
L* meat		32.03	32.30	30.93	31.33	1.88	0.48
a* meat	H72	9.61	10.26	10.67	10.31	1.01	0.13
b* meat		12.56	12.49	13.17	12.36	1.38	0.66
DWL (%)	H24	2.19[ab]	2.34[ab]	3.28[a]	1.75[b]	0.911	0.02
	H48	4.71	4.39	5.55	3.88	1.38	0.14
	H72	7.95[ab]	6.76[b]	8.99[a]	7.61[ab]	1.19	0.01
SF (kg)		3.07[b]	3.60[ab]	4.79[a]	3.74[ab]	0.93	0.01

¥ Treatments: Control: 0.0, T1: 1.5, T2: 3.0, T3: 4.5 as % inclusion (BD) of the diet. SEM= standard error of the mean, DWL= drip water loss, SF= shear force.
ab Different literal in the same row indicates statistical differences ($P \leq 0.05$).

Discussion

Productive behavior

Dry matter intake (DMI). The DMI is one of the most critical parameters when using ingredients rich in phenolic compounds (condensed tannins, hydrolyzable tannins, and flavonoids) as part of the animals' diet. Previous studies carried out by this research group on the same CDFAf samples found that they contain phenolic compounds, such as ethyl gallate, methyl gallate, gallic acid, naringin, and naringenin[14,15]. These compounds could be interacting in the metabolism of dietary nutrients and modifying the productive response of animals; this coincides with other studies that have reported that the total phenols of *A. farnesiana* fruits may be around 397.5 g/kg of dry matter[31]. In general, phenolic compounds have different bioactive effects when consumed by animals, for example, flavonoids, such as naringin and naringenin that were found in the same dried pods of *A. farnesiana* that were used in the present study and those of previous studies[14,15], could increase the digestion of structural carbohydrates (cellulose and hemicellulose) of the plant cell walls, and they could also modify microbial protein synthesis, favoring cellulolytic species and inhibiting methanogenic protein[32]. Biologically, this can be explained by the fact that phenolic compounds, such as flavonoids and condensed tannins, due to their molecular weight, can form complexes with dietary proteins and carbohydrates through four chemical reactions: a) hydrogen bonds between the hydroxyl radicals of the phenolic groups and the oxygen of the amide groups of the peptide bonds of the proteins, which can be reversible depending on the pH of the medium, b) hydrophobic interactions between the aromatic ring of the phenolic compounds and the hydrophobic regions of the protein, c) ionic bonds between the phenolate ion of gallic acid and the cationic site of the protein; this type of complexes are exclusive to hydrolyzable tannins and are reversible[33]. The gallic acid found in CDFAf is part of the chemical structure of hydrolyzable tannins and could form complexes with dietary proteins, modifying the site of digestion and absorption of nutrients, and finally, d) when polyphenols oxidize to quinones, they can form complexes with dietary proteins through covalent bonds; this type of complex is reversible[33]. Considering that the highest inclusion level of CDFAf in the present study was 45 g/kg DM and 39.7 % of that amount is total phenols, it is estimated that the diet only had 17.86 g of bioactive compounds, a concentration that could have a synergistic effect with the metabolism of dietary nutrients. Additionally, in the present study, the addition of CDFAf did not show differences between treatments for dry matter consumption in both production stages (Table 2), which could be related to the levels of inclusion in the diet of the bioactive compounds that were used, as they were below 50 g/kg DM, a concentration that has been considered beneficial as it does not have a negative effect on the voluntary consumption of animals. Intakes greater than this amount can negatively affect dry matter intake and,

consequently, the productive response of animals[33]. Other studies have also reported no negative effect of including *A. farnesiana* in the diet when 120 to 240 g/kg of DM were used; on the contrary, when the inclusion was 300 g/kg of DM, consumption in sheep was increased[7,34]. It is important to consider that, in this type of study, the specific bioactive compounds found in the DM of plants must be determined because the biological response in animals will depend on the chemical nature and concentration of each of them. In this sense, Quiroz-Cardoso *et al*[9] mention that the condensed tannins in fruits of *A. farnesiana* do not influence consumption or palatability index, but the amount of total phenols does affect this parameter. The concentration of secondary compounds in *A. farnesiana* can be variable and depends on the state of maturity of the fruits, the soil and environmental conditions in which they develop, and the morphological and chemical nature of the type of compound[9,10,35]. Therefore, for future research, the state of ripeness of the fruits and the quantification of specific bioactive compounds should be considered.

Weight gain. Both daily weight gain (DWG) and total weight gain (TWG) in the growing stage were positively affected by treatments that included CDFAf, which were reflected in the final live weight (FLW) of the animals. The DWG found in this study was 260, 330, 330, and 360 g/d for the growing period and 330, 330, 350, and 340 g/d in the finishing stage for the control, T1, T2, and T3, respectively. The increase in production variables during the growing stage could be attributed to the fact that the animals consumed a diet higher in protein, and if it is considered that the phenolic compounds (ethyl gallate, methyl gallate, gallic acid, naringin, and naringenin) present in CDFAf are chemical compounds of high molecular weight[4] and complex chemical structure with a large number of hydroxyl groups, which can form complexes[36] with the amino acid proteins and reserve and structural polysaccharides of the diet[6], which at neutral pH are insoluble, which, when passed to the abomasum, dissociate due to the effect of acidic pH[37-38], increasing the pool of metabolizable protein to the duodenum, thus increasing the availability of amino acids for muscle protein synthesis, which translates into greater weight gain, as happened in the animals that received CDFAf in the present research.

Another mechanism may be the alteration that they generate on the bacterial populations of the rumen since they can inhibit the growth of protozoa and fibrolytic bacteria, and in turn, stimulate the proliferation of amylolytic bacteria such as *Succinimonas amylolytica* and *Selenomonas ruminantium*, which produce propionate[39]. Likewise, adding saponins from some species improves the efficiency of microbial protein synthesis, leading to a more energy-efficient fermentation process[39]. In this context, future studies on rumen fermentation parameters, bacteria count, and secondary metabolites in rumen will be considered. In many tropical and subtropical regions of Mexico and the world, there are various trees and shrubs rich in these bioactive compounds, which could be used as a nutraceutical strategy to improve animal productivity in rural areas of the world where the use of concentrated protein or energy feeds is not widely available. There are other studies that have included tree and shrub forages in animal feed; specifically, when *Guazuma ulmifolia*[40] was included, there was an improvement in weight gain; the same

happened in García-Winder *et al*'s[7] research when including 12 % of *A. farnesiana* fruits in the diet of growing Pelibuey ewe lambs.

Carcass traits, primal cuts, and viscera weight

Carcass quality is one of the most important parameters to evaluate in sheep production and marketing processes because it largely determines the selling price.

Carcass color. In the present study, the color of the carcass was similar to that reported by Jaborek *et al*[41] for L* with 40.91; in this sense, L* values are affected by myoglobin concentration, which varies with the age of the animals[41]; in contrast, the values of a* and b* differ from those reported by Jaborek *et al*[41]. This variation in the results found in this study and previous studies is possibly due to the age of the animals and the type of diet[26].

Fat color. The color of the fat in this study did not show significant differences between treatments compared to the control; the values found for L* coincide with those reported in other studies[41], while the values of a* of the same authors (8.63) were higher than those found in this study, an effect attributed to the type and level of energy in the diet and age and sex of the animals evaluated; however, they also mention that handling during slaughter can be important. Regarding the values of b*, those found in the present study were slightly higher than those found by Jaborek *et al*[41]; this yellowish color could be attributed to the carotenoids and xanthophylls commonly present in all green forages, which cause yellowing in fat; these compounds could be present in the CDFAf that were used in the present study.

PH. The carcass pH values (5.65-5.83) obtained in this study at 24 h for all treatments were similar to those reported by other studies[41] for sheep from the Dorset x Hampshire cross. In contrast, they were slightly higher than those observed by Partida de la Peña *et al*[24], who reported average pH values of 5.5 at 24 h after slaughter. The variation in this parameter depends on different factors, such as the handling of the animals at the time of slaughter and the age of the animals[42-44]. Similarly, some authors have reported variation due to the type of diet since carcasses from animals finished with high-grain diets may present higher values compared to animals whose diet was based on forage[41].

Back fat. The fat cover in the carcass is the main factor that determines its commercial value since it prevents the carcass from drying out, influences the tenderness and juiciness of the meat, and, in the case of sheep, interferes with the aroma and flavor of the meat[26]. The back fat obtained in this experiment was low (2.43, 2.79, 3.26, and 2.92 mm, respectively) compared to what was reported in another study[45], where they obtained 6.33 mm. This characteristic is due to the exit weight of the animals, which indicates that the slaughter weights can be increased[45]. In this sense, the Mexican standard for the classification of carcasses allows up to 6.9 mm of subcutaneous fat cover in heavy lambs to be considered in the "MEX EXT" classification.

GR Grades. The thickness of the dorsal subcutaneous fat is an objective parameter highly correlated with most of the tissue of the carcass, mainly the three pieces of the highest commercial value[26]. In this sense, the measurements of the GR point are another alternative related to the amount of fat in the entire carcass, which facilitates its implementation without interfering with the slaughter line of the animals[46]. In this study, no differences (P>0.05) were found for this variable due to the effect of the treatments (10.94, 12.06, 12.75, and 12.48) for T0, T1, T2, and T3, respectively; these results are within the ranges proposed by Bianchi[47] for carcasses weighing 18.5 to 22 kg.

Rib eye area. The *Longissimus dorsi* muscle is an important variable in determining carcass quality since it is highly correlated with the total amount of muscle in the carcass and corresponds to the rack and loin, which are the pieces with the highest economic value[45]. In this study, although no differences were found (P>0.05), there was an increase in the treatments compared to the control (Table 4). Likewise, the values found in the present study for this parameter are higher than those reported in a study with Katahdin sheep (17.4 cm^2) and the average data reported by Partida de la Peña *et al*[24] for intensively finished sheep, which is due to the genotype of the animals studied.

Carcass yields. The yields found in this study are largely consistent with Partida de la Peña *et al*[24], who reported an average of 50.9 % of carcass yields for sheep finished in intensive systems, which coincides with what was found in this study for T0; the yields of T1, T2, and T3 were higher than those reported by these authors.

Primal cuts. The yield of primal cuts is an important factor for the marketing of sheep meat when it comes to cuts since each of them receives a different value. In this study, the weights of the primal cuts were similar (P>0.05) for legs, neck, shoulder, rack, and ribs (Table 6), which coincides with what was reported in Pelibuey sheep fed with waste chickpeas[48] and in hair sheep fed with different proportions of *Tithonia diversifolia*[49]. Nonetheless, statistical differences were observed in the weight of the loin (P<0.05) (Table 6). The weight and yield of primal cuts are associated with the slaughter weight and the feeding system[49].

Morphometry. The measurements corresponding to this item agree with what has been reported by other authors[45] for carcasses from the Katahdin-Charollais cross and with Partida de la Peña *et al*[24] in carcasses from intensively finished sheep. In this sense, these variables were not affected by the levels of inclusion of *A. farnesiana* pods since these depend to a large extent on the breed and age of the animals[24].

Viscera. It is believed that the workload of absorption, rather than the amount or characteristics of the digestion in the small intestine, has a significant impact on the intestinal mass, just as the weight, texture, or chemical composition of the digestion affects the mass of the gastrointestinal tract[50]. In this study, the weight of the different components of the digestive tract was not affected, which is consistent with various studies that included supplementation with legume fruits[42,51]. The weight of the different

organs (heart, liver, lungs) was not affected by the inclusion of *A. farnesiana* fruit meal, which indicates that it is a suitable supplement for animal consumption. The weight of total fat was high due to the use of isoenergetic and isoprotein diets, which resulted in the accumulation of fat in the kidneys and pericardium[52].

Meat quality

Flesh color. In this study, no significant differences (P>0.05) were observed in the values of L*, a*, and b* for treatments containing *A. farnesiana* fruits. Nevertheless, the L* color values were lower than those reported by Smeti *et al*[53], a* values were below the standards mentioned by Alberti *et al*[54], and b* values were within the standards[29] of the UNE 48-103-94 standards, where they indicate the thresholds of the color of pink flesh L* 44.0-51.6, a* 11.6-15.1, b* 9.8-17.6, and DFD (dark – firm – dry) meat L*25.1-32.8, a* 17.0-21.3, b* 7.2-16.9. These color changes after cutting (hour 0) will vary when they come into contact with oxygen and reach their maximum values after 48 h[54]. Other authors point out that the acceptable standard for the color of lamb meat is equivalent to a L*value 34-35 and a* value < 19; these color thresholds indicate that lamb meat with an L chromatic value between 34 and 35 (showing lightness) and a redness value (a) below 19 (indicating less redness) would be acceptable[55]. However, these criteria may differ according to the rating of regional standards and consumer preferences[56,57]. This performance coincides with that reported by Luciano *et al*[58] for b*; although the pH of the meat was not recorded, the pH of the carcass at 24 h was >5.6, possibly due to the stress of transport and *pre mortem* handling of the sheep, which affected the pH and DFD meat as a consequence[59]. On the other hand, other authors[53] reported a similar behavior for a* values on the ninth day of maturation. With storage time, b* values correlate positively with sensory appreciation of meat degradation, while a* correlates negatively with sensory color degradation[58]. These color changes in meat can be affected by the oxidative processes of myoglobin in contact with oxygen (the amount of myoglobin in the muscle determines the color saturation: purple-red myoglobin, bright red oxymyoglobin, brown metmyoglobin)[60]. The color of the fat will be the deposit of pigments from the food (xanthophylls, carotenes, etc.)[54].

Drip water loss. According to the results obtained in the present research, the lowest level of CDFAf inclusion presented the lowest values of drip water loss (hour 72) if it is considered that the water in meat can be found bound, immobilized, and free and the distribution of its electrons is not neutral but has a positive and a negative charged end, which means that they can be associated with reactive groups of various chemical compounds, such as proteins and phenolic compounds of *A. farnesiana*, due to their complex chemical structure and the large number of free hydroxyl groups, they can form complexes with water at the tissue level and increase the water retention capacity of the muscle[36].

Shear force. Texture is a variety of sensations related to chewing, cutting, and penetration of meat and is the parameter most respected by consumers[61]. The differences found in

this study show a tendency to increase the shear force as the levels of inclusion of *A. farnesiana* fruits increased. As the shear force in the treatments where CDFAf was included increased, it can be hypothesized that intramuscular fat decreased since this gives tenderness to the meat; these findings are good from the point of view of lean meats. It is considered that one of the most critical factors influencing meat quality is the oxidative process, which is decreased when the meat has less fat, increasing shelf life, which has been reported to be possible by including antioxidant phytochemicals in the animals' diet[62]. Other studies support this scientific justification by mentioning that phenolic compounds in the diet have been proposed to be effective in improving the antioxidant status of meat, contributing to the stabilization of color and flavor, and preventing rancidity[6,63].

Conclusions and implications

According to the results obtained in this study, CDFAf can be included in diets for growing and finishing sheep because no adverse effect was found on dry matter intake; it can improve weight gain in the growing stage and increase the weight of cuts of high commercial value, such as loin. The use of the fruits of this shrub species represents a potential option as a feed to improve ruminant production and reduce the use of feed inputs external to the production unit.

Conflict of interest

The authors of this paper declare that there is no conflict of interest.

Financing

This work was funded by the Autonomous University of the State of Mexico under the UAEM 4585/2018/CIP project. The first author received a scholarship from CONAHCyT Mexico with reference number 577419.

Acknowledgments

This study is part of M.C. Miguel Ángel Zarza-Albarrán's thesis work to obtain the degree of Doctor in Agricultural Sciences and Natural Resources (Autonomous University of the State of Mexico) under the direction of Dr. Rolando Rojo-Rubio, Dr. Agustín Olmedo-Juárez, and Dr. Jaime Mondragón Ancelmo.

Literature cited:

1. Chetroiu R. Results and potential in the economic efficiency of breeding young sheep for meat. Scientific Papers: Management, Economic Engineering in Agr Rural Develop 2020;20(2):127-132.

2. Vasta V, Luciano G. The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. Small Ruminant Res 2011;101:150–159.

3. Haile A, Tolemariam T. The feed values of indigenous multipurpose trees for sheep in Ethiopia: The case of *Vernonia amygdalina*, *Buddleja polystachya* and *Maesa lanceolata*. Livestock Res Rural Develop 2008;20(3):1-7.

4. Vélez-Terranova, M, Campos-Gaona R, Sánchez-Guerrero, H. Uso de metabolitos secundarios de las plantas para reducir la metanogénesis ruminal. Trop Subtrop Agroecosist 2014;17:489-499.

5. Oh J, Wall EH, Bravo DM, Hristov AN. Host-mediated effects of phytonutrients in ruminants: A review. J Dairy Sci 2017;100(7):5974-5983.

6. Mendel M, Chłopecka M, Dziekan N, Karlik W. Phytogenic feed additives as potential gut contractility modifiers a review. Anim Feed Sci Technol 2017;230:30-46; http://dx.doi.org/10.1016/j.anifeedsci.05.008.

7. García-Winder LR, Goñi-Cedeño S, Olguín-Lara PA, Díaz-Salgado G, Arriaga-Jordán CM. Huizache (*Acacia farnesiana*) whole pods (flesh and seeds) as an alternative feed for sheep in Mexico. Trop Anim Health Prod 2009;41(8):1615-21.

8. Degen AA, El-Meccawi S, Kam M. Cafeteria trials to determine relative preference of six desert trees and shrubs by sheep and goats. Livestock Sci 2010;132(1-3):19-25.

9. Quiroz-Cardoso F, Rojas-Hernández S, Olivares-Pérez J, Hernández-Castro E, Jiménez-Guillén R, Córdova-Izquierdo A, Villa-Mancera A, *et al*. Composición nutricional, consumo e índices de palatabilidad relativa de los frutos de tres acacias en la alimentación de ovejas y cabras. Arch Med Vet 2015;47(1):33-38.

10. Barrientos-Ramírez L, Vargas-Radillo JJ, Rodríguez-Rivas A, Ochoa-Ruíz HG, Navarro-Arzate F, Zorrilla J. Evaluación de las características del fruto de huizache (*Acacia farnesiana* (L.) Willd.) para su posible uso en curtiduría o alimentación animal. Madera y Bosques 2012;18(3).

11. Cuchillo HM, Puga DC, Wrage-Mönning N, Espinosa MJG, Montaño BS, Navarro-Ocaña A, *et al.* Chemical composition, antioxidant activity and bioactive compounds of vegetation species by goats on semiarid rangelands. J Anim Feed Sci 2013;22:106–115.

12. Sosa-Pérez G, López-Ortiz S, Pérez-Hernández P, Cortez-Romero C, Gallegos-Sánchez J. Uso de frutos tropicales (Fabaceae) para complemento alimenticio de pequeños rumiantes. Agroproductividad 2017;10(2):37-41.

13. Qin S, Hou D. The biofunctions of phytochemical and their application in farm animals: the Nrf2/Keap 1 system as target. Engineering 2017;(3):738-752.

14. Zarza-Albarrán MA, Olmedo-Juárez A, Rojo-Rubio R, Mendoza-de Gives P, González-Cortazar M, Tapia-Maruri D, *et al*. Galloyl flavonoids from *Acacia farnesiana* pods possess potent anthelmintic activity against *Haemonchus contortus* eggs and infective larvae. J Ethnopharmacol 2020;249:12402.

15. Olmedo-Juárez A, Zarza-Albarrán MA, Rojo-Rubio R, Zamilpa A, González-Cortazar M, Mondragón-Ancelmo J, *et al*. *Acacia farnesiana* pods (plant: Fabaceae) possesses anti-parasitic compounds against *Haemonchus contortus* in female lambs. Exp Parasitol 2020;218:217.

16. Delgadillo-Puga C, Cuchillo-Hilario M, Espinosa-Mendoza GE, Medina-Campos O, Molina-Jijón E, Díaz-Martínez M, *et al*. Antioxidant activity and protection against oxidative-induced damage of *Acacia shaffneri* and *Acacia farnesiana* pods extracts: *in vitro* and *in vivo* assays. BMC Compleme Altern Med 2015;15:435.

17. García E. Modificaciones al sistema de clasificación climática de Köppen. 4ª ed. Instituto de Geografía. Universidad Nacional Autónoma de México. México D.F; 1988.

18. NRC. Nutrient requirements of small ruminants, sheep, goats, cervids, and New World camelids. Animal Nutrition Series. The National Academy Press. Washington, DC, USA; 2007.

19. AOAC (Association of Official Analytical Chemists). Official Methods of Analysis, 16th ed. AOAC, Arlington, VA, USA; 1997.

20. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and non-starch carbohydrates in relation to animal nutrition. J Dairy Sci 1991;74:583-597.

21. Colomer-Rocher F, Morand-Fehr P, Kirton AH. Standard methods and procedures for goat carcass evaluation, jointing and tissue separation. Livestock Prod Sci 1987;17:49-159.

22. Cañeque V, Pérez C, Velasco S, Dıaz MT, Lauzurica S, Álvarez I, *et al*. Carcass and meat quality of light lambs using principal component analysis. Meat Sci 2004;67(4):595-605.

23. Centre Internationale de LÕEclairage Colorimetry. 2nd ed. Vienna: Publication CIE 1986:15.2.

24. Partida de la Peña JA, Ríos-Rincón FG, De la Cruz-Colín L, Domínguez-Vara IA, Buendía-Rodríguez G. Caracterización de las canales ovinas producidas en México. Rev Mex Cienc Pecu 2017;8(3):269-277.

25. Rust RE, Olson DG, Kratzer DD, Schuler RO, Vetter RL. M. *Longissimus* area of lamb carcasses—a Comparison of four measurement techniques and the evaluation of operator differences. J Anim Sci 1970;30(1):36-39.

26. Ruiz de Huidobro F, Miguel E, Cañeque V, Velasco S. Clasificación y conformación de la canal ovina. En: estandarización de las metodologías para evaluar la calidad del producto (animal vivo, canal, carne y grasa) en los rumiantes. Monografías INIA: Serie ganadera; 2005:143-169.

27. Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat Sci 1998;49:447–457. doi:10.1016/S0309- 1740(98)00034-5.

28. Bratzler LJ. Determining the tenderness of meat by use of the Warner-Bratzler method. Proc Recip Meat Conf 1949;2:117-121.

29. Centre Internationale de L'Eclairage (CIE). 'Definition dun space de coleur por deux coordonees de cromaticite et la luminosite. Supplement 2 to CIE publication no 15 (E-1–3-1) 1971/ (TC-1–3).' (Cente Internationale de L'Eclairage: Paris) 1976.

30. SAS, SAS Online Doc Version 9.1.3.SAS, NC. USA, Cary.: 2014.

31. Olivares-Pérez J, Rojas-Hernandez S, Camacho-Diaz LM, Cipriano-Salazar M, AZM. Salem. Fruits chemical composition and potential ruminal digestion of nine tree species in dry tropic region of Mexico. Agroforest Syst 2019;93:665–674. https://doi.org/10.1007/s10457-017-0161-y.

32. Alexander G, Singh B, Sahoo A, Bhat TK. *In vitro* screening of plant extracts to enhance the efficiency of utilization of energy and nitrogen in ruminant diets. Anim Feed Sci Technol 2008;145:229–244.

33. Frutos P, Hervás G, Giráldez FJ, Mantecón AR. Tannins and ruminant nutrition. Spanish J Agric Res 2004;2(2):191-202.

34. Velázquez AJ, González M, Perezgrovas R, Bórquez J, Domínguez I. Producción, digestibilidad y rentabilidad en corderos de dietas con vainas de *Acacia farnesiana*. Arch Zootec 2011;60 (231):479-88.

35. Musharaf K, Farrukh H. Palatability and animal preferences of plants in Tehsil Takht-e-Nasrati, District Karak, Pakistan. African J Agric Res 2012;7(44):5858-5872.

36. Cortés JE, Moreno B, Pabón ML, Ávila P, Kreuzer M, Hess HD, *et al*. Effects of purified condensed tannins extracted from *Calliandra*, *Flemingia* and *Leucaena* on ruminal and postruminal degradation of soybean meal as estimated *in vitro*. Anim Feed Sci Technol 2009;151:194–204.

37. Dentinho TP, Belo AT, Bessa RJB. Digestion, ruminal fermentation and microbial nitrogen supply in sheep fed soybean meal treated with *Cistus ladanifer* L. tannins. Small Ruminant Res 2014;119:57–64.

38. Orlandi T, Kozloski GV, Alves TP, Mesquita FR, Ávila SC. Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of *Acacia mearnsii* tannin extract. Anim Feed Sci Technol 2015;210:37–45.

39. Castro-Montoya JM, Makkar HPS, Becker K. Chemical composition of rumen microbial fraction and fermentation parameters as affected by tannins and saponins using an *in vitro* rumen fermentation system. Can J Anim Sci 2011;91:433-448.

40. Gómez-Gurrola A, Partida-Hernández M, Ramírez-Duran R, Ramírez-Ramírez JC, Gómez-Gurrola JA, González-Mormita M, *et al*. Efecto de la inclusión del fruto de G*uazuma ulmifolia* como sustituto de maíz en la dieta sobre el comportamiento productivo y rendimiento en canal de ovinos Pelibuey. Trop Subtrop Agroeco 2014;17:215–222.

41. Jaborek JR, Zerby HN, Moeller SJ, Wick MP, Fluharty FL, Garza III H, *et al*. Effect of energy source and level, and animal age and sex on meat characteristics of sheep. Small Ruminant Res 2018;166:53–60.

42. Beriain MJ, Purroy A, Treacher T, Bas P. Effect of animal and nutritional factors and nutrition on lamb meat quality. Sheep and goat nutrition: Intake, digestion, quality of products and rangelands, 2000;75-86.

43. McGeehin B, Sheridan JJ, Butler F. Factors affecting the pH decline in lamb after slaughter. Meat Sci 2001;58(1):79-84.

44. Knapik J, Ropka-Molik K, Pieszka M. Genetic and nutritional factors determining the production and quality of sheep meat–a review. Ann Anim Sci 2017;17(1):23-40.

45. Vázquez-Soria ET, Méndez-Medina D. Comportamiento productivo y características de la canal en corderos provenientes de la cruza de ovejas Katahdin con machos de cuatro razas cárnicas especializadas. Rev Mex Cienc Pecu 2011;2(3):247-258.

46. Kirton AH, Feistand CL, Duganz DM. Prediction of ewe mutton carcass composition from carcass weight, GR and C measurements, and the Hennessy grading probe. Proc NZ Soc Anim Prod 1986;46:59-61.

47. Bianchi J. Un vistazo al sistema de tipificación de canales ovinas y su relación con la calidad del producto. El país agropecuario; 2008:26-29.

48. Ríos-Rincón FG, Barragán HB, Cerrillo-Soto MA, Estrada-Angulo A, Juárez-Reyes AS, Obregón JF, *et al*. Carcass characteristics, primal cut yields and tissue composition of Katahdin x Pelibuey lambs fed cull-chickpeas. Rev Mex Cienc Pecu 2012;3(3):357-371.

49. Gómez-Gurrola A, Del Sol-García G, Loya-Olguín L, Benítez-Meza A, Hernández-Ballesteros A. Rendimiento en canal de corderos de pelo, alimentados con diferentes proporciones de *Tithonia diversifolia* y *Pennisetum* spp. Aba Vet 2017;7(2):34-42. http://dx.doi.org/10.21929/abavet2017.72.3

50. Yamazaki A, Choki S, Kakizaki T, Matsuura A, Irimajiri M, Hodate K. Comparison of passage rate, structure and motility of the reticulo-rumen in two sheep breeds. In: Ruminant physiology. Leiden, The Netherlands: Wageningen Academic; 2009; 404-405.

51. Mireles EJ, Rodríguez D, Jordán H, Valdivia M, Ramírez A, García A, *et al*. Profile of fatty acids of *Longissimus dorsi* muscle and productive indicators of sheep, supplemented with pods of *Acacia cochliacantha*, in grasslands native to dry tropics. Cuban J Agr Sci;2015;49(3):329-388.

52. Preziuso G, Russo C, Casarosa L, Campodoni G, Piloni S, Cianci D. Effect of diet energy source on weight gain and carcass characteristics of lambs. Small Ruminant Res 1999;3(1):9-15.

53. Smeti S, Atti N, Mahouachi M, Munoz F. Use of dietary rosemary (*Rosmarinus officinalis* L.) essential oils to increase the shelf life of Barbarine light lamb meat. Small Ruminant Res 2013;113:340–345.

54. Albertí P, Panea B, Ripoll G, Sañudo C, Olleta JL, Hegueruela I, Campo MM, Serra X. Medición del color. Estandarización de las metodologías para evaluar la calidad del producto (animal vivo, canal, carne y grasa) en los rumiantes. Madrid, España: MICYT-INIA: Ganadera 2005;3:216-25.

55. Hopkins DL, Toohey ES, Warner RD, Kerr MJ, Van de Ven R. Measuring the shear force of lamb meat cooked from frozen samples: comparison of two laboratories. Anim Prod Sci 2010;50(6):382-385.

56. Corlett MT, Pethick DW, Kelman KR, Jacob RH, Gardner GE. Consumer perceptions of meat redness were strongly influenced by storage and display times. Foods 2021;10(3):540.

57. Novoselec J, Šalavardić ŽK, Samac D, Ronta M, Steiner Z, Sičaja V, Antunović Z. Slaughter indicators, carcass measures, and meat quality of lamb fattened with spelt (*Triticum aestivum* spp. Spelta L.). Foods 2021;10(4):726.

58. Luciano G, Monahan FJ, Vasta V, Biondi L, Lanza M, Priolo A. Dietary tannins improve lamb meat colour stability. Meat Sci 2009;81:120–125.

59. Cam MA, Olfaz M, Kirikci K, Tufekci H, Mercan L, Kilic U. Effects of pre-slaughter stress on meat quality characteristics of male lambs of Hemsin and of sheep breeds. J Anim Plant Sci 2021;47:8445-8459.

60. Li X, Zhang Y, Li Z, Li M, Liu Y, Zhang D. The effect of temperature in the range of -0.8 to 4°C on lamb meat color stability. Meat Sci 2017;134:28-33.

61. Holman BWB, Alvarenga TI, Van de Ven RJ. Hopkins DL. A comparison of technical replicate (cuts) effect on lamb Warner–Bratzler shear force measurement precision. Meat Sci 2015;105:93-95.

62. Mireles-Arriaga AI, Ruiz-Nieto JE, Hernández-Ruiz J, Hernández-Marín JA. Fitoquímicos antioxidantes alimentarios como estrategia de promoción de la estabilidad oxidativa de la carne de conejo. (*Oryctolagus cuniculus* L.). Agroproductividad 2018;11(6):91-96.

63. Ortuno J, Serrano R, Banón S. Use of dietary rosemary diterpenes to extend the preservation of sulphited-lamb products. Small Ruminant Res 2015;123:269–277.

https://doi.org/10.22319/rmcp.v15i4.6628

Article

Prediction model for productive life extension in censored records of Holstein cattle from Mexico

Sandra Giovanna Núñez-Soto [a,c]

Adriana García-Ruiz [b]

Hugo Oswaldo Toledo Alvarado [c]

Felipe de Jesús Ruiz-López [b*]

[a] Universidad Nacional Autónoma de México. Facultad de Medicina Veterinaria y Zootecnia. Programa de Maestría y Doctorado en Ciencias de la Producción y de la Salud Animal. Unidad de Posgrado, Edificio "B" Primer Piso Circuito del Posgrado, Ciudad Universitaria, Delegación Coyoacán, 04510 México D.F. México.

[b] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal, Querétaro, México.

[c] Universidad Nacional Autónoma de México. Facultad de Medicina Veterinaria y Zootecnia, Departamento de Genética y Bioestadística, Ciudad de México, México.

[*]Corresponding author: ruiz.felipe@inifap.gob.mx

Abstract:

The objective of this study was to predict months in production at 84 mo of age (MIP84) to include information from still-living animals in the genetic evaluation of longevity based on productive and reproductive information to establish complete longevity as MIP84. The records were obtained from animals born between 1986 and 2020 from the Holstein Association of Mexico. To predict MIP84, a linear regression model was fitted for 1st, 2nd, 3rd, 4th, and 5th calving before 84 mo of age. The model included information from cows with complete longevity, such as milk production in kilograms adjusted to 305 d ME (SP) at current calving, cumulative months in production before current calving (MACL), months in

production at current calving (MLCC), pregnancy index at current calving (PRI), lactation status index at current calving (LAS), and age at first calving in months (AFC) in its linear and quadratic effects (AFC^2). The model explained 44 to 98 % of the variation observed in MIP84. Most regression coefficients for expanding longevity were significant and positive ($P<0.01$). The mean coefficient for PRI was negative in all calving's (-0.7159 ± 0.0171 and -2.0632 ± 0.0732). The proposed model allowed the inclusion of cows that have not yet finished their productive life, being of interest in genetic longevity assessments.

Keywords: Prediction model, Longevity, Holstein cows.

Received: 31/01/2024

Accepted: 17/07/2024

Introduction

Longevity in dairy cattle is an important economic characteristic that presents genetic variability, generally low but sufficient for genetic progress in subsequent generations[1,2]. Countries that evaluate longevity in dairy cattle have measured it as herd life[3], productive life[3,4], functional longevity[5,6], true longevity[5,6], productive lifespan[7], longevity at 84 mo of age[8], longevity before culling or censorship[9], permanence[10], life expectancy[11,12], and milking life[13]. The low heritability estimates (0.02 to 0.11)[14] are the result of relatively high residual variability, which can be explained by the complexity of the trait and the considerable influence of environmental factors such as management. The productive life of dairy cows is difficult to improve genetically because, among other factors, complete data are available too late for the animals of interest, so an early selection of longevity, which would be appropriate, is impossible. Different authors have mentioned that improving longevity by identifying superior animals early is possible using correlated traits, as is the case of Maugan *et al*[15], who included, in their model, characteristics correlated with each other and with functional longevity, such as udder composition, fertility, somatic cell index, and incidence of mastitis, in order to include young animals in the early genetic evaluation of longevity in Holstein cattle; on the other hand, 13 type characteristics have been studied in Italian Montbeliarde cows, which were correlated with survival, allowing their early prediction[16]; other researchers used correlations of 15 type characteristics with herd life at 48 mo of age in Guernsey cattle[17]. Early selection can also be achieved with a nonlinear evaluation of censored data[18] or using predicted longevity for live cows in addition to complete longevity data[8,12]. The latter methodology is used in the United States of

America[8] to evaluate the longevity of dairy cattle measured as months in production at 84 mo of age (MIP84) because it allows the use of incomplete records from cows that are still alive at the time of evaluation, commonly called censored records. The process is based on the phenotypic prediction of MIP84 of animals not yet discarded based on population regressors to extend the productive lifespan and their subsequent adjustment to homologate variances, a process similar to that applied to the extension of incomplete lactations for the milk production trait[19]. In addition, since MIP84 is a continuous variable, it better represents the lifespan of a cow and brings the distribution of the variable closer to the normal distribution, allowing to have both complete data until the disposal of very old cows and censored data from younger cows[8]. In Mexico, the evaluation of longevity in Holstein cattle is done only for males using a survival model[20]; this is a limitation because early life indicators are needed to help farmers in the selection of animals that are more likely to reach their full potential; therefore, the use of MIP84 and a linear model will not only allow the evaluation of females to be carried out directly but will also allow genomic information to be included shortly. The first step to implement the evaluation of MIP84 in the Holstein population of Mexico is the prediction of the variable in animals that are still active or those whose true longevity is unknown for any reason; therefore, the objective of this study was to predict the months in production at 84 mo of age based on complementary productive and reproductive information in records of Holstein cows from Mexico, using the simple linear regression model developed by VanRaden and Klaaskate[8] and evaluating the fit of this model.

Material and methods

Information from the production control system of the Holstein Association of Mexico was used. The information included corresponded to the observed productive life of a total of 70,314 cows with 1 to 5 calving's because there were no cows that started their sixth lactation before 84 mo. The dependent variable was established as the months in production at 84 mo of age, establishing a maximum of 10 mo in production for each lactation so as not to indirectly favor cows with extended lactations[8]. In order to predict MIP84, the independent variables included in Van Raden and Klaasklate's[8] statistical model and those available at the end of each calving from 1 to 5 were used, which consisted of the accumulated months in production (MACL), months in production at the last calving (MLCC), the lactation status at the time of culling or termination of lactation (LAS), the pregnancy index at the time of culling or termination of lactation (PRI), the interaction of the herd-year and season of first calving and the age at first calving, and milk production measured in kilograms adjusted to 305 d mature equivalent (SP). Although this model[8] included the variable of dry days, these were not included in the model because they showed significant unexplained variations

(analysis not presented in this study). The PRI was equal to 1 if the cow was pregnant; the cow was considered pregnant if it was more than 70 d after being artificially inseminated, it had a diagnosis of pregnancy, or it had a subsequent calving to the one analyzed, or zero in any other case. The lactation status index (LAS) was coded as zero when the cow was dry or in milking for more than 305 d and as one if the cow was in milking within 305 d. Age at first calving in months was also considered in its linear and quadratic effects.

Five scenarios were considered for the calculations, which represented the amount of information that the cow had for its prediction and depended on the number of complete lactations it had. That is, if it had finished its first or second lactation and so on until its fifth lactation. The number of cows that presented a complete calving was 26,704, two complete calvings 18,351, three complete calvings 10,496, four complete calvings 5,115, and up to five complete calvings 3,065. This differentiates the MIP84 calculated in this study from that obtained in the population of the United States of America, where the information they considered was obtained from different age groups[8]. In other words, separate models were fitted for animals removed during their second, third, fourth, or fifth lactation, using the information generated up to the previous calving. Current calving was considered to be lactation during which the cow was removed.

The statistical model used for the prediction of MIP84 was as follows:

$$MEP84_{ijklmnopq} = \mu + hyfc_i + \beta_1 macl_j + \beta_2 mlcc_k + \beta_3 pri_1 + \beta_4 las_m + \beta_5 sp_n + \beta_6 afc_o + \beta_7 afc^2_p + \varepsilon_{ijklmnopq}$$

Where,
hyfc is the herd-year of first calving,
macl are the months in production accumulated until the previous calving,
mlcc are the months in production at the current calving,
pri is the pregnancy index at the current calving,
las is the lactation status at the current calving,
sp is the standardized milk production at 305 days ME at the current calving,
afc is the age at first calving,
$\boldsymbol{afc^2}$ is the quadratic effect of age at first calving.
$\boldsymbol{\varepsilon_{ijklmnopq}}$ is the random error.
β_1, β_2, β_3, β_4, β_5, β_6, and β_7, are the coefficients of linear regressions for the variables described above. The GLM procedure of the SAS statistical software[21] was used to perform the analyses.

Results and discussion

Table 1 shows the regression coefficients, their probability value, and the coefficients of determination obtained from the model to predict MIP84 based on information from the first, second, third, fourth, and fifth calving. The model explained 98, 96, 92, 79, and 44 % of the variation from the effects included for MIP84 for the first to fifth calving, respectively.

Table 1: Coefficients of determination, regression coefficients, and *P*-value of the variables used in the prediction model for MIP84 in the first five calvings

Variable	MIP84				
	First calving	**Second calving**	**Third calving**	**Fourth calving**	**Fifth calving**
	Regression coefficient				
MACL, m	0.8951***	0.8188***	0.6669***	0.4123***	0.1414***
MLCC, m	0.0034^{NS}	0.0265***	0.0714***	0.1329***	0.1348***
LAS (0,1)	-0.0579**	0.0166^{NS}	0.1330^{NS}	0.1801^{NS}	0.1781^{NS}
PRI (0,1)	-0.7159***	-1.1221***	-1.7292***	-2.0632***	-1.4743***
SP, kg	0.0001***	0.0001***	0.0001***	0.0002***	0.0002**
R^2, %	98	96	92	79	44

MACL= months in production accumulated until the current calving, MLCC= months in production at the current calving, LAS= lactation status index (0= milking, 1= dry), PRI= pregnancy index (0= empty, 1= pregnant), SP= milk production in kg adjusted to 305 d ME at the current calving, R^2= coefficient of determination.

***= less than 0.001, **= between 0.001 and 0.01, *= between 0.011 and 0.05, NS= above 0.05

To explain the effect of the independent variables used in the model to predict MIP84 through the five calvings, MIP84 are shown directly since they are months in production already completed, and this is reflected in an increase in the MIP84 forecast. One-month increases were reported for the same variable[8]. The MLCC were significant from the second calving to the fifth and indicated that when increasing one month in milk in the current calving, the MIP84 increased by 0.026 for the second calving, by 0.071 for the third calving, by 0.133 for the fourth calving, and by 0.135 for the fifth calving; it may be due to two factors: one is the relationship between days in production and total milk production, since the higher the milk production, the lower the probability of discarding; and the other is that a cow with more months in production in the last lactation is closer to reaching the end of it and the possibility of starting a new lactation increases, and with this, the expectation of a higher MIP84 increases, in addition to the fact that, in general, these cows have a lower probability of having locomotion or health problems, they become pregnant more easily and have higher milk productions, which is consistent with what Dallago *et al*[22] stated.

Figure 1: Effect of the months in production accumulated until the current calving (MACL) on MIP84

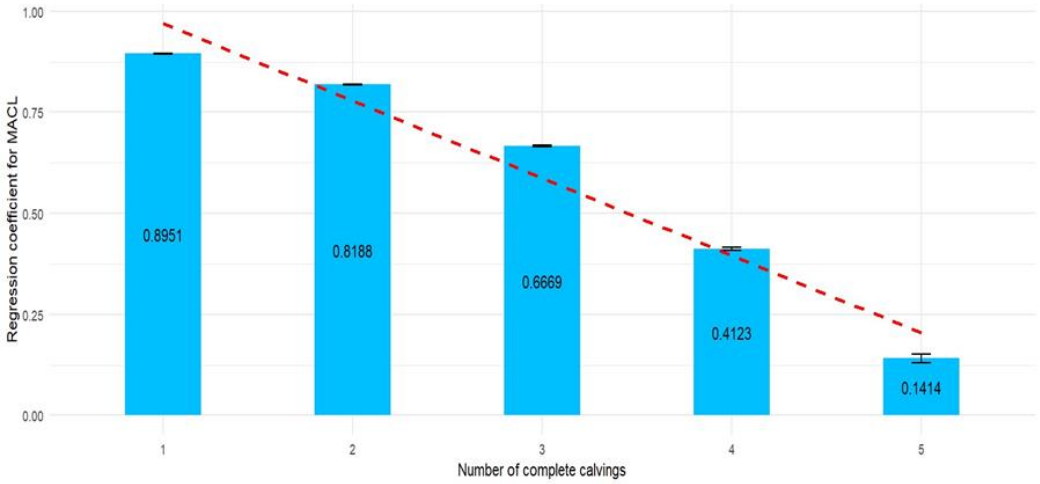
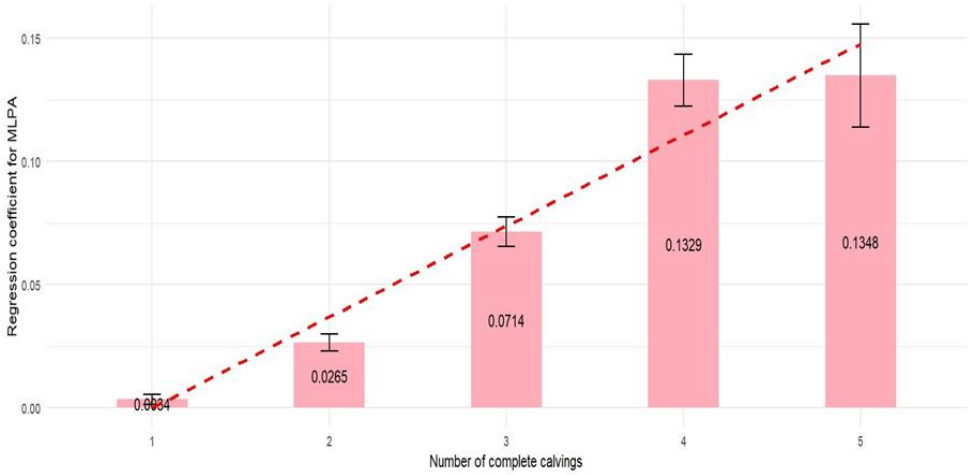


Figure 2: Effect of months in production at current calving (MLCC) on MIP84



The MLCC was not significant at first calving probably because of the distance in time between the MLCC of the first lactation and the date of discarding the cow. As the cow approaches the end of its productive life, MLCC tends to be important in predicting MIP84 because having more months of production in the previous lactation would predict that the cow stayed longer in the productive herd because it had a high production or greater fertility or better health; on the contrary, when there is a cow with a short previous lactation, in principle, it should have lower MIP84; this could be because the productive or reproductive conditions within the herd were not the best for the cow and caused it to have fewer months in production with a greater probability of being culled in the subsequent lactation. Although lactation status (LAS) regression coefficients were similar in magnitude to those of other characteristics, such as MLCC for calvings 2 to 5, they were only significant for the first

calving. In the case of the first calving, when the cow is in milking, the prediction of MIP84 decreases by 0.06 months compared to when the cow is in the dry period (Figure 3). This could be because when the cow is primiparous and finishes its lactation without drying off, it is at a disadvantage against cows that end their cycle and become dry because they are less prepared to start a new productive cycle and thus decrease their expectation of MIP84. Contradictorily, when the cow finished its third lactation without drying off, the MIP84 prediction increased by 0.13 mo (P<0.05), probably because the cow is already close to reaching its actual MIP84 measurement, and the fact that it does not have information on the date of dry-off at this time is not as critical in the prediction of MIP84. On the other hand, when the cow was not pregnant (PRI) at the end of the previous lactation, the prediction of MIP84 was negative in the five calvings (P<0.0001), with a trend that indicates that it decreases as the number of calvings increases until it decreases by 2.06 mo at the fourth calving (Figure 4), which suggests that the fact that the cow has not ensured an upcoming calving at the time of prediction drastically decreases the predicted MIP84, which is consistent with what has been reported by several authors[1,23,24]. Finally, the effect of SP on MIP84 was significant in the five calvings, indicating that when milk production increases by one kilogram, the predicted MIP84 increases by 0.0001 mo for the first three calvings and 0.0002 mo for the fourth and fifth calvings (Figure 5). This may reflect the fact that when cows have higher milk production, farmers tend to give them more opportunities to stay in the cowshed, increasing their MIP84[20]. However, the large variation that exists in this variable makes the effect small compared to those of the other variables in the study. Age at first calving in its linear and quadratic effect was not significant in any calving.

Figure 3: Effect of lactation status (LAS) on MIP84

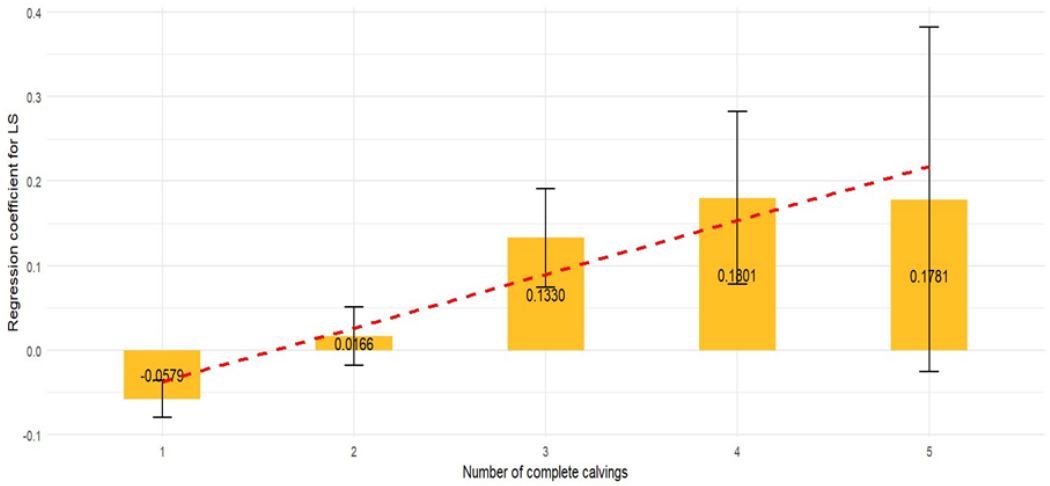


Figure 4: Effect of pregnancy index (PRI) on MIP84

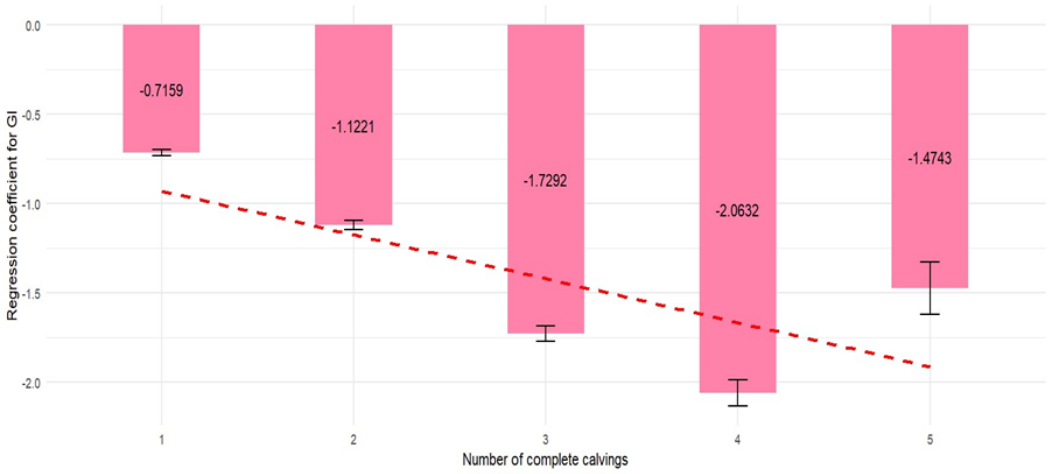
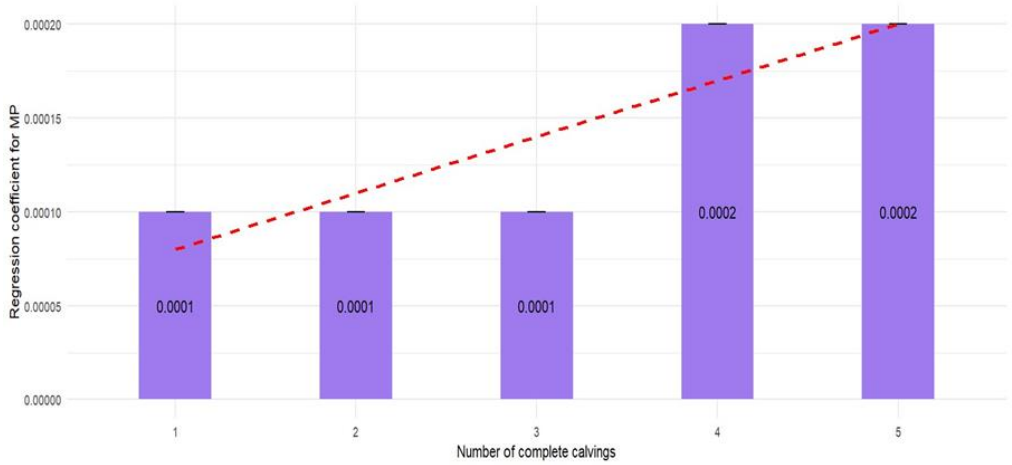


Figure 5: Effect of milk production at 305 days of ME (SP) on MIP84



Conclusions and implications

According to the results obtained in this study, it is possible to predict with high accuracy MIP84 in Holstein dairy cows, with at least one lactation completed, based on the milk production in kilograms adjusted to 305 days ME, the accumulated months in production, the months in production of the last lactation, whether the cow is in production or dry and whether it is pregnant or not, common variables in milk production controls. On the other hand, the predicted MIP84 was higher for cows with more months in production at their last recorded full lactation, cows that were in production at their last record (except second-lactation cows), or cows that were pregnant at the end of the last lactation. The prediction of

MIP84 will allow animals that have not finished their productive life to be included in the genetic evaluation of this population, information that will help producers in the genetic improvement of longevity in their herds.

Acknowledgements and financial source

Research conducted thanks to funding from the CONACYT grant 47153. Thanks to the Holstein Association of Mexico and the CENIDFyMA-INIFAP for the information and facilitations provided to carry out this work.

Literature cited:

1. Hu H, Mu T, Ma Y, Wang X, Ma Y. Analysis of longevity traits in Holstein cattle: A Review. Front Genet 2021;(12):1-15. doi:10.3389/fgene.2021.695543.

2. De Vries A. Economic trade-offs between genetic improvement and longevity in dairy cattle. J Dairy Sci 2017;100(5):4184-4192. doi:10.3168/jds.2016-11847.

3. Tsuruta S, Misztal I, Lawlor TJ. Changing definition of productive life in US Holsteins: Effect on genetic correlations. J Dairy Sci 2005;88(3):1156-1165. doi.org/10.3168/jds.S0022-0302(05)72782-X.

4. Costa A, Bovenhuis H, Penasa M. Changes in milk lactose content as indicators for longevity and udder health in Holstein cows. J Dairy Sci 2020;103(12):11574-11584. doi.org/10.3168/jds.2020-18615.

5. Vukasinovic N, Moll J, Künzi N. Analysis of productive life in Swiss Brown cattle. J Dairy Sci 1997;80(10):2572-2579. doi.org/10.3168/jds.S0022-0302(97)76213-1.

6. Sasaki O, Aihara M, Hagiya K, Nishiura A, Ishii K, Satoh M. Genetic evaluation of the longevity of the Holstein population in Japan using a Weibull proportional hazard model. Anim Sci J 2011;83(2):95–102. doi:10.1111/j.1740-0929.2011.00943.

7. Caraviello DZ, Weigel KA, Gianola D. Prediction of longevity breeding values for US Holstein sires using survival analysis methodology. J Dairy Sci 2004;87(10):3518–3525. doi:10.3168/jds.S0022-0302(04)73488.

8. VanRaden PM, Klaaskate EJH. Genetic evaluation of length of productive life including predicted longevity of live cows. J Dairy Sci 1993;(76):2758-2764.

9. Raguz N, Jovanovac S. Analysis of the relationships between type traits and longevity in Croatian Simmental cattle using survival analysis. Agric Conspec Sci 2011;76(3):249-253.

10. Brickell JS, Wathes DC. A descriptive study of the survival of Holstein-Friesian heifers through to third calving on English dairy farms. J Dairy Sci 2011;(94):1831-1838. http://dx.doi.org/10.3168/jds.2010-3710.

11. Van Pelt M, Meuwissen THE, De Jong G, Veerkamp RF. Genetic analysis of longevity in Dutch dairy cattle using random regression. J Dairy Sci 2015;98(6):4117-4130. https://doi.org/10.3168/jds.2014-9090.

12. Brotherstone S, Veerkamp RF, Hill WG. Predicting breeding values for herd life of Holstein-Friesian dairy cattle from lifespan and type. Anim Sci 1998;(67):405–411.

13. Zhang H, Liu AY, Wang H, Luo X, Yan X, Guo X, Li L, Liu, Su G. Genetic parameters and genome-wide association studies of eight longevity traits representing either full or partial lifespan in Chinese Holsteins. Front Genet 2021;(12):634986. https://doi.org/10.3389/fgene.2021.634986.

14. Forabosco F, Jakobsen JH, Fikse WF. International genetic evaluation for direct longevity in dairy bulls. J Dairy Sci 2009;(92):2338–2347.

15. Maugan LH, Rostellato R, Tribout T, Mattalia S, Ducrocq V. Combined single-step evaluation of functional longevity of dairy cows including correlated traits. Genet Sel Evol 2023;55(75):1-15. https://doi.org/10.1186/s12711-023-00839-6.

16. Kern EL, Cobuci JA, Costa CN, McManus CM, Campos GS, Almeida TP, *et al.* Genetic association between herd survival and linear type traits in Holstein cows under tropical conditions. Ital J Anim Sci 2014;(13):665–672.

17. Harris BL. Linear programming applied to dairy cattle selection [doctoral thesis]. Iowa, USA: University of Iowa State; 1992. https://lib.dr.iastate.edu/rtd/9997.

18. Mészáros G, Sölkner J, Ducrocq V. The Survival Kit: Software to analyze survival data including possibly correlated random effects. Computer methods and programs in biomedicine 2013;110(3):503-10. https://doi.org/10.1016/j.cmpb.2013.01.010.

19. VanRaden PM, Dematawewa CM, Pearson RE, Tooker ME. Productive life including all lactations and longer lactations with diminishing credits. J Dairy Sci 2006;89(8):3213-3220. doi:10.3168/jds.S0022-0302(06)72596-6.

20. Abadía JR, Ruíz FJ, Vega VE, Montaldo HH. Análisis genético para vida productiva en ganado Holstein de México. Rev Mex Cienc Pecu 2016;7(1):1-14. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-11242016000100001&lng=es.

21. SAS. SAS/STAT User´s Guide (version 9.4 ed.). Cary NC, USA: SAS Inst. Inc. 2010.

22. Dallago GM, Wade KM, Cue RI, McClure JT, Lacroix R, Pellerin D, *et al.* Keeping dairy cows for longer: a critical literature review on dairy cow longevity in high milk-producing countries. Animals 2021;(11):808. https://doi.org/10.3390/ani11030808.

23. Pipino DF, Piccardi M, Lopez-Villalobos N, Hickson RE, Vázquez MI. Fertility and survival of Swedish Red and White × Holstein crossbred cows and purebred Holstein cows. J Dairy Sci 2023;106(4):2475-2486. doi:10.3168/jds.2022-22403.

24. Essl A. Longevity in dairy cattle breeding: A review. Livest Sci 1998;(57):79–89. doi .org/10 .1016/ S0301-6226(98)00160-2.

https://doi.org/10.22319/rmcp.v15i4.6440

Article

In vitro anthelmintic evaluation of curcumin against the eggs and larvae of three *Haemonchus contortus* isolates with different susceptibility to ivermectin

Lisandro Alberto Encalada-Mena [a]

Raquel López-Arellano [b]

María Eugenia López-Arellano [c]

María Gabriela Mancilla-Montelongo [d]

Carlos Alfredo Sandoval-Castro [e*]

Agustín Olmedo-Juárez [c]

Juan Felipe de Jesús Torres-Acosta [e]

[a] Universidad Autónoma de Campeche, Facultad de Ciencias Agropecuarias, Campeche, México.

[b] Universidad Nacional Autónoma de México. FES-Cuautitlán, Laboratorio de Ensayos de Desarrollo Farmacéutico, Estado de México, México.

[c] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, Morelos, México.

[d] CONAHCYT-Universidad Autónoma de Yucatán. Facultad de Medicina Veterinaria y Zootecnia, Yucatán, México.

[e] Universidad Autónoma de Yucatán, Facultad de Medicina Veterinaria y Zootecnia, Km 15.5 Carr. Mérida-Xmatkuil, 97315, Mérida, Yucatán, México.

*Corresponding author: carlos.sandoval@correo.uady.mx

Abstract:

Increasing gastrointestinal nematode resistance to conventional anthelmintics (AH) is a growing worldwide problem. Among various sources, the search for alternative AH has focused on plant secondary metabolites such as curcumin. The *in vitro* AH activity of curcumin (CUR) was evaluated against three isolates of the ruminant nematode *Haemonchus contortus* with different susceptibilities to conventional AH. Four *in vitro* tests were run: egg hatching inhibition (EHI), larval migration inhibition (LMI), larval exsheatment inhibition (LEI) and 72 h mortality of unsheathed L_3. Curcumin (CUR) concentration range was 0 - 8.5 µg CUR/mL in the EHI, LMI and mortality tests. In the LEI test it was 0 - 17.3 µg CUR/mL. Concentration-response curves were generated using a log-logistic regression. Experimental design was completely random and results were analyzed with an ANOVA. Curcumin did not exhibit AH activity in the EHI, LMI and mortality tests, but had a significant AH effect in the LEI test. This effect was strongest against the FMVZ-UADY isolate (EC_{50}= 1.9 µg/mL, 95%CI= 1.58-2.31), followed by the Paraíso isolate (EC_{50}= 3.2 µg/mL, 95%CI = 2.69-3.81) and the CENID-SAI, INIFAP isolate (EC_{50}= 7.0 µg/mL; 95%CI= 6.58-7.43). At the evaluated doses, curcumin had an AH effect against exsheatment of *H. contortus* L_3, but no effect on egg hatching, L_3 migration or mortality of exsheathed L_3.

Key words: Polyphenol, Anthelmintic, Larval exsheathed, Polymeric stabilizers.

Received: 26/04/2023

Accepted: 03/07/2024

Introduction

Gastrointestinal nematodes (GIN) are a major health problem in grazing ruminants[1,2]. Small ruminants with high GIN infections suffer digestive disorders that cause growth retardation or decrease productivity parameters. These parasites can also cause anemia, edema and, in very severe cases, death[3,4]. *Haemonchus contortus* is one of the most important GIN due to its pathogenicity and worldwide distribution in tropical and subtropical regions[5].

Control of *H. contortus* is based on commercial dewormers that can, over time, select for populations with anthelmintic resistance (AR)[6]. Increasing AR is the driving force behind the search for alternative helminth control measures[7]. Plant secondary metabolites, particularly polyphenolic compounds, have an anthelmintic (AH) effect against different *H.*

contortus life stages[8,9]. Turmeric *Curcuma longa* L., a member of the Zingiberaceae family and native to Asia, contains polyphenols. The main polyphenol in *C. longa* extracts is curcumin (CUR) (60-75 %), although smaller proportions of desmethoxycurcumin and bisdesmethoxycurcumin are also present[10,11]. Curcumin has confirmed pharmacological activities such as antioxidant, anti-inflammatory, anticancer, antiviral, antibacterial, and antiparasitic[12-18]. Its antiparasitic activity has been evaluated using extracts produced with solvents of different polarity, and different elements of *C. longa* plants. These extracts' AH activity has been evaluated using different concentrations (mg/mL) against *H. contortus* adults, L_3 and eggs[19-21]. High doses are used because CUR exhibits low hydrosolubility, poor absorption, and rapid degradation, which reduce its bioavailability[10].

Research has focused on increasing CUR solubility by encapsulating secondary metabolites in lipid nanoparticles, nanoemulsions, nanoliposomes, biodegradable polymers and dendrimers, and hydrogels, with the use of casein and cyclodextrins[22,23]. A combination of CUR and polymeric stabilizers such as polyvinylpyrrolidone (PVP) in a solid dispersion (CUR/PVP) has recently been proposed[24,25]. This combination improves CUR solubility and provides low toxicity in cells and tissues[26-28]. Few studies have evaluated the *in vitro* AH activity of *C. longa*, and none mention the CUR metabolite concentrations used in the bioassays; indeed, they assume that any observed AH effect is caused by CUR and other curcuminoids[19-21]. Therefore, the AH activity of CUR against *H. contortus* has yet to be unequivocally demonstrated. The present study objective was to evaluate the *in vitro* AH activity of CUR against three *H. contortus* isolates with different AR status.

Material and methods

Experimental ethics

All experimental animals were handled following applicable laws for germplasm collection (NOM-051-ZOO-1995 and NOM-062-ZOO-1999) (www.gob.mx/senasica). The experimental design and procedures followed the ethical guidelines of the Bioethics Committee of the Faculty of Veterinary Medicine and Zootechny of the Autonomous University of Yucatan (Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Yucatán - FMVZ-UADY) (Permit No. CB-CCBA-D-2021-005).

Study area

Production of *H. contortus* in donor animals with monospecific infections, and all *in vitro* bioassays, were performed at the FMVZ-UADY, Xmatkuil, Mérida, Mexico.

Haemonchus contortus isolates

Three *H. contortus* isolates were used in the tests. The AH resistance status of each was determined before their use.

(1) The Paraíso isolate originated from a commercial sheep farm in Umán, Yucatan, Mexico. It is reported to exhibit resistance to ivermectin (IVM; fecal egg count reduction: 64 %), albendazole sulfoxide (ABZ; fecal egg count reduction: 0%), and levamisole (LEV; fecal egg count reduction: 92 %)[29];

(2) The FMVZ-UADY isolate originated from a farm in Merida, Yucatan, Mexico. It has been reported as resistant to ABZ (fecal egg count reduction: 89 %) and LEV (fecal egg count reduction: 87 %)[30], but susceptible to IVM (fecal egg count reduction: 99 %)[29];

(3) The CENID-SAI-INIFAP isolate was provided by the Helminth Department of the National Center for Disciplinary Research in Animal Health and Food Safety (Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad - CENID-SAI) of the National Institute of Forestry, Agriculture and Livestock Research (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias - INIFAP). *In vitro* resistance assays were performed at the FMVZ-UADY. Based on the egg hatch methodology described by Von Samson-Himmelstjerna *et al*[31], this isolate was found susceptible to thiabendazole (TBZ) (EC_{50}= 0.050 μg/mL), and using the larval migration technique[32] it was found susceptible to IVM (EC_{50}= 1.09 μM)[33]. In a separate study using the *in vitro* mortality technique, it was found susceptible to IVM (79.22 % mortality at 11.42 mM)[34].

Donors and collection of monospecific *Haemonchus contortus* isolates

Donor animals were six goats aged 3 to 4 months, 15 kg live weight (LW), raised GIN-free from birth. For each *H. contortus* isolate, two donor animals were infected with 7,000 L_3 *per*

os[35]. At all times, the animals were kept in individual cages with raised floors located inside individual pens with concrete floors at the FMVZ-UADY facilities.

Beginning at 24 d post-infection, fecal samples were collected directly from the rectum of each donor animal in new polyethylene bags[36]. The fecal samples were processed following the McMaster technique to quantify the number of eggs *H. contortus* eggs and quantify them as eggs per gram of feces (EPG)[37]. Each test was performed with 2 g of feces and 28 mL saturated sugar solution (density = 1.28, 50 EPG sensitivity). Third stage larvae (L_3) were obtained from stool cultures maintained in clean plastic jars incubated at 28 °C for 5 to 6 days. The larvae were recovered using the Baermann technique[38], and identified as *Haemonchus* using morphological keys[39].

Haemonchus contortus egg collection

Egg collection was done following the MAFF (1986) procedure[38]. Feces were collected directly from the rectum of donor animals using polyethylene bags. In the laboratory, the feces were placed in a plastic strainer over a porcelain mortar. For every 10 g of feces, 100 mL water was added and the mixture macerated. The feces/water suspension was filtered through a double layer of gauze placed in a funnel, and the liquid recovered in a flask. This liquid was filtered through a 25 μm mesh with a minimal amount of dechlorinated water. Eggs were recovered from the mesh and placed in 50 mL tubes (5810R, Eppendorf, Germany). These were centrifuged at 453 *g* for 5 min, and the supernatant removed. A saturated sugar solution (25 mL, density= 1.28) was added to the sediment, The latter was resuspended in a vortex mixer and the tubes centrifuged again. The surface layer of the solution was recovered with a bacteriological loop and placed in another plastic tube containing water purified by reverse osmosis. This procedure was repeated several times to recover the largest possible number of eggs. Concentration per milliliter was estimated and adjusted until attaining a 200 eggs/mL suspension.

Haemonchus contortus L_3 production

Third stage larvae (L_3) were produced in feces collected every 24 h in polyethylene bags from a mesh placed under each cage. In the laboratory, the feces were washed with running water to remove detritus, and the recovered washed feces were used to perform separate coprocultures for each isolate with a Baermann apparatus[38], and placed in culture bottles with ventilated lids. The bottles were identified with harvest date, isolate name and

concentration (L_3/mL). The larvae were stored under refrigeration (6-10 °C) until used in the *in vitro* migration and exsheathing inhibition tests, and the exsheathed L_3 larvae mortality test.

Curcumin dispersion in polyvinylpyrrolidone (CUR/PVP)

Curcumin has poor solubility[16,40]. Therefore, prior to the *in vitro* studies, an established procedure was applied to improve turmeric E solubility and absorption[25]. Turmeric E (52.28% CUR; Laboratorios Mixim S.A. de C.V., Naucalpan, Mexico) was combined with PVP K30 (Agrimer K-30 Ashland, Columbus, Ohio, U.S.) at a 1:7 ratio. This produced a turmeric E dispersion with a 6.2 % final CUR concentration (CUR/PVP). This procedure was done at the Pharmaceutical Development Testing Laboratory (LEDEFAR), Cuautitlán Higher Education Faculty, National Autonomous University of Mexico (Universidad Nacional Autónoma de México – UNAM).

Preparation of CUR/PVP dispersion stock solutions

For the egg hatching inhibition (EHI), larval migration inhibition (LMI) and 72 h mortality tests of exsheathed L_3, a stock solution was prepared with 32.4 mg CUR/PVP dispersion in 20 mL purified water and stirred with a magnetic bar for 2 h. This suspension was centrifuged (BHG, Germany) at 1.057 *g* for 5 min and the supernatant used in the different bioassay concentrations. For the larval exsheathing inhibition (LEI) test, a stock solution was prepared with 64.8 mg CUR/PVP dispersion in 20 mL purified water.

Curcumin concentration

Because of CUR's low solubility, real CUR content was quantified in the supernatant suspension used in the bioassays. Suspension CUR concentration was measured following methodologies described in Buchi note No. 747[41] and FSSAI[42]. Briefly, the supernatant was analyzed with a UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, Beaconsfield, UK) at 425 nm transmittance. A calibration curve (0 - 6 mg/L) of CUR (Sigma Aldrich® standard cat. C7727, 91% purity) in ethanol was prepared. Pure ethanol (Sigma Aldrich® cat. E7148) was used to correct the background reading. The stock solution prepared with 32.4

mg CUR/PVP was found to contain 11.3 µg CUR/mL, and the stock solution prepared with 64.8 mg CUR/PVP contained 23 µg CUR/mL.

Egg hatching inhibition test

The EHI test was run following Coles *et al*[43]. The egg suspension (200 eggs/mL) was evenly distributed in 24-well plates (1 mL per well). Using the CUR/PVP stock solution containing 11.3 µg/mL CUR, serial dilutions (%) were made to four final concentrations in the wells: 2.3 (20 %), 4.0 (35 %), 5.7 (50 %) and 8.5 (75 %) µg/mL CUR in a 2,000 µL final volume. The positive control was 10 µL Lugol's solution (0.5 % incubated volume), and the negative control was 1,000 µL purified water. The plate was incubated at 28 ºC in a bacteriological oven. After 48 h, egg hatching was stopped by adding 50 µL Lugol's solution per well. A total of two replicates were performed with three repetitions per concentration. The content of each well was counted using McMaster chambers and a compound microscope (10x). In the bottom of the chamber, per sample counts were done of morulated eggs (ME), eggs containing unhatched larvae (UL) and L_1 larvae. The following formula was used:

$$\%\ Inhibition\ hatching = \frac{unhatched\ eggs}{unhatched\ eggs + \ L_1} \times 100$$

Larval migration inhibition

The LMI test was run to evaluate IVM in dimethyl sulfoxide according to Demeler *et al*[32], modified as follows for testing with CUR/PVP. The 11.3 µg CUR/mL concentration of CUR/PVP stock solution was used. A serial dilution (%) was done of the stock solution to produce seven final concentrations in the wells: 0, 0.6 (5 %), 1.1 (10 %), 2.0 (17.5 %), 3.7 (32.75 %), 5.7 (50 %) and 8.5 (75 %) µg CUR/mL. In a 24-well plate, 0 - 500 µL purified water plus 0 - 750 µL stock solution were placed in each well. The positive control was 100 µL (10 %) Lugol's solution, and the negative was 500 µL purified water. In wells with an 8.5 µg CUR/mL final concentration, 250 µL of a larval suspension containing 600 L_3/mL was added, with a 1,000 µL final volume. In the remaining wells, 500 µL of a larval suspension containing 300 L_3/mL was added, with a 1,000 µL final volume. Two replicates and three repetitions were done for each CUR concentration, and the positive and negative controls. The plates were incubated at 28 °C for 24 h. To prepare the migration plates, 500 µL 1.5 %

bacto agar were added to alternating rows, that is, one row with bacto agar and the next empty. A 25 µm mesh was placed in each well containing bacto agar. After incubation in CUR, the L_3 content of the working wells and control wells was transferred onto the mesh. The migration plates were incubated at 28 °C for 24 h to allow the L_3 to migrate through the mesh. After incubation, the mesh was removed, leaving the migrated L_3 larvae in the corresponding wells. Those L_3 that did not migrate and remained in the mesh were transferred to the empty wells in the adjacent rows of the same plate. The mesh was washed with 1,000 µL purified water to recover all L_3 in the corresponding well. One drop Lugol's solution was added to all wells, and the contents of each well poured into a McMaster chamber to count L_3 per well. The number of migrated and non-migrated L_3 in each concentration was counted and percentage of migration calculated using the formula described by Demeler *et al*[32]:

$$\%\ migration\ inhibition = \frac{non\ migrated\ L_3}{migrated\ L_3 + \ non\ migrated\ L_3} \times 100$$

Larval exsheathment inhibition

Inhibition of larval exsheathing (LEI) was quantified following Jackson and Hoste[44]. A suspension containing 1,000 L_3 per mL, and CUR/PVP dispersion stock solution containing 23.0 µg CUR/mL were used. The L_3 were incubated for 3 h at 23 ºC in seven different concentrations, obtained by serial dilution of the stock solution in 15 mL tubes: 0.6 (2.5 %), 2.3 (10 %), 3.5 (15 %), 4.6 (20 %), 8.1 (35 %), 11.5 (50 %) and 17.3 (75 %) µg CUR/mL. Additionally, L_3 were incubated in the respective concentrations of PVP K-30 to rule out any AH activity from the polymer. In the positive control, L_3 were incubated with LEV (120 mg/mL, Laboratorios Aranda S.A. de C.V., Mexico), and in the negative, they were exposed to purified water. The tubes were centrifuged at 453 *g* for 5 min and washed with purified water three times. Larvae exposed to the different treatments were divided into four 200 µL aliquots containing 200 L_3 each. Prior to the LEI test, calibration curves were generated for each isolate by inducing gradual exsheatment in five dilutions of a sodium hypochlorite (2 %) and sodium chloride (16.5 %) solution (1/300, 1/400, 1/480, 1/600 and 1/800) in phosphate buffer solution (PBS, pH 7.4). Exsheatment was monitored every 20 min (0, 20, 40 and 60 min) in 50 µL aliquots (25-50 L_3) using a microscope (10x and 40x). Exsheatment was stopped by flaming the slides covered with coverslips containing the L_3. Four replicates were run for each CUR concentration, and %LEI calculated with the formula:

$$\%\ Inhibition\ of\ exsheathing\ = \frac{L_3\ not\ exsheathed}{L_3\ not\ exsheathed + L_3\ exsheathed} \times 100$$

Mortality in exsheathed L_3

Mortality in exsheathed L_3 caused by CUR was tested following Reyes-Guerrero *et al*[34], with some modifications. When the L_3 of each *H. contortus* isolate were 3 to 15 wk of age, five mortality trials were run using exsheathed L_3 with 0.187 % commercial sodium hypochlorite[45]. Using a CUR/PVP dispersion stock solution containing 11.3 µg/mL CUR, exsheathed L_3 were exposed to five different concentrations: 1.1 (10 %), 2.3 (20 %), 4.0 (35 %), 5.7 (50 %) and 8.5 (75 %) µg CUR/mL. Lugol's solution (10 µL) was used as the positive control and purified water (50 µL) as the negative control. Using 96-well microtiter plates, two replicates and three repetitions were done for each CUR concentration, and the positive and negative controls. In wells with an 8.5 µg/mL CUR final concentration, 25 µL containing 100 L_3 were added to each well. In the remaining wells, 50 µL containing 100 L_3 were added to each well. Final volume in all wells was 100 µL. The plates were incubated at 28 °C for 72 h. After incubation, the live and dead L_3 in each well were counted by collecting the entire well in 10 µL drops that were deposited on slides for counting with an optical microscope (4x). Mortality was calculated as a percentage using the formula:

$$\%\ Mortality = \frac{dead\ L_3}{dead\ L_3\ \ +\ live\ L_3} \times 100$$

Data analysis

The data produced in the assays involving eggs (EHI) or L_3 (LMI, LEI and mortality) were used to generate concentration-response curves in a log-logistic regression with two parameters; the drc extension in the RStudio software was used[46,47]. Each test's EC_{50} and 95% CI were calculated. The percentage data for EHI, LMI, LEI and mortality were analyzed per test with a completely randomized design, using an ANOVA and the GLM function of the mass extension in the RStudio software[42,43]. Two factors were included: the three isolates (Paraíso, FMVZ-UADY and CENID-SAI, INIFAP) and the CUR concentrations in

each test. Comparison of the means was run with a Bonferroni test at a $P<0.05$ significance level.

Before running the ANOVA, a Kolmogorov-Smirnov normality test was run and confirmed that the EHI, LMI and LEI data did not meet the assumption of normality. A Breusch-Pagan heteroscedasticity test found that the same data did not comply with homogeneity of variance. Therefore, the data was transformed with the Box-Cox transformation using the mass extension of the RStudio software[47,48]. Analyses were then done using the transformed values, after confirming the assumptions of normality and homogeneity of variance. However, the results are presented transformed to normal values to facilitate interpretation.

Results

CUR inhibition of egg hatching and L_1 development

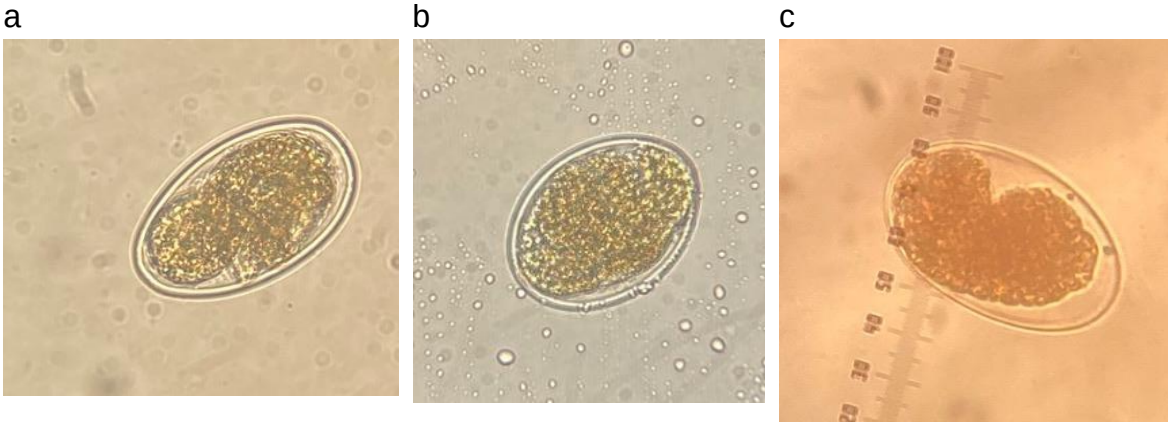
Of the four tested CUR concentrations, only the 5.7 µg/mL CUR achieved 99 % inhibition of hatching in the Paraíso and FMVZ-UADY isolates (Table 1); morulated eggs and larvae were observed (Figure 1 a y b). Inhibition did not decrease significantly for these two isolates even at the highest (8.5 µg CUR/mL) concentration (Table 1). At this same concentration, the L_1 exhibited morphological changes that may be associated with larval damage or non-viability (Figure 2 a and b). In the CENID-SAI, INIFAP isolate, the EHI percentages were low (< 10 %) at all concentrations. The EC_{50} could not be calculated for any of the tested *H. contortus* isolates.

Table 1: Egg hatching inhibition (%, average ± standard deviation) at five curcumin (CUR) concentrations in three *Haemonchus contortus* isolates with different susceptibilities to commercial anthelmintics

Isolate	0 µg CUR/mL	2.3 µg CUR/mL	4 µg CUR/mL	5.7 µg CUR/mL	8.5 µg CUR/mL
Paraíso	5.1 ± 2.5	6.6 ± 3.6	16.4 ± 10.5	100 ± 0 [a]	34.8 ± 50.6
FMVZ-UADY	3.3 ± 0.9	8.3 ± 4.9	7.4 ± 2.4	99.8 ± 0.3 [a]	8.7 ± 3.5
CENID-SAI INIFAP	3.2 ± 1.2	6.5 ± 2.2	7.6 ± 2.1	6.5 ± 1.9 [b]	6.6 ± 1.2

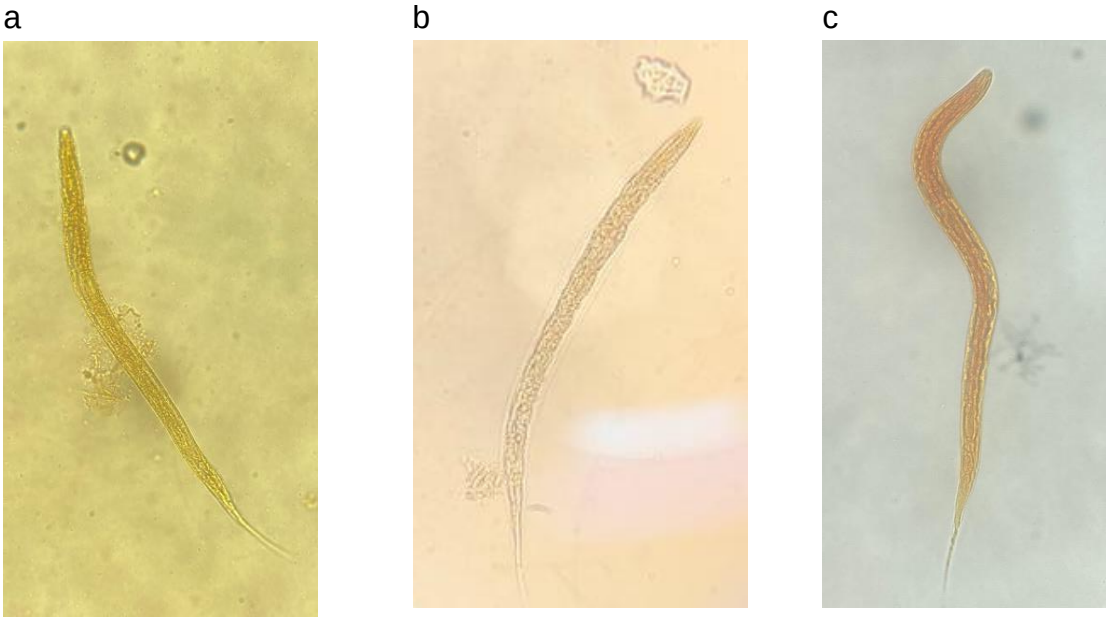
ab Different letter superscripts in the same column indicate significant difference ($P<0.05$).

Figure 1: Eggs of the *Haemonchus contortus* Paraíso isolate (40x) in 5.7 µg/mL CUR concentration in egg hatching inhibition test



(a) Larvated egg, (b) morulated egg, (c) egg from positive control containing thiabendazole.

Figure 2: L_1 larvae of the *Haemonchus contortus* Paraíso isolate (40x) in 8.5 µg/mL CUR concentration in egg hatching inhibition test



(a) Degraded larva, (b) degraded larva with cuticle separated from body, (c) normal L_1 larva in negative control.

CUR inhibition of L_3 migration

Of the six tested concentrations, only the 5.7 µg/mL CUR concentration notably inhibited migration compared to the negative control (P<0.05): 64 % inhibition against the Paraíso isolate and 53 % against the FMVZ-UADY isolate (Table 2). The highest inhibition against the CENID SAI, INIFAP isolate was only 20.3 % in the 8.5 µg/mL CUR concentration, which did not differ from the negative control. As occurred with the EHI results, the EC_{50} could not be calculated for CUR inhibition of migration in the three isolates.

Table 2: L_3 migration inhibition (%, average ± standard deviation) at seven curcumin (CUR) concentrations in three *Haemonchus contortus* isolates with different susceptibilities to commercial anthelmintics

Isolate	0 µg CUR/mL	0.6 µg CUR/mL	1.1 µg CUR/mL	2.0 µg CUR/mL	3.7 µg CUR/mL	5.7 µg CUR/mL	8.5 µg CUR/mL
Paraíso	11.4 ± 3.6	15.2 ± 3.6	18.8 ± 14	13.2 ± 1.8	12.7 ± 3.8	64.1 ± 22.1 [a]	18.1 ± 7.1
FMVZ-UADY	11.1 ± 4.5	19.2 ± 10.5	10.8 ± 3.5	10.8 ± 3.5	12.1 ± 6.8	53 ± 23.1 [a]	18.9 ± 12.9
CENID-SAI INIFAP	8.4 ± 3.1	9.3 ± 7.4	8.4 ± 2.7	8.7 ± 2.4	14.6 ± 4.3	13.6 ± 2.4 [b]	20.3 ± 12.8

[ab] Different letter superscripts in the same column indicate significant difference (P<0.05).

CUR inhibition of exsheatment in L_3

Exsheatment was inhibited in L_3 from 60 to 100 % at 8.1, 11.5 and 17.3 µg CUR/mL. The EC_{50} of each isolate differed from the others since their 95%CI did not overlap. The lowest values were for the FMVZ-UADY isolate (EC_{50}= 1.9 µg/mL, 95%CI = 1.58-2.31), followed by the Paraíso isolate (EC_{50}= 3.2 µg/mL, 95%CI= 2.69-3.81), and CENID-SAI, INIFAP (EC_{50}= 7.0 µg/mL; 95%CI= 6.58-7.43).

CUR-caused mortality in exsheathed L_3

Mortality in exsheathed L_3 of all three isolates was less than 10 % at all the tested CUR concentrations. Therefore, CUR had no apparent effect on mortality in exsheathed L_3 in these isolates.

Discussion

CUR inhibition of egg hatching

All but one of the tested CUR concentrations had no AH effect on hatching of *H. contortus* eggs; indeed, no EC_{50} could be determined for CUR in the three isolates. However, a 99% decrease in hatching was observed with the 5.7 µg CUR/mL concentration in the Paraíso and FMVZ-UADY isolates. Apparently, conditions at this concentration were favorable for solubility of CUR in water and its diffusion through the layers protecting the *H. contortus* egg. This effect was not observed at the highest concentration (8.5 µg CUR/mL). Why this occurred is unclear. It may be due to high solute saturation in the bioassay liquid, or to a hormetic effect. In the latter, a biphasic response occurs in which low doses have no effect, moderate doses cause change, and high doses exhibit little or none of the expected effect (when graphed it has the shape of an inverted "J" or "U")[49]. This phenomenon is a central theme in the biological, adaptive and repair response, and has implications in pharmacology and toxicology[50]. Hormesis is known to occur in different cell types as curcumin concentrations change[51,52]. A *C. longa* methanol:water extract (70:30) is reported to have an AH effect on *H. contortus* eggs, with an EC_{50} of 69.75 µg/mL[20]. In another study, *H. contortus* eggs were affected by a hydroalcoholic (1:9) extract of *C. longa* rhizome (EC_{50} = 100.9 mg/mL), as well as by an aqueous one (EC_{50}= 83.7 mg/mL)[53]. In neither of these two studies was the role of CUR determined in the AH effect of the *C. longa* extracts against *H. contortus* eggs. The solvents used in both studies for compound extraction were of varying polarity and the extracts were not purified for identification of the main compounds. Therefore, any effect of curcumin can only be assumed, since this is the main component in *C. longa* extracts. However, a synergistic effect between different curcuminoids cannot be discounted. For instance, in a study of curcuminoids' effects on *Toxocara canis*, CUR alone exhibited an AH effect on L_2, but this was augmented when CUR was combined with desmethoxycurcumin, bisdesmethoxycurcumin and cyclocurcumin, suggesting that synergism improved its effectiveness[54].

Of note in the present EHI results is that CUR may cause loss of cellular continuity in L_1 at the internal organ level, and these changes may undermine viability in this larval stage. This effect on L_1 has not been reported previously, suggesting the need to use the larval development methodology to evaluate the effects of CUR on larvae at different stages[55].

CUR anthelmintic activity against L_3

In both the LMI and EHI tests, CUR had no AH effect and the EC_{50} could not be calculated for any of the three evaluated isolates. As in the EHI test, the best inhibition of L_3 migration was with the 5.7 µg CUR/mL concentration, though even at this concentration inhibition percentages were only 50 to 60 %. Results in the EHI and LMI tests were inconsistent, possibly due to degradation of CUR. Indeed, the CUR solution in the wells had changed from a light yellow at the beginning of the bioassay to a brown color at the end (48 h)[56,57]. As far as is known, no previous research exists on *in vitro* LMI testing evaluating any *C. longa* extract against *H. contortus* L_3.

The most outstanding result is that the evaluated CUR extracts blocked exsheathment in *H. contortus* L_3. This blockage, which is known to occur in ruminal fluid[58], may prevent L_3 from invading the crypts in the abomasum, and consequently make larvae incapable of transitioning to later stages, including L_4, L_5 and adult[59]. Blockage of exsheathment is associated with the activity of polyphenols in plant extracts from leaves and other elements[8,60]. Curcumin is a polyphenol structurally related to caffeic acid and ferulic acid[61]. Both these acids have been evaluated against *H. contortus* in EHI and LEI bioassays[8]. Ferulic acid has ovicidal activity at 200-400 µg/mL, and in the LEI test caffeic acid exhibited activity at 7.8 µg/mL and ferulic acid at 20.6 µg/mL. In the present results, CUR inhibited L_3 exsheathment at concentrations < 7 µg/mL, lower than polyphenols mentioned above.

The mechanism by which CUR inhibits exsheathment in the three evaluated *H. contortus* isolates is unknown. The effectiveness of CUR's inhibition of exsheathment varied between the three isolates. How effective CUR is at blocking exsheathment may depend on how it interacts with enzymes, proteins, nucleic acids, biomolecules and different receptors types in the sheath of *H. contortus*[62]. A recent metabolomics study reported a panel of metabolites directly responsible for L_3 exsheathment, which were associated with amino acids and with the purine and pyruvate metabolism pathways in *H. contortus*[63]. Further research is needed on the possibility that CUR's effects on one or more of these metabolic pathways is responsible for its inhibition of exsheathment.

The present results confirm that CUR does not affect mortality in exsheathment L_3. The low mortality observed here in exsheathment L_3 (<10 %) coincides with the low mortality (<10 %) reported elsewhere with a *C. longa* extract at 100 µg/mL[20], and the absence of any effect on L_3 mortality with two *C. longa* extracts[53]. These studies contrast with the work of Nasai *et al*[21], in which an ethanolic *C. longa* extract increased L_3 mortality (78 %) at doses of 200 mg/mL. However, a large amount of extract was used in this study, which is unfeasible under *in vivo* conditions. Nonetheless, even though no EC_{50} could be estimated for CUR in

the L_3 mortality assay, the EHI test demonstrated that CUR can cause changes compatible with non-viability of *H. contortus* L_1.

Finally, the results confirm that *H. contortus* isolates from different geographic regions exhibit different *in vitro* susceptibility to natural plant compounds, both in the EHI and LEI tests[64,65]. These previous studies suggest parasites implement an adaptive process to survive in the presence of secondary compounds with AH activity ingested by the ruminant during grazing. In the present study, the isolates had varying responses at the evaluated CUR concentrations in the different *in vitro* bioassays. This was most evident in the LEI tests in which the three isolates' responses differed notably from each other. It is unclear why the CENID-SAI, INIFAP isolate exhibited the lowest sensitivity to the CUR extract concentrations compared to the two isolates from Yucatan.

Conclusions and implications

The evaluated curcumin extract had no *in vitro* anthelmintic activity against egg hatching, L_3 migration or mortality in exsheathed L_3 in any of the evaluated *H. contortus* isolates (FMVZ-UADY, Paraíso, CENID-SAI, INIFAP). However, it did inhibit exsheathment in L_3, with variable responses in each of the isolates.

Conflict of interests

The authors declare no conflicts of interest.

Acknowledgements

The research reported here was funded by the CENID SAI, INIFAP through project 20454534898, and forms part of the PhD project of Lisandro Encalada Mena at the FMVZ-UADY. Lisandro Encalada Mena received a graduate studies scholarship from the PRODEP-SEP program (UNACAM-122). Production of *H. contortus* eggs and larvae of the three isolates was done using material from the FMVZ-UADY GIN ceparium, a product of the Cátedras CONAHCYT No. 692 project.

Literature cited:

1. Encalada-Mena LA, Corbala-Bermejo JA, Vargas-Magaña JJ, García-Ramírez MJ, Uicab-Brito L, del Río-Rodríguez J. Gastrointestinal nematode prevalence in dual purpose systems in the municipality of Escárcega, Campeche, México. Agrociencia 2009;43(6):569–576.

2. Rinaldi L, Catelan D, Musella V, Cecconi L, Hertzberg H, Torgerson PR. *et al. Haemonchus contortus*: spatial risk distribution for infection in sheep in Europe. Geospat Health 2015;19:325–331.

3. Coop RL, Holmes PH. Nutrition and parasite interaction. Int J Parasitol 1996;26(8-9):951-962.

4. Arsenopoulos KV, Fthenakis GC, Katsarou EI, Papadopoulos E. Haemonchosis: A Challenging parasitic infection of sheep and goats. Animals 2021;11:1–28.

5. Besier RB, Kahn LP, Sargison ND, Van Wyk JA. The Pathophysiology, Ecology and Epidemiology of *Haemonchus contortus* Infection in Small Ruminants. Adv Parasitol 2016;93:95-143.

6. Rose H, Rinaldi L, Bosco A, Mavrot F, De Waal T, Skuce P. *et al.* Widespread anthelmintic resistance in European farmed ruminants: a systematic review. Vet Rec 2015;176(21):546-546.

7. Torres-Acosta JFJ, Hoste H, Sandoval-Castro CA, Torres-Fajardo RA, Ventura-Cordero J, González-Pech P. *et al.* The art of war against gastrointestinal nematodes in sheep and goat herds of the tropics. Rev Académica Cienc Anim 2019;17:39-46.

8. Mancilla-Montelongo G, Castañeda-Ramírez GS, Torres-Acosta JFJ, Sandoval-Castro CA, Borges-Argáez R. Evaluation of cinnamic acid and six analogues against eggs and larvae of *Haemonchus contortus*. Vet Parasitol 2019;270:25-30.

9. Lima CS, Pereira MH, Gainza YA, Hoste H, Regasini LO, de Souza Chagas AC. Anthelmintic effect of *Pterogyne nitens* (Fabaceae) on eggs and larvae of *Haemonchus contortus*: Analyses of structure-activity relationships based on phenolic compounds. Ind Crops Prod 2021;164:113348.

10. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The essential medicinal chemistry of curcumin. J Med Chem 2017;60(5):1620–1637.

11. Tsuda T. Curcumin as a functional food-derived factor: degradation products, metabolites, bioactivity, and future perspectives. Food Funct 2018;9(2):705-714.

12. Mimche PN, Taramelli D, Vivas L. The plant-based immunomodulator curcumin as a potential candidate for the development of an adjunctive therapy for cerebral malaria. Malar J 2011;10(Suppl 1):1-9.

13. Rauf A, Imran M, Orhan IE, Bawazeer S. Health perspectives of a bioactive compound curcumin: A review. Trends Food Sci Technol 2018;74:33–45.

14. Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza-Chaverrí J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol 2013;1(1):448–456.

15. Xue X, Yu JL, Sun DQ, Zou W, Kong F, Wu J. *et al.* Curcumin as a multidrug resistance modulator - A quick review. Biomed Prev Nutr 2013;3(2):173–176.

16. Mahmood K, Zia KM, Zuber M, Salman M, Anjum MN. Recent developments in curcumin and curcumin based polymeric materials for biomedical applications: A review. Int J Biol Macromol 2015;81:877–890.

17. De Aguiar DP, Moscardini MBM, Morais ER, De Paula RG, Ferreira PM, Afonso A. *et al.* Curcumin generates oxidative stress and induces apoptosis in adult *Schistosoma mansoni* worms. PLoS One 2016;11(11):1–25.

18. Cervantes-Valencia ME, Alcalá-Canto Y, Sumano-Lopez H, Ducoing-Watty AM, Gutierrez-Olvera L. Effects of *Curcuma longa* dietary inclusion against *Eimeria spp*. in naturally-infected lambs. Small Ruminant Res 2016;136:27-35.

19. Singh A, Varshneya C, Telang RS. *In vitro* anthelmintic effect of *Curcuma longa*. Indian Vet J 2005;82(6):594–596.

20. Ullah S, Khan MN, Sajid MS, Muhammad G. Comparative anthelmintic efficacy of *Curcuma longa, Citrullus colocynthis* and *Peganum Harmala.* Glob Vet 2013;11(5):560–567.

21. Nasai NB, Abba Y, Abdullah FFJ, Marimuthu M, Tijjani A, Sadiq MA. *et al*. *In vitro* larvicidal effects of ethanolic extract of *Curcuma longa* Linn on *Haemonchus* larval stage. Vet World 2016;9(4):417–420.

22. Yallapu MM, Jaggi M, Chauhan SC. Curcumin nanoformulations: a future nanomedicine for cancer. Drug Discov 2012;17(1-2):71-80.

23. Rafiee Z, Nejatian M, Daeihamed M, Jafari SM. Application of curcumin-loaded nanocarriers for food, drug and cosmetic purposes. Trends Food Sci Technol 2019;88:445-458.

24. Paradkar A, Ambike AA, Jadhav BK, Mahadik KR. Characterization of curcumin–PVP solid dispersion obtained by spray drying. Int J Pharm 2004;271(1-2):281-286.

25. Hernandez-Patlan D, Solis-Cruz B, Pontin KP, Latorre JD, Baxter MF, Hernandez-Velasco X. *et al.* Evaluation of a solid dispersion of curcumin with polyvinylpyrrolidone and boric acid against *Salmonella enteritidis* infection and intestinal permeability in broiler chickens: A pilot study. Front Microbiol 2018;9:1–10.

26. Teodorescu M, Bercea M. Poly(vinylpyrrolidone) – A versatile polymer for biomedical and beyond medical applications. Poly Plast Technol Eng 2015;54(9):923–943.

27. Sadeghi F, Ashofteh M, Homayouni A, Abbaspour M, Nokhodchi A, Garekani HA. Antisolvent precipitation technique: A very promising approach to crystallize curcumin in presence of polyvinyl pyrrolidon for solubility and dissolution enhancement. Colloids Surf B 2016;147:258-264.

28. Guo L, Shi M, Song N, Wan Z, Liu H, Liu L. Anchorage of curcumin onto PVP enhances anti-tumor effect of curcumin. Med Chem Res 2019;28(5):646–656.

29. Sepúlveda-Vázquez J, Lara-Del Rio MJ, Vargas-Magaña JJ, Quintal-Franco JA, Alcaraz-Romero RA, Ojeda-Chi MM. *et al.* Frequency of sheep farms with anthelmintic resistant gastrointestinal nematodes in the Mexican Yucatán peninsula. Vet Parasitol: Reg Stud Rep 2021;24:1–7.

30. Mancilla-Montelongo MG, González-Pech PG, Miranda-Miranda DY, Castañeda-Ramírez GS, Encalada-Aguilar PA, Can Celis AC. *et al.* Targeted treatment strategies for the control of gastrointestinal nematodes in a goat flock with anthelmintic resistant worm populations and poor nutrition. Small Rumin Res 2023;227:107062.

31. Von Samson-Himmelstjerna G, Coles GC, Jackson F, Bauer C, Borgsteede F, Cirak VY. *et al.* Standardization of the egg hatch test for the detection of benzimidazole resistance in parasitic nematodes. Parasitol Res 2009;105(3):825–834.

32. Demeler J, Küttler U, von Samson-Himmelstjerna G. Adaptation and evaluation of three different *in vitro* tests for the detection of resistance to anthelmintics in gastro intestinal nematodes of cattle. Vet Parasitol 2010;170:61–70.

33. Encalada-Mena LA, López-Arellano, R, López-Arellano ME, Mancilla-Montelongo M G, Sandoval-Castro CA, Olmedo-Juárez A. *et al.* Curcumin-PVP improves the *in vitro* efficacy of ivermectin against resistant and susceptible *Haemonchus contortus*. Exp Parasitol 2024;256:108670.

34. Reyes-Guerrero DE, Cedillo-Borda M, Alonso-Morales RA, Alonso-Díaz MA, Olmedo-Juárez A, Mendoza-de-Gives P. *et al.* Comparative study of transcription profiles of the P-glycoprotein transporters of two *Haemonchus contortus* isolates: Susceptible and resistant to ivermectin. Mol Biochem Parasitol 2020;238:111281.

35. Ramos-Bruno E, Sandoval-Castro CA, Torres-Acosta JFJ, Sarmiento-Franco LA, Torres-Fajardo R, Chan-Pérez, JI. *et al.* Nitrogen retention in hair sheep lambs with a gradient of *Haemonchus contortus* infection. Vet Parasitol 2021;296:109488.

36. Mendoza de Gives P, Torres-Acosta JF, Figueroa-Castillo JA, Soberanes-Céspedes N, Mancilla-Montelongo MG, Jasso-Villazul C. *et al.* Diagnóstico y control sustentable de nematodos gastrointestinales en ovinos y caprinos en la era de la resistencia antihelmíntica. Primera ed. Mérida, Yucatán, México: Editorial Universidad Autónoma de Yucatán; 2023.

37. Bauer BU, Pomroy WE, Gueydon J, Gannac S, Scott I, Pfister K. Comparison of the FLOTAC technique with the McMaster method and the Baermann technique to determine counts of *Dictyocaulus eckerti* L_1 and strongylid eggs in faeces of red deer (*Cervus elaphus*). Parasitol Res 2010;107:555-560.

38. Ministry of Agriculture, Fisheries and Food (MAFF). Manual of veterinary parasitological laboratory techniques. 3rd ed. London. Her Majesty´s Stationery Office (HMSO); 1986.

39. van Wyk JA, Mayhew E. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. Onderstepoort J Vet Res 2013;80(1):1–14.

40. Liu W, Zhai Y, Heng X, Che FY, Chen W, Sun D. *et al.* Oral bioavailability of curcumin: problems and advancements. J Drug Target 2016;24(8):694–702.

41. Buchi (2021) Application note No. 747/2021. Determination of curcuminoids in turmeric powder. https://assets.buchi.com/image/upload/v1623829658/pdf/ShortNotes/SN_747_2021_Determination_of_Curcuminoids_in_turmeric_powder.pdf.

42. Food Safety and Standards Authority of India (FSSAI). Manual of methods of analysis of foods: spices, herbs and condiments. New Delhi, Government of India, Ministry of Health and Family Welfare; 2012.

43. Coles GC, Jackson F, Pomroy WE, Prichard RK, Von Samson-Himmelstjerna G, Silvestre A. *et al.* The detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 2006;136(3–4):167–185.

44. Jackson F, Hoste H. *In vitro* methods for primary screening of plant products for direct activity against ruminant nematodes. In: Vercoe PE, *et al* editors. *In vitro* screening of plant resources for extra- nutritional attributes in ruminants: Nuclear and related methodologies. Springer;2010:25–45.

45. Liébano-Hernández E, López-Arellano ME, Vázquez-Prats V, Mendoza de Gives P. Cryopreservation of infective larvae of *Haemonchus contortus*. Rev Latinoam Microbiol 1996;38(2):111-114.

46. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-response analysis using R. PLoS One 2015;10(12):1–13.

47. R Core Team. A language and environment for statistical computing Vienna, Austria. 2020.

48. Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A. Firth D, Ripley MB. Package 'mass'. Cran R 538. 2013;113-120.

49. Calabrese EJ. Hormesis: path and progression to significance. Int J Mol Sci 2018;19(10):2871.

50. Calabrese EJ, Mattson MP. How does hormesis impact biology, toxicology, and medicine?. NPJ Aging Mech 2017;3(1):13.

51. Moghaddam NSA, Oskouie MN, Butler AE, Petit PX, Barreto GE, Sahebkar A. Hormetic effects of curcumin: What is the evidence? J Cell Physiol 2019;234(7):10060–10071.

52. Rainey NE, Moustapha A. Petit PX. Curcumin, a multifaceted hormetic agent, mediates an intricate crosstalk between mitochondrial turnover, autophagy, and apoptosis. Oxid Med Cell Longev 2020;3656419.

53. Cruz-Aguila J. Pruebas *in vitro* de extractos vegetales contra *Haemonchus contortus* [tesis licenciatura]. México, Tabasco: Universidad Autónoma Chapingo; 2018.

54. Kiuchi F, Goto Y, Sugimoto N, Tsuda Y, Akao N, Kondo K. Nematocidal activity of turmeric: synergistic action of curcuminoids. Chem Pharm Bull 1993;41(9):1640–1643.

55. Olmedo-Juárez A, Rojo-Rubio R, Arece-García J, Salem AZ, Kholif AE, Morales-Almaraz E. *In vitro* activity of *Pithecellobium dulce* and *Lysiloma acapulcensis* on exogenous development stages of sheep gastrointestinal strongyles. Ital J Anim Sci 2014;13(4):3104.

56. Li B, Konecke S, Wegiel LA, Taylor LS, Edgar KJ. Both solubility and chemical stability of curcumin are enhanced by solid dispersion in cellulose derivative matrices. Carbohydr Polym 2013;98(1):1108-1116.

57. Rathnayaka RMMP, Madhusankha GDMP, Perera ODAN. Curcumin ethanolic extraction and its' stability in food applications. In 2019 From Innovation to Impact (FITI), 1-6.

58. Marin-Tun CG, Mancilla-Montelongo MG, Capetillo-Leal CM, Sandoval-Castro CA, Hoste H, Borges-Argáez R, Torres-Acosta JFJ. Adapting the *in vitro* rumen incubation method to evaluate the effect of a plant extract on the exsheathment inhibition of *Haemonchus contortus* infective larvae. Vet Parasitol 2024;327:110135.

59. Sommerville RI. The exsheathing mechanism of nematode infective larvae. Exp Parasitol 1957;6(1):18-30.

60. Spiegler V, Liebau E, Hensel A. Medicinal plant extracts and plant-derived polyphenols with anthelmintic activity against intestinal nematodes. Nat Prod Rep 2017;34(6):627-643.

61. Hardman WE. Diet components can suppress inflammation and reduce cancer risk. Nutr Res Pract 2014;8(3):233-240.

62. Ali R, Rooman M, Mussarat S, Norin S, Ali S, Adnan M, Khan SN. A systematic review on comparative analysis, toxicology, and pharmacology of medicinal plants against *Haemonchus contortus*. Front Pharmacol 2021;12:644027.

63. Palevich N, Maclean PH, Subbaraj AK, Cao M. A multimodal metabolomics approach to elucidate the trigger components of rumen fluid for larval exsheathment in a model gastrointestinal parasitic nematode, *Haemonchus contortus*. bioRxiv 2021. [preprint]

64. Chan-Pérez JI, Torres-Acosta JF, Sandoval-Castro CA, Hoste H, Castañeda-Ramírez GS, Vilarem G. *et al. In vitro* susceptibility of ten *Haemonchus contortus* isolates from different geographical origins towards acetone:water extracts of two tannin rich plants. Vet Parasitol 2016;217:53–60.

65. Chan-Pérez, JI, Torres-Acosta JFJ, Sandoval- Castro CA, Castañeda-Ramírez GS, Vilarem G, Mathieu C. *et al.* Susceptibility of ten *Haemonchus contortus* isolates from different geographical origins towards acetone:water extracts of polyphenol-rich plants. Part 2: Infective L_3 larvae. Vet Parasitol 2017;240:11-16.

https://doi.org/10.22319/rmcp.v15i4.6630

Article

Effect of spent coffee grounds aqueous extract as an antioxidant in raw pork patties during refrigerated storage

Juan Luis Murillo Hernández [a]

Rey David Vargas Sánchez [b]

Brisa del Mar Torres Martínez [b]

Nelson Huerta Leidenz [c]

Gastón Ramón Torrescano Urrutia [b]

Armida Sánchez Escalante [b*]

[a] Instituto Tecnológico de Estudios Superiores de Zamora. Ingeniería en Industrias Alimentarias. Michoacán, México.

[b] Centro de Investigación en Alimentación y Desarrollo (CIAD). Coordinación de Tecnología de Alimentos de Origen Animal (CTAOA). Carretera a La Victoria Km. 0.6, 83148 Sonora, México.

[c] Texas Tech University. Department of Animal and Food Sciences. Texas, USA.

*Corresponding author: armida-sanchez@ciad.mx

Abstract:

The effect of spent coffee grounds (SCG) aqueous extract and butylated hydroxytoluene (BHT) on color deterioration, lipid oxidation, and antioxidant status of uncooked pork patties during refrigerated storage (4 °C/9 days, under dark) was investigated. Polyphenols content and antiradical activity of SCG extract were evaluated. Pork patties were evaluated for pH, color parameters and lipid oxidation (LOX), as well as total antioxidant activity of meat. Results showed that SCG extract is an important source of polyphenols and exerts antioxidant activity. Their inclusion in meat samples mitigated undesirable changes in pH, color, and

LOX values and increased the antioxidant stability during storage (P<0.05). In conclusion, using SCG extract as a natural antioxidant can improve raw pork patties' quality and shelf life.

Keywords: Natural extract, Coffee residues, Antioxidant activity, Pro-oxidation, Meat quality.

Received: 29/01/2024

Accepted: 25/09/2024

Introduction

Lipid oxidation (LOX) of meats leads to the development of undesirable compounds that undermine nutrient composition (e.g., essential amino acids and fatty acids) and sensory attributes (color, odor, flavor, and texture), thus compromising the purchase intentions and acceptability of ultimate consumers. Therefore, to reduce LOX process in meat and meat products, synthetic antioxidants (e.g., butylated hydroxytoluene and butylated hydroxyanisole) have been used extensively. Nevertheless, increasing concerns about the health risks posed by synthetic antioxidants are forcing their replacement for natural antioxidant compounds[1].

Natural antioxidant compounds have been extracted from each anatomical structure of plants, such as flowers, fruits, leaves, among others. However, by-products derived from the fruit processing industry have also been considered an important source of polyphenols with this property[2]. The insoluble residue obtained after filtering the drink is a by-product commonly known as spent coffee grounds (SCG). SCG is usually discarded when it is not used as a fertilizer[3].

Instead of treating SCG as a waste, other processing industries have taken advantage of this raw material as a substrate for fungal growth[4] and as a food additive for bakeries[5]. In this context, roasted ground coffee residue extract added at 15 % to salted mackerels has decreased LOX during 15 d of refrigerated storage[6]. SCG has also been proposed as an ingredient for the meat industry to reduce LOX[1]. However, the assessment of SCG and their extracts as an antioxidant additive for developing novel meat products to enhance shelf life needs further examination.

Therefore, the inclusion of SCG aqueous extract as a functional ingredient to enhance antioxidant status of raw pork patties during refrigerated storage was investigated.

Material and methods

Polyphenols extraction

SCG was procured (Caffenio®, dark *Coffea arabica* L.) and subjected to thermal sterilization. Polyphenols compounds from SCG were extracted with water as a solvent by an ultrasound-assisted method (42 KHz/25 °C/30 min), using a 1:10 SCG-solvent ratio (ultrasound bath, Bransonic 3800; Jeju, Korea). The resultant mixture was filtered (Whatman 1 filter paper) under vacuum (vacuum pump, MVP 6; Jeju, Korea), evaporated at 100 rpm/60 °C (Yamato RE301BW; Tokyo, Japan), and dried (Yamato DC401; Tokyo, Japan). The resulting SCG extract was stored at -20 °C/under dark conditions[7].

Polyphenols content

Chlorogenic acid content (CAC) was determined, as reported previously[8]. SCG extract (100 µl, 500 µg/mL) was mixed with 200 µL of urea (0.17 M) and 200 µL of glacial acetic acid (0.1 mol/L), then 500 µL of dH_2O were added. The resultant mixture was homogenized with 500 µL of $NaNO_2$ (0.14 mol/L) and 500 µL of NaOH (0.5 mol/L) and centrifuged (2,250 xg/4 °C, 10 min). At 510 nm was measured the absorbance and results displayed as mg of chlorogenic acid equivalents (CAE)/per gram of extract.

Total phenol content (TPC) was determined by the Folin-Ciocalteu procedure[9]. SCG extract (10 µL, 500 µg/mL) was mixed with 80 µL of dH_2O and 60 µL of Na_2CO_3 (7 %, w/v), then 40 µL of Folin-Ciocalteu reagent (2 M) was added. The resultant mixture was homogenized with 80 µL of dH_2O and incubated (25 °C/1 h, under dark). At 750 nm was measured the absorbance and results displayed as milligrams of gallic acid equivalents (GAE)/g.

Total flavonoids content (TFC) was determined by the $NaNO_2$-$Al(NO_3)_3$-NaOH procedure[10]. SCG extract (500 µL, 500 µg/mL) was homogenized with 1 mL of $NaNO_2$ (5 %, w/v), 10 mL of NaOH (1 mol/L), and 1 mL of $AlCl_3$ (10 %, w/v). Then 25 mL of ethanol (70 %, v/v) was added. The resultant solution was incubated (25 °C/15 min, under

dark). At 510 nm was measured the absorbance and results displayed as mg of rutin equivalents (RE)/g.

Total tannins content (TTC) was determined by the vanillin procedure[11]. SCG extract (0.2 g) was mixed with 10 mL of methanol and centrifuged (10,000 xg/4 °C, 20 min). Then 180 µL of the supernatant was mixed with 900 µL of vanillin (1 %, w/v) and 900 µL of HCl (8 %, v/v) and incubated (25 °C/20 min, under dark). At 500 nm was measured the absorbance and results were displayed as mg of (+)-catechin equivalents (CE)/g.

Antioxidant assays

Free-radical scavenging activity was determined by the radical DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) procedure[12]. Then 100 µL of SCG extract (500 µg/mL) was homogenized with 100 µL of radical solution (300 µM/kg) and incubated (25 °C/30 min, under dark). At 520 nm was measured the absorbance and results displayed as (%) inhibition: DPPH$^{\bullet}$ (%) = [(Radical absorbance at 0 min) – (Antioxidant-radical absorbance at 30 min) / (Radical absorbance at 0 min)] × 100.

Radical-cation scavenging activity was evaluated by the radical ABTS$^{\bullet+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation) procedure[13]. The radical cation (0.8 absorbance) was mixed with SCG extract (500 µg/mL) in a ratio of 99:1 and incubated (25 °C/6 min, under dark). At 734 nm was measured the absorbance and results displayed as (%) inhibition: ABTS$^{\bullet+}$ (%) = [(Radical absorbance at solution at 0 min) – (Antioxidant-radical absorbance at 6 min) / (Radical absorbance at solution)] × 100.

Reducing power was determined by the Ferric-reducing antioxidant power (FRAP) procedure[14]. Then 5 µL of SCG extract (500 µg/mL) were homogenized with 150 µL of FRAP solution [10:1:1, 300 mM/kg of buffer sodium acetate in glacial acetic acid and 10 mM/kg of TPZ reagent in 40 nM/kg of HCl and 20 mM/kg $FeCl_3$] and incubated (25 °C/8 min/under dark). At 595 nm was measured the absorbance and results displayed as mg of iron equivalent (Fe^{2+})/g.

Reducing power was also determined by the RPA procedure[14]. Then 100 µL of SCG extract (500 µg/mL) were homogenized with 300 µL of phosphate buffer (0.2 mol/L, pH 6.6) and 300 µL of $C_6FeK_4N_6$ (1 %, w/v). The resultant mixture was incubated at 50 °C for 20 min. After that, 300 µL of TCA (10 %, w/v) were added, and the samples were centrifuged at 4,200 xg/4 °C, 15 min (Sorvall ST18R, Thermo Fisher Scientific; Waltham, USA). The

supernatant was homogenized with 100 µL of dH_2O and 250 µL of $FeCl_3$ (0.1 %, w/v). At 700 nm was measured the absorbance and results displayed as absorbance.

Pork patties elaboration

Fresh pork minced meat (*Semimembranosus* muscle) was procured (Norson®) and mixed with 1.5 % salt (NaCl, w/w) and back fat (20 %, w/w). Pork patties were assessed in four treatments (in triplicate) as follows: Control (samples without antioxidant); T1 (samples with 0.05 % of SCG extract, w/w); T2 (samples with 0.1 % of SCG extract, w/w); T3 (samples with 0.02 % of BHT, w/w). Sixteen patties (40 g per patty) per treatment were elaborated, packaged in Styrofoam™ trays (expanded polystyrene), and overwrapped with polyvinyl chloride film (17,400 cm^3 O_2/m^2/23 °C, 24 h). The packaged patties were refrigerated (4 °C/9 days/under the dark), and on each sampling day, four packages per treatment were opened for due analysis.

Meat quality measurements

The proximate chemical composition (moisture, fat, protein, ash, and carbohydrate content) of the meat product was determined following standard procedures[15].

The pH of the meat product was determined by mixing the samples with dH_2O (1:10 ratio) at 4,500 rpm/5 °C, 1 min (T25, IKA®; Staufen, Germany), and using a potentiometer (pH211, Hanna; RI, USA)[15].

Thiobarbituric acid reactive substances procedure was used to measure the lipid oxidation (LOX)[16]. The meat product (1 g) was homogenized with 2,000 µL of TCA (10 %, w/v) (4,500 rpm/5 °C, 1 min) and centrifuged (2,300 xg/4 °C, 20 min). Then 200 µL of the filtered (Whatman 1 filter paper) solution was homogenized with 200 µL of TBA reagent (0.02 mol/kg) and incubated at 98 °C, 20 min. At 531 nm was measured the absorbance and results displayed as mg of malondialdehyde (MDA)/g.

The meat product color was measured spectrophotometrically (CM-508d, Konica Minolta Inc.; Tokyo, Japan). Samples were exposed to O_2 under refrigeration at 4 °C, 30 min. After that, 10 readings were performed on the samples surface to record: L*, lightness; a*, redness; b*, yellowness; C*, chromaticity; h*, hue angle[17].

The meat homogenate was obtained after pork patties were homogenized with dH_2O (1:10 ratio) at 4,500 xg/4 °C, 10 min. Thereafter, the supernatant was filtered and subjected to polyphenols content, antiradical and reducing power activity measurements.

Statistical analysis

Polyphenols and antioxidant activity data (n=6) were analyzed by a one-way ANOVA, while meat quality measurements were subjected to a two-way ANOVA. A Tukey test was performed ($P<0.05$). In addition, a multivariate analysis was used to determine the relationship among all parameters (SPSS version 21).

Results and discussion

Polyphenols content and antioxidant activity of SCG extract

The presence of polyphenols in SCG extract was demonstrated by the obtained results, including CAC (205.03 ± 4.13 mg CAE/g), TPC (562.71 ± 20.04 mg GAE/g), TFC (756.38 ± 11.82 mg RE/g), and TTC (12.50 ± 3.33 mg CE/g). In addition, mean values of antioxidant activity also showed that SCG extract displays high $DPPH^{\bullet}$ (84.95 ± 0.61 %) and $ABTS^{\bullet+}$ antiradical activity (43.93 ± 2.08 %), although the standard (BHT) showed the highest ($P<0.05$) antioxidant values respect to SCG extract (89.12 ± 2.10 % and 81.20 ± 1.15 %, respectively). Furthermore, SCG extract displays moderate FRAP (0.21 ± 0.10 mg Fe^{2+}/g) and RPA (0.11 ± 0.01 abs) values concerning BHT (0.53 ± 0.15 mg Fe^{2+}/g and 0.60 ± 0.10 abs, respectively) ($P<0.05$).

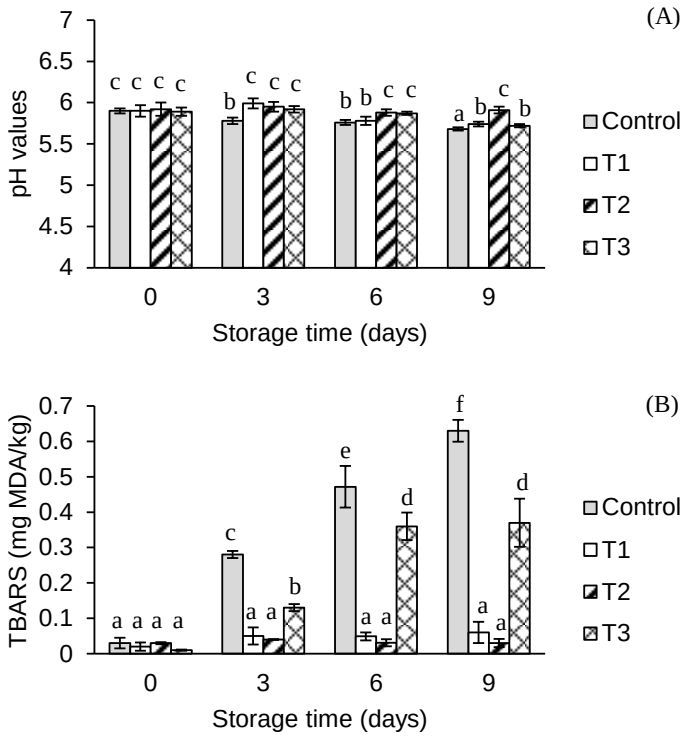
In previous works, it has been demonstrated that these agro-industrial by-products are a key source of polyphenols, including flavonoids and phenolic acids, widely correlated with their *in vitro* antioxidant effect[2]. However, the absence or presence of these components in coffee residue extracts could be associated with the variety (*C. arabica* and *C. robusta*), extraction system (solid-liquid, Soxhlet, among others), and the solvent type (water or ethanol) used for the compound extraction[18,19]. In addition, the presence of polyphenols in SCG extract is related to their antiradical effect; hence, a promissory strategy to enhance the antioxidant stability of pork meat products during storage could be the addition of SCG extract.

Pork patties quality

According to the results, the proximate composition of meat samples did not vary with the inclusion of 0.05 and 0.1 % of SCG extract (P>0.05). The average values obtained were 55.73 % (moisture), 19.5 % (protein), 22.5 % (fat), 1.8 % (ash) and 0.53 % (carbohydrates). It had been observed that adding 1 and 2 % of extracts from agro-industrial residues in formulations of pork patties did not significantly affect the original proximate composition[20].

Figure 1 illustrates raw pork patties' pH and LOX changes (A and B, respectively). The treatment × storage time interaction significantly affected (P<0.05). On the initial day of storage (d 0), the incorporation of antioxidant treatments did not affect pH and LOX values (P>0.05). However, pH values decreased, and LOX values increased during storage time (P<0.05). At d 9 (end of storage), meat samples treated with T1 and T2 exerted the highest) pH values, and the lowest LOX values (P<0.05).

Figure 1: pH (A) and LOX (B) values (Mean ± SD, n = 6) of raw pork patties during storage time



T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

[abcdef] Lowercase superscripts indicate significant differences when considered treatment x storage time interaction effect (P<0.05).

Meat quality evaluation indicates *antemortem* and *postmortem* changes occurring in slaughter animals; pH and LOX are key properties in meat quality perceptions influencing the meat purchase intention associated with quality losses in meat industry products[16,21]. However, it has also been demonstrated that the inclusion of non-synthetic sources rich in polyphenols improves food quality[22].

In this investigation, initial pH values in pork patties remained within the typical range for fresh pork meat (pH 5.5-5.9). A reduction in pH values of pork patties was observed during cold storage when synthetic or natural (date pits) antioxidant extracts were added[23]. Additionally, in agreement with the results of this study, a 42 % decrease in LOX of cooked pork patties added with light and dark SCG extract (1 g/kg or 10 %), stored under freezing conditions for three months, was observed[24]. When adding 0.05 and 0.1 % of SCG ethanol extract reduced LOX in raw pork-meat system stored at 37 °C/12 h[19]. Also, a LOX reduction of ground (top round) beef added with 0.1 % of ground roasted coffee (light, medium, and dark) through storage (4 °C/6 d) has been demonstrated[25].

According to the obtained results (Table 1) the treatment × storage time interaction had a significant effect on color values (P<0.05). At d 0, the antioxidant incorporation did not affect these parameters (P>0.05). However, L*, a*, b*, and C* values were reduced during storage time, while h* values were increased (P<0.05). On d 9, meat samples treated with T1 and T2 exerted the highest L*, a*, b*, and C* values and the lowest h* values (P<0.05). Color is another key parameter in meat quality perceptions[21]. In agreement with the study, a reduction in L*, a*, and b* values through refrigerated storage (4 °C/6 d) has been reported in control meat samples compared to their counterparts treated with 0.1 % of ground roasted coffee[25].

Table 1: Colour changes of raw pork patties treated with the aqueous extract of SCG during storage time

Item	Treatment	Storage time days			
		0	**3**	**6**	**9**
L*	Control	57.14 ± 1.36^{c}	51.51 ± 1.57^{a}	51.77 ± 1.36^{a}	49.39 ± 1.35^{a}
	T1	56.83 ± 1.16^{c}	54.40 ± 1.74^{b}	54.06 ± 1.93^{b}	54.63 ± 1.56^{b}
	T2	56.97 ± 0.86^{c}	53.61 ± 0.90^{b}	53.24 ± 1.39^{b}	53.18 ± 1.98^{b}
	T3	56.35 ± 1.56^{c}	56.01 ± 0.63^{c}	54.31 ± 1.86^{b}	53.25 ± 1.31^{b}
a*	Control	10.56 ± 1.37^{c}	7.55 ± 1.39^{b}	6.27 ± 0.97^{b}	4.15 ± 0.94^{a}
	T1	9.60 ± 0.88^{c}	9.33 ± 1.14^{c}	8.28 ± 1.39^{bc}	7.25 ± 0.83^{b}
	T2	10.79 ± 1.18^{c}	10.12 ± 1.67^{c}	8.25 ± 0.74^{bc}	8.52 ± 1.01^{bc}
	T3	9.22 ± 0.68^{c}	9.10 ± 1.79^{c}	7.68 ± 0.82^{b}	4.75 ± 1.38^{a}
b*	Control	18.09 ± 1.37^{c}	15.68 ± 1.43^{b}	14.56 ± 0.95^{a}	12.28 ± 1.65^{a}
	T1	18.20 ± 0.97^{c}	16.67 ± 1.05^{b}	16.34 ± 1.01^{b}	15.84 ± 1.35^{b}
	T2	19.08 ± 1.21^{c}	17.21 ± 1.65^{bc}	16.95 ± 1.40^{b}	16.11 ± 1.50^{b}
	T3	17.62 ± 1.03^{bc}	16.16 ± 1.34^{b}	15.75 ± 0.90^{b}	14.95 ± 1.11^{a}
C*	Control	21.15 ± 1.49^{c}	17.35 ± 1.77^{b}	16.74 ± 1.29^{b}	13.54 ± 0.86^{a}
	T1	20.35 ± 1.08^{c}	18.62 ± 1.61^{bc}	18.45 ± 1.80^{bc}	16.77 ± 1.32^{b}
	T2	21.25 ± 1.29^{c}	19.45 ± 1.60^{c}	18.84 ± 1.44^{bc}	17.78 ± 1.71^{b}
	T3	19.06 ± 1.17^{c}	17.52 ± 1.69^{b}	16.51 ± 1.72^{b}	15.64 ± 1.68^{ab}
h*	Control	61.41 ± 1.16^{a}	63.62 ± 1.51^{a}	66.17 ± 1.72^{b}	72.75 ± 1.02^{c}
	T1	63.80 ± 1.60^{a}	63.10 ± 2.06^{a}	65.74 ± 1.46^{ab}	67.83 ± 1.88^{b}
	T2	62.21 ± 1.53^{a}	62.47 ± 1.90^{a}	63.01 ± 1.46^{a}	64.45 ± 1.49^{a}
	T3	62.51 ± 1.86^{a}	65.09 ± 1.84^{ab}	66.44 ± 1.51^{b}	71.73 ± 2.06^{c}

Results expressed as mean ± SD (n = 6); T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

^{abc} Lowercase superscripts indicate significant differences when considered treatment x storage time effect (P<0.05).

Polyphenols content and antioxidant activity of meat homogenates

According to the obtained results (Table 2), the treatment × storage period interaction significantly affected the meat homogenates' polyphenols content (P<0.05). Non-differences (P>0.05) were detected in TTC (average value 0.87 mg CE/g), $ABTS^{\bullet+}$ (49.58 %), and RPA values (0.87 abs) during storage. At d 0, chlorogenic acid (CGA), TPC, and TFC values increased (P<0.05) in meat samples treated with SCG extract (T2>T1). Yet CGA, TPC, and TFC values significantly decreased (P<0.05) during storage period, and at day 9, the highest (P<0.05) CGA, TPC, and TFC values corresponded to T2.

Table 2: Polyphenols content of pork patties treated with the aqueous extract of SCG during storage time

Item	Day	Treatments			
		Control	**T1**	**T2**	**T3**
CGA,	0	31.01 ± 0.50^{a}	85.99 ± 1.50^{d}	110.15 ± 2.78^{e}	31.43 ± 0.50^{a}
mg CAE/g	9	31.20 ± 0.62^{a}	33.10 ± 0.42^{b}	75.72 ± 0.33^{c}	31.60 ± 0.44^{a}
TPC,	0	31.70 ± 3.01^{c}	33.17 ± 2.50^{c}	42.84 ± 2.10^{e}	38.67 ± 1.15^{d}
mg GAE/g	9	23.20 ± 1.04^{a}	21.20 ± 1.30^{a}	27.10 ± 1.23^{b}	26.62 ± 1.00^{b}
TFC,	0	34.01 ± 0.90^{d}	34.46 ± 1.50^{d}	46.20 ± 0.80^{e}	22.27 ± 1.98^{b}
mg RE/g	9	15.60 ± 1.13^{a}	20.30 ± 1.55^{b}	31.91 ± 2.56^{c}	20.16 ± 1.13^{b}
TTC,	0	1.00 ± 0.50	0.96 ± 0.15	1.06 ± 0.42	0.78 ± 0.28
mg CE/g	9	0.80 ± 0.45	0.74 ± 0.31	0.79 ± 0.30	0.84 ± 0.33

Results expressed as mean ± SD (n= 6); T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

^{abcde} Lowercase superscripts indicate significant differences when considered treatment x storage time interaction effect (P<0.05).

According to the obtained results (Table 3) the treatment × storage period interaction significantly affected the meat homogenates' antioxidant status (P<0.05). Regarding the antioxidant activity, at d 0, DPPH$^{\bullet}$ and FRAP values increased (P<0.05) in meat samples due to SCG extract. However, antioxidant values were reduced (P<0.05) during storage in the Control and T3 samples. On d 9, T1 and T2 showed the highest DPPH$^{\bullet}$ and FRAP values (P<0.05).

Table 3: Antioxidant status of pork patties treated with the aqueous extract of SCG during storage time

Item	Day	Treatments			
		Control	**T1**	**T2**	**T3**
DPPH$^{\bullet}$, %	0	30.10 ± 2.70^{c}	41.97 ± 1.00^{d}	48.65 ± 1.54^{e}	43.92 ± 2.99^{d}
	9	13.50 ± 0.55^{a}	43.70 ± 1.60^{d}	45.08 ± 2.04^{de}	15.37 ± 1.02^{b}
ABTS$^{\bullet+}$, %	0	50.40 ± 1.55^{a}	51.98 ± 1.83^{a}	48.68 ± 2.75^{a}	48.52 ± 3.00^{a}
	9	49.10 ± 1.52^{a}	48.95 ± 1.45^{a}	49.25 ± 3.22^{a}	49.83 ± 2.99^{a}
FRAP,	0	4.64 ± 0.52^{a}	12.09 ± 1.13^{b}	17.03 ± 0.50^{c}	3.89 ± 0.55^{a}
mg Fe^{2+}/g	9	3.97 ± 0.50^{a}	10.62 ± 0.55^{b}	18.42 ± 1.74^{c}	4.64 ± 0.52^{a}
RPA (Abs)	0	1.10 ± 0.30	1.03 ± 0.05	0.91 ± 0.30	0.92 ± 0.25
	9	0.80 ± 0.20	0.70 ± 0.30	0.81 ± 0.30	0.70 ± 0.30

Results expressed as mean ± SD (n= 6); T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

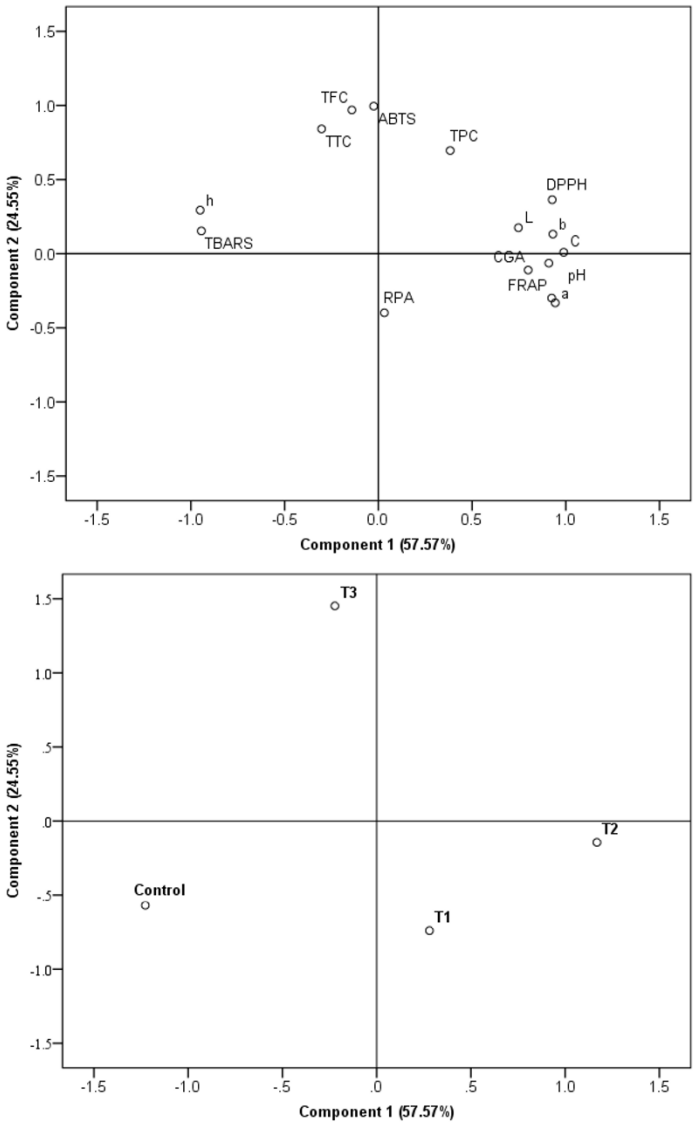
^{abcd} Lowercase superscripts indicate significant differences when considered treatment x storage time interaction effect (P<0.05).

Oxidative reactions in foods, including meat and meat products, are considered the principal non-microbial cause of quality deterioration, and it is associated with a loss of endogenous antioxidants *postmortem*. Animal species, breed, muscle type, and anatomical location can influence endogenous antioxidant content[22]. Concerning exogenous antioxidant content, the presence of phenolic compounds in meat and meat products may result from the animal´s diet[26], while the extraction and incorporation of bioactive compounds from natural sources into meat formulations can increase the antioxidant status of meat products[22]. In this context, the phenolic content and antioxidant status of raw and cooked pork patties stored (4 °C/6 d) and added with 2 % of a natural ethanol extract was increased[27].

Multivariate analysis

Figure 2 shows a principal component analysis to determine the differences among analyzed variables and treatments. The first and second components showed a 57.57 and 24.55 % variance, respectively. In this context, 82.12 % of the total variation was explained by both components. In addition, a separation of the treatments concerning the variables was observed; for example, the T2 treatment, loaded towards the right quadrant, presented the highest polyphenols and antioxidant activity content.

Figure 2: Principal component analysis of evaluated parameters and treatments



Control, samples without-antioxidant; T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

Conclusions and implications

SCG extract is a novel source of antioxidant components, including polyphenols. SCG extract incorporation into pork patties elicits desirable responses in pH values, color, and lipid oxidation stabilities during storage times. Moreover, SCG extract increases polyphenols content and the antioxidant status of meat samples. SCG extract can be used in the formulation of pork patties to prevent oxidation reactions and mitigate meat quality losses during refrigerated storage.

Acknowledgements and conflict of interest

We gratefully acknowledge the fellowship received from "Investigadoras e Investigadores por México" program – CONAHCYT. We also thank the technical support of Melina Estrada-Alanis.

Literature cited:

1. Shah MA, Bosco SJD, Mir SA. Plant extracts as natural antioxidants in meat and meat products. Meat Sci 2014;98(1):21-33.

2. Oswell NJ, Thippareddi H, Pegg RB. Practical use of natural antioxidants in meat products in the US: A review. Meat Sci 2018;145:469-479.

3. Anastopoulos I, Karamesouti M, Mitropoulos AC, Kyzas GZ. A review for coffee adsorbents. J Mol Liq 2017;229:555-565.

4. Besufekad Y, Mekonnen A, Girma B, Daniel R, Tassema G, Melkamu J, Asefa M, Fikiru T, Denboba L. Selection of appropriate substrate for production of oyster mushroom (*Pleurotus ostreatus*). J Yeast Fungal Res 2020;11(1):15-25.

5. Martínez-Saez N, García AT, Pérez ID, Rebollo-Hernanz M, Mesías M, Morales FJ, *et al*. Use of spent coffee grounds as food ingredient in bakery products. Food Chem 2017;216:114-122.

6. Song EJ, Kim JY, Lee SY, Kim KBWR, Kim SJ, Yoon SY, *et al*. Effect of roasted ground coffee residue extract on shelf-life and quality of salted mackerel. J Korean Soc Food Sci Nutr 2009;38(6):780-786.

7. Pérez-Hernández LM, Chávez-Quiroz K, Medina-Juárez LÁ, Meza NG. Phenolic compounds, melanoidins, and antioxidant activity of green coffee bean and processed coffee from *Coffea arabica* and *Coffea canephora* species. Biotecnia 2013;15(1):51-56.

8. Griffiths DW, Bain H, Dale MFB. Development of a rapid colorimetric method for the determination of chlorogenic acid in freeze-dried potato tubers. J Sci Food Agric 1992;58(1):41-48.

9. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc 2007;2:875-877. https://doi.org/10.1038/nprot.2007.102.

10. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64(4):555-559.

11. Price ML, Butler LG. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J Agric Food Chem 1977;25(6):1268-1273.

12. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004;26(2):211-219.

13. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 1999;26(9-10):1231-1237.

14. Berker KI, Güçlü K, Tor İ, Demirata B, Apak R. Total antioxidant capacity assay using optimized ferricyanide/prussian blue method. Food Anal Methods 2010;3:154-168.

15. AOAC. Official methods of analysis. 18th ed.; Gaitherburg, MD, USA: Association of Official Analytical Chemists. 2005.

16. Pfalzgraf A, Frigg M, Steinhart H. Alpha-Tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage. J Agric Food Chem 1995;43(5):1339-1342.

17. Hernández B, Sáenz C, Alberdi C, Diñeiro JM. CIELAB color coordinates versus relative proportions of myoglobin redox forms in the description of fresh meat appearance. J Food Sci Technol 2016;53:4159-4167.

18. Bravo J, Monente C Juániz I, De Peña MP, Cid C. Influence of extraction process on antioxidant capacity of spent coffee. Food Res Int 2013;50(2):610-616.

19. Kim JH, Ahn DU, Eun JB, Moon SH. Antioxidant effect of extracts from the coffee residue in raw and cooked meat. Antioxidants 2016;5(3):21.

20. Schmidt MM, Kubota EH, Prestes RC, Mello RO, Rosa CS, Scapin G, Ferreira S. Development and evaluation of pork burger with added natural antioxidant based on extract of banana inflorescence (*Musa cavendishii*). CyTA J Food 2016;14(2):280-288.

21. Hughes JM, Clarke FM, Purslow PP, Warner RD. Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. Compr Rev Food Sci Food Saf 2020;19(1):44-63.

22. Falowo AB, Fayemi PO, Muchenje V. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. Food Res Int 2014;64:171-181.

23. Sayas-Barberá E, Martín-Sánchez AM, Cherif S, Ben-Abda J, Pérez-Álvarez JÁ. Effect of date (*Phoenix dactylifera* L.) pits on the shelf life of beef burgers. Foods 2020;9(1):102-116.

24. Jully KMM Toto CS, Were L. Antioxidant effect of spent, ground, and lyophilized brew from roasted coffee in frozen cooked pork patties. LWT-Food Sci Technol 2015;66:244-251.

25. Lin C, Toto C, Were L. Antioxidant effectiveness of ground roasted coffee in raw ground top round beef with added sodium chloride. LWT-Food Sci Technol 2015;60(1):29-35.

26. Beghelli D, Cosmo AD, Faeti V, Lupidi G, Bailetti L, Cavallucci C, Polidori P. *Origanum vulgare* L. and *Rosmarinus officinalis* L. aqueous extracts in growing-finishing pig nutrition: effects on antioxidant status, immune responses, polyphenolic content and sensorial properties. J Food Res 2019;8(2):90-99.

27. Vargas-Sánchez RD, Torrescano-Urrutia GR, Torres-Martínez BDM, Pateiro M, Lorenzo JM, Sánchez-Escalante A. Propolis extract as antioxidant to improve oxidative stability of fresh patties during refrigerated storage. Foods 2019;8(12):614.

https://doi.org/10.22319/rmcp.v15i4.6647

Article

Prevalence and diversity of zoonotic intestinal parasites in household dogs in urban areas of the Colombian Caribbean

Luz Adriana Sarmiento-Rubiano [a]*

Yina García Toscano [b]

Julieannie Paola Ruiz [c]

Lucena Delgado Soraca [b]

Alfonso Bettin Martínez [b]

Jimmy Becerra Enriquez [a]

[a] Metropolitan University. Food and human behavior research group, Calle 76 42 78, Barranquilla, Atlantico. Barranquilla, Colombia.

[b] Metropolitan University. Caribbean Research Group on Infectious Diseases and Microbial Resistance, Barranquilla, Colombia.

[c] BIOVET Specialized Veterinary Diagnostic Center. Barranquilla, Colombia.

* Corresponding author: lusarru@hotmail.com

Abstract:

Dogs offer multiple benefits in their relationship with humans, but they can also be carriers of zoonotic parasites that affect human and animal health. Zoonoses account for about 58% of all human infectious diseases. The objective of this study was to assess intestinal parasitism in dogs with owners in the City of Barranquilla in the years 2016 to 2018. A retrospective descriptive study was carried out that included 3,279 reports of parasitological evaluation of feces from a clinical laboratory that serves a network of veterinary services in the city of Barranquilla. 49.2 % of the dogs had some type of intestinal parasite. The most frequent were helminths: *Strongyloides* sp. 9.6 %, *Toxocara canis* 7.7 % and *Ancylostoma*

caninum 6.2 %; and the protozoa: *Entamoeba* spp. 10.0 %, *Isospora* spp. 6.9 % and *Giardia* spp. 5.7 %. The Principal Component Analysis of the parasite profiles by year showed significant differences. The presence of zoonotically transmitted intestinal parasites in dogs evidenced the need to establish corrective and preventive measures in the field of public health that allow their control, since they constitute a significant risk of disease in the community.

Keywords: Intestinal parasites, Giardia, Blastocystis, Helminths, Zoonoses, Protozoa.

Received: 22/02/2024

Accepted: 05/08/2024

Introduction

Companion animals such as dogs offer multiple benefits in their relationship with humans, but in this close and millennial association of collaboration and affection between species, there are also zoonotic transmission parasites that can represent a potential risk to human and animal health. Zoonoses account for about 58 % of human infectious diseases, and of the 177 pathogens considered by the WHO to be reemerging, 73 % are related to human contact with an animal source[1,2]. The transmission of zoonotic parasites between humans and pets, such as dogs, is linked to the inadequate handling of excreta. The presence of dog feces in parks, streets, and public spaces where pets walk with their owners is an important source of contamination for humans and animals. The physical contact of children and adults during the game with their pets allows the exchange of parasites present in the hair and paws of the animals. In the feces of dogs that live with humans in both rural and urban environments, it is possible to find, in addition to canine intestinal parasites such as *Toxocara canis*, *Ancylostoma caninum*, *Echinococcus* sp., and *Dipylidium caninum*, among others, parasites typical of humans such as *Ascaris lumbricoides* or *Strongyloides stercolaris* as occasional findings[3].

Protozoa such as *Giardia* sp., *Entamoeba histolytica*/dispar, *Cyclospora*, and *Cryptosporidium* sp., commonly found in the world human population as the cause of gastrointestinal disorders and diarrhea in both healthy and immunologically compromised people, are considered parasites of zoonotic transmission[4,5]. Intestinal parasites are a global public health problem that has social, economic, and cultural effects associated with poverty[6]. In Colombia, the national parasitism surveys of the years 1965, 1980, and 2014 coincide in reporting intestinal parasitism in more than 80 % of the population[7].

The impact of zoonoses on human health makes it pertinent and opportune to conduct studies that help to understand and define the possible risks of transmission of these pathologies, even more so when they involve animals that live as closely with our families as dogs. In Colombia, according to data from the National Administrative Department of Statistics (DANE), pet ownership increased significantly in the last few years. Actuality 57 % of households live with at least one pet (4.4 million families); dogs are the preferred pet in 71 % of these households[8]. The climatic and environmental conditions of the Colombian Caribbean, where the city of Barranquilla is located, are appropriate for the transmission of intestinal parasites. The objective of this work was to determine the frequency of intestinal parasites in dogs with owners, including 3279 results of coprological analyses performed on pets during the years 2016 to 2018 in the city of Barranquilla, Colombia.

Material and methods

Description of the study area

Barranquilla, located in the northeastern vertex of the department (province) of Atlántico, Colombia. This urban center borders the Caribbean Sea to the north, the Magdalena River to the east, and other municipalities to the southwest. It has a dry tropical climate; the average temperature ranges between 24 °C and 28 °C, and humidity ranges between 65 % and 85 %[9]. There are no exacting data on the dog population in Barranquilla in 2016 – 2018 period, but it is calculated that in 2016 there were 82,386 dogs[10], and if it is considered that the estimated human population was 1’223,616 people in Barranquilla for the same year according to the National Department of Statistics[11], it can be inferred that there is a dog for every 15 inhabitants, approximately.

Sample collection and evaluation

A retrospective descriptive study in which were linked 3,279 reports of feces parasitological analysis of dogs with owner from a clinical laboratory that attends a network of veterinarian services in the city of Barranquilla. The fecal samples were provided by the dog owners as a routine control examination of their pets and the parasitological diagnosis was carried by an expert bacteriologist through direct microscopic examination of the fecal samples with saline and lugol solution to for the identification of parasitic forms.

Statistical analysis

For the analysis of the results, the dogs were classified into two categories: mongrels, as those animals of unknown ancestry with characteristics of two or more types of breeds, and purebred animals, according to the classification of the International Cynological Federation

(FCI) of the Organization Canine World (Table 1). An exploratory descriptive analysis of the results was carried out to establish the absolute and relative frequency of the parasites present in the samples and to compare, using the Chi-square test for categorical variables, the results between purebred and mixed-breed dogs and between the years evaluated. (It is considered significant if the value of $P<0.05$) Using a Principal Component Analysis (PCA), the parasite profiles by breed and by year were compared (XLSTAT program for Excel Addinsoft Inc., Paris, France).

Table 1: Classification of the dog population according to the breed groups established by the International Cynological Federation (FCI)

Animals (n)	Sections by group (n)
Total dogs (3,279) Mixed-breed dogs (861) Purebred dogs (2,418)	
GROUP I. Sheepdogs and Cattledogs (74)	Sheepdogs (74)
GROUP II. Pinscher and Schnauzer (574)	Molosoides (150) Pinschers and Schnauzers (424)
GROUP III. Terriers (344)	Companion Terriers (258) Bull Terriers (86)
GROUP IV. Dachshunds (6)	dachshunds (6)
GROUP V. Spitz and Primitive(192)	Alaskan Malamute (6) Nordic sled dogs (95) Asian Spitz and similar (16) European Spitz (72) Primitive Type - Hunting Dogs (3)
GROUP VI. Hound and trail (115)	Hound type dogs (115)
GROUP VII. Scent hounds and related breeds (16)	Continental samples (13) Sample English and Irish (3)
GROUP VIII. Retrievers - Flushing dogs (289)	Hunting Retrievers (200) Hunting lifting dogs (89)
GROUP IX. Companion and toy dogs (808)	Bichons and similar breeds (42) Poodle (404) Chihuahua (43) Small Molossian type Dogs (157) Tibetan breeds (162)

The table shows the number of animals for each group and section in the FCI classification.

Results

Of the 3,279 fecal samples analyzed, 73.7 % came from dogs of breeds identified and classified by the FCI (n= 2,418) and 26.3% (n= 861) were mixed-breed dogs. 49.2 % of the animals had intestinal parasites, without significant differences between purebred and mixed-breed dogs, only in the *Toxocara canis* helminth was a significantly higher prevalence observed in mixed-breed dogs in relation to purebred dogs (*P*=0.010 Chi square) (Table 2). Table 3 shows the parasite profiles for the total population studied, purebred and mixed-breed dogs, and by year.

Table 2: Prevalence of parasitism in dogs by race and year of evaluation

Parasitism	Total % (n 3,279)	Purebred (n 2,418)	Mixed-breed (n 861)	2016 (n 997)	2017 (n 1,428)	2018 (n 854)
Positive	49.2 (1,614)	49.0 (1,184)	49.9 (430)	51.2 (510)	47.8 (683)	49.3 (421)
1 parasite	41.8 (1,371)	41.4 (1,002)	42.9 (369)	43.2 (431)	41.0 (586)	41.5 (354)
2 parasite	6.6 (215)	6.9 (167)	5.6 (48)	7.7 (77)	5.7 (82)	6.6 (56)
3 ≥ parasites	0.9 (28)	0.6 (15)	1.5 (13)	0.2 (2)	1.1 (15)	1.3 (11)
Helmintes	28.2 (925)	27.4 (662)	30.5 (263)	23.1[a] (230)	31.5[b] (450)	28.7[b] (245)
1 helminte	25.5 (836)	25.0 (604)	26.9 (232)	20.6 (205)	27.6 (394)	27.8 (237)
2 helmintes	2.3 (77)	2.2 (52)	2.9 (25)	2.5 (25)	3.3 (47)	0.6 (5)
3 helmintes	0.4 (12)	0.2 (6)	0.7 (6)	0.0 (0)	0.6 (9)	0.4 (3)
Protozoa	24.2 (794)	24.9 (602)	22.3 (192)	31.3[a] (312)	18.6[b] (265)	25.4[c] (217)
1 protozoa	22.3 (731)	22.8 (551)	20.9 (180)	29.0 (289)	17.5 (250)	22.5 (192)
2 protozoa	1.8 (60)	2.0 (49)	1.3 (11)	2.2 (22)	1.0 (14)	2.8 (24)
3 ≥ protozoa	0.1 (3)	0.1 (2)	0.1 (1)	0.1 (1)	0.1 (1)	0.1 (1)

[abc] Frequencies (%) in the same row that do not share the superscript letter are different (*P*< 0.05).

Table 3. Prevalence of parasitism by groups of breeds and by year

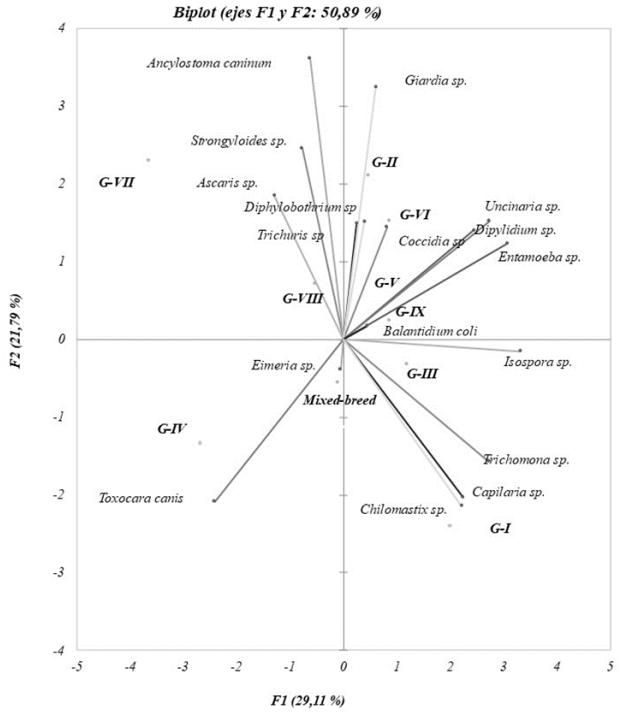
Parasite	Total n (3,279)	Purebred n (2,418)	Mixed-breed n (861)	2016 n (997)	2017 n (1,428)	2018 n (854)
Nematodes						
Toxocara canis	7.7 (254)	7.0[a] (170)	9.8[b] (84)	9.2[a] (92)	8.2[b] (117)	5.3[c] (45)
Trichuris spp.	0.1(3)	0.1(3)	0.0 (0)	0.0 (0)	0.2 (3)	0.0 (0)
Ancylostoma caninum	6.2 (200)	6.0 (140)	6.2 (54)	3.1[a] (31)	7.8[b] (112)	6.7[c] (57)
Diphylobothrium spp	0.0 (1)	0.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.1 (1)
Capillaria spp.	0.3 (11)	0.3 (7)	0.5 (4)	0.4 (4)	0.4 (6)	0.1 (1)
Strongylus spp	9.6 (314)	9.0 (218)	11.1 (96)	6.9[a] (69)	12.8[b] (183)	7.3[a] (62)
Uncinaria spp.	6.1 (200)	6.0 (144)	6.5 (56)	5.7 (57)	6.4 (91)	6.1 (52)
Ascaris spp.	1.1 (35)	1.2 (28)	0.8 (7)	0.0 (0)	0.1 (1)	4.0 (34)
Cestodes						
Dipylidium caninum	0.3 (9)	0.4 (9)	0.0 (0)	0.2 (2)	0.2 (3)	0.5 (4)
Protozoa						
Entamoeba spp	10.0 (329)	10.5 (253)	8.8 (76)	13.3[a] (133)	7.1 (102)	11.0[a] (94)
Giardia spp.	5.7 (188)	6.0 (144)	5.1 (44)	7.4[a] (74)	3.6[b] (52)	7.3[a] (62)
Isospora canis.	6.9 (225)	6.7 (163)	7.2 (62)	7.6 (76)	7.1 (101)	5.6 (48)
Coccidia sp*	3.0 (99)	3.3 (81)	2.1 (18)	4.4[a] (44)	1.3[b] (19)	4.2[a] (36)
Balantidium coli	0.0 (1)	0.0 (1)	0.0 (0)	0.1 (1)	0.0 (0)	0.0 (0)
Eimeria spp.	0.0 (1)	0.0 (0)	0.1 (1)	0.1 (1)	0.0 (0)	0.0 (0)
Thichomonas spp.	0.3 (11)	0.4 (9)	0.2 (2)	0.3 (3)	0.5 (7)	0.1 (1)
Chilomastix spp.	0.2 (4)	1.4 (1)	0.2 (2)	0.4 (4)	0.0 (0)	0.2 (2)

* Different species of *Coccidia* infect dogs: *Isospora burrowsi*, *I. canis*, *I. neorivolta*, and *I. ohioensis*, only *I. canis* can be identified by the oocyst structure; the others were classified as *Coccidia* sp.

[abc] Frequencies (%) in the same row that do not share the superscript letter are different ($P<0.05$).

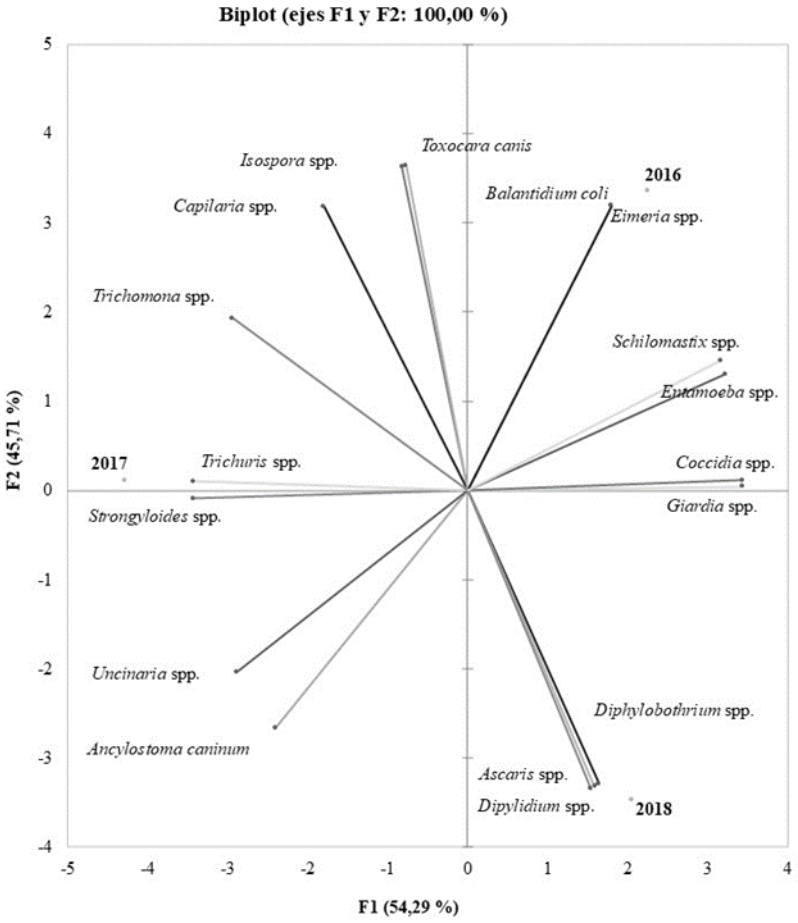
Principal component analysis (PCA) allows to summarize and to visualize the relationship between the parasite profiles and dog breeds according to FCI and mixed-breed dogs using the Pearson correlation coefficient. Figure 1, is the graphical representation of factors 1 and 2 with an accumulated variance of 50.89 %; the main components of factor 1 (variance 29.11 %) are *Isospora* sp. (17.9 %), *Entamoeba* (15.5 %), *Coccidia* sp. (12.2 %), and *Trichomonas* (12.1 %); the main components of factor 2 (Variance 21.79 %) were *Ancylostoma* sp. (21.3 %), and *Giardia* (17.2 %); the addition of factor 3 results in an accumulated variance of 68.1 % for the components *Ascaris* sp. (20.0 %) and *Strongyloides* sp. (17 %). It is not observed that there are relationships between the parasitic profiles of the dog groups of breeds.

Figure 1. PCA analysis relates parasite profiles between the groups of dogs studied



When parasitism by year was compared, it was observed that there are no significant differences in the number of animals with intestinal parasites per year; however, contamination by helminths and by trophozoans shows significant differences (P<0.05 Chi-square test). 2017 is the year with the highest prevalence of helminthiasis, and 2016 is the year with the highest contamination with protozoa (Table 2). The main helminths that establish the difference between years are *Toxocara canis*, *Ancylostoma caninum*, and *Strongyloides* sp.; the protozoa *Entamoeba* spp., *Giardia* spp., and *Coccidia* sp. show significant differences between the years evaluated (P<0.05) (Table 3). The PCA analysis of the parasitary profiles by year (Figure 2), shows that 100 % of the variance is reached with factors 1 and 2, being the main variables of factor 1 (variance 59.3 %), *Giardia* sp. 10.8 %, *Strongyloides* sp. 10.8 %, *Trichuris* sp. 10.8 %, and *Coccidia* sp. 10.8 %; and for factor 2 (variance 45.7 %), *Isospora* sp. 12.2 %, *Toxocara canis* 12.1 %, *Dipylidium* 10.3 %, and *Ascaris* sp. 10.1 %. It is observed that the parasite profiles per year are different.

Figure 2: PCA analysis of the parasite profiles by year



Discussion

Recently, a large number of studies have been carried out in many parts of the world to determine the presence of intestinal parasites in companion animals that live with human families. The results of these studies have been heterogeneous and largely dependent on environmental and climatic factors that facilitate the transmission of parasites. Furthermore, the socioeconomic conditions of poverty and poor hygiene facilitate the transmission of zoonotic parasites. La Torre *et al*[12] found in urban areas such as the city of Rome a prevalence of parasitism of 9.7 % when evaluating 493 dogs with owners, with *Trichuris vulpis* (5.5 %) and *Toxocara canis* (4.3 %) being the most frequent parasites[12]. In the city of Villahermosa in Tabasco, Mexico, it was observed that 26.5 % of the 302 evaluated feces of domestic dogs contained gastrointestinal parasites, with *Ancylostoma caninum* being the

most common parasite[13]. And in Argentina, the evaluation of 1,944 samples of dog feces collected in rural and urban areas showed the presence of parasites in 37.86 % of the samples, with rural areas having 40.06 % and urban areas having 33.44 %, with significant differences between the parasite profiles of both areas[14].

In Barranquilla city, was found 49.2 % of parasitism prevalence in dogs with owners; the most frequent parasites were the helminths *Strongylus* sp., *Toxocara canis*, and *Ancylostoma caninum*, and the protozoa *Entamoeba* spp., *Isospora* spp., and *Giardia* spp. One previous study in Barranquilla in the year 2015 found parasitism in 73.3 % of 925 dogs analyzed; the most prevalent parasites were *Entamoeba* sp. 34.1 %, *Isospora* sp. 21.1 %, *Giardia* 18.1 %, and *Toxocara canis* 12.3 %, showing a reduction in the number of parasitized animals in comparison with the actual study but maintaining a similar parasite profile[15]. The public health importance of the protozoa found in this study is evidenced by their zoonotic potential and their significant pathogenicity in both humans and animals. *Isospora canis* and *Isospora ohioensis* are the most common species of coccidia that affect dogs. *I. canis* in the canine gastrointestinal tract results in enteritis and mucosal damage causing hemorrhagic diarrhea, vomiting, tenesmus, inappetance, and respiratory and neurologic signs. Human infection is associated with ingesting fecally contaminated food with dog feces[16].

The prevalence of Giardia in people and dogs as asymptomatic carriers and as a cause of pathology represents a constant risk to the health of both species; continuous treatment and reinfection can cause resistance to antiparasitic that make it increasingly difficult to eliminate the parasite[17]. Giardiasis is the cause of diarrhea and malnutrition in children and has a global distribution, with more than 200 million cases annually. Giardia has been included in the "*neglected diseases initiative*" by the World Health Organization[18]. A systematic review reporting what the prevalence of Giardia in Colombian, analyzed by microscopy is between 0.9 and 48.1 %[19]. In the metropolitan área of Barranquilla, the prevalence of giardiasis in 2015 was 15.2 % in children under 10 yr of age[20]. *Entamoeba* is the third most common parasitic disease responsible for mortality worldwide; it is the cause of human amoebiasis and invasive liver abscesses. About 90 % of human amoebiasis cases are asymptomatic, leading to continuous transmission of the parasite. *Entamoeba* is a zoonotic protozoan that colonizes the digestive tract of humans and animals and is considered a worldwide public health problem[21]. The prevalence of *Entamoeba* spp. in the Barranquilla human population in 2015 was 6.1 %[20]. *Toxocara* infection in humans may cause visceral larva migrans and, together with *Ancylostoma* spp., is associated with cutaneous larva migrans in poor communities[22]. The dogs are susceptible to experimental infection with *S. stercoralis* of human origin, although infection from dogs to humans has not been fully demonstrated[23].

Balantidium coli is considered a neglected zoonotic disease in tropical areas. This protozoan infects the intestinal tract, causing severe diarrhea and other gastrointestinal abnormalities in domestic animals. *B. coli* is considered a finding of zoonotic significance[24]. In this study,

one of the animals had *Balantidium*, which represents a risk to the health of the pet and its owners. Other infrequent findings in this study include *Eimeria* sp., which was found in one of the animals, this species of coccidia parasite, which causes diarrhea and gastrointestinal disorders mainly in immunosuppressed people, was also found in domestic dogs in Peru with a prevalence of 10.68 %[25]. *Trichomonas* that are occasionally observed in the feces of dogs with diarrhea, although considered opportunistic, were found in eleven of the samples analyzed in this study even though the feces were not diarrheal. Using molecular and sequencing techniques, Gookin *et al*[26], demonstrated that *Trichomonas fetus* and *Pentatrichomonas hominis* are present in canine samples and that this protozoan causes human gastrointestinal infections.

The PCA analysis of the parasite profiles for each year evaluated showed significant differences, which could be attributed to slight variations in climatic conditions from one year to another in the same geographical area. This has been previously observed in the same geographic area, but when comparing human parasite profiles in areas with very similar environmental and cultural characteristics[20]. Cultural factors could also cause variations and favor or inhibit the possibility of infection at each moment of the complex life cycle of each parasite. The proper disposal of human and animal excrement is essential. Diverse studies have demonstrated in urban and rural areas of different countries that the presence of infective parasitic forms in the soil from the feces of humans and parasitized animals is a key factor in the infection of pets. In Chile, in 48.3 % of 83 parks in the city of Temuco, parasite eggs were found with *Toxocara* sp. (12.4 %) were the most frequent[27]. In the Tunja city, Colombia, 60.7 % of canine fecal samples collected in city parks and 100 % of soil samples had parasite eggs and larvae, mainly *Toxocara* sp., *Ancylostoma* spp., *Trichuris* sp., and *Strongyloides* sp.[28]. The viability of *Toxocara* sp. in the soil depends on factors such as temperature, pH, humidity, among others. However, it is known that they are very resistant to climatic conditions and that, depending on the condition, they could be infective for 6 to 12 mo or even up to several years at low temperatures[29].

Conclusions and implications

This study found that 49.2 % of the animals had intestinal parasites. It is not observed that there are relationships between the parasitic profiles of the dog groups for breeds, but it is observed that the parasite profiles per year are statistically different. The presence of intestinal parasites with zoonotic potential found in owned dogs observed in this work demonstrates the need for new studies to define the factors associated with this public health problem, and implement corrective and preventive measures to control them.

Literature cited:

1. Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said M. Zoonosis emergence linked to agricultural intensification and environmental change. PNAS USA. 2013;110(21):8399-8404.

2. Woolhouse MEJ, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. Emerg Infect Dis 2005;11(12):1842.

3. Ritossa L, Viozzi G, Flores V. The state of knowledge on intestinal helminths in free-roaming dogs in southern South America. Canine Genet Health Med. 2021. https://www.intechopen.com/online-first/the-state-of-knowledge-on-intestinal-helminths-in-free-roaming-dogs-in-southern-south-america. Accessed Nov 22, 2023.

4. Sotiriadou I, Pantchev N, Gassmann D, Karanis P. Molecular identification of *Giardia* and *Cryptosporidium* from dogs and cats. Parasite 2013;20(8):1-8.

5. Dantas-Torres F, Otranto D. Dogs, cats, parasites, and humans in Brazil: Opening the black box. Parasite Vectors 2014;7(1):22.

6. Inter-American Development Bank, Pan American Health Organization, Sabin Vaccine Institute. A Call to Action: Tackling soil-transmitted helminths in Latin America and the Caribbean 2011. http://www.paho.org/hq/in. Accessed Nov 30, 2023.

7. Ministry of Health and Social Protection, University of Antioquia. National Survey of Intestinal Parasitism in the School Population 2012 – 2014. Medellín: National Faculty of Public Health, University of Antioquia. 2015. https://www.minsalud.gov.co/sites/.../encuesta-nacional- of-parasitism-2012-2014.pdf. Accessed Nov 25, 2023.

8. DANE National Administrative Department of Statistics. National Quality of Life Survey 2023. https://www.dane.gov.co/index.php/sistema-estadistico-nacional-sen.

9. Ramírez-Cerpa E, Acosta-Coll M, Vélez-Zapata J. Analysis of weather conditions of short-term rainfall in urban areas: case study, Barranquilla, Colombia. Idesia 2017;35(2). https://www.cabdirect.org/cabdirect/abstract/20183232316. Accessed Nov 30, 2023.

10. Ministry of Health and Social Protection. Anti-rabies vaccination coverage by departments. 2016. https://www.minsalud.gov.co/sites/rid/Lists/Biblioteca Digital/RIDE/VS/PP/SA/Cobertura-vacunacion-antirrabica-departamentos-2016.pdf Accessed Nov 30, 2023.

11. DANE National Administrative Department of Statistics. Population projections of the National Administrative Department of. Statistics –DANE- 2005-2020. https://www.dane.gov.co/files/investigaciones/poblacion/.../8 Tablasvida1985_2020.pdf. Accessed May 30, 2023.

12. La Torre F, Di Cesare A, Simonato G, Cassini R, Traversa D, Frangipane di Regalbono A. Prevalence of zoonotic helminths in Italian house dogs. J Infect Dev Ctries 2018; 12(8):666-672.

13. Torres-Chablé OM, García-Herrera RA, Hernández-Hernández M, Peralta-Torres JA, Ojeda-Robertos NF, Blitvich BJ. Prevalence of gastrointestinal parasites in domestic dogs in Tabasco, southeastern Mexico. Rev Bras Parasitol 2015;24(4).

14. Soriano SV, Pierangeli NB, Roccia I, Bergagna HFJ, Lazzarini LE, Celescinco A, Basualdo JA. A Wide diversity of zoonotic intestinal parasites infects urban and rural dogs in Neuquén, Patagonia, Argentina. Vet Parasitology 2010;167(1):81-85.

15. Sarmiento-Rubiano LA, Delgado L, Ruiz JP, Sarmiento MC, Becerra J. Intestinal parasites in dogs and cats from owners from the city of Barranquilla, Colombia. Rev Investig Vet Perú 2018;29(4).

16. Dubey JP, Lindsay DS. Coccidiosis in dogs (*Canis familiaris*) In: Coccidiosis in livestock, poultry, companion animals, and humans, CRC Press: Boca Raton; 2020;245-254.

17. Fantinatti M, Gonçalves-Pinto M, Lopes-Oliveira LA, Da-Cruz AM. Epidemiology of *Giardia duodenalis* assemblages in Brazil: there is still a long way to go. Memórias do Instituto Oswaldo Cruz. 2021;115:e200431.

18. Buret AG, Cacciò SM, Favennec L, Svärd S. Update on *Giardia*: Highlights from the Seventh Int *Giardia* and *Cryptosporidium* Conf. Parasite 2020;27:49.

19. Fusaro C, Chávez-Romero YA, Prada SL, Serrano-Silva N, Bernal JE, González-Jiménez FE, Sarria-Guzmán Y. Burden and epidemiology of human intestinal *Giardia duodenali*s infection in Colombia: a systematic review. Trop Med Infect Dis 2022;7(10): 325.

20. Sarmiento-Rubiano LA, Filott M, Gómez L, Suarez-Marenco M, Sarmiento MC, Becerra JE. Comparative study of the intestinal parasitism profiles between communities across the five municipalities of the Barranquilla metropolitan area, Colombia. Osong Public Health Res Perspect 2021;12(5):333-341.

21. Fu X, Zhong Y, Chen L, Ge M, Yu M, Sun Y, Shen L. Global burden and trends of the *Entamoeba* infection-associated diseases from 1990 to 2019: An observational trend study. Tropica Act 2023;240:106866.

22. Leung AK, Barankin B, Hon KL. Cutaneous larva migrans. Recent patents on inflammation and allergy drug discovery. 2017;11(1):2-11.

23. Streit A. Strongyloidiasis: Really a zoonosis? In: Strube C, Mehelhorn H, editors. Dog parasites endangering human health. Cham Springer; 2021:195-226.

24. Ahmed A, Ijaz M, Ayyub RM, Ghaffar A, Ghauri HN, Aziz MU, Javed MU. *Balantidium coli* in domestic animals: An emerging protozoan pathogen of zoonotic significance. Tropic Act 2020;203:105298.

25. Torrel TS, Vargas LA, Murga CA, Huaman V. Prevalence of *Eimeria* sp, and morphometric study of oocysts found in domestic dogs in Peru. Rev Vet 2022;33(1):59-62.

26. Gookin JL, Birkenheuer AJ, John S, Spector VM, Levy MG. Molecular characterization of trichomonads from feces of dogs with diarrhea. J Parasitol 2005;91(4):939–943.

27. Pacheco-Ortega G A, Chan-Pérez JI, Ortega-Pacheco A, Guzmán-Marín E, Edwards M, Brown MA, Hernández-Cortazar IB. Screening of zoonotic parasites in playground sandboxes of public parks from subtropical Mexico. J Parasitology Res 2019;7409076.

28. Díaz-Anaya AM, Pulido-Medellín MO, Giraldo-Forero JC. Nematodes with zoonotic potential in public parks in the city of Tunja, Colombia. Public Health of Mexico. 2015;57(2):170-176.

29. Rostami A, Ma G, Wang T, Koehler AV, Hofmann A, Chang BC, Gasser RB. Human toxocariasis–a look at a neglected disease through an epidemiological 'prism'. Infect Genet Evol 2019;74:104002.

https://doi.org/10.22319/rmcp.v15i4.6609

Article

Spatial and vertical transmission of milk prices from the international market to Mexico's regional and national markets

Benigna González Ortiz [a]

José Luis Jaramillo Villanueva [a*]

Samuel Vargas López [a]

Ángel Bustamante González [a]

Juan de Dios Guerrero Rodríguez [a]

Vinicio Horacio Santoyo Cortés [b]

[a] Colegio de Postgraduados, Campus Puebla. Boulevard Forjadores de Puebla No. 205, Santiago Momoxpan, Municipio de San Pedro Cholula, 72760. Puebla, México.

[b] CIESTAAM. Centro de Investigaciones Económicas, Sociales y Tecnológicas de la Agroindustria y la Agricultura Mundial. México.

*Corresponding author: jaramillo@colpos.mx

Abstract:

When a country imports a good or service, it is subject to prices determined by the world market; therefore, domestic market prices change when international prices do. This study aimed to estimate the degree of price transmission between the producer price of milk in Mexico at the national and regional levels and that of the United States (spatial transmission) and between the retail price of milk and the producer price in Mexico (vertical transmission). An econometric analysis of monthly time series of milk prices from January 1990 to December 2021 was performed, applying unit root tests, cointegration tests, and an error correction vector model. The results indicate that there is a long-term relationship between United States prices and producer prices at the national and regional levels, as well as between the retail price and the producer price. It was found that the spatial transmission of

international prices to the producer price at the national level and in the regions of Jalisco and Veracruz is symmetrical and asymmetrical with the producer price in the state of Coahuila. There are differences between regions in the speed of adjustment when international prices increase and when they decrease. The vertical transmission was also symmetrical, unidirectional, from the producer to the retail market, and incomplete.

Keywords: Price transmission, Milk market, Error correction model.

Received: 07/12/2023

Accepted: 20/07/2024

Introduction

Historically, Mexico has been an importer of powdered milk because domestic production is insufficient to cover domestic demand. In 2022, imports accounted for 23.3 % of national consumption[1]. In 2021, imports of powdered milk represented 40 % of the total value of dairy imports. Mexico's main supplier of these imports is the United States[2,3,4]. For the national dairy subsector, imports historically exceed exports, i.e., the trade balance is in deficit[2,3].

When a country imports a good, it is subject to prices determined by the world market; therefore, domestic market prices change when international prices do. In an import scenario, those who benefit are the consumers, and these benefits represent a loss for the producer because the imported products are obtained in the international market at lower prices than what is paid in the domestic market[5,6]. The United States can offer competitive prices of powdered milk to the international market since it has high productivity: the yield (liters of milk/cow/d) in Mexico in 2021 was 13.3, and for the United States in the same year, it was 29.78[7]. In addition, both production and marketing in the United States receive high subsidies, which distorts product prices in world markets[3,8].

In Mexico, there is a high concentration of both production and industry. Fifty-three (53) percent of milk production is concentrated in four states, Jalisco (21 %), Coahuila (11.3 %), Durango (11.4 %), and Chihuahua (9.4 %)[2]; within the milk chain, in the industrialization link, there are 130 companies that process 86 % of the national production, with an employed staff of 42 thousand people[9]. The dairy industry is not only an important source of price

change but also acts as a mediator of price signals originating from different parts of the food chain[10].

The national milk industry is an oligopoly characterized by a high degree of concentration of firms, significant barriers to entry, and dynamic product differentiation[11]. In the latter, prices determine the allocation of resources and the production decisions of economic agents[12].

Price transmission is the process through which information is transmitted between market participants[10]; its study allows to know if the markets are integrated. A theoretical framework used in the literature is the law of one price, which states that when there is a commercial exchange between two spatially separated regions, under conditions of perfect competition, price shocks in one market are transmitted completely and symmetrically to the other market and equilibrium prices differ only in transfer costs[13-16]. Markets that transmit price information quickly and comprehensively are said to be perfectly integrated and often efficient[13].

Specifically, price transmission studies analyze the form and speed of adjustment of domestic prices in the face of changes in international prices[14-17]. The speed with which prices are transmitted, the magnitudes of the transmission, and the non-linear behavior in the transmission of prices are indicators of market inefficiency[18].

The results obtained through a meta-analysis[19] suggest that asymmetric price transmission in producer-retailer relationships is more likely in sectors in countries with a more fragmented agricultural structure and greater government support. In Mexico, studies have been carried out on the transmission of fluid milk prices from the international market to the national market and even to the regional and local markets[14,15], but in a single geographical point, and there is no study of transmission in the largest producing regions, both spatial and vertical. This study aimed to estimate the degree of spatial transmission between United States milk import prices and the producer price of milk in Mexico at the national level and in the main producing regions and between the retail price of milk and the producer price at the national level (vertical transmission).

Material and methods

The econometric analysis performed used monthly time series of fluid milk prices from 1990:01 to 2022:12. The data for Mexico at both the national and regional levels are those paid to the producer (average rural price), obtained from the website of the Agri-Food and Fisheries Information Service of the Secretariat of Agriculture[20]. The regional prices

correspond to those paid to the producer in the states of Jalisco, Coahuila, and Veracruz. The states that rank first in the production of bovine milk in their respective regions were selected; Jalisco is the first producing state in the Central-Western region and ranks first nationally (21 %), Coahuila is the first producing state in the Northeast region, ranking second nationally (11.8 %), and Veracruz is the first producing state in the South-Southeast region and ranks sixth nationally (6 %)[21].

The international milk price corresponds to the export prices of skimmed milk powder from the United States to Mexico, transformed into milk equivalent, obtained from the USDA-AMS website[22]. Consumer prices correspond to the average consumer price per liter of pasteurized fluid milk provided by the National System of Information and Integration of Markets[23] of the Secretariat of Economy and adjusted by the national consumer price index[24]. The price series were expressed in dollars per liter using the exchange rate published by the Bank of Mexico[25]. The data were transformed into natural logarithms in order to perform the econometric analysis and interpret the coefficients as elasticities.

The stationarity tests used were the Augmented Dickey-Fuller (ADF)[26] and Phillips-Perron (PP)[27] tests, with which the order of integration of each series was verified. The long-term relationship was then estimated using two-stage cointegration[28] and confirmed with the Johansen test[29]. Finally, it was estimated an Asymmetric Vector Error Correction Model (AVECM), a test to select the order of lag for an AVECM, and an F-test for equality of ECT+ and ECT- coefficients (positive and negative changes in the error term, respectively). The null hypothesis (H_0) of symmetry is rejected if β_2^+ and β_2^- (price adjustment coefficients) differ significantly.

Cointegration tests

The cointegration test was applied in both the spatial and vertical price transmission models. The cointegration between variables – once the existence of unit roots has been demonstrated – is a necessary condition for the existence of a long-term equilibrium relationship in the series. A vector of variables that have a unit root is cointegrated if a linear combination of these variables is stationary[26]. The two-step Engle-Granger cointegration test[28] and the Johansen test[29] were used to test the long-term relationship. The first approach is to estimate the cointegration regression (equation 1) using OLS:

$$p_t^{out} = a + b_1 p_t^{in} + m_t \quad (1)$$

Where p_t^{out} is a firm's production price in period t, p_t^{in} and is the price of inputs in period t. The residual $\hat{U}_t$ is obtained from the cointegration regression, and an ADF unit root test is applied to it. The failure to reject the null hypothesis of non-stationarity allows us to estimate equation 2.

$$D\mu_t = a + b_1 \mu_{t-1} + b_2 D\mu_{t-1} \quad (2)$$

A negative coefficient of the error term (between -2 and zero) confirms a long-run relationship between the milk price paid to the producer and the international milk price. On the other hand, the Johansen test derived the distribution of two test statistics for the null hypothesis of non-cointegration: the tests of eigenvalues and trace[29]. Once the cointegration between prices was verified, an Error Correction Model (ECM) was applied to capture the short- and long-run effect of p_t^{in} on p_t^{out}, and the rate of adjustment at which p_t^{out} returns to equilibrium after a change in p_t^{in}.

Asymmetric spatial transmission of prices between international and domestic prices

Considering that producer and international prices are cointegrated, an Asymmetric Vector Error Correction Model (AVECM) was estimated to investigate the possible interdependence of import prices on domestic prices (spatial transmission). The division of the Error Correction Term (ECT) into positive and negative components allowed to verify the existence of asymmetric price transmission; following this approach, equation 3 was used to study spatial asymmetric price transmission[12].

$$\Delta p_t^{farm} = \alpha + \sum_{j=1}^{k} (\beta_j^+ D^+ \Delta p_{t-j+1}^{int}) + \sum_{j=1}^{L} (\beta_j^- D^- \Delta p_{t-j+1}^{int}) + \emptyset^+ ECT_{t-1}^+ + \emptyset^- ECT_{t-1}^- + \gamma_t \quad (3)$$

Where Δ is the first difference of the operator; P_t^{farm}= producer price; P_t^{int}=import price, D^+ and D^- are fictitious variables by means of which the price of inputs is divided into a variable that includes only increasing prices and another that includes only decreasing prices; β_j^+ and β_j^- are price adjustment coefficients; $\emptyset^+$ and $\emptyset^-$ are constant parameters; ECT is the error correction term. An F-test was used to verify the null hypothesis of symmetry.

Asymmetric vertical price transmission

Equation 4 was used to estimate asymmetric vertical transmission and an F-test was used to verify the null hypothesis of symmetry[12].

$$\Delta p_t^{farm} = \alpha + \sum_{j=1}^{k} (\beta_j \Delta p_{t-j+1}^{int}) + \emptyset^{+} ECT_{t-1}^{+} + \emptyset^{-} ECT_{t-1}^{-} + \gamma_t \quad (4)$$

Results and discussion

According to the results of the ADF and PP unit root tests on the price series, the statistical value of t does not allow to reject the null hypothesis of unit root with a confidence level of 95 %, i.e., the price series are non-stationary (Table 1). Recent studies of milk price transmission obtained similar results of non-stationarity between time series[15,17,30]. The non-stationarity result of the time series justifies the use of cointegration tests. Cointegration allows a combination of non-stationary variables to be stationary. It can be seen as a long-term equilibrium relationship between variables despite the fact that in the short term, they go through situations of disequilibrium[31]. To determine if the series are cointegrated (long-term equilibrium), the residuals (u_t) of the cointegration regression must be stationary; this is achieved by applying the ADF test to determine the stationarity of the time series.

Table 1: Results of ADF and PP tests on the milk price series corresponding to imports, domestic producer, regional producer, and consumer

Price series	ADF test	5% critical value	PP test	5% critical value
Import price	-2.328	-3.425	-20.865	-21.406
Consumer price	-2.032	-3.425	-13.350	-21.406
Production price, National	-3.409	-3.425	-37.672	-21.406
Production price, Jalisco	-3.339	-3.425	-24.447	-21.402
Production price, Coahuila	-2.830	-3.425	-15.919	-21.402
Production price, Veracruz	-3.301	-3.425	-22.482	-21.402

Long-term co-integration of the national and regional spatial model

The results of the ADF test of the error term indicate that the null hypothesis of non-stationarity is rejected (Table 2), which means that the import price series is cointegrated in the long run with both the national and regional price series.

Table 2: Results of the ADF test of the error term

Price pairs	ADF test of the error	5% critical value
P^{Imp}-$P^{national\ prod}$	-4.174	-2.875
P^{Imp}-P^{Jal}	-3.450	-2.875
P^{Imp}-P^{Coah}	-3.344	-2.875
P^{Imp}- P^{Ver}	-3.598	-2.875

Engle and Granger[28] confirmed a long-term relationship between the producer milk price at the national level and the milk import price (Table 3), as well as between the producer price in Jalisco, Coahuila, and Veracruz and the international price (Table 4).

Table 3: Results of the two-step Engle-Granger cointegration test of the spatial model between the import price and the domestic producer price

Variable	Coefficient	Standard error	t-value	P>|t|
μ_{t-1}	**-0.118624**	0.0179831	-6.60	0.000
$D\mu_{t-1}$	.4825031	0.044505	10.84	0.000
Constant	0.0000821	0.0021071	0.04	0.969
F-test	69.26			
R-squared	0.2677			

Table 4: Results of the two-stage Engle-Granger cointegration test of the spatial model between the import price and the regional producer price

	Jalisco				Coahuila				Veracruz			
Variable	Coef.	SE	t value	P>|t|	Coef.	SE	t value	P>|t|	Coef.	SE	t value	P>|t|
μ_{t-1}	-0.0657	0.0169	-3.87	0.000	-.0640	.0169	-3.77	0.000	-.0692	.018	-3.82	0.00
$D\mu_{t-1}$	.1426	0.0510	2.79	0.006	.1366	.0512	2.67	0.008	.0762	.051	1.48	0.13
Constant	-0.0005	0.0024	-0.21	0.831	-.0004	.0021	-0.21	0.832	-.0007	.002	-0.29	0.77
F-test	9.94				9.27				7.66			
R-squared	0.506				0.42				0.39			

SE= standard error.

Like the Engle-Granger test, the Johansen test determines the existence of a stable and long-term equilibrium relationship. The results of the Johansen test of cointegration of the import and domestic producer price series yielded a trace statistical value (5.9169) higher than the critical value of 5 % (3.76), as did the values for the series of prices in Jalisco, Coahuila, and Veracruz (6.8901, 4.7077, 5.0788, respectively); therefore, the null hypothesis of non-cointegration between prices is rejected, i.e., import prices influence the behavior of producer prices at the national level and in the states of Jalisco, Coahuila, and Veracruz in the long term.

Similar studies[14,15] conducted in Mexico with milk price data confirmed the long-term cointegration between the import price and the price paid to the producer, suggesting that the import price influences the behavior of producer milk prices in the long run. In addition to the long-term relationship of Mexican prices with prices in the United States, this relationship with prices in Oceania and the European Union was also verified[15]. This relationship of cointegration of import prices with domestic prices does not always follow a long-term relationship, especially in countries where exports are higher than milk imports[16].

Given the confirmation of cointegration between the import price and the producer price of milk at the national and regional levels, an Error Correction Model (ECM) was estimated, which relates the changes in P_t^{int} with the changes P_t^{farm} in the case of the spatial model and the so-called error correction term (ECT), the lagging residuals of the cointegration equation were calculated.

Spatial vector error correction model: national

The values of ECT_{t-1}^{+} and ECT_{t-1}^{-} reflect that when the import price changes, the producer price at the national level changes in different proportions when it increases compared to when it decreases. When import prices increase, producer prices increase by 8 %, but when import prices decrease, producer prices decrease by 14 % (Table 5).

Table 5: Results of the spatial vector error correction model (VECM) of the import price series and producer price series at the national level

	Symmetric spatial model				Asymmetric spatial model			
Independent variable	**Coef.**	**SE**	**t**	**p>|t|**	**Coef.**	**SE**	**t**	**p>|t|**
$\mathrm{P}int$	0.1140	0.0365	3.12	0.002	---	---	---	
$\mathrm{P}int_t^-$	---	---	---		0.2438	0.1004	2.43	0.016
$\mathrm{P}int_t^+$	---	---	---		0.2493	0.0949	2.63	0.009
$\mathrm{P}farm_{t-1}$	0.5741	0.0486	11.82	0.000	0.5685	0.0486	11.68	0.000
$\mathrm{P}farm_{t-2}$	0.1047	0.0503	-2.08	0.038	-0.097	0.0504	-1.94	0.053
$\mathrm{P}int_{t-1}$	0.0105	0.0402	0.26	0.793	0.0149	0.0459	0.33	0.745
$\mathrm{P}int_{t-2}$	-0.0428	0.0376	-1.14	0.255	-0.0385	0.0376	-1.03	0.306
ECT_{t-1}	-0.1135	0.0182	-6.22	0.000	---	---	---	
ECT_{t-1}^+	---	---	---		-0.0861	0.0235	-3.66	0.000
ECT_{t-1}^-	---	---	---		-0.1487	0.0262	-5.66	0.000
Constant	0.00031	0.0019	0.16	0.875	-0.0003	0.0019	-0.16	0.876
Normality: (Prob>z)	0.963				0.96389			
LM test (Prob>Ji^2)	0.863				0.049			
DW test	2.012				1.9148			
R-squared	0.3937				0.3985			
Test: $H_0: b_1^+ = b_1^-$	---				F(1,374) = 2.35			
Test: $H_0: b_2^+ = b_2^-$	---				F(1, 372) = 3.41			

SE= standard error.

Similar results are reported in Chile[32] in a study of the spatial transmission of international prices to domestic prices, where negative effects or shocks are passed on more quickly than positive effects, which could be explained by the oligopsonic structure in the reception of fluid milk. As reported in previous studies, for the 1990-2016 period[14], in this study, the contemporaneous exchange coefficients are significantly less than one in both equations, indicating that producer prices do not fully react in one month to changes in international prices. The F-test indicates that the null hypothesis of symmetry $H_0: b_2^+ = b_2^-$ is accepted at a significance level of 5 % (Prob > F=0.065).

Studies of milk price transmission in Mexico are scarce[14,15] and have yielded different results in error correction models. In the present study, the speed of adjustment of the national price in the event of deviations from equilibrium shows a lower value than in the preliminary studies (-0.113). The evidence of symmetry in the spatial transmission of prices at the national level differs from that found by previous studies in Mexico for the period 1990-2016[14]. This is probably because the period of analysis is different. Nonetheless, the findings

of the present research coincide with other recent research in Mexico[15] for the period 2001-2019. On the other hand, in a similar study carried out in Chile[32], they found evidence of asymmetry in the spatial price transmission between markets.

Spatial vector error correction model: regional

The values of the coefficients associated with the ECT of the regional models were negative and significant. The result of the F-test indicates that the null hypothesis of symmetry: $H_o: \beta_2^+ = \beta_2^-$ is accepted at the significance level of 5 % for the response of producer prices in Jalisco (F=1.01) and Veracruz (F=0.02); however, it is rejected for the case of Coahuila (F=7.71; P>F= 0.0058), suggesting evidence of asymmetry in the price response in this state (Table 6). The symmetry results for the transmission of international prices to the producer in Jalisco coincide with what was reported by a recent study carried out in the same region[15], in which they analyzed the transmission of prices from three international markets to the national market, regional market in Jalisco and local market in Chicontepec, Veracruz.

The present study also found differences in the transmission of producer prices from one region to another; the values of ECT_{t-1}^{+} and ECT_{t-1}^{-} reflect that when the import price changes, the producer price in Jalisco, Coahuila, and Veracruz changes in different proportions when it increases compared to when it decreases.

Unlike national and regional (Jalisco) prices, import prices affect prices in the state of Coahuila in different ways; when import prices increase, producer prices in Coahuila increase by 10 %, but when import prices decrease, producer prices decrease by 1 %, i.e., the speed of adjustment is significantly greater when prices rise than when they decrease. The speed of adjustment for producer prices in Veracruz did not show significant differences when prices rise or when they fall (Table 6).

These variations in price transmission and in the response of producer prices in the three regions to changes in import prices may be associated with the market structure within each region. Coahuila is part of La Comarca Lagunera, which together with the state of Durango contribute 22.5 % of the national production[21]; in this region, the specialized system predominates and the market structure is of the oligopsony type; here are the two companies with the largest share in the dairy market in Mexico, LALA and Alpura. Both companies maintain a close relationship with their partners from whom they buy milk at a comparatively high price[33], which could explain why positive changes in import prices are reflected faster than negative changes in producer prices. The importance of the study of market structure as part of the analysis of price transmission in the milk market has already been addressed in

other studies[34]. Studies carried out in Mexico[35] and in the milk market between countries that make up a sector at the international level[36] have emphasized the need to consider the analysis of price transmission at the regional level since differences in the response to price transmission between regions can be identified.

Long-term cointegration of the vertical model

For the vertical transmission model between the producer price of milk (P^{prod}) and the retail price (P^{con}), the hypothesis that the retail price is caused by the producer price was tested. Since the time series were non-stationary, was necessary to proceed to perform the long-term cointegration tests using equation (1).

The results of the estimation of equation (1) showed an R^2 of 0.14, a statistical value of t of 16.72, and a statistical value of F of 279.58. The ADF test of the error term showed a test statistic of -3.646, compared to the 5 % critical value of -2.875, indicating that the null hypothesis of non-stationarity is rejected. The results of the two-stage Engle-Granger cointegration test show a negative coefficient of error, confirming the long-term relationship between prices (Table 7).

Table 7: Results of the two-stage Engle-Granger cointegration test for the vertical price transmission model

Variable	Coefficient	Standard error	t-value	P>|t|
η_{t-1}	-0.0963809	0.0155752	-6.19	0.000
$D\eta_{t-1}$	0.5261937	0.0435508	12.08	0.000
Constant	0.000011	0.0021777	0.01	0.996
F-test	81.75			
R-squared	0.3014			

A study of vertical price transmission in the milk market in Russia[37] found that there is no long-term cointegration relationship between producer prices and retail prices; nevertheless, a change in retail price has a significant effect on producer price and vice versa, i.e., there is a bidirectional effect.

The value of the trace statistic (3.3776) of the Johansen test was less than the 5 % critical value (3.76), which does not allow us to reject the null hypothesis of cointegration, i.e., it confirms that the price series are cointegrated.

Vertical vector error correction model

Once the cointegration of retail and producer milk prices was verified, a Vector Error Correction Model[12] was estimated and an F-test was used to test the null hypothesis of symmetry. Error correction models allow to quantify what proportion of the price is transmitted throughout the marketing chain and the speed with which this occurs[12]. The result of the F-test indicates that the null hypothesis of symmetry ($H_o: \beta_2^+ = \beta_2^-$) is accepted at the significance level of 5 % (P>F= 0.5534); this result differs from that found in a previous study in Mexico[14] for the 1990-2016 period, in which they identified evidence of asymmetry in the transmission response of producer prices to the retail market. In contrast to this study, the ECT_{t-1}^- values obtained induce a slightly greater change in the retail price than the ECT_{t-1}^+ (Table 8).

Table 8: Results of the error correction model: symmetrical and asymmetric vertical

	Symmetric model				**Asymmetric model**			
Independent variable	**Coef.**	**SE**	**t**	**p>|t|**	**Coef.**	**SE**	**t**	**p>|t|**
$Pprod_t$	0.0326	0.0593	0.55	0.583	---	---	---	
$Pprod_{t-1}$	0.6234	0.0489	12.75	0.000	0.6248	0.0489	12.75	0.000
$Pprod_{t-2}$	-.1419	0.0511	-2.77	0.006	-0.1441	0.0513	-2.81	0.005
$Pcon_{t-1}$	0.0572	0.0596	0.96	0.338	0.0454	0.0642	0.71	0.480
$Pcon_{t-2}$	-0.0222	0.0592	-0.38	0.708	-0.0222	0.0593	-0.38	0.708
ECT_{t-1}	-0.0780	0.0153	-5.08	0.000	---	---	---	
ECT_{t-1}^+	---	---	---		-0.0692	0.0211	-3.28	0.001
ECT_{t-1}^-	---	---	---		-0.0865	0.0213	-4.06	0.000
Constant	0.0002	0.0020	0.13	0.899	0.00007	0.0020	0.04	0.969
Normality test (Prob>z)	0.99330				0.9933			
LM test (Prob>Ji^2)	1.011				0.996			
DW test	2.013912				2.0144			
R-squared	0.3440				0.3451			
Test: $H_0: b_2^+ = b_2^-$	---				F (1,372) = 0.35			

SE= standard error.

Studies of the vertical transmission of milk prices in other countries such as Slovakia[38,39], Hungary[40], and Uruguay[30] found evidence of asymmetry in the transmission of prices in different links of the chain. One of the factors causing asymmetry, common in these studies, is the market power of the industry; however, the fact that producers are more integrated into the production chain (being part of the industry through cooperatives, for example) makes them react more quickly to changes in prices[30].

Conclusions and implications

There is a long-term cointegration relationship between import prices of powdered milk and the producer price at the national level and in the regions of Jalisco, Coahuila, and Veracruz, and between retail and domestic producer prices. Import prices of powdered milk are transmitted symmetrically to the producer at the national level and in the regions of Jalisco and Veracruz, indicating that there are no significant differences in the response of the producer price whether import prices increase or decrease. Nevertheless, evidence of asymmetry in the transmission of international prices to producer prices in the state of Coahuila was found, where an increase is transmitted more quickly than a decrease. The speed of adjustment to deviations in long-run equilibrium behaved differently between regions. No evidence of asymmetry was found in the vertical price transmission between the retail price and the domestic producer price; the adjustment speed shows that the response of retail prices is faster when producer prices decrease than when they increase. Understanding the dynamics of the spatial and vertical transmission of prices can guide public policy designers to design more comprehensive and regionally differentiated dairy support programs, thereby ensuring a better distribution of welfare and income throughout the chain. This study contributes to the literature on the transmission of milk prices in Mexico; likewise, it also identifies possible differences in the spatial transmission of milk import prices at the regional level, highlighting the importance for public policy designers to consider regional differences when formulating strategies to serve the sector to ensure a better distribution of welfare and income along the chain. Finally, given the constraints of the model used, it is suggested, in subsequent studies, to extend the linear VECM model to a threshold VECM incorporating the Momentum-Threshold Autoregressive (M-TAR) model since they allow the identification of profound changes in the price series, in addition to the fact that asymmetries in price adjustments can be obtained in the face of positive or negative deviations.

Acknowledgements

C. Faustino Zaragoza López of the National System of Information and Integration of Markets (SNIIM, for its acronym in Spanish) of the Secretariat of Economy is thanked for providing historical information on consumer prices of milk. We declare that there is no conflict of interest.

Table 6: Results of the Regional Asymmetric Spatial Model

	Jalisco				Coahuila				Veracruz			
Independent variable	**Coef.**	**SE**	**t**	**p>|t|**	**Coef.**	**SE**	**t**	**p>|t|**	**Coef.**	**SE**	**t**	**p>|t|**
$\mathrm{P}\,\mathrm{int}_t^-$	-0.0326	0.126	-0.26	0.796	-0.0028	0.105	-0.03	0.978	-0.0258	0.134	-0.19	0.847
$\mathrm{P}\,\mathrm{int}_t^+$	-0.0284	0.119	-0.24	0.796	0.0029	0.099	0.03	0.976	-0.0318	0.126	-0.25	0.801
$\mathrm{P}farm_{t-1}$	0.1563	0.052	2.99	0.003	0.1086	0.052	2.07	0.039	0.0838	0.053	1.59	0.114
$\mathrm{P}farm_{t-2}$	-0.0337	0.052	-0.65	0.516	-0.0182	0.051	-0.36	0.723	-0.0029	0.052	-0.06	0.954
$\mathrm{P}\,\mathrm{int}_{t-1}$	0.0648	0.057	1.13	0.259	0.0768	0.047	1.62	0.107	0.0762	0.061	1.25	0.210
$\mathrm{P}\,\mathrm{int}_{t-2}$	-0.0220	0.046	-0.48	0.631	-0.0158	0.038	-0.42	0.678	-0.0470	0.048	-0.97	0.334
ECT_{t-1}^+	-0.0497	0.023	-2.14	0.033	-0.1037	0.022	-4.70	0.000	-0.0737	0.024	-3.10	0.002
ECT_{t-1}^-	-0.0847	0.027	-3.17	0.002	-0.0105	0.025	-0.42	0.674	-0.0690	0.028	-2.47	0.014
Constant	-0.0008	0.002	-0.33	0.743	-0.0008	0.002	0.39	0.697	-0.0004	0.003	-0.17	0.867
Normality test (Prob>z)	0.8032				0.8554				0.7778			
LM test (Prob>Ji^2)	1.546				1.706				0.303			
DW test	1.511				1.668				0.296			
R-squared	0.637				0.855				0.550			
Test: $H_0 : b_2^+ = b_2^-$	F(1, 366) = 1.01				F(1, 366) = 7.71				F(1, 366) = 0.02			

SE= standard error.

Literature cited:

1. FIRA. Fideicomisos Instituidos en relación con la Agricultura (2021). Panorama Agroalimentario. https://www.fira.gob.mx. Consultado 20 Ago, 2022.

2. CANILEC. Cámara Nacional de Industriales de la Leche (2022). Estadísticas del Sector lácteo 2011-2021. https://www.canilec.org.mx/estadisticas.htm. Consultado 05 Ago, 2022.

3. SIAP. Servicio de Información Agroalimentaria y Pesquera. (2021). Panorama Agroalimentario. https://nube.siap.gob.mx/panorama_siap/pag/2021/Panorama-Agroalimentario-2021. Consultado 10 de Ago, 2022.

4. FAO. Food and Agriculture Organization of the United Nations (2022). Dairy Market Review. Overview of global dairy market and policy developments in 2021. Rome.

5. Parkin M, Loría DE. Microeconomía. Versión para Latinoamérica. 9a ed. México: PEARSON EDUCACIÓN; 2010.

6. Robledo PR. Producción de leche en México y su comercio de lácteos con países del APEC. In: Teoría, impactos externos y políticas públicas para el desarrollo regional. Universidad Nacional Autónoma de México y Asociación Mexicana de Ciencias para el Desarrollo Regional A.C, Coeditores, México: 2018.

7. FAOSTAT. Food and Agriculture Organization of the United Nations (FAO). https://www.fao.org/faostat/es/#home(1997). Consultado 29 Jul, 2023.

8. SE. Secretaría de Economía. Análisis del sector lácteo en México. Dirección General de Industrias Básicas. 2012. https://www.economia.gob.mx/files/comunidad_negocios/industria_comercio/informacionSectorial/analisis_sector_lacteo.pdf.

9. SAGARPA. Secretaría de Agricultura, Ganadería, Pesca y Alimentación. https://www.gob.mx/agricultura/colima/articulos/crece-la-produccion-de-leche-en-mexico-sagarpa-158944?idiom=es#:~:text=Dentro%20de%20la%20cadena%20de,380%20mil%20-millones%-20de%20pesos. Consultado 05 Ene, 2023.

10. Lloyd T. Forty years of price transmission research in the food industry: Insights, challenges and prospects. J Agr Econ 2017;68(1):3-21.

11. Caballero GMA. La industria de la leche en México: conductas y tendencias. [Tesis doctorado]. Texcoco, Estado de México: Colegio de Postgraduados, Campus Montecillos; 2010. Disponible en: http://colposdigital.colpos.mx:8080/jspui/handle/10521/83.

12. Meyer J, Crammon-Taubadel SV. Asymmetric Price transmission: A survey. J Agr Econ 2004;55(3):581-611.

13. Bakucs Z, Benedek Z, Fertö I. Spatial Price Transmission and Trade in the European Dairy Sector. AGRIS on-line Papers Econ Inf 2019;11(2):13-20.

14. Jaramillo-Villanueva JL, Palacios-Orozco A. Transmisión de precios vertical y espacial en el mercado mexicano e internacional de leche. Rev Mex Cienc Pecu 2019;10(3):623-642.

15. Martínez-Marcos D, Barrios-Puente G, Pérez-Soto Francisco, Ruiz-Hernández JA, Lara-Reimers EA. Transmisión espacial de precios desde el mercado internacional al mercado mexicano de leche. Ecosist Recur Agropecu 2023;10(1):e3022.

16. Arcos CA. Transmisión de precios internacionales en el mercado lácteo chileno. Chilean J Agric Anim Sci 2020;36(1):86-94.

17. Jaramillo-Villanueva JL, Benítez-García E. Trasmisión de precios en el mercado mexicano e internacional de café (*Coffea arabica L.*): un análisis de cointegración. Agrociencia 2016;50(7):931-944.

18. Ramsey AF, Goodwin BK, Hahn WF, Holt MT. Impacts of COVID-19 and Price transmission in U.S. Meat Markets. Agr Econ 2021;52(3):441-458.

19. Bakucs Z, Fałkowski J, Fertő I. Does market structure influence price transmission in the Agro-food sector? A Meta-analysis perspective. J Agric Econ 2013;65(1):1-25.

20. SIAP-SADER. Servicio de Información Agroalimentaria y Pesquera. Indicadores económicos. https://www.gob.mx/siap/acciones-y-programas/produccion-pecuaria. Consultado 15 Abr, 2022.

21. SIAP-SADER. Servicio de Información Agroalimentaria y Pesquera (2022). Panorama Agroalimentario. https://www.gob.mx/siap/acciones-y-programas/panorama-agroalimentario-258035. Consultado 01 de Ago, 2023.

22. USDA-AMS. United States Department of Agriculture. http://www.fas.usda.gov/ustrade/. Consultado 10 Mar, 2022.

23. SNIIM. Sistema Nacional de Información de Mercados, Secretaría de Economía (SE). http://www.economia-sniim.gob.mx/. Consultado 18 Feb, 2022.

24. INEGI. Instituto Nacional de Estadística y Geografía. Índice Nacional de Precios al Consumidor y al Productor. http://www.inegi.org.mx/. Consultado 15 Mar, 2022.

25. Banco de México. Indicadores económicos. http://.banxico.org.mx. Consultado 20 Mar, 2022.

26. Dickey DA, Fuller WA. Likelihood ratio statistics for autoregressive time series with a unit root. Econometrica 1981;49(4):1057-1072.

27. Phillips PCB, Perron P. Testing for unit root in time series regression. Biometrika 1988;75(2):335-346.

28. Engle RF, Granger CWJ. Cointegration and error correction: Representation, estimation and testing. Econometrica 1987;55(2):251-276.

29. Johansen S. Estimation and hypothesis testing of cointegration vectors in Gaussian vector autoregressive models. Econometrica 1991;59(6):1551-1580.

30. Bertamini F, Carriquiry M. Incidencia del mercado internacional y de la estructura del mercado doméstico en la transmisión de precios en la cadena láctea: evidencia desde Uruguay. Udelar. FCEA. Lecon 2021. Serie Documentos de Trabajo; 12/21.

31. Jiménez TR. Orígenes y evolución de la teoría de la cointegración y del análisis de la transmisión de los precios en mercados. Historia de la probabilidad y la estadística (V) 2011:249-262.

32. Labra-Hernández J, Cabas-Monje J, Velasco-Fuenmayor J. Efectos del precio internacional de la leche sobre el precio pagado a productor chileno: un análisis de cointegración. Rev Científica 2017;XXVII(6):385-392.

33. García HLA. Estrategias de las agroindustrias lecheras latinoamericanas. Estudio comparativo ante el proceso de globalización económica. Rev Mex Agron 2001;5(9):263-273.

34. Acosta A, Ihle R, Cramon-Taubadel SV. Combining market structure and econometric methods for Price transmission analysis. Food Sec 2019;11:941-951.

35. Rojas-Juárez LA, Jaramillo-Villanueva JL, Vargas-López S, Bustamante-González Á, Guerrero-Rodríguez JDD. Transmisión vertical y horizontal de precios en el subsector ganado carne de bovino en México. Ecosist Recur Agropecu 2022;9(3):e3307.

36. Bakucs Z, Benedek Z, Fertö I. Spatial price transmission and trade in the European dairy sector. AGRIS on-line Papers in Economics and Informatics 2019;11(2):13-20.

37. Kharin S. Price transmission analysis: The case of milk products in Russia. AGRIS on-line Papers in Econ and Inf 2018;10(1):15-23.

38. Lajdová Z, Bielik P. Vertical price transmission analysis: The case of milk in the Slovak dairy sector. APSTRACT: Applied Studies in Agribusiness and Commerce 2013;7(4-5):89-96.

39. Weldesenbet T. Asymmetric price transmission in the Slovak liquid milk market. Agric Econ Czech 2013;59(11):512-524.

40. Ben-Abdallah M, Fekete-Farkas M, Lakner Z. Analysis of dairy product price transmission in Hungary: A nonlinear ARDL model. Agriculture 2020;10(6):217.

https://doi.org/10.22319/rmcp.v15i4.6530

Article

Competitiveness and comparative advantage in beef cattle production in the Sierra Norte of Puebla, Mexico

Lissete Abigail Rojas-Juárez [a]

José Luis Jaramillo-Villanueva [a*]

Samuel Vargas-López [a]

Juan Cabas-Monje [b]

[a] Colegio de Postgraduados. Boulevard Forjadores de Puebla No. 205, Santiago Momoxpan, Municipio de San Pedro Cholula, 72760, Puebla, México.

[b] Universidad del Bío-Bío. Facultad de Ciencias Empresariales. Chile.

* Corresponding author: jaramillo@colpos.mx

Abstract:

Beef cattle production systems in Mexico are socially and economically important because they contribute to economic development by generating employment and income. Profitability and competitiveness in this subsector have been negatively affected by structural changes in government support as part of the economic integration of Mexico with the United States and Canada. An evaluation was done of the competitiveness and comparative advantages of beef cattle production systems in the Sierra Norte region of the state of Puebla, Mexico. Technical and productive information from 116 beef cattle production units were used in the policy analysis matrix method to identify benefits, restrictions and opportunities. Three production systems were identified: cow-calf (79 %), grower (13 %) and mixed (8 %). The private cost ratios (0.22 for cow-calf systems, 0.45 for grower systems, and 0.23 for mixed systems) indicated high competitiveness. The internal resources cost ratios (0.11 for cow-calf systems, 0.08 for grower systems, and 0.14 for mixed systems) implied they all have a comparative advantage. The effective protection quotients (0.55 for breeding systems, 0.16 for grower systems, and 0.64 for mixed systems)

indicated that beef cattle production in this region lacks protection. The studied beef cattle production systems are profitable for the producers and for Mexico, but could clearly benefit from policy modifications aimed at generating positive incentives for production.

Key words: Cattle, Effective Protection, Private Profitability, Input Use.

Received: 17/07/2023

Accepted: 12/02/2024

Introduction

Livestock production in Mexico generates employment and income, which support national economic development, and provides food security, and household subsistence[1,2]. In 2022, national beef production was 2,175,576 t, valued at 163,811,754 thousand Mexican pesos (MXP) (approximately USD 9.1 billion)[3]. Since this production does not meet national beef demand, about 8.5 % of the beef consumed in Mexico is imported, mostly from the United States of America, Nicaragua, and Canada[4,5]. This production deficit is the result of structural changes and adverse public policies applied to the livestock sector in the form of trade liberalization[6], privatization of government-owned companies, a reduction in credit, and elimination of subsidies[7]. Over the last ten years, these policy changes have created disadvantages such as high input costs, livestock theft, higher transportation costs, and limited extension services[8]. COVID-19 containment measures further aggravated these tendencies[9,10]. Beef cattle production dynamics in these main importing countries directly impact the beef cattle subsector in Mexico[11], affecting producer competitiveness and productivity, and consumer purchasing power[12]. Understanding the actual situation of producer competitiveness and their comparative advantages is essential to implementing policy instruments that help to minimize the impact of foreign competition on different types of producers in Mexico, especially small-scale producers[13].

In beef cattle production, competitiveness is defined as the capacity of this sector to face international competition, remain in international markets (mainly the United States), maintain production quality and efficiency, and generate greater profits from available resources. Other factors to consider include exchange rate fluctuations, marketing infrastructure availability and availability of relatively low-cost productive factors[14,15].

Various methods exist to measure competitiveness, but the policy analysis matrix (PAM) is a robust methodology that encompasses different approaches for measuring competitiveness and comparative advantage[16]. It is widely used and accepted[17-21]. This tool is fundamentally based on analysis of budgets at market prices and social prices (opportunity cost) to quantify the competitiveness and comparative advantages of production systems and the policy instruments that affect that competitiveness[13]. Research to date on competitiveness and comparative advantages in different economic sectors in Mexico is insufficient[22]. Existing studies using PAM to measure competitiveness in dual-purpose livestock[23] and in feedlot beef production systems[24] have shown negative competitiveness, good competitiveness or developing competitiveness.

Analyses need to be done of the trade policies that impact the competitiveness of regional beef production systems so as to identify efficient production and pricing patterns and therefore make relevant recommendations to policy makers. In the Sierra Norte region of the state of Puebla, Mexico, there is an apparent lack of protection for beef cattle producers, and their competitiveness and comparative advantages are negatively impacted by the costs of feed input, internal factors, and production system technical services. The present study objective was to use income, costs, tradable inputs prices, and internal factors to evaluate competitiveness and comparative advantages in beef cattle production systems in the Sierra Norte region of Puebla.

Material and methods

The study was done among calf breeders and growers in six municipalities in the Sierra Norte region (20°34' and 20°51' N, 97°44' and 98° 01' W; 60 to 940 m asl). Regional climate is warm humid with 1,400 to 2,600 mm annual rainfall, and 22 to 25 °C average annual temperature[25,26]. Using National Livestock Registry information for the Sierra Norte of Puebla[27], a structured questionnaire was applied to a random sample of beef cattle production units; reliability was 95 % and accuracy was 9 %. The sample was distributed proportionally among the six selected municipalities which account for the largest livestock inventory in the region (45.5 %): 4.4 % of the sample was in Francisco Z. Mena; 6.6 % in Pantepec; 4.4 % in Venustiano Carranza; 6.2 % in Xicotepec de Juárez; 7.1 % in Jopala; and 9.3 % in Jalpan. Sample size was calculated with the formula:

$$n = \frac{(p)(q)(N)(z^2)}{E^2(N-1) + z^2(p)(q)}$$

Where N= total population of study area (4,453 production units), n= sample size, p= estimated positive variability (%): 50%, q= 100-p (negative variability), E= error or allowed estimation accuracy (9%), Z= confidence level, and Z from tables = 1.96
Inserting the appropriate values generated this calculation:

$$n = \frac{(0.50)(0.50)(4{,}453)(1.96^2)}{0.09^2(4{,}453-1)+1.96^2(0.50)(0.50)} = 116\ interviews$$

Final sample size was 120 producers. Questionnaire items addressed technical production data, income, costs and profits of each production system (Table 1). The data was organized into five categories: tradable inputs, production factors, materials, indirectly tradable inputs and expenses. All were expressed in their corresponding units to identify the technical coefficient matrices of the private budget. The private prices were then specified, which, based on the collected data, were the market prices received or paid by the producer to carry out the activity, both for inputs, and products and by-products. Within this budget, the loan for supplies represented the short-term refinancing loan producers can obtain to acquire inputs, raw materials and materials, pay wages and salaries, as well as other direct production expenses; with the approximate figures used by the Agricultural Trust Funds (Fideicomisos Instituidos Relacionados con la Agricultura - FIRA), the cumulative value of these concepts was MXP 280,000 (USD 15,556). The surveyed producers stated that banks offered an 18.23 % average rate. The costs of livestock depletion were calculated using the private prices considered in the internal factors, which include initial values, the use life of each system and annual recovery. The private budget was then generated by multiplying the amounts by the prices, and including income from total sales, total costs and producer profits.

Calculation of the social budget was done using the coefficients recorded initially and replacing the private prices with the economic or social efficiency prices of inputs, products and by-products. These are the prices that would exist in the absence of policy interventions and market distortions of factors and products[28]. For inputs, yellow corn was considered to be the main component of balanced feed, and the costs per dose for internal and external dewormers were estimated separately. For this purpose, the world-wide prices in the Agricultural Marketing Service[29] were used, adjusted for freight, insurance and tariff costs. Border (CIF - Cost, Insurance and Freight) prices were calculated to generate import parity prices. The FoB (Free on Board) prices for cattle, as well as the costs for bridge tolls, transport to distribution center and delivery were then added in. To this end, a balanced exchange rate was used calculated based on a 20.1 MXP/USD nominal exchange rate[30]; a rate adjustment was done for 2022 with 2018 as the base year, and referencing the producer and consumer price indexes for Mexico and the USA.

Long-term expected values were utilized to prevent data distortion due to global fluctuations and foreign policies. For the internal production factors, social value was estimated at a national level equivalent to its opportunity cost focused on the best alternative use, such as sheep farming. Unlike the private budget, short-term credit for supplies in the social budget considered the interest and inflation rates of Mexico (24 % and 7.82 %, respectively)[31], and the United States (4.75 % and 6.50 %, respectively)[32]; the resulting nominal parity interest rate was 14.36 %. The economic cost of water was quantified as the equivalent of payment of a fixed annual fee for livestock activities levied by the Agua de Puebla water company[33]. Finally, livestock import parity prices were calculated assuming entry at the Texas, USA, border[34]. This represented a producer's cost to import livestock to the location of consumption, that is, the social prices of this activity's products. After including the subsidies, taxes and exchange rate distortions that affect products and input prices, the quantities were multiplied by the prices to generate the social budget ([1]The complete calculations of the systems' private or social budget values are available).

The resulting data was processed with the PAM (Table 2). The values calculated from the previous budgets were replaced. First, private profitability (D) was calculated as the difference between total income (cattle sales and available stock in total herd: surplus heifers, culled calves and bulls) and costs (tradable and indirectly tradable inputs), and internal factors and other miscellaneous materials or expenses. Social profitability (H) was calculated as the difference between income and costs, but evaluated using social prices to analyze comparative advantage. Eliminating these effects allowed calculation of national beef cattle production profitability, which could be evaluated versus that of other countries to determine if it is competitive or not. Finally, the effects of policy (I, J, K and L) were estimated as the differences between the private and social evaluations for income, costs and profits. Under this premise, the differences between the private prices and social prices can be explained by the effects of policy distortions or imperfect markets.

Table 2: Policy Analysis Matrix

	Income	Costs		Profits
		Tradable Inputs	Internal Factors	
Private prices	A	B	C	D[1]
Social prices	E	F	G	H[2]
Effects of differences and efficient policy	I[3]	J[4]	K[5]	L[6]

[1] Private profits, D= A–B– C; [2] Social profits, H= E–F–G; [3] Product transfer, I= A– E; [4] Input transfer J= B–F; [5] Factor transfer, K= C–G; [6] Total transfer, L= D–H = I–J–K.
Source: Monke & Pearson[21].

Once private profitability was calculated, the private cost ratio (PCR) could be calculated. This is the quotient between the costs of production internal factors and the value added in private prices [PCR = C/(A-B)], which shows how much the system can afford to pay for internal factors and consequently if the producer is competitive.

Using each system's net social profitability, the internal resource cost ratio (RCR) was calculated by dividing the cost of internal factors valued at social prices (without subsidies) by the social value added [RCR = G/(E-F)]. This is the difference between a product's internationally-priced production value and the costs of tradable inputs at international prices. It indicates if the value of domestic resources is lower or higher than the value of earned or saved foreign exchange, and thus if there is any comparative advantage in beef cattle production.

Finally, an estimation of whether or not pricing policies encourage domestic beef cattle production was done by contrasting product market prices with product social prices (Pi/Pi* or A/E) using the Nominal Protection Coefficient for products (pNPC). The contrast of tradable inputs at private and social prices (Pj/Pj* or B/F) was done using the Nominal Protection Coefficient for inputs (iNPC). The Effective Protection Quotient (EPQ), another indicator of incentives, was defined as the ratio between value added at private prices and at social prices (i.e. without subsidies) [EPQ = (A-B)/(E-F)]. Finally, the Producer Support Estimate (PSE) was calculated as a proportion of total gross income to private prices (L/A) as a way of showing net policy transfer.

Results

Beef cattle production systems

Three beef cattle production systems were identified in the Sierra Norte. Most (79%) of the surveyed producers were engaged in cow-calf systems (CCS). This involves intermediate management with sufficient space for pasture rotation with an average stocking rate of 65 head. Minimal feed supplementation is used and calves are weaned at between 160 and 180 kg. A smaller proportion (13%) were using grower systems (GS). They purchase calves and finish them in intensive (6 mo), intermediate (12 mo) or slow (18 mo) systems. Finished animals are sold at 300 to 350 kg, and average general inventory was 33 head per producer. The smallest proportion (8%) were using a mixed system (MS) which combines the cow-

calf and grower systems. This allows them to manage the complete lifecycle in the same production unit. It requires a good finishing plan, and can have an average of up to 90 head.

The dominant breeds in all three systems were zebu crosses with Swiss and Brahman, used in an attempt to maximize reproductive efficiency through continuous natural mating. Feed was based on grazing grass directly in pastures, with very few production units using supplementation with balanced feed and mineral salts. Consequently, most units reported low weight gain. Water was supplied from natural sources (rivers, streams and springs), was freely available to the animals and thus represented no cost to producers. Disease control depended largely on application of vaccines against rabies, brucellosis, blackleg, malignant edema, and clostridial infections. Parasites were controlled internally and externally through permanent and continuous doses of dewormers, and flea/tick baths. Producers incurred no electricity costs because they used grazing systems. Neither were there fuel costs because both weaned and grown cattle were sold at the ranch gate; purchasers directly assumed transportation costs. However, a transport cost for materials and inputs to the production unit was included.

Profitability and competitiveness

The assessment of production process profitability identified the main production costs for the resources and factors used in each system (Table 3). Tradable inputs (feed, medicine) were the highest cost item per kilogram of meat in all three production systems (CCS, GS and MS), accounting for from 56 to 60 % of total costs. Internal factors such as labor represented 25 % in the CCS, 17% in the GS and 26 % in the MS. Labor included total daily wages per hectare for pasture maintenance (i.e., hoeing, herbicide and fertilizer application), as well as daily wages for herd care and management.

Table 3: Average production costs for inputs in beef cattle production in the Sierra Norte of Puebla, at private prices in constant values

Concept	Private budget					
	CCS[§]		GS		MS	
	$	(%)	$	(%)	$	(%)
Tradable inputs	476,433.3	56.1	408,558.5	59.6	680,558.8	59.6
Feed	461,747.3	54.4	398,709.9	58.2	661,339.6	58.0
Medicine	14,686.0	1.7	9,848.6	1.4	19,219.2	1.7
Internal factors	213,044.0	25.1	116,722.0	17.0	299,444.0	26.2
Labor	162,000.0	19.1	91,200.0	13.3	248,400.0	21.8
Credit	51,044.0	6.0	25,522.0	3.7	51,044.0	4.5
Water		0.0	0.0	0.0	0.0	0.0
Misc. materials	2,137.6	0.3	2,443.0	0.4	2,137.6	0.2
Indirectly tradable inputs	153,523.3	18.1	155,945.6	22.8	154,878.8	13.6
Breeding stock	93,869.3	11.1	123,407.4	18.0	70,368.8	6.2
Installations	59,653.9	7.0	32,538.2	4.7	84,510.0	7.4
Administration and services	5,700.0	0.7	3,900.0	0.6	6,200.0	0.5
Total Income	1,632,141.0		831,600.0		2,159,879.0	
Total cost (excluding land)	848,700.5	100.0	685,126.1	100.0	1,141,081.6	100.0
Net profit (excluding land)	783,440.5		146,473.9		1,018,797.4	

[§]CCS = Cow-calf system; GS = Grower system; MS = Mixed system.

Indirectly tradable inputs accounted for 18 % in the CCS, 23 % in the GS, and 14 % in the MS. This parameter considers depreciation values of breeding stock and calf values, as well as the recovery costs of equipment, assets and some implements not marketed internationally.

The highest effective income from cattle sales (47.9 %) was observed in the CCS, followed by the MS (47.1 %), and the GS (17.3 %) (Table 4). Both the CCS and MS were the most profitable systems because they sold breeding stock and controlled the complete weaning and/or finishing cycle, thus limiting and exploiting some input costs.

Table 4: Policy analysis matrix for beef cattle production systems in the Sierra Norte of Puebla

		Costs		
	Income	**Tradable inputs**	**Internal factors**	**Profits (net)**
Cow-calf System				
Private budget	1,632,141	635,657 (38.9%)	215,182 (13.2%)	781,303 (47.9%)
Social budget	2,426,316	604,038 (24.9%)	207,789 (8.6%)	1,614,489 (66.5%)
Divergences	(794,175)	31,618	7,393	(833,186)
Grower System				
Private budget	831,600	568,404 (68.4%)	119,165 (14.3%)	144,031 (17.3%)
Social budget	2,035,388	747,034 (36.7%)	137,268 (6.7%)	1,151,086 (56.6%)
Divergences	(1,203,788)	(178,630)	(18,103)	(1,007,056)
Mixed System				
Private budget	2,159,879	841,638 (39.0%)	301,582 (14.0%)	1,016,660 (47.1%)
Social budget	2,843,329	791,749 (27.8%)	294,189 (10.3%)	1,757,391 (61.8%)
Divergences	(683,450)	49,889	7,393	(740,731)

In the CCS, total production costs (tradable inputs and internal factors) accounted for 52 % of the budget, resulting in a 48 % profit. In the MS, these costs accounted for 53 % of the budget, resulting in a 47 % profit. However, in the GS these costs were 82.7 %, with particularly high tradable inputs costs ($568,404), resulting in less than half the profits of the other systems (17.3 %). In the private budgets, the profitability indicators showed that all three systems were profitable due mainly to the use of up-to-date technology, current market prices, and transfers or taxes generated by economic policy measures.

The prices of yellow corn and medicine (triple bacterin, amitraz, and ivermectin) were included in the social budget analysis. The best social budget among the three systems was for the CCS, which had an almost 67 % profit and just 25 % tradable inputs costs. In the GS, the tradable inputs costs (36.7 %) kept profits below 57 %, even considering price changes due to exchange rate adjustments.

The above shows that the GS exhibited negative transfers of both products (I) and inputs (J). This was due to two main distorting policies that cause divergences between observed prices and world prices. The first are taxes, subsidies and commercial policies applied to breeding or growth calves in Mexico, which affect private profitability. The second refers to the social foreign exchange rate policy which differed from the observed rate such that it was undervalued by -0.08 %, resulting in a real exchange rate of 18.57 MXP/USD. This constitutes an implicit support for producers since it generates an indirect saving on inputs.

In summary, the sum of the negative incomes for the inputs (J) and internal factors (k) divergences reflect a positive net transfer (L) to the system.

This is confirmed by the PCR and RCR, as well as the EPQs. With values near zero, the PCRs of all three systems confirm that the surveyed producers were competitive (Table 5), mainly because sales of calves and breeding stock allowed them to pay the value of the production factors and still generate a profit.

Table 5: Profitability and protection indicators for beef cattle production systems in the Sierra Norte of Puebla

Concept	Production system		
	Cow-calf	Grower	Mixed
Private Cost Ratio	0.22	0.45	0.23
Internal Resources Cost Ratio	0.11	0.11	0.14
Producer Support Estimate	(0.51)	(1.21)	(0.34)
Product Nominal Protection Coefficient	0.67	0.41	0.76
Inputs Nominal Protection Coefficient	1.05	0.76	1.06
Effective Protection Quotient	0.55	0.20	0.64

Given that their PCR was the lowest (0.22), the CCS were the most competitive of the three studied systems. The higher PCR (0.45) for the GS showed that their profits were negatively affected by the extended growth time in the corral.

Both the CCS and GS had a low RCR (0.11), highlighting their social profitability. Their low RCR indicates they have comparative advantages, and that beef cattle production can be profitable in Mexico if resources are used efficiently; in other words, the value of the internal resources required for production was lower than the savings in foreign currency.

All three system types had EPQ values lower than 1.0, confirming the disincentives originating in policy interventions and the consequent lack of protection. The negative PSE values in all three system types represent their high private costs versus social costs due to prevailing economic policies. For example, gross income taxes imposed on GS producers were 121 %, an artifact of policy distortions and market imperfections in internal production factors.

Overall, the pNPC values were less than one, confirming a lack of protection for beef cattle production in the study area and suggesting that current policies inhibit national production. For the iNPC, both CCS and MS had values greater than one, indicating protection of

negative price policies; that is, they benefit from an indirect subsidy not reflected in the iNPC for the GS.

Discussion

Beef cattle production systems

Beef cattle production systems in the Sierra Norte region of Puebla are similar to others reported in Mexico. In one study, full cycle and cow-calf-to-weaning systems in Tizimín municipality in the state of Yucatan[35] were reported to use zebu breeds and crosses with European breeds, extensive grazing and corral feeding, although the systems were stratified based on number of head and stocking rate. Another study, of small and medium production units in the southern portion of the State of Mexico[36], identified three systems based on livestock production surface; all the producers used zebu, Brahman and Swiss breeds and crosses between them, and, generally, grazing with complementary supplementation.

Profitability and competitiveness

The present results for the percentage participation of costs is similar to that reported in a study of corral-grown cattle production in Tejupilco and Amatepec, in the State of Mexico[24]. In this study, costs were primarily (80 %) tradable inputs, followed by internal factors (10 %) and the remaining production costs (10 %). Feed, health and fuel costs represented more than 86% of variable costs in a study of dual-purpose cattle production systems in Jamapa municipality, in the state of Veracruz[37]. Labor costs accounted for up to 60 % of fixed costs, meaning that these factors generated the greatest economic and productive impact. The CCS had the highest effective income compared to MS and GS. This is supported by a study on calf grazing in the state of Sonora[38] which observes that traditional extensive cow-calf systems better utilize natural land conditions as long as they are extensive enough. They allow for adequate management because calves are left in pastures after weaning where they can gain various kilograms per day.

Indeed, as supported in the present results, grazing is an effective way of controlling total costs. This is further supported by a study of the beef production model in Chile, in which estimates showed that in mixed systems grazing is an effective and economic source of feed[39]. In these systems, supplementation is only needed in months of low pasture biomass

production or during the finishing stage to ensure efficient weight gain and maintain producer profitability.

Among the three systems studied here, the GS had the lowest percentage of effective income. This is similar to a report on intensive, semi-intensive, and extensive cattle grower systems in Gowa, Malaysia[19]. In these systems, total income from cattle sales varied according to sale price, and price was influenced by growth duration. Income was consequently highest for producers using a slow growth strategy (48 %), compared to those using intermediate (33 %) and intensive (18 %) strategies; suggesting that the strategy used may affect profits.

The profitability results for the three systems studied here are similar to those reported for three types of corral grower systems in the southern portion of the State of Mexico[28]. As observed in the present results, each producer type used efficient input management to generate additional income for each peso invested, meaning all production factors contributed to creating added value.

The present social budget results are comparable to those in a study of grower systems in Bali, Indonesia[18] in which profits were generated at both the private and social levels. However, non-tradable input costs were much higher in this study due to producer dependence on internal production inputs.

The negative transfers observed here in both the products (I) and inputs (J) affecting private profitability were probably the result of changes in federal agricultural policies in Mexico after ratification of the North America Free Trade Agreement (NAFTA) in 1993. Tariffs were reduced, and support and subsidies for agricultural activity were progressively withdrawn. This treaty has prevented strengthening of beef cattle production in Mexico through public policy[5].

Although all three studied systems were deemed economically efficient, their competitiveness results were lower than the 0.51 to 0.52 PCR reported for three types of corral cattle systems in Tejupilco[24].

The comparative advantages for the three studied systems were similar to the 0.31 reported in a study of ruminant systems in Malaysia[20]; as observed in the present results, these advantages were created through efficient use of domestic inputs and resources in production, and the saving or earning of foreign currency.

The present EPQs results were similar to a study using PAM[40] to identify federal livestock policy as an example of weak governance, since, beginning in the 1980s, the Mexican

government has eliminated or reduced support for livestock and agricultural production. This contrasts with the 1.71 EPQ reported for grower systems in Gowa[19], a manifestation of the impact of government policies supporting domestic productive activities. In this study, the ESP was positive (0.15) indicating that government policies allowed the studied grower systems to incur lower private costs than social costs.

Overall, the present PAM results confirmed that the studied CCS, GS and MS are efficient and profitable for producers since they have a comparative advantage and are competitive. However, despite their efficiency, government policy exploits their comparative advantage to keep prices low, meaning they are relatively unprotected. This lack of protection may be due mainly to an absence of public policy support for the livestock subsector aimed at strengthening national production. The absence of support allows foreign products to at least partially substitute domestic production, negatively affecting producers in Mexico.

Conclusions and implications

Application of the policy analysis matrix to beef cattle production systems in the Sierra Norte region of Puebla identified the cow-calf systems as having the best private profitability, the grower systems as having the highest comparative advantage and the cow-calf systems as the most competitive. In all three systems, tradable inputs (food and medicine) accounted for most of the costs in the production cost structure per kilogram of meat. The ratio between production value at domestic market prices and international prices showed that pricing policies discourage domestic production. Through changes in policy that distort efficiency, as well as direct subsidies, domestic production systems could improve their market participation in private and social terms, with a consequent increase in income.

Table 1: Technical and production data

Variables	Measurement unit	Definition	Interpretation	Formula
Tradable inputs	pesos/ton pesos/treatment	Inputs required for cattle production, available domestically and internationally	These coefficients show the amounts used and prices paid by the producer in the regional market for inputs, products and byproducts,	(+) feed (+) medicine
Internal factors	pesos/work day pesos pesos/m^3 pesos/equipment	Production factors without an international price (land, labor, capital)		(+) labor (+) credit (+) water (+) misc. materials
Indirectly tradable inputs	pesos/head pesos/infrastructure	Inputs untradable internationally (e.g. implements and basic equipment)		(+) breed stock (+) installations
Administration and services	pesos/hour pesos/service pesos/ha	Factors with no international price, needed to manage and support production		(+) containers (+) veterinarians (+) hauling (+) taxes
Total income		Income from sale of breeding stock, grown calves (slow, intermediate or intensive), surplus or culled animals	Financial resources received by producers from cattle sales	(+) sale weaned calves (+) sale grown calves (+) other sales
Total cost	pesos	Total value paid for goods and services required for production	Sum of all input and product costs for production	(+) tradable inputs (+) internal factors (+) indirectly tradable inputs (+) administration and services
Profit		Total difference between income and production cots	Financial profit or earnings in beef cattle production units	(+) total income (-) total cost

Literature cited:

1. Figueroa D, Galicia L. Ganadería bovina con menor costo ambiental: un desafío entre lo personal y lo político. Soc Amb 2021;24:1-17.

2. Rodríguez DI, Anríquez G, Riveros JL. Food security and livestock: The case of Latin America and the Caribbean. Cienc Inv Agr 2016;43(1):5-15.

3. SIAP. Anuario Estadístico de la Producción Ganadera. Información, Producción Ganadera 2022. Carne en canal. México. Servicio de Información Agroalimentaria y Pesquera. 2022. https://nube.siap.gob.mx/cierre_pecuario/ Consultado 15 Ene, 2023.

4. COMECARNE. Compendio estadístico 2022. Consejo Mexicano de la Carne. México. 2022. https://comecarne.org/compendio-estadistico-2022/ Consultado 15 Nov, 2022.

5. Montaño MIE, Avendaño RB, Ávila AA, González MD. Competitividad y el desequilibrio comercial de México en el mercado mundial de carne de bovino, 1990-2016. Cienc Tecnol Agr 2021;22(1):1-19.

6. Rojas JLA, Jaramillo VJL, Vargas LS, Bustamante GA, Guerrero RJ. Transmisión vertical y horizontal de precios en el sub-sector ganado carne de bovino en México. Ecosistemas Rec Agr 2022;9(3):1-11.

7. Málaga JE, Williams GW. La competitividad de México en la exportación de productos agrícolas. Rev Mex Agroneg 2010;27:295-309.

8. Sarma PK, Raha SK, Jørgensen H. An economic analysis of beef cattle fattening in selected areas of Pabna and Sirajgonj Districts. J Bangladesh Agric Univ 2014;12(1):127-134.

9. Ingram PB, Arce CBA. Impacto en la pandemia COVID-19 en la ganadería bovina a nivel nacional y estatal (Veracruz). Rev Cienc Adm 2021;2:49-62.

10. Salas C, Quintana L, Mendoza MA, Valdivia M. Distribución del ingreso laboral y la pobreza en México durante la pandemia de la Covid-19. Escenarios e impactos potenciales. El trimestre Econ 2020;87(348):929-962.

11. Rinconada CF, Serna HJA, Valdez RRI. Competitividad de la carne de res fresca mexicana en el mercado estadounidense, 1967-2020. Anál Econ 2023;38(97):129-148.

12. Hernández RIK, Merchán ADM, Miranda FJD, Morales LPA, Cala MN. Influencia del COVID-19 en las dinámicas de exportación, producción y consumo de carne vacuna en Colombia y el mundo: Una revisión monográfica. Spei Domus 2022;18(1):1-21.

13. Salcedo BS. Competitividad en la agricultura en América Latina y el Caribe. Matriz de análisis de política. Santiago Chile. 2007. https://www.cepal.org/sites/default/files/courses/files/03_3_map_manual_fao.pdf.

14. Magaña MMA, Leyva MCE, Alonzo SJF, Leyva PCG. Indicadores de competitividad de la carne bovina de México en el mercado mundial. Rev Mex Cienc Pecu 2020;11(3):669-685.

15. Durán ME, Ruiz MA, Sánchez VV. Competitividad de la ganadería de doble propósito en la costa de Oaxaca, México. Rev Mex Agroneg 2018;43:77-88.

16. Monke EA, Pearson SR. The policy Analysis Matrix. Chapter 2. In: The policy analysis matrix for agricultural development. 1st ed. New York, USA. Cornell University Press; 1989.

17. Tossou BW, Chogou SK, Sossou CH. Analysis of the competitiveness of soybean production in Benin. Sci African 2023;19:e01491.

18. Bukifan M, Krisnamurthi B. The effect of policy on business competitiveness of Bali cattle farming in Kupang District, East Nusa Tenggara, Indonesia. J Management Agribusiness 2021;18(3):308-315.

19. Mohd YHH, Iwana IN, Hirawaty KN. Assessing the comparative advantage of integrated farming and feedlot production system of the ruminant sector in Malaysia: A policy analysis matrix approach. Asian J Agric Rural Develop 2020;10(1):227-238.

20. Nurlaelah S, Insani AN, Magfirah N. Level of competitiveness of cattle fattening business in Gowa Regency. IOP Conference Series: Earth Environ Sci 2020;492(1):012147.

21. Rouf AA, Daryanto A, Fariyanti A. Competitiveness of smallholder beef cattle farming in Gorontalo District, Gorontalo. Buletin Peternakan 2019;43(3):199-206.

22. Galván VE, Santos GG. Análisis de la elasticidad del precio y ventaja comparativa revelada del sector de cítricos en México. Mercados y Negocios 2019;39:87-104.

23. Durán ME, Ruiz MA, Sánchez VV. Competitividad de la ganadería de doble propósito en la costa de Oaxaca, México. Rev Mex Agroneg 2018;43:77-88.

24. Hernández MJ, Rebollar RA, Rebollar RS, Guzmán SE, Gómez TG. Análisis comparativo de la competitividad de la producción bovina en corral en Tejupilco y Amatepec, México (2009-2014). Agric Soc Des 2019;16(4):414-427.

25. INEGI-A. Compendio de información geográfica municipal 2010, Francisco Z. Mena. Puebla. Clave geo estadística 21064. Instituto Nacional de Estadística y Geografía. México. 2010. Consultado 12 Sep, 2022 https://www.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/21/21064.pdf.

26. INEGI-B. Compendio de información geográfica municipal 2010, Jalpan. Puebla. Clave geo estadística 21086. Instituto Nacional de Estadística y Geografía. México. 2010. Consultado 12 Sep, 2022. https://www.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/21/21086.pdf

27. Padrón Ganadero Nacional. Estadística pecuaria. Bovinos. Puebla. Consultado 12 Dic, 2022. http://www.pgn.org.mx/estadistica-rep.php.

28. Hernández MJ, Rebollar RA, Mondragón AJ, Guzmán SE, Rebollar RS. Costos y competitividad en la producción de bovinos carne en corral en el sur del Estado de México. Invest Cienc Univ Autón Ags 2016;69:13-20.

29. USDA. Cattlemen´s Livestock Auction-Dalhart, TX. AMS, Livestock, Poultry, & Grain Market News. Texas Dept of Ag Mrkt News. Agricultural Marketing Service. 2023. Consultado 17 Ene, 2023. https://www.ams.usda.gov/mnreports/ams_1953.pdf.

30. BANXICO. Tipos de cambio diarios. Sistema de Información Económica. México. 2023. Consultado 12 Ene, 2023. https://www.banxico.org.mx/SieInternet/consultarDirectorioInternetAction.do?accion=consultarCuadro&idCuadro=CF102&sector=6&locale=es.

31. IPC Financiera. Crédito habilitación y avío. Identidad crediticia para mexicanos. Consultado 16 Ago, 2022 https://www.ipcfinanciera.com/productos/credito-habilitacion-y-avio.

32. USDA. Farm Service Agency. Farm Loan Programs. Current FSA Loan Interest Rates. Consultado 10 Ago, 2022 https://www.fsa.usda.gov/programs-and-services/farm-loan-programs/index.

33. Agua de Puebla. Tarifas de la prestación del servicio de agua potable. Uso pecuario. México 2022. Consultado 15 Nov, 2022. https://www.aguapuebla.mx/wp-content/uploads/TARIFAS.pdf.

34. USDA. Texas Weekly Cattle Auction Summary. AMS Livestock, Poultry, & Grain Market News. Texas Dept of Ag Mrkt News. https://www.texasagriculture.gov/Home/Production-Agriculture/Livestock-Export-Pens/Livestock-Facility-Fees.

35. Gamboa-Mena JV, Magaña-Magaña MA, Rejón-Ávila M, Martínez VP. Eficiencia económica de los sistemas de producción de carne bovina en el municipio de Tizimín, Yucatán, México. Trop Subtrop Agroecosystems 2005;5(2):79-84.

36. Morales-Hernández JL, González-Razo FJ, Hernández J. Función de producción de la ganadería de carne en la zona sur del Estado de México. Rev Mex Cienc Pecu 2018;9(1):1-13.

37. Gudiño ERS, Tapia SLI, Villagómez CJA. Costo de producción y rentabilidad de ganado vacuno en sistema de doble propósito en el municipio de Jamapa, Veracruz, México. Rev Mex Agroneg 2022;51(2022):277-288.

38. Retes LR, Moreno MS, Martin RMH, Ibarra FFA, Suárez SNE. Determinación de la rentabilidad del repasto de becerros en Sonora, ciclo 2018. Rev Mex Agroneg 2019;45(2019):395-405.

39. Acuña WK, Castro LA. Modelo de negocio para producción de carne bovina en una región de Chile. Rev Acad Neg 2019;4:61-70.

40. Álvarez-Macías A, Santos-Chávez VM. Alcances de la política pecuaria en México. El caso del Progan 2008-2013. Estudios sociales. Rev Aliment Contemp Desarro Reg 2019;29(53):1-26.

https://doi.org/10.22319/rmcp.v15i4.6313

Article

Seasonal dynamics of sideoats grama [*Bouteloua curtipendula* (Mich.) Torr.] in Chihuahua, Mexico: a geostatistical approach

Ireyli Zuluamy Iracheta-Lara [a]

Carlos Raúl Morales-Nieto [b]*

Gerardo Méndez-Zamora [c]

Jaime Neftalí Márquez-Godoy [d]

Federico Villarreal-Guerrero [b]

[a] Universidad Autónoma de Chihuahua. Facultad de Ciencias Agrotecnológicas, Campus 1, Av. Pascual Orozco SN, 31350 Chihuahua, Chihuahua, México.

[b] Universidad Autónoma de Chihuahua. Facultad de Zootecnia y Ecología, Periférico R. Almada km 1. 31000 Chihuahua, Chihuahua, México.

[c] Universidad Autónoma de Nuevo León. Facultad de Agronomía. General Escobedo, Nuevo León, México.

[d] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Campo Experimental Valle de Culiacán, Culiacán, Sinaloa, México.

*Corresponding author: cnieto@uach.mx

Abstract:

Phenological data observed on Earth, along with satellite data, are crucial tools for identifying the growing season of vegetation. Using a geostatistical approach, this study aimed to determine the seasonal dynamics of sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.] in Chihuahua and their relationship with climate variability. The metrics of the start (SOS) and end (EOS) of the growing season of this species in the state of Chihuahua were calculated. In addition, the effect of air temperature and precipitation on the dynamics of SOS

and EOS during the 2000-2010 and 2011-2019 periods was evaluated. The treatments considered the three ecological regions (desert, central valleys, and mountain) and the years of recording. The state was studied through three ecological zones: desert (D), central valleys (CV), and sierra (S) for comparison. The SOS and EOS of sideoats grama in each zone were defined annually from Landsat data during the 2000-2019 period based on the dynamics of the Normalized Difference Vegetation Index (NDVI). The SOS ranged from May to June [average Julian day (doy)=174], while the EOS ranged from October to November [average Julian day (doy)=283]. There was a delay in SOS in zone D; the stunted growth of sideoats grama in the spring season may be due to a relative scarcity of water; however, the higher temperature in spring makes it easier to meet the thermal requirements for the species' growth. These findings suggest that climate variability significantly impacts the seasonal dynamics of sideoats grama, which may influence the management strategies of these ecosystems.

Keywords: Geomatics, Environmental variability, NDVI, Chihuahua.

Received: 12/09/2022

Accepted: 30/07/2024

Introduction

Climate variability, including changes in air temperature and precipitation, significantly impacts the seasonal dynamics of vegetation. Seasonal dynamics refers to the periodic changes in the biological processes of plants throughout the year, influenced by environmental factors[1]. Recent studies have shown that variability in rainfall and temperatures can significantly alter plant growth and senescence patterns[2,3]. It is highlighted how the increase in global temperature and the decrease in rainfall[4] has led to an extension of the growing season in various regions, highlighting the need to adapt agricultural practices to these new conditions[5,6].

For vegetation monitoring, typical methodologies include recording species-level terrestrial data to perform plant-specific analyses at the local scale[7]. At the regional and global levels, satellite data are used to define characteristics and periods of vegetation or landscapes[8,9]. Both *in situ* and satellite phenological data are commonly used to identify the growth phase of vegetation at different scales. Several satellite-derived vegetation indices, such as the normalized difference vegetation index (NDVI) and the enhanced vegetation index (EVI),

have been developed to extract phenological parameters from vegetation[10,11]. The abovementioned indices focused on analyzing the spatial-temporal differences of phenological phenomena between different biomes or geographical zones[12,13].

In the state of Chihuahua, grasslands, including sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.], are essential for providing resources for livestock and the ecological stability of the region. Research has shown that changes in patterns, such as flowering and fruiting time, are early signs of the effects of climate variability[14]. Conversely, in places where consecutive rainfall events occur sporadically, the increase in temperature may not have significant effects on spring phenology[15,16]. Increased rainfall has not shown significant effects on triggering the flowering phase in grasslands[17]; in contrast, reduced rainfall induced earlier growth and flowering of herbaceous species in field experiments[18]. Therefore, the study of phenology and its relationships with climatic factors would serve to adopt appropriate strategies for grazing activities with a view to the sustainable use of grasslands.

In Chihuahua, rainfall in 2011 was only 156 mm, representing a third of the annual average (i.e., 470 mm), causing huge economic losses. In addition, frost events recorded during the same period in the state were not typical. These events destroyed pastures and caused the death of more than 300,000 head of cattle[19,20]. The objectives of this research were to analyze the phenological metrics of the start (SOS) and the end (EOS) of the growing season during 2000-2010 and 2011-2019, which are periods before and after 2011. Additional objectives included determining which climate factor is involved in the dynamics of the SOS and identifying possible trends of change in SOS and EOS during the 2000-2010 and 2011-2019 periods. This will make it possible to assess whether a disturbing phenomenon determines or alters seasonal dynamics and affects grassland growing periods, providing vital information for their sustainable management in the region.

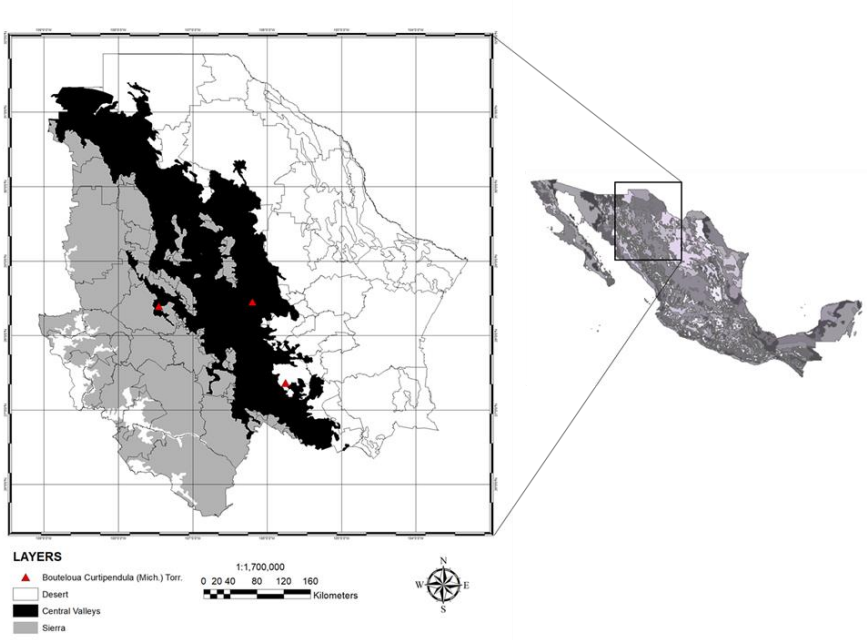
Material and methods

Study area

To organize the information, it was decided to classify the state of Chihuahua into ecological zones: Desert (D), Central Valleys (CV), and Sierra (S) according to the climatic conditions they present (Figure 1). Zone D has a dry and semi-warm climate, with daily maximum temperatures of 40 ºC or higher in the summer and daily minimum temperatures of -5 ºC or lower in the winter. On the other hand, the CV zone has a temperate semi-desert to subhumid

climate, with daily minimum temperatures of up to -20 ºC in winter, commonly the most extreme minimum temperatures in the state. Annual rainfall ranges between 300 and 550 mm in this region. Finally, the S has a climate with a humid, semi-cold-to-temperate summer and winter with snowfall. Minimum temperatures can drop to -10 °C, while maximum temperatures can reach 30 °C. The climate responds, among other factors, to the altitude above sea level, which on average is 2,400 m, and plains surrounded by peaks 200 to 1,000 m above the plain floor[21].

Figure 1: Study area



Data collection

Precipitation and temperature data were obtained from CONAGUA[22] for the period from 2000 to 2019. On the other hand, the Normalized Difference Vegetation Index (NDVI) was calculated from scenes obtained from the Landsat ETM+7 and Landsat OLI8 (https://glovis.usgs.gov/). In addition, 196 verified records of the presence of sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.] in each of the three ecological regions into which the state was divided were used.

The calculation of the NDVI (equation 1) was performed on the Google Earth Engine (http://earthengine.google.com/) platform. Scene information was obtained every 16 days for the 2000-2019 period at a spatial resolution of 30 m (WRS Path/Row 32/34 38/42).

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

Where: NDVI is the Normalized Difference Vegetation Index. NIR and Red are the reflectance values in the near-infrared and red ranges of the electromagnetic spectrum, respectively.

NDVI was obtained from compounds of maximum values, choosing observations with minimal cloud cover and near-nadir views[23]. Additional corrections were applied for atmospheric gases, clouds, and aerosols[24]. Time series were created with valuable information from NDVI. Although the highest-quality reflectance measurements were selected, likely, the low-quality measurements will still be part of the time series due to cloudy periods. Removing these low-quality values in the NDVI time series was crucial to obtaining a credible phenological metric. There are usually some sequential low points that occur at the beginning and end of the time series, as well as abrupt and extreme low points in the middle of the growing period. First, the issue of the abrupt points [NDVI (t)] corresponding to the middle of the station was addressed; if the difference between NDVI (t) and NDVI (t ± 1) was greater than (NDVImax - NDVImin)/2, then NDVI (t) was replaced by the mean of NDVI (t - 1) and NDVI (t + 1). The entire time series was then smoothed with a 9-point moving average filter. Finally, the low values at the beginning and end of the time series were replaced by the average NDVI value for March, when most NDVI values are similar to the NDVI value of bare soil, due to the limited presence of vegetation on the surface. In this case, NDVI values during the non-growing season are assumed to be constant.

Analysis of phenological function

The typical logistic function retrieves phenological metrics during spring and fall separately; in contrast, the dual logistic function can extract phenological events of spring and fall simultaneously. In this study, equations 2 and 3 were used to obtain SOS and EOS, respectively[25]:

$$SOS = \frac{2ln(\sqrt{3}-\sqrt{2})}{I} + S \qquad \text{(Equation 2)}$$

$$EOS = \frac{2ln(\sqrt{3}-\sqrt{2})}{D} + E \qquad \text{(Equation 3)}$$

Where SOS and EOS denote the day of the year (doy) of the start and end of the season, I and D represent the maximum ascending and descending slopes (inflection points) on the

adjusted NDVI curve, respectively, while S and E represent doy when I and D occur on the adjusted NDVI curve.

Statistical analysis

SOS and EOS data were compared at the start and end of the study period rather than calculating the mean multi-pixel trends of SOS and EOS. This was decided to reduce miscalculations. To eliminate the effects of abnormal years on SOS and EOS, the global mean of SOS and EOS during 2000-2010 and 2011-2019 was first obtained separately for each time series. To infer the possible causes of the variations in SOS and EOS, temperature and precipitation records were obtained from the meteorological stations closest to the sampling point[26]. The records corresponded to dates before SOS and EOS and the 2000-2010 and 2011-2019 periods.

SPD and EPD data were compared for their differences between zones D, CV, and S with a univariate analysis of variance (ANOVA). This was done through a 3x2 factorial arrangement, three regions: D, CV, and S, and two periods: 2000 to 2010 and 2011 to 2019. Of the 196 records of the presence of grasslands, a coordinate of each ecological zone was randomly selected in order to compare the vegetation in different areas and how it responds to changes in climatic conditions (Figure 1). In addition, a correlation analysis was performed to evaluate the relationship between SPD and EPD with the climatic variables of each region and period.

Results and discussion

Environmental conditions

The environmental conditions average (precipitation and temperature) during the 2000-2019 period showed that the spring temperature in zone D, where the record of the sideoats grama was analyzed, was higher than in zones CV and S. Meanwhile, the precipitation of the spring season was higher in zone D than in zones CV and S.

As reported in previous studies, spring phenological events are particularly sensitive to temperature, and the warming experienced in recent decades has already shown effects on phenology, triggering earlier spring phenological events and extending the growing

period[27]. Zone D was also warmer and drier in autumn than zones CV and S. In all three ecological zones, autumn is cooler and wetter than spring (Table 1). Authors stated[28] that temperature is the main climatic factor affecting plant phenology. They also reported that increased air temperature affects phenology and can be easily detected in phenological data.

Table 1: Environmental conditions in the Sierra, Central Valleys, and Desert

Ecological zone	ST (°C)	SP (mm)	AT (°C)	AP (mm)
Sierra	22.27	48.56	21.6	117.2
Central Valleys	24.42	64.52	20.7	56.76
Desert	21.14	20.51	15.02	106.16

ST= spring temperature; SP= spring precipitation; AT= autumn temperature; AP= autumn precipitation.

General comparison of SOS and EOS between ecological zones

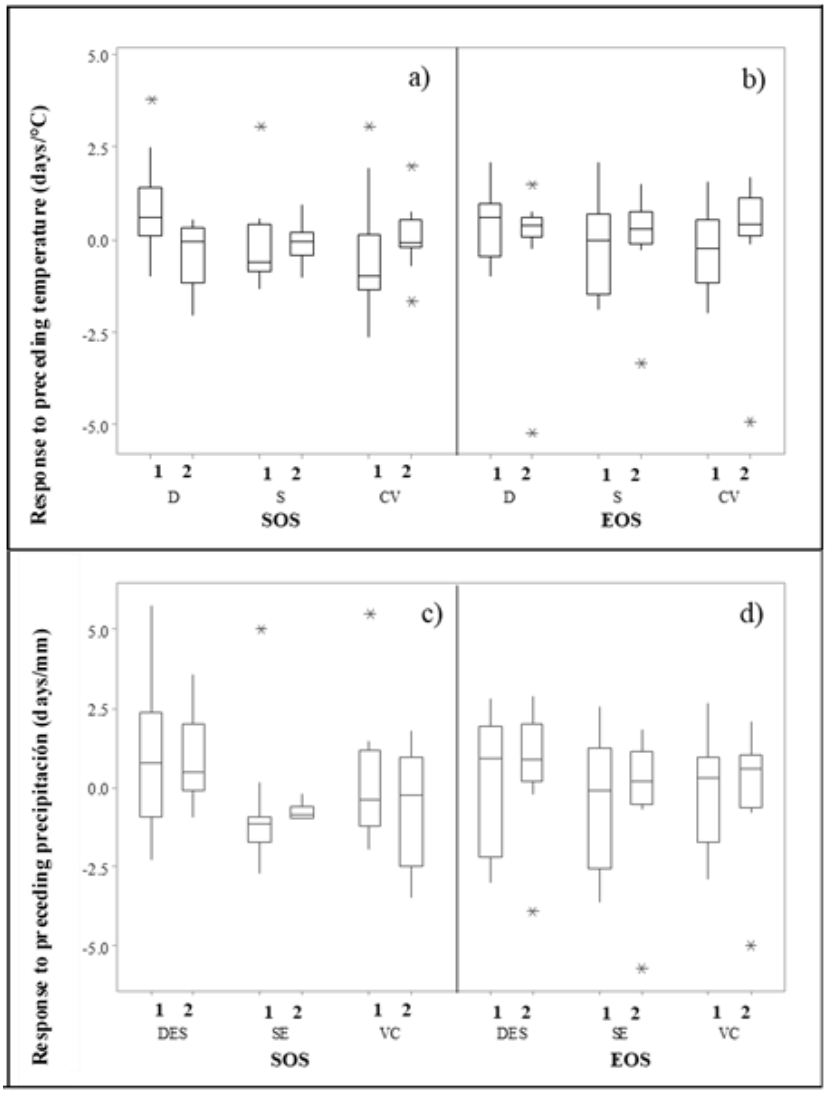
The averages for SPD and EPD were on the 174th and 283rd days of the year, respectively; locating these days in months, they would be May-June and October. In zone D, SPD and EPD occurred on the 172nd and 265th d of the year, respectively, earlier (P<0.05) in the year compared to the other two zones (doy 180 and 286). The SOS occurred earlier in zone D than in zones CV and S. Both SOS and EOS occurred significantly (P<0.05) earlier in zone D (doy 172 and 265, respectively) than in zones CV (doy 180 and 286, respectively) and D (doy 179 and 297, respectively). Regarding the annual fluctuations of SOS and EOS, the mean standard deviation (SD) of EOS (25.47 d) was greater than that of SOS (18.53 d). The highest SDs of SOS and EOS corresponded to zone D (21.6 and 25.15 d, respectively) compared to zones CV (19.5 and 25.05 d, respectively) and S (11.5 and 12.41 d, respectively). Phenological models based on satellite data indicate water availability as a determining environmental condition in SPD in North American meadows[7]; for its part, temperature was a determining factor in triggering spring phenological events in woody plants of temperate ecosystems[29] and herbaceous plants of alpine ecosystems[30].

SOS and EOS response rates to temperature and precipitation

Although there was a 0.33-d delay for SOS in zone D during 2000-2019, SOS occurred 0.20 and 0.09 d earlier for zones CV and S, respectively (Figure 2a). Meanwhile, the decrease in spring precipitation, which happened before the start of the season, caused an SOS delay of

0.42, 0.24, and 0.32 d mm^{-1} for zones D, CV, and S, respectively (Figure 2b). For zone D, the sensitivity of SOS to the preceding temperature and precipitation, which were recorded before the start of the season, was significantly higher than in zones CV and S. In autumn, warmer temperatures delayed SOS at rates of 0.94 and 0.35 d $ºC^{-1}$ for zones CV and S, respectively, while extending SOS by 0.72 d $ºC^{-1}$ for zone D (Figure 2c).

Figure 2: SOS response to preceding temperature (a) and precipitation (c), and SOS response rates due to preceding temperature (b) and precipitation (d) at EOS



1, period from 2000 to 2010; 2, period from 2011 to 2019. D= desert; S= sierra; CV= central valleys.

Some authors[31] reported that, during 1959-1996, the spring phenological events of a large number of species were 6.3 days early on average, while those of autumn experienced a delay of 4.5 days on average; thus, the growing season extended by 10.8 days on average[32,33]. The reduction in rainfall caused a delay of 0.89, 0.02, and 0.45 d mm^{-1} in EOS for zones D, CV, and S, respectively (Figure 2d). Finally, zone D showed a significantly lower sensitivity to

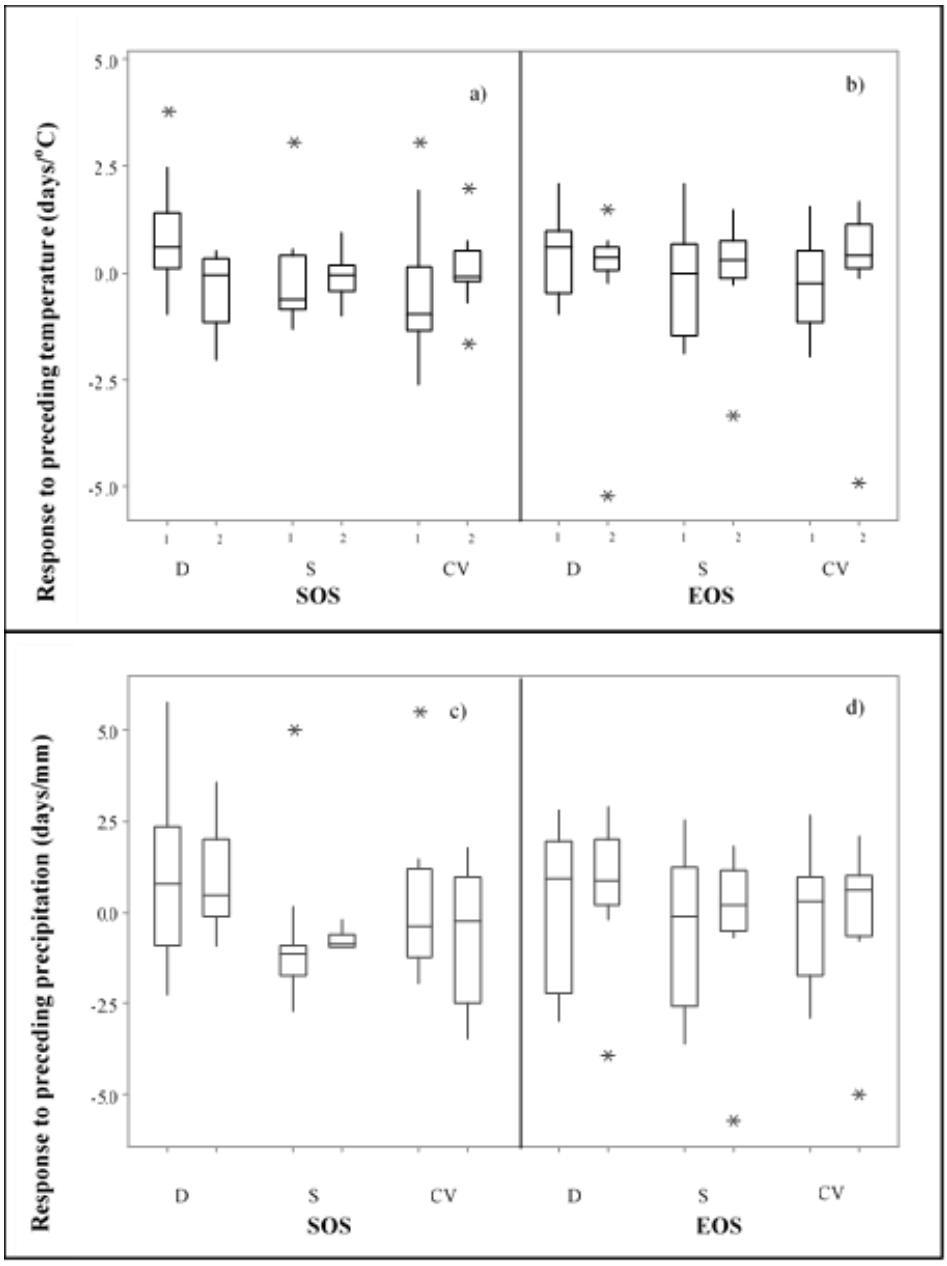
the preceding temperature but a significantly higher sensitivity to the preceding precipitation than zones CV and S. Anticipated phenological patterns could alter the distribution of sideoats grama plant resources, have implications for pollination systems, and increase the size, species richness, and intraspecific genetic diversity of the soil seed bank[34].

SOS and EOS change trends

To report overall SOS and EOS changes in the ecological zones studied, the annual mean SOS and EOS between 2000-2010 and 2011-2019 were compared. During 2010-2019, SOS and EOS were delayed by 16 ($P<0.05$) and 18 ($P<0.05$) days, respectively, compared to 2000-2019 (Figure 3).

The sideoats grama results showed that the mean 2011-2019 SOS was 16 d later ($P<0.05$) than the 2000-2010 SOS mean for zone D (Figure 3). On the other hand, SOS was 42 d early for zone CV ($P<0.05$) and 3 d early for zone S ($P<0.05$). Meanwhile, EOS showed no significant changes ($P>0.05$) when comparing the two periods for zones CV and S. Nonetheless, a significant lag was identified for both SOS and EOS from 2000-2010 to 2011-2019 ($P<0.05$) in zone D. During 2011-2019, SOS and EOS were 6 ($P<0.05$) and 18 ($P<0.05$) days late, respectively, compared to 2000-2010.

Figure 3: General changes in SOS and EOS in three ecological zones affecting the species. 1, period from 2000 to 2010; 2, period from 2011 to 2019.



1, period from 2000 to 2010; 2, period from 2011 to 2019. D= desert; S= sierra; CV= central valleys.

This study found significant negative correlations (P<0.05) between SOS and preceding precipitation and temperature. This means that higher temperatures and rainfall recorded before the start of the season can bring forward the start of the growing season (SOS). Nevertheless, the zones showed significant correlations (P<0.05) between SOS and the preceding precipitation than between EOS and the antecedent temperature. Therefore, recorded precipitation rather than temperature may primarily control the start of the growing

season in most of the study area. This is consistent with the results of previous studies based on data from greening dates, derived from satellites, and terrestrial meteorological data[35]. On the other hand, some experimental and phenology modelling studies have highlighted the critical effects of water availability on SOS in the North American meadows[7,36] and Mongolian meadows[2,11]. Temperature has been reported to be a key factor in triggering spring phenological events of plants in temperate ecosystems[37] and herbaceous species in alpine ecosystems[38]. The predominant role of temperature in SOS has also been observed in grasslands in the Middle East and in the Tibetan Plateau[39,40].

Likewise, the ecological zones of the study area showed significant positive correlations (P<0.05) between EOS and the preceding temperature, indicating that temperature may be the most critical factor in regulating seasonal vegetation dynamics in grasslands. Although temperature was the dominant factor, precipitation was also positively correlated with EOS. Therefore, warmer and wetter autumn conditions could lead to a delay in the process of leaf senescence.

Behavior of SOS and EOS between ecological zones during 2000-2010 and 2011-2019

Factor analysis revealed that the average day of the year (doy) for the start of the growing season (SOS) of the species was 160.55 (P<0.05). Significant effects in the factorial design depended on the zone (D, CV, S) for both SOS and the end of the growing season (EOS). The factor that caused the biggest change in SOS and EOS (i.e., variability in doy) was the ecological zone in which the vegetation grows. The interaction between the ecological zones and the periods analyzed significantly influenced both SOS and EOS (P<0.005).

Conclusions and implications

This research has provided a detailed understanding of the seasonal dynamics of sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.] in different ecological zones of the state of Chihuahua, Mexico, using a geostatistical approach supported by satellite data and *in situ* phenological observations. The results show that climate variability, particularly in temperature and precipitation, affects the start (SOS) and end (EOS) of its growing season. It was observed that in the desert zone (D), SOS occurs earlier due to higher temperatures. In addition, decreased precipitation delayed SOS in all zones, while in autumn, warmer temperatures extended EOS in zone D. Understanding the seasonal dynamics of this species

is essential to develop sustainable management practices that ensure the resilience of these ecosystems to climate change. The results obtained provide a robust scientific basis for informed decision-making in the management of natural resources in the region, promoting sustainability and adaptation to new environmental conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

Literature cited:

1. IPCC. Climate change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ Press; 2014.

2. Smith J, Doe J, Clark H. Temporal and spatial variability in phenological responses of grass species. J Ecol 2020;108(5):1234-1245.

3. Liu Q, Fu YH, Zeng Z, Huang M, Li X, Piao S. Temperature, precipitation, and insolation effects on autumn vegetation phenology in temperate China. Glob Change Biol 2019;25(3):644-655.

4. Sánchez, DH, Navarro-Gómez CJ, Rentería M, Rose JF, Sánchez-Navarro JR. Evolution of the groundwater system in the Chihuahua-Sacramento aquifer due to climatic and anthropogenic factors. J Water Clim Change 2022;13(2):645-663.

5. Jones MB, Donnelly A. Responses of Irish vegetation to future climate change. Biol Environ Proc R Ir Acad 2018;118(2):157-168.

6. Williams K, Clark, J. Global warming extends the growing season across ecosystems. Environ Res Lett 2021;16(1):14-23.

7. Schwartz MD. Phenology: An integrative environmental science. Berlin, Germany Springer; 2013:170-171.

8. Badeck FW, Bondeau AK, Böttcher D, Doktor W, Lucht J, Schaber S. Responses of spring phenology to climate change. New Phytol 2004;162:295-309.

9. Richardson AD, Keenan TF, Migliavacca M, Ryu Y, Sonnentag O, Toomey M. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agric For Meteorol 2017;169:156-173.

10. Crimmins TM, Crimmins MA, Bertelsen C. Complex responses to climate drivers in onset of spring flowering across a semi-arid elevation gradient. J Ecol 2010;98:042-1051.

11. Lesica P, Kittelson P. Precipitation and temperature are associated with advanced flowering phenology in a semi-arid grassland. J Arid Environ 2010;74:1013-1017.

12. Zhang X, Friedl MA, Schaaf CB, Strahler AH, Hodges JCF, Gao F, Reed BC, Huete A. Monitoring vegetation phenology using modis. Remote Sens Environ 2003;84:471-475.

13. Delbart N, Picard G, Le Toan T, Kergoat K, Quegan S, Woodward I, Dye D, Fedotova V. Spring phenology in boreal Eurasia over a nearly century time scale. Glob Change Biol 2008;14:603-614.

14. Stumpf F, Schneider MK, Keller A, Rentschler T, Meuli RG, Liebisch, F. Spatial monitoring of grassland management using multi-temporal satellite imagery. Ecol Indic 2020;113:106-121.

15. Schwartz MD. Phenology: An integrative environmental science. Springer 2013;1:20.

16. Piao S, Tan J, Chen A, Fu YH, Ciais P, Liu Q, Janssens IA, Vicca S, Zeng Z, Jeong SJ. Leaf onset in the Northern Hemisphere triggered by daytime temperature. Nat Commun 2015;6:69-81.

17. Bloor JM, Pichon P, Falcimagne R, Leadley P, Soussana JF. Effects of warming, summer drought, and CO_2 enrichment on aboveground biomass production, flowering phenology, and community structure in an upland grassland ecosystem. Ecosystems 2010;13:888-900.

18. Hovenden MJ, Wills KE, Vander-Schoor JK, Williams AL, Newton PC. Flowering phenology in a species-rich temperate grassland is sensitive to warming but not elevated CO_2. New Phytol 2008;178:815-822.

19. Zelikova TJ, Williams DG, Hoenigman R, Blumenthal DM, Morgan JA, Pendall E. Seasonality of soil moisture mediates responses of ecosystem phenology to elevated CO_2 and warming in a semi-arid grassland. J Ecol 2015;103:1119-1130.

20. Piazza V. Estimación satelital de la productividad primaria neta aérea de la vegetación herbácea del Caldenal [disertación doctoral]. Buenos Aires, Argentina; 2006.

21. Morales-Nieto CR, Quero-Carrillo A, Pérez-Pérez J, Hernández-Garay A, Le-Blan O. Caracterización morfológica de poblaciones nativas de pasto banderita [(Michx.) Torr.] en México. Agrociencia 2008;42(7):767-775.

22. Breman H, De Wit C. Rangeland productivity and exploitation in the Sahel. Science 1983;221:1341–1347.

23. Butt B, Turner MD, Singh A, Brottem L. Use of modis NDVI to evaluate changing latitudinal gradients of rangeland phenology in sudano-sahelian West Africa. Remote Sens Environ 2011;115:3367-3376.

24. Arzani H, Zohdi M, Fish E, Zahedi-Amiri G, Nikkhah A, Wester D. Phenological effects on forage quality of five grass species. J Range Management 2004;57:624–629.

25. Gorlier A, Lonati M, Renna M, Lussiana C, Lombardi G, Battaglini L. Changes in pasture and cow milk compositions during a summer transhumance in the Western Italian Alps. J Appl Bot Food Qual 2013;(85):216-231.

26. Ortega-Gaucin D, Velasco I. Aspectos socioeconómicos y ambientales de las sequías en México. Aqua-LAC 2013;5(2):78-90.

27. Gonsamo A, Chen JM. Continuous observation of leaf area index at fluxnet-Canada sites. Agric For Meteorol 2014;189:168-174.

28. INEGI. Instituto Nacional de Estadística, Geografía e Informática 2003. Síntesis de Información Geográfica del Estado de Chihuahua, 1a.ed. Editorial INEGI. México. 2013.

29. CONAGUA. Comisión Nacional del Agua. Capacidad de Almacenamiento y Uso de las Principales Presas de México. http://www.conagua.gob.mx/atlas/atlas.html?seccion=2mapa=5. Consultado 4 Abr, 2021.

30. Chen X, Li J, Xu L, Liu L, Ding D. Modeling green up date of dominant grass species in the inner Mongolian grassland using air temperature and precipitation data. Int J Biometeorol 2014;58:463-471.

31. Vermote E. Vermeulen. Atmospheric correction algorithm: Spectral reflectances (MOD09), ATBD version 4; Department of Geography, University of Maryland: College Park, MD, USA. 1999.

32. Busetto L, Colombo R, Migliavacca M, Cremonese E, Meroni M, Galvagno M, *et al*. Remote sensing of larch phenological cycle and analysis of relationships with climate in the Alpine region. Glob Change Biol 2010;16:2504-2517.

33. Menzel A. Trends in phenological phases in Europe between 1951 and 1996. Int J Biometeorol 2000;44:76–81.

34. Peñuelas J, Filella I. Responses to a warming world. Science 2001;294:793–795.

35. Chen X, Xu L. Phenological responses of *Ulmus pumila* (Siberian Elm) to climate change in the temperate zone of China. Int J Biometeorol 2012;56:695-706.

36. Chen X, An S, Inouye MD. Temperature and snowfall trigger alpine vegetation green-up on the world's roof. Glob Change Biol 2015;21:3635-3646.

37. Peñuelas J, Filella I. Responses to a warming world. Science 2001;294(3):793-795.

38. Roetzer T, Wittenzeller M, Haeckel H, Nekovar J. Phenology in central Europe–differences and trends of spring phenophases in urban and rural areas. Int J Biometeorol 2000;44:60–66.

39. Walkovszky A. Changes in phenology of the locust tree (*Robinia pseudoacacia L*.) in Hungary. Int J Biometeorol 1998;41:155–160.

https://doi.org/10.22319/rmcp.v15i4.6470

Article

Productive behavior of a Mombasa-Kudzu association at different times of the year

Eduardo Daniel Bolaños-Aguilar [a*]

Javier Francisco Enríquez-Quiroz [b]

Abraham Fragoso-Islas [c]

Roberto Omar Castañeda-Arriola [d]

Maribel Montero-Lagunes [b]

Julio César Vinay-Vadillo [b]

[a] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP). Campo Experimental Huimanguillo, 86600, Huimanguillo, Tabasco, México.

[b] INIFAP, Campo Experimental La Posta. Paso del Toro Medellín, Veracruz, México.

[c] INIFAP, Sitio Experimental Las Margaritas. Hueytamalco, Puebla, México.

[d] INIFAP, Sitio Experimental Pichucalco. Pichucalco, Chiapas, México.

*Corresponding author: bolanos.eduardo@inifap.gob.mx

Abstract:

The objective was to evaluate the effect of the legume kudzu (*Pueraria phaseoloides*) on the dry matter yield and nutritional value of the pasture and the weight gain of grazing heifers. The study was conducted from Dec-19-2019 to Jul-21-2020 on 4 ha at INIFAP-Pichucalco, Chis., with 2 ha of Mombasa Guinea grass (*Megathyrsus maximus* var. Mombasa) and 2 ha of the Mombasa-kudzu association. Each pasture had six heifers in rotational grazing. The dry matter yield, protein, neutral detergent fiber, lignin, and daily weight gain were evaluated. A completely randomized split-plot design was used for dry

matter yield and nutritional value, and a completely randomized design was used for animal weight gain. The lowest dry matter yield was in the dry season with 2,963 kg ha^{-1} for mombasa, and 3,771.5 kg ha^{-1} for mombasa-kudzu. The highest yield was in rainfall with 10,092 kg ha^{-1} for mombasa and 8,977 kg ha^{-1} for the association. Kudzu registered the highest yield in the north winds season with 763.4 kg ha^{-1} and 19.4 % cover. Kudzu registered 1.7 times more protein than mombasa, maintaining its concentration at 146.26 g kg^{-1} DM during the study period mombasa-kudzu registered 31.9 g kg^{-1} DM more protein than mombasa, and higher neutral detergent fiber (44.3 g kg^{-1} DM more) and lignin (8 g kg^{-1} DM more). The highest animal weight gain was registered in the north winds season, in association with 504 g $animal^{-1}$ d^{-1} *vs* 333 g $animal^{-1}$ in monoculture.

Keywords: Animal production, Dry matter yield, Grazing, Dry matter yield, *Megathyrsus maximus*, Nutritional value, *Pueraria phaseoloides*.

Received: 23/05/2023

Accepted: 27/08/2024

Introduction

Seasonal forage production is the main issue for the efficient utilization of forage grasses by grazing animals. In tropical areas, grasslands are the basis of cattle feed for beef, milk, or calf production; however, they have a marked seasonality of biomass production due to climate variations during the year. Most pasture production occurs during the rainy season, characterized by high temperatures in June through September, after which forage production decreases over the months[(1)]. There are records of up to four times less forage yield reduction in the dry season (mid-March to mid-June) compared to the rainy season[(2)]. This leads to changes in the availability of forage and animal-carrying capacity of the pastures during the year. Another problem with seasonality is that forage plants of the same age may have different nutritional values depending on the time of year, which leads to variations in animal production[(3)].

Different strategies have been evaluated for managing the seasonal distribution of forage production. One of them is the use of associated pastures, in which the use of legumes associated with grasses is usually considered[(4)]. In soils of low fertility, the evaluation of the dry matter yield (DMY) and protein concentration in a *Brachiaria humidicola* pasture in monoculture and in association with the legume *Arachis pintoi* showed that, during the dry

season, both pastures had the lowest yield of the year, but the association registered a higher DMY with 0.8 t ha^{-1} more than the monoculture pasture[1]. Also, the humidicola grass in association recorded 19.21 g kg^{-1} of DM more protein than in monoculture. This decrease in the DMY of the associated grassland and the higher protein concentration of the associated grass had been previously reported[5] under the same environmental conditions when studying a humidicola-*Stylosantes guianensis* association. Although forage legumes are well known to have a higher concentration of protein (and minerals) than grasses[6], previous research shows that legumes also benefit when accompanying grasses with nitrogen, which is taken from the atmosphere and fixed to the soil by bacteria found in the root nodules of these species[7].

The decrease in the DMY or the greater stability of production of a grass-legume association results from the genetic diversity of the species that make up the association, since the association of different species exhibits short-term morphological changes, giving the grassland greater stability over time[8,9]. As a result of their higher protein content and greater DMY stability, grass-legume associations can improve animal nutrition and production in pasture[10]. In this regard, it has been observed that young cattle attain higher values with the grass-legume associations, yielding higher daily weight gains in steers (602 g d^{-1}) than in heifers (573 g) with the grazing of *Andropogon gayanus* associated with *Stylosanthes capitata*[11]. The effect of the time of year on weight changes was not measured. Studies on animal production with grazing of associated grass-legume pastures under tropical conditions are still limited. Forage legumes are hypothesized to improve the stability of grassland biomass production and protein availability for livestock. Therefore, the objective of the present study was to evaluate the impact of legumes on the dry matter yield and chemical composition of the pasture, and on the weight gain of grazing animals at different times of the year.

Material and methods

Environmental conditions and evaluated grasslands

The study was conducted at the "Pichucalco" Experimental Site belonging to Instituto Nacional de Investigaciones Foestales, Agrícolas y Pecuarias (INIFAP) and located in Pichucalco, Chiapas. The soil was loam textured, high in organic matter (3.59 %), Fe (83 ppm), Zn (5.05 ppm), Mn (39.4 ppm), and Cu (6.37 ppm), and low in Al (3.93 ppm) and Mg (164 ppm), with a bulk density of 1.2 g cm^{-3} and a slightly acidic pH (6.0). In June through August 2018, over an area of 4 ha, secondary vegetation was eliminated by cutting with

machetes and burning of the cut material. In August of the same year, the invasive grass *Paspalum virgatum* was eliminated with a glyphosate herbicide [N-(phosphonomethyl) glycine], applying 1 kg of the active ingredient per hectare. Once the weeds were removed in September, seeds of two types of grass were sown: Mombasa guinea grass in monoculture (*Megathyrsus maximus* var. *mombaza*) and Mombasa guinea grass in association with a legume (*Mombaza-Pueraria phaseoloides*). The planting was carried out with seeds both of the grass and the legume. As it was a hilly terrain, the two pastures were sown with a spiked stick with a separation of 50 cm between strokes (approximately 5 cm deep) on a line drawn with sisal twine, and at 1 m between rows.

Two hectares were used for each pasture. The monoculture pasture was sown with 7 kg ha^{-1} of commercial seed. In the associated pasture, the sowing rate of Mombasa was 6 kg ha^{-1} and that of kudzu was 3 kg ha^{-1}; the grass was sown mixed with the legume. In October, there was an ant attack on the seeds of both pastures; the seeds were carried out of the study area, which strongly affected their establishment. Consequently, the pastures were reseeded in November, and the seeds were impregnated with an organophosphate/pyrethroid insecticide powder and received no fertilizers. Both the monoculture and the associated pastures were established in June 2019. Table 1 shows the climate data that prevailed during the study period.

Table 1: Average weather data during the study period at three times of the year

	Season of the year		
	North winds (2019 and 2020)	**Dry (2020)**	**Rains (2020)**
Period covered	Nov-Feb	Mar-May	Jun-Jul
Accumulated rains, mm	580	250	450
Average monthly rains, mm	145	84	225
Maximum temperature, °C	27	34	34
Minimum temperature, °C	19	24	24

Note: Maximum and minimum temperatures are seasonal averages.

In July through September 2019, it was proceeded to the perimeter delimitation of the entire experimental area and the separation of the two types of grasslands with barbed wire, using four wires, thus forming two plots of 2 ha each: one plot for monoculture grassland (mombasa), and one plot for associated grassland (mombasa+kudzu). In October and November, each type of pasture was divided into four paddocks for rotational grazing within each pasture using electric fences with two wires per fence posts spaced 10 m (33 ft) apart from each other.

Animal management

A total of 12 Brahman (*Bos indicus*) calves averaging 9 mo of age were selected and divided into two homogeneous groups of 6 animals; each group was randomly assigned to one of the two plots. Each calf was considered a repeat. Group 1 averaged 206 kg ± 22 kg and was assigned to the mombasa-kudzu association plot, and Group 2 averaged 210 kg ± 25 kg and was assigned to the mombasa monoculture plot. From December 9 to 18, adaptive grazing was carried out. The evaluation began on December 19, 2019, and ended on July 21, 2020. Grazing was rotational and consisted of 14 d of occupation and 42 d of rest, and the animals were weighed at the end of each grazing cycle, i.e., every 42 d. Due to failures of the electronic livestock scale, the first weighing was carried out 48 d after the start of the study.

Response variables

Animal weights were measured at the end of the north winds season (February-March), the dry season (March to May), and the beginning of the rainy season (end of May-June). Samples of the forage available for each treatment were taken every 42 d at the entrance of the animals to each of the paddocks that made up the pastures; that is, at the end of each grazing cycle. In this manner, the available forage, after a 42-d pasture rest, was determined by the m^2 method[12], which consists of cutting the forage within four steel squares of 1.0 m^2 each, randomly assigned in each of the paddocks of the Mombasa pasture in monoculture, as well as in each of the paddocks with the mombasa-kudzu association. Thus, four replicates were used as the harvests (or samples) of both types of grasslands; this meant collecting four replicates per treatment on the dates of December 19, 2019, January 23 and February 28, 2020, for the north winds season; April 4 and May 10 for the dry season, and June 15 and July 21 for the rainy season.

The sampling consisted of harvesting all green biomass or green matter (GBM) within each square with cuts at 15 cm above ground level in both grasslands. When m^2 covered individual kudzu plants, they were harvested at 7 cm above ground level. Additional samples of kudzu were also collected from the associated pasture for their individual evaluation in terms of yield, chemical composition, and their proportion in the associated pasture. The harvested plant material was weighed on a portable electronic scale with a capacity of 10 ± 0.001 kg. To determine the dry matter yield (DMY, kg ha^{-1}) of each paddock, subsamples of 300 g of VM were separated, dried in forced-air ovens at 65 °C for 48 h, and dried at 65 °C for 48 h. Thus, the available dry matter (ADM) yield was calculated based on the dry matter yield of the 300 g of VM as well as on the total green matter yield of 1 m^2. A second subsample of

200 g separated into grass and legume components was utilized to determine the DM yield (kg ha^{-1}) or proportion of legume in the total biomass. The proportion of the legume (%) in the total biomass was obtained by dividing the dry weight of the legume by the dry weight of the total biomass (grass + legume).

Concentrations (g kg^{-1}DM) of protein, neutral detergent fiber (NDF), and lignin were determined only on three sampling dates (December 19, 2019, May 10, and July 21, 2020) representative of each time of year, the samples consisting of leaves and stems. These determinations were quantified from dry samples of 300 g of MV, which were previously ground to a particle size of 1 mm in a Wiley Mill. The protein concentration was determined by the Kjeldhal method[13], by multiplying the N content by the conversion factor 6.25. The NDF was determined using sodium lauryl sulfate at neutral pH[14].

Statistical analysis

The analysis of variance applied to determine the available forage and the nutritional value and weight gain of the calves was based on the SAS GLM software[15]. The effects of the harvest date, grassland type, and date × DMY interaction were analyzed in a totally random design in a split-plot arrangement; the large plot was the harvest date, and the small plot was the grassland type. For the study of the chemical composition, a representative date was analyzed for both treatments and for each of the three seasons of the year. Thus, the dates analyzed for the chemical composition were January 19, May 10, and July 21, 2020, in relation to the north winds, dry, and rainy seasons. The means were compared using Tukey's method ($P \leq 0.05$). The calf weight gain was analyzed separately under a completely randomized design with six replicates, in which the effects of treatments (pasture types) on the average daily gain were analyzed. The animals were used as replicates, with a comparison of means with Tukey's test ($P \leq 0.05$).

Results and discussion

The climate conditions varied significantly during the grazing period. The total accumulated rainfall in the north winds season was 330 mm higher than in the dry season and 130 mm higher than in the rainy season. However, the average temperature was higher and similar in the dry and rainy seasons, while the average temperature in the north winds season was 6 °C lower than in the dry and rainy seasons (Table 1).

Available dry matter (ADM) yield

Data are presented for the DMY of mombasa in monoculture, mombasa-kudzu association, and kudzu separately. There was a harvest date × grassland type interaction ($P \leq 0.05$) for the available DMY (Table 2). The chronological pattern of the available DMY was similar in both grassland types throughout the evaluation period.

Table 2: Mean squares per pasture of available dry matter (DMY) yield, associated pasture, legume, and chemical composition of available forage, at regrowth age 42 days after grazing, harvested on seven different dates and in two types of pastures

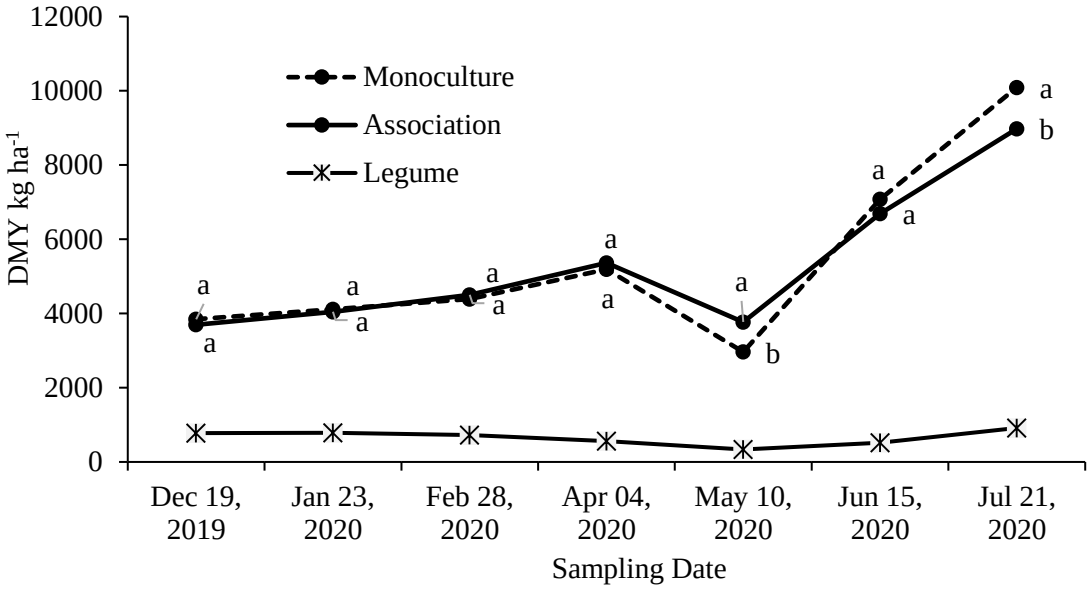
Variables measured	Mean	Date (D)	Pasture (P)	D x P
DMY, kg ha^{-1}	5337.07	383 x 10^{5} NS	107 x 10^{3}***	685 x 10^{3}*
Legume, kg ha^{-1}	660.12	156 x 10^{3}***	----------	----------
DF		6	1	6
Protein, g kg^{-1} DM	98.75	2975.9***	4585.6***	635.4**
NDF, g kg^{-1} DM	716.42	43.44 NS	8831.2***	805.72*
Lignin, g kg^{-1} DM	52.87	610.33***	11.20 NS	162.00 NS
DF				

NDF= neutral detergent fiber. DF= degrees of freedom. *, **, *** Significance level at 0.05, 0.01, and 0.001, respectively. NS= not significant.

From December 19 to April 4 and at harvest on June 15, there were no differences ($P>0.05$) in DMY availability between both types of pasture (Figure 1), with average DM yields of 3,769, 4,079, 4,444, and 5,279 kg ha^{-1}, with respect to the dates Dec 19,2019, Jan 23, 2020, Feb 28, 20--20 and Apr 04, 2020, and with a yield of 6,885 kg ha^{-1} for the June 15 date.

Differences in DMY between the two types of pasture occurred in the dry season (May 10 harvest), and in the rainy season (July 21 harvest). During the dry season, the mombasa-kudzu association was the pasture with the highest MSY, with a value of 3,771 *vs* 2,963 kg of DM ha^{-1} of the grassland consisting of Mombasa (Figure 1). When comparing an associated *Brachiaria humidicola-Arachis pintoi pasture vs B. humidicola* in monoculture, it was observed that the associated pasture was more stable because it registered a higher DMY than the monoculture pasture in the dry season of the year in very acid soils (pH of 4.6) of low fertility[(1)].

Figure 1: Dry matter yield (DMY) of a Mombasa pasture in monoculture, Mombasa in association with kudzu, and kudzu separately, at the regrowth age of 42 days after grazing at different sampling dates



[ab] Different letters within a date indicate significant differences ($P \leq 0.05$).

A greater production stability of the associated pastures during periods of difficult plant growth had also been observed in other studies[16,17]. This production stability of the association was explained above[9]: The diversity of species in a pasture has been proven to regulate its productivity and stability by inducing complementary effects. On the other hand, the fixation of nitrogen to the soil by the legume may have been used by the grass resulting in a higher DMY in association with kudzu than in monoculture[18]. During the rainy season, particularly in the July 21 harvest, the DMY were reversed; the monoculture pasture then registered the highest yield, with 1,115 kg ha^{-1} more than the yield recorded in the associated pasture (Figure 1). Similar results were reported before[5], when 1,200 kg ha^{-1} more of DM production was registered in a *Brachiaria humidicola* pasture in monoculture than in the *B. humidicola-Stylosanthes guianensis* association during the rainy season.

When observing the behavior of DMY between harvest dates, changes were recorded throughout the study period for the two types of grasslands (Table 3). In the first stage, there was stability in the DMY of both types of pasture from Dec19, 2019 to Apr 04, 2020, i.e., no differences ($P>0.05$) in DMY were observed between harvest dates in this period. Subsequently, this pattern of available DMY included a decline from April to May (dry season of the year), which coincided with a decrease of more than 300 mm of accumulated rainfall in both grassland types. The DMY of the monoculture pasture decreased by 2,232 kg ha^{-1} from April 4 to May 10 (42-d period), while in this same period the associated pasture registered a smaller decrease, of 1,593 kg ha^{-1} (42-d period). This meant an DMY reduction rate of 53 kg ha^{-1} d^{-1} for the monoculture pasture, and only 38 kg for the associated pasture.

This higher DMY of the association in the dry season was also found by another study[19], in which higher yields were observed in the dry season when grass and legume mixtures were utilized. At the end of the dry season, particularly from May 10, 2020 to July 21, 2020 (rainy season), the monoculture pasture registered the greatest increase in DMY, of 7,129 kg ha^{-1}, with the lowest increase for the associated pasture in this same period, of 5,206 kg ha^{-1}. This increase coincided with an increase of 200 mm in cumulative rainfall, compared to the cumulative rainfall of the dry season.

Table 3: Available forage (DM) in two types of pasture, available legume forage in the pasture associated with protein, neutral detergent fiber (NDF), and lignin of the available forage on seven harvesting dates

Type of pasture	Sampling date	Available forage (kg DM ha^{-1})	Legume (kgDM ha^{-1})	Chemical composition (g kg^{-1} DM)		
				Protein	NDF	Lignin
Monoculture Mombasa	Dec-19-2019	3843.3 ^{c}		80.4 ^{b}	704.3 ^{a}	60.8 ^{a}
	Jan-23-2020	4111.3 ^{cde}		------	------	------
	Feb-28-2020	4385.5 ^{cd}		------	------	------
	Apr-04-2020	5195.0 ^{c}		------	------	------
	May-10-2020	2963.0 ^{e}		96.7 ^{a}	691.8 ^{a}	41.2 ^{b}
	Jun-15-2020	7076.5 ^{b}		------	------	------
	Jul-21-2020	10092.0 ^{a}		71.2 ^{c}	686.7 ^{a}	43.7 ^{b}
	Mean	5380.8		82.7	694.2	48.6
Mombasa associated with kudzu	Dec-19-2019	3695.8 ^{d}	773.8 (21) ^{a}	121.8 ^{b}	722.3 ^{a}	67.8 ^{a}
	Jan-23-2020	4047.3 ^{d}	788.2 (20) ^{a}	------	------	------
	Feb-28-2020	4503.0 ^{dc}	728.3 (17) ^{ab}	------	------	------
	Apr-04-2020	5364.3 ^{c}	561.1 (11) ^{bc}	------	------	------
	May-10-2020	3771.5 ^{d}	335.1 (9) ^{d}	142.7 ^{a}	744.6 ^{a}	49.4 ^{b}
	Jun-15-2020	6694.3 ^{b}	519.0 (8) ^{dc}	------	------	------
	Jul-21-2020	8977.0 ^{a}	915.2 (11) ^{a}	79.53 ^{c}	748.73 ^{a}	54.16
	Mean	5293.2	660.10	114.7	738.5	57.1

Numbers in parentheses indicate the proportion of the legume in the associated pasture, calculated based on the harvested samples and measured as a percentage.

^{abcd} Averages with different letters within a column by pasture type are statistically different ($P \leq 0.05$).

Kudzu, present in the associated pasture, maintained a low DMY ―which implied a low proportion of this legume in the pasture― through the various harvesting dates (Table 3). During the months with minimum temperatures and favorable environmental humidity (Dec19, 2019 to Feb 28, 2020), kudzu registered the highest DMY, averaging 763.4 kg ha^{-1}, which amounted to 19.4 % of the pasture's yield. This proportion declined considerably during the dry period of the year, from February 28 to May 10, when the DMY decreased to 335 kg ha^{-1} (9%). Subsequently, at the end of the study (July) and during the rainy season, the kudzu DMY increased to 915 kg ha^{-1} but maintained its proportion in the pasture at 11.0 % due to the high increase in biomass of the pasture (mombasa + kudzu), which went from 3,771.5 kg ha^{-1} in the dry period to 8,977 kg DM ha^{-1} at the end of the study (in the rainy

season). Similar results in terms of legume yield have been reported in the past[20], with a three-fold increase in kudzu from the dry season to the rainy season. However, the mombasa grass (plant C4), expressed its potential to convert photoassimilates into biomass more efficiently under favorable conditions of humidity, compared to kudzu (C3).

Chemical composition

There was a harvest date x pasture type interaction for protein and NDF concentration, but not for lignin concentration (Table 2). In the case of protein, the interaction originated in the rainy season (Jul 21, 2020), when the two types of pastures exhibited no differences in protein concentration, both of them averaging 75 g kg^{-1} of DM. conversely, in the north winds season (Dec 19, 2019) as well as the dry season (May 10, 2020), the protein concentration was higher in the associated pasture, registering 41 and 40 g kg^{-1} of DM plus protein than the monoculture pasture, in those periods, respectively (Figure 2). The mombasa-kudzu pasture registered a higher protein concentration, as a result of kudzu having 1.7 times more protein than mombasa grass, whereby this legume maintained a high protein concentration throughout the study, with an average of 146.2 g kg^{-1} of DM (Table 4).

Between harvest dates, variations in the protein concentration differed between the monoculture and associated pastures (Table 3). Both pastures registered the highest concentrations in the dry season (May 10, 2020), and the lowest in the rainy season (July 21, 2020). In the monoculture pasture, average values of 16 and 25 g kg^{-1} DM of protein were higher in the dry season than in the north winds and rainy seasons, respectively. The decrease in protein in the two types of pastures in the rainy period of the year may have resulted from the higher plant growth induced by the higher rainfall, which caused a dilution of protein with the increase in grassland biomass. This phenomenon of protein dilution has been explained in tropical pastures[21] and consists in a decrease of the concentration of protein per unit of dry matter accumulated in the plant during its growth.

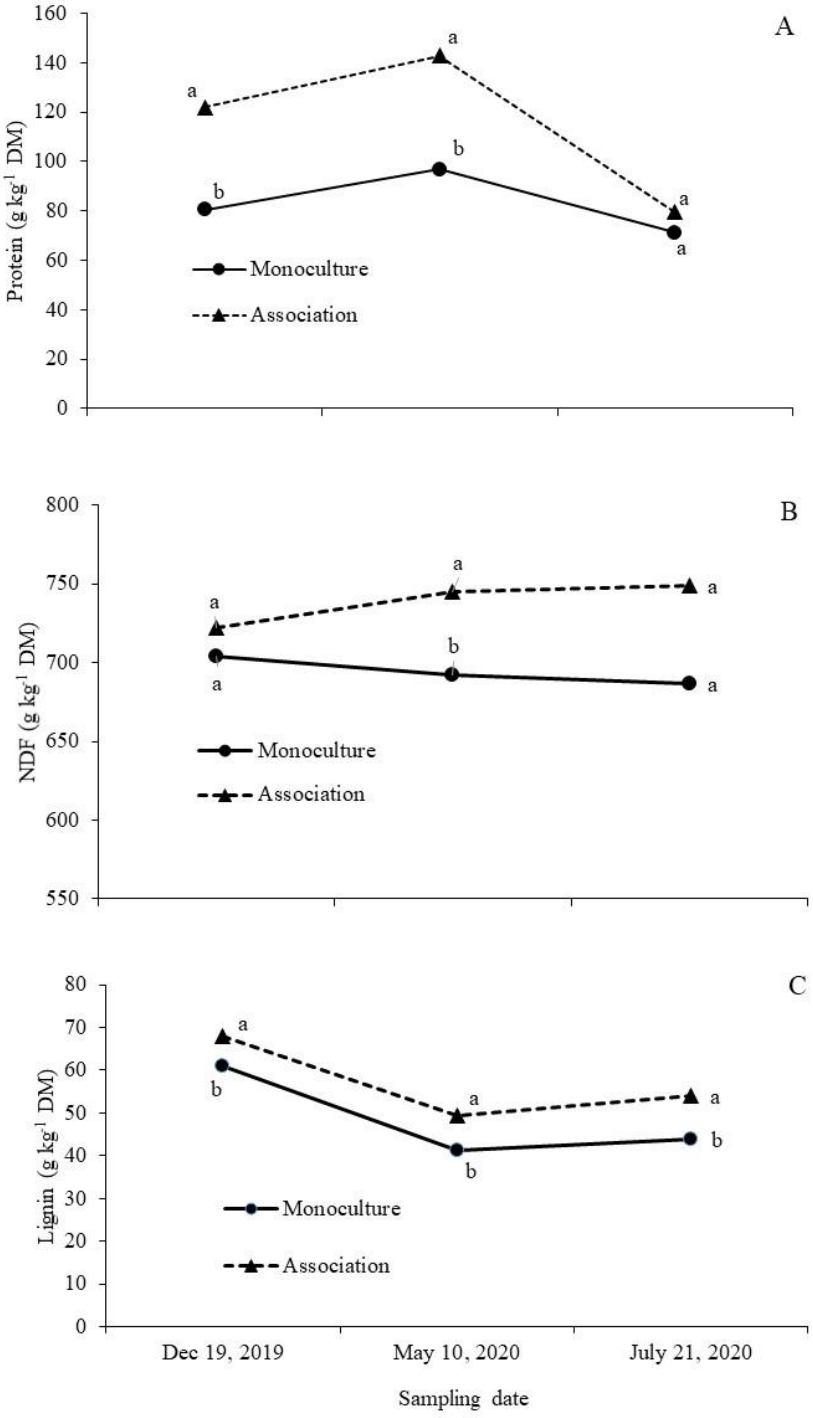
The harvest date x pasture type interaction for NDF (Table 2) was recorded in the dry season (May 10, 2020), as the associated pasture then had a higher concentration and there were no differences between the two pastures in the two remaining seasons of the year. In the dry season, the associated pasture registered 52.8 g kg^{-1} of DM more NDF than the monoculture pasture. In the north and rainy seasons, there were no differences in NDF concentration between the two types of pasture, with average values of 713.3 and 717.3 g kg^{-1} of DM for Dec19, 2019 and Jul 21, 2020, respectively (Figure 2). Between harvest dates or times of the year, the mombasa pasture both in monoculture and associated with kudzu, exhibited no

change in its NDF concentration (Table 3). Throughout the study period, the DM averaged 694.5 g kg^{-1} in the monoculture pasture, and the NDF, 738.5 g in the associated pasture.

Given the absence of harvest date × grassland type interaction for lignin concentration (Table 2), the highest concentrations throughout the study period were recorded in mombasa-kudzu grassland (Figure 2). This associated pasture recorded 7.0, 8.2 and 10 g kg^{-1} of DM more lignin than the monoculture pasture for the north, dry and rainy seasons, respectively. The higher lignin concentration in the associated pasture is the result of the high lignin concentration recorded in kudzu, registering an average value of 89.5 g kg^{-1} DM for the three seasons of the year (Table 4). These high lignin values are in agreement with another study[22] where the nutritional value of kudzu was evaluated at different ages for animal consumption, with the result that, at 40 d of age, kudzu registered average concentrations of 90 g kg^{-1} of DM, with a lower concentration of lignin before 40 d of age.

Variations in lignin concentration between sampling dates were observed in both grassland types. In both monoculture and association pastures, lignin concentration was higher in the north winds and was similar in the dry and rainy seasons (Table 3). This higher concentration in the northeast was due to the favorable availability of water in the soil, which probably led to an increase in the number of stems of the plants, but with slow growth due to the minimum temperatures during this period. Slow-growing stems accelerate the process of lignification of vegetative organs[23]. The monoculture pasture recorded 18.3 g kg^{-1} of DM more lignin in the north winds compared to the average value (42.4 g) of the dry and rainy seasons. In addition, the associated grassland recorded 16 g more lignin in the north winds season than the average (51.7 g) recorded in the dry and rainy seasons.

Figure 2: Concentration of protein, neutral detergent fiber (NDF), and lignin of a mombasa pasture in monoculture, and of the mombasa + kudzu association, at the regrowth age of 42 days after grazing in three sampling dates, representative of the north winds season (Dec 19, 2019), dry (May 10, 2020) and rainy (Jul 21, 2020) seasons



Different letters within a date indicate statistically different differences ($P \leq 0.05$).

Table 4: Crude protein, neutral detergent fiber (NDF), and lignin of the association kudzu at 42 days of regrowth on three different harvest dates

Sampling dates		Protein	NDF	Lignin
			g kg^{-1} DM	
December 19, 2019		152.4 [a]	667.7 [ab]	89.4 [a]
May 10, 2020		144.9 [b]	663.5 [b]	88.6 [a]
July 21, 2020		141.4 [b]	679.7 [a]	90.4 [a]
Mean		146.2	670.3	89.5
	DF	**Mean squares**		
Harvesting date	2	93.65**	210.68*	2.44 NS

DF= degrees of freedom.

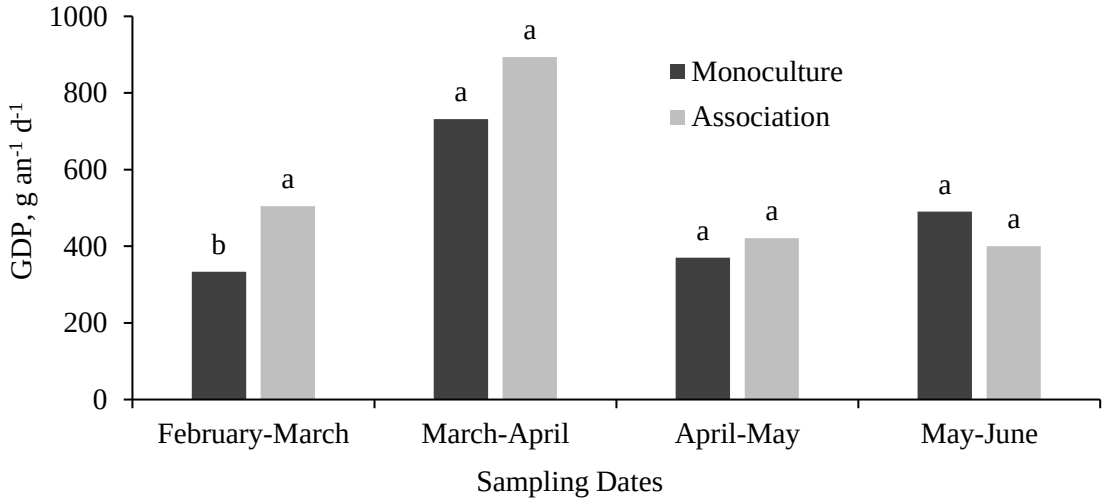
[ab] Averages with different letters within a column are different ($P \leq 0.05$); *, **, *** Significance level at 0.05, 0.01, and 0.001, respectively. NS= not significant.

Daily animal weight gain (DWG)

Changes in nutritional value and available forage yield as the seasons progressed were reflected in the DWG of grazing calves (Figure 3). In the February-March grazing cycle, the DWG differed ($P \leq 0.05$) between the two types of pasture, with gains of 333 and 504 g animal^{-1} d^{-1} for the monoculture and association pastures, respectively. In the following grazing cycle, in the months of March to April, there were no differences in weight gain ($P > 0.05$), but the average of both pastures was high, with 813 g animal^{-1} d^{-1} In the two subsequent cycles, April-May and May-June, DWG declined by 416 and 367 g, respectively, and averaged over the two pasture types. From March to early May, the two pastures registered an increase in the protein concentration and a reduction in the lignin concentration (Table 3). This increase in the values of chemical composition may have been due to the increase in temperature and the favorable availability of soil moisture, especially in March and April, since, in these areas, the soils still maintain sufficient moisture until April, which may have induced an increase in the resprouting or the sprouting of new young plants with higher nutritional value. In the May-June cycle, despite the high increase in forage production brought about by the increased rainfall (200 mm more than in the previous cycle, Table 1), the calves maintained a low DWG (445 g animal^{-1} d^{-1}). This was related to a decrease in protein concentration (Figure 3), and an increase in NDF (Table 4). With these changes in the chemical composition of the pasture, it can be deduced that the higher forage production that occurred in this last grazing cycle consisted mainly of stems, that is, in a decrease in the leaf/stem ratio, as has been observed in other studies[24,25]. But also, it could be a consequence of the impoverishment in the proportion of the legume in the pasture over time. Only in the first grazing cycle (north winds season), the mombasa-kudzu pasture registered the highest DWG of calves, with a value of 171 g d^{-1} above that of the calves that grazed the mombasa

monoculture pasture. This was due to the higher kudzu DMY registered in February (728.4 kg ha^{-1}) compared to April (561 kg) or May (335 kg). In the remaining cycles, there were no differences in DWG between the two types of pasture (Figure 3). The average total gain per animal for the associated pasture was 80.26 kg *vs* 70.08 kg for the monoculture pasture; however, this difference was not significant (P>0.05).

Figure 3: Daily weight gain (DWG) of calves grazing Mombasa in monoculture pastures and in association with kudzu on four grazing dates



Conclusions and implications

The mombasa-kudzu association showed greater stability in the production of dry matter throughout the experimental period and a higher protein concentration than the Mombasa pasture in monoculture during the north winds and dry seasons, unlike during the rainy season. The concentration of neutral detergent fiber was similar in the north winds and the rainy seasons between the two types of pastures, and the concentration of lignin was higher in the association in all three seasons. The favorable results of the associated pasture in terms of yield and protein concentration were reflected in the higher daily weight gain of the animals at the end of the north winds season (February-March).

Acknowledgments

The authors would like to thank INIFAP for its financial support, through tax resources, for the development of the project "*Potencial forrajero y producción animal en pastoreo en praderas asociadas gramínea-leguminosa en el trópico de México*" ("Forage potential and

grazing animal production in associated grass-legume pastures in the Mexican tropics"), from which the present research arose.

Literature cited:

1. Pardo-Aguilar N, Bolaños-Aguilar ED, Lagunes-Espinoza LC, Enríquez-Quiroz JF, Fragoso-Islas A. Efecto de una asociación pasto-leguminosa en el rendimiento de materia seca y concentración de proteína de la pradera fertilizada con fósforo. Agro Product 2020;13(7):53-60.

2. Reyes-Purata A, Bolaños-Aguilar ED, Hernández-Sánchez D, Aranda-Ibañez EM, Izquierdo-Reyes F. Producción de materia seca y concentración de proteína en 21 genotipos del pasto *Brachiaria humidicola*. Rev Univ y Cienc 2009;25(3):213-224.

3. Mayberry DA, Hatcher S, Cowley F. New skills, networks and challenges: the changing face of animal production science in Australia. Anim Prod Sci 2021;(61):201-207.

4. García A, Hernández A, Quero A, Guerrero J, Ayala W, Zaragoza J, Trejo C. Persistencia de *Dactylis glomerata* L. solo y asociado con *Lolium perenne* L. y *Trifolium repens* L. Rev Mex Cienc Agríc 2017;7(4):885-889.

5. Domínguez-Pérez D, Bolaños-Aguilar ED, Lagunes-Espinoza LC, Salgado-García S, Ramos-Juárez J, Guerrero-Rodríguez JD. Rendimiento de materia seca y concentración de fósforo de una asociación *Brachiaria humidicola-Stylosanthes guianensis*. Rev Mex Cienc Agríc 2017;8(8):1705-1717.

6. Lüscher A, Mueller-Harver I, Soussana JF, Rees RM, Peyraud JL. Potential of legume-based grassland-livestock systems in Europe: a review. Grass Forage Sci 2014;(69):206-228.

7. Yates RJ, Harrison RJ, Loi A, Steel EJ, Edwards TS, Nutt BJ, Porqueddu C, Gresta F, Howieson JG. Sourcing *Rhizobium leguminosarum* biovar viciae strains from Mediterranean centers of origin to optimize nitrogen fixation in forage legumes grown on acid soils. Grass Forage Sci 2021;76(1):33-43.

8. Zuppinger-Dingley D, Schmid B, Petermann JS, Yadav V, De Deyn GB, Flynn DFB. Selection for niche differentiation in plant communities increases biodiversity effects. Nature 2014;515(7525):108-111.

9. Prieto I, Violle C, Barre P, Durand JL, Ghesquiere M, Litrico I. Complementary effects of species and genetic diversity on productivity and stability of sown grasslands. Nat Plants 2015;(10):1038.

10. Olivera Y, Machado R, Ramírez F, Castañeda L. Evaluación del establecimiento de una colección de accesiones de *Brachiaria brizantha* asociadas con *Stylosanthes guianensis* CIAT-184. Pastos y Forrajes 2012;(35):153-164.

11. Ramírez-Restrepo CA, Vera RR. Bodyweight performance estimated carcass traits and methane emissions of beef-cattle categories grazing *Andropogon gayanus*, *Melinis minutiflora* and *Stylosanthes capitata* mixed swards and *Brachiaria humidicola* pasture. Anim Prod Sci 2019;(59):729-740.

12. Toledo JM, Schutze-Kraft R. Metodología para evaluación agronómica de pastos tropicales. Toledo JM (Ed.). Manual para la evaluación agronómica. Cali, Colombia. CIAT; 1982:91-110.

13. Horwitz W, Latimer GW. Official methods of analysis of AOAC International. 18th edition, Association of official Analytical Chemistry International. Gaithersburg, Maryland, USA; 2005.

14. Van Soest PJ. Nutritional ecology of the ruminant; LTTIECA and London: Comstock Publishing Associates, Cornell University Press; 1994.

15. SAS Institute. User's Guide: Statistics, version 9.3. SAS Inst. Inc. Cary, N. C. USA; 2010

16. Sleugh B, Moore KJ, George R, Brummer EC. Binary legume-grass mixture improve forage yield, quality and seasonal distribution. Agron J 2000;92(1):24-29.

17. Tilman D, Reich PB, Knops JMH. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 2006;(441):629-632.

18. Rasmussen J, Soegaard K, Pirhofer-Walzl EJ. N2-fixation and residual N effect of four legumes species and four companion grass species. Eur J Agron 2012;36(1):66-74.

19. Portillo LPA, Meneses HB, Lagos BE, Duter EN, Castro RE. Valor nutritivo de mezclas forrajeras en épocas seca y de lluvias en Nariño, Colombia. Agron Mesoam 2021;32(2):556-572.

20. García-Ferrer L, Bolaños-Aguilar ED, Ramos-Juárez J, Osorio-Arce M, Lagunes-Espinoza LC. Rendimiento y valor nutritivo de leguminosas forrajeras en dos épocas del año y cuatro edades de rebrote. Rev Mex Cienc Pecu 2015;6(4):453-468.

21. Juárez-Hernández J, Bolaños-Aguilar ED, Vargas-Villamil LM, Medina S, Martínez-Hernández PA. Curvas de la dilución de la proteína en genotipos del pasto *Brachiaria humidicola* (Rendle) Schweick. Rev Cub Cienc Agríc 2011;45(3):321-331.

22. Akoutey AM, Kpodekon T, Bannelier C, Gidenne T. Nutritive value of sun-dried *Pueraria phaseoloides* for rabbits under tropical conditions. World Rabbit Sci 2012; (20):209-213.

23. Borrás L, Maddonni GA, Otegui ME. Leaf senescence in maize hybrids:plant population, row spacing and kernel set effects. Field Crops Res 2003;(82):13-26.

24. Bolaños-Aguilar ED, Émile JC. Efecto de la distancia entre surcos y densidad de siembra en el rendimiento y calidad del forraje de sorgo. Rev Mex Cienc Pecu 2013;4(2):161-176.

25. Wade RN, Seed P, McLaren E, Wood E, Christin PA, Thompson K, Rees M, Osborne CP. The morphogenesis of fast growth in plants. New Phytologist 2020;228(4):1306-1315.

https://doi.org/10.22319/rmcp.v15i4.6551

Review

Characteristics of lactation curves in ewes and factors influencing their variation: A review

Gabriela Castillo-Hernández [a]

Ana Erika Ochoa-Alfaro [b]

Manuel Antonio Ochoa-Cordero [c]

Jorge Alonso Maldonado Jáquez [d]

Glafiro Torres-Hernández [a*]

[a] Colegio de Postgraduados-Campus Montecillo. Programa de Ganadería. 56264, Montecillo, Edo. de México. México.

[b] Universidad Autónoma de San Luis Potosí. Facultad de Química. San Luis Potosí. México.

[c] Iniciativa Privada. San Luís Potosí. México.

[d] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental La Laguna. Coahuila. México.

* Corresponding author: glatohe@colpos.mx

Abstract:

Dairy sheep breed genetic improvement programs have responded to the increasing market demand and popularity, especially for cheeses made from sheep's milk. These milk derivatives are an important source of bioactive substances for human health. Therefore, it is very important to learn about milk production (MPROD) and the factors that influence its variation. The typical pattern of MPROD during the period when an ewe is lactating is known as the lactation curve (LC), and this can be typical (TLC) or atypical (ACL). TLCs are characterized by reaching a maximum MPROD (lactation peak, LP) within a few days after parturition, and then gradually decreasing until the end of lactation, or lactation drying, is

reached. ALCs are those that show some deviation from the normal pattern. It is important to know the graphical representation of lactation behavior, as, in addition to predicting MPROD, it makes it possible to identify health and feeding issues, as well as to select females that will excel in MPROD. Persistence of lactation (PER) has been defined as the rate of decline in MPROD after the LP was reached, and it is highly desirable for ewes to have a high PER. Mathematical models have been developed for the study of LCs and PER. There are genetic and environmental factors that influence LCs.

Keywords: Milk production, Peak lactation, Persistence, Typical curves, Sheep breeds.

Received: 11/09/2023

Accepted: 30/07/2024

Introduction

Over the last 150 yr, genetic selection and improvements in management have led to improved breeds of sheep for milk production (MPROD), responding to growing market demand and popularity, especially for cheeses made from sheep's milk[1]. There are currently an estimated 1 billion sheep in the world[2]; the main breeding areas are located within latitudes 35-55 degrees north in Europe and Asia, as well as between 30 and 45 degrees south in South America, Australia and New Zealand[3]. Products derived from sheep's milk, such as cheese, cottage cheese, yogurt, etc., constitute the typical diet of sheep farmers[4] and are an important source of bioactive substances that benefit human health[5]. About 1,500 sheep breeds have been described; of these, only 180 are identified as milking breeds because of their zootechnical purpose (milk), although many are local breeds used for meat, wool, and milk production where milk is not the main product of interest[6]. Some of the most important breeds of dairy sheep in the world are East Friesian[7], Lacaune[8], Chios[9], Sarda[10], and Manchega[11].

The productive level of the ewe is the most important economic characteristic in the flock, as it provides information used in the estimation of biological indexes that facilitate selection decisions in genetic improvement programs[12]. Therefore, one of the most important criteria for evaluating female productivity is MPROD, since it directly affects the efficiency of the production system and has very important effects on farm profitability[13]. Therefore, knowledge of the behavior of the lactation curve (LC) is very important, since it will allow adequate planning of general management and genetic improvement programs. The objective

of this review is to describe the main characteristics of LCs and to enumerate the factors that influence their variation. This review has included studies conducted in sheep; however, the vast majority of studies in the scientific literature that address this topic are focused on describing LCs in dairy cattle.

Definition of lactation curve

MPROD during the lactation period in mammals and domestic ruminants is the result of physiological processes developed by specialized cells of the mammary gland, which synthesize and secrete organic and inorganic compounds through active and passive blood filtration[14]. MPROD begins when gestation is nearing completion through expansion of the mammary gland tissue, and ends when the mammary gland volume decreases, due to secretory regression that ends with the cessation of lactation, or drying[15]. All these physiological mechanisms result in a typical pattern of MPROD over time known as the "lactation curve" (LC), which can be defined as the graphical representation of the time period in which MPROD occurs, although it is also expressed as a continuous physiological function describing milk secretion over time[16]. According to the criteria of certain authors[17], and taking Assaf dairy ewes as an example, lactation can be divided into three periods: early lactation, which considers the period from lambing until month 2, mid lactation, which covers months 3 to 7, and late lactation, from month 8 to dry-off.

Importance of knowing lactation curves

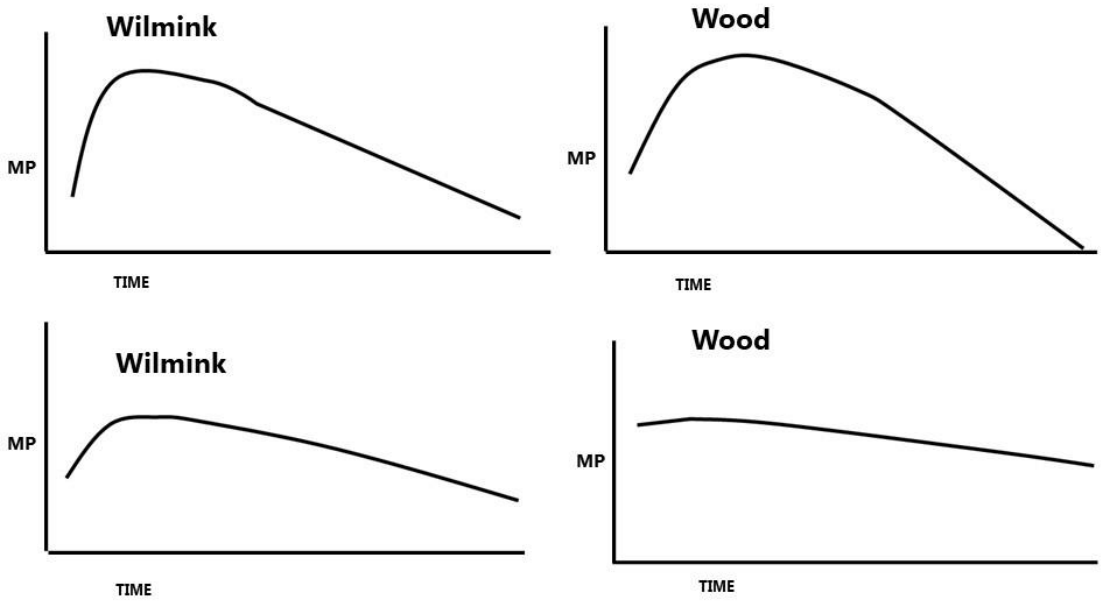
Knowledge of a LC allows prediction of the total milk production[18], the characteristics of the curve (discussed below), and, finally, the future performance of the breeding animals (cattle, sheep, goats) or their progeny[19]. In addition, by understanding the behavior of the LC's shape, it is possible to make decisions regarding such aspects as nutrition, health, and management of the herd. Above all, knowledge of these curves is useful for identifying and selecting superior ewes for MPROD and, therefore, valuable for the producers[20].

Lactation curve types

According to their shape, there are two types of LCs: typical (TLC) and atypical[21] (ALC). A TLC reaches its maximum milk production (peak production, lactation peak, LP) a few

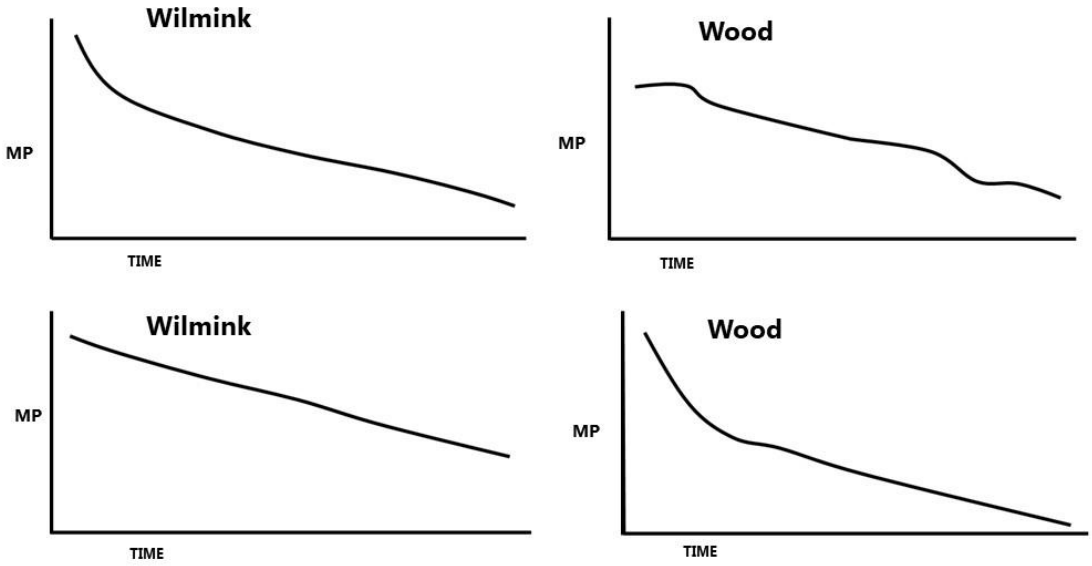
days after lambing (2-6 weeks), and thereafter shows a steady decline until it reaches the drying stage, or end of lactation[22]. The typical pattern of a LC (Figure 1) is regular and continuous, and constitutes the expression of physiological mechanisms from the onset of MPROD[23]. An ALC is one represented by slight deviations from the TLC due, for example, to the presence or absence of an inflection point in the decreasing lactation; others decline steadily and lack the LP[24], as shown in Figure 2; they are represented by deviations from the regular pattern, which can be attributed to various factors such as nutrition, health status of the animal, and environmental disturbances[23]. For example, in Wood's model[25], a characteristic that differentiates TLCs from ALCs is that the "b" and "c" parameters are positive in TLCs, and negative in ALCs[26].

Figure 1: Schematization of typical lactation curves, according to Wilmink's and Wood's models



Adapted from Palacios Espinosa *et al*[21]

Figure 2: Schematization of atypical lactation curves, according to Wilmink and Wood's models



Adapted from Palacios Espinosa *et al*[21]

Stages (phases) of a lactation curve

The typical stages of a LC are: an initial gradual increase from lambing until reaching a point of maximum milk production that represents the LP is reached, which occurs within the first days after lambing (or days in milk), generally in the range between 2 and 6 wk, and which is also a criterion used in the selection of breeding females[28]. Subsequently, the decreasing phase begins until the MPROD ceases, or until the drying of the animal, when the MPROD is minimal. Drying, in dairy sheep breeds such as the East Friesian, generally takes between 180 and 210 d, and, in exceptional cases, it can take up to 260 d[29]. Great care must be taken with the method used to dry the animal, due to the possibility of infections in the mammary gland, such as mastitis[30,31]. Drying can be abrupt: stopping milking on a given day, or gradual, with a reduction of the frequency of milking over days or weeks[32]. In dairy cows, some management practices have been recommended to carry out the drying process[33,34], which could also be put into practice with sheep. At the end it has a CL that graphically represents the total MPROD, which can be estimated based on the area under the curve, defined as the total amount of milk produced during the whole lactation and determined by the shape of the curve[35].

Lactation curve models

The first mathematical models to characterize LCs were developed in studies of dairy cows; however, several of these have also been used to characterize LCs of sheep and goats. These models are classified as a) empirical and b) mechanistic. In relation to lactation, empirical models are based on actual MPROD data; e.g., test day records, whereas mechanistic models are based on the biology of lactation; e.g., mammary gland growth and regression, or nutrient flux[36]. In other words, the theory of the empirical model refers to the level of reality in which the phenomenon under consideration is expressed, while the mechanistic one is characterized by a deeper theoretical assumption[37]. Describing and discussing these models is not an objective of this review. Therefore, and for illustrative purposes only, Tables 1 and 2 show examples of empirical and mechanistic models, respectively.

Table 1: Examples of empirical models and their parameters used in sheep lactation curves, expressed as a function of t

Model	**Parameters**	**Author(s)**
$Y = ae^{-bt}$	2	Brody *et al.* (1923)
$Y_t = at^b \exp(-ct)$	3	Wood (1967)
$Y_t = a - bt - a \exp(-ct)$	3	Cobby & Le Du (1978)
$Y_t = at^{bc} \exp(-ct)$	3	Dhanoa (1981)
$Y_t = a + bt + c \exp(-wt)$	4	Wilmink (1987)
$Y_t = a_0 + a_1 t + a_2 t^2 + a_3 \log(1/t) + a_4 \log(1/t)^2$	5	Ali & Schaeffer (1987)
$Y_t = \sum[a_1 b_1 [1 - tanh^2(b_1(t - c_1))]]$	3 per phase	Grossman & Koops (1988)
$Y_t = \exp(a + bt + ct^2 + d/t)$	4	Morant & Gnanasakthy (1989)
$Yt = a_1 b_1 (tanh^2(b_1(t - c_1))) + a_2 b_2 (1 - tanh^2(b_2(t - c_2)))$	6	Gipson & Grossman (1989)

$Y_t = at^{bexp(-ct)}$	3	Cappio-Borlino *et al*. (1995)
$Y_t = {}^{t}/_{a} + bt + ct^2$	3	Nelder (1996)
$Y_t = \sum_{i=1}^{n} \alpha_1 P_j$	5	Brotherstone *et al.* (2000)

Adapted from Bilgin et al.[38] and Macciotta et al.[37]

Table 2: Examples of mechanistic models and their parameters used in sheep lactation curves

Model	**Parameters**	**Author(s)**
$\int_0^{t_L} R_M(t)dt.$	14	Neal & Thornley (1983)
dY/dt = a{ exp[-exp(G_0 – bt)]} [exp (-ct)]	4	Emmans & Fisher (1986)
$Y_t = a\ exp^{[b(1-exp-ct)/c - dt]}$	4	Dijkstra *et al*. (1997)
Y_t=a{1/[1+(1-b)/b exp^{-cn}]-1/[1+(1-d)/d exp^{-gn}]}	5	Pollot (2000)
$I = SE^{L}(de^{-k_2 t} + l_6 e^{w_6 t} + l_7 e^{w_7 t})$	8	Vetharaniam *et al*. (2003)

Source: Neal & Thornley[39], Friggens *et al*[40], Adediran *et al*[41], Vetharaniam *et al*[42].

In order to carry out genetic improvement programs for CL, it is necessary to know the magnitude of the additive genetic variance of CL parameters. Based on the above, some studies have been carried out in sheep to estimate the heritability (h^2) of CL parameters. Pollot and Gootwine[43] found in improved Awassi ewes low values of the additive genetic variance for LP and day on which the LP occurs (DPL), resulting in h^2 values of 0.11 for PL and 0.032 for DPL, explaining that these results indicate that environmental factors exert a greater effect on the manifestation of these parameters. In the USA, a group of researchers(20) analyzed first lactations of Dorset, Romanov, Targhee, Rideau Arcott, Polypay, Booroola Merino, Suffolk, Rambouillet, Finnsheep and East Friesian ewe crosses to investigate genetic variation in CL parameters using a Bayesian analysis of Wood's model[25]. The h^2 values obtained for parameters “a”, “b”, and “c” were 0.35, 0.35, and 0.27, respectively, so these authors concluded that part of the variation in lactation curves among ewes is heritable. In another study on Yankasa sheep[44], and also with Wood’s model[25], h^2 values of 1.4, 0.3, and 0.2 were found for parameters “a”, “b”, and “c”, respectively. With

respect to the irregular value of the parameter "a", these authors explained that this value could be subject to large sampling errors and, moreover, overestimated, due to the participation of non-additive genetic effects. Reviewing the magnitude of the h^2 estimators in the previous studies, it is inferred that, by virtue of being in the low-medium range, a positive response to CL selection in ewes could be expected.

Lactation persistence

A phase of lactation closely related to CL is what is known as "lactation persistency" (or milk production, PER), which was initially defined as "the rate of milk secretion indicating the initial value at parturition and its change with advancing lactation"[45], and whose first numerical measure was given, in cattle, as a percentage of the MPROD in the previous month. Subsequently, it was defined as[46] "a function of CL flattening"; i.e., a female has a higher PER the more flattened her CL is. One year later another definition was published in the literature[47]: "the ability to maintain the level of MPROD during lactation" and that it can be extended to milk components, including fat and protein. Finally, with a different approach[48], PER was said to be: "the rate of decrease in MPROD after reaching the LP".

Most of the information on lactation PER, especially mathematical models, comes from larger species, particularly dairy livestock[37]. However, in studies with dairy sheep, PER has been studied with the same approach as dairy livestock[49,50]. Under this scenario, PER has an important impact on dairy cattle, which has benefits both in feed costs[51], as well as reproductive aspects[52]. Therefore, the current trend in MPROD in cattle is to improve the PER and extend it, rather than to increase MPROD in the LP[53], which also applies to sheep and goats.

Lactation persistence models

Different criteria have been proposed to measure the PER[35] which involve the use of different mathematical models. However, as in the case of the LC models, describing and discussing PER models is not an objective of this review. Therefore, for illustrative purposes, Table 3 shows some mathematical models that have been proposed for cattle, according to the definition of PER.

Table 3: Some mathematical models and their parameters for measuring lactation persistence in dairy cows

Persistence model	**Reference**
$P = (3 + 4 + 5th\ months\ yield) - (7 + 8 + 9th\ months\ yield)/12$ $P = Total\ yield\ (sum\ of\ 7\ months) /milk\ yield\ of\ last\ 3\ months$	Ludwin (1942)
$P = \sum(\gamma_i - S_i) \times (d_i - d_0)$	Cole & VanRaden (2006)
$P = EBV_{290} - EBV_{90}$	Cobuci *et al*. (2007)
$P = \sum_{i=61}^{300} EBV - 240 \times EBV_{60}$	Harder *et al*. (2006)
$P = \sum_{i=61}^{305} EBV - 245 \times EBV_{60}$ $P = (milk_{270}\ / milk_{90}) \times 100$	DeRoos *et al*. (2001)
$P = (milk_{225}\ / milk_{45}) \times 100$ $P = (\sum_{i=1}^{150} milk\ /maximum\ milk\ yield) \times 100$	Weller *et al*. (2006)
$P = 305\ day\ milk\ yield\ /\ the\ first\ 50\ day\ milk\ yield$	Yilmaz & Koc (2013)
$P = maximum\ milk\ yield\ /\ average\ milk\ yield$	Atashi *et al*. (2006)
$P = EBV_{280}\ /\ EBV_{65}$ $P = \sum_{i=66}^{280} EBV\ /\ \sum_{i=5}^{65} EBV$	Togashi & Lin (2004)
$P = (((EBV_{280} - EBV_{60}) + Y_{280})/ Y_{60}) * 100$	Mostert *et al*. (2008)
$P = \sum_{i=61}^{280} milk_{280} - milk_{60}$	Jamrozik *et al*. (1997)
$P = {}^{1}/_{55} \sum_{i=255}^{i=350} milk\ yield\ i - {}^{1}/_{21} \sum_{i=50}^{i=70} milk\ yield\ i$	Kistemaker (2003)

$P = \sum_{i=101}^{200} milk \,/\, \sum_{i=1}^{100} milk \quad P = \sum_{i=201}^{305} milk \,/\, \sum_{i=1}^{100} milk$ $P = \sum_{i=1}^{100} milk \,/\, (MAX \sum_{i=1}^{100} milk \times 200)$	Johansson & Hansson (1940)
$P = \sum_{DIM=60}^{279} D_{DIM} - D_{280} \qquad P = EBV_{280} - EBV_{60}$	Jakobsen *et al*. (2002)
$P = -(b+1)1n\ c$	Wood (1970)
$P = 100\ (1 + 2\gamma_i)$	Kamidi (2005)

Source: Torshizi *et al*[54]

As in the case of LCs, studies have also been carried out in sheep to estimate the h^2 of PER, although for this parameter, in smaller numbers compared to dairy cows. In order to estimate the h^2 of PER, a group of researchers in Greece[55] used Sfakia dairy ewes using MMP2:MMPP1 (MPROD month 2:MPROD month 1), MMP3: MMP1 (MPROD month 3:MPROD month 1), MMP4: MMP1 (MPROD month 4: MPROD month 1), MPR (measure of the reduction in an ewe's MPROD relative to MPROD level in early lactation, in percent), and VC (measure associated with the variation in an ewe's MPRODs on the test day, in percent), with results, respectively, of 0. 26, 0.16, 0.14, 0.24, y 0.28. In a study with improved Awassi ewes[43] the h^2 of PER was estimated, measured as the daily loss of MPROD between DPL and the end of lactation, thus obtaining a value of 0.11. Kominakis *et al*[56] estimated the h^2 of PER in Boutsiko dairy ewes from Greece, for which they used three measures of PER: $\hat{\beta}$ (measures the rate of decline of MPROD following an ewe's LP, in kg/day), in addition to the MPR and VC measures (already described), having obtained values of 0.15, 0.10, and 0.13, for the $\hat{\beta}$, MPR, and VC measures, respectively. As with LCs, h^2 estimators of PER are in the low-medium range, which is encouraging for use in selection programs to improve PER in ewes.

Factors affecting the lactation curve

Genetic

Lactation behavior is largely determined by the genotype of the individual; i.e., the shape of the CL is genetically determined[57]. A group of researchers[58] used a mechanistic

mathematical model of the milk secretion process, based on the physiological theory of the mammary gland, where the model output can be a monoexponential or biexponential function. Using 64 Sarda dairy ewes, the biexponential function fitted regular LCs (R^2=0.87), while the monoexponential fitted decayed LCs (R^2=0.80). The authors concluded that LC dimorphism was not due to environmental factors (production level, type of birth, and udder health status), but did have a genetic influence.

A study using crosses between several dairy sheep breeds researched genetic variation in LC traits[20] using a three-stage Bayesian hierarchy: 1) Wood's model was utilized, 2) inter-sheep variation was described, and 3) *a priori* distributions of all unknown parameters were included. The results showed that some of the variation in LCs between ewes is heritable. On the other hand, genetic correlations were negligible, suggesting that there is sufficient scope for modifying LCs genetically.

The MPRODs of Araucana and Romney Marsh ewes were tested[59], also characterizing their LCs and relating MPROD to the growth of their lambs. The LCs in both breeds were typical; however, the MPROD of Araucana ewes was characterized by an ascending phase until d 30, with a maximum production of 2.18 L d^{-1}, while Romney Marsh ewes reached the LP on day 20 of lactation, with a maximum MPROD of 2.47 L d^{-1}.

Komprej *et al*[60] analyzed the LCs for daily MPROD, fat, and protein content in Bovec, improved Bovec, and Istrian Pramenka dairy ewes, estimated with a repeatability animal model that included records of the test days. The shape of the LCs for the daily milk production of Bovec and improved Bovec ewes was a good fit (51.35 %) for the general lactation curve of dairy ewes. In Istrian Pramenka ewes, the shape of the LCs was more or less atypical, with a lower peak production and a decreasing daily MPROD during almost the whole lactation. The shapes of the LCs for fat and protein contents were opposite to those of the LCs for daily MPROD in all three breeds.

In order to determine the MPROD and the LC characteristics, 863 weekly MPROD records from 70 lactations were analyzed[61] in six genetic groups of ewes: East Friesian (EF), Criollo (Cr), ½ EF x ½ Cr, ¾ EF x ¼ Cr, ½ Suffolk x ½ Cr, and Corriedale (C). Wood's function (WF) was used to calculate the total observed MPROD (TLP^{obs}) and the estimated 180-d MPROD (TLP^{180}), the peak lactation (PL), the time to peak lactation (TPL), and the PER. The genetic group significantly (P<0.05) influenced the TLP^{obs}, TLP^{180}, LP, and parameter "b" of Wood's model, with higher values in ½ EF x ½ Cr ewes. In all cases, the LCs were typical, although with varying degrees of PER. The authors concluded that differences in productive performance due to the genetic group may be associated with the adaptability of EF ewes to local climatic conditions.

Environmental

Before addressing the results found in the literature concerning this type of factors, a group of researchers[37] in dairy cattle pointed out that linear mixed models are an adequate mathematical tool for the evaluation of environmental effects, as they can take into account factors that could affect each test-day record differently. These authors presented the basic structure of these models as follows: y = HTD + F + DIM + L + e; where y= daily MPROD; HTD= interaction between herd and test date taking into account the peculiar effects of a specific date; F= fixed factor (lambing season, production region, lambing number); DIM= fixed effect of days on MPROD groups, whose least squares solutions allow generating lactation curves corrected for other effects included in the model; L= individual random effect of the cow (ewe, goat) associated with a variance component (σ^2L); e= residual random effect associated with the variance component σ^2_L.

In a study on Sarda dairy ewes[62], LCs were estimated and predicted LCs by age at lambing, in addition to seasonal effects for milk, fat, and protein yields. Trends in seasonal effects showed a spring peak for MPROD, milk, fat, and protein yields. The seasonal effects on fat content were very irregular, while in the case of the protein content they were small and constant over time. The predicted LCs showed an increasing effect of age at lambing on all variables. From these results, the authors concluded that the trend of seasonal effects on milk yields within herd-years could be an important tool for improving management techniques.

Using Sarda dairy ewes with different levels of milk production (in grams), lambing type, and udder health, a modified nonlinear version[63] of Wood's model ($y=at^{bexp(-ct)}$) was tested The results showed that the modified version ("a"=702.3 + 56.2, "b"=1.29 ± 0.09, "c"=0.133 ± 0.013) of the model fitted the LC very well (R^2= 0.905; residual standard deviation= 145.3) with few iterations required for convergence (<5). Milk yield, production level, and lambing type influenced all the parameters, while udder health only influenced parameter "a".

In a study on Comisana dairy ewes[64], MPROD data were fitted with Wood's model, and the effect of environmental factors on the LC was assessed. The interaction between the lambing number and the lambing season had a strong influence on the lactation parameters. The LCs for winter-lambing ewes had a higher LP than for those for fall-lambing ewes. The lambing number correlated positively with the peak milk production and negatively with the milk production decrease (MPD) and PER. The lambing type did not significantly influence the shape of the LC.

In the case of Valle del Belice dairy ewes[65], test-day models were used to estimate the LC and assess the influence of environmental factors on MPROD and fat and protein percentages. Three flocks were analyzed. In each flock, two groups of ewes were formed;

one group received no feed supplement, while the other group received 500 g d^{-1} of a commercial concentrate. The lambing number affected the LC for MPROD, which was lower and flatter for first-time ewes; the effects on the fat and protein contents were smaller. The time of the lambing affected all variables. Seasonal productivity had the greatest effect on the milk composition, resulting in an imbalance between fat and protein percentages. Herd and dietary supplementation effects affected only the LC for MPROD.

A study was conducted in Mexico[66] with crossbred dairy ewes from four commercial farms to research those environmental factors that influence LC parameters using Pollot's 5-parameter additive model. The crossbred ewes were the progeny of East Friesian as the paternal line, and Suffolk, Pelibuey, Blackbelly, and Hampshire as the maternal line. The parameters estimated were the maximum milk secretion potential (MSmax), the relative rate of decline in cell number (DR), and the proportion of dead parenchyma cells at delivery. The effects of birth type, lambing number, herd, and lambing season on the total milk yield (TMY), lactation length, and estimated parameters of the Pollot model were analyzed. The herd had a significant effect ($P<0.05$) on most of the analyzed variables; the TMY was higher ($P<0.05$) in double lambing lactations than in those of single lambing. First-lambing ewes had a lower TMY than fourth-lambing ewes ($P<0.01$).

Likewise, in order to characterize the LC of ewes from the Bulgarian synthetic dairy population, taking into account the MPROD of the test day and the number of lambing, the following MPROD records of the Agricultural Institute-Shumen during the 2009-2019 period herd were analyzed[67]. For this purpose, a linear mixed model was used where the analytical hypothesis included the effects of year and month of lactation, lambing number, lambing type, test day (related to the LC), lactation period, permanent effect of environmental changes, genetic value of the animal, and residual environmental effects. A typical, relatively flat curve was found, which varied according to the day of the test and the number of births.

Climatic factors such as temperature, humidity, wind speed, and radiation are environmental elements that influence animal welfare and stress[68] and can affect various productive aspects such as growth, reproduction, and MPROD in ruminants[69]. In a study with Churra dairy ewes[70], these same factors, in addition to precipitation, affected the total milk production and milk quality, which exerted a direct influence on the LC.

A study was carried out in the Mediterranean region[71] to investigate the effect of heat stress on the MPROD of Valle del Belice sheep. The results indicated that there was an antagonistic effect between MPROD and heat stress, as the selection to increase the MPROD reduced the heat tolerance.

Similar results to the previous study were found in dairy cows from two regions of the USA[72], given that the selection to increase the MPROD remained constant up to a certain

point (threshold) and then exhibited a linear decreasing behavior as the value of the temperature-humidity index (THI), designed to measure heat stress, increased.

Cold stress has also been found to have a significant effect on the MPROD. In a study with Mediterranean Manchega dairy ewes[73] the effect of the exposure to adverse climate conditions (exposure to heat and cold) on MPROD was analyzed to measure the thermotolerance capacity of the sheep, as well as the degree of decrease in MPROD outside the thermal comfort zone. The results showed that cold stress had a greater negative effect on MPROD than heat stress.

Conclusions and implications

A lactation curve is the graphical representation of the behavior of the level of milk production of an individual, or a group of individuals, throughout lactation. The knowledge of a lactation curve is of utmost importance since it allows predicting the total milk production and making decisions on nutrition, health, and farm management, but, above all, it allows identifying the superior females in terms of milk production to be used in the herd as breeders. According to their shape, lactation curves can be typical (normal) and atypical. Lactation persistency is a phase closely linked to the lactation curve and represents the rate of decline in milk production after the peak lactation has been reached. Mathematical models have been developed to characterize lactation curves and study lactation persistence, mostly in cattle, although several have also been used in sheep. For selection purposes, both for the lactation curve and persistence, the heritability estimates reported in sheep show values that are in the low-medium range, which gives the confidence to expect positive responses in genetic improvement programs; these should be designed with clear, well-defined objectives, in addition to using the appropriate program methodology, based on the characteristics of the variables to be measured, the animal population, and the environment, and, finally, they should consider the potential influence of genetic and environmental factors in the response of the sheep to lactation curves. The scarcity of information on the lactation curves in ewes indicates the need to carry out more research on this species —not only on ewes of dairy breeds, but also on ewes of meat-producing breeds—, since the maternal ability to produce milk significantly influences the pre-weaning growth and survival of the offspring and impacts the profitability of the production system directly.

Acknowledgments

The authors are grateful to CONHACYT (National Council for Humanities, Science, and Technology of Mexico) for the scholarship granted to the first author (GCH) to pursue her PhD in Animal Science. This article belongs to the Knowledge Generation and Application Line (*LGAC*) *"Innovación Tecnológica y Seguridad Alimentaria en Ganadería"* ("Technological Innovation and Food Safety in Livestock").

Conflict of interest

The authors declare that they have no conflicts of interest.

Literature cited:

1. Haenlein GFW, Wendorff WL. Sheep Milk. In: handbook of milk of non-bovine mammals. Chapter 3: Young W. Park GFH. Haenlein, and William L. Wendorff, editors. Blackwell Publishers; 2006:137-194. https://doi.org/10.1002/9780470999738.ch7.

2. FAOSTAT. Producción de ovinos en el mundo. 2020. Consultado 31 Ago, 2022. http://www.fao.org/faostat/es/#data/QL/visualize.

3. Morris ST. Overview of sheep production systems. In: Ferguson D, Lee C, Fisher A, ediytors. Advances in sheep welfare; 1st. ed. Duxford, United Kingdom. Woodhead Publishing; 2017:19-35. https://doi.org/10.1016/C2015-0-00983-2.

4. Pulina G, Milán MJ, Lavín MP, Theodoridis A, Morin E, Capote J, *et al.* Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. J Dairy Sci 2018;101(8):6715-6729. https://doi.org/10.3168/jds.2017-14015.

5. Flis Z, Molik E. Importance of bioactive substances in sheep's milk in human health. Int J Mol Sci 2021;22(9):4364. https://doi.org/10.3390/ijms22094364.

6. Thomas DL, Haenlein GFW. Sheep Milk. 3.1 Production of sheep milk. In: Handbook of milk of non-bovine mammals. Young W. Park GFH Haenlein, and William L Wendorff, editors. John Wiley & Sons Ltd; 2017:181-209. https://doi.org/10.1002/9780470999738.ch7.

7. Hamann H, Horstick A, Wessels A, Distl O. Estimation of genetic parameters for test day milk production, somatic cell score and litter size at birth in East Friesian ewes. Livest Prod Sci 2004;87(2-3):153-160. https://doi.org/10.1016/j.livprodsci.2003.09.015.

8. Barillet F, Marie C, Jacquin M., Lagriffoul G, Astruc JM. The French Lacaune dairy sheep breed: use in France and abroad in the last 40 years. Livest Prod Sci 2001;71(1):17-29. https://doi.org/10.1016/S0301-6226(01)00237-8.

9. Gelasakis AI, Arsenos G, Valergakis GE, Oikonomou G, Kiossis E, Fthenakis GC. Study of factors affecting udder traits and assessment of their interrelationships with milking efficiency in Chios breed ewes. Small Ruminant Res 2012;103(2-3):232-239. https://doi.org/10.1016/j.smallrumres.2011.09.045.

10. Sanna SR, Carta A, Casu S. Genotype by environment interaction for milk yield in Sarda dairy sheep. J Anim Breed Genet 2002:119:190-199. https://doi.org/10.1046/j.1439-0388.2002.00337.x.

11. Ramón M, Legarra A, Ugarte E, Garde JJ, Pérez-Guzmán MD. Economic weights for major milk constituents of Manchega dairy ewes. J Dairy Sci 2010;93(7):3303-3309. https://doi.org/10.3168/jds.2009-2787.

12. Pérez-Álvarez JG, Rodríguez-Almeida FA, Domínguez-Viveros J. Genetic parameter estimates for productivity of the Katahdin and Hampshire ewe and its components. J Anim Sci 2016;94(5):836-848. https://doi.org/10.2527/jam2016-1715.

13. Bayril T, Akdemir F, Baran MS, Orhan C, Yildirim IH, Yildiz AŞ, Çelik R. Effects of lamb sex, parity, and birth type on milk yield, lactation length, and milk components in Zom ewes raised under semi-intensive conditions. South African J Anim Sci 2023; 53(2):185-196. http://dx.doi.org/10.4314/sajas.v53i2.05.

14. Akers RM. Lactation and the mammary gland. Wiley-Blackwell; 2016.

15. Lérias JR, Hernández-Castellano LE, Suárez-Trujillo A, Castro N, Pourlis A, Almeida AM. The mammary gland in small ruminants: major morphological and functional events underlying milk production–a review. J Dairy Res 2014;81(3):304-318. https://doi.org/10.1017/S0022029914000235.

16. Dongre VB, Gandhi RS, Avtar S, Atul G. A brief review on lactation curve models for predicting milk yield and different factors affecting lactation curve in dairy cattle. Int J Agric Res Review 2011;1(1): 6-15.

17. Abecia JA, García A, Castillo L, Palacios C. The effects of weather on milk production in dairy sheep vary by month of lambing and lactation phase. J Anim Behav Biometeorol 2017;5(2):56-63. http://dx.doi.org/10.26667/2318-1265jabb.v5n2p56-63.

18. Wood PDP. A note on the estimation of total lactation yield from production on a single day. Anim Prod 1974;19(3):393-396. https://doi.org/10.1017/S0003356100022972.

19. Yépez-Ruidías H, Rúa-Bustamante CV, Idárraga-Idárraga Y, Arboleda-Zapata E, Calvo Cardona SJ, Montoya Atehortúa A, *et al.* Estimación de las curvas de lactancia y producción de leche de cabras del departamento de Antioquia, usando controles lecheros quincenales y mensuales. Rev CES Med Vet Zoot 2010;5(2):30-35.

20. Chang YM, Rekaya R, Gianola D, Thomas DL. Genetic variation of lactation curves in dairy sheep: A Bayesian analysis of Wood's function. Livest Prod Sci 2001;71(2-3):241-251. https://doi.org/10.1016/S0301-6226(01)00185-3.

21. Palacios-Espinosa A, González-Peña Fundora D, Guerra-Iglesias D, Espinoza-Villavicencio JL, Ortega-Pérez R, Guillén-Trujillo A, *et al*. Curvas de lactancia individuales en vacas Siboney de Cuba. Rev Mex Cienc Pecu 2016;7(1):15-28. http://www.scielo.org.mx/scielo.php?.

22. Ángeles-Hernández JC, Aranda-Aguirre E, Muñoz-Benítez AL, Chay-Canul AJ, Albarrán-Portillo B, Pollott GE, *et al*. Physiology of milk production and modelling of the lactation curve. CAB Reviews 2021;60(056):1-22. doi: 10.1079/PAVSNNR202116056.

23. Macciotta NPP, Dimauro C, Steri R, Cappio-Borlino A. Mathematical modelling of goat lactation curves. In*:* Dairy goats feeding and nutrition. Cannas A, Pulina G, Dias Francesconi AH, editors. CABI International 2008:31-46.

24. Druet T, Jaffrézic F, Boichard D, Ducrocq V. Modeling lactation curves and estimation of genetic parameters for first lactation test-day records of French Holstein cows. J Dairy Sci 2003;86(7):2480-2490. https://doi.org/10.3168/jds.S0022-0302(03)73842-9.

25. Wood PDP. Algebraic model of the lactation curve in cattle. Nature 1967;216:164-165. https://doi.org/10.1038/216164a0.

26. Shimizu H, Umrod S. An application of the weighted regression procedure for constructing the lactation curve in dairy cattle. Jap J Zootech Sci 1976;47(12):733-738.

27. Waheed A, Khan MS. Lactation curve of Beetal goats in Pakistan. Arch Anim Breed 2013;56(1):892-898. https://doi.org/10.7482/0003-9438-56-089.

28. Aytekin I, Boztepe S. The key to successful dairy cattle: days in milk (DIM). Selcuk J Agric Food Sci 2017;31(3):184-188. doi:10.15316/SJAFS.2017.53.

29. Fahmy MH, Shrestha JNB. Dairy Animals/Sheep Breeds. In*:* Encyclopedia of dairy sciences. Roginski, H, editor. Academic Press. 2002: 598-613. https://doi.org/10.1016/B0-12-227235-8/00112-7.

30. Chaffer M, Leitner G, Zamir S, Winkler M, Glickman A, Ziv N, Saran A. Efficacy of dry-off treatment in sheep. Small Ruminant Res 2003;47(1):11-16. https://doi.org/10.1016/S0921-4488(02)00194-3.

31. Petridis IG, Mavrogianni VS, Fragkou IA, Gougoulis DA, Tzora A, Fotou K, *et al*. Effects of drying-off procedure of ewes' udder in subsequent mammary infection and development of mastitis. Small Ruminant Res 2013;110(2-3):128-132. http://dx.doi.org/10.1016/j.smallrumres.2012.11.020.

32. Sougaris S, Brozos CN, Petridou E, Papadopoulos T, Kiossis E. Abrupt and gradual drying-off procedure and intramammary dry treatment: Impact on udder health status of Chios breed dairy sheep. J Hellenic Vet Med Soc 2022;73(2):4031-4040. https://doi.org/10.12681/jhvms.26304.

33. Vilar MJ, Rajala-Schultz PJ. Dry-off and dairy cow udder health and welfare: Effects of different milk cessation methods. The Veterinary J 2020;262:105503 http://dx.doi.org/10.1016/j.tvjl.2020.105503.

34. Persson-Waller K, Landin H, Nyman AK. Herd routines and veterinary advice related to drying-off and the dry period of dairy cows. Dairy 2022;3(2):377-399. https://doi.org/10.3390/dairy3020029.

35. Grossman M, Hartz SM, Koops WJ. Persistency of lactation yield: A novel approach. J Dairy Sci 1999;82(10):2192-2197. https://doi.org/10.3168/jds.s0022-0302(99)75464-0.

36. Grossman M, Koops WJ. Modeling extended lactation curves of dairy cattle: A biological basis for the multiphasic approach. J Dairy Sci 2003;86(3):988-998. https://doi.org/10.3168/jds.S0022-0302(03)73682-0.

37. Macciota NPP, Dimauro C, Rassu SPG, Steri R, Pulina G. The mathematical description of the lactation curves in dairy cattle. Italian J Anim Sci 2016;10:4. https://doi.org/10.4081/ijas.2011.e51.

38. Bilgin OC, Esenbuga N, Davis ME. Comparison of models for describing the lactation curve of Awassi, Morkaraman and Tushin sheep. Arch Anim Breed 2010;53(4):447-456. https://doi.org/10.5194/aab-53-447-2010.

39. Neal HDSC, Thornley JHM. The lactation curve in cattle: a mathematical model of the mammary gland. J Agric Sci Camb 1983;101:389-400. http://dx.doi.org/10.1017/S00218596000337710.

40. Friggens NC, Emmans GC, Veerkamp RF. On the use of simple ratios between lactation curve coefficients to describe parity effects on milk production. Livest Prod Sci 1999;62(1):1-13. https://doi.org/10.1016/S0301-6226(99)00110-4 .

41. Adediran SA, Ratkowusky DA, Donaghy DJ, Malau-Aduli AEO. Comparative evaluation of a new lactation curve model for pasture-based Holstein-Friesian dairy cows. J Dairy Sci 2012;95:5344-5356. http://dx.doi.org/10.3168//jds.2011-4663.

42. Vetharaniam I, Davis SR, Upsdell M, Kolver ES, Pleasants AB. Modeling the effect of energy status on mammary gland growth and lactation. J Dairy Sci 2003;86(10):3148-3156. https://doi.org/10.3168/jds.S0022-0302(03)73916-2.

43. Pollott GE, Gootwine E. A genetic analysis of complete lactation milk production in Improved Awassi sheep. Livest Prod Sci 2004;71:37–47. http://dx.doi.org/10.1016/S0301-6226(01)00239-1.

44. Afolayan RA, Abubakar BY, Dim NI, Osinowo OA. Inheritance of growth and lactation parameters in Yankasa sheep. Nigerian J Anim Prod 2001;28(1):9-13.

45. Gaines WL. Measures of persistency of lactation. J Agric Res 1927;34(4):373-383.

46. Sturtevant EL. Influence of distance from calving on milk yield. N.Y. State Agr Expt Sta Ann Rept 1887;5:26-28.

47. Gengler N. Use of mixed models to appreciate the persistency of yields during the lactation of milk cows [Ph.D. Diss]. Gembloux, Belgium: Faculté Univ Sci Agron. 1995.

48. Gengler N. Persistency of lactation yields: a review. Interbull Bulletin 1996;12:87-96.

49. Cole JB, Null DJ. Genetic evaluation of lactation persistency for five breeds of dairy cattle. J Dairy Sci 2009;92(5):2248-2258. https://doi.org/10.3168/jds.2008-1825.

50. Gutiérrez JP, Legaz E, Goyache F. Genetic parameters affecting 180-days standardised milk yield, test-day milk yield and lactation length in Spanish Assaf (Assaf. E) dairy sheep. Small Ruminant Res 2007;70(2-3):233-238. https://doi.org/10.1016/j.smallrumres.2006.03.009.

51. Dekkers JCM, Ten Hag, JH, Weersink A. Economic aspects of persistency of lactation in dairy cattle. Livest Prod Sci 1998;53:237-252. https://doi.org/10.1016/S0301-6226(97)00124-3.

52. Dobson H, Smith RF, Royal MD, Knight CH, Sheldon IM. The high producing dairy cow and its reproductive performance. Reprod Dom Anim 2007;42(Suppl 2):17-23. https://doi.org/10.1111%2Fj.1439-0531.2007.00906.x.

53. Schutz MM, Hansen LB, Steuernagel GR, Kuck AL. Variation of milk, fat, protein, and somatic cells for dairy cattle. J Dairy Sci 1990;73:484-493. https://doi.org/10.3168/jds.S0022-0302(90)78696-1.

54. Torshizi ME, Mashhadi MH, Farhangfar H. Different aspects of lactation persistency in dairy cows. Indian J Anim Sci 2019;89(6):607-614. https://doi.org/10.56093/ijans.v89i6.91098.

55. Kominakis AP, Volanis M, Rogdakis E. 1999. Genetic and phenotypic parameters of milk yield in Sfakia sheep. In: Proc 15th Scient Meet Hellenic Zootech Soc Chania, Greece. November 3-5, 1999.

56. Kominakis AP, Rogdakis E, Koutsotolis K. Genetic aspects of persistency of milk yield in Boutsico dairy sheep. Asian-Aust J Anim Sci 2002;15(3):315-320. https://doi.org/10.5713/ajas.2002.315.

57. Bonnier G. Is the shape of the lactation curve genetically determined?. Hereditas 1935;10:199-213.

58. Cappio-Borlino A, Macciotta NPP, Pulina G. The shape of Sarda ewe lactation curve analysed by a compartmental model. Livest Prod Sci 1997;51(1-3):89-96. https://doi.org/10.1016/S0301-6226(97)00102-4.

59. Millanao I, Herdener N, Parada D, Sepúlveda N. Producción de leche, curvas de lactancia y crecimiento de sus corderos, en dos razas de ovejas en la región de la Araucanía, Chile. APPA – ALPA, Cusco, Perú. Sitio Argentino de Producción Animal 2007;1-4.

60. Komprej A, Gorjanc G, Kompan D, Kovač M. Lactation curves for milk yield, fat, and protein content in Slovenian dairy sheep. Czech J Anim Sci 2012;57(5):231-239. doi: 10.17221/5921-CJAS.

61. Ángeles-Hernández JC, Guerrero-Loredo A, Solís-Guzmán DA, Ramírez-Pérez AH, Ángeles-Campos S, González-Ronquillo M. Efecto del grupo genético sobre las características de la curva de lactación ovina. Ecosist Rec Agrop 2018;5(14):327-333. https://doi.org/10.19136/era.a5n14.1425.

62. Carta A, Sanna SR, Casu S. Estimating lactation curves and seasonal effects for milk, fat and protein in Sarda dairy sheep with a test day model. Livest Prod Sci 1995;44:37-44. https://doi.org/10.1016/0301-6226(95)00051-L.

63. Cappio-Borlino A, Pulina G, Rossi G. A non-linear modification of Wood's equation fitted to lactation curves of Sardinian dairy ewes. Small Ruminant Res 1995;18(1):75-79. https://doi.org/10.1016/0921-4488(95)00713-U.

64. Portolano B, Spatafora F, Bono G, Margiotta S, Todaro M, Ortoleva V, *et al.* Application of the Wood model to lactation curves of Comisana sheep. Small Ruminant Res 1997;24(1):7-13. https://doi.org/10.1016/0921-4488(95)00842-X.

65. Cappio-Borlino A, Portolano B, Todaro M, Macciotta NPP, Giaccone P, Pulina G. Lactation curves of Valle del Belice dairy ewes for yields of milk, fat, and protein estimated with test day models. J Dairy Sci 1997;80(11):3023-3029. https://doi.org/10.3168/jds.S0022-0302(97)76269-6.

66. Ángeles-Hernández JC, Pollot G, Albarrán-Portillo B, Ramírez-Pérez AH, Lizarazo-Chaparro A, Castelán-Ortega OA, *et al*. The application of a mechanistic model to analyze the factors that affect the lactation curve parameters of dairy sheep in Mexico. Small Ruminant Res 2018;164:58-63. . https://doi.org/10.1016/j.smallrumres.2018.05.003

67. Stancheva N, Angelova T, Yordanova D, Krastanov J. Lactation curve of the sheep from Bulgarian dairy synthetic population. Trad Mod Vet Med 2021;6(2):64-71. doi: 10.5281/zenodo.5741398.

68. Naskar S, Gowane GR, Chopra A, Paswan C, Prince LLL. Chapter 13: Genetic adaptability of livestock to environmental stresses. In: Sejian V, *et al*, editors. Environmental stress and amelioration in livestock production. Springer Berlin Heidelberg;2012:317-378. https://doi.org/10.1007/978-3-642-29205-7-13.

69. Silanikove N. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest Prod Sci 2000;67:1-18. https://doi.org/10.1016/S0301-6226(00)00162-7.

70. González-Ronquillo M, Abecia JA, Gómez R, Palacios C. Effects of weather and other factors on milk production in the Churra dairy sheep breed. J Anim Behav Biometeorol 2021:9:2125. https://doi.org/10.31893/jabb.21025.

71. Finocchiaro R, van Kaam JBCHM, Portolano B. Effect of weather conditions on somatic cell score in Sicilian Valle del Belice ewes. Ital J Anim Sci 2007;6(Suppl. 1):130-132. https://doi.org/10.4081/ijas.2007.1s.130.

72. Bohmanova J, Misztal I, Cole JB. Temperature-humidity indices as indicators of milk production losses due to heat stress. J Dairy Sci 2007;90:1947-1956. https://doi.org/10.3168/jds.2006-513.

73. Ramón M, Díaz C, Pérez-Guzmán MD, Carabaño MJ. Effects of exposure to adverse climatic conditions on production in Manchega dairy sheep. J Dairy Sci 2016;99(7):5764-5779. https://doi.org/10.3168/jds.2016-10909.

https://doi.org/10.22319/rmcp.v15i4.6599

Technical note

Carrying capacity of white-tailed deer (*Odocoileus virginianus texanus*) in northeast Mexico

Fernando Isaac Gastelum Mendoza [a]

Fernando Noel González Saldívar [a]

Eloy Alejandro Lozano Cavazos [b]

José Isidro Uvalle Sauceda [a]

Guillermo Romero Figueroa [c]

Enrique de Jesús Ruíz Mondragón [c]

César Martín Cantú Ayala [a*]

[a] Universidad Autónoma de Nuevo León. Facultad de Ciencias Forestales. Linares, 67700 Nuevo León, México.

[b] Universidad Autónoma Agraria Antonio Narro. Saltillo, Coahuila, México.

[c] Universidad Autónoma de Baja California. Facultad de Ciencias, Ensenada, Baja California, México.

*Corresponding author: cantu.ayala.cesar@gmail.com

Abstract:

White-tailed deer (*Odocoileus virginianus texanus*) is widely distributed in the desert scrublands of Mexico and is both ecologically and economically important. Knowledge about forage production in deer habitat is essential to designing effective management plans. It was used aboveground biomass production in microphyllous desert scrub to calculate carrying capacity (K) for white-tailed deer as a reference in extensive management of this species in the municipality of Monclova, Coahuila, in northern Mexico. Data were collected at the

Rancho San Juan Wildlife Conservation Management Unit during a full seasonal round: October 2018 to August 2019. The Adelaide method was used to estimate production in high, middle and low vegetation strata. Estimation of K was based on the Holechek model. Average seasonal biomass production was 621.19 kg DM ha^{-1}. Production was highest in the middle stratum (377.77 ± 73.92 kg DM ha^{-1}), and lowest in the high stratum (37.59 ± 23.59 kg DM ha-1). Production was highest in summer (744.35 kg DM ha^{-1}) and fall (607.93 kg DM ha^{-1}). Estimated K was 4.94 ha per deer annually, equivalent to 209 deer in a 1,030 ha area. Aerial census of the deer population in October 2020 recorded a density of 1.77 ha per deer, or 582 deer in the same area. Although this is more than double the calculated K for the study area, it highlights the role of supplementary feed and water in maintaining deer population growth.

Key words: Aerial biomass, Density, Microphyllous desert scrub, Adelaide method, Holechek model.

Received: 13/05/2024

Accepted: 28/06/2024

Sustainable management of white-tailed deer (*Odocoileus virginianus texanus*) habitat involves assessing its carrying capacity (K). This parameter, linked to habitat conditions, is defined as the number of animals that rangeland can support per unit of surface area without causing deterioration in the plant community or other resources[1]. It is useful for identifying fluctuations in natural forage production and quality[2]. Carrying capacity varies constantly in time and space. It is influenced by density-independent factors such as the availability of food, water, cover and usable space, as well as by density-dependent factors related to animal density per unit area[3].

Of the models used to estimate K, those that incorporate forage production generate values useful to rangeland managers in northern Mexico[1,3]. Trends in plant biomass production and forage utilization, the latter defined as the amount of organic matter per unit of surface area used as an energy source by herbivores[1], are significant elements in the concept of K because, for deer, a decline in forage production indicates that animal density is exceeding K[4]. Unlike climate, the habitat elements of forage availability and production can be influenced by habitat improvement techniques. Of course, model results are estimates[3]. It is also best to produce region-specific estimates to calculate K in managed white-tailed deer populations and, because forage production conditions vary by region, to avoid utilizing K results from one region in other regions and ecosystem types[4].

White-tailed deer population density varies geographically. For example, in the United States, the Edwards Plateau in the state of Texas boasts the highest density of white-tailed deer in the world: more than 0.45 deer ha^{-1}[3]. In contrast, parts of the High Plains and Rolling Plains regions support less than 0.15 deer ha^{-1}. Deer densities in the Cross Timbers and South Texas Plains regions range from 0.15 to 0.30 deer ha^{-1}[3]. Although poaching and habitat degradation in northern Mexico drove white-tailed deer near extinction in the 1970s, current population densities are estimated to range between 0.10 and 0.20 deer ha^{-1}[5]. In Tlachichila in the Sierra El Laurel mountains, in the state of Zacatecas, Mexico, deer density is 0.03 deer ha^{-1}[6].

Carrying capacity also varies within Mexico. In the La Michilía Biosphere Reserve, in the state of Durango, estimated K is 0.22 deer ha^{-1}[7]. In dry tropical forest in the state of Jalisco, estimated K is 0.16 to 0.18 deer ha^{-1}[8], and in the Mixteca region, it is 0.10 deer ha^{-1}[9]. White-tailed deer may be one of the most studied herbivore species in North America, but it is still essential to individually assess each area where it is managed extensively. This is particularly relevant in fragmented habitats because K is influenced by the size and connectivity of preserved habitat fragments[8].

Available forage directly influences herbivore population trends, so K based on forage production provides the most practical conceptual foundation for extensive deer management[6,9]. The present study objective was to estimate seasonal forage production in microphyllous desert scrub vegetation in eastern Coahuila, Mexico, and calculate K for white-tailed deer to produce data for use in management plans and habitat conservation strategies for this species in northeastern Mexico.

The study was done in the Rancho San Juan Wildlife Conservation Management Unit (UMA) (26° 49’ 31.11” N, 101° 01’ 57.77” W), in the municipality of Monclova, in the state of Coahuila, Mexico. This UMA is located 38 km east of the municipal seat and 43 km west of the municipality of Candela. The predominant vegetation types in this area are microphyllous desert scrub and rosetophyllous desert scrub, with some open medium grassland associations[10]. The climate is dry (BSohw), with an average temperature of 21°C. Annual precipitation varies from 200 mm to 900 mm, and elevation ranges from 600 to 1,000 m asl[11]. Extensive management of a population of Texas white-tailed deer is done in a 1,030 ha area at this UMA.

To estimate K values, forage production was evaluated over a full seasonal round: fall (October 2018), winter (February 2019), spring (May 2019) and summer (August 2019). Using the Adelaide method, forage production was expressed as the amount of aerial biomass per plant stratum[12]. Forage production in the high stratum (>1.5 m) was quantified in 18 parcels covering 50 m^2, and for the middle stratum (<1.5 m) 18 plots of 25 m^2 were used. To estimate the number of units of each specimen and the sampled species in each plot, a

representative unit of each plant per species was selected considering its leaf shape and density. In the lower stratum, grasses and herbaceous plants, production was quantified by completely harvesting 18 plots of 1 m^2, according to the methodology proposed by Chávez[13]. All biomass samples (grasses and herbaceous plants, plus the reference units) were placed in paper bags, labeled and dried in an oven (120VAC 60HZ INOX) at 75 °C to constant weight. After drying, sample dry weight was measured using a scale (ENTRIS 8201-1S). These data were used to calculate biomass production with the equation[12]:

$$Bt = Wd * ni$$

Where: Bt = total aerial biomass (kg DM ha^{-1}); Wd = dry weight of each manual sample; ni = number of replicates of species i inside each parcel.

Seasonal differences in biomass production were calculated using a non-parametric Kruskal-Wallis test ($\alpha \leq 0.05$) run with RStudio[14].

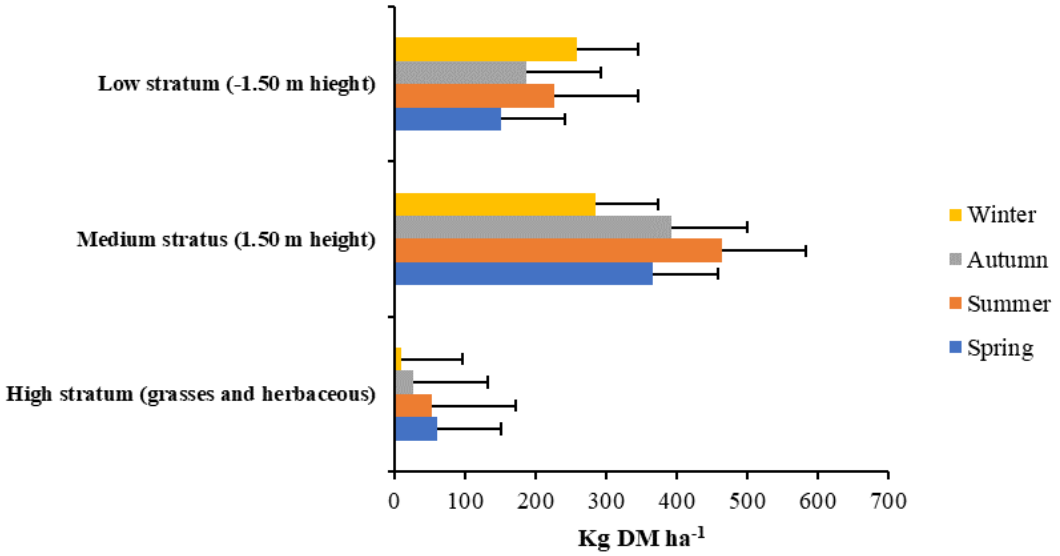
Carrying capacity was calculated with the forage production results, and expressed as the number of animals per hectare (deer ha^{-1}) per year. Only 25 % of the area's total biomass production was used in the calculation because this conservative value allows for a sustainable estimation of animal load with the goal of maintaining long-term, stable forage production[1]. Calculation of K was done using study area surface area, available biomass, live deer weight and daily dry matter intake (%), with the equation[1]:

$$K = \frac{LW \times DIDM \times GC}{DMP \times 0.25}$$

Where: K= carrying capacity (deer ha^{-1}); LW= animal live weight (kg); DIDM= daily intake dry matter (3 % LW); GC= grazing cycle (365 d); DMP= dry matter production (kg ha^{-1}); 0.25= forage utilization percentage (25 %).

Average annual forage production in the studied microphyllous desert scrub was 2,484.77 kg DM ha^{-1}. Estimated average biomass production per season was 621.20 ± 85.08 kg DM ha^{-1}. Biomass production was highest in the summer (744.36 ± 44.20 kg) and fall (607.93 ± 57.77 kg), and lowest in the winter (553.36 ± 50.12 kg) (Figure 1). All these seasonal production levels are relatively low compared to the 1,501 ± 492.35 kg DM ha^{-1} per season reported in a study in the state of Tamaulipas[15], and the 929.2 ± 401.64 kg reported in Zacatecas[6].

Figure 1: Aerial biomass production by vegetation stratum and season at the Rancho San Juan UMA, Monclova, Coahuila, Mexico.



Lines extending to right of colored bars indicate standard error.

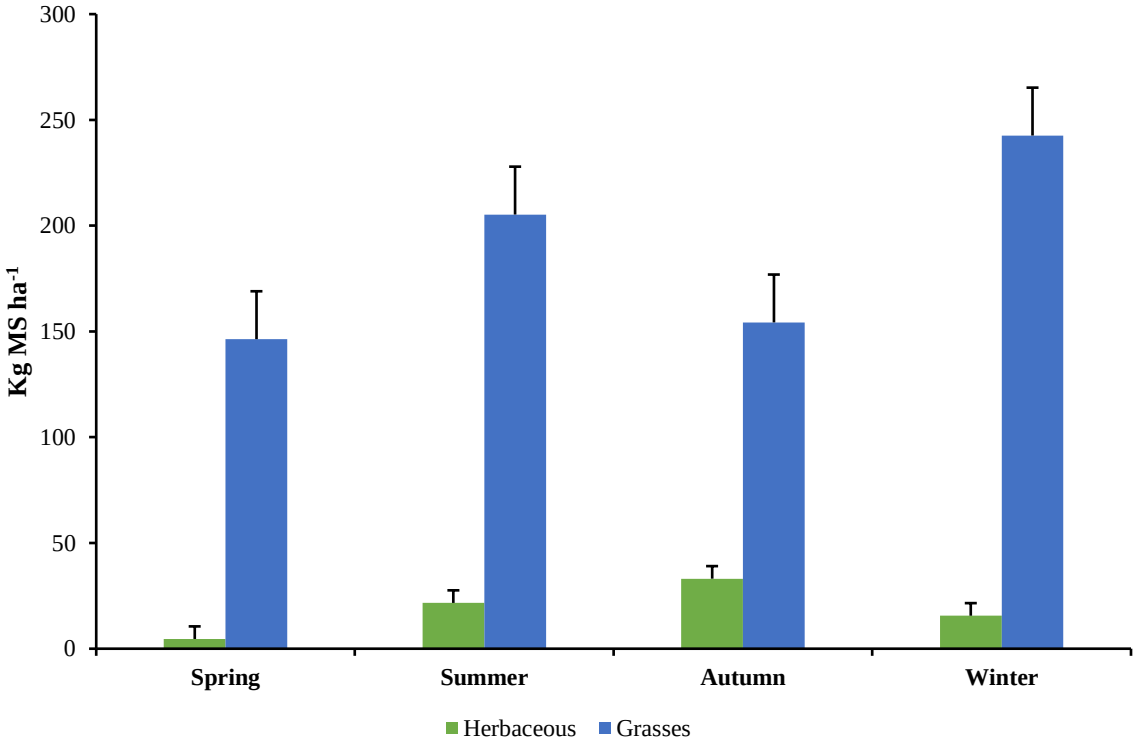
Habitat biomass production directly affects deer development and, therefore, the low production observed here had a direct impact on vegetation K. An adult deer requires a daily biomass intake of 2 to 4 % of its body weight (60 kg average for adult animal and 0.13 animal unit)[3,4,16]. However, this intake varies in response factors such as physiological state, animal age, nutritional value of available plants, forage species composition and forage spatial distribution[17].

An example of this is that adult male body weight increases rapidly during spring, which increases their forage intake[16]. But this coincides with the season of lowest natural forage availability (Figure 1). To stabilize the deer population during this time of year, food supplementation strategies are implemented at the Rancho San Juan UMA. A total of 39 feeders are installed to supplement nutrients from natural sources at critical times of the year, normally from the first half of March to the end of October. During this period, the forage species preferred by deer are scarce. At the feeders, deer have free access to feed consisting of pellets containing 18 % crude protein (CP) and cotton seed. This feed helps to promote muscle gain, antler development, and milk production[4].

The seasonal variations in above-ground biomass production observed here (Figure 1) coincide with seasonal fluctuations in forage intake by deer reported for South Texas. In this region, a drop in forage intake occurs in the summer, followed by an increase in the fall, and another decline in the winter[18].

Among the three vegetation strata, the middle stratum contributed the most to aboveground biomass in all four seasons. This stratum's contribution was highest in the summer (Figure 1; 1,858.52 kg DM ha^{-1}), largely due to relatively high rainfall (80 mm) in July 2019. The low stratum (grasses and herbaceous plants) produced the largest amount of aboveground biomass during the winter (Figure 2; 1,032.70 kg DM ha^{-1}). The high stratum produced the lowest contribution overall: ≤ 250 kg (Figure 1).

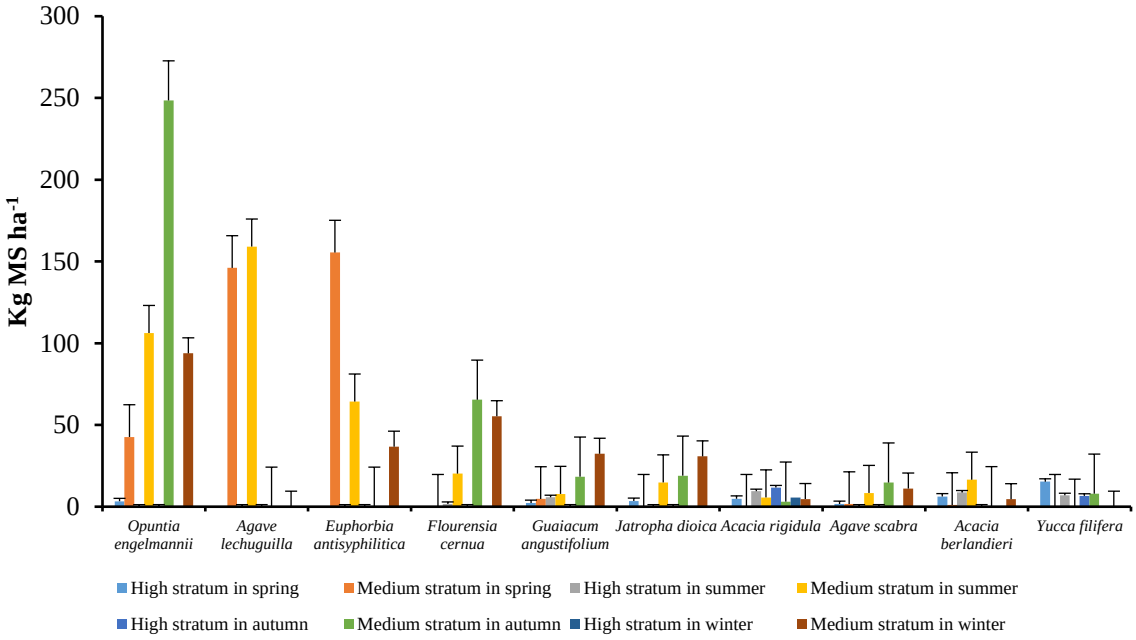
Figure 2: Seasonal dry matter production of grasses and herbaceous plants at the Rancho San Juan UMA, Monclova, Coahuila, Mexico



Lines extending to right of colored bars indicate standard error.

During the spring, the plants *Euphorbia antisyphilitica* and *Agave lechuguilla* provided the highest vegetal biomass contribution (Figure 3), but in conjunction only represented 3.86 % of the white-tailed deer diet in this season[19]. However, the cactus *Opuntia engelmannii*, an important forage species for white-tailed deer in northeastern Mexico[4,19], was among the ten species with the highest biomass production in spring. In contrast, the perennial shrub *Eysenhardtia texana*, another important deer forage plant, made the lowest biomass contribution (0.06 kg DM ha^{-1}) in spring.

Figure 3: The ten high- and middle-stratum plant species with the highest seasonal biomass contribution at Rancho San Juan UMA, Monclova, Coahuila, Mexico



Lines extending above the colored bars indicate standard error.

In summer, *A. lechuguilla* and *O. engelmannii* made the greatest contribution to biomass (Figure 3; 265.35 kg DM ha^{-1}), although *O. engelmannii* accounted for a relatively low proportion (4.29 %) of the deer diet[19]. *Acacia rigidula*, another important forage species in the summer (12.57 %)[19], did not have a particularly high biomass production.

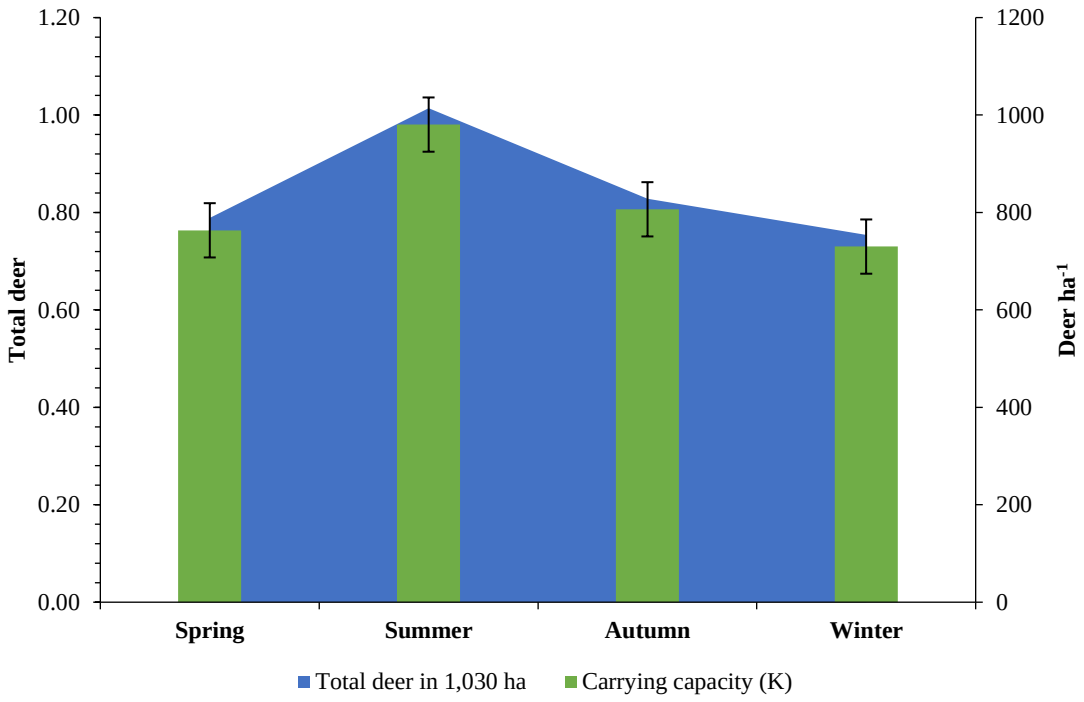
In autumn, *O. engelmannii* contributed most to total biomass production (40.87 %) (248.49 kg DM ha^{-1}) and accounted for 15.40% of the deer diet (Figure 3)[19]. In winter, *O. engelmannii* contributed 16.95 % (93.80 kg) of the biomass production, and 14.90 % of the deer diet[19]. Although important as a deer forage in autumn[4], *A. rigidula* contributed only 2.44 % (14.85 kg DM ha^{-1}) of the biomass production in this season. Similarly, *Leucophyllum frutescens* provided only 0.01 % (3.07 kg DM ha^{-1}) of biomass production in autumn but was 10.61% of the deer diet[19,20].

White-tailed deer show a marked preference for consumption of shrub leaves and stems since these plant elements have a higher protein content and relatively lower fiber and lignin levels[4]. Scrubland is a major source of shrub biomass year round (Figure 1), but primarily in the summer, when does require more forage to produce milk for fawns[3]. Grass and herbaceous plant production is also essential since deer resort to them when facing competition for food[4]. Grasses with high lignin content and a low digestibility percentage (1.4) show a higher average aboveground biomass production (187.11 ± 45.28 kg DM ha^{-1})

per season, and represent up to 43.84 % of total winter biomass production (Figure 2). Of note is that *Acacia berlandieri* and *A. rigidula*, both scrubland plants preferred by deer[4,19,20], have average seasonal biomass production (18.79 ± 15.13 kg DM ha^{-1}) that is <5.5 % of their average seasonal aerial aboveground biomass production (621 ± 85.08 kg DM ha^{-1}).

Aboveground biomass production was used in estimating K for white-tailed deer. In the present results, overall K was calculated as 0.2 (± 0.15) deer ha^{-1}, equivalent to 209 deer in 1,030 ha (Figure 4). It was higher in spring (0.76 deer ha^{-1}) and winter (0.73 deer ha^{-1}). An aerial deer population survey done in October 2020 (parallel N/S transects of variable length at 200 m spacing) estimated a density 0.57 deer ha^{-1} (i.e., 582 deer) in 1,030 ha. This deer density is over twice the calculated K for this UMA. The studied population has apparently grown beyond habitat K in response to the supplementary feed and water supplied by unit managers, however, deer in this UMA have been documented eating native grasses, suggesting forage overuse[3]. In addition, plant species known to be key in the deer diet, such as *A. rigidula* and *A. berlandieri*, contributed significantly less to biomass production than the grasses.

Figure 4: Carrying capacity (K) for white-tailed deer at the Rancho San Juan UMA, Monclova, Coahuila, Mexico



Lines extending above the bars indicate standard error.

The K calculated in the present study (0.2 deer ha^{-1}) is lower than the 0.03 deer ha^{-1} reported for the Sierra El Laurel, in Tlachichila, Zacatecas[6], but similar to the 0.22 deer ha^{-1} reported for the La Michilía Biosphere Reserve, Durango[7]. Lower K values have been reported for dry tropical forest in Jalisco (0.16 and 0.18 deer ha^{-1})[8] and the Mixteca region (0.16 and 0.18 deer ha^{-1})[9]; in both these regions deer population density is below K.

Sampling methods can introduce error into estimates of K based on forage biomass. For example, sampling strategies often do not consider herbivore grazing patterns[21]. In addition, the 25 % standard forage use value (considered a conservative value because it prevents overestimation of the number of animals per unit area that a habitat can sustainably support)[22,23] included in calculation of K does not contemplate losses due to trampling, and forage contamination by feces, rodents and insects. In a study done in the state of Utah, United States of America, 23 % trampling losses of available forage were reported[5], and in a study in desert grasslands, small mammals were found to consume up to 20 % of the forage available to large herbivores[4].

Estimates of K should be considered as approximations, but are useful as indicators to guide management decisions for white-tailed deer habitat[3]. Management decisions should be based on temporal trends in K capacity; a decrease in K means animal load could need to be reduced[24]. In addition, calculations of the relationship between forage production in forage species and forage nutritional value are useful in refining estimates of K, and should be done annually.

Estimates of animal carrying capacity and aboveground biomass production are valuable in extensive management of white-tailed deer populations in northeastern Mexico. Forage production varies by vegetation stratum and season of the year, with the highest values observed in the middle stratum in summer and fall. The carrying capacity calculated for the Rancho San Juan UMA during the study period was higher than some previous reports in Mexico. Although the population density exceeded the ecosystem's carrying capacity, feed supplementation strategies may have been acting as buffers, preventing forage overuse and consequent population decline. Future research can focus on evaluating how the use of supplemental feed and water might affect ecosystem carrying capacity.

Acknowledgements

Thanks to Gerardo Benavides Pape, owner of Rancho San Juan, for his help and support. Thanks are also due ranch operations personnel, especially Vladimir Lara Ramírez. Thanks also to the technical personnel at the Faculty of Forestry Sciences, Universidad Autónoma de Nuevo León, particularly Leonel Reséndiz Dávila and Dr. Eduardo Estrada Castillón.

Conflict of interests

The authors declare no conflict of interests.

Literature cited:

1. Holechek JL, Pieper RD, Herbel CH. Range management: principles and practices. 4th. ed. Upper Saddle River, NJ: Prentice Hall; 2001.

2. Gastelum-Mendoza FI, Estrada-Castillón EA, Cantú-Ayala CM, González-Saldívar FN, Serna-Lagunes R, Salazar-Ortíz J. Methodologies for estimating wildlife habitat quality: review and examples. Agroproductividad 2020;13(6):37–42.

3. Fulbright TE, Ortega JA. White-tailed deer habitat: ecology and management in rangelands. Texas A&M University Pres. College Station, TX, USA; 2006.

4. Ramírez-Lozano RG. Alimentación del venado cola blanca: biología y ecología nutricional. Facultad de Ciencias Biológicas, Alimentos-Universidad Autónoma de Nuevo León. San Nicolás de los Garza, Nuevo León, México; 2012.

5. Villarreal JG. Venado cola blanca (manejo y aprovechamiento cinegético). Unión Ganadera Regional de Nuevo León. Impresora Monterrey. Monterrey, NL., México; 2000.

6. Navarro JA, Olmos G, Palacio J, Clemente F, Vital C. Dieta, población y capacidad de carga del venado cola blanca (*Odocoielus virginianus*) en dos condiciones de hábitat en Tlachichila, Zacatecas, México. Agroproductividad 2018;11(6):15–23.

7. Gallina SA. White-tailed deer and cattle diets in La Michilía, Durango, Mexico. J Range Management 1993;46(6):487–492.

8. Mandujano S. Precipitación, capacidad de carga y potencial de uso de los ungulados en un bosque tropical seco. En Espinosa ME, Lorenzo C, editores. Avaneces Mastozoológicos (637-660). Vol. 2. AMMAC, México, DF; 2008.

9. Villarreal-Espino O. El venado cola blanca en la mixteca poblana: conceptos y métodos para su conservación y manejo. Fundación Produce Puebla, Puebla, México; 2006.

10. Miranda F, Hernández X E. Los tipos de vegetación de México y su clasificación. Boletín de la Sociedad Botánica de México1963;(28,29):179.

11. García E. Modificaciones al régimen de clasificación climática de Köppen, México. Instituto de Geografía. Universidad Nacional Autónoma de México. México, DF., México; 1988.

12. Foroughbakhch R, Reyes G, Alvarado MA, Hernández J, Rocha A. Use of quantitative methods to determine leaf biomass on 15 woody shrub species in northeastern Mexico. For Ecol Manage 2005;216(1-3):359–366.

13. Chávez GO. Determinación de la calidad del hábitat, dieta y calidad de forraje para tres especies de cérvidos en Montemorelos, Nuevo León. [tesis Maestría]. Colegio de Postgraduados, Montecillo, Edo. de Méx., México; 2000.

14. RStudio Team. RStudio: Integrated Development for R. (Online) RStudio, Inc. Boston, MA, USA; 2016.

15. Olguín C, González F, Cantú C, Rocha L, Uvalle J, Marmolejo J. Competencia alimentaria entre el venado cola blanca y tres herbívoros exóticos en el noreste de Tamaulipas, México. Rev Mex Cienc Forest 2017;8(42):7–27.

16. Kie JG, White M, Drawe D. Condition parameters of white-tailed deer in Texas. J Wildl Manag 1983;47(3):583–594.

17. Stocker M, Gilbert FF. Vegetation and deer habitat relations in southern Ontario: application of habitat classification to white-tailed deer. J Appl Ecol 1977;14(2):433–444.

18. Wheaton C, Brown RD. Feed intake and digestive efficiency of South Texas white-tailed deer. J Wildl Manag 1983;(47):442–450.

19. Gastelum-Mendoza FI, González-Saldívar FN, Cantú-Ayala CM, Uvalle-Sauceda JI, Guerrero-Cárdenas I, Lozano-Cavazos EA. Forage diversity and selection in white-tailed deer (*Odocoileus virginianus Texanus* MEARNS) in Coahuila, Mexico. Agroproductividad 2023;16(1): 97–107.

20. Lozano-Cavazos EA, Gastelum-Mendoza FI, Reséndiz-Dávila L, Romero-Figueroa G, González-Saldívar FN, Uvalle-Sauceda JI. Composición de la dieta del venado cola blanca (*Odocoileus virginianus texanus* Mearns) identificada en contenido ruminal en Coahuila, México. Agroproductividad 2020;13(6):49–54.

21. Krausman PR. Introduction to wildlife management: the basics. Upper Saddle River, NJ: Prentice Hall; 2002.

22. Granados D, Tarango L, Olmos G, Palacio J, Clemente F. Disponibilidad de forraje del venado cola blanca *Odocoileus virginianus thomasi* (Artiodactyla: Cervidae) en un campo experimental de Campeche, México. Rev Biol Trop 2014;62(2):699–710.

23. Barrera-Salazar A, Mandujano S, Villarreal O, Jiménez-García D. Classification of vegetation types in the habitat of white-tailed deer in a location of the Tehuacán-Cuicatlán Biosphere Reserve, Mexico. Trop Conserv Sci 2015;8(2):547–563.

24. Gallina SA, Mandujano S, Villarreal O. Monitoreo y manejo del venado cola blanca: conceptos y métodos. México: Instituto de Ecología; 2014.

https://doi.org/10.22319/rmcp.v15i4.6758

Technical note

The management of irrigated elephant grass intercropped with legumes in the semi-arid region

Jéssica Daisy do Vale Bezerra [a]

João Virgínio Emerenciano Neto [ab*]

Tamiris da Cruz da Silva [a]

Patrick Bezerra Fernandes [c]

Stela Antas Urbano [b]

Felipe Pontes Teixeira das Chagas [b]

Gelson dos Santos Difante [d]

Marcone Geraldo Costa [b]

Antonio Leandro Chaves Gurgel [e]

Francisco Israel Lopes Sousa [b]

Rodrigo da Silva Santos [a]

Ana Beatriz Graciano da Costa [a]

[a] Universidade Federal do Vale do São Francisco. Petrolina, PE, Brazil.

[b] Universidade Federal do Rio Grande do Norte. Macaíba, RN, Brazil.

[c] Instituto Federal de Educação Ciência e Tecnologia Goiano. Rio Verde, GO, Brazil.

[d] Universidade Federal de Mato Grosso do Sul. Campo Grande, MS, Brazil.

[e] Universidade Federal do Piauí. Bom Jesus, PI, Brazil.

Corresponding author: jv.em.neto@gmail.com

Abstract:

The present study aimed to evaluate the production and chemical composition of elephant grass (*Pennisetum purpureum* syn. *Cenchrus purpureus* cv. Mott) intercropped with *Cajanus cajan* (Mandarim and Fava Larga) and *Stylosanthes guianensis* (Bela) compared to its cultivation in monoculture under irrigated management in the semi-arid region. The experiment was conducted at the Campus of Agricultural Sciences of the Federal University of the São Francisco Valley, Petrolina, Brazil. The experiment consisted of the following treatments: elephant grass intercropped with each legume and two monoculture types, one with nitrogen fertilization (200 kg ha^{-1}) and the other without. The legumes helped to improve the quality of the forage canopy, with high levels of crude protein. Nitrogen fertilization increased the mass of forage produced by elephant grass. The cumulative analysis of all the cuts showed that the intercropping between elephant grass and the Bela cultivar achieved the highest yield, with 13.49 Mg ha^{-1}, mainly due to the increase in the population of the Bela, which proved to be superior to the other legumes over the cuts. Based on the results, the intercropping of elephant grass with the Bela cultivar is recommended as the most effective strategy for maximizing forage production in the semi-arid region.

Keywords: *Cajanus*, Stylo, Grass, Forage mass, Crude protein.

Received: 05/08/2024

Accepted: 09/09/2024

Semi-arid regions suffer from seasonality in forage production caused by water scarcity and irregular rainfall[1]. In addition, the soils of this region exhibit little organic matter, which reduces the fraction of essential nutrients and soil moisture[2], aggravating the production of food for the nutrition of domestic ruminants. Another point of attention is the limited number of native forage resources with phenotypic plasticity for this region.

To overcome this situation, it is crucial to introduce cultivated forage plants. One promising option is elephant grass (*Pennisetum purpureum* syn. *Cenchrus purpureus*). When the sum of all cuts is performed to evaluate forage yield, up to 40 Mg ha^{-1} of forage mass can be obtained[3]. Another advantage is its versatility, which can be used as fresh or preserved fodder animal feed[4,5].

However, elephant grass is very demanding in its fertilization management since it exhibits maximum production potential when between 100 and 200 kg ha^{-1} of nitrogen fertilization is provided[3,6]. Therefore, maintaining this grass can be costly for the production system. Despite this, there is a strategy that can be adopted to reduce the maintenance costs of

elephant grass: the introduction of forage legumes from tropical climates, as this type of forage can increase nitrogen in the soil by 120 to 150 kg ha^{-1} yr^{-1}[7], reducing dependence on chemical fertilizers, as well as promoting sustainability in the production system.

The introduction of forage legumes, in addition to improving the chemical composition of the soil, will positively influence other agronomic parameters, as observed by Rezende *et al*[8]. When pigeon pea (*Cajanus cajan*) was intercropped with Paiaguas palisade grass (*Urochloa brizantha*, cv. BRS Paiaguás) in the Brazilian Cerrado region, it was they found that this cultivation strategy boosted the efficiency of macronutrient use in the grass, generating increases in forage yield[8]. In the same region, Epifanio *et al*[9] observed that the intercropping of *Stylosanthes* with two cultivars of *Urochloa brizantha* (Piata palisade grass and Paiaguas palisade grass) promoted increases in the forage mass values of the grasses, as well as improvements in the chemical composition of the forage produced.

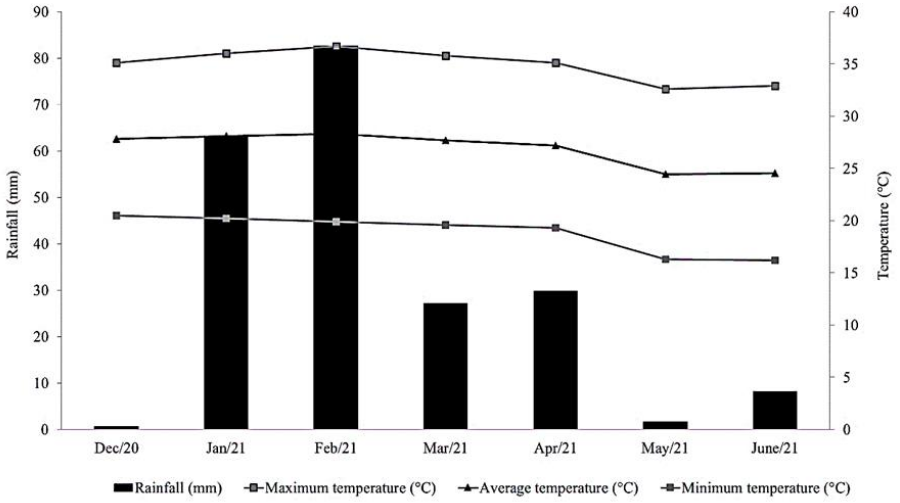
Based on the benefits provided by the forage legumes in the aforementioned systems, the following hypothesis was formulated: tropical climate forage legumes (*Cajanus cajan* and *Stylosanthes*), when intercropped with elephant grass will promote increases in forage yield compared to grass monoculture. In addition, the intercropping will positively impact the chemical composition of the forage produced in the semi-arid region.

This study aimed to evaluate forage availability and the chemical composition of elephant grass intercropped with forage legumes, compared to the monoculture managed irrigated in the semi-arid region.

The experiment was conducted in the experimental area at the Campus of Agricultural Sciences of the Federal University of the São Francisco Valley (UNIVASF), in Petrolina, Brazil (09º23'55" S, 40º30'03" W, an altitude of 391 m). The experiment began in December 2020 and ended in June 2021.

The climate of the region is semi-arid, with rainfall concentrated in the summer, low annual rainfall (435 mm), high potential evapotranspiration rates (1,520.9 mm), and a significant water deficit over the year. The weather data for the period studied (Figure 1) was monitored by the UNIVASF weather station, located approximately 50 m from the experimental area.

Figure 1: Rainfall and average, maximum, and minimum temperatures during the experimental period



The experimental design was randomized blocks with four blocks (replication) and five cultivation systems associated with four cuts. The treatments were: elephant grass (*Pennisetum purpureum* Schum., cv Mott) intercropped with the Mandarim cultivar (*Cajanus cajan* cultivar Mandarin); Elephant grass intercropped with Fava Larga (*Cajanus cajan* cv. Fava Larga); Elephant grass intercropped with Bela (*Stylosanthes guianensis* cv. Bela); Elephant grass in monoculture with nitrogen fertilization (200 kg^{-1} ha^{-1} yr^{-1}); Elephant grass in monoculture without fertilization.

The soil in the area is classified as Argissolo Amarelo, sandy/medium texture[10]. For the chemical characterization of the soil, samples were collected at the 0-20 cm soil layer at random points in the area. These were sent for analysis in the laboratory to determine the chemical parameters. Based on the analysis results, there was no need to correct the active acidity of the soil (Table 1).

Table 1: Soil chemical characteristics in the 0-20 cm layer

pH	OM	P	K	Ca	Mg	Na	Al	H+Al	SB	CEC	V
	g kg^{-1}	mg dm^{-3}	cmol dm^{-3}								%
6.80	8.80	31.0	0.07	2.30	0.60	0.04	0.00	0.33	2.98	3.31	90

pH= active acidity; OM= organic matter, P= phosphorus, K= potassium, Ca= calcium, Mg= magnesium, Na= sodium, Al= aluminum, H+Al= potential acidity, SB= sum of bases, CEC= cation exchange capacity, V= base saturation.

The elephant grass was established in March 2018 utilizing horizontal cuttings in furrows 20 cm deep and spaced 100 cm apart. The legumes were sown in October 2020 between the rows of elephant grass in a system of pits with a spacing of 20 cm between pits. Five seeds

were sown per pit for the cultivars of pigeon pea (Madarim and Fava Larga). For the stylo (Bela), 0.5 grams of seed were sown per pit. The area of the plots was 2 x 5m (10 m²).
The evaluation cuts were made at 45-d intervals, totaling four cuts in 6 mo. Irrigation was conducted by drip irrigation, with two rows per block arranged 0.5 m from the edge and 1.0 m between rows, with an average flow rate of 1.3 L h^{-1}, applying an average water depth of 6.5 mm h^{-1}. The irrigation shift was 24 h, four hours at a time, five days a week.

Morphogenesis evaluations were conducted on three tillers of elephant grass in each experimental unit, starting seven days after each cut, with a 7-d interval between evaluations. Each tiller was marked with a colored ribbon and new tillers were selected at each cut.

The data collected was the number of live leaves (expanded and expanding), the number of senescent and dead leaves by manual counting, the length of the pseudostem (stem + ligule) from the base of the soil to the ligule of the last expanded leaf, and the length of the leaf blade (expanded and expanding), from the ligule to the apex of the leaf blade. The data collected was used to estimate the Leaf appearance rate (LAR, leaf tiller d^{-1}) - the difference between the number of final and initial leaves divided by the interval of days between measurements; the leaf elongation rate (LER, cm tiller d^{-1}) - calculated as the difference between the sums of the final and initial leaf lengths (expanded and expanding) divided by the interval of days between measurements; stem elongation rate (SER, cm tiller d^{-1}) - calculated as the difference between the final and initial length of the stem divided by the interval of days between measurements; leaf lifespan (LLS, days) - the interval from leaf emergence to 50 % senescence; and phyllochron (Phyl, days) being the inverse of the LAR rate.
To analyze the structural characteristics, the tiller population density (TPD, m²) was first measured by manual counting at three different points in a known area (0.25 m² quadrant), and six tillers close to the ground were collected from each experimental unit. The following were measured on the tillers: number of live leaves (NLL, leaves $tiller^{-1}$) by manual counting and final leaf length (FLL, cm) from the base of the ligule to the end of the leaf blade using a graduated ruler.

For the height assessments, the canopy height (CH, cm) of the elephant grass and the plant height (PH, cm) of the legumes were measured using a stick graduated in centimeters at three representative points in each plot. The CH corresponded to the average height of the curvature of the leaves around the stick from ground level. The PH corresponded to the apical bud of the highest branch.

The cutting height adopted for the grass was close to the ground, while the legume cultivars were 20 cm above the ground. All the material contained in the central rows (5 m²) of the plot was collected and weighed to quantify the green weight. From this, a sub-sample of

approximately 1 kg was taken for each cultivar to determine the dry mass and separate it into leaf blade, stem, and senescent material fractions.

After separation, the components were placed in a forced circulation oven at 55 °C for 72 h. Once the dry weight was obtained, the dry matter (DM) content was calculated, and the forage mass (FM, kg ha^{-1}) and botanical components were determined: leaf forage mass (LFM, kg ha^{-1}); stem forage mass (STFM, kg ha^{-1}); dead material forage mass (DMF, kg ha^{-1}); forage mass to stem forage mass ratio (F:ST). During the experimental period, four cuts were made, so at the end of data collection, the four cycles were added together to quantify the production of the systems evaluated (Mg ha^{-1}).

The chemical composition of the forage was assessed on the whole plant obtained by cutting and drying in a forced circulation oven for 72 h. After drying, they were ground in a mill, identified, and submitted for analysis at the Multiuser Animal Nutrition Laboratory at the Jundiaí Agricultural School, Specialized Academic Unit in Agricultural Sciences of the Federal University of Rio Grande do Norte. The samples were evaluated for dry matter (DM), crude protein (CP, g kg^{-1}), ash (g kg^{-1}), neutral detergent fiber (NDF, g kg^{-1}), acid detergent fiber (ADF, g kg^{-1}), and lignin (g kg^{-1}). All the analyses followed the recommendations of Detmann *et al*[11].

The data was analyzed using three models: model I ($Y_{ijk} = \mu + G_i + B_k + e_{ijk}$) was used to analyze the characteristics of the elephant grass; model II ($Y_{ijk} = \mu + L_i + B_k + e_{ijk}$) was used to analyze the characteristics related to the forage legumes; model III ($Y_{ijk} = \mu + A_i + B_k + e_{ijk}$) was used to analyze the production of forage mass during the experimental period.

The model parameters are represented by: Y_{ijk} represents the characteristic evaluated; μ model constant; G_i effect of the cultivation system (Elephant grass + Mandarin, Elephant grass + Fava Larga, Elephant grass + Bela, Elephant grass with fertilizer, Elephant grass without fertilizer); B_k block effect (I, II, III, IV); L_i forage legume effect (Mandarin, Fava Larga, and Bela); A_i represents the cumulative effect of the intercropping and monocultures; e_{ijk} random error observed in each model.

The factors of cropping systems and forage leguminous plants were considered fixed effects, while the block was considered as a random effect. Mixed model analyses were then conducted using the *lme4* package[12]. The means were calculated by least squares using the *emmeans* package[13], and when a statistically significant effect was observed ($P<0.05$), the means were compared using the Tukey test. All analyses were conducted using R software[14].

The condition with fertilizer generated the highest FLL and SER values compared to the other systems evaluated (Table 2). There was no cultivation system effect for the other

characteristics. Therefore, for elephant grass, the average values obtained were as follows: LAR of 0.166 leaf tiller d^{-1}, LER of 7.05 cm tiller d^{-1}, Phyl of 6.78 d, LLS of 57.54 d, NLL of 8.65 leaves $tiller^{-1}$ and TPD of 194 m^2.

Table 2: Morphogenesis and tiller structure of elephant grass in different cropping systems

Cultivation systems	LAR (leaf tiller d^{-1})	LER (cm tiller d^{-1})	SER (cm tiller d^{-1})	Phyl (days)	LLS (days)	NLL (leaves $tiller^{-1}$)	FLL (cm)	TPD (m^2)
Elephant grass + Mandarin	0.168[a]	6.86[a]	0.172[ab]	6.96[a]	56.20[a]	8.34[a]	40.10[ab]	185.10[a]
Elephant grass + Fava Larga	0.164[a]	6.96[a]	0.149[b]	6.79[a]	59.30[a]	8.88[a]	40.50[ab]	184.70[a]
Elephant grass + Bela	0.159[a]	6.29[a]	0.143[b]	7.03[a]	58.30[a]	8.44[a]	37.40[b]	196.50[a]
Elephant grass with fertilizer	0.178[a]	8.37[a]	0.206[a]	6.16[a]	54.30[a]	8.92[a]	44.30[a]	204.70[a]
Elephant grass without fertilizer	0.160[a]	6.76[a]	0.139[b]	6.99[a]	59.60[a]	8.67[a]	39.20[ab]	197.30[a]
SEM	0.004	0.376	0.011	0.205	1.50	0.099	0.870	7.50
P-value	0.375	0.060	0.001	0.424	0.546	0.209	<0.001	0.292

LAR= leaf appearance rate; LER= leaf elongation rate; SER: Stem elongation rate; Phyl= Phyllochron; LLS= leaf lifespan; NLL= number of live leaves; FLL= final leaf length; TPD= tiller population density. *P*-value= probability of significant effect. SEM= Standard error of the mean.

[ab] Means followed by distinct lowercase letters in the column differ by the Tukey test.

When checking the CH, FM, LFM, STFM, and DMF of elephant grass, the scenario with fertilization generated the highest values (Table 3). The cultivation systems had no effect on F:ST, with an average value of 2.98.

Table 3: Structural and botanical characteristics of elephant grass in different cultivation systems

Cultivation systems	CH (cm)	FM (kg ha^{-1})	LFM (kg ha^{-1})	STFM (kg ha^{-1})	DMF (kg ha^{-1})	F:ST
Elephant grass + Mandarin	39.10^{b}	1238^{c}	832^{c}	355^{b}	51.10^{b}	2.94^{a}
Elephant grass + Fava Larga	37.50^{b}	1333^{bc}	901^{bc}	380^{b}	51.60^{b}	2.97^{a}
Elephant grass + Bela	40.30^{b}	1227^{c}	828^{c}	345^{b}	53.90^{b}	3.16^{a}
Elephant grass with fertilizer	45.50^{a}	2209^{a}	1475^{a}	651^{a}	83.30^{a}	2.79^{a}
Elephant grass without fertilizer	40.20^{b}	1681^{b}	1125^{b}	484^{ab}	71.80^{ab}	3.03^{a}
SEM	0.757	80.93	46.08	35.02	4.94	0.136
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.452

CH= canopy height; FM= forage mass; LFM= leaf forage mass; STFM= stem forage mass; DMF= forage mass of dead material; F:ST= ratio of forage mass to stem forage mass. *P*-value: probability of significant effect. SEM: Standard error of the mean.

^{abc} Means followed by distinct lowercase letters in the column differ by the Tukey test.

The Mandarin and Fava Larga cultivars had the highest PH values, while the Bela cultivars had high LFM, PD, and FSM values (Table 4). The cropping systems did not affect LFM, with an average value of 1,041 kg ha^{-1}.

Table 4: Structural and botanical characteristics of forage legumes intercropped with elephant grass

Cultivation systems	PH (cm)	PD (m^2)	FM (kg ha^{-1})	LFM (kg ha^{-1})	FSM (kg ha^{-1})
Elephant grass + Mandarim	104.90^{a}	6.99^{b}	1551^{b}	987^{a}	564^{b}
Elephant grass + Fava Larga	98.90^{a}	6.87^{b}	1482^{b}	983^{a}	499^{b}
Elephant grass + Bela	55.00^{b}	59.77^{a}	2145^{a}	1153^{a}	991^{a}
SEM	4.27	0.279	121.56	56.42	3,70
P-value	<0.001	<0.001	0.008	0.268	<0.001

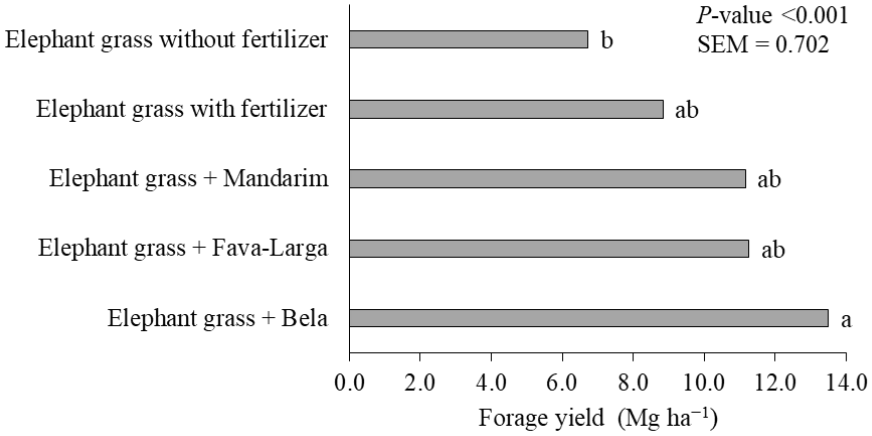
PH= plant height; PD= plant density; FM= forage mass; LFM= leaf forage mass; FSM= forage stem mass. *P*-value: probability of significant effect. SEM: Standard error of the mean.

ab Means followed by distinct lowercase letters in the column differ by the Tukey test.

The cultivation systems affected the forage yield (Figure 2), where it can be seen that the intercropping involving elephant grass and the Bela cultivar had the highest cumulative

production (13.49 Mg ha^{-1}). When no fertilization was applied, the elephant grass in monoculture generated the lowest yield, with a value of 6.72 Mg ha^{-1}.

Figure 2: Forage yield obtained in the cultivation systems during the evaluation period. Lowercase letters in the columns differ by Tukey test



P-value: probability of significant effect. SEM: standard error of the mean.

The highest ash value is observed when elephant grass is intercropped with the Bela cultivar (Table 5). As for the other chemical characteristics of the grass in the different cultivation systems, averages of 73.10 g kg^{-1} of CP, 676.20 g kg^{-1} of NDF, 374.80 g kg^{-1} of ADF, and 62.12 g kg^{-1} of lignin. The Mandarim and Fava Larga cultivars had the highest CP, ADF, and lignin values. On the other hand, there were increases in ash values for the Bela cultivar. The highest concentration of NDF was found in the Mandarim cultivar.

By analyzing the morphogenesis and structure characteristics of elephant grass, such as LAR, LER, Phyl, LLS, and TPD, the cultivar evaluated (Elephant grass - Mott) in the semi-arid region shows remarkable phenotypic plasticity, enabling it to integrate effectively with different forage resources. This adaptability is highly advantageous, as it significantly expands the opportunities for diversification in forage production. In different climatic and soil contexts, as found by Silva *et al* [15] and Seibt *et al* [16], elephant grass has demonstrated its ability to coexist with other forage legumes, such as forage peanuts (*Arachis pintoi*), arrowleaf clover (*Trifolium vesiculosum*), and Asian pigeonwings (*Clitoria ternatea*).

The FLL is one canopy characteristic that reflects leaf area gain[17]. In the case of elephant grass, it was observed that the greatest leaf length was achieved under monoculture conditions and with chemical nitrogen fertilization. In this management, the absence of other forage species that could restrict leaf area dynamics allowed for an increase in leaf size. In addition, the supply of chemical nitrogen fertilizer guarantees the immediate availability of

this nutrient, optimizing the dynamics of leaf area[18] and SER[19], justifying the higher values of FM, LFM, and STFM in the monoculture.

As some researchers mentioned[20,21], the NLL is a genetically predetermined trait. Therefore, the tillers maintain a constant number of leaves in ideal conditions without stresses that could inhibit the plant potential. Even in monoculture management without nitrogen fertilization, when the soil has good fertility parameters (Table 1), creating an environment conducive to expressing this constant pattern in the NLL is possible. Likewise, the NLL measurement observed is similar to the results of other authors[22], who obtained a value of 8.58 leaves tiller^{-1} in elephant grass, cultivar Pioneiro (*Pennisetum purpureum* Schum. cv. Pioneiro).

When adding up the FM of elephant grass and legumes, it can be seen that the potential of monoculture is limited, as the intercropping of elephant grass and Bela had the highest forage production (Figure 2). In the literature, it is reported that intercropping between different forage resources promotes increases in the efficiency of utilization of abiotic resources, resulting in increases in plant production (grain and biomass) and soil utilization efficiency[23].

This increase in production can only be achieved by carefully selecting the forage resources that make up the intercropping, so when selecting the forage plants to form this production system, the decision must be based on various agronomic parameters. In the case of the Bela cultivar, despite having the lowest PH, there was a higher PD compared to the guandu bean cultivars, which allowed the stylo cultivar to increase its FM.

This shows that this cultivar can be good option for forage production in the semi-arid region when intercropped with elephant grass. Another advantage of using stylo in intercropping with grasses is the positive residual effect on the soil. Even if this legume disappears from the area, the increase in nitrogen and organic matter ensures forage production in future crops[24].

Grasses from tropical climates naturally exhibit a lower protein fraction in the composition of the FM, and it is common for this group of plants in the vegetative phase to exhibit a CP value ranging from 72.43 g kg^{-1} to 119.30 g kg^{-1}[25,26]. On the other hand, high values of NDF and ADF are observed in the chemical composition of DM from tropical climate pastures[27,28,29].

Higher NDF and ADF values generate a forage resource, a potential limiting factor for forage consumption when fed to animals. In addition, increases in fibrous and lignin fractions are associated with forages with high STFM fractions in the forage canopy, impacting a low F:ST ratio[30].

In a study by Lima *et al*[31], different genotypes of elephant grass showed F:ST values ranging from 0.95 to 1.43 when the forage was harvested 56 d after regrowth from the previous cut. In the cutting management adopted for elephant grass intercropped and monocultures in the semi-arid region, a better F:ST ratio was observed when cutting was conducted at the height of 20 cm above the ground, reducing the share of stem in FM. These results indicate that this management strategy was suitable for harvesting elephant grass in the different cropping systems evaluated.

The legumes are expected to have a higher CP content in their composition, making it possible to produce a better-quality feed for animal nutrition. Ligoski *et al*[32] found that the intercropping of pigeon pea (*Cajanus cajan* cv. Super N) with Xaraes palisade grass (*Urochloa brizantha* cv. BRS Xaraés) and maize (*Zea mays*) not only had a higher protein content compared to maize monocropping, but also resulted in a forage that contributes to lower methane emission rates.

The intercropping between the Bela cultivar and elephant grass increased ash fractions for the grass and the legume. Prado *et al*[33] observed that the intercropping of the Bela cultivar with Tamani guinea grass (*Megathyrsus maximus* cv. BRS Tamani) produced FM with higher ash values than monocultures. The introduction of forage legumes through intercropping with other forage resources directly impacts the chemical composition of the soil, where this form of cultivation promotes increases in the bioavailability of essential nutrients. As a result, the grasses in this type of cultivation develop in an environment that enables greater nutrient absorption for the aerial part, which alters the mineral composition of the produced forage[8,34].

It was recommended to intercrop–elephant grass with the cultivar Bela, as it resulted in significant increases in total forage production, which highlights the potential of this cultivation system to optimize forage production in the semi-arid region.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES – Finance Code 001) and National Council for Scientific and Technological Development (CNPq). We thank UNIVASF and UFRN for the support granted for the realization of this research.

Competing interests

The authors declare there are no conflicts of interest.

Table 5: Chemical composition of elephant grass and forage legumes (g kg^{-1})

	*Elephant grass					*Forage legumes				
Cultivation systems	**CP**	**Ash**	**NDF**	**ADF**	**Lignin**	**CP**	**Ash**	**NDF**	**ADF**	**Lignin**
Elephant grass + Mandarin	74.60[a]	124.00[ab]	681[a]	381[a]	61.40[a]	168[a]	45.40[b]	616[a]	434[a]	178.0[a]
Elephant grass + Fava Larga	76.20[a]	124.00[ab]	669[a]	371[a]	58.50[a]	165[a]	38.8[c]	611[ab]	430[a]	178.90[a]
Elephant grass + Bela	72.70[a]	135.00[a]	673[a]	370[a]	62.30[a]	147[b]	77.20[a]	557[b]	357[b]	99.30[b]
Elephant grass with fertilizer	71.10[a]	112.00[b]	683[a]	375[a]	67.60[a]	-	-	-	-	-
Elephant grass without fertilizer	70.90[a]	118.00[ab]	675[a]	377[a]	60.80[a]	-	-	-	-	-
SEM	0.743	2.59	6.40	3.34	1.79	6.58	3.65	10.02	11.95	8.31
P-value	0.108	0.010	0.894	0.250	0.371	<0.001	<0.001	0.006	<0.001	<0.001

* Values expressed concerning dry matter. CP= crude protein, NDF= neutral detergent fiber; ADF= acid detergent fiber. *P*-value: probability of significant effect. SEM: Standard error of the mean.

[abc] Means followed by distinct lowercase letters in the column differ by the Tukey test.

Literature cited:

1. Pereira GF, Emerenciano Neto JV, Difante GS, Assis LCS, Lima PO. Morphogenic and structural characteristics of tropical forage grasses managed under different regrowth periods in the Brazilian semi-arid region. Semina: Ciênc Agrár 2019;40(1):283.

2. Costa ABG, Difante GS, Campelo BAM, Gurgel ALC, Costa CM, Theodoro GF, *et al*. Morphogenetic, structural and production traits of Marandu grass under nitrogen rates in Neo soil. Arq Bras Med Vet Zootec 2021;73:658-664.

3. Oliveira LE, Oliveira FH, Silva GG, Bezerra MGS, Morais ÉG, Bezerra GF, *et al*. Nitrogen and phosphorus fertilizer application to Elephant grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*) cultivar 'Cameroon' in an arenosol in Rio Grande do Norte, Brazil. Trop Grassl Forrajes Trop 2022;10(3):280-287.

4. Figueiredo MRP, Teixeira ACB, Bittencourt LL, Moreira GR, Ribeiro AJ, Silva FSG, *et al*. Elephant grass silage with addition of regional by-products. Acta Sci Anim Sci 2022;44.

5. Islam MR, Garcia SC, Islam MA, Bashar MK, Roy A, Roy BK, Sarker NR, Clark CEF. Ruminant production from napier grass (*Pennisetum purpureum* Schum): A review. Animals (Basel) 2024;14:467.

6. Bueno AM, Andrade AF, Viçosi KA, Flores RA, Sette CR, Cunha TQG, Santos GG. Does nitrogen application improve elephant grass yield and energetic characteristics of biofuels? Bioenerg Res 2021;14:774-784.

7. Boddey RM, Casagrande DR, Homem BGC, Alves BJR. Forage legumes in grass pastures in tropical Brazil and likely impacts on greenhouse gas emissions: A review. Grass Forage Sci 2020;75:1-15.

8. Rezende PR, Rodrigues LM, Backes C, Teodoro AG, Santos AJM, Fernandes PB, Giongo PR, Ribon AA, Bessa SV. Productivity and nutrient extraction by Paiaguás palisadegrass submitted to doses of nitrogen in single cultivation and intercropped with pigeon pea. Arq Bras Med Vet Zootec 2022;74:1151-1160.

9. Epifanio PS, Costa KAP, Severiano EC, Souza WF, Teixeira DAA, Silva JT, Aquino MM. Productive and nutritional characteristics of *Brachiaria brizantha* cultivars intercropped with *Stylosanthes* cv. Campo Grande in different forage systems. Crop Pasture Sci 2019;70:718-729.

10. Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbrera JF, Coelho MR, *et al*. Sistema brasileiro de classificação de solos. 5th ed. Brasília: Embrapa CNPS; 2018.

11. Detmann E, Souza MA, Valadares Filho SC. Métodos para análise de alimentos – INCT - Ciência animal. Visconde do Rio Branco: Suprema; 2012.

12. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-Effects models using lme4. J Stat Softw 2015;67:1-48.

13. Lenth R, Singmann H, Love J, Buerkner P, Herve M. Emmeans: estimated marginal means, aka least-squares means. R Package Version 1.4.8. Retrieved from https://CRAN.R-project.org/package=emmeans.

14. R Core Team. R: A Language and Environment for Statistical Computing. Retrieved from https://www.R-project.org/.

15. Silva PH, Santos MV, Mello AC, Silva TBS, Neto DES, da Silva VJ, Dubeux Jr JCB, Coelho JJ, Souza EJO, Cunha MV. Agronomic responses and herbage nutritive value of elephant grass (*Cenchrus purpureus*) genotypes grown as monocrops and mixed with butterfly pea (*Clitoria ternatea*). Crop Pasture Sci 2023;74:1210-1222.

16. Seibt DC, Olivo CJ, Alessio V, Sauter CP, Bratz VF, Aguirre PF. Forage mass and nutritional value of elephant grass intercropped with forage legumes. Rev Ceres 2021;68:429-440.

17. Fernandes PB, Barbosa RA, Morais MG, Medeiros-Neto C, Gurgel ALC, Costa CM, Costa ABG, Santana JCS, Silva MGP, Difante GS. Evaluation and reparametrization of mathematical models for prediction of the leaf area of *Megathyrsus maximus* cv. BRS Zuri. Trop Grassl Forrajes Trop 2020;8:214-219.

18. Euclides VPB, Montagner DB, Araújo AR, Pereira MA, Difante GS, Araújo IMM, Barbosa LF, Barbosa RA, Gurgel ALC. Biological and economic responses to increasing nitrogen rates in Mombaça guinea grass pastures. Sci Rep 2022;12:1937.

19. Rodrigues LF, Santos AC, Junior OS, Santos JGD, Faria AFG, Coelho BPL. Morphogenic and structural characteristics of Marandu grass cultivated under grazing management and nitrogen fertilization. Semina: Ciênc Agrár 2019;40(5Supl1):2331-2340.

20. Lemaire G, Agnusdei M. Leaf tissue turn-over and efficiency of herbage utilization. In: Lemaire G, Hodgson J, Moraes A, et al. editors. Grassland ecophysiology and grazing ecology. Wallingford: CAB International; 2000:265-288.

21. Difante GS, Nascimento Junior DD, Da Silva SC, Euclides VPB, Montagner DB, Silveira MCT, Pena KS. Características morfogênicas e estruturais do capim-marandu submetido a combinações de alturas e intervalos de corte. Rev Bras Zootec 2011;40:955-963.

22. Sales ECJ, Saraiva CRS, Reis ST, Rocha Júnior VR, Pires DAA, Vitor CMT. Morphogenesis and productivity of Pioneiro elephant grass under different residual heights and light interceptions. Acta Sci Anim Sci 2014;36:137-143.

23. Chapagain T, Riseman A. Intercropping wheat and beans: effects on agronomic performance and land productivity. Crop Sci 2014;54:2285-2293.

24. Menezes RC, Alves EB, Casagrande DR, Lara MAS, Evangelista AR, Bernardes TF. Xaraés palisadegrass remains productive after the disappearance of stylo in tropical legume-grass pasture. Sci Agric 2015;72(6):484-488.

25. Ferreira EA, Abreu JG, Martinez JC, Braz TGS, Ferreira DP. Cutting ages of elephant grass for chopped hay production. Pesqui Agropecu Trop 2018;48(3):245-253.

26. Coca FOCG, Gomes ENO, Junges L, Itavo LCV, Nonato LM, Gomes FK, Itavo CCBF, Difante GS, Dias AM. Protodioscin content, degradation kinetics, and *in vitro* digestibility of Marandu palisadegrass hay as were affected by cutting interval of the canopy. Trop Anim Sci J 2022;45:299-307.

27. Euclides VPB, Macedo MCM, Valle CBD, Difante GDS, Barbosa RA, Cacere ER. Valor nutritivo da forragem e produção animal em pastagens de *Brachiaria brizantha*. Pesqui Agropecu Bras 2009;44:98-106.

28. Rodrigues JG, Difante GS, Ítavo LCV, Pereira MG, Gurgel ALC, Costa ABG, Véras ELL, Monteiro GOA, Dias AM, Ítavo CCBF. Forage Accumulation and Nutritional Characteristics of *Brachiaria* Cultivars Grown in a Semi-arid Environment. Trop Anim Sci J 2023;46:85-96.

29. Costa RRGF, Costa KAP, Souza WF, Epifanio PS, Santos CB, Silva JT, Oliveira SS. Production and quality of silages pearl millet and paiaguas palisadegrass in monocropping and intercropping in different forage systems. Biosci J 2018;34:957-967.

30. Brâncio PA, Euclides VPB, Nascimento Júnior D, Fonseca DM, Almeida RG, Macedo MCM, Barbosa RA. Avaliação de três cultivares de *Panicum maximum* Jacq. sob pastejo: disponibilidade de forragem, altura do resíduo pós-pastejo e participação de folhas, colmos e material morto. Rev Bras Zootec 2003;32:55-63.

31. Lima ES, Silva JFC, Vásquez HM, Araújo SAC, Lista FN, Costa DPB. Produção de matéria seca e proteína bruta e relação folha/colmo de genótipos de capim-elefante aos 56 dias de rebrota. Rev Bras Zootec 2007;36:1518-1523.

32. Ligoski B, Gonçalves LF, Claudio FL, Alves EM, Krüger AM, Bizzuti BE, Lima PMT, Abdalla AL, Paim TP. Silage of intercropping corn, palisade grass, and pigeon pea increases protein content and reduces *in vitro* methane production. Agronomy 2020;10:1784.

33. Prado LG, Costa KAP, Silva LM, Costa AC, Severiano EC, Costa JVCP, Habermann E, Silva JAG. Silages of sorghum, Tamani guinea grass, and *Stylosanthes* in an integrated system: production and quality. Front Sustain Food Syst 2023;7:1-15.

34. Silva LM, Costa KAP, Oliveira KJ, Costa AC, Silva JAG, Costa JVCP, *et al*. Fermentation dynamics and quality of maize silage with Pigeon pea. Sem Ci Agr 2023;44:567-584.

https://doi.org/10.22319/rmcp.v15i4.6402

Technical note

In vitro evaluation of a protected ruminant nitrate source: effect on dry matter degradation and methane production

María Elizabeth Rendón-Correa [a]

Sandra Lucia Posada-Ochoa [a]

Jaime Ricardo Rosero-Noguera [a*]

[a] Universidad de Antioquia. Facultad de Ciencias Agrarias. Grupo de Investigación en Ciencias Agrarias – GRICA Calle 70 No. 52 – 21, Apartado aéreo 1226. Colombia.

[*]Corresponding author: jaime.rosero@udea.edu.co

Abstract:

The objective was to evaluate a method to reduce the calcium nitrate release rate in a simulated rumen fermentation environment, and to determine its effect on dry matter degradation and methane production. In the *in vitro* experiment, kikuyu grass (*Cenchrus clandestinus, Hochst ex Chiov*) (KK) was used as the base feed and the addition of protected nitrate (PN), free nitrate (FN) and urea (KU) to the fermentation environment. The amount of nitrate added corresponded to 3 % of the incubated dry matter. The data were analyzed with repeated measures over time considering treatment and time as fixed effects and the rumen inoculum donor animal as a random factor. After 24 h of incubation, FN and PN reduced dry matter degradation by 11.4 and 15 %, respectively. The addition of nitrate significantly reduced methane production. The difference in methane production rates expressed in ml/g of degraded dry matter between the FN (21.0) and PN (31.2) treatments at 48 h of incubation indicates a lower nitrate release rate as a consequence of the protection method employed. The results of this trial show that the inclusion of protected nitrates at levels corresponding to 3 % of the incubated dry matter can reduce methane production by 53 %.

Keywords: Additives, Encapsulated Nitrate, Methane.

Received: 20/01/2023

Accepted: 03/09/2024

Among the greenhouse gases (GHG) caused by human activity, methane (CH_4) is the second most emitted gas, after carbon dioxide (CO_2), although CH_4 remains in the atmosphere for a shorter period of time and is emitted in smaller quantities. Its global warming potential is 25 to 34 times greater than that of CO_2[1]. CH_4 accounts for 30 % of the global enteric emissions of this gas. Because CH_4 is a short-lived climate pollutant, reducing enteric CH_4 emissions can help mitigate climate change within our current lifetime[2].

In ruminants, CH_4 production occurs during the enteric fermentation of organic matter, due to the need to remove hydrogen from the rumen in order to maintain a low redox potential at the fermentation site. Nitrate (NO^{3-}), an electron acceptor, has been studied as a potential pathway to route reduced equivalents away from methanogenesis, presenting itself as a hydrogen dissipating pathway that is useful to the animal and to the environment[3]. In the rumen, NO_3^- is reduced to nitrite (NO^{2-}) ($NO^{3-} + H_2 \rightarrow NO^{2-} + H_2O$), which in turn is reduced to the ammonium ion (NH_4^+) ($NO^{2-} + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O$) —a process that captures four moles of hydrogen per mole of reduced NO^{3-}[4]. The reduction of NO^{3-} to NO^{2-} has a ΔG= -130 kJ, while that of NO^{2-} a NH_4^+ exhibits a ΔG= -371 kJ, which is energetically more favorable than the production of methane (ΔG= -67 KJ)[5]. The reduction of NO_2^- to NH_4^+ is a slow step, due to the low production of the enzyme nitrite reductase by rumen microorganisms, which can lead to an increase in nitrites at the rumen level. These nitrites cross the rumen wall and pass into the blood circulation, binding to hemoglobin and forming methemoglobin, which affects oxygen transport in the blood and may eventually lead to death by hypoxia[6]. Considering that the supply of pure NO^{3-} can present risks to animal health, several studies have been carried out with encapsulated NO^{3-} to release it slowly to ruminal microorganisms and reduce its potential toxic effect[7,8,9]. The purpose of this work was to evaluate a method for reducing the calcium nitrate release rate in a simulated rumen fermentation environment and determining its effect on dry matter degradation and methane production.

The experiment was carried out in the Nutrilab-Grica laboratory, at the University Research Headquarters (SIU) of the University of Antioquia - Colombia.

A sample of kikuyu grass (*Cenchrus clandestinus*, Hochst ex Chiov) at 45 d of regrowth was collected at the "La Montaña" farm located in the municipality of San Pedro de los Milagros (Antioquia - Colombia), at an altitude of 2,470 m asl and an average temperature of 16 ºC, corresponding to a Low Montane Humid Forest life zone.

The grass sample was partially dried in a forced ventilation oven at 60 ºC for 72 h, ground through a 1 mm sieve and stored for subsequent chemical analysis. Dry matter (DM), crude protein (CP), and ash concentrations were determined on the partially dried grass sample[10]. The proportions of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were

determined as described by Van Soest *et al*[10]. Table 1 describes the chemical composition of kikuyu grass.

Table 1: Chemical composition of kikuyu grass (*Cenchrus clandestinus*)

Chemical composition	Value
Dry matter (DM), %	23.5
Crude protein, % DM	20.6
Neutral detergent fiber, % DM	57.2
Acid detergent fiber, % DM	30.3
Ashes, % DM	11.5

The NO_3- source utilized in this study was calcium nitrate (CALCINIT, 15.5-0-0, YARA, Bogotá, Col.). The NO^{3-} was protected using a handmade soap produced by the saponification of commercial soybean oil with sodium hydroxide (NaOH, Merck N^o 106462)

The treatments evaluated were kikuyu grass incubated as a control treatment (KK), kikuyu grass + nitrate without protection (FN), kikuyu grass + protected nitrate (PN) and kikuyu grass + urea (KU). NO_3- treatments were adjusted to provide 3% nitrate/g DM incubated. The urea treatment was included as a control to demonstrate the effect of nitrogen addition on dry matter degradation and CH_4 production.

The nitrogen content present in the additives was determined by the Kjeldahl method[10]; for urea 48.1, unprotected nitrate 13.0, and protected nitrate 3.9.

The rumen fluid for *in vitro* incubation was obtained from three non-lactating adult Holstein cows, equipped with the one-stage ruminal cannula described by Castillo and Hernandez[11]. Donor animals were managed in a rotational grazing system with kikuyu grass, free access to fresh water, and mineral supplementation. The ruminal fluid was collected in the morning hours (0600) and transported to the laboratory in thermal containers previously heated with water at 39 °C. The ruminal fluid was gassed with CO_2 and filtered through four layers of absorbent cotton and kept in a water bath at 39 °C for the inoculation process.

One day prior to the start of the experiment, a buffer solution was prepared as described by McDougall[12]. This solution was mixed with each of the collected inocula at a 9:1 ratio (buffer: inoculum). A 0.5 g sample of kikuyu grass and the additives to be evaluated were weighed and placed in 100 ml glass bottles. Subsequently, a volume of 50 ml of the buffer-inoculum solution was added to each flask; during the process, it was continuously gassed with CO_2 to ensure anaerobic conditions and sealed with rubber stoppers. The sealed flasks were kept in a forced ventilation oven at 39 °C and removed from the incubation process at 24 and 48 h post-incubation to determine DM degradation and CH_4 production.

A total of 60 flasks were incubated: 48 flasks with substrate and inoculum (4 treatments * 3 repeats/treatment * 2 reading times * 2 repeats/schedule) and 12 flasks corresponding to the blanks (2 reading times * 3 inocula * 2 blanks per schedule). The blanks are flasks with buffer solution and inoculum without substrate or additive, whose function is to correct gas production and DM degradation generated by the inoculum.

Total gas production was measured at 24 and 48 h of incubation by measuring the pressure generated in each flask using a digital transducer (Ashcroft 2089QG- Precision Digital Test Gauges, USA) described by Posada *et al*[13]. After the measurement, a gas sample was taken to determine the concentration of CH_4 gas. A valve with three outlets was used. The first outlet was connected to a needle (0.6 mm); the second, to the pressure transducer, and the third, to a plastic syringe that was used to extract the gas sample. The needle attached to the valve was inserted through the rubber cap for pressure measurement, and, subsequently, the gases accumulated at the top of the bottle were withdrawn with the syringe to the point where the pressure recorded on the transducer reached zero. The gas collected in the syringe was stored in Clear Flex type co-extruded polyolefin bags (Baxter, USA). After finishing the sampling, CH_4 concentrations were measured by gas chromatography. A subsample of 100 μL of gas was taken from each bag with the help of a syringe to be injected into a Thermo Trace GC Ultra gas chromatograph (Thermo Scientific, USA). CH_4 production was established as the product of the total gas volume recorded over the incubation time (24 and 48 h) and the CH_4 concentration determined in the sample by gas chromatography.

After gas sampling at each measurement time, the flasks were opened to measure the degraded dry matter (DDM), determined by the difference in weight between the incubated DM (IDM) and the residue after incubation. In order to determine the DDM by gravimetry, the contents of each vial were filtered through glass crucibles (porosity 1, 100 - 160 μm) using a vacuum pump. The crucible-residue set was dried in a forced ventilation oven at 60 ºC for 48 h and subsequently weighed. After deducting the weight of the crucible, the value of the degraded DM was obtained as the difference between the DM of the residue and the DM of the blank, divided by the value of the initially incubated DM[14]. The liquid fraction of each incubation bottle was preserved by adding sulfuric acid (98 % v/v) drop by drop until an average pH of 2 was achieved; each sample was centrifuged at 4,000 rpm for 10 min and, finally, a subsample of 1.5 ml of supernatant was collected for the measurement of volatile (acetic, propionic, and butyric) fatty acids (VFA) by gas chromatography. The remaining liquid fraction was used to determine the ammoniacal nitrogen ($N\text{-}NH_3$) concentrations with the Kjeldahl method[10].

The effect of treatments on gas production, CH_4 and DM degradation were analyzed with a repeated measures model over time, using the PROC MIXED procedure of SAS[15] where the fixed effects corresponded to treatment and time (schedules), and the random effect corresponded to the source of rumen inoculum (animal). Comparison of means was

performed with the Tukey - Kramer test ($P<0.05$). Differences between treatments with respect to VFA and N-NH_3 production at 24 h were measured with a completely randomized model, using the GLM procedure of SAS[15]. Differences between means were determined with Duncan's multiple comparisons test ($P<0.05$).

The effect of NO_3- on DM degradation, gas production and CH_4 production *in vitro* at the 0 to 24 h and 0 to 48 h measurement intervals are presented in Table 2. NO_3- treatments showed a reduction in DM degradation at 24 h of incubation. The PN caused a 24 % reduction of DDM, while the reduction caused by the FN was 18 % compared to the control treatment (KK). At 48 h of incubation, there were no differences ($P>0.05$) between treatments. When the DDM was expressed in percentage terms, clearly the treatments that included nitrates exhibited lower degradations at 24 and 48 h of incubation ($P<0.01$) than the KK treatment ($P<0.01$). The comparison between the KU and KK treatments shows that the addition of urea to the fermentation environment had no effect on DDM or CH_4 production, indicating that the nitrogen supply in the control treatment (KK) was sufficient to maintain microbial activity during the incubation process.

Table 2: Effect of nitrate with and without protection on total gas production, methane production and degraded dry matter (DDM) in two *in vitro* fermentation schedules

Variable	Schedule	Treatments				Effects		
		KK	FN	PN	KU	T	Ti	Tx Ti
DDM, g	0 a 24 h	0.266^{a}	0.218^{bc}	0.202^{c}	0.248^{ab}	0.01	0.01	0.03
	0 a 48 h	0.271	0.27	0.267	0.30			
DDM, %	0 a 24 h	55.5^{ab}	49.2^{bc}	47.0^{c}	56.1^{a}	0.01	0.01	0.33
	0 a 48 h	64.6^{ab}	58.5^{b}	60.5^{b}	67.9^{a}			
Gas production, ml	0 a 24 h	46.9^{a}	35.2^{ab}	23.4^{b}	45.1^{a}	0.01	0.01	0.01
	0 a 48 h	84.2^{a}	57.1^{b}	60.1^{b}	84.4^{a}			
Gas production, ml/g DDM	0 a 24 h	177.8^{a}	161.0^{ab}	115.3^{b}	181.5^{a}	0.01	0.01	0.01
	0 a 48 h	313.8^{a}	211.2^{c}	225.6^{b}	279.7^{ab}			
Mehtane, ml	0 a 24 h	8.2^{a}	2.6^{b}	1.6^{b}	7.8^{a}	0.01	0.01	0.01
	0 a 48 h	17.7^{a}	5.7^{b}	8.3^{b}	16.35^{a}			
Methane, ml/100 ml gas	0 a 24 h	17.5^{a}	7.4^{b}	6.8^{b}	17.2^{a}	0.01	0.01	0.05
	0 a 48 h	21.0^{a}	9.7^{b}	13.8^{b}	19.4^{a}			
Methane, ml/g DDM	0 a 24 h	30.9^{a}	12.0^{b}	7.8^{b}	31.2^{a}	0.01	0.01	0.01
	0 a 48 h	66.0^{a}	21.0^{b}	31.2^{b}	54.2^{a}			

KK= kikuyu grass (*Cenchrus clandestinus*); FN= kikuyu grass + free nitrate; PN= kikuyu grass + protected nitrate; KU= kikuyu grass + urea; T= effect of the treatment; Ti= effect of the incubation; TxTi= effect of the interaction between the treatment and the incubation schedule.

^{abc} Means of treatments with different letters in the same row show differences ($P<0.05$).

Total gas production was significantly reduced (P<0.001) with the PN treatment at 24 h of incubation, compared to the KK and KU treatments. After 48 h of incubation *in vitro*, PN and FN treatments decreased total gas production by an average of 30 % compared to KK and KU treatments (P<0.05). When gas volume was expressed in ml/g DDM, the PN treatment produced 35 % and 28 % less gas than KK during 24 and 48 h *in vitro*.

The FN and PN treatments reduced total CH_4 production by 68 and 80 % with respect to KK (P<0.05) at 24 h (P<0.05). At the end of 48 h, the FN treatment maintains a 68 % reduction in CH_4 volume, and PN achieves a 53 % reduction compared to the control.

Table 3 shows the effect of the addition of protected and unprotected NO_3- on the production of VFA and ammonia nitrogen (N-NH_3) in an *in vitro* fermentation system. The production of VFA and N-NH_3 was not affected by the addition of NO_3- or urea to the fermentation environment (P>0.05).

Table 3: Effect of nitrate on the production of volatile fatty acids, and ammoniacal nitrogen (N-NH3) with and without protection at 24 hours of *in vitro* fermentation

Variables	**Treatments**				
	KK	**FN**	**PN**	**KU**	***P* value**
Acetic, mmol/L	62.9	58.9	76.7	98.2	0.29
Propionic, mmol/L	16.9	14.5	12.3	20.3	0.44
Butyric, mmol/L	8.5	7.7	7.1	8.5	0.20
N-NH_3, mg/L	14.0	10.5	10.5	11.7	0.69

KK= kikuyu grass (*Cenchrus clandestinus*); FN= kikuyu grass + free nitrate; PN= kikuyu grass + protected nitrate; KU= kikuyu grass + urea.

The decrease in DDM occurring with the PN treatment may be due to the soap used for protection. There is evidence that soy soap has a high dissociation in mediums with a pH of approximately 6.5[16]. This characteristic of soybean soap may lead to an increase in the polyunsaturated fatty acid content, which significantly depresses cell wall digestibility[17]. When a high dissociation of the soap has occurred, it enhances the release rate of NO_3-, potentially reducing the DDM[17]. Therefore, the use of PN may have prompted an additive effect of the unsaturated fatty acids and NO_2- on the reduction of DDM. On the other hand, the decrease in DDM with the FN and PN treatments may have been caused by the toxic effect of nitrites (NO_2-), which inhibit growth and promote the abundance of methanogens and other bacteria, such as *F. succinogenes* and *R. flavefaciens*, that play an important role in the degradation of dry matter in the rumen[18,19].

Mitigation of the CH_4 production through the inclusion of NO_3- *in vitro* has been reported by in previous research[8,20,21]. In the present study, the use of a 3 % dose of NO_3- with the FN treatment reduced the production of CH_4 /g DDM by 68 % during the 48 h of incubation. The

reduction in the CH_4 production observed with FN may be a consequence of the high reducing capacity of NO_3- in anaerobic media[22,23]. NO_3- behaves as an alternative hydrogen sink in the rumen, through its reduction to NH_4+ —an energetically more favorable process (ΔG =-501 kJ) than the reduction of CO_2 to CH_4 (ΔG= -67 KJ)[5]. Furthermore, the NO_2- resulting from the reduction of NO_3- may have exerted a toxic effect on the population of methanogens and certain cellulolytic bacteria[19,23], as mentioned above, which may have favored the trend in the reduction of the DDM.

The PN treatment brought about a 74 % reduction in CH_4 production in ml/g of DM, affecting DM degradation by 21 % at 24 h, compared to the control treatment (KK). Natael *et al*[18], assessed a similar dose of PN (3 % of the incubated DM), in an 80:20 (forage/concentrate) diet and found a 10 % reduction in CH_4 production during the same incubation time that did not affect the degradation of the incubated organic matter. With a 15 % inclusion of PN in 24 h *in vitro*, Lee *et al*[8] obtained a 45 % reduction in the produced CH_4 volume with respect to the control.

The purpose of utilizing PN is to slow down the dissolution rate of NO^{3-} in order to favor the growth of NO^{3-} and NO^{2-} that reduce the bacteria which accelerate NH_4+ formation[8]. The increase in this type of bacteria favors the reduction rate of NO_3- to NO^{2-} as well as of NO^{2-} to NH_4+, which would imply a decrease in the risk of NO_2- toxicity for both for ruminal microorganisms and the host animal. Theoretically, the reduction of 0.015 g of NO^{3-} should result in a decrease of CH_4 by 5.32 ml[24,25]; however, with PN, a total reduction of 9.4 ml of CH_4 was obtained, which is 76 % more than expected. This behavior was possibly due to a factorial effect of polyunsaturated fatty acids resulting from the dissociation of soap and NO^{2-} from the reduction of NO^{3-}, on the decrease in DDM, which finally favored the reduction in CH_4 production *in vitro*. The dissociation of the soap made with soybean oil may have favored the reduction in CH_4 production, as was the case in another study[26] where a strong correlation was found between the high degree of soybean oil establishment and a significant reduction in the number of methanogens and density of rumen protozoa. This correlation favored the reduction in CH_4 production by 60 % with respect to the control at 36 h *in vitro*. In an analysis of eight *in vitro* and four *in vivo* experiments on the potential of medium-chain fatty acids on CH_4 production, Machmüller[27] reported a significant decrease in the number of methanogens and a reduction of up to 40 % in CH_4 release with the use of soybean oil.

The present study found no significant differences (P>0.05) in the fermentation profile due to the use of NO_3-; however, there was a numerical difference of 28 % in the production of propionic acid with FN, and 40 % with PN, compared to the treatment with urea (Table 3). The addition of NO_3- in the rumen can reduce the production of CH_4 and of propionate, as it diminishes the availability of hydrogens, since many NO^{3-} reducing bacteria can utilize them as a substrate[28]; Therefore, this may generate competition not only with methanogenesis but also with propiogenesis[29]. The inclusion of protected NO_3- at the rate of 3 % of the incubated

DM in an 80:20 (concentrate/forage) diet reportedly[18] resulted in a linear reduction in propionic acid production and an increase in acetic acid production. Contrary to this, Lund *et al*[30] report that VFA production was not statistically affected by the addition of NO_3- at any of the concentrations used (6.66, 13.3, and 20 g/kg DM).

The concentration of N-NH_3 at 24 h of incubation did not vary between treatments. Contrary to what was found in other studies[8,26], where diets containing urea showed an increase in N-NH_3 concentration compared to treatments containing FN and PN. The KU treatment did not show a significant increase in NH_3 concentration after 24 h possibly because, although urea is a highly available source of nitrogen, it is rapidly hydrolyzed to NH_3 and is utilized by ruminal microorganisms for growth and development during the first three hours of incubation[31]. This causes a reduction of NH_3 levels and, possibly, an increase in the bacterial population and fermentative activity —a behavior that coincides with the increase in DDM observed with KU.

The fact that there were no differences in N-NH_3 concentration between the FN and PN treatments with respect to the control may be due to the type of metabolism of NO^{3-}. The rumen NO^{3-} is metabolized mainly by assimilatory reduction to NH_3; however, depending on the balance of enzymatic activities, nitrous oxide (N_2O) can be formed through denitrification. Because the rumen inoculum used in the current study was obtained from animals that were not adapted to NO^{3-}, NO^{2-} may have been accumulated in the system and, instead of being reduced to NH_3, it was diverted to the denitrification pathway, converting NO^{2-} to N_2O; this is the main source of N_2O under anaerobic conditions[32]. With an inclusion of 2 and 2.5 % NO^{3-} in the total DM incubated for 24 h, Welty *et al*[33] observed that NO^{3-} had a minimal effect on NH_3 concentration, which registered a significant increase only one hour after starting the *in vitro* test, while the values decreased the rest of the time and remained low during incubation.

The results of this *in vitro* test show that the inclusion of protected nitrates at levels corresponding to 3 % of the incubated dry matter can reduce methane production by 53 % after 48 h of *in vitro* incubation. The use of soaps with soybean oil as a nitrate protection method should be considered in greater detail, as the dissociation of the soap with a pH of approximately 6.5 favors the release of unsaturated fatty acids, potentially altering thereby the dynamics of the fermentation and degradation of feed in the rumen.

Acknowledgments

The authors are grateful to the Ministry of Science, Technology, and Innovation (Minciencias) of the Republic of Colombia for funding this work through project 66737 (call for proposals 836-2019).

Literature cited:

1. IPCC. Climate Change 2013: The physical science basis. Inter-governmental Panel on Climate Change, Working Group I Contribution to the IPCC Fith Assessment Report. Cambridge Univ Press, New York, USA. 2013. https://www.ipcc.ch/pdf/assessmentreport/ar5/wg1/WG1AR5_ALL_FINAL.pdf.

2. FAO. Organización de las Naciones Unidas para la Alimentación y la Agricultura. Soluciones ganaderas para el cambio climático. 2018. https://openknowledge.fao.org/server/api/core/bitstreams/4c38936f-8175-4752-bb66-32710168079e/content.

3. Lee C, Beauchemin KA. A review of feeding supplementary nitrate to ruminant animals: nitrate toxicity, methane emissions, and production performance. Can J Anim Sci 2014;94(4):557-570.

4. Hino T, Asanuma N. Suppression of ruminal methanogenesis by decreasing the substrates available to methanogenic bacteria. CAB Reviews. Perspect Agric Vet Sci Nutr Nat Resour 2003;73:1R-8R.

5. Ungerfeld EM, Kohn RA. The role of thermodynamics in the control of ruminal fermentation. Ruminant Physiol 2006;55–85.

6. Bruning-Fann CS, Kaneene JB. The effects of nitrate, nitrite, and N-nitroso compounds on animal health. Vet Hum Toxicol 1993;35(3):237–253.

7. Lee C, Araujo RC, Koenig KM, Beauchemin KA. Effects of encapsulated nitrate on enteric methane production and nitrogen and energy utilization in beef heifers. J Anim Sci 2015;93(5):2391–2404.

8. Lee C, Araujo R, Koenig KM, Beauchemin KA. *In situ* and *in vitro* evaluations of a slow-release form of nitrate for ruminants: Nitrate release rate, rumen nitrate metabolism and the production of methane, hydrogen, and nitrous oxide. Anim Feed Sci Technol 2017;231:97-106.

9. Mamvura CI, Cho S, Mbiriri DT, Lee HG, Choi NJ. Effect of encapsulating nitrate in sesame gum on *in vitro* rumen fermentation parameters. J Anim Sci 2014;27(11):1577–1583.

10. AOAC. Official Methods of Analysis. 18th ed. Washington, DC, USA: Association of Official Analytical Chemists. 2005.

11. Castillo C, Hernández J. Ruminal fistulation and cannulation: A necessary procedure for the advancement of biotechnological research in ruminants. J Anim 2021;11(7):1870.

12. McDougall EI. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem J 1948;43(1):99–109.

13. Posada SL, Rosero-Noguera R, Bolívar D. Relación entre presión y volumen para la implementación de la técnica *in vitro* de producción de gases en Medellín, Colombia. Rev Colomb Cienc Pecu 2006;19(4):407-414.

14. Posada SL, Ramírez-Agudelo JF, Rosero-Noguera R. Producción de metano y digestibilidad de mezclas kikuyo (*Pennisetum clandestinum*) - papa (*Solanum tuberosum*). Rev Agron Mesoam 2014;25(1):141-150.

15. SAS Inst. Inc. 2019. http://www.sas.com/en_us/software/university-edition.html.

16. Sukhija PS, Palmquist DL. Dissociation of calcium soaps of long-chain fatty acids in rumen fluid. J Dairy Sci 1990;73(7):1784–1787.

17. Jenkins TC, Palmquist DL. Effect of added fat and calcium on *in vitro* formation of insoluble fatty acid soaps and cell wall digestibility. J Anim Sci 1982;55(4):957–963.

18. Natel AS, Abdalla AL, de Araujo RC, McManus C, Paim T, de Abdalla FAL, *et al*. Encapsulated nitrate replacing soybean meal changes *in vitro* ruminal fermentation and methane production in diets differing in concentrate to forage ratio. J Anim Sci 2019;90(10):1350–1361.

19. Zhao L, Meng Q, Li Y, Wu H, Huo Y, Zhang X, Zhou Z. Nitrate decreases ruminal methane production with slight changes to ruminal methanogen composition of nitrate-adapted steers. BMC Microbiol 2018;18:21.

20. Zhou Z, Yu Z, Meng Q. Effects of nitrate on methane production, fermentation, and microbial populations in *in vitro* ruminal cultures. Bioresour Technol 2012;103(1):173–179.

21. Hulshof RB, Berndt A, Gerrits WJ, Dijkstra J, van Zijderveld SM, Newbold JR, Perdok HB. Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. J Anim Sci 2012;90(7):2317–2323.

22. Leng R. Interactions between microbial consortia in biofilms: A paradigm shift in rumen microbial ecology and enteric methane mitigation. Anim Pros Sci 2014;54:519-543.

23. Zhou Z, Meng Q, Yu Z. Effects of methanogenic inhibitors on methane production and abundances of methanogens and cellulolytic bacteria in *in vitro* ruminal cultures. Appl Environ Microbiol 2011;77(8): 2634–2639.

24. Lin M, Schaefer DM, Zhao GQ, Meng QX. Effects of nitrate adaptation by rumen inocula donors and substrate fiber proportion on *in vitro* nitrate disappearance, methanogenesis, and rumen fermentation acid. J Anim 2013;7(7):1099–1105.

25. Patra A, Yu Z. Effects of adaptation of *in vitro* rumen culture to garlic oil, nitrate, and saponin and their combinations on methanogenesis, fermentation, and abundances and diversity of microbial populations. Front Microbiol 2015;6:1434.

26. Wang M, Wang R, Yang S, Deng JP, Tang SX, Tan ZL. Effects of three methane mitigation agents on parameters of kinetics of total and hydrogen gas production, ruminal fermentation and hydrogen balance using *in vitro* technique. J Anim Sci 2016;87(2):224-232.

27. Machmueller A. Medium-chain fatty acids and their potential to reduce methanogenesis in domestic ruminants. Agric Ecosyst Environ 2006;112(2):107-114.

28. Zumft WG. The biological role of nitric oxide in bacteria. Arch Microbiol 1993;160(4):253–264.

29. van Zijderveld SM, Gerrits WJ, Apajalahti JA, Newbold JR, Dijkstra J, Leng RA, Perdok, HB. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J Dairy Sci 2010;93(12):5856–5866.

30. Lund P, Dahl R, Yang H, Hellwing A, Cao B, Weisbjerg M. The acute effect of addition of nitrate on *in vitro* and *in vivo* methane emission in dairy cows. Anim Prod Sci 2014;54(9):1432-1435.

31. Leng R, Emeritus D. The potential of feeding nitrate to reduce enteric methane production in ruminants. A report to the department of climate change, Canberra, Australia. 2008.

32. Kaspar HF, Tiedje JM. Dissimilatory reduction of nitrate and nitrite in the bovine rumen: nitrous oxide production and effect of acetylene. Appl Environ Microbiol 1981;41(3):705-709.

33. Welty CM, Wenner BA, Wagner BK, Roman-Garcia Y, Plank JE, Meller RA, *et al*. Rumen microbial responses to supplemental nitrate. II. Potential interactions with live yeast culture on the prokaryotic community and methanogenesis in continuous culture. J Dairy Sci 2019;102(3):2217–2231.

https://doi.org/10.22319/rmcp.v15i4.6439

Technical note

Color, moisture, and pollen content of honeys from the mangrove ecosystem of the coast of Tabasco, Mexico

Hugo Alvarado-Díaz [a]

Juan Manuel Zaldívar-Cruz [a*]

Ángel Sol-Sánchez [a]

Said Cadena-Villegas [a]

Jorge Ismael Tucuch Haas [b]

Rocío Guadalupe Acosta-Pech [a]

[a] Colegio de Postgraduados. Campus Tabasco, Periférico Carlos A. Molina S/N Km. 3, 86500 Tabasco, México.

[b] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. INIFAP, Mocochá, Yucatán, México.

[*]Corresponding author: zaldivar@colpos.mx

Abstract:

The physical characteristics of honeys are important attributes for consumers when choosing one honey over another; because of this, knowing these parameters and their botanical origin are essential to determine their quality and price. The present study determined the color, moisture, and pollen content of honeys collected in the mangrove area on the coast of the state of Tabasco, Mexico. Seventeen (17) samples were collected and their color and moisture content were determined, and a sample from each locality was selected to perform a melissopalynological analysis. Five honey colors were found with values from 12 to 120 mm Pfund, with extra light and white amber being the predominant colors. In relation to moisture content, there were samples with values of 18 to 23 %, of which 53 % of them comply with the limit (20 %) established in the regulations; finally, all the samples were multifloral, with

the Fabaceae, Poaceae, and Asteraceae being the most important botanical families. In conclusion, in the mangroves of the coast of Tabasco, Mexico, multifloral honeys of light shades are produced, where those of extra light amber and white colors predominate, with a moisture content between 18 and 23 %; therefore, these honeys could be suitable for entering more specialized markets if a maximum moisture of 20 % is ensured.

Keywords: Ecosystem, Brix, Mangroves, Pollen, Honey.

Received: 04/04/2023

Accepted: 22/02/2024

Honey is a natural product that bees produce from the nectar of flowers, secretions of living parts of plants, or from excretions of plant-sucking insects that remain on them and that bees collect, combine with their own specific substances, deposit, dehydrate, and store in the honeycomb so that it matures and ages[1]. This process gives honey unique properties that define its physicochemical, sensory, and microbiological characteristics[2].

The study of the organoleptic properties and botanical origin of honeys has been important in recent years since their analysis provides commercial added value and allows to know the interaction of *Apis mellifera* with plants[3], facilitating their characterization since these parameters are decisive for consumers when choosing a honey[4]. The color of honey is determined by its botanical origin, the composition of the nectar of the flower of origin, the extraction process, temperature, time, and storage conditions[2]. Nonetheless, geographical origin, climatic conditions, soil conditions of the plant of origin, exposure to light, heat treatment, and crystallization processes, as well as the content of minerals, antioxidants, and sugars, also influence this attribute[5,6].

In recent years, there has been an increase in reports linking honey color with the presence of phytochemicals, such as ascorbic acid, phenolic compounds, amino acids, enzymes, tocopherols, carotenoids, and flavonoids, with dark-colored honeys having the highest content of pigments with greater antioxidant potential[7,8]. In color determination, the Pfund technique is one of the most widely used due to its fast, economical, and simple characterization; it classifies honeys into seven shades of amber (water white, extra white, white, extra light amber, light amber, amber, and dark)[6,9]. In Mexico, the study of the color of honeys has revealed that this attribute varies from month to month, demonstrating the change in floral resources throughout the year, with the lightest honeys coming from the months with the highest flow of nectar (October-November)[10]. Likewise, a strong correlation has been found between antioxidant activity and color, with dark honeys having

the highest capacity[11]; this is similar to what has been reported in honeys from Tabasco[12], where they show extra-light amber and light amber colors[13]. Another fundamental factor in the physicochemical characteristics of honeys is the pollen content[2] since, as it is distinguished by its high content of proteins, vitamins, minerals, carotenes, xanthophylls, phenols, and antioxidants, among others, its analysis is decisive to evaluate the quality of the honey and botanical origin[14].

The state of Tabasco has several geographical areas that offer an important beekeeping potential, where the mangrove is one of the ecosystems that can be exploited since it is possible to obtain sweet and perfumed honey, even with a salty and bitter touch[15]. According to CONABIO[16], Tabasco has 49,225 ha of mangrove, where red mangrove (*Rhizophora mangle* L.), black mangrove (*Avicennia germinans* L.), and white mangrove (*Laguncularia racemosa* L.) are the species with the greatest presence, developing on Solonchak soils and histosols generally rich in organic matter and nutrients[17]. These mangroves are distributed in the municipalities of Huimanguillo, Cárdenas, Comalcalco, Jalpa de Méndez, Paraíso, and Centla, where they offer important environmental and socioeconomic benefits, developing as important productive systems[18]. Due to the fact that there are gaps in information in research that documents and characterizes the honeys produced in these ecosystems, the color, moisture, and pollen content in honeys from the mangrove area on the coast of the state of Tabasco were determined and their characteristics are described in order to classify them according to these parameters.

The honeys were collected from July to September 2022 through random sampling, considering, for this purpose, the beekeepers of the Beekeepers Registry registered in the Office of the Secretariat of Agriculture and Rural Development of the state of Tabasco, who are located in the mangrove areas, as well as their availability to collaborate in this study. The mangrove on the coast of the state is located approximately between the coordinates 18° 00' 31" and 18° 38' 53" N and 92° 25' 26" and 94° 07' 40" W; it is bordered to the north by the Gulf of Mexico, to the south by Plan Chontalpa, to the east by the San Pedro and San Pablo Rivers, and to the west by the Tonalá River.

The samples were stored in 500 ml translucent plastic containers, labeled according to their geographical origin, and transferred to the Food Laboratory of the College of Postgraduates, Tabasco Campus, for analysis. The color was determined using a Hanna colorimeter, model C 221, with direct readings in mm Pfund. The equipment was calibrated using glycerin as a reference target and the readings were taken in triplicate. The honeys were classified according to NOM-004[19], which classifies them according to the values of mm Pfund as water white (0-8), extra white (9-16), white (17-34), extra light amber (35-50), light amber (51-84), amber (85-114), and dark (115-140). Moisture percentage was measured in triplicate using an ATAGO PAL-22S digital refractometer, Honey Moisture (12~30 %). The data

obtained were used to perform a descriptive analysis of the qualitative variables of interest (color and moisture) through the statistical software of R Core Team[20].

To characterize the pollen, a sample was chosen from each locality (10 in total), of which 50 g of each one was taken and analyzed using the melissopalynological method described by NOM-004[19]. The identification of pollen grains was carried out by comparison with the help of the pollen keys from the Reference Collection of the Palynology Laboratory of CIATEJ (Center for Research and Assistance in Technology and Design of the State of Jalisco, A.C.) and specialized scientific papers[21-29]. Honeys were characterized as monofloral when their composition presented a pollen species with a percentage ≥ 45 % or multifloral with several pollen species present, sub-classified into: (a) oligofloral, dominated by two or more taxa of a plant family with 16 to 44 %, (b) bifloral, with two relevant taxa from different botanical families present from 16 to 44 %, and (c) strictly multifloral, with three or more taxa from different families with percentages ≥ 10 %. Finally, the most important pollen families were identified based on their dominance in each sample.

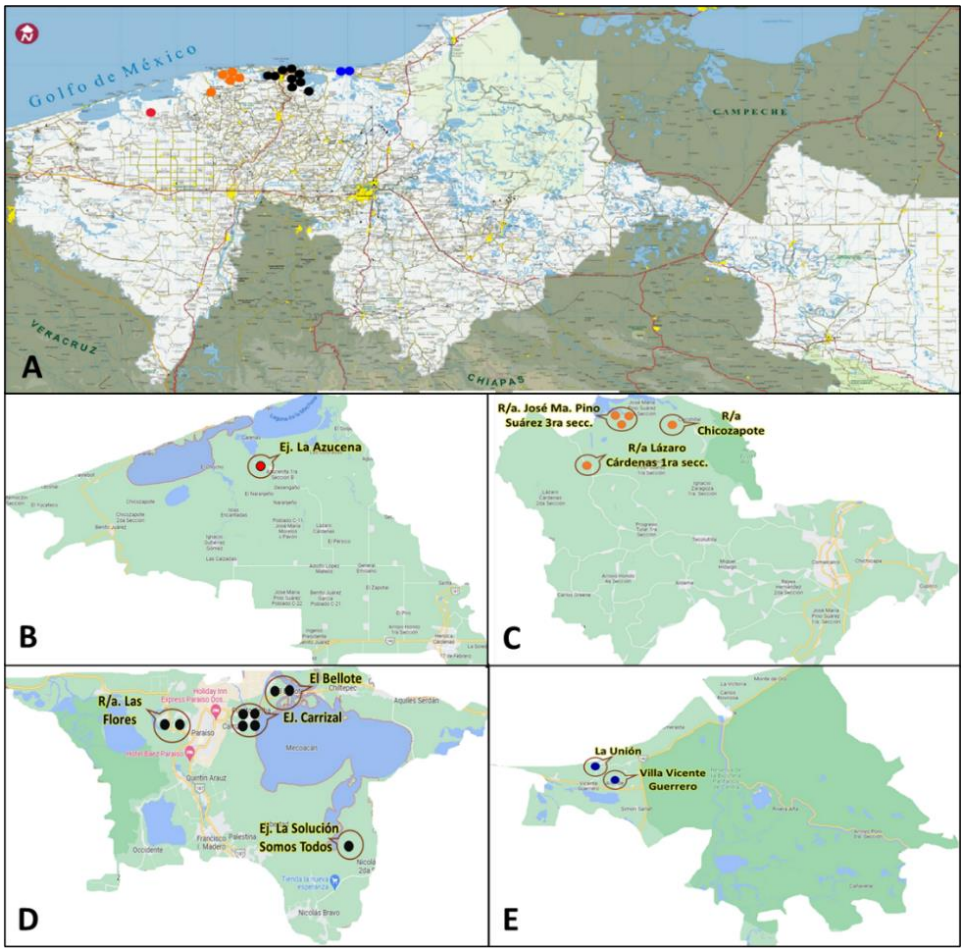
To determine color and moisture, 17 samples were collected in the municipalities of Paraíso, Comalcalco, Centla, and Cárdenas; the localities of origin and the percentage of samples by municipality is reported in Table 1.

Table 1: Number of honey samples collected, percentage by municipality, and localities of origin

Municipality	No. of samples	Percentage	Localities
Paraíso	9	53	R/a. Las Flores 1st section, Ejido Carrizal Puerto Ceiba, El Bellote, Ejido La Solución Somos Todos.
Comalcalco	5	29	R/a. Lázaro Cárdenas 1st section, R/a. José Ma. Pino Suárez 3rd section R/a. Chicozapote.
Centla	2	12	La Unión, Villa Vicente Guerrero.
Cárdenas	1	6	Ejido la Azucena 2nd section.

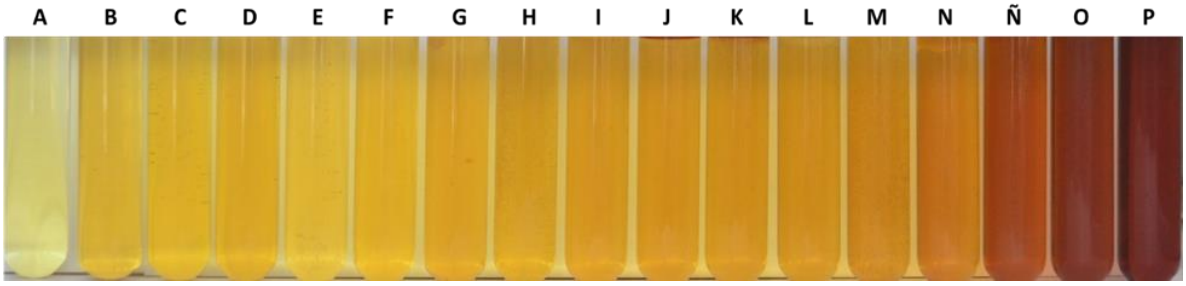
Figure 1 shows the location of the localities where the honey samples were collected, as well as the municipalities of origin.

Figure 1: A) Location of the localities where the honey samples were collected on the coast of the state of Tabasco (Modified from SOTOP, 2019), B) Cárdenas, C) Comalcalco, D) Paraíso, E) Centla.



According to the values of mm Pfund in the honeys analyzed, the following colors were found: extra light amber (47 % of the samples), white (29 %), light amber (12 %), amber (6 %), and dark (6 %); this is similar to what was reported for multifloral honeys from Guerrero, Mexico, where the variety of shades is attributed to the diversity of nectar-polliniferous plants and the metabolites they contain[11], originating from the floristic composition of the ecosystem that changes throughout the year[10]. Figure 2 shows the shades of the honeys collected: white (A, B, C, D, E), extra light amber (F, G, H, I, J, K, L, M), light amber (N, Ñ), amber (O), and dark (P).

Figure 2: Colors of honey collected in the mangrove area of the state of Tabasco



The lightest shade was found in a white honey (12±0.6 mm Pfund), whereas the darkest shade was for a dark-colored honey (120±1.2 mm Pfund) from Paraíso, as shown in Table 2. These values are similar to those reported in honeys from Peru, where it was found that the color of the honeys varied from extra light amber (44 mm Pfund) to dark amber (107 mm Pfund), attributing this variation to melanoidins (pigments generated in the Maillard reaction), which were also found in light, light amber, and dark honeys from Poland, these being the ones that establish the differences between a light and a dark honey[30,31].

The light shades are characteristic of black mangrove (*A. germinans*) honeys[32], accompanied by sweet and bitter flavors, even a little salty[33]. In Tabasco, extra-light amber and light amber honeys (46 to 68 mm Pfund) from different geographical areas have been reported[13]. These light shades have been linked to low mineral content, mild flavors, and subtle aromas, whereas dark shades are associated with strong flavors and aromas, and high content of pigments, antioxidants, and minerals[10]. Therefore, the color of honey can be used as an indicator of certain phytochemical compounds[11,33,34].

Table 2: Color and moisture of honey samples collected in the mangrove area of the state of Tabasco

Sample	mm Pfund (Mean ± SD)	Color[16]	Moisture, % (Mean ± SD)
A	12 ± 0.6	White	19.8 ± 0.2
B	21 ± 0.0	White	19.6 ± 0.1
C	28 ± 0.0	White	20.4 ± 0.2
D	32 ± 0.0	White	19.3 ± 0.2
E	32 ± 1.2	White	21.3 ± 0.3
F	38 ± 0.0	Extra light amber	21.9 ± 0.2
G	40 ± 0.6	Extra light amber	20.2 ± 0.5
H	42 ± 5.2	Extra light amber	22.8 ± 0.1
I	45 ± 0.0	Extra light amber	18.6 ± 0.1
J	45 ± 1.0	Extra light amber	19.3 ± 0.2
K	46 ± 6.4	Extra light amber	20.5 ± 0.3
L	48 ± 5.5	Extra light amber	20.5 ± 0.1
M	49 ± 3.1	Extra light amber	20.8 ± 0.1
N	57 ± 9.8	Light amber	19.1 ± 0.5
Ñ	76 ± 9.0	Light amber	19.6 ± 0.1
O	114 ± 0.6	Amber	18.9 ± 0.1
P	120 ± 1.2	Dark	18.9 ± 0.2

The honeys analyzed had an average moisture of 20.1 %; however, 47.1 % of these (Table 2) do not comply with the provisions of NOM-004[19] and the Codex Alimentarius[1] (20 % maximum for *Apis mellifera* honeys). The highest values were for samples H, F, and E (22.8 ± 0.1, 21.9 ± 0.2, and 21.3 ± 0.3 %, respectively), which could indicate that they were harvested from uncapped honeycombs[30], causing a short shelf life due to fermentation problems[35]. The variability in the moisture content (18.6 ± 0.1 to 22.8 ± 0.1) of the samples could be influenced by the moisture of the nectar of the floral source and the conditions of the environment since, in addition to the fact that the honeys are bifloral and multifloral (Table 3), they come from the 2022 spring harvest, so the increase in temperatures and the onset of rains in June[36] increase the relative humidity of the environment and therefore that of the nectar of the floral source[2].

In relation to the light shades of the honeys produced by beekeepers in this area, 59 % of them mention that this characteristic generates distrust in local consumers, so they have resorted to mixing them with darker honeys from other apiaries, areas, or seasons; nevertheless, it is important to preserve their original characteristics since each type of honey has its market and price, so these honeys could be suitable for markets such as the United

States, where they are preferred[37]. Therefore, even though it represents more work for beekeepers, it is important to keep honey from different apiaries, hives, and microregions separate, allowing a wide variety of shades and physical, chemical, and microbiological properties, targeting more specialized and demanding markets[10,38]. In addition to this, good beekeeping practices must be guaranteed to avoid dark tones generated by bad beekeeping practices, such as heating[5,14].

The melissopalynological analysis allowed to identify 34 pollen types, belonging to 7 plant families; none of the samples was considered monofloral (with some dominant taxon ≥ 45 %), so all the samples were multifloral (Table 3), of which samples H and M were subclassified as bifloral, where Fabaceae, Poaceae, and Asteraceae were the most important botanical families (16 to 44 %); finally, sample P was subclassified as strict multifloral with three taxa from different botanical families, where Myrtaceae, Fabaceae, and Sapindaceae presented percentages ≥ 10 %.

Table 3: Pollen classification of honey harvested in the mangrove area of the state of Tabasco

Municipality	Sample	Locality	Pollen classification	Pollen families
Paraíso	A	El Bellote	Multifloral	-
	E	Ejido Carrizal Puerto Ceiba	Multifloral	-
	H	R/a. Las Flores 1st section	Bifloral	Fabaceae, Poaceae
	P	Ejido La Solución Somos Todos	Strict multifloral	Myrtaceae, Fabaceae, and Sapindaceae
Comalcalco	I	R/a. Lázaro Cárdenas 1st section	Multifloral	-
	K	R/a. José Ma. Pino Suárez 3rd section	N/ID	-
	N	R/a. Chicozapote	Multifloral	-
Centla	M	La Unión	Bifloral	Poaceae and Asteraceae
	O	Villa Vicente Guerrero	Multifloral	-
Cárdenas	C	Ejido la Azucena 2nd section	N/ID	-

N/ID= not identified due to low pollen content.

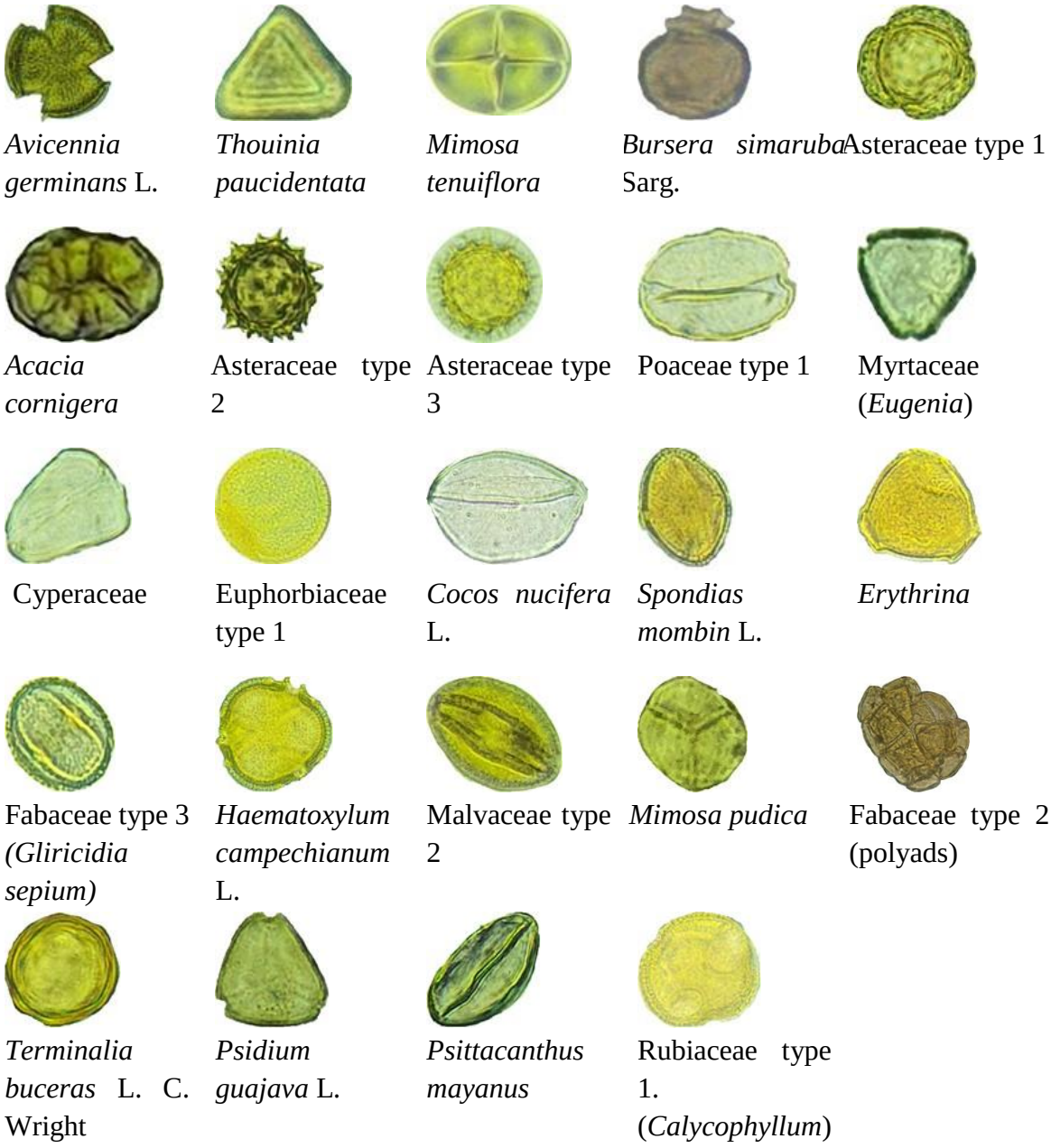
In Paraíso, there were multifloral samples that were subclassified as bifloral and one strict multifloral (Table 3), which differs from what was reported in previous research for honeys

in this municipality[26], where two monofloral samples of *Cocos nucifera* and *Mimosa albida* were identified, respectively; nonetheless, a bifloral sample was also found, with *C. nucifera* and *Psidium guajava* being the important pollen species. In Comalcalco and Cárdenas, there were honeys that were classified as multifloral, whereas for the municipality of Centla, a bifloral sample was found, where the Poaceae and Asteraceae were the important botanical families (16 to 45 %). In the case of samples K and C, they did not contain enough pollen particles to determine their frequency, so they could not be classified. Figure 3 shows some of the pollen types identified in the samples analyzed.

Black mangrove pollen was present in seven samples from the four municipalities, being found as secondary pollen in sample E (26.6 %), which is a white honey from Paraíso, as well as in sample N (17 %), which has a light amber coloration and is from Comalcalco. No pollen from other mangrove species was found, which could be attributed to the abundance of flowers from other plant species in the area and season since bees tend to discriminate and select flowers by color, smell, or by type of pollen. Because of this, it is necessary to carry out studies that allow the identification of these granules in the harvests of other seasons.

According to the total diversity of palynomorphs found in the honeys collected from the coastal mangroves, nine important taxa were found, considering their presence in the honeys in a percentage ≥10 %, the most representative being Poaceae type 1 (Poaceae), Asteraceae type 1 (Asteraceae), *Eugenia* (Myrtaceae), *Avicennia germinans* L. (Acanthaceae), *Mimosa tenuiflora* (Fabaceae), *Mimosa albida* Humb. & Bonpl. Ex Willd. (Fabaceae), *Bursera simaruba* Sarg. (Burseraceae), *Thouinia paucidentata* Radlk. (Sapindaceae), and *Lonchocarpus punctatus* Kunth (Fabaceae). Previous studies have identified important taxa in these areas, such as Acanthaceae, Poaceae, and Fabaceae, among others[26].

Figure 3: Pollen types found in honey samples collected in the mangrove area of the state of Tabasco



In conclusion, the honeys collected in the mangrove area of the coast of Tabasco and from the 2022 spring harvest are multifloral honeys with light shades, where those of extra-light amber and white colors predominate, with an average moisture content of 20.1 % (between 18 and 23 %), so these honeys could be suitable for entering into more specialized markets if the moisture content established by the regulations to guarantee their quality from

production to consumption is complied with. Likewise, although they could not be considered as mangrove honeys in terms of pollen, black mangrove pollen grains were found in 70 % of the samples analyzed, and as secondary pollen in 2 of them, so the mangrove is an ecosystem that can be considered important for beekeeping production in the area.

Literature cited:

1. Codex Alimentarius. Norma para la miel CXS 12–1981. Adoptada en 1981. Revisada en 1987 y 2001. Enmendada en 2019. 2019. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B12-1981%252FCXS_012s.pdf. Consultado 7 Mar, 2023.

2. Campo-Barrera OI, Hincapié-Llanos GA. Factores que determinan las propiedades fisicoquímicas de la miel de abejas: Revisión Sistemática de Literatura. Rev Mutis 2023;13(1):1-28.

3. Baena-Díaz F, Chevez E, Ruiz DMF, Porter-Bolland L. *Apis mellifera* en México: producción de miel, flora melífera y aspectos de polinización. Revisión. Rev Mex Cienc Pecu 2022;13(2):525-548.

4. Luna-Rivera I, Paz-Calderón Y, Flores-Castillo LA. Comercialización de miel en Huajuapan de León: Desafíos y oportunidades. NOVUN 2020;1(10):124-146.

5. Vegh R, Puter D, Vaskó A, Csóka M, Mednyanszky Z. Examination of the nutrient content and color characteristics of honey and pollen samples. J Food Invest 2022;68(1):3793-3806.

6. Bodor Z, Benedek C, Urbin A, Szabo A, Sipos L. Color of honey: can we trust the Pfund scale? – An alternative graphical tool covering the whole visible spectra. LWT-Food Sci Technol 2021;149:1-9.

7. López-Velasco DS, Sosa-Montes E, Pro-Martínez A, González-Cerón F, Vargas-Galicia AJ. Efecto antioxidante de la miel de abeja sobre la carne de conejo almacenada en refrigeración. Ciencia UAT 2021;5(2):135-143.

8. Roy S, Mandal S. Color intensity and antioxidative capacity of four natural monofloral honey from Malda, India. Asian J Pharm Clin Res 2019;12(9):111-115.

9. Hasnul HMH, Jern KP, Thiviyanathan VA, Hoon TSG, Sing LY, Jing LH, *et al*. The amber-colored liquid: A review on the color standards, methods of detection, issues and recommendations. Sensors 2021;21:38.

10. Quintero-Domínguez R, Reyes-Carrillo JL, De la Cruz-Larios L, González-Eguiarte DR. Bee honey color variation throughout the year in Huejotitán, Jalisco, México. Sust Agri Food Environ Res 2018;6(3):1-9.

11. Mendoza-Bacilio CI, Epifanio-Gómez R, Yam-Puc A, Ávila-Caballero LP, Palemón-Alberto F, Torres-Guzmán F, Bello-Martínez J. Color influence on phenolic compounds and bioactive properties of honey from Guerrero, Mexico. SANUS 2022;24(2):5-11.

12. Balcázar-Cruz L, Valadez-Villarreal A, López-Naranjo JI, Ochoa-Flores AA, Rodríguez-Blanco L, López-Hernández E. Relación del contenido de flavonoides y color en miel de abeja (*Apis mellifera*) originaria del estado de Tabasco, México. Investig Desarro Cienc Tecnol Aliment 2019;4:818-825.

13. López-González MM, Moo-Huchín VM, Sauri-Duch E, Zaldívar Cruz JM. Determinación del color de las mieles tabasqueñas. Cetzal-Ix W, *et al*. editores. Agrecosistemas tropicales: Conservación de recursos naturales y seguridad alimentaria. México. Tecnológico Nacional de México 2019;97-103.

14. Ormeño-Luna J, Santander-Riuz WE. Dominancia cromática de carga de polen corbicular y mieles colectadas por *Apis mellifera* L. en el Bajo Mayo, San Martin. Rev Agrotec Amaz 2023;3(1):11.

15. Cunill FJM, Nettel HA, Tovilla HC. Manglares entre el mar y la tierra prometida. Ecofronteras 2018;22(63):22-25.

16. CONABIO. (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad). Actualización de la cartografía del Sistema de Monitoreo de los Manglares de México 2020. México. 2021. https://www.gob.mx/conabio/prensa/el-sistema-de-monitoreo-de-los-manglares-de-mexico-presenta-nueva-cartografia-de-la-distribucion-de-manglares-en-2020-262804?idiom=es. Consultado 7 Mar, 2023.

17. Domínguez-Domínguez M, Zavala-Cruz J, Rincón-Ramírez JA, Martínez-Zurimendi P. Management strategies for the conservation, restoration and utilization of mangroves in Southeastern Mexico. Wetland 2019;39:907-919.

18. Domínguez-Domínguez M, Martínez-Zurimendi P. Beneficios ambientales, usos forestales maderables y no maderables del manglar en la planicie costera. En: Cruz AA, Cruz MJ, Valero PJ, Rodríguez RFP, Daniela ME, Mata ZEE, Palma LDJ. La biodiversidad en Tabasco. Estudio de Estado. México, CONABIO. 2019;243-247.

19. NOM-004-SAG/GAN. Producción de miel y especificaciones. 2018 https://www.dof.gob.mx/nota_detalle.php?codigo=5592435&fecha=29/04/2020#gsc.tab=0 Consultado 7 Mar, 2023.

20. RStudio Team. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. 2020. http://www.rstudio.com Consultado 7 de marzo, 2023.

21. Souza DRH, De Jesús SV, Magalhães e Silva FH, Dias SM. Floristic characterization and pollen morphology of plants visited by *Apis mellifera* L. in caatinga areas in Bahia, Brazil. Acta Botanica Brasilica 2023;37:1-21. https://doi.org/10.1590/1677-941X-ABB-2022-0264.

22. Briceño-Santiago CI, Cano-Sosa J, Ramos-Díaz AL, Noriega-Trejo R, Couoh-May DI. Estudio de la flora presente en apiarios de tres municipios en el estado de Yucatán, México. Polibotánica 2022;53:13-34. https://doi.org/10.18387/polibotanica.53.2

23. Reis HS, Araújo SO, Lima LCL, Silva FHM, Diogo IJS, Saba MD. Qualitative analysis of *Apis mellifera* L. honey in an ecotone area in the Bahian semiarid. Sci Plena 2021;17(5):1-18. https://doi.org/10.14808/sci.plena.2021.051502.

24. Villanueva-Gutiérrez R, Moguel-Ordóñez YB, Echazarreta-González CM, Arana-López G. Monofloral honeys in the Yucatan Peninsula, Mexico. Grana 2009;48:214-223. https://doi.org/10.1080/00173130902929203.

25. Ramos-Díaz A, Noriega-Trejo R, Sánchez-Contreras A, San Román-Ávila D, Góngora-Chin R, Rodríguez-Buenfil I. Catálogo de los principales tipos polínicos encontrados en las mieles producidas en la península de Yucatán. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ) Unidad Sureste, Mérida, Yucatán, México. 2015;110.

26. Castellanos-Potenciano BP, Ramírez-Arriaga E, Zaldívar-Cruz JM. Análisis del contenido polínico de mieles producidas por *Apis mellifera* L. (Hymenoptera: Apidae) en el estado de Tabasco, México. Acta Zool Mex (n.s.). 2012;28(1):13-36. ISSN 0065-1737.

27. Reyes-Carrillo JL, Muñoz-Soto R, Cano-Ríos P, Eischen FA, Blanco-Contreras E. Atlas del polen de la Comarca Lagunera, México. México DF; Guzmán Editores: 2009.

28. Martínez-Hernández E, Cuadriello-Aguilar JI, Téllez-Valdez O, Ramírez-Arriaga E, Sosa-Nájera MS, Melchor-Sánchez JEM, *et al*. Atlas de las plantas y el polen utilizados por las cinco especies principales de abejas productoras de miel en la región del Tacaná, Chiapas, México. Universidad Nacional Autónoma de México, Instituto de Geología, México DF. 1993:105.

29. Palacios CHR, Ludlow-Wiechers B, Villanueva GR. Flora palinológica de la reserva de la biosfera de Sian Ka´an, Quintana Roo, México. Centro de Investigaciones de Quintana Roo, Chetumal, Quintana Roo, México. 1991:321.

30. Coronado JMF, Ormeño LJ, Barrera LM, Castillo DT. Caracteres fisicoquímicos en mieles del ecosistema del Bajo Mayo, región San Martín Perú. Arnaldao 2019;26(2):607-622.

31. Starowicz M, Ostaszyk A, Zieliński H. The relationship between the browning index, total phenolics, color, and antioxidant activity of Polish-originated honey samples. Foods 2021;10:1-12.

32. Carmejo EM, González AM. Clasificación de mieles uniflorales cubanas a partir de sus propiedades físico-químicas. Rev CENIC 2005;36(Esp):7.

33. González CR, Toledo NB, May CMI. Culturas y territorios: un mundo en una gota de miel. Ecofronteras 2021;25(73):14-17.

34. Al-Farsi M, Al-Amri A, Al-Hadhrami A, Al-Belushi S. Color, flavonoids, phenolics and antioxidants of Omani honey. Heliyon 2018;4(10):1-14.

35. Grajales-Conesa P, Velázquez-Aguilar JM, Ricón-Rabanales M, Sánchez-Guillén D. Caracterización físico-química de mieles de *Apis mellifera* de tres paisajes forestales de Chiapas. Quehacer Científico de Chiapas 2013;8(2):12-17.

36. INECC. (Instituto Nacional de Ecología y Cambio Climático). Visión estatal: Vulnerabilidad, riesgos y peligros Tabasco. 2021. https://cambioclimatico.gob.mx/estadosymunicipios/Vulnerabilidad/V_27.html#:~:text=Climatolog%C3%ADa%20estatal,es%20septiembre%20(360.6%20mm).Consultado 7 Ene, 2024.

37. Dulce EV. Estrategia de inserción internacional de la miel de abeja en el mercado de Estados Unidos. Rev Agrotec Amaz 2023;3(1):13.

38. Molaveisi M, Beigbabaei A, Akbari E, Shahidi NM, Mohamadi M. Kinetics of temperature effect on antioxidant activity, phenolic compounds and color of Iranian jujube honey. Heliyon 2019;5(1):e01129.

Revista Mexicana de Ciencias Pecuarias

Rev. Mex. Cienc. Pecu. Vol. 15 Núm. 4, pp. 762-1005, OCTUBRE-DICIEMBRE-2024

CONTENIDO
CONTENTS

ARTÍCULOS / ARTICLES — Pags.

Momento de la suplementación sobre carga animal y producción de leche por hectárea de vacas Holstein en pastoreo
The effects of supplementation timing on stocking rate and milk production per hectare in grazing Holstein dairy cows
Citlali Anais Castro Jaime, Rodolfo Ramírez Valverde, Juan Andrés Burgueño Ferreira, Jacinto Efrén Ramírez Bribiesca, Luis Alberto Miranda Romero, Ricardo Daniel Améndola Massiotti.....762

Respuesta productiva, características de la canal y calidad de la carne de ovinos alimentados con niveles crecientes de frutos secos triturados de Acacia farnesiana
Productive response, carcass traits, and meat quality of sheep fed with increasing levels of crushed dry fruits of Acacia farnesiana
Miguel Ángel Zarza-Albarrán, Agustín Olmedo-Juárez, Pedro Mendoza- de Gives, Jaime Ancelmo- Mondragón, Javier Arece-García, Francisca Aviles-Nova, Benito Albarrán-Portillo, Rolando Rojo-Rubio.....778

Modelo de predicción para la extensión de la vida productiva en registros censurados de ganado Holstein de México
Prediction model for productive life extension in censored records of Holstein cattle from Mexico
Sandra Giovanna Núñez-Soto, Adriana García-Ruiz, Hugo Oswaldo Toledo Alvarado, Felipe de Jesús Ruiz-López.....800

Evaluación antihelmíntica in vitro de curcumina contra huevos y larvas de aislados de Haemonchus contortus con distinta susceptibilidad a ivermectina
In vitro anthelmintic evaluation of curcumin against the eggs and larvae of three Haemonchus contortus isolates with different susceptibility to ivermectin
Lisandro Alberto Encalada-Mena, Raquel López-Arellano, María Eugenia López-Arellano, María Gabriela Mancilla-Montelongo, Carlos Alfredo Sandoval-Castro, Agustín Olmedo-Juárez, Juan Felipe de Jesús Torres-Acosta.....811

Effect of spent coffee grounds aqueous extract as an antioxidant in raw pork patties during refrigerated storage
Efecto del extracto acuoso de granos de café gastado como antioxidante en hamburguesas de cerdo crudas durante el almacenamiento refrigerado
Juan Luis Murillo Hernández, Rey David Vargas Sánchez, Brisa del Mar Torres Martínez, Nelson Huerta Leidenz, Gastón Ramón Torrescano Urrutia, Armida Sánchez Escalante.....833

Prevalence and diversity of zoonotic intestinal parasites in household dogs in urban areas of the Colombian Caribbean
Prevalencia y diversidad de parásitos intestinales zoonóticos en perros domésticos en un área urbana en el Caribe colombiano
Luz Adriana Sarmiento-Rubiano, Yina García Toscano, Julieannie Paola Ruiz, Lucena Delgado Soraca, Alfonso Bettin Martínez, Jimmy Becerra Enriquez.....848

Transmisión espacial y vertical de los precios de leche del mercado internacional a mercados regionales y nacional de México
Spatial and vertical transmission of milk prices from the international market to Mexico´s regional and national markets
Benigna González Ortiz, José Luis Jaramillo Villanueva, Samuel Vargas López, Ángel Bustamante González, Juan de Dios Guerrero Rodríguez, Vinicio Horacio Santoyo Cortés.....861

Competitividad y ventaja comparativa de ganado bovino de carne en la Sierra Norte de Puebla, México
Competitiveness and comparative advantage in beef cattle production in the Sierra Norte of Puebla, Mexico
Lissete Abigail Rojas-Juárez, José Luis Jaramillo-Villanueva, Samuel Vargas-López, Juan Cabas- Monje.....879

Dinámica estacional de pasto banderita [Bouteloua curtipendula (Mich.) Torr.] en Chihuahua, México: enfoque geoestadístico
Seasonal dynamics of sideoats grama [Bouteloua curtipendula (Mich.) Torr.] in Chihuahua, Mexico: a geostatistical approach
Ireyli Zuluamy Iracheta-Lara, Carlos Raúl Morales-Nieto, Gerardo Méndez-Zamora, Jaime Neftalí Márquez-Godoy, Federico Villarreal-Guerrero.....898

Comportamiento productivo de una asociación Mombaza-Kudzú en diferentes épocas del año
Productive behavior of a Mombasa-Kudzu association at different times of the year
Eduardo Daniel Bolaños-Aguilar, Javier Francisco Enríquez-Quiroz, Abraham Fragoso-Islas, Roberto Omar Castañeda-Arriola, Maribel Montero-Lagunes, Julio César Vinay-Vadillo.....913

REVISIONES DE LITERATURA / REVIEWS

Características de las curvas de lactación en ovejas y factores que influyen en su variación: Revisión
Characteristics of lactation curves in ewes and factors influencing their variation: A review
Gabriela Castillo-Hernández, Ana Erika Ochoa-Alfaro, Manuel Antonio Ochoa-Cordero, Jorge Alonso Maldonado Jáquez, Glafiro Torres-Hernández.....930

NOTAS DE INVESTIGACIÓN / TECHNICAL NOTES

Capacidad de carga del venado cola blanca (Odocoileus virginianus texanus) en el noreste de México
Carrying capacity of white-tailed deer (Odocoileus virginianus texanus) in northeast Mexico
Fernando Isaac Gastelum Mendoza, Fernando Noel González Saldívar, Eloy Alejandro Lozano Cavazos, José Isidro Uvalle Sauceda, Guillermo Romero Figueroa, Enrique de Jesús Ruíz Mondragón, César Martín Cantú Ayala.....951

The management of irrigated elephant grass intercropped with legumes in the semi- arid region
Manejo del pasto elefante de riego intercalado con leguminosas en la región semiárida
Jéssica Daisy do Vale Bezerra, João Virgínio Emerenciano Neto, Tamiris da Cruz da Silva, Patrick Bezerra Fernandes, Stela Antas Urbano, Felipe Pontes Teixeira das Chagas, Gelson dos Santos Difante, Marcone Geraldo Costa, Antonio Leandro Chaves Gurgel, Francisco Israel Lopes Sousa, Rodrigo da Silva Santos, Ana Beatriz Graciano da Costa.....963

Evaluación in vitro de una fuente de nitrato protegido para rumiantes: efecto sobre la degradación de la materia seca y producción de metano
In vitro evaluation of a protected ruminant nitrate source: effect on dry matter degradation and methane production
María Elizabeth Rendón-Correa, Sandra Lucia Posada-Ochoa, Jaime Ricardo Rosero-Noguera.....979

Color, humedad y contenido polínico de mieles del ecosistema manglar de la costa de Tabasco, México
Color, moisture, and pollen content of honeys from the mangrove ecosystem of the coast of Tabasco, Mexico
Hugo Alvarado-Díaz, Juan Manuel Zaldívar-Cruz, Ángel Sol-Sánchez, Said Cadena-Villegas, Jorge Ismael Tucuch Haas, Rocío Guadalupe Acosta-Pech.....991

Revista Mexicana de Ciencias Pecuarias Rev. Mex. Cienc. Pecu. Vol. 15 Núm. 4, pp. 762-1005, OCTUBRE-DICIEMBRE-2024

inifap
Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias