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EFFECTO DEL ESTRÉS TÉRMICO SOBRE LA PRODUCCIÓN LÁCTEA Y EL
ESTADO REPRODUCTIVO DE VACAS HOLSTEIN EN UN ENTORNO ÁRIDO:
UN ENFOQUE DE SERIES DE TIEMPOS

Tesis

Que presenta IRENE CONCEPCION CHAVARRIA NERI
como requisito parcial para obtener el grado de
DOCTOR EN CIENCIAS EN PRODUCCIÓN AGROPECUARIA

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Elaborada por IRENE CONCEPCION CHAVARRIA NERI como requisito parcial
para obtener el grado de Doctor en Ciencias en Producción Agropecuaria con la
supervisión y aprobación del Comité de Asesoría



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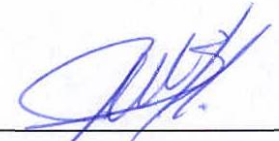
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CARTA DE ACEPTACIÓN DE LOS ARTÍCULOS

Carta aceptación artículo 1

Decision on submission to Journal of Thermal Biology

Journal of Thermal Biology

Time: Tue, Aug 6, 2024 at 5:54 AM

Message Body

Manuscript Number: **TB-D-24-00130R1**

Unmasking seasonal cycles in a high-input dairy herd in a hot environment: How climate shapes dynamics of milk yield, reproduction, and productive status

Dear Dr Mellado,

Thank you for submitting your manuscript to Journal of Thermal Biology.

I am pleased to inform you that your manuscript has been accepted for publication.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Journal of Thermal Biology and hope you will consider us again for future submissions.

We encourage authors of original research papers to share the research objects – including raw data, methods, protocols, software, hardware and other outputs – associated with their paper. More information on how our open access Research Elements journals can help you do this is available at https://www.elsevier.com/authors/tools-and-resources/research-elements-journals?dgcid=ec_em_research_elements_email.

Kind regards,
Dominic McCafferty
Editor-in-Chief

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Parece que este mensaje está en inglés

Dear PhD. Mellado,

I am pleased to inform you that your manuscript "A time series analysis of monthly Holstein milk yield and composition in a hot environment" has been accept for publication in Mljekarstvo. This paper will be published in January-March 2025 / Vol. 75 No. 1

Kind regards,

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RESUMEN

Efecto del estrés térmico sobre la producción láctea y el estado reproductivo de vacas Holstein en un entorno árido: un enfoque de series de tiempos

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Director de tesis

Este estudio integró información productiva y reproductiva de un hato Holstein de alta producción expuesto a estrés térmico prolongado en un ambiente árido, con registros de 2014 a 2020. Mediante modelos ARIMA se evaluó la influencia del clima sobre la producción láctea, la composición de la leche y el estatus reproductivo, además de predecir tendencias futuras. Los resultados mostraron una marcada estacionalidad: la proporción anual de producción fue mayor en invierno, alcanzando su punto máximo en febrero, y menor en verano, especialmente en agosto. Las predicciones indicaron diferencias importantes entre estaciones, con una caída de hasta 8.4 kg en la producción diaria durante los meses cálidos. Los componentes lácteos (sólidos totales, grasa, proteína y lactosa) fueron más altos en enero y disminuyeron conforme aumentó la temperatura. En el ámbito reproductivo, el mayor porcentaje de vacas gestantes se presentó en mayo y el menor en noviembre, reflejando la sensibilidad del ciclo reproductivo al ambiente térmico. También se observaron variaciones estacionales en los recuentos bacterianos, con mayores valores en otoño e invierno. En conjunto, el estrés por calor afectó la productividad, la calidad de la leche y los parámetros reproductivos. Los modelos ARIMA demostraron ser una herramienta sólida para pronosticar tendencias y apoyar la toma de decisiones en sistemas lecheros de climas cálidos.

Palabras claves: Leche, Clima, Reproducción, Estacionalidad, ARIMA

ABSTRACT

Effect of Heat Stress on Milk Production and Reproductive Status of Holstein Cows in an Arid Environment: A Time-Series Approach

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Thesis's Director

This study integrated productive and reproductive data from a high-input Holstein dairy herd exposed to prolonged thermal stress in a hot-arid environment, using records from 2014 to 2020. ARIMA time-series models were applied to evaluate climate effects on milk yield, milk composition, and reproductive status, and to forecast future trends. Results revealed strong seasonality: annual milk yield proportions peaked in winter, with February showing the highest values, and declined markedly in summer, particularly in August. Forecasts showed substantial seasonal differences, including up to an 8.4-kg drop in daily milk yield during the hottest months. Milk components (total solids, fat, protein, and lactose) were highest in January and decreased as temperature increased. Reproductive variables showed similar climatic sensitivity, with the highest percentage of pregnant cows in May and the lowest in November. Seasonal shifts were also evident in bacterial counts, which were higher in autumn and winter. Overall, heat stress negatively affected productivity, milk quality, and reproductive performance. ARIMA models proved to be a reliable tool for forecasting key parameters and supporting management strategies in dairy systems operating under hot climates.

Keywords: Climate, Milk, Reproduction, ARIMA, Seasonality

INTRODUCCIÓN

La producción de leche en sistemas intensivos representa un de los pilares fundamentales de la ganadería en México y en el mundo (Camacho et al., 2017; Brito et al., 2021). En muchas zonas del mundo, las vacas Holstein experimentan temperaturas extremas, alta radiación solar y baja humedad, lo que conduce a fenómenos de estrés por calor que tienen impactos negativos en su bienestar, salud, nivel de producción y eficiencia reproductiva (Bernabucci et al., 2014; Polsky and von Keyserlingk, 2017; Sammad et al., 2020). Este estrés, cuya carga térmica supera la capacidad del animal para disiparla, produce una disminución en el consumo de materia seca (Baumgard y Rhoads, 2012; Chen et al., 2024), alteraciones metabólicas y ruminales (Abeni et al., 2007; Kaufman et al., 2020; Akköse and Vural, 2026), disfunciones de la glándula mamaria (Tao et al., 2011; Ouellet et al., 2021), reflejadas en una menor producción y calidad de la leche (Chavarría et al., 2025) y parámetros reproductivos negativamente afectados (Mellado et al., 2013; Stefanska et al., 2024). En climas cálidos como el norte de México, donde las temperaturas pueden alcanzar más de 40 °C, junto con un índice de humedad térmica (ITH) > 72, resultan en un estrés por calor continuo (Das et al., 2016), lo que deteriora la recuperación nocturna y la reproducción de las vacas lecheras (MbulTHa et al., 2021; Pérez-Reboloso et al., 2025).

El sector lácteo es particularmente sensible a las temperaturas ambientales elevadas, ya que el estrés térmico conlleva un aumento de la incidencia de enfermedades infecciosas (Mellado et al., 2023) y metabólicas (Skibieli, 2024), una menor producción de leche y una disminución de la fertilidad de las vacas lactantes con el tiempo (Stefanska et al., 2024). Cuando las vacas lecheras se exponen a temperaturas superiores al umbral de su zona termoneutral (16 a 25 °C) (Dovolou et al., 2024), sufren estrés. Una humedad relativa elevada, en combinación con el aumento de las temperaturas, puede exacerbar el grado de estrés calorífico en las vacas.

Para evaluar con precisión las condiciones climáticas que favorecen el bienestar de las vacas, se ha adoptado el índice de temperatura y humedad (ITH). Según el ITH, las vacas se encuentran en la zona de confort con un $ITH < 68$, con estrés leve con un ITH entre 69 y 78, con estrés moderado con un ITH entre 79 y 89, y con estrés severo con un $ITH > 90$ (Bernabucci et al., 2010). Sin embargo, cabe señalar que los umbrales establecidos para definir el confort térmico pueden variar según diversos factores (raza, nivel de producción de leche, color de piel, etc.). Casi todas las razas europeas de vacas lecheras son extremadamente vulnerables al estrés térmico debido a que estas razas se han criado tradicionalmente en climas más fríos y carecen de adaptaciones genéticas para afrontar el estrés térmico (Becker et al., 2020; Habimana et al., 2023). Además, su tasa metabólica extremadamente alta, asociada a la síntesis de leche, aumenta significativamente la producción interna de calor (Wolfenson et al., 2019). Las altas temperaturas y la humedad también pueden tener efectos residuales en los cambios conductuales y fisiológicos de las vacas lecheras de alta producción. Si bien la disminución del comportamiento debido al estrés por calor se asocia típicamente con los meses de verano, de junio a septiembre, las consecuencias negativas pueden extenderse hasta el otoño, incluso después de que las vacas ya no presenten estrés por calor (De Rensis and Scaramuzzi, 2003). En consecuencia, el manejo y la prevención del estrés por calor se han convertido en un desafío importante debido al creciente número de animales de producción con altos comportamientos de leche en zonas con altas temperaturas ambientales (Pérez-Reboloso et al., 2025).

Se ha reportado una estacionalidad en la producción de leche en regiones templadas, con picos durante el invierno y las producciones más bajas registradas en verano (Heck et al., 2009; Ferreira et al., 2020), pero hay una falta de información de áreas bajo hipertermia crónica como la observada en el noreste de México, donde sus pérdidas económicas se reflejan en menor producción de leche, bajo porcentaje de sólidos, menores

tasas de fertilidad y mayor descarte (Jeong et al., 2018; Chavarría et al., 2024). En este contexto, los modelos de series temporales como ARIMA (promedio móvil integrado autorregresivo) se utilizan para analizar patrones históricos y prever las variaciones en el desempeño productivo y reproductivo de los animales mediante el uso de datos secuenciales (Box, 2013; Hyndman and Athanasopoulos, 2018).

Su uso en la ganadería intensiva ayuda a predecir declives en la temporada más calurosa, planificar recursos y ajustar el manejo reproductivo y nutricional (Ferreira et al., 2016; Li y Cabrera, 2021). Además, el análisis de correlación cruzada entre ITH y parámetros zootécnicos ayuda a establecer los retrasos de los efectos climáticos en la productividad (Guo et al., 2018).

La tesis se basa en estudios realizados en hatos comerciales ubicados en el norte de México, con más de 2,500 vacas Holstein y propone: analizar la dinámica estacional de la producción, composición de la leche y estado reproductivo utilizando modelos ARIMA; predecir los procesos ARIMA como herramienta de predicción para mejorar la eficiencia productiva bajo condiciones de estrés ambiental por calor.

Se presentan dos hipótesis: (1) existe una fluctuación anual significativa en la composición de la leche que está afectada por el clima, y (2) las circunstancias de La Laguna modifican la composición y contenido bacteriano de la leche en sistemas intensivos.

REVISIÓN DE LITERATURA

1. Bases conceptuales del estrés térmico en bovinos

1.1. Concepto y factores ambientales-fisiológicos determinantes del estrés térmico

El estrés por calor es una condición fisiológica en las vacas lecheras que resulta de un desequilibrio entre la carga de calor interna producida durante la actividad metabólica y la capacidad del animal para liberar el exceso de calor al ambiente (Giannone et al., 2023). Este proceso ocurre cuando las condiciones ambientales, que consisten en temperatura, índice temperatura-humedad (ITH), radiación solar y velocidad del viento, tienen valores superiores a los que representan la zona de confort térmico del animal (Oliveira et al., 2025).

Este equilibrio es particularmente precario en las vacas lecheras de alta producción de leche porque la cantidad de calor metabólico producido para secretar leche es considerable (West, 2003; Nzeyimana et al., 2023). El ganado lechero de producción excepcionalmente alta es susceptible debido a que su zona termoneutral es limitada en comparación con las vacas de baja producción. El estrés por calor es una forma de hipertermia (temperatura corporal elevada) en la que los sistemas fisiológicos del cuerpo no logran regular la temperatura corporal dentro de un rango normal. El estrés por calor es la influencia ambiental adversa más generalizada en la salud y el comportamiento animal a nivel mundial, aunque numerosos elementos ambientales pueden afectar el sistema inmunitario y la producción de un animal (Dahl et al., 2020).

La zona térmica neutral de las Holstein es de 5-25 °C, en función de la humedad relativa y el viento. Cuando el índice de temperatura y humedad (ITH) supera los 68, pueden mostrar signos de estrés por calor, que incluyen una temperatura rectal más alta, jadeo, disminución de la ingesta de

alimento y producción de leche (Țogoe and Mincă, 2024). En un ambiente más árido como la Comarca Lagunera en el norte de México, donde las temperaturas de verano son alrededor de 40 °C o más y los valores de ITH alcanzan niveles críticos (>78), el período en el que ocurre el estrés por calor se prolonga de la primavera al otoño (Mellado et al., 2013).

Otro factor que influye junto con la temperatura ambiental es el microambiente dentro del establo que también es determinante para considerar la carga radiante de cubiertas o alambres, densidad de población, ventilación y flujo de aire en la parte posterior y acceso a sombra que definen el gradiente térmico y la evaporación de agua cutánea y pulmonar (Becker et al., 2020; Frigeri et al., 2023).

Durante el estrés por calor, las vacas lecheras corren el riesgo de sufrir acidosis ruminal (Sammad et al., 2020) debido a la disminución del pH ruminal causada por la alta concentración y acumulación de ácido láctico (Yadav et al., 2013). Las condiciones de pH bajo disminuyen la abundancia de bacterias fibrolíticas, lo que reduce la digestibilidad de la fibra (Baek et al., 2020).

Una alta ingesta de concentrado en lugar de forraje (Uyeno et al., 2010) disminuye la motilidad ruminal y la rumia, lo que reduce la producción de saliva, que actúa como agente amortiguador en el rumen (Das et al., 2016), disminuyendo así el pH ruminal. En consecuencia, un aumento de lactato y una disminución de las especies productoras de acetato en la microbiota intestinal constituyen una posible causa de la disminución en la producción de leche (Zhao et al., 2019).

El ganado responde al estrés térmico mediante la adaptación de diversas respuestas conductuales y fisiológicas. Las respuestas del ganado bovino a cualquier cambio en el clima o el ambiente se denominan, respectivamente, proceso de aclimatación y adaptación (Yadav et al., 2021). La aclimatación y la adaptación a las condiciones climáticas cálidas implican cambios fenotípicos que son cruciales para la producción ganadera sostenible y pueden extenderse a lo largo de varias generaciones (Hansen,

2004). Las condiciones climáticas cálidas inducen cambios en la expresión génica y respuestas adaptativas endocrinas y metabólicas para mejorar la termotolerancia (Farooq et al., 2010), pero pueden ir acompañadas de una reducción del potencial productivo.

El proceso de aclimatación, una respuesta coordinada a varios factores de estrés térmico individuales simultáneamente, como la temperatura y la humedad, comienza entre tres y cuatro días después de la exposición a las altas temperaturas (Maggiolino et al., 2025). En un episodio agudo de estrés térmico de uno o dos días, el ganado adopta mecanismos adaptativos a corto plazo, como beber más agua, aumentar la respiración y evaporación, para disipar el calor corporal. Sin embargo, en episodios de estrés térmico crónico, al fallar las adaptaciones a corto plazo, la temperatura corporal aumenta gradualmente, lo que resulta en una menor ingesta de alimento y una producción de leche reducida. El aumento de la frecuencia cardíaca, la tasa de sudoración, el tiempo que el cuerpo pasa de pie o acostado y el patrón de alimentación pueden indicar estrés térmico crónico (Zhou et al., 2022).

En cuanto a los determinantes fisiológicos, la producción de leche es el principal factor que intensifica el estrés por calor principalmente en vacas altas productoras de leche que son las que generan más calor metabólico por litro de leche producido, reduciendo así el margen térmico de seguridad (Bernabucci et al., 2014). La capacidad de conducción térmica, así como los recursos energéticos para invertir en termorregulación, son modificados por la paridad y el estado de lactación (inicio de la lactación) y la condición corporal (Becker et al., 2020).

Otro factor fisiológico que influye en el estrés calórico es el fenotipo (color y longitud del pelo, pigmentación de la piel) por la capacidad de absorción y de disipación de la radiación; siendo los animales de color más oscuro expuestos a un entorno radiante tienen una mayor carga de calor (Ghassemi Nejad et al., 2017; Becker et al., 2020).

Por lo tanto, las vacas para defenderse del calor generan respuestas termorreguladoras al calor, como el aumento de la respiración (hasta 120–140 respiraciones/min) y el jadeo (enfriamiento evaporativo), vasodilatación periférica (pérdida a través de convección/radiación), disminución de la sudoración y menor ingesta de materia seca para disminuir el calor producido por la digestión (Polsky and von Keyserlingk, 2017; Becker et al., 2020). Estas respuestas conllevan costos fisiológicos: la redistribución de sangre del tracto gastrointestinal a otros tejidos, cambios en la motilidad ruminal y microbioma que afecta la eficiencia fermentativa y la integridad de la barrera intestinal (Habimana et al., 2023; Kim et al., 2022).

Las vacas poseen mecanismos fisiológicos y conductuales para adaptarse a los desafíos que les presenta el entorno en el que viven. Una alta carga térmica en el entorno animal puede inducir cambios en el comportamiento, incluyendo la actividad motora, la frecuencia con la que se acuestan y el tiempo que pasan descansando y de pie, el comportamiento reproductivo, la ingesta de alimento y agua, agresividad, miedo y un aumento o alteración de la vocalización (Herbut et al., 2021).

De igual manera el sistema neuroendocrino actúa como mediador para el estrés por calor produciendo una activación del eje hipotálamo-hipófisis-adrenal; haciendo que los niveles de cortisol aumenten, al igual que las alteraciones de la insulina y las hormonas tiroideas que son las favorecen la disipación del calor realizando repartición de nutrientes hacia la glándula mamaria (Becker et al., 2020; Togoe and Mincă, 2024). A nivel celular, se regula al alza la expresión de HSP70/HSP90, afectando la estabilidad de las proteínas y membranas, y se inician las vías de respuesta inflamatoria/oxidativa (Giannone et al., 2023; Kim et al., 2024).

A lo que respecta un efecto en la reducción de la ingesta de materia seca, disminución de la producción y cambios en la composición de la leche, disfunción reproductiva (tasa de ovulación, calidad del ovocito y viabilidad del embrión) y aumento de la susceptibilidad a enfermedades (mastitis, cojera) (Becker et al., 2020; Giannone et al., 2023).

Resumiendo, el riesgo térmico es una consecuencia de la interacción entre el clima y el microclima (determinantes ambientales) y la fisiología animal (determinantes fisiológicos), por lo que su evaluación debe integrar indicadores ambientales (ITH, carga radiante y viento), así como las constantes fisiológicas (temperatura rectal, frecuencia respiratoria, ritmo cardíaco e ingesta o actividad) para el monitoreo del bienestar animal en condiciones de estrés por calor (Becker et al., 2020; Oliveira et al., 2025).

2. Impacto del estrés térmico sobre la producción láctea

2.1. Efecto del calor sobre la ingesta, metabolismo y comportamiento lácteo

La disminución sustancial en la producción y calidad de la leche es uno de los problemas de producción más notables asociados con el estrés por calor en vacas lecheras lactantes (Min et al., 2017). Debido al rápido aumento de las temperaturas globales, se prevé que las vacas lecheras que sufren de estrés por calor representen un problema a gran escala en el futuro (Zhao et al., 2019). Anteriormente se suponía que la disminución en el consumo de alimento debido al estrés por calor era la responsable directa de la reducción en la producción de leche (Souza et al., 2023); sin embargo, la evidencia mostró que la reducción en el consumo de alimento solo contribuyó a aproximadamente el 35 % de la disminución en la producción de leche inducida por el estrés por calor (Rhoads et al., 2009).

Investigaciones posteriores sugirieron que hasta el 50% de la reducción en el consumo de alimento inducida por el estrés por calor podría afectar la lactancia (Sammad et al., 2020). Más bien, otras alteraciones independientes de la ingesta inducidas por el HS, en particular aquellas relacionadas con la partición de nutrientes, pueden estar asociadas con cambios en el metabolismo de la glucosa y los lípidos (Wheelock et al., 2010).

Los efectos adversos del estrés por calor se ven influenciados a nivel de la vaca

por el genotipo, el estado de salud y la inmunidad. Además, los efectos adversos del estrés por calor se ven influenciados por la temperatura atmosférica, la humedad, el flujo del viento y la radiación solar (Pragna et al., 2017). La alteración de las funciones fisiológicas normales de las vacas lecheras debido al estrés por calor provoca una mayor incidencia de problemas de salud en la ubre, especialmente durante el verano (Turk et al., 2015). Asimismo, la alta temperatura ambiente y la alta humedad relativa durante el verano favorecen el crecimiento de microorganismos responsables de las infecciones de la glándula mamaria, lo que compromete la capacidad de defensa mamaria e induce la colonización bacteriana de la glándula (Lemal et al., 2025).

La lactancia en vacas lecheras es un proceso fisiológico altamente exigente a nivel metabólico, particularmente durante el pico de producción de leche. Bajo esas condiciones térmicas extremas donde el ambiente impone un exceso de calor y limita la disipación, la fisiología de la glándula mamaria, así como los mecanismos que apoyan la producción de leche, se ven gravemente alterados (Tao et al., 2018). Se ha demostrado que un ITH por encima del rango crítico está relacionado con una disminución en la producción de leche, de la cual es el resultado de una menor ingesta de materia seca (Chen et al., 2024).

Desde un punto de vista mamario, la agresión térmica influye en las células epiteliales mamarias, así como su actividad funcional, en estudios *in vitro* mostró un aumento de la apoptosis y una reducción de la proliferación, lo que indica un "número efectivo" reducido de células productoras dentro de la glándula al experimentar estrés térmico (Castellani et al., 2024). De manera similar, la expresión de proteínas de choque térmico (HSPs) y los cambios en los transportadores de glucosa (GLUT1) sugieren que la glándula mamaria sacrifica parte de su metabolismo en la supervivencia celular debido a la acción del calor, en lugar de sólo sintetizar leche (Zeng et al., 2024).

En situación de estrés por calor, la reducción del consumo de materia seca es uno de los primeros eventos en una cadena de procesos que lleva a una

disminución de la producción de leche. La baja ingesta de alimento conduce a un suministro reducido de energía, aminoácidos y precursores para la producción de leche, lo cual a su vez afecta negativamente la gluconeogénesis hepática, la lipogénesis mamaria y la síntesis de lactosa, grasa y proteína de la leche (Kadzere et al., 2002). Además, bajo estrés por calor, el flujo sanguíneo se redistribuye, restringiendo la perfusión del tejido mamario y, en consecuencia, el suministro de nutrientes, de ser desviado hacia la piel para el enfriamiento corporal (Tao et al., 2018).

Así, la fisiología de la lactancia bajo condiciones térmicas muy altas se describe como baja disponibilidad de sustratos, células del tejido mamario disfuncionales, distribución de recursos hacia la disipación del calor y la defensa en lugar de la producción de leche (Kulaz and Ser, 2022).

2.2. Cambios en la composición de la leche

La reducción de la producción de leche y las alteraciones en la composición de la leche son efectos universales del estrés por calor. En condiciones bajo ITH altos, la leche de las vacas experimenta reducciones de grasa y proteína que, en muchos casos, son incluso mayores que la reducción del número en proporción de tratamientos implementados en umbrales más bajos, lo que indica una alta sensibilidad de la síntesis de sólidos a la carga de calor ambiental (Maggiolino et al., 2020; Moore et al., 2023). Los estudios poblacionales y meta analíticos también han confirmado que el estrés por calor impacta en la producción de leche y en el contenido de grasa, proteína y lactosa de la leche, causando cambios funcionales medibles en el precio de la leche (Moore et al., 2023; Habimana et al., 2023).

Para las vacas Holstein en ambientes de verano cálidos y húmedos, se ha descrito que el ITH crítico predice que los parámetros de la producción diaria sean de 72, pero el recuento de células somáticas comienza a aumentar bajo estimaciones de ITH ~78, lo que indica una mayor presión inflamatoria y una infección subclínica al convertirse en calor (Besteiro et al., 2025). Estos resultados están de acuerdo con el hecho de que la calidad

higiénica parece disminuir también durante las olas de calor prolongadas, y con un mayor deterioro de los sólidos (grasa, proteína y lactosa) (Moore et al., 2023; Lovarelli et al., 2024).

Mecánicamente, aproximadamente la mitad de la pérdida de leche bajo estrés por calor se debe a la disminución de la ingesta de materia seca, mientras que las otras contribuciones son de naturaleza endocrino-metabólica y de perfusión, ya que reducen la disponibilidad de sustratos y energía hacia la termorregulación. Esto incluye la depresión de la síntesis de lactosa (conductor osmótico de volumen) y la síntesis de ácidos grasos dentro de la glándula mamaria (Habimana et al., 2023; Chen et al., 2024). Diversos estudios publicados indican que el estrés por calor provoca estrés oxidativo con altos niveles de radicales libres en los tejidos. Además, se propone que la participación de especies reactivas de oxígeno (ROS) en la inducción de apoptosis, la reducción del número de células epiteliales mamarias y las alteraciones endocrinas son los principales mecanismos por los cuales el estrés oxidativo inducido por el estrés por calor modifica la síntesis de proteínas de la leche. Sin embargo, aún existen dificultades para determinar los niveles de apoptosis in vivo, así como para rastrear las fuentes de formación de ROS. Por lo tanto, se requieren más investigaciones para dilucidar este punto (Guo et al., 2021).

2.2.1. Efecto del estrés térmico sobre la composición grasa de la leche en vacas Holstein

El estrés por calor induce cambios metabólicos que afectan la movilización y utilización de los lípidos corporales. Además del balance energético negativo (NEB) posparto, las vacas no movilizan los ácidos grasos no esterificados bajo condiciones de hipertermia, debido al aumento de la secreción de insulina y a una sensibilidad periférica significativamente menor a esta hormona (Wheelock et al., 2010). Esto significa que los ácidos grasos derivados del cuerpo están insuficientemente disponibles para la

síntesis de grasa de la leche, lo que, debido a la mayor internalización de este precursor, se produce en menores cantidades.

De igual manera, se ha demostrado una reducción en la actividad de la enzima acetil-CoA carboxilasa y de la sintasa de ácidos grasos en la glándula mamaria, lo cual, debido a la reducida síntesis de *novo*, produce menos ácidos grasos de cadena corta y media (Collier et al., 2018).

Bajo las condiciones de carga de calor, la composición de la microflora ruminal también cambia, es decir, se produce una alteración en la proporción de bacterias celulolíticas y amiloidales (Zhao et al., 2019). Este tipo de fermentación reduce la producción de acetato y aumenta la síntesis de propionato, lo que permite la producción de glucosa a expensas de la lipogénesis (Baumgard et al., 2012). En consecuencia, la proporción acetato-propionato, que está directamente relacionada con la síntesis de grasa de la leche, se reduce significativamente bajo estas condiciones (Baumgard et al., 2012). Esto se intensifica por la reducción de la rumia y la secreción salival, predisponiendo a los animales a desarrollar acidosis subclínica, alterar la biohidrogenación de ácidos grasos insaturados en el rumen y disminuir la disponibilidad de precursores de grasa saludable, de manera similar (Moore et al., 2023).

Basado en estos factores, la grasa de la leche producida durante la época de verano tiene un contenido de grasa relativamente reducido, ácidos grasos saturados de cadena larga y menos ácidos grasos de cadena corta y media, específicamente C4:0, C6:0 y C8:0 (Liu et al., 2017). Esto no solo reduce el valor nutricional, sino también las propiedades de procesamiento, como la estabilidad de la emulsión y la consistencia de los productos lácteos (Bernabucci et al., 2014).

En lo que respecta a la grasa láctea, los lípidos constituyen un componente fundamental, con una porción sustancial de lípidos polares que actúan como emulsionantes y sirven como elementos estructurales primarios de la membrana del glóbulo graso. Son esenciales para que el sistema de emulsión de la leche se mantenga estable (Ouellet et al., 2019). Liu et al.

(2017) observó que el contenido de lípidos polares se redujo un 43 % tras la exposición al estrés térmico durante dos días, disminuyendo posteriormente hasta un 52 % cuatro días después de la exposición.

Tras un periodo de recuperación de cinco días, el contenido de lípidos polares volvió a su nivel basal. Además, en Tejaswi et al. (2020) observó que las vacas expuestas a altas temperaturas durante uno o más días consecutivos producían menos grasa láctea que las vacas que no estuvieron sometidos a estrés térmico.

2.2.2 Efecto del estrés térmico en la composición de proteínas de la leche en vacas Holstein

Dado que la fracción de proteínas de la leche, compuesta por caseínas en forma de α_1 , α_2 , β , y κ , y suero con β -lactoglobulina, α -lactalbumina, albúmina sérica y lactoferrina, juegan un papel esencial en la influencia de la capacidad de coagulación y las propiedades tecnológicas del producto, la industria láctea enfrenta desafíos relacionados con ello. Según Bernabucci et al. (2015), informaron que el estrés térmico cambió los genes y, como resultado, redujo la calidad de la leche y el comportamiento industrial asociado con la industria láctea.

El impacto del calor en el contenido de las proteínas de la leche ocurre principalmente debido a la reducción de la ingesta de materia seca y al cambio del metabolismo energético de la vaca. En el caso de estrés por calor, la ingesta de materia seca puede reducirse hasta un 10 % a 30 %, lo que lleva a un déficit energético y a la incapacidad de proporcionar suficientes aminoácidos precursores para la síntesis de proteínas mamarias (Baumgard and Rhoads, 2013). Sin embargo, la disminución del contenido no solo ocurre debido a la ingesta, sino debido a las alteraciones endocrinas y metabólicas que ocurren en el estado de estrés por calor (Collier et al., 2017). Se produce una reducción en las concentraciones plasmáticas de GH e IGF-1 y un aumento en la insulina plasmática, lo que cambia la

partición de nutrientes hacia la glándula mamaria y reduce la síntesis de proteínas de la leche (Collier et al., 2018).

A nivel celular, el calor excesivo puede interrumpir directamente la función de las células epiteliales mamarias. Estas células disminuyen su capacidad para sintetizar caseínas debido al estrés oxidativo y al daño estructural de las proteínas del retículo endoplasmático. Un aumento en los ROS induce la expresión de proteínas como HSP, HSP70, y HSP90, que actúan como chaperonas moleculares, pero cuando se sobre expresan, alteran la traducción normal de las proteínas de la leche (Dado-Senn et al., 2019). Además, la alteración de la expresión de los genes de la leche, como *CSN2* (β -caseína) o *LALBA* (α -lactoalbúmina) disminuye sus niveles de expresión bajo condiciones de estrés por calor crónico (Liu et al., 2021).

En la composición específica, el calor reduce la concentración total de proteínas y la relación de caseínas a proteínas del suero. Esta situación resulta en la alteración de la estabilidad coloidal de la micela de caseína y una disminución en la capacidad de coagulación, lo que también afecta la calidad tecnológica del queso y el yogur (Zhao and Corredig et al., 2020). Además, se ha encontrado un aumento relativo de β -lactoglobulina y lactoferrina debido a respuestas fisiológicas de protección antioxidante e inmunológica contra el estrés térmico (Tu et al., 2022). Sin embargo, tales aumentos no compensan la pérdida de proteínas totales ni el deterioro en el comportamiento tecnológico de la leche.

Existen diversas explicaciones posibles para la disminución de las proteínas de la leche, algunas de las cuales podrían ser inherentes a la glándula mamaria. Cowley et al. (2015) reveló que la disminución de las proteínas de la leche observada en vacas sometidas a estrés térmico se debe a una regulación negativa específica de la síntesis de proteínas en la glándula mamaria y no a una disminución general de la producción de leche. Los autores determinaron que el estrés térmico influyó exclusivamente en las proporciones de las fracciones de masa de caseína individuales, lo que conllevó un aumento de la α S1-caseína y una disminución de la α S2-

caseína. Gao et al. (2017) investigaron el impacto de las altas temperaturas en la generación de proteínas de la leche, el ganado expuesto al estrés térmico mostró una disminución del 17,1% en la cantidad total de aminoácidos en su plasma. Esta reducción afectó tanto a los aminoácidos esenciales como a los no esenciales.

Desde el punto de vista productivo, las reducciones en el contenido de proteínas de la leche varían entre un 5% y un 15% en periodos cálidos, y su intensidad depende de varios factores, como la gravedad o duración de los eventos estresantes. Estas pérdidas evitables afectan el beneficio financiero del productor, ya que la proteína representa la variable que más influye en los vectores de pago basados en la calidad de la industria láctea (Bernabucci et al., 2015).

2.2.3. Efecto del estrés calórico en el contenido de lactosa en la leche de vacas Holstein

El estrés por calor constituye uno de los desafíos más serios para la producción lechera en regiones cálidas porque provoca cambios profundos en la homeostasis fisiológica y endocrina de las vacas. Una de las sustancias más sensibles a la variación térmica es la síntesis de lactosa, que es el principal regulador osmótico del volumen de leche y el determinante directo de la producción total (Bernabucci et al., 2014). La lactosa está presente a una tasa de aproximadamente 4.8% del volumen total de leche de vacas lactantes y depende de la disponibilidad adecuada de glucosa, la actividad enzimática de la lactosa sintetasa y la integridad funcional de los epitelios alveolares mamarios (Bernabucci et al., 2014; Chen et al., 2023).

Durante los procesos de disipación del calor (sudoración, jadeo, vasodilatación periférica) que se generan durante el estrés térmico debido al aumento de la temperatura corporal, se consume activamente energía y oxígeno, los cuales se restan del metabolismo productivo en forma de

termorregulación (Baumgard and Rhoads, 2013 Zhou et al., 2022). La ingesta de materia seca disminuye como consecuencia, resultando en una disminución de la glucosa sistémica (Wheelock et al., 2010). La glándula mamaria depende principalmente de la glucosa sanguínea sistémica para la síntesis de lactosa. Por lo tanto, una disminución en el suministro de energía resulta en una disponibilidad reducida del sustrato principal necesario para la galactopoyesis (Wheelock et al., 2010).

Además de los efectos metabólicos mencionados, el estrés por calor deteriora la regulación hormonal de la lactogénesis. Los estudios han demostrado una reducción en las concentraciones de insulina y somatotropina, y una elevación del cortisol plasmático bajo condiciones de calor (Rhoads et al., 2009). Estos cambios endocrinos alteran la captación de glucosa por los tejidos periféricos y disminuyen la eficiencia del transporte de glucosa a la glándula mamaria, el sitio desde el cual se polimeriza la lactosa, producida por el complejo sintasa de lactosa compuesto por galactosiltransferasa y α -lactoalbúmina, en presencia de un contenido adecuado de UDP-galactosa y glucosa (Tao et al., 2018).

Tao y Dahl (2013) informaron que la exposición de vacas Holstein a temperaturas superiores a 30 °C condujo a la supresión de la expresión génica de la α -lactalbumina y los transportadores de glucosa GLUT1 y GLUT8 en el tejido mamario. Posteriormente, se comprometió la capacidad de síntesis de lactosa. Estas alteraciones moleculares reducen la presión osmótica intraluminal y el volumen de leche secretada.

Al mismo tiempo, el estrés por calor también afecta la integridad de las uniones estrechas entre las células epiteliales, alterando la permeabilidad mamaria y la estabilidad de la secreción (Becker et al., 2020). Desde un punto de vista fisiológico, la disminución en la concentración de lactosa ocurre junto con una menor tasa de flujo de leche y un cambio en la composición iónica de la leche (Bouraoui et al., 2002). Además, estudios recientes demuestran que la hiperlactacidemia y un cambio en el

metabolismo de carbohidratos hacia la termogénesis no glucolítica reducen el suministro de glucosa a la glándula mamaria (Mehaba et al., 2021).

En conjunto, estos procesos representan un patrón jerárquico complejo de reordenamiento metabólico en el que se da prioridad biológica a la termogénesis sobre la producción.

Florio et al. (2022) observaron una reducción en la concentración de grasa y proteína en la leche durante los meses de verano, mientras que se observó un aumento en la concentración durante el invierno; sin embargo, se encontró que la lactosa se mantuvo constante a lo largo de las estaciones. Contrariamente a este hallazgo, en el trabajo de Farrell et al. (2004) se observó que el nivel de lactosa en el ganado de pastoreo disminuye durante el verano en comparación con el invierno.

La lactosa está vinculada a la producción de α -lactosiltransferasa (α -La), una coenzima necesaria para su formación. Esta proteína específica del suero se sintetiza en la glándula mamaria, donde la α -La interactúa con la β -1,4-galactosiltransferasa en el aparato de Golgi de las células epiteliales de la glándula mamaria para facilitar la formación de la enzima lactosa sintasa. Como se indica en el trabajo de Sánchez-Juanes et al. (2009) α -La altera la especificidad del sustrato de la β -1,4-galactosiltransferasa para permitir la creación de lactosa a partir de glucosa y UDP-galactosa.

2.3 Estacionalidad y patrones productivos en entornos cálidos

La estacionalidad es un fenómeno bien conocido en la determinación de la productividad de los sistemas lecheros, especialmente en regiones cálidas y áridas. En estas regiones, las variaciones climáticas afectan directamente las variables fisiológicas, reproductivas y productivas del ganado lechero. Los desequilibrios homeotérmicos creados por las altas temperaturas, junto con la intensa radiación solar en vacas Holstein de alta producción, alteran los patrones productivos a lo largo del año, con una reducción en la eficiencia alimentaria, los comportamientos de leche y la fertilidad.

(Bernabucci et al., 2010). Tales variaciones estacionales están altamente correlacionadas con el ITH, uno de los métodos de cuantificación de estrés térmico más comúnmente utilizados (Oliveira et al., 2025).

Después del estrés térmico mencionado durante el verano, la expresión del celo, la calidad de los ovocitos y la tasa de concepción también se reducen, prolongando los intervalos entre nacimientos y aumentando los días abiertos (Giannone et al., 2023). Estudios de series de tiempo demostraron previamente que tanto las variables productivas como reproductivas mostraron una clara ciclicidad anual asociada al comportamiento estacional del clima, con bajas productivas en verano y recuperaciones en otoño e invierno (Jeon et al., 2023). Desde una perspectiva metabólica estricta, la estacionalidad en ambientes cálidos genera un cambio drástico en la partición de nutrientes. Comenzando con los meses de temperaturas más altas, las vacas reducen drásticamente la ingesta voluntaria de materia seca, lo que suprime la disponibilidad de sustratos para la síntesis de lactosa y caseína (Moore et al., 2023).

Al mismo tiempo, aumenta la producción de especies reactivas de oxígeno, perjudicando la integridad celular y reduciendo la eficiencia bioenergética. Todos estos efectos resultan en una menor producción de leche, una persistencia de la lactancia más baja y un importante descenso en la calidad de la leche (Moore et al., 2020).

3. Efectos del estrés térmico sobre el estado reproductivo

El estrés por calor es uno de los principales factores que afectan la eficiencia reproductiva y reducen la productividad en los animales de la granja (Chawicha and Mummed, 2022). El concepto se caracteriza por el hecho de que un organismo no logra mantener su temperatura corporal en un rango normal, lo que provoca alteraciones metabólicas, hormonales y celulares (Takahashi, 2011). El exceso de calor en las hembras deteriora la maduración del ovocito (De Rensis et al., 2021), la función luteínica (Nanas

et al., 2021) y la implantación embrionaria (Kasimanickam and Kasimanickam, 2021), lo que lleva a un descenso de las tasas de concepción, así como a mayores pérdidas embrionarias tempranas (Roth, 2020; Dovolou et al., 2023; Togoe et al., 2024).

En las vacas lecheras, por ejemplo, las tasas de concepción caen en verano al 27%, mientras que en invierno superan el 40% (Wolfenson y Roth, 2018). Rodríguez-Godina et al. (2024) observó que la tasa de preñez para todos los servicios fue mayor en las vacas que no sufrieron estrés térmico durante el período seco (60.2 %) y menor (42.6 %) en las vacas con estrés térmico severo durante dicho período. Las vacas que no experimentaron estrés térmico durante el período seco requirieron un promedio de 5.6 servicios por preñez, en comparación con 6.5 en las vacas expuestas a un índice de temperatura y humedad (ITH) > 80 durante el período seco.

Así mismo, Mellado et al., 3013) observaron que el aumento del índice de temperatura y humedad (ITH) de ≤ 70 a ≥ 95 unidades se asociaron con una disminución de la tasa de preñez por inseminación artificial del 47 % al 26%. La tasa de preñez en vacas inseminadas en días extremadamente calurosos (ITH = 85 a 90) precedidos de temperaturas más frescas fue seis puntos porcentuales mayor que en vacas expuestas a altas temperaturas antes del apareamiento. La tasa de preñez fue mayor de enero a marzo (39% a 41 %) en comparación con el resto del año (27% a 35 %). El número promedio de inseminaciones por gestación fue mayor de mayo a julio (3.0 a 3.4) en comparación con los demás meses (2.1 a 3.0).

El estrés térmico desencadena una respuesta endocrina que incluye niveles elevados de cortisol en el suero sanguíneo y una modificación del eje hipotálamo-pituitario-gonadal (Menta et al., 2022); así como la inducción de las proteínas de choque térmico que pueden afectar la supervivencia celular (Carvajal et al., 2021). Estos cambios disminuyen la intensidad del estro, retrasan el inicio de la ovulación y afectan el ambiente uterino de tal manera que reducen la supervivencia embrionaria, así como en los gametos de las vacas lecheras (LeBlanc et al., 2019; Nascimento et al., 2025).

Por lo tanto, el estrés térmico induce una degradación general del estado reproductivo en la mayoría de los niveles, tanto fisiológica como económicamente hablando, especialmente en regiones áridas y calientes con esquemas de producción intensivos.

3.1 El eje hipotálamo–hipófisis–ovario y su alteración térmica

El eje hipotálamo-pituitario-ovárico (HPO) media el control reproductivo de la vaca lechera y está regulado por la liberación pulsátil de la hormona liberadora de gonadotropinas (GnRH) desde el hipotálamo y la posterior secreción de hormona luteinizante (LH) y hormona foliculoestimulante (FSH) desde la pituitaria anterior. Estas gonadotropinas actúan sobre el ovario para iniciar el desarrollo folicular, la secreción de E2, y la ovulación (Roth, 2020).

Este eje se vea afectado cuando el ITH es elevado, comprometiendo la pulsación de GnRH, la amplitud/frecuencia de LH, y el desarrollo folicular dominante (Țogoe and Mincă, 2024).

A nivel del hipotálamo-hipófisis, el estrés térmico agudo causa una adaptación manifestada por un aumento en la producción de cortisol como resultado de la estimulación del eje HPA (hipotálamo-hipófisis-adrenal). Este mecanismo de retroalimentación negativa del cortisol elevado (disminuye la secreción de GnRH y reduce la reserva hipofisaria por corticosteroides) causa una reducción en la liberación de LH/FSH (Dovolou et al., 2023).

La limitación de la ingesta de materia seca con altas temperaturas también intensifica el efecto negativo al crear un balance energético negativo, lo cual modula negativamente el eje HPO (Țogoe and Mincă, 2024).

En la hipofisis anterior, la secreción de la hormona luteinizante se reduce tras la estimulación con estrógenos en vacas expuestas a cargas de calor, y el número de pulsos de LH antes de la ovulación disminuye, retrasando o impidiendo la liberación del oocito (Roth, 2020; Dovolou et al., 2023). Esta

modificación determina la fase de crecimiento del folículo dominante, que se presenta con una masa disminuida de células de la granulosa y baja producción de hormonas estrogénicas, lo que también resulta en un estro silencioso o menos intenso (Țogoe and Mincă, 2024).

Con el estrés calórico, el ovario, la menor estimulación gonadotrópica limita la luteogénesis que afecta la formación del cuerpo lúteo es menos eficiente y la liberación de progesterona postovulación es menor, limitando así el apoyo endocrino para la preimplantación del blastocisto (Țogoe and Mincă, 2024). Esta disfunción se asocia con una mayor mortalidad embrionaria temprana y tasas de concepción reducidas (Roth, 2020).

Además, el aumento de la temperatura puede actuar directamente sobre los folículos en desarrollo: la termólisis ovárica (pérdida del gradiente entre el folículo y el ambiente) afecta la maduración del oocito y su capacidad para ser fertilizado; mecanismo que también se asocia con la disrupción del eje HHO (Zhang et al., 2025). Así, los cambios en el eje HHO bajo estrés por calor combinan efectos centrales (hipotálamo/hipófisis) y periféricos (ovario) que se coordinan entre sí para inducir la regresión reproductiva.

En resumen, el estrés por calor induce una disrupción en el eje HHO a nivel de la liberación disminuida de GnRH y la respuesta deficiente de la hipófisis (LH/FSH), así como en la dinámica folicular, la luteogénesis y la secreción de progesterona en vacas lecheras. Es a partir de esta cascada fisiológica que las vacas expuestas al calor tienen tasas de ovulación más bajas, expresión de estro reducida y fertilidad disminuida. Comprender estos cambios es esencial para el diseño de prácticas de manejo reproductivo adaptadas al estado de carga térmica en vacas lecheras.

3.2. Impacto del estrés calórico en la calidad ovocitaria y la tasa de fertilización

El estrés por calor induce alteraciones significativas en la fisiología reproductiva de las vacas lecheras, interfiriendo con el HHO, así como con

la dinámica folicular, la competencia de los ovocitos y la tasa de fertilización. Un índice de ITH crónico puede reducir la frecuencia/amplitud de los pulsos de GnRH/LH, deprimir la esteroidogénesis folicular y acortar o incluso interrumpir las ondas foliculares, lo que lleva a folículos dominantes más pequeños con menor producción de estradiol (Wolfenson and Roth, 2018; Dovolou et al., 2023).

A nivel del folículo ovárico, el estrés térmico induce estrés oxidativo y respuesta de choque térmico, mientras altera la viabilidad de las células de la granulosa y la teca, así como la expresión génica que regula la función esteroidogénica y el control del ciclo (Dovolou et al., 2023). Cambios como estos causan un deterioro de la condición del ambiente folicular (contenido de factores tróficos, equilibrio redox) y llevan a influencias directas en la maduración nuclear y citoplasmática del oocito (Miętkiewska et al., 2022).

La capacidad del ovocito para ser fecundado y nutrir el desarrollo inicial del embrión es particularmente susceptible al calor. El estrés térmico afecta la estructura y función de las mitocondrias, causa fragmentación del ADN, cambia el perfil transcriptómica materno y conduce a tasas de segmentación disminuidas y desarrollo en blastocistos viables (Miętkiewska et al., 2022; Feng et al., 2024).

El efecto del estrés térmico en la tasa de concepción está de acuerdo con estos mecanismos. Se encontró que el periodo peri-inseminación era un periodo destacado en el que, cuando el ITH promedio de los días -21 a -1 antes de la inseminación estaba por encima del 73%, la tasa de concepción disminuía (del 31% al 12%) (Schüller et al., 2014).

Esta disminución no solo se atribuye a una menor detección del estro, sino también a la disfunción de los ovarios, la menor calidad de los ovocitos y un microentorno uterino que favorece menos la fertilización y la viabilidad embrionaria (Wolfenson and Roth, 2018; Dovolou et al., 2023).

El efecto del estrés térmico en la tasa de concepción se encontró que el periodo de peri-inseminación era un periodo destacado en el que, cuando el ITH estaba por encima de los 73 en los días -21 a -1 antes de la

inseminación esta disminuía del 31% al 12% (Schüller et al., 2014). Esta disminución no solo se atribuye a una menor detección del estro, sino también a la disfunción de los ovarios, la menor calidad de los ovocitos y un microentorno uterino que desfavorece la fertilización y la viabilidad embrionaria (Wolfenson and Roth, 2018; Dovolou et al., 2023; Capela et al., 2025). Prácticamente, esto significa que, a pesar de la inseminación oportuna, la tasa de fecundación y la competencia embrionaria temprana se reducen en olas de calor o periodos de verano prolongados (Wolfenson and Roth, 2018).

3.3 Efectos del estrés térmico sobre los días abiertos

Los “días abiertos”, o el intervalo entre el parto y la concepción subsiguiente, constituyen una de las medidas más significativas del comportamiento reproductivo en las vacas lecheras. Este indicador caracteriza indirectamente la eficiencia del manejo reproductivo, el estado fisiológico posparto y los factores ambientales que influyen en la fertilidad (De Rensis and Scaramuzzi, 2019). En las regiones cálidas o áridas, el calor y el estrés térmico —debido a su impacto negativo en la actividad endocrina, la función ovárica, la calidad de los gametos y la viabilidad embrionaria— son las principales causas de la expansión del rango de días abiertos (De Rensis and Scaramuzzi, 2019).

El estrés térmico es una situación donde la temperatura ambiente y la humedad relativa están por encima de la capacidad del animal para disipar el calor, y se mide usando el índice de temperatura y humedad. Cuando el ITH alcanza valores umbral específicos, en el rango de 68-72 para vacas Holstein, las vacas experimentan graves alteraciones fisiológicas, afectando el metabolismo energético, el comportamiento y la capacidad reproductiva (Zhao et al., 2023). Como resultado, la variable de días abiertos se expande significativamente, con duraciones totales que varían

de 20 a 40 días entre el estrés por calor y las condiciones termoneutrales (Ravagnolo and Misztal, 2002).

El calor interfiere con la disminución de los días abiertos, en gran medida debido a la ineficiencia del ciclo estral. En particular, las vacas bajo estrés calórico tienen una reducción en la frecuencia y duración del estro (Szalai, et al., 2025), y una reducción en la secreción pulsátil de la hormona luteinizante y FSH, lo que lleva a una limitación en la maduración folicular y la ovulación (Wolfenson et al., 2015). Además, se observa una disminución en la concentración plasmática de estradiol y progesterona, hormonas necesarias para la sincronización del ciclo y la implantación del embrión (Hansen, 2019). Con la supresión del comportamiento estral, la detección del estro se vuelve más difícil, lo que retrasa la inseminación artificial y es una de las razones para aumentar el intervalo entre partos y la concepción.

Debido a la exposición al calor, la calidad de los gametos se ve directamente afectada. En los oocitos de vacas sometidas a estrés por calor, su competencia para la fertilización se redujo, caracterizándose por un aumento en la tasa de degeneración y una reducción en el número de blastocistos viables en desarrollo (Ferreira et al., 2021). Por otro lado, los embriones producidos durante el verano muestran una mayor susceptibilidad al estrés oxidativo y a los cambios epigenéticos, lo que reduce su viabilidad. Estas situaciones reducen la tasa de concepción, aumentando el retorno al estro y, por lo tanto, el número de servicios por gestación y los días abiertos (Silva et al., 2022).

Otro factor determinante es el balance energético ya que la reducción del consumo de alimento limita la disponibilidad de energía para la síntesis de hormonas del ciclo hipotálamo-pituitaria-ovárico, lo que conduce a cambios en su funcionamiento. La redistribución del flujo sanguíneo hacia la periferia también limita el suministro de nutrientes y oxígeno al útero y a los ovarios, deteriorando el microambiente sexualmente reproductivo (Baumgard y Rhoads, 2013; Rhoads et al., 2021).

El estrés por calor impacta sustancialmente los días abiertos de las vacas Holstein a través de alteraciones endocrinas, metabólicas y gaméticas que reducen las tasas de concepción y prolongan el tiempo de retorno a la fertilidad. Como resultado, el comportamiento reproductivo se ve comprometido, y la eficiencia y rentabilidad de la empresa lechera se ven amenazadas.

ARTÍCULOS

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Unmasking seasonal cycles in a high-input dairy herd in a hot environment: How climate shapes dynamics of milk yield, reproduction, and productive status

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ABSTRACT

This study aimed to predict the annual herd milk yield, lactation, and reproductive cycle stages in a high-input dairy herd in a zone with prolonged thermal stress. Also, the impact of climatic conditions on milk yield and productive and reproductive status was assessed. An autoregressive integrated moving average (ARIMA) model was used in data fitting to predict future monthly herd milk yield and reproductive status using data from 2014 to 2020. Based on the annual total milk output, the highest predicted percentage of milk yield based on the yearly milk production was in February (9.1%; 95% CI = 8.3–9.9) and the lowest in August (6.9%; 95% CI = 6.0–7.9). The predicted highest percentage of pregnant cows for 2021 was in May (61.8; 95% CI = 53.0–70.5) and the lowest for November (33.2%; 95% CI = 19.9–46.5). The monthly percentage of dry cows in this study showed a steady trend across years; the predicted highest percentage was in September (20.1%; CI = 16.4–23.7) and the lowest in March (7.5%; 4.0–11.0). The predicted days in milk (DIM) were lower in September (158; CI = 103–213) and highest in May (220; 95% CI = 181–259). Percentage of calvings was seasonal, with the predicted maximum percentage of calvings occurring in September (10.3%; CI = 8.0–12.5) and the minimum in April (3.2%; CI = 1.0–5.5). The highest predicted culling rate for the year ensuing the present data occurred in November (4.3%; 95% CI = 3.2–5.4) and the lowest in April (2.5%; 95% CI = 1.4–3.5). It was concluded that meteorological factors strongly influenced rhythms of monthly milk yield and reproductive status. Also, ARIMA models robustly estimated and forecasted productive and reproductive events in a dairy herd in a hot environment.

1. Introduction

Ideally, regular all-year-round calvings are required to maximize profitability in intensive dairy farms. This strategy generates an even milk production pattern throughout the year, allowing dairy farmers to spread the workload and meet the demands of milk by the market. However, parturition patterns and milk yield are seasonal throughout the United States (Ferreira et al., 2020), regardless of the climatic conditions (Ferreira and De Vries, 2015; Salfer et al., 2019).

One of the primary causes of seasonality in milk yield is thermal

stress (Tao et al., 2020; Lim et al., 2021). Although not totally clear, mechanisms that mediate the decreased milk production by heat stress include reduced feed intake (Polsky and von Keyserlingk, 2017), altered metabolism (Sammad et al., 2020b), activation of immune system and inflammation (Bagath et al., 2019; Molinari et al., 2023), altered function and mammary gland development (Tao et al., 2018), alterations in feeding behavior (Becker et al., 2020) and alteration of the rumen microbiome fermentation (Kaufman et al., 2020). The effects of an endogenous circannual rhythm on milk yield and component percentages have also been documented (Salfer et al., 2020).

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Additionally, the adverse effect of heat stress on milk yield can be mediated by a depressed reproductive function of dairy cows, which reduces the percentage of milking cows (Sammad et al., 2020b). Heat stress disrupts several reproductive processes, resulting in a pronounced depression of conception rate in dairy cows worldwide (Wolfenson and Roth, 2019). Decreased fertility in dairy cows results in an increased seasonal calving pattern in the herd, which results in a variation in the average days in milk throughout the year, partially explaining seasonal milk production (Shock et al., 2015). These responses significantly impact dairy cows' productivity despite the heat abatement equipment widely used in the dairy industry (de Rensis and Scaramuzzi, 2003).

Hence, reduced fluctuation in milk production is an integral part of an intensive dairy farming system, particularly in areas with unfavorable climate conditions. Measurements of the level of milk yield over the year allow us to have an in-depth understanding of the past milk production of the herd to evaluate the strengths and weaknesses of strategies adopted in the past, and provide the necessary basis for designing management strategies appropriate to the area for setting future milk production targets.

The reduced fertility of dairy cows increases calving interval, which is reflected in lower milk yield (Giordano et al., 2012; Galvao et al., 2013). Also, reduced reproductive performance is a significant motive for involuntary culling (Dallago et al., 2021), which decreases profitability by augmenting replacement costs (Schuster et al., 2020). Besides the effects on profitability, decreased cow longevity due to involuntary culling has raised global cow welfare concerns (Olteneacu and Algers, 2005).

Despite the number of reports describing and explaining causes of calving and culling seasonality (Hadley et al., 2006) as well as seasonal trends in monthly pregnant cows and days in milk (De Vries and Risco, 2005; Refsdtal, 2007), only a few studies have attempted to report how these metrics are distributed across the year in a zone of chronic hyperthermia. Also, little is known about the effect of chronic heat stress on annual herd calvings and culling, as well as percentage of cows pregnant and dry. This information is helpful for management planning and decision-making because seasonality in calving patterns and milk production changes cash flows throughout the year. Also, milk yield seasonality may result in supply and demand imbalances, which are expensive (Washington et al., 2002).

Accurate forecasts of milk yield, reproductive performance, and herd composition are crucial to secure a stable milk supply to the market, reducing logistics costs and greenhouse gas emissions. Therefore, accuracy in the milk production forecast benefits farmers as total milk production influences optimum farm configurations and cash flow/income. Several studies aiming at forecasting milk yield have been conducted in several countries using time series models (ARIMA model), which have shown good fitting models for forecasting cow milk yield (Perez-Guerra et al., 2023; Samson et al., 2023).

The temporal variation in productive and reproductive status and milk yield in hot environments has had incomplete documentation; thus, data from regions with chronic heat stress need to be investigated. Also, in hot-dry environments, there are no empirical models for forecasting the dynamics of milk yield, reproductive performance, and herd structure.

Therefore, the present study aimed: (1) to quantify herd milk production trends and seasonal patterns in a commercial dairy farm in a hot environment; (2) to assess how heat stress changes seasonal dynamics of a dairy herd milk production and productive and reproductive status; and (3) summarize the general pattern for milk yield and reproductive status of cows for future management in a high-input commercial dairy farm. We hypothesized that trends calculated from time series are accurate forecasting methods to describe seasonality of milk yield and stages of the lactation cycle in a dairy herd in a location with prolonged thermal stress. An additional hypothesis was that heat stress leads to a substantial fluctuation in herd productive and reproductive traits in a year-round calving dairy herd.

2. Material and methods

2.1. General herd information

Data were from a single commercial dairy herd (2560–3300 milking cows in seven years) in Northeastern Mexico (Latitude: N 25° 31' 42"), comprising milk yield and cow's stages of the lactation cycle from 2014 to 2020. The average annual rainfall is 210 mm, and the mean annual temperature is 22.7 °C. Cows were kept in open, dirt-based pens with soil/dry manure substrate. Due to the scarce precipitation, mud was not an issue. Shade was placed in the middle of the corrals and feed bunks with 4.5 square meters per cow. The shaded feed bunk was fitted with sprinkler nozzles and fans, which were turned on at air temperature ≥ 25 °C.

Cows were fed as a group a total mixed ration (TMR) based on corn silage, alfalfa hay, and grain concentrate (soybean meal, cottonseed meal, steam-flaked corn grain, and a mineral premix) twice daily (equal quantities; $\approx 08:00$ and $16:00$ h), with an expected 3% refusal of the total amount offered daily. Throughout the study, diets contained at least 18% CP, 31% NDF, and an NE_L of 1.67 Mcal/kg adjusted for 23 kg of DMI. The forage-to-concentrate ratio was 50:50, and the ration met the requirements of 650 kg Holstein cows producing 36 kg of 3.5% fat-corrected milk (National Research Council (NRC), 2001). TMR was adjusted to milk yield at different stages of lactation (0–30, 30–150, 150–210, and 210 to >305 days in milk). Cows had ad libitum access to water. Cows were milked three times daily at 01:00, 09:00, and 17:00 h. The herd's annual daily milk yield varied from 27.6 (summer) to 33.0 kg/day (winter), and the herd's mean annual culling rate was 27.5%.

2.2. Reproductive management

Calves were vaccinated against brucellosis and revaccinated against this disease yearly after that. Cows were immunized with a 10-way clostridial vaccine and against bovine respiratory syncytial virus, infectious bovine rhinotracheitis, parainfluenza 3, bovine viral diarrhoea, and leptospirosis (five serovars). All cows were periodically tested for brucellosis (card tests), and seropositive reactors were culled.

Estrus was detected by direct observation twice daily. Cows were artificially inseminated when estrus was detected after the 50-day voluntary waiting period following the a.m./p.m. guideline. In non-pregnant cows, inseminations continued until 300 days in milk; therefore, involuntary extended lactations (≥ 500 days) were common. The Ovsynch program was used in all repeat-breeding cows. Frozen-thawed semen from high genetic merit bulls from the USA was used. Pregnancy diagnoses were performed at 45 ± 3 days from their last recorded artificial insemination by transrectal palpation of the uterine contents.

2.3. Milk yield and days in milk recording

The cows were milked with a milking machine with monovac, pulsation rate of 60 cycles/min, pulsation ratio 70/30, and vacuum of 42 kPa. An automated milking system was used to register individual cow milk yield at each milking. Also, daily herd milk yield data from the cooling tanks was recorded. Cows were dried off 42–60 days before the expected calving (220 ± 3 d of pregnancy) or when daily milk yield was <18 kg.

2.4. Meteorological data

Seven-year climatic records (from 2014 to 2020) were obtained from an official meteorological station located approximately 2-km away from the dairy farm. Ambient temperature (°C) and relative humidity (%) data were used for environmental monitoring. This information was used to calculate THI using the following equation: Temperature-humidity index (THI) = $(0.8 \times \text{ambient temperature}) + [\% \text{ relative humidity} / 100 \times (\text{mean ambient temperature} - 14.4)] + 46.4$.

2.5. Statistical analyses

For forecasting herd milk yield and cows' lactation cycle stages, we set out a back-testing framework for two years of forecasting using a time series approach. Statgraphics Centurion XV software (Statpoint Technologies Inc., Warrenton, VA, USA) was employed to select a forecasting model that compared multiple models and automatically picked one that maximized the specified information criterion. A usual practice of this procedure is to choose an ARIMA model of the ARMA(p, p-1) form, which, unlike most control diagrams, does not assume independence among sequential measurements. Cycles of 30 days were chosen as the measurement unit to analyze the month effect on monthly herd milk yield, productive and reproductive status, and percentage of calving and culling cows. This interval is commonly used in the dairy industry to monitor milk production. ARIMA is a mixture of autoregressive and moving average, represented by p, d, and q. Where p represents the number of lag observations incorporated in the model, d denotes the number of times raw observations undergoing differencing, and q is the degree of MA. The model is as follows:

$$Y_t = \phi_1 Y_{t-1} + \phi_2 Y_{t-2} + \dots + \phi_p Y_{t-p} + \epsilon_t$$

$$\theta_1 \epsilon_t - 1 + \theta_2 \epsilon_t - 2 + \dots + \theta_q \epsilon_t - q$$

The Box-Jenkins procedure was used to estimate p, d, and q values.

The cross correlation between herd milk yield and THI over time was calculated utilizing the Wessa software (2017) that computes the cross-correlation function for univariate time series. Also, the association between the variables analyzed and months was assessed using the CurveExpert Professional 2.5.6 software (Hyams Development, Madison, AL, USA). For all statistical analyses, values with $p < 0.05$ were regarded as statistically significant.

3. Results

The autocorrelation function (ACF) and partial autocorrelation function (PACF) showed the stationarity nature of herd milk yield data over time (ACF and PACF between -0.5 and 0.5). Fig. 1A shows the pattern of herd milk yield. A well-defined trend could be recognized over the seven years. Based on the annual total milk output, the highest predicted percentage of milk yield based on the yearly milk production was in February (9.1%; 95% CI = 8.3–9.9) and the lowest in August (6.9%; 95% CI = 6.0–7.9). Fig. 1B shows how monthly herd milk yield plummeted in the summer months. Based on the determination coefficient, the sinusoidal functions presented a very good adjustment, indicating that the sinusoidal model had a high predictability for future herd milk yield.

THI (input variable) and monthly herd milk yield (%) (output variable) were significantly negatively correlated in the same month

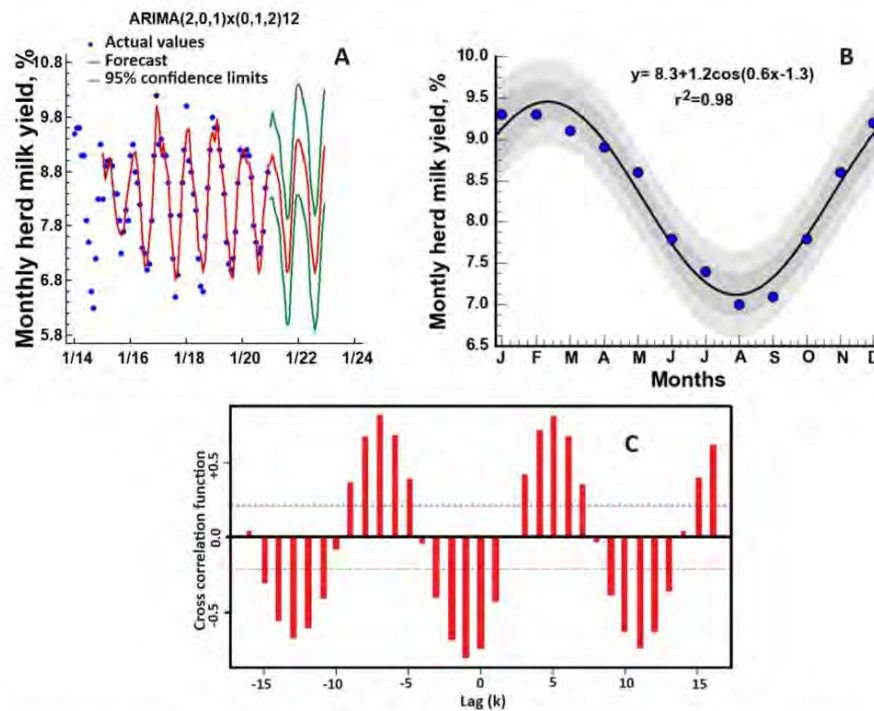


Fig. 1. A: The time series for monthly herd milk yield (percentage of the yearly milk yield). Each point denotes the mean value of a single herd (~2900 lactating cows) from 2014 to 2020. Values are fitted and predicted values. B: The association between monthly herd milk yield (percentage from total annual milk production) and months of the year. C: Cross-correlation function of the time-series of monthly temperature-humidity index and proportion of monthly milk yield based on yearly milk yield in a hot environment: 2014–2020. The spikes denote the cross-correlation coefficients. The dashed lines represent the 95% confidence limits (CL). Spikes outside the CL are significant cross-correlation coefficients.

(Fig. 1C). Correlations were significant and negative for these variables at lags +1, -1, -2, and -3, indicating that THI and milk yield were negatively correlated 2.5 months earlier and 2.5, 5, and 7.5 months later, reaffirming the strong negative influence of thermal stress on milk yield.

Regarding percentage of cows pregnant, the ARIMA model showed that the time plot looked similar at different points along the time axis, which implies homogeneity in how the series behave regardless of time (series stationary; Fig. 2A). The predicted highest percentage of pregnant cows for 2021 was in May (61.8; 95% CI = 53.0–70.5) and the lowest for November (33.2%; 95% CI = 19.9–46.5). Fig. 2B showed that pregnant cows (%) had a marked seasonal periodicity, peaking during summer. The R^2 of almost 1 indicated a perfect fit of the model, which indicated that most of the variation in the percentage of pregnant cows was explained by months of the year.

Results of cross-correlation function analysis for time series between the monthly THI and percentage of pregnant cows showed a significant positive association on the same month, and the previous 2.5, 5, 7.5, and 9 months (lags +1 to +4; Fig. 2C), which implied a strong effect of cooler conditions on higher percentages of pregnant cows.

Time series plot of monthly percentage of dry cows in this study showed a steady trend across years (Fig. 3A). The predicted highest percentage of dry cows was in September (20.1%; 95% CI = 16.4–23.7)

and the lowest in March (7.5%; 95% CI = 4.0–11.0). Fig. 3B shows a percentage of dry cow cycle consisting of a steady percentage of dry cows from January to June and a rapid increase in June. The best model for determining the percentage of dry cows was sinusoidal, which was highly efficient in determining the percentage of dry cows based on the month of the year.

Also, the cross-correlation plot between THI and percentage of dry cows showed that these variables were significantly positively correlated in the same month and 2.5, 5, and 7.5 months later (Fig. 3C). From Fig. 4A, it was noticed that the predicted days in milk (DIM) for the following year of the data presented was lower in September (158; 95% CI = 103–213) and highest in May (220; 95% CI = 181–259). Fig. 4B shows that the highest days in milk occurred in summer months and the lowest in winter months. The coefficient of determination was almost 1. Thus, it may be inferred that accuracy of days in milk is outstanding when considering the month of the year.

The cross correlation between monthly THI and monthly DIM showed a significantly positive correlation in the same month and the previous 2.5 and 5 months (Fig. 4C).

Fig. 5A shows the time series of monthly percentage of calvings from 2014 to 2020. From the ARIMA model, the rate of calvings was related to seasonality, with the predicted maximum percentage of calvings occurring in September (10.3%; 95% CI = 8.0–12.5) and the minimum

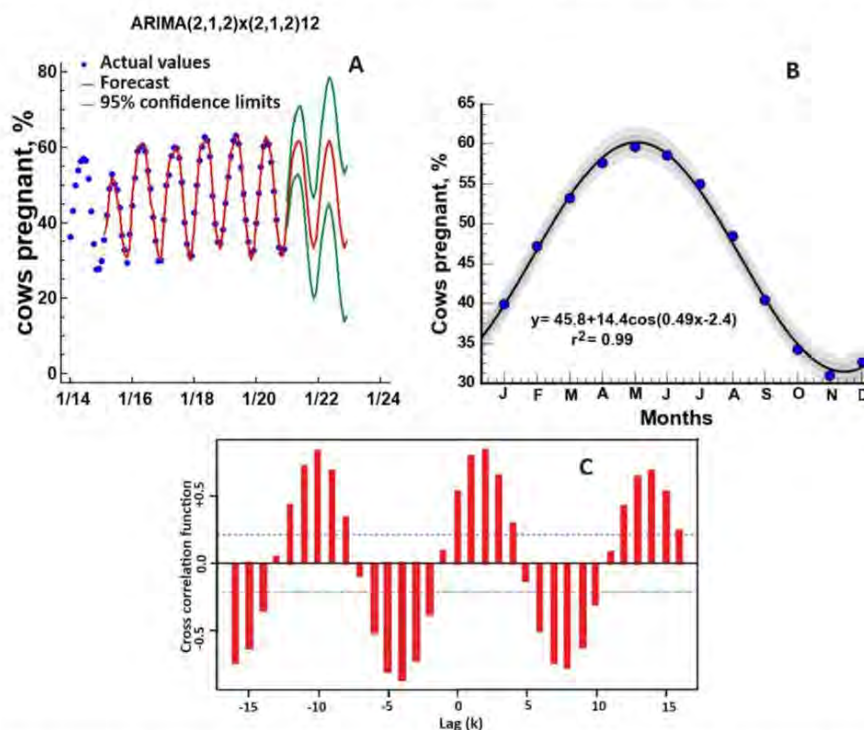


Fig. 2. A: The time series for the monthly percentage of cows pregnant in the herd (considers the population of milking and dry cows currently in the herd). Each point denotes the mean value of a single herd (~2900 lactating cows) from 2014 to 2020. Values are fitted and predicted values. B: The association between monthly percentage of cows pregnant in the herd (considers the population of all cows currently in the herd) and months of the year. C: Cross-correlation function of the time-series of monthly temperature-humidity index and proportion of monthly milk yield based on yearly milk yield in a hot environment: 2014–2020. The spikes denote the cross-correlation coefficients. The dashed lines represent the 95% confidence limits (CL). Spikes outside the CL are significant cross-correlation coefficients.

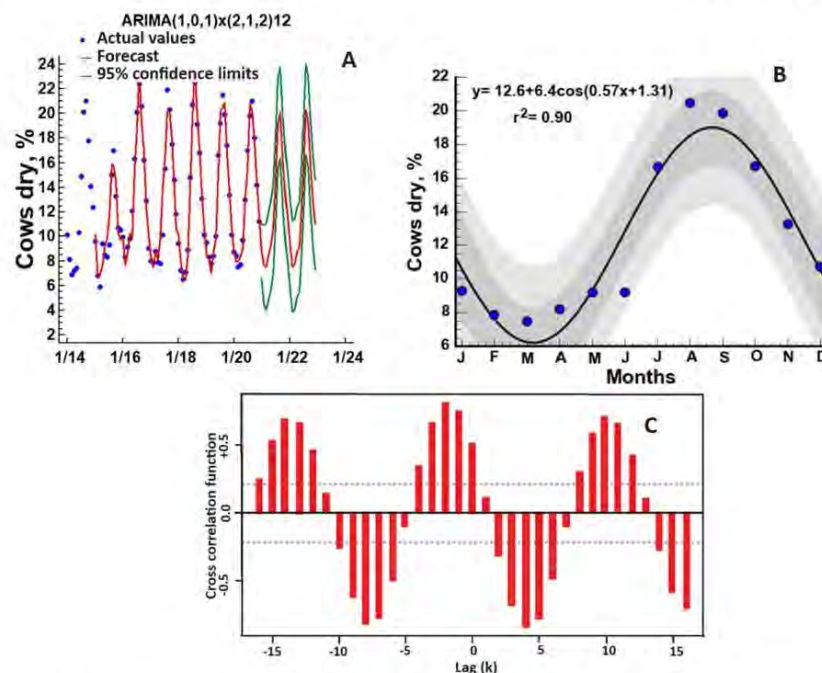


Fig. 3. A: The time series for the monthly percentage of dry cows (average monthly percent of the total herd dry every month). Each point denotes the mean value of a single herd (~2900 lactating cows) from 2014 to 2020. Values are fitted and predicted values. B: The association between the average monthly percent of the total dry herd every month and the months of the year. C: Cross-correlation function of the time-series of monthly temperature-humidity index and proportion of monthly percentage of dry cows in a hot environment: 2014–2020. The spikes denote the cross-correlation coefficients. The dashed lines represent the 95% confidence limits (CL). Spikes outside the CL are significant cross-correlation coefficients.

in April (3.2%; 95% CI = 1.0–5.5). The lowest calving percentages occurred in spring months and the highest in autumn months (Fig. 5B). The sinusoidal functions presented reasonable adjustment based on the coefficient of determination ($R^2 = 92$). Thus, calving percentages could be estimated accurately from month of the year information.

The THI and monthly percentage of calvings were not significantly correlated in the same month, but the correlations were significant and positive 2.5–10 months later (Fig. 5C). From the decomposition of multiplicative time series (Fig. 6A), there was a long-term trend and a clear seasonality in the percentage of culled cows from 2014 to 2020. The highest predicted culling rate for the year ensuing the present data occurred in November (4.3%; 95% CI = 3.2–5.4) and the lowest in April (2.5%; 95% CI = 1.4–3.5). The first seven months of the year had a similar culling rate, but the percentage of culled cows concentrated in the last trimester (Fig. 6B). Cross-correlation between the monthly THI and percentage of cows culled was not significantly correlated in the same month, but it was significantly negatively correlated from 2.5 to the 10 previous months (Fig. 6C). This situation indicates a prolonged lag relationship between the percentage of culled cows and heat stress.

4. Discussion

This study predicted seasonal dynamics of herd milk yield and productive and reproductive status with high accuracy from ARIMA(p, d, q) modeling. The herd monthly milk yield percentage was depressed by 2.3 percentage points of the annual total, with increased THI during summer

and fall. This milk yield seasonality has also been well-documented across the United States, with greater seasonality in the southern zones of this country (Ferreira et al., 2020). These data reaffirm previous reports (West et al., 2003; Bernabucci et al., 2014), where daily milk yield declined linearly with increasing ambient temperature. The authors mentioned above found a lag (2–8 days) between the high ambient temperature and its most decisive influence on milk yield. In the present study, the monthly herd milk yield occurred immediately and exhibited a prolonged delayed response in the subsequent months following the initial heat insult. Thus, in the zone where this study took place, milk yield presents an inherently seasonal fluctuation due to hyperthermia in spring, summer, and fall.

Feed intake is negatively correlated with heat stress (Hill and Wall, 2017; Sammad et al., 2020a), and the decreased intake is a response to decreased metabolic rate within two days of the onset of thermal stress (West et al., 2003). Reduced feed intake explains at least half of the reduction in milk yield caused by heat stress (Tao et al., 2020). These highlight the importance of adequate intake to maximize milk yield during hot months. Other mechanisms that mediate the declined milk yield by thermal stress, in addition to feed intake, include but are not limited to altered homeostatic mechanisms to make a shift in the energy metabolism to diminish metabolic heat production (Baumgard and Rhoads, 2012), potential activation of the immune system (Dahl et al., 2020) and inflammation (Most and Yates, 2021), changes in feeding behavior (Becker et al., 2020), and altered mammary gland development and function (Tao et al., 2018). Also, recently, the effects of an

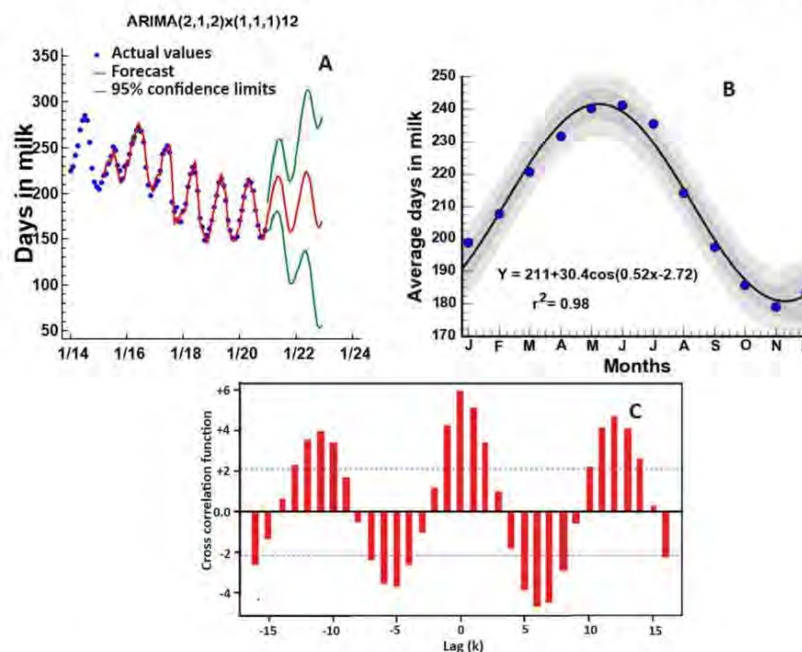


Fig. 4. A: The time series for the monthly average of days in milk. Each point denotes the mean value of a single herd (~2900 lactating cows) from 2014 to 2020. Values are fitted and predicted values. B: The association between monthly average of days in milk and months of the year. C: Cross-correlation function of the time series of monthly temperature-humidity index and monthly average of days in milk from 2014 to 2020. The spikes denote the cross-correlation coefficients. The dashed lines represent the 95% confidence limits (CL). Spikes outside the CL are significant cross-correlation coefficients.

endogenous circannual rhythm on milk production have been documented (Salfer et al., 2020). At the herd level, annual fluctuations in calvings across the year also influence monthly milk yield (Ferreira et al., 2020). The present data allow us to reasonably predict the expected monthly milk production patterns for intensive dairy herds in hot, arid environments of Northeastern Mexico.

There was a strong tendency for a higher percentage of pregnant cows in the summer months and a marked plummet of pregnant cows in winter. Seasonal patterns of pregnancy outcomes were consistent throughout the seven years included in this study and suggest that thermal stress, through a carry-over effect, created a time lag in the cows' pregnancy outcome. These results indicate that fertility (reduced ovarian function, estrus expression, oocyte health, and embryonic development; Wolfenson and Roth, 2019) was significantly decreased at high THI and moderate THI in the fall months, demonstrating the disruption of fertility even at moderate thermal stress. Therefore, high-producing cows require more effective reproductive strategies to ensure a more stable percentage of pregnant cows throughout the year (Negrón-Pérez et al., 2019). These results align with previous studies that documented trends over time in annual reproductive performance in dairy herds in the Southeastern United States (Washburn et al., 2002; De Vries and Risco, 2005; Ferreira et al., 2020). In the present study, cows did not suspend or delay breeding of cows during the summer and fall months; therefore, the marked fluctuations in percentage of pregnant cows over the year were basically due to climatic conditions. Since heat stress is a significant component of reproductive seasonality in dairy farms, ameliorating seasonal depression of reproductive efficiency

is a great challenge for the dairy industry in hot, arid areas (Roth, 2020).

There was a consistent annual rhythm for the monthly percentage of dry cows, with less than 10% monthly dry cows in the first semester and about 20% in autumn. These situations explain, in part, the herd's seasonal calving pattern. This response is linked to the accumulation of cows with prolonged days open due to the inability to get pregnant in the summer months (Mellado et al., 2013). However, this sharp increment in the percentage of dry cows in the second semester of the year was not associated with reduced milk yield at the end of the year. This response indicates that herd milk yield reduction in the summer months was not due to a greater proportion of dry cows in the herd but basically to the reduced feed intake of cows due to hyperthermia (Liu et al., 2019). Increase in the monthly percentage of dry cows was the response to a long-term effect of hyperthermia expressed during at least 10 months following the change in ambient temperature. The proportion of the herd producing milk versus the nonproductive dry cows affects total herd profitability (Cabrera, 2021). Thus, dairy herd managers in this zone must set a target of keeping a herd composition with nearly 90% monthly percentage of milking cows via increased reproductive success in months with intense heat stress.

The forecast model demonstrated the seasonal pattern of average monthly DIM. Average days in milk were markedly increased (33%) in summer months compared to winter months. An average DIM greater than 180 indicates a reproductive failure, resulting in a lower lifetime milk production per cow due to prolonged lactations and milking in the late lactation phase when milk production is lower. In the intensively managed dairy herd, repeat breeding is a significant factor in infertility

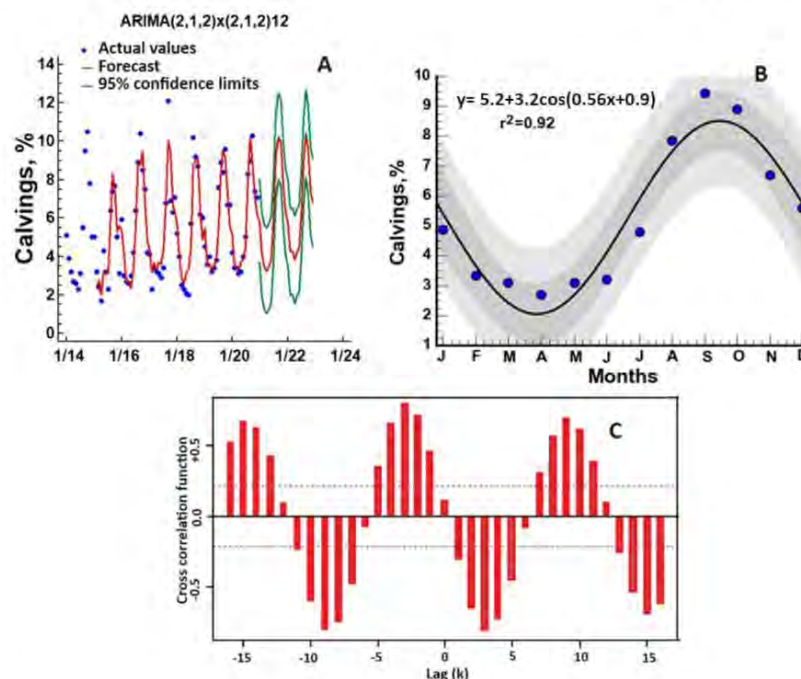


Fig. 5. A: The time series for the percentage of monthly calvings relative to the total annual calvings in the herd. Each point denotes the mean value of a single herd (~2900 lactating cows) from 2014 to 2020. Values are fitted and predicted values. B: The association between percentage of monthly calvings relative to annual calvings and months of the year. C: Cross-correlation function of the time series of monthly temperature-humidity index and monthly calvings from 2014 to 2020. The spikes denote the cross-correlation coefficients. The dashed lines represent the 95% confidence limits (CL). Spikes outside the CL are significant cross-correlation coefficients.

(Mellado et al., 2012). The greater DIM in summer reflects the conception failure of cows in the spring months due to thermal stress. This increase in DIM with higher ambient temperature explains, in part, the reduction of milk yield in the summer months due to the presence of too many cows in late lactation, where milk production is generally lower yet increases the number of lactating days relative to dry days.

Time series indicated that calving data experienced regular and predictable changes that recurred every calendar year. Percentage of calvings relative to yearly total calvings in the present study was highest in the fall, indicating that most conceptions occurred in winter. These results are in line with studies in southeastern USA states where breeding during the cool months affected little the conception rates of high-producing cows (De Vries and Risco, 2005; Huang et al., 2009), but in these states with the greatest number of heat stress days per year (Perrera et al., 2016) in the USA, hyperthermia constitute a major factor for calving seasonality in dairy herds (West et al., 2003). Results of the present study align with observations of Oseni et al. (2003), who found well-defined patterns across the year for days open and calvins in the Southeastern USA. These calving patterns could be associated with the present herd that had suboptimal reproductive success the previous summer and fall and experienced a rebound in conception rate over the winter. In the present study, management decisions did not lead to a seasonal calving pattern; therefore, high ambient temperatures several months before breeding, according to the cross-correlation between THI and monthly calving percentage, were responsible for the greatest

proportion of cows calving in the fall.

In the present study, culling rates were disproportionately higher in fall and winter. This response is basically due to the great decline in pregnancy success during spring and summer due to chronic heat stress. Heat stress increases days open (Sammad et al., 2020b), and this reproductive variable is a major reason for culling dairy cattle. Cows with poor fertility increase the producer's costs daily (St-Pierre et al., 2003) due to longer days in milk, increased calving intervals, increased culling and replacement costs, loss of genetic gain through the augmented generation intervals, and multiple services per conception (Jordan, 2003; Jeong et al., 2018). Thus, culling of cows in the present study was not scrutinized to control costs and achieve profitable milk yield; instead, most cullings were involuntary, forced by climatic infertility. Results of the present study help describe patterns of culling cows in this hot environment.

5. Conclusions

Based on ARIMA models, it was robustly estimated and forecasted monthly herd milk yield, percentage of pregnant cows and dry cows, days in milk, monthly percentage of calvings, and culling rate, demonstrating the valid potentials of time series modeling of retrospective data to forecast future development trends and patterns in dairy herds in hot environments. The study also revealed that actual and residual heat stress caused a severe distortion in the monthly herd milk yield

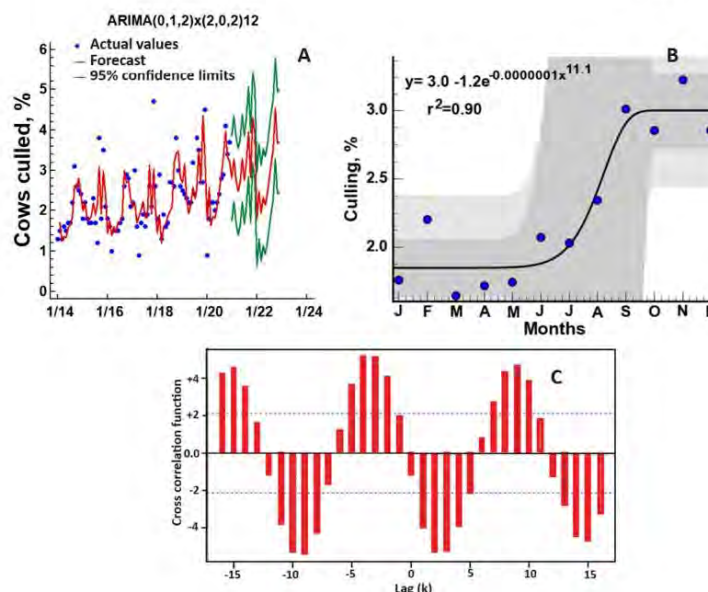


Fig. 6. A: The time series for the percentage of culled cows relative to the total annual culled cows in the herd. Each point denotes the mean value of a single herd (~2900 lactating cows) from 2014 to 2020. Values are fitted and predicted values. B: The association between percentage of monthly culling, relative to annual culling, and months of the year. C: Cross-correlation function of the time series of monthly temperature-humidity index and monthly culling relative to the total annual culled cows from 2014 to 2020. The spikes denote the cross-correlation coefficients. The dashed lines represent the 95% confidence limits (CL). Spikes outside the CL are significant cross-correlation coefficients.

percentage and reproductive and productive status. Characterizing dynamics of milk yield, reproduction, and culling would help shape the continuous benchmarking processes to identify high-impact improvements in the dairy herd.

In addition, the study reaffirmed the determinant role of thermal stress in the production and some reproductive events in this hot environment. This study stresses the need for further investigation to explore the role played by meteorological/bio-meteorological factors on the dynamics of the herd milk yield and some reproductive variables to determine the weather transition period that has the most significant effect on productive traits in the study area, and also to know the lead-time for assist in preparedness and early cautioning intervention to alleviate the impact of thermal stress on dynamics of the herd milk yield, reproduction, and productive status.

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CRediT authorship contribution statement

I. Chavarría: Data curation. A.S. Alvarado: Validation. U. Macías-Cruz: Investigation, Funding acquisition. L. Avendaño-Reyes: Formal analysis. O. Ángel-García: Validation. V. Contreras: Visualization, Validation. D.I. Carrillo: Validation, Supervision. M. Mellado: Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported.

Data availability

Other (please explain: e.g. 'I have shared the link to my data as an attachment').

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Artículo 2

I. Chavarría et al.: Monthly variation in Holstein milk yield and composition

Original scientific paper

UDK: 637.112.2

A time series analysis of monthly Holstein milk yield and composition in a hot environment

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Abstract

This study aimed to evaluate the effects of thermal stress on milk yield and milk composition using the time series approach to forecast the trend of daily milk yield and milk components in Holstein cows in a hot-arid environment. Also, this study evaluated the effect of season on the microbiology of raw bulk Holstein cow milk. The obtained data comprised the average daily milk yield, composition, and bacterial counts from a single high-input dairy herd in northern Mexico, registered for seven consecutive years (2014–2020). The autoregressive integrated moving average (ARIMA) procedure showed a distinct pattern for the daily milk yield. A two-year forecasting showed 8.4 kg difference in daily milk yield between winter and summer months. The predicted future values based on past values showed that milk solids were highest in January (12.09 %; CI= 11.86–12.31 %) and the lowest in August (12.02; CI= 11.75–12.29). The mean predicted fat content was highest in January (3.60 %; CI= 3.48–3.71 %) and lowest in July (3.44%; CI= 3.29–3.59 %). Following the repeated observations, forecasting values for milk protein percentage were 3.22 % (CI=3.15–3.28 %) in January and 3.07 (CI=2.98–3.1) in April. The ARIMA method forecasted milk lactose as highest in January (4.87 %; CI=4.79–4.95) and lowest in June (4.77 %; 4.68–4.86 %). Standard plate count was higher ($p<0.01$) in autumn and winter than spring and summer. During winter, the average thermotolerant count (TDC) in raw bulk milk was higher (12.5 ± 2.01) compared with TDC in the spring (9.7 ± 1.5 ; $p<0.01$). The somatic cell count did not differ between seasons. The coliform bacteria count was highest in autumn (77.8 ± 28.3 cfu/mL; $p<0.01$) and lowest in spring (53.7 ± 1.3 cfu/mL). It was concluded that hotter months diminished daily milk yield and its components. The ARIMA model can be used to accurately forecast daily milk yield and composition, which would allow to develop long-term strategies for managing milk production.

Keywords: heat stress; milk bacterial count; milk fat; milk lactose; milk protein

Introduction

Thermal stress has a great impact on milk production in temperate (Lim et al., 2021; Ogundejiet al., 2021) and tropical and subtropical zones (Dikmen et al., 2020; Mbuthia et al., 2021). Climatic conditions in zones of northern Mexico are hot for most of the year. Thus, chronic heat stress adversely impacts milk yield in intensive dairy farming (Rodríguez-Venegas et al., 2022). Seasonal patterns and milk production per cow greatly differ across geographical zones (Guinn et al., 2019). Therefore, a precise milk yield and composition projections by particular zones would benefit dairy farm managers and the market in making policy decisions considering seasonal milk yield and composition. Noticing whether milk yield is more likely to happen on a particular calendar date would improve the understanding of daily milk yield variation in hot environments, its causes, and suitable responses.

High-quality milk is essential for processing high-quality dairy products. Therefore, milk processing plants use bulk tank milk analysis to reward farmers who produce high-quality milk. Hence, composition of cow's milk is economically crucial to both, milk producers and processors. Milk is nutritionally vital to consumers worldwide due to its high-quality proteins, high calcium and phosphorus content, some microelements, and significant amounts of fat-soluble and water-soluble vitamins, especially vitamin B (Pereira, 2014; Antunes et al., 2022).

Many factors affect milk composition, one of them being heat stress. Heat stress has been associated to a decline in total protein and fat content (Bernabucci et al., 2015; Hill and Wall, 2015; Ouellet et al., 2019). Additionally, heat stress changes the triacylglycerol profile and reduces phospholipids, which modify the physical properties of milk fat as well as the nutritional value of milk (Liu et al., 2017).

Most studies on the effect of thermal stress on milk yield and quality have been conducted in zones with short, warm periods. In some areas of northern Mexico, numerous consecutive months of heat-stress conditions exist. These consecutive days of thermal stress significantly impact milk yield and composition, reducing the chance for cows to dissipate body heat at night. Consequently, the body temperature surge is accumulated daily for months, leading to significant declines in milk yield, reproductive performance, and animal health (Polsky and von Keyserlingk, 2017).

Because of the combined effects of the current and past milk yield and milk component levels on future lactation, it is vital to accurately forecast the milk yield and its quality at the herd level. Therefore, this study aimed to predict trends, seasonal effects, and future yields in cow's milk in a hot environment through time series analysis. Another objective was to evaluate the seasonal microbial properties of raw milk throughout the year.

Material and methods

Dairy herd

All experimental procedures complied with The Guide for Care and Use of Agricultural Animals of the Autonomous Agrarian University Antonio Narro (#3001-2423). A commercial dairy farm in northern Mexico (25 °N, elevation 1140 m, mean annual temperature 24.6 °C, mean annual rainfall 230 mm) was used. During the study period, the maximum ambient temperature ranged from 1 °C to 41.4 °C, and the relative humidity ranged from 13 % to 81 %. The herd ranged from 2256 to 3299 lactating Holstein cows (mean = 2931; median = 2973; standard deviation = 234). Animals were kept outdoors in open-dirt pens with fans operating during the warm weather. Pens were equipped with ample shade structures and feeding alleys. Cows were fed diets formulated to meet or exceed the suggested daily nutrients for 670 kg dairy cows producing 38 kg/day of 3.5 % fat-corrected milk (NRC, 2001). Some cows in this study initiated their lactation with an abortion, and about 18 % of the cows had prolonged lactations (>400 days).

Cows were fed a total mixed diet containing ground-shelled corn and soybean meal in the concentrate mix (50 % of the diet); the forage portion of the diet was corn silage and alfalfa hay. Diets are pretty homogeneous all year round in terms of ingredients and nutrient content. Cows were fed two times per day. Animals had a free access to water at all times.

Milk yield recording

The lactation number of cows included into the study varied from one to eight. Cows were milked thrice daily (04:00, 12:00, and 20:00 hours), and milk yield was recorded electronically at each milking for individual cows. The daily milk yield per cow was estimated as the sum of the harvested milk from midnight to midnight divided by the number of milked cows. The daily milk yield's mean, standard deviations, median, and range were 30.4, 2.5, 30.1, and 25.3-35.1, respectively. Cows completed their tenure when they reached 210 days of pregnancy or produced ≤20 kg of milk/day. The actual 305-day rolling herd average for the six dairy herds was about 10350 kg, and the percentage of culled cows per year varied from 26 % to 33 %. Milk yield and composition were recorded for seven consecutive years (2014–2020).

Collection and processing of bulk tank milk samples (BTM)

BTM samples were obtained weekly for 365 days to record milk components. A volume of 50 mL of milk was collected at 4 °C no later than eight hours after milking. Milk collection was performed by using sterile plastic screw-cap centrifuge tubes after an agitation cycle. The tubes were placed into an icebox and transferred to the laboratory for analysis within 15 min. The total solids (TS), fat, protein and lactose content

in milk were measured by near-infrared spectroscopy using a MilkoScan FT 120 (Foss, Hillerød, Denmark). Milk was tested for urea nitrogen using a FOSS 4000 analyzer (Foss North America, Eden Prairie, Minnesota, USA). All analyses of raw milk were carried out in triplicate.

Bacteriological analysis of raw milk

The milk was collected daily and was a mix of three consecutive milkings. Raw milk samples from the bulk tank were placed in sterile 50-mL tubes. The milk samples were mixed thoroughly several times, and 20 mL of this milk was transferred to a snap-cap vial to determine BTM somatic cell count. The remaining part of the milk sample was used for bacteriological analysis and milk components.

Milk samples were examined for the standard plate count (SPC) by a pour plating method using standard growth medium followed by aerobic incubation for 48 h at 32 °C, after which bacterial colonies were enumerated and expressed as colony-forming units per mL (cfu/mL). Coliform count (CC) was assessed by spreading a milk sample onto a MacConkey Agar followed by incubation at 32 °C for 24 h; colonies were expressed as cfu/mL. The thermophilic psychrotrophic count (TC) was assessed by placing milk samples into sterile 20 x 125-mm test tubes and incubated in a water bath at 63 °C for 30 min. Samples were cooled in an ice-water bath for 10 min, placed in duplicate on plate count agar (Remel Inc., Lenexa, KS), and incubated at 7 °C for 10 days. Somatic cell counts (SCC) were performed using a Bentley Somacount 300 (Chaska, MN) according to manufacturer's procedures.

Statistical analyses

For forecasting daily milk yield and milk components, we set out a back-testing framework for two years of forecasting using a time series approach. STATGRAPHICS software was employed to select a forecasting model that compared multiple models and automatically picked the model that maximized the specified information criterion. A usual practice of this procedure in Six Sigma is to choose an ARIMA model of the ARMA(p,p-1) form, which, unlike most other control diagrams, does not assume independence among sequential measurements.

ARIMA is a mixture of auto-regressive and moving average, represented by p, d, and q. Where p represents the number of lag observations incorporated in the model, d denotes the number of times raw observations undergo differencing, and q is the degree of moving average. The model is as follows:

$$Y_t = \phi_1 Y_{t-1} + \phi_2 Y_{t-2} + \dots + \phi_p Y_{t-p} + \epsilon_t \\ \epsilon_t = 1 + \theta_1 \epsilon_{t-1} + \theta_2 \epsilon_{t-2} + \dots + \theta_q \epsilon_{t-q}$$

The final automatic model selection provided the optimal model based on the lowest value of the Akaike information criterion. When the model was constructed, the autocorrelation function (ACF) and partial autocorrelation function (PACF) of model residuals were depicted to confirm

autoregressive and the moving average parameters and evaluate the goodness of fit. The Box-Jenkins procedure was used to estimate p, d, and q values. 30 days cycles were chosen as the measurement unit for analysis of season effect on milk yield and its components because this interval is commonly used in the dairy industry for monitoring milk production. The cross correlation between the milk yield and the THI over time was calculated utilizing the Wessa software (2017) that computes the cross-correlation function for univariate time series.

Also, analysis of the milk components was conducted using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), with month included in the model as a random effect to account for the clustering of samples within month. The PDIF option of SAS using the Bonferroni test (PDIF ADJUST=BON) was used for multiple comparisons of means.

One-way ANOVA (PROC GLM of SAS) was performed on log₁₀ values (for better homogeneity of the distribution of residuals) for each bacterial and cell somatic count. For this analysis, seasons were classified as winter (December to February), spring (March to May), summer (June to August), and autumn (September to November). The PDIF option of SAS was used to compare means.

Results and discussion

Figure 1 shows a seasonal variation in the time series within years. Also, tendencies suggest an additive model as the seasonal fluctuations are roughly constant over time. A well-defined trend for this trait could be recognized over the seven years. Predicted monthly daily milk yield of cows was highest in January (34.5; IC=32.9-36.1) and lowest in July (26.1; CI=23.7-28.5). The results indicate marked differences in average daily milk yield in cows during periods of high ambient temperatures compared to cooler temperatures during the year. Production determinants such as the feeding program, genetic merit, and health management were indistinguishable among cows, so the only apparent difference was the daily temperature and humidity. Climatic conditions were the most likely cause of the ample milk production levels during the year. These results align with previous studies in intensive dairy operations in temperate climates where an annual rhythm of milk yield has been established at the cow level (Vallimont et al., 2010) or country level (Salfer et al., 2019; Ferreira et al., 2020) data.

Figure 2 depicts the cross-correlations between THI (input variable) and daily milk yield (output variable). Correlations were significant and negative for the THI and the daily milk yield in the same month and at lags -1, +1, +2, and +3, indicating that the THI and monthly daily milk yield in cows were negatively correlated 2.5, 5, and 7.5 months earlier, and 2.5 months later, reaffirming the strong negative influence of thermal stress with residual and preceding effects on daily milk yield.

These results confirmed that heat stress is the primary driver of milk yield seasonality in intensive dairy operations in a hot-arid environment. Our model found unequivocally,

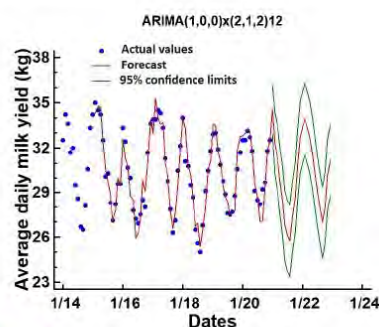


Figure 1. The differences of time series of mean daily milk yield of Holstein cows in a hot environment between 2014 and 2020, based on 12-month intervals. The forecasts are shown in red, and the green lines are 95 % confidence intervals

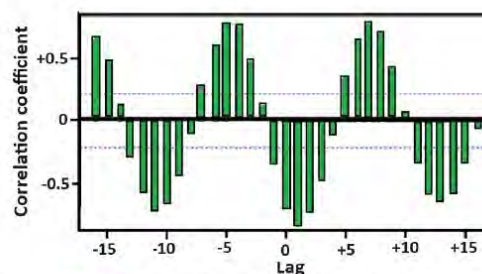


Figure 2. The cross-correlation functions between daily milk yield and monthly temperature-humidity index (THI) average. The spikes denote the cross-correlation coefficients. The dashed lines represent the bounds of cross-correlation functions. Spikes outside the bounds are significant cross-correlation coefficients

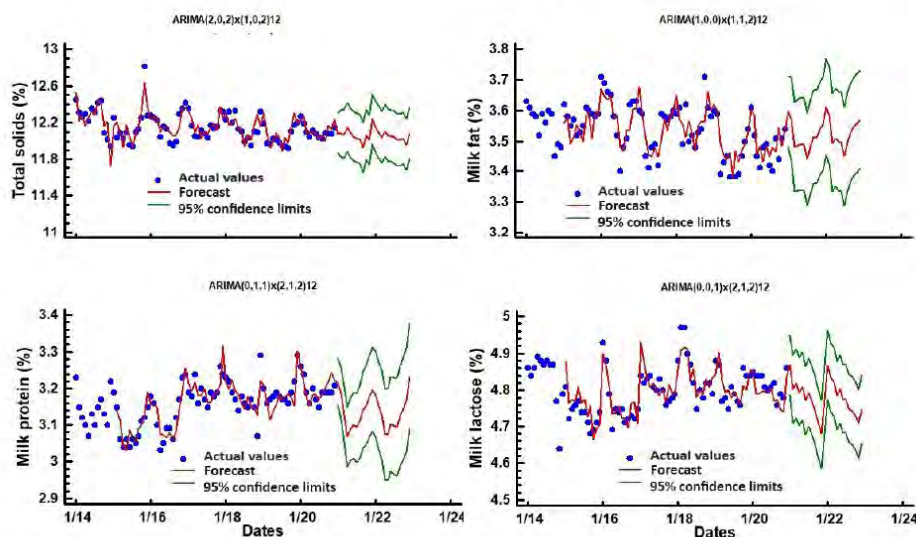


Figure 3. The differences of time series of mean milk total solids, milk fat percentage, milk protein percentage, and milk lactose percentage of Holstein cows in a hot environment between 2016 and 2020, based on 12-month intervals. The forecasts are shown in red, and the green lines are 95 % confidence intervals

in terms of peak timing, amplitude, or seasonal shape, that thermal stress alone explains milk yield seasonality. The predicted future value based on the past values showed a depression of 8.4 kg in daily milk yield in the hottest part of the year, which bears a significant economic loss in large dairy herds in this hot environment. Our study contributes to improving projections of future milk yield, which is essential for dairy operation planning. This forecasting can determine

budget allocation implementation of spatial (e.g., housing, infrastructure, labor) policies.

Figure 3 shows the monthly mean values of total solids, fat, lactose, and protein content of raw milk of Holstein cows for seven consecutive years. Starting in January, the total solid content of milk steadily declined as the year progressed, with the lowest value in July (12.05 %; $p < 0.01$), and then gradually started to increase to a maximum peak in November (12.31 %).

A perusal of Figure 2 indicates an increasing trend in the total solids in the milk in the winter months. P-values for the root mean square error, the mean absolute error, the mean absolute percentage error, the mean error, and the mean percentage error were <0.01 , reaffirming the occurrence of a defined trend for milk total solids. The predicted future values based on past values (ARIMA procedure) showed the highest value in January (12.09%; CI= 11.86–12.31 %) and the lowest in August (12.02; CI= 11.75–12.29).

The values of the total solids in milk were a consequence of the reduction in protein fat and lactose during the warmest months, which is in agreement with the findings of Kadzere et al. (2002), who observed an 18.9 % decrease in solids-not-fat when Holstein cows were transferred from an air temperature of 18 to 30 °C. Chen et al. (2014) and Lohaj and Sulejmani (2020) observed a reduction in total solids content from winter to summer in dairy farms in Europe, which is also in line with the current study. Using the autoregressive integrated moving average (ARIMA), we forecasted the depression of total milk solids during two future years, with annual peaks detected.

Months also affected ($p<0.01$) the concentration of milk fat, with the highest mean predicted fat content observed in January (3.60%; CI= 3.48–3.71%) and the lowest in July (3.44%; CI= 3.29–3.59 %). The statistical significance of the terms in the forecasting model was <0.01 . The drastic decline in raw milk fat in July is generally in line with various studies in temperate zones (Hammami et al., 2015; Garcia et al., 2015). The reduction of milk fat may be partly a result of a decrease in dry matter intake in cows subjected to mild (Gomiak et al., 2014) or severe (Spiers et al., 2004) heat stress, which alters ruminal fermentation reducing acetate and butyrate, substrates required for de novo milk fat synthesis.

Additionally, partitioning of energy in thermoregulation-related mechanisms in response to heat challenge diminishes anabolic activities such as synthesis of fatty acids in mammary tissue, which may also reduce milk fat content during warm weather (Das et al., 2016; Moore et al., 2023). Also, in cows undergoing thermal stress, the circulating non-esterified fatty acid levels are reduced (Abeni et al., 2007), and the adipose tissue is less sensitive to catabolic signals in heat-stressed animals (Baumgard and Rhoads, 2012). In the current study, milk fat showed an increasing consistent trend during the coldest months, as indicated by the time series analysis. The predicting values revealed an annual peak, and these variations, although minimal (0.16 percent points), are likely to be meaningful for commercial dairy herds in this hot environment.

Month had a significant effect ($p<0.01$) on milk proteins, which varied from 3.12 to 3.20 %. Following previous observations, the forecast values for milk protein percentage were 3.22 % (CI= 3.15–3.28 %) in January and 3.07 % (CI= 2.98–3.1) in April. All terms in the forecast model (ARIMA) were significant ($p<0.0001$), which confirms the seasonality of milk protein percentage attributed to ambient temperatures.

Chen et al. (2014) observed that the total protein content was higher in spring than during summer and autumn in European countries. Similarly, Lindmark-Mansson (2003) and Heck et al. (2009) reported that the raw milk protein content was the highest in Sweden and the Netherlands during January and December. Karlsson et al. (2017) observed the highest protein content in November in Swedish raw milk. Similar to the studies mentioned above, in the present study, milk protein content presented minimum values in summer months and maximum values at the end of autumn and during the winter months. Given that cows in the present study were fed the same diets throughout the study period, monthly changes in the main raw milk components were not likely due to dietary variation. Yang et al. (2013) also found that seasonal changes in milk components of Holstein cows under intensive conditions were not due to diet changes, as these researchers found a distinctive milk component depression in summer without changes in the ration offered to cows.

Rhoads et al. (2009) also reported a decreased milk protein content in heat-stressed cows due to a lower synthesis of casein formation enzymes. The detrimental effect of heat stress on the milk protein reduction seems to arise, in part, from the fact that heat stress changes amino acids profile of dairy cows so that more amino acids are required for maintenance (immune response and gluconeogenesis) but not for milk protein synthesis under heat stress (Guo et al., 2018). Also, Guo et al. (2021) suggested that oxidative stress as a consequence of heat stress contributes to a reduction in milk proteins by causing apoptosis in mammary gland tissue and hampering milk protein synthesis by disrupting signalling pathways. The reduction in milk protein and fat content in hot months is particularly harmful in countries that use cow milk for cheese production.

Milk produced in July had the lowest ($p<0.01$) lactose percentage, with a slight variation in spring, autumn, and winter months compared to July. Even so, the ARIMA method detected that the average value in summer months differed from that in winter months. The extreme forecasting values were 4.87 % CI= 4.79–4.95) for January and 4.77 % (4.68–4.86 %) in June. These results are surprising as lactose is well known to be one of the least variable milk components (Heck et

Table 1. Seasonal bacterial content of raw milk from Holstein cows in a hot environment (means $\pm$ SD)

Season	Standard plate count (cfu/mL)	Thermotolerant count (cfu/mL)	Coliform count (cfu/mL)	Somatic cell count (cell $\times 10^3$ /mL)
Spring	5613 $\pm$ 3951 ^a	9.7 $\pm$ 1.5 ^a	53.7 $\pm$ 21.3 ^a	229 $\pm$ 53
Summer	5485 $\pm$ 2916 ^a	10.1 $\pm$ 0.8 ^a	66.1 $\pm$ 7.5 ^{ab}	249 $\pm$ 56
Autumn	7540 $\pm$ 5400 ^{ab}	12.2 $\pm$ 3.4 ^b	77.8 $\pm$ 28.3 ^b	261 $\pm$ 83
Winter	8947 $\pm$ 10579 ^b	12.5 $\pm$ 2.0 ^b	57.7 $\pm$ 15.2 ^b	267 $\pm$ 128

cfu= colony forming units

^{a,b} Within columns, means with different superscript differ ($p<0.05$)

al., 2009; Chen et al., 2014). However, Garcia et al. (2015) documented a reduction of milk lactose in Holstein cows under heat stress in a tropical climate. A metabolomics study by Tian et al. (2016) suggests that either less blood glucose is available to the mammary gland or lactose synthesis is disrupted in heat-stressed cows, which would explain the reduced lactose content in summer in the present study. Raw milk's mean urea nitrogen content over the year did not significantly vary with months (12.9 ± 1.8 mg/dL). The yearly mean SPC was 6896 cfu/mL for the seven years included in the study. Season affected the SPC, with winter milk having a significantly higher SPC than spring and summer milk (Table 1). The mean TC was higher ($p < 0.01$) in raw milk obtained in autumn and winter than in spring and summer milk. CC was higher ($p < 0.01$) in milk sampled in autumn than in other seasons. Finally, SCC was below the standards outlined in the U.S. Pasteurized Milk Ordinance (Food and Drug Administration, 2015). Thus, the month of sampling did not significantly affect the somatic cell count (mean = $252 \times 103 \pm 82 \times 103$ cells/mL). Contrary to other studies where TDC in raw cow's milk reached the highest values during the summer months (Vithanage et al., 2016; Stocco et al., 2023), in the present study, autumn and winter months favored the proliferation of these bacteria in raw milk. The reduced standard deviation for this variable suggests that cleaning practices in the dairy herd in this study did not substantially affect the presence of thermophilic bacteria in raw milk. These results indicate the need for dairy producers to pay distinctive attention to their cleaning-in-place procedures during the autumn and winter months to avoid contamination with thermophilic bacteria, as the primary sources of thermophiles in milk are contamination of the teat skin from bedding and soil (Gleeson et al., 2013). No seasonal effect was observed on SCC, which is contrary to the findings of other authors in zones characterized by high ambient temperature in summer (Bertocchi et al., 2014; Bernabucci et al., 2015) or milder summer temperatures (Green et al., 2006; Olde Riekerink et al., 2007), where the higher SCC occurs in summer compared to other seasons. However, some other authors have not observed seasonal fluctuations in SCC in hot semi-arid environments (Lima et al., 2019). Bulk milk SCC in a herd is affected by subclinical

and clinical mastitis, which depends on many factors, among which season is the most critical one (Zucali et al., 2011). Because the epidemiology of each microorganism causing mastitis is distinct, the effect on bulk milk SCC and its relationship to climatic and environmental factors might differ. Given the strong association between udder hygiene and bacterial counts in BTM (Elmoslemany et al., 2009), these data suggest that cows were dirtier in a particular season.

Conclusions

Long-term trends based on time series evidenced a considerable effect of the seasonality of the average daily milk yield with a predicted depression of 8.4 kg in daily milk yield in the hottest part of the year. Also, results of this study showed a considerable variation throughout the year in concentrations of the main bulk tank milk components in a high-input dairy herd of northern Mexico, with the highest depression of these components in the summer months. The ARIMA procedure was reasonably accurate in forecasting milk yield and components as the year progressed. Autumn and winter were the most critical seasons for standard plate count and thermophilic count in raw milk, with no changes in somatic cell counts in different seasons. Thus, seasonal fluctuations must be considered when processing milk, as they greatly affect the bacterial microflora in raw milk. Additionally, applying time series provided a practical methodology to distinguish seasonal patterns across time. It is essential knowledge for dairy producers to allow them to make ideal decisions from an economic perspective in this zone of intense and prolonged thermal stress.

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Analiza vremenskog niza mjesečne količine i sastava mlijeka holstein krava u vrućem okolišu

Sažetak

Cilj ovog istraživanja bio je ispitati učinke toplinskog stresa na količinu i sastav mlijeka korištenjem pristupa vremenskog niza za predviđanje trenda dnevne količine i sastava mlijeka kod holstein krava u vrućem i sušnom okolišu. Također, u ovom je istraživanju određen učinak sezone na mikrobiološku kvalitetu sirovog mlijeka holstein krava. Dobiveni podaci su uključivali prosječni dnevni prinos mlijeka, sastav i broj bakterija iz jednog stada s visokom proizvodnjom u sjevernom Meksiku, a koji su prikupljeni tijekom sedam uzastopnih godina (2014.-2020.). Postupak autoregresijskog integriranog pomičnog prosjeka (ARIMA) pokazao je jasan uzorak za dnevnu količinu mlijeka. Dvogodišnja prognoza pokazala je 8,4 kg razlike proizvodnje u dnevnoj mliječnosti između zimskih i ljetnih mjeseci. Predviđene buduće vrijednosti temeljene na prošlim vrijednostima pokazuju da je ukupna suha tvar mlijeka bila najveća u siječnju (12,09 %; CI=11,86-12,31 %), a najniža u kolovozu (12,02; CI=11,75-12,29). Srednji predviđeni udio masti bio je najviši u siječnju (3,60 %; CI= 3,48-3,71 %), a najniži u srpnju (3,44 %; CI= 3,29-3,59%). Nakon ponovljenih promatranja, prognozirane vrijednosti udjela proteina u mlijeku bile su 3,22 % (CI=3,15-3,28 %) u siječnju i 3,07 % (CI=2,98-3,1) u travnju. Metoda ARIMA predviđa da je udio laktoze u mlijeku najviši u siječnju (4,87 %; CI=4,79-4,95), a najniži u lipnju (4,77 %; 4,68-4,86 %). Ukupan broj aerobnih mezofilnih bakterija bio je veći ($p<0,01$) u jesen i zimu nego u proljeće i ljeto. Prosječni broj termorezistentnih bakterija (TDC) u sirovom mlijeku bio je veći ($12,5\pm2,01$) zimi u usporedbi s TDC u proljeće ($9,7\pm1,5$; $p<0,01$). Broj somatskih stanica nije se razlikovao između godišnjih doba. Broj koliformnih bakterija bio je najveći u jesen ($77,8\pm28,3$ cfu/mL; $p<0,01$), a najmanji u proljeće ($53,7\pm21,3$ cfu/mL). Zaključno, topliji mjeseci smanjuju dnevni prinos mlijeka i udjele pojedinih sastojaka mlijeka. ARIMA model može se koristiti za točnu prognozu dnevne količine i sastava mlijeka, što bi omogućilo razvoj dugoročnih strategija za upravljanje proizvodnjom mlijeka.

Ključne riječi: toplinski stres; broj bakterija u mlijeku; mliječna mast; laktoza; proteini mlijeka

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CONCLUSIÓN GENERAL

Ambos estudios analizados, centrados en definir la influencia de la exposición al calor en la producción, reproducción y composición de la leche en ganado Holstein, contribuyen consecuentemente a ampliar y complementar entre sí a cómo el estrés por calor altera las dinámicas productivas o biológicas del ganado criados en áreas áridas. Los dos estudios logran destacar la utilidad del ARIMA como un predictor confiable para tendencias futuras y en explicar la estacionalidad cíclica involucrada en sistemas de producción lechera bajo situaciones climáticas severas.

Por lo tanto, se demuestra que el estrés térmico es el principal contribuyente a la estacionalidad de la producción de leche. La dinámica estacional en ganado en climas cálidos, estudiada mediante el porcentaje mensual de producción de leche, muestra una disminución de más del 2% tanto en verano como en otoño (cuando el ITH es mayor) sobre un total anual. Además, la comparación de los datos de monitoreo de leche a nivel diario demostró una depresión de 8,4 kg/vaca por día de invierno a verano y claramente indica que el estrés por calor prolongado deprime el rendimiento productivo de las vacas lecheras, incluso de aquellas bajo instalaciones y sistemas intensivos con provisiones de enfriamiento.

En ambos estudios muestran que el calor no solo actúa de inmediato, sino que también tiene influencias residuales y prolongadas. La correlación cruzada entre el índice ITH y la producción de leche muestra que la disminución en el rendimiento puede ocurrir meses después de la primera exposición al calor, lo que indica la presencia de huellas fisiológicas y reproductivas a medio plazo, como una menor percepción del estrés térmico debido al calentamiento del organismo. Una tendencia similar se siguió en el rendimiento reproductivo: la proporción de vacas preñadas mostró picos y valles estacionales bien definidos, con reducciones notables en los meses más calurosos, y un subsiguiente aumento o el llamado "rebote reproductivo" asociado a las ventajas ambientales que ofrece el invierno.

Para el análisis de la leche especialmente para grasa, proteína, lactosa y sólidos totales las reducciones fueron mayores durante la temporada cálida, lo que puede indicar que la formación de componentes de la leche se ve más afectada por el estrés calórico debido a alteraciones en el metabolismo, disponibilidad reducida de energía y cambios en la microflora ruminal. La disminución del contenido de proteínas y grasas no es muy pronunciada en verano, pero tiene implicaciones importantes para la industria de producción de queso y el valor de la leche.

No obstante, ambos estudios coinciden en que el estrés calórico también afecta los parámetros estructurales del hato, relacionados con los días en leche, la proporción de vacas secas, la estacionalidad de los partos y las tasas de sacrificio. Estas variables siguieron patrones cíclicos a lo largo del año, presentando mayores tasas de vacas secas y sacrificio en los meses posteriores al verano, reflejando el efecto acumulativo de la infertilidad inducida por el calor. En conclusión, los dos documentos ofrecen evidencia consistente de que el clima cálido crónico restringe la productividad, la reproducción y la economía de los hatos lecheros en tierras áridas. También muestran que los modelos ARIMA son útiles para predecir estas fluctuaciones y ajustar la gestión, la nutrición, la reproducción y el control del estrés calórico. La síntesis de estos hallazgos destaca la necesidad de sistemas más robustos, tecnologías de enfriamiento que consuman menos energía y programas de reproducción adaptados a entornos cálidos continuos.

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