

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA

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**PREDICCIÓN DE LA TEMPERATURA RECTAL EN VAQUILLAS
HOLSTEIN USANDO MEDIDAS TERMOGRÁFICAS,
FISIOLÓGICAS Y CLIMÁTICAS EN UNA ZONA ÁRIDA**

TESIS

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PRESENTA

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La presente tesis “**PREDICCIÓN DE LA TEMPERATURA RECTAL EN VAQUILLAS HOLSTEIN USANDO MEDIDAS TERMOGRÁFICAS, FISIOLÓGICAS Y CLIMÁTICAS EN UNA ZONA ÁRIDA**”, realizada por la **M.C. Chilove Theusme** y dirigida por el **Ph.D. Leonel Avendaño Reyes**, ha sido evaluada y aprobada por el Comité Particular abajo indicado, como requisito parcial para obtener el grado de:

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Resumen

La temperatura rectal (TR) es una de las variables fisiológicas más utilizadas para monitorear cambios en la temperatura corporal, vigilando así la salud y bienestar del ganado lechero. En la práctica, la TR se registra introduciendo un termómetro en el recto, procedimiento que requiere la sujeción del animal y provoca estrés de manejo que altera la medición final. Por lo anterior, la termografía infrarroja (TIR) ha emergido como una tecnología para registrar la TR de forma no invasiva. Por tanto, este estudio plantea dos objetivos: 1) predecir la temperatura rectal en vaquillas Holstein usando variables fisiológicas y climáticas, y 2) determinar el efecto del color del pelaje en variables fisiológicas de vaquillas Holstein, así como predecir la TR por color del pelaje en las mismas. Se utilizaron 200 vaquillas Holstein puberales (edad 12-14 meses; peso promedio 297.0 ± 12 kg y condición corporal promedio 3.5 ± 1.1 unidades) durante dos veranos consecutivos (2019 y 2020), las cuales se dividieron por color de pelaje predominante: negro ($n_1=60$), blanco ($n_2=62$) y mixto ($n_3=78$, 50 % blanco y 50 % negro). Se midió la frecuencia respiratoria (FR) y las temperaturas de la superficie corporal (TSC) colectadas con TIR en las siguientes regiones: paleta, vientre, grupa, cuello, cabeza, frente, nariz, lomo, pierna, vulva, ojo, flanco derecho, y tronco del cuerpo derecho. Las variables climáticas registradas fueron TA, humedad relativa (HR) e índice temperatura-humedad. Se usó análisis de correlación para determinar el grado de asociación lineal entre las variables, así como análisis de regresión lineal múltiple para desarrollar ecuaciones de predicción de TR. El efecto del color del pelaje sobre variables fisiológicas se estimó por medio de análisis de varianza. Las variables TR y FR se correlacionaron alta y positivamente ($r = 0.73$; $P < 0.01$), mientras que las TSC tuvieron correlaciones de bajas a moderadas ($0.15 < r < 0.55$) con TR y FR. La temperatura de la frente mostró la correlación más alta ($r = 0.58$; $P < 0.01$) con TR. Las vaquillas negras tuvieron mayor FR y TR ($P < 0.01$) que las vaquillas mixtas y de pelaje blanco; las vaquillas blancas tuvieron una TR similar pero menor ($P < 0.05$) FR que las vaquillas mixtas. En general, las vaquillas de pelaje negro y mixto tuvieron TSC más altas ($P < 0.01$) que las vaquillas blancas en la mayoría de las regiones anatómicas medidas. La mejor ecuación de predicción fue $TR = 35.55 + 0.033 (FR) + 0.030 (TSC_{\text{frente}})$, ya

que obtuvo la más alta R^2 (60%) combinada con los indicadores de variabilidad más bajos. Basado en el color del pelaje, los modelos de predicción seleccionados fueron: $TR_{\text{negro}}=35.59 - 0.013(\text{HR}) + 0.045(\text{FR}) + 0.019(\text{TSC}_{\text{oreja}})$ [$R^2= 71 \%$]; $TR_{\text{blanco}}=35.29 + 0.035(\text{FR}) + 0.033(\text{TSC}_{\text{frente}})$ [$R^2 = 71 \%$]; y $TR_{\text{mixto}}=35.07 + 0.022(\text{FR}) + 0.038(\text{TSC}_{\text{cabeza}})$ [$R^2 = 44 \%$]. Se concluye que las vaquillas con color de pelaje oscuro mostraron constantes fisiológicas más altas que las vaquillas blancas, y que la predicción de la TR fue más precisa en vaquillas con color de pelaje bien definido (negras o blancas). La termografía infrarroja es una tecnología que puede ser utilizada como técnica no invasiva para predecir la TR en ganado lechero.

Palabras clave: Ganado Holstein, Temperatura corporal, Estrés calórico, Color del pelaje, Termografía infrarroja, Modelos de regresión.

Abstract

Rectal temperature (RT) is one of the physiological variables most used to screen changes in body temperature, monitoring health and the animal welfare. In practice, RT is recorded by introducing a thermometer in the rectum, procedure that requires the restraint of the animal, triggering stress that lead to alterations in the final output. Thus, infrared thermography (IRT) arises as a new technology to register RT in a non-invasive way. Therefore, this study proposes two objectives: 1) to predict RT in Holstein heifers using physiological and climatic variables, and 2) to estimate the effect of coat color on physiological variables of Holstein heifers, as well as to predict RT by coat color of those animals. Two hundred pubertal Holstein heifers (age 12-14 months; average body weight 297.0 ± 12 kg, and average body condition score 3.5 ± 1.1 points) were used during two consecutive summers (2019 and 2020) and were divided by coat color into black ($n_1=60$), white ($n_2=62$), and mixed ($n_3=78$, 50% black and 50% white). Respiratory frequency (RF) and body surface temperatures (BST) using TIR were collected considering the following body regions: shoulder, belly, rump, leg, neck, head, forehead, nose, loin, leg, vulva, eye and flank, lateral zone (right side). Climatic variables were ambient temperature, relative humidity, and the temperature-humidity index. Correlation analysis was used to determine the degree of association among variables and multiple linear regression analysis to construct the prediction equations of RT. To estimate the effect of coat color on physiological variables, an analysis of variance was used. There was a high and positive correlation ($r = 0.73$; $P < 0.01$) between RT and RF, while the BST had from low to moderate correlation ($0.15 < r < 0.55$) with RT and RF. Heifers with black coat color had more RF and RT ($P < 0.01$) than heifers with white and mixed coat color; although white heifers had similar RT and lower ($P < 0.05$) RF than mixed coat color heifers. In general, black and mixed coat color heifers showed higher ($P < 0.01$) BST than white heifers in the majority of the anatomical regions measured. The equation $RT = 35.55 + 0.033 (RF) + 0.030 (BST_{\text{forehead}})$ was considered the best regression model to predict RT based on the adjustment ($R^2 = 60\%$) and the lowest value in indicators of variability. The prediction equations according coat color were: $RT_{\text{black}} = 35.59 - 0.013(RH) + 0.045(RF) + 0.019(BST_{\text{ear}})$ [$R^2 = 71\%$]; $RT_{\text{white}} = 35.29 +$

$0.035(RF) + 0.033(BST_{\text{forhead}})$ [$R^2=71\%$]; and $RT_{\text{mixed}}=35.07 + 0.022(RF) + 0.038(BST_{\text{head}})$ [$R^2=44\%$]. It is concluded that black coat color heifers showed higher physiological constant than white coat color heifers, likewise the RT prediction was more precise in heifers with well-defined coat color (black or white). Infrared thermography can be used as a non-invasive technique to predict RT.

Keywords: Holstein cattle, Body temperature, Heat stress, Coat color, Infrared Thermography, Regression models.

CAPÍTULO I. INTRODUCCIÓN

En México, aproximadamente el 53% de su superficie pertenece a regiones áridas o semiáridas ubicadas en la zona centro y norte del país; se caracterizan por presentar escasa precipitación pluvial, vegetación con regular o bajo valor forrajero y temperaturas extremosas, siendo los veranos particularmente cálidos (González, 2017). Sin embargo, alrededor del 45% de la producción de leche a nivel nacional se produce en zonas áridas y semiáridas, lo cual representó 5'606, 846 L de leche en el año 2020 (SIAP, 2021). Esto significa que gran parte de la industria lechera está establecida bajo condiciones donde las altas temperaturas y una intensa radiación solar son imperantes durante gran parte del año. En este sentido, el ganado bovino productor de leche en estas zonas sufre de estrés calórico (EC) hasta por 6 meses cada año, condición en la cual es incapaz de mantener normotermia (38 – 38.5°C), por lo que debe gastar parte de su energía en regular su temperatura corporal en vez de dirigirla a producir leche de alta calidad (Correa-Calderón et al., 2022).

Por lo anterior, el monitoreo de parámetros fisiológicos como la temperatura corporal es un aspecto clave dentro del sector lechero en estas regiones, ya que es un indicador importante sobre salud y bienestar animal (Godyń et al., 2019). Este parámetro puede ser medido en distintas regiones del cuerpo del bovino como el recto, la oreja, la vagina (George et al., 2014), así como en el rumen (Boehmer et al., 2015). No obstante, la temperatura rectal (TR) es una de las variables fisiológicas más utilizadas para monitorear cambios en la temperatura corporal, ya que se relaciona de manera muy cercana con el estado de salud del animal o cualquier tipo de estrés que se presente en el mismo (Lees et al., 2018) al presentar una correlación alta con la temperatura corporal interna (Vickers et al., 2010).

La forma tradicional de medir la TR es introduciendo un termómetro por el recto, lo que resulta en una metodología invasiva que puede comprometer el bienestar animal (Godyń et al., 2019) debido al contacto con el equipo usado y/o la presencia de personal para la inmovilización del animal (Zhang, 2019). Además, esta técnica puede sobrestimar el valor real de la TR porque implica el sometimiento

del animal, produciendo un estrés extra que aumenta la producción de calor metabólico (Vicente-Pérez et al., 2016). Es necesario destacar que en los últimos años ha habido un creciente interés por el bienestar de los animales, ya que los consumidores prestan mayor atención a las condiciones de crianza de los mismos, especialmente cuando las prácticas de manejo utilizadas implican dolor y sufrimiento (Godyń et al., 2013). Por tanto, se recomienda usar métodos no invasivos que eviten el contacto físico con el animal, a fin de no afectar su bienestar.

En los últimos años, varios estudios han demostrado que la termografía infrarroja (TIR) puede ser una herramienta útil para evaluar el estrés sin comprometer el bienestar animal (Stewart et al., 2005). La TIR es una tecnología que puede ser útil para el análisis térmico no invasivo, capaz de analizar distintas funciones fisiológicas relacionadas con el control de temperatura de la piel. Actualmente, la cámara termográfica infrarroja se puede aplicar en estudios tanto de humanos como de animales, ya que es un dispositivo que permite tomar una imagen y después cuantificar su temperatura (Salles et al., 2016). Esta tecnología no invasiva se ha utilizado también para monitorear otros estados clínicos como fiebre, diarrea, cojera y mastitis en ganado bovino (Schaefer et al., 2012; Alsaad et al., 2015; Sathiyabarathi et al., 2016; Stewart et al., 2017), así como para detectar signos tempranos de enfermedad respiratoria bovina en ganado en crecimiento, o bien la eficiencia alimenticia en corrales de engorda (Montanholi et al., 2015; Schaefer et al., 2007). McManus et al. (2016) señalaron que las imágenes termográficas pueden indicar cambios en el flujo sanguíneo como resultado del aumento de la temperatura corporal relacionado con situaciones estresantes por las condiciones ambientales.

Sin embargo, algunos estudios reportan variaciones hasta de 1 °C en algunas partes de la superficie del cuerpo (Salles et al., 2016; Peng et al., 2019), lo que se debe a que algunas regiones corporales muestran temperaturas superiores a otras, como el caso del ojo y la ubre (Peng et al., 2019). Debido a la variación en la temperatura de la piel, es difícil señalar una región exacta que corresponda a la temperatura total del cuerpo. Por lo anterior, varios autores han propuesto

ecuaciones para predecir la temperatura rectal usando la TIR. Chung et al. (2010), Martello et al. (2010) y Niedermann et al. (2014) desarrollaron modelos predictivos de TR a partir de la temperatura corporal utilizando algunas regiones de la piel en cerdos, vacas lecheras y humanos, respectivamente.

En el área de Ciencia Animal, la mayoría de los estudios sobre predicción de TR en ganado lechero utilizando métodos no invasivos se han desarrollado en vacas lactantes (Daltro et al., 2017), por lo que no consideran otras etapas fisiológicas como el ganado joven. Asimismo, varios de estos estudios se realizaron bajo condiciones tropicales o subtropicales, careciendo de suficiente información en regiones áridas y semiáridas (Martello et al., 2010). Las vaquillas prepúberes constituyen el reemplazo de las vacas que se desechan en los hatos lecheros, representando así la futura producción de leche en el rebaño. Dado que las vaquillas generan menos calor metabólico que vacas lactantes, se consideran más tolerantes al EC (Satori et al., 2009). No obstante, la literatura científica ha demostrado que el EC impacta en las variables fisiológicas, variables productivas y reproductivas de terneros y novillas (Wang et al., 2020). Por lo tanto, es fundamental monitorear el estado térmico de las vaquillas en ambientes cálidos, donde las temperaturas extremas pueden comprometer la capacidad termorreguladora de los animales.

Otro aspecto importante a considerar es el hecho que los estudios mencionados previamente sobre predicción de TR en ganado lechero solamente desarrollaron ecuaciones generales, sin considerar las características físicas de la cobertura del pelaje. Estudios recientes señalan que el color del pelaje puede proporcionar información sobre cómo algunas razas de ganado tienen más probabilidad de adaptarse a un entorno particular, ya que esta característica está directamente influenciada por el ambiente (Anzures-Olvera et al., 2019). Isola et al. (2020) informaron que los animales de pelaje blanco absorben entre un 40 y 50 % menos radiación solar que los animales de pelaje negro. Asimismo, Hillman et al. (2013) expusieron vacas Holstein blancas y negras a la radiación solar directa durante 90 minutos en condiciones de calor, reportando que las vacas blancas absorbieron el 66 % y las negras el 89 % de la radiación solar. Por tanto, se

recomienda que los modelos de predicción desarrollados en el área de producción animal tomen en cuenta la ubicación geográfica, el estado fisiológico y, particularmente, el color del pelaje del ganado.

1.1. Hipótesis

- 1) Las vaquillas Holstein que predominantemente tienen un color de pelaje blanco presentan mejor capacidad de termorregulación fisiológica que las vaquillas de pelo predominantemente negro, o bien que aquellas que no tienen un color dominante bajo condiciones climáticas áridas.
- 2) La temperatura rectal de vaquillas Holstein puede predecirse a partir de ecuaciones desarrolladas con variables predictoras climáticas, así como fisiológicas obtenidas por métodos no invasivos tales como termografía corporal y frecuencia respiratoria; además, la precisión y exactitud de predicción de esas ecuaciones puede ser mejorada considerando el color del pelaje.

1.2. Objetivos

- 1) Determinar el efecto del color del pelaje en variables fisiológicas de vaquillas Holstein bajo condiciones naturales de una zona árida.
- 2) Desarrollar modelos de regresión lineal múltiple para predecir la temperatura rectal en vaquillas Holstein utilizando variables fisiológicas y climáticas y por color del pelaje en una zona árida.

CAPÍTULO II. REVISIÓN DE LITERATURA

2.1. Ganado lechero en climas cálidos

Las zonas áridas y semiáridas se caracterizan por poseer condiciones climáticas desfavorables para la producción animal, particularmente para el ganado lechero (Ammer et al., 2017). Aunado a esto, las proyecciones sobre el clima global indican que estas condiciones se intensificarán debido a variaciones en diferentes variables atmosféricas, lo que conducirá a un aumento en la temperatura de la superficie del planeta de 1.5°C entre 2030 y 2052 (IPCC, 2018). De acuerdo con estos escenarios climáticos, durante el siglo XXI se prevé que la proporción de regiones cálidas y secas aumenten debido a una excesiva concentración atmosférica de gases de efecto invernadero (GEI) y el consecuente calentamiento global (Al-Ghussain, 2019). Otro estudio sobre condiciones ambientales en regiones áridas muestra una tendencia a exceder el límite de confort para la mayoría de los especies domésticas, provocando la presencia de EC por periodos más prolongados (Theusme et al., 2021). Así, se han cuantificado pérdidas económicas entre 897 a 1,500 millones de dólares para la industria lechera asociadas al problema del EC (St-Pierre et al., 2001).

En ganado lechero, cuando la temperatura del ambiente (TA) y la humedad relativa (HR) rebasan de la zona termoneutral (5 y 25 °C; Bernabucci et al., 2010), la eficiencia productiva y reproductiva están comprometidas porque los nutrientes se desvían hacia el mantenimiento de la eutermia, ya que preservar la normotermia se convierte en la máxima prioridad, restando importancia al proceso de formación de carne y leche (Baugmand et al., 2013). De esta manera, la producción de leche y síntesis de grasa en la misma se reducen por un efecto directo de las altas temperaturas sobre la actividad sintética y secretora de la glándula mamaria en las vacas (Silanikove, 1992). Se estima una disminución en la producción de leche del 15%, además de una caída del 35% en la eficiencia energética con fines productivos en vacas Holstein lactantes cuando la temperatura del aire oscila entre 18 y 35°C (McDowell et al., 1976). También se han documentado efectos negativos del EC

sobre la calidad de la leche, reportándose una disminución en el contenido de grasa y proteína de 39.7 y 16.9 %, respectivamente. En general, Johnson (1976) atribuyó de 3 al 10 % de la varianza en la producción de leche a factores climáticos.

Las variables TA y HR son las más importantes para cuantificar el EC, y ambas se combinan para estimar el índice de temperatura-humedad (ITH), con lo cual se puede determinar el grado de EC que experimenta el ganado lechero en producción (Herbut et al., 2018). Así, el EC en vacas lactantes empieza a un ITH de 72 unidades, y se calcula una reducción en la producción de leche entre 0.20 y 0.32 kg por unidad de incremento en el ITH (Ravagnolo et al., 2000). Smith et al. (2013) reportaron que cuando el ITH sobrepasó de 72 unidades, las vacas Holstein experimentaron una reducción en la producción de leche desde 35.6 a 34.2 kg/d (-3.9%). Cabe mencionar que diversos estudios de las últimas dos décadas señalan que el ganado de leche actual es más sensible al aumento de la TA por la alta presión de selección para la variable de producción de leche, comenzando a experimentar una reducción en la síntesis láctea alrededor de un ITH de 68 unidades (Zimbelman et al., 2009). Las vacas lecheras de alta producción generan más calor metabólico que las vacas lecheras de baja producción, por lo tanto, se vuelven más sensibles al EC (Berman, 2005). De esta manera, la elevada producción de calor metabólico que implica la producción láctea en combinación con el EC conducen a una disminución en la producción de leche (Berman, 2005; Kadzere et al., 2002).

Cabe mencionar que el ganado lechero con la producción más alta es *Bos taurus* (Holstein), seguido de otras razas de origen similar, por lo que también son afectadas por esta variabilidad climática. Por ejemplo, la raza Holstein se desarrolló en Europa, específicamente en Países Bajos, posteriormente se dispersó a regiones muy diferentes de su origen; áridas, semiáridas y subtropicales (Avendaño-Reyes, 2014). Por lo que, para sobrevivir en condiciones ambientales extremas, las vacas Holstein tratan de modificar su conducta y fisiología para soportar situaciones de calor intenso. En particular los animales expuestos a altas temperaturas y radiación solar aumentan su temperatura corporal, tratando de que el exceso de

calor corporal pueda ser eliminado. Esto causa respuestas fisiológicas intensas como aumento en la frecuencia respiratoria, en la frecuencia cardíaca, en la sudoración y en la temperatura corporal general (Habebe et al., 2020; Wijffels et al., 2020).

2.2. Respuestas fisiológicas en ganado lechero en zonas áridas

La capacidad de un animal para adaptarse a un medio contribuye a su capacidad de tolerancia al clima adverso y su variabilidad. Dentro de la evaluación de la fisiología del estrés en ganado lechero, la frecuencia respiratoria (FR) y la temperatura corporal (TC) se consideran los parámetros fisiológicos más importantes, aunque no son los únicos (Marai et al. 2007; Sejian et al., 2018).

2.2.1. Frecuencia respiratoria

El proceso de respiración consiste en la entrada de oxígeno (O_2) y la eliminación de dióxido de carbono (CO_2), que en condiciones del EC su frecuencia conduce a la evaporación y disipación del calor corporal para así mantener el equilibrio térmico (da Silva et al., 2017). Shaji et al. (2016) mencionan que la FR en bovinos se detecta fácilmente observando los movimientos de los flancos y contando el número de respiraciones registradas por unidad de tiempo, generalmente en respiraciones por minuto (rpm). La FR es utilizada como una medida de regulación de la TC en ganado lechero, ya que es un indicador fisiológico de la disipación del exceso de calor en condición de hipertermia (Sailo et al., 2017). En condiciones de EC el animal tiende a aumentar la FR y la sudoración con el objeto de transferir el exceso de calor corporal hacia el ambiente mediante el mecanismo físico conocido como evaporación (Polsky et al., 2017). Una FR alta indica mayor sensibilidad a altas temperaturas ambientales, demostrando una condición no adaptativa y respuestas severas al EC en bovinos. Sin embargo, se debe recordar que el ganado raza Holstein tiene mayor sensibilidad en comparación con las demás razas debido su alta productividad. Romanello et al. (2018) en condiciones tropicales reportaron que la FR fue de 40.8 rpm en *Bos taurus* y de 38.2

rpm en *Bos indicus*. Otros autores han reportado que la termorregulación del ganado *Bos taurus* puede ser afectada cuando se encuentra en condiciones de clima tropical, ya que la FR supera 80 rpm, especialmente en las horas más calurosas del día (Baena et al., 2019). Los animales *Bos indicus* son más tolerantes a altas temperaturas y el promedio de FR puede variar de 33 a 41 rpm, según la temperatura del aire y la hora del día (Cardoso et al., 2015). En regiones donde las estaciones del año están bien definidas, como es el caso de las latitudes medias, la FR puede variar dependiendo de la severidad de la temperatura, humedad relativa, velocidad del viento y la radiación solar (Sailo et al., 2017).

En general se considera un rango normal de FR de 20 a 60 rpm, por lo que un valor mayor a 60 rpm indica que los animales están tratando de mantener su homeostasis disipando la carga de calor corporal ganada (Nienaber et al., 2007). Se ha observado que con el aumento del ITH, la FR también incrementa, siendo evidente a partir de un valor de ITH de 73 unidades (Schüller et al., 2014). Valente et al. (2015) reportaron que la FR fue significativamente más alta cuando la TA superó 37.7 °C. Igualmente, Indu et al. (2015) encontraron que la FR superó 80 rpm en animales en condiciones de EC. Se ha determinado que un incremento en la FR requiere de 7 a 25 % de gasto extra de energía debido al movimiento del músculo tracto respiratorio, ya que el proceso de vaporización de agua a través de la piel y del tracto respiratorio demanda un gasto de energía de 2.43 J/mL de agua evaporada (Kadzere et al., 2002). De hecho, se ha documentado que la FR está asociada a un 18% de incremento en el calor metabólico (Mader et al., 2006). También se ha observado una correlación negativa de la producción de leche con FR, ya que un aumento en FR puede ser un indicador de la pérdida de leche en vacas lecheras lactantes que padecen EC. Según Kumar et al. (2016), la disipación de la carga de calor adicional mediante un mecanismo de enfriamiento respiratorio vaporiza más humedad al medio ambiente, lo cual involucra gastos energéticos.

Por otro lado, en vacas lactantes con EC, la FR y el jadeo conducen a una mayor pérdida de CO₂ a través de ventilación pulmonar, así como a una reducción en la concentración de ácido carbónico en sangre, lo que modifica su proporción,

es decir, el bicarbonato necesario para mantener el pH de la sangre, resultando eventualmente en alcalosis respiratoria (Wang et al., 2020). En zonas cálidas, el flujo sanguíneo se redistribuye a los periféricos y tejidos, lo que asegura que el calor corporal sea transportado desde los órganos a la superficie del cuerpo (Hooda et al., 2015). No obstante que la FR es utilizada como medida termorregulatoria confiable en condición de hipertermia, la TC es, dentro de los parámetros fisiológicos, el indicador clave de la salud y el bienestar de los animales (Li et al., 2020).

2.2.2. Temperatura corporal

Los rumiantes son animales homeotermos, por lo que son capaces de mantener su TC en un rango estrecho a pesar de las variaciones de la TA mediante procesos de termorregulación (Godyn et al., 2019). La TC se encuentra bajo control neuronal, y las neuronas sensibles a la temperatura (caliente y fría) se localizan en la región preóptica del hipotálamo anterior y se consideran un termostato con un punto de ajuste (Fecteau y White, 2014). No obstante, los sensores de temperatura también se encuentran en la piel y en los tejidos profundos del cuerpo como el tórax, alrededor de las grandes venas del abdomen y en las vísceras abdominales (Robinson, 2002). Los mecanismos de termorregulación empiezan a funcionar cuando las sensaciones térmicas aferentes son percibidas por estos termorreceptores, los cuales transfieren la información en la región hipotalámica y dan respuesta a estímulos externos como el cambio de temperatura (Taylor, 2014). La regulación de la TC es uno de los mecanismos más importantes para mantener la homeostasis en todos los organismos vivos, y se considera un indicador fisiológico fundamental para reflejar el estado de bienestar de los animales (Sejian et al., 2018).

La TC refleja la acumulación de calor en el cuerpo del animal, pudiendo ser las fuentes de calor la fiebre o la hipertermia generada por la ganancia de calor exógena o endógena (Collier et al., 2017). La fiebre es una reacción compleja a los pirógenos que no solo hacen que aumente el punto de ajuste termorregulador del cuerpo, sino también estimulan una reacción de fase aguda y activa en numerosos

sistemas y procesos metabólicos, endocrinológicos e inmunológicos (Wrotek et al., 2011; Fecteau y White, 2014). Por su parte, la hipertermia representa una falla en la termorregulación (producción de calor descontrolada, mala disipación de calor o una carga de calor externa), no implicando un punto de ajuste termorregulador (Wrotek et al., 2011; Fecteau y White, 2014), por lo que los productos microbianos y las citoquinas pirogénicas no están directamente involucradas (Wrotek et al., 2011). También el funcionamiento normal de órganos como el cerebro, el corazón y algunas vísceras (hígado y riñones) generan la mayor parte del calor corporal (alrededor del 60%), por lo tanto, ellos son importantes para definir la temperatura central del cuerpo (Sellier et al., 2014).

En general, el proceso de termorregulación consiste en generar un complejo equilibrio entre la producción de calor, especialmente por parte de los músculos, y el proceso de dispersión y eliminación del mismo alojado en el cuerpo (Sellier et al., 2014). Como consecuencia de la complejidad de este sistema termorregulador, se genera un cambio en la TC que puede estar asociado con enfermedades, lesiones, EC, exposición a toxinas u otros problemas relacionados con la salud y las condiciones ambientales extremas (Koltjes et al., 2018).

Una respuesta inminente a la presencia de EC en ganado lechero es el aumento en la TC debido a una acumulación del calor ambiental que entro al cuerpo (Collier et al., 2017). Dependiendo del tipo (agudo o crónico) y grado de EC (ligero, moderado o severo), será la secuencia de activación de los mecanismos de termorregulación fisiológicos (Correa et al., 2022). Conforme el EC es más severo y prolongado, las pérdidas de calor corporal por medios no evaporativos disminuyen, mientras que las pérdidas evaporativas (sudor y jadeo) incrementan (Correa et al., 2022). Maia et al. (2005) observaron 85 % de pérdidas de calor corporal debido a la evaporación cutánea, y solamente 15 % a través del tracto respiratorio en vacas Holstein expuestas a TA >30 °C; en ese estudio se activaron las pérdidas de calor corporal, tanto por medios no evaporativos como evaporativos. Todos los animales tienen una zona de confort térmico, rango de TA que es conveniente para sus funciones fisiológicas (Bernabucci et al., 2014). En ganado

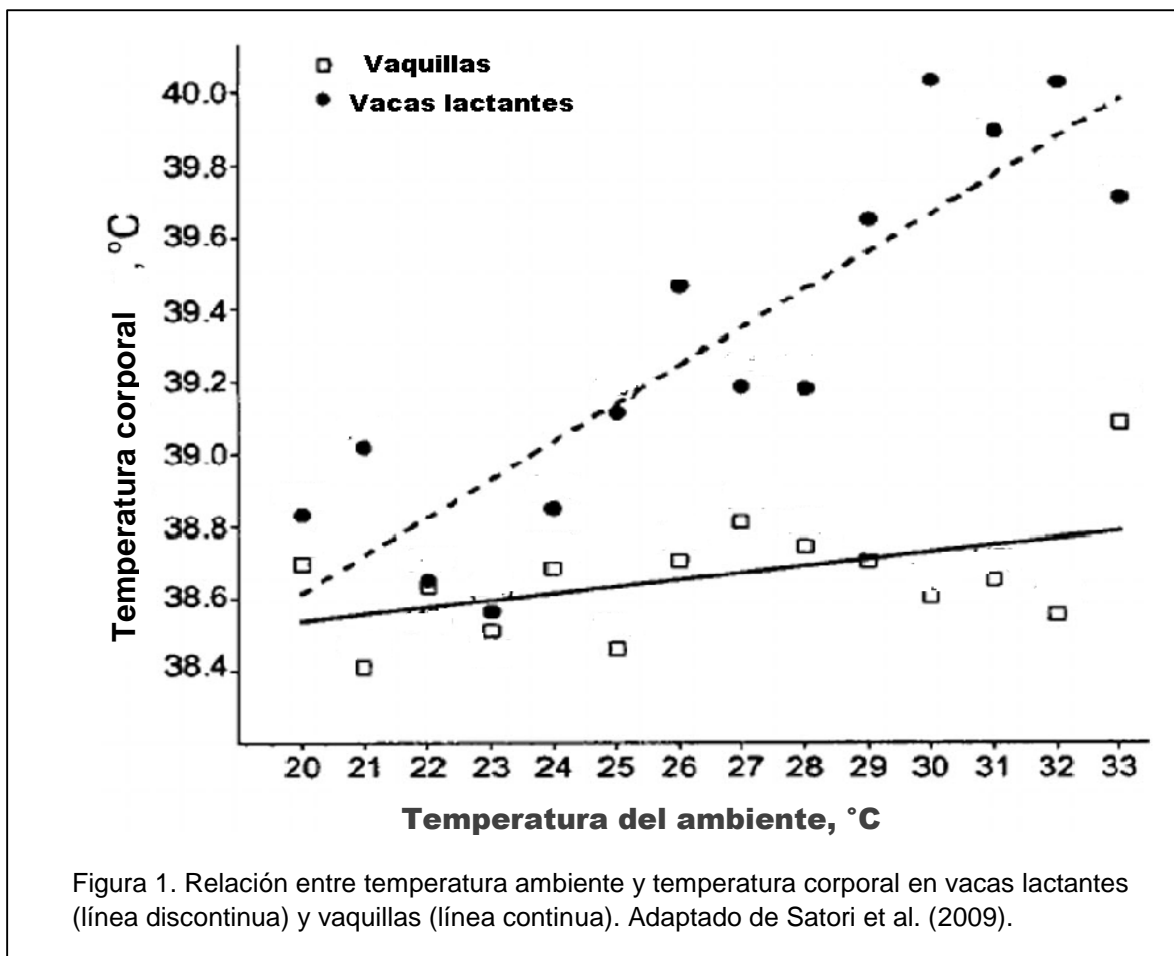
lechero, el nivel óptimo de TA oscila entre 13 y 20 °C, siendo su zona de confort entre 4 y 24 °C (Herbut et al., 2018). Durante el día, el ganado mantiene una temperatura corporal dentro de un rango de $\pm 0.5^{\circ}\text{C}$ (Henry et al., 2012). Cuando la temperatura aumenta más allá de la temperatura crítica superior del rango (varía según el tipo de especie), los animales comienzan a sufrir EC (Herbut et al., 2018). Algunos estudios reportan un incremento en la TC de 0.4 °C en ganado lechero cuando está expuesto a una TA elevada en comparación con vacas en condiciones termoneutrales (Fabris et al., 2020).

Diversos investigadores han reportado que las condiciones ambientales como TA, HR, velocidad del viento y radiación solar tienen fuerte impacto en la TC (Lowe et al., 2001; Faurie et al., 2001; Berry et al., 2003; Davis et al., 2003; Green et al., 2005). Otros factores fisiológicos, ambientales o genéticos tales como presencia de celo, estado de preñez, raza y características fenotípicas, también influyen en la variabilidad de la TC (Lefcourt et al., 1999). La medición continua de la TC pueden ser una herramienta interesante y eficiente para monitorear el EC (Kendal et al., 2009; Vickers et al., 2010; Collier et al., 2017), o realizar mejoramiento genético a través de la selección de animales termotolerantes a condiciones ambientales extremas (Dikmen et al., 2009). Además, la TC puede ser útil para establecer programas reproductivos y de bienestar apropiados en ganado lechero que impliquen la implementación de estrategias de mitigación de los efectos negativos de las condiciones ambientales (Kaufman et al., 2018). Existen evidencias que indican que la TC de las vacas lactantes parece estar más influenciada por niveles elevados de TA que en las vaquillas (Satori et al., 2009), como se muestra en la Figura 1.

2.2.3. Respuestas fisiológicas de vaquillas en ambiente cálidos

La TC de las vaquillas comparada con la de vacas lactando está menos influenciada por los cambios en la TA, siendo más notoria esta diferencia conforme los valores promedio de esta variable climática aumentan (Figura 1; Satori et al., 2009). En este sentido, se considera a las vaquillas más resistentes al EC debido a

una menor producción de calor metabólico y una mayor eficiencia de disipación de calor, no obstante, sí son afectadas negativamente a nivel productivo bajo este escenario climático (Wang et al., 2020). Las evidencias muestran que el EC tienen impacto sobre variables fisiológicas, eficiencia alimenticia y motilidad ruminal (Wang et al., 2020).



Igual que las vacas, las vaquillas en condiciones de EC entran en proceso de termorregulación a través de una serie de cambios fisiológicos. Se ha observado un aumento en la FR de 23 a 58 rpm en vaquillas cuando la TA aumentó de 28 a 33°C (Nonaka et al., 2008). También se reportó un aumento de 1.2°C en la TC por encima

de lo normal en las vaquillas cuando se tuvo una TA de 33°C y con HR superior a 80 % (Purwanto et al., 1993; Nonaka et al., 2012). Otros estudios demostraron que el consumo en las vaquillas empieza a disminuir cuando la TA alcanza 38°C (Colditz y Kellaway, 1972). Igualmente, Baccari et al. (1983) reportaron menor consumo de alimento y eficiencia alimenticia de vaquillas Holstein en condiciones de EC (TA; de 32.5 a 34 °C) comparado con condiciones termoneutrales (TA; 18 a 20 °C). Asimismo, Nonaka et al. (2012) encontraron que el consumo de la materia seca en novillas Holstein pre-púberes se redujo en 9 % a una TA de 33 °C. En comparación con vacas lactantes, algunos autores señalan que el inicio del EC en vaquillas ocurre a un ITH de 75 unidades (Silanikove, 2000), 8 unidades más arriba que en las lactantes. De hecho, la TC de las vaquillas superan los 39°C hasta que el ITH excede de 83 unidades (Correa et al., 2014), y reducen el consumo de alimento cuando el ITH rebasa las 75 unidades (Broucek et al., 2009).

Cabe destacar que la respuesta de los parámetros fisiológicos debido a la hipertermia da lugar alteraciones hormonales y metabólicas (Al-Dawood, 2017). En terneros y vaquillas estresadas por calor, se observa un aumento en insulina y una disminución en las hormonas tiroideas (T_3 y T_4), las cuales al estar correlacionadas positivamente con el aumento de peso y desarrollo de tejido, el crecimiento de las hembras puede ser comprometido (Bernabucci et al., 2010). Debido al ajuste fisiológico para mantener el equilibrio térmico, las vaquillas también tienden a reducir la motilidad ruminal para disminuir el calor metabólico, por lo tanto, reducen la tasa de pasaje de la ingesta y, en consecuencia, el consumo de alimento (Schneider et al., 1986). Nonaka et al. (2008) reportaron que las vaquillas prepúberes sujetas a 33 °C tuvieron 56 % menos tasa de pasaje ruminal en comparación con las vaquillas sujetas a 20 °C. Asimismo, algunas investigaciones informaron que vaquillas bajo TA mayor a 30 °C disminuyeron la cantidad de ácidos grasos volátiles (AGV; Tajima et al., 2007). Estos cambios ruminales pueden contribuir a reducir la tasa de crecimiento al ser los AGV fuente de energía primaria (Wheelock et al., 2010).

El desbalance térmico en vaquillas compromete su actividad reproductiva al activarse el eje adrenal, el cual inactiva el eje reproductivo causando una

disminución la producción de hormonas como hormona luteinizante, estradiol y gonadotropinas (Kim et al., 2019). Esto a su vez se refleja en alteraciones en la ciclicidad normal del estro, deprime el desarrollo folicular y embrionario, lo que finalmente conduce a una baja tasa de concepción (Kim et al., 2019). Las vaquillas puberales representan el reemplazo en los hatos lecheros, y por ende el futuro de la producción de leche en el hato (Donovan et al., 2003). Por lo anterior, es fundamental evaluar la salud y el estado térmico de las vaquillas en zonas áridas, que se caracterizan por condiciones climáticas extremas, con temperaturas ambientales muy altas en verano que comprometen la capacidad termorreguladora de los animales (Wang et al., 2020). Además de las condiciones ambientales que comprometen la capacidad termorregulatoria en las vaquillas, existen otros factores como las características fenotípicas, particularmente el color de pelaje. Se ha documentado que la TC es más alta en vacas con pelaje mayormente negro comparado con el pelaje mayormente blanco (Anzures-Olvera et al., 2019). Sin embargo, datos científicos que abordan el impacto del EC sobre respuestas fisiológicas en vaquillas en relación con el color de pelaje en zonas áridas son escasos.

2.2.4. Respuestas fisiológicas por color del pelaje

El color del pelaje en el ganado bovino, como en otros mamíferos, se determina en gran parte por genes que controlan la síntesis de melanina (Fernández et al., 2004), principalmente por la feomelanina que es responsable de la pigmentación rojo y amarillo, así como la eumelanina que es responsable de la pigmentación negra a través de los melanocitos ubicados en el pelo, piel y mucosas (Dikmen et al., 2017). Las mutaciones en el gen receptor de melanocortina 1 (MCR1) son las principales responsables de la variación en el color de pelaje y contiene alelos existentes en múltiples razas, entre los que se tienen: MC1RD (negro; dominante), MC1RBR (tipo salvaje; negro rojizo) y MC1Re (rojo; recesivo) (Switonski et al., 2013). Las diferentes zonas del cuerpo del animal que no cuentan con melanocitos exhiben ausencia de coloración y, por lo tanto, la presencia de manchas blancas (Fernández et al., 2004). La raza Holstein generalmente tiene dos

fenotipos, blanco y negro (negro sobre un cuerpo blanco) y rojo y blanco (marrón rojizo sobre un cuerpo blanco) (Isola et al., 2020).

El color del pelaje puede tener un impacto importante en el balance de la TC (Hansen et al., 1990). El pelo más claro es capaz de reflejar mayor cantidad de radiación solar, lo que a su vez permite que se transmita menos calor al animal (Cena y Monteith, 1975; Maia et al., 2005). Stewart (1953) encontró valores de absorción de alrededor del 40 % para las manchas blancas y de 90 % para las manchas negras en ganado Holstein. Se ha estimado que los animales de pelaje blanco absorben entre 40 y 50 % menos radiación solar que los animales de pelaje negro (Brown-Brandl et al., 2016). Así, el color del pelaje es una característica que puede tener una función importante en la habilidad del bovino para resistir los efectos adversos del EC asociado con alta incidencia de radiación solar directa (Hansen, 1990; Stewart, 1953). Hillman et al. (2013) expusieron vacas Holstein blancas y negras a la radiación solar directa durante 90 minutos en condiciones de calor, reportando que las vacas blancas aumentaron la TR a razón de 0.8 °C/h, mientras que las vacas negras a razón de 1.3 °C/h. Además, la temperatura de la superficie de las vacas negras aumentó en promedio 4.8 °C, y las vacas blancas solo 0.7°C. Estos resultados se atribuyeron a que las vacas blancas absorbieron el 66% de la radiación solar y las negras el 89%.

La relación entre el color del pelaje y los parámetros productivos en el ganado lechero comenzó a documentarse a principios de la década de los 90's (Becerril et al., 1993). Posteriormente, hubo mayor interés en clasificar a las vacas según el color de su pelaje como un rasgo potencial para la adaptación a climas cálidos (King et al., 1988). Según estudios más recientes, el color del pelaje puede proporcionar información sobre cómo algunas razas de ganado tienen más probabilidad de adaptarse a un entorno particular, ya que esta característica está directamente influenciada por el ambiente (Anzures-Olvera et al., 2019). Estudios en raza Holstein señalan una mayor producción de leche en vacas predominantemente blancas en comparación con vacas predominantemente negras (Anzures-Olvera et al., 2019; Maia et al., 2005). Valle (1988) concluyó que un menor porcentaje de color negro

mejoró el balance termorregulador y el comportamiento productivo y reproductivo, con 10% más de producción de leche en vacas con menos de 50% de color negro. Becerril et al. (1994) indicaron que el porcentaje de color blanco del pelaje tuvo una asociación lineal con la producción de leche, con una respuesta de 2.75 kg de leche por cada incremento de unidad porcentual de color blanco en vacas Holstein de primera lactancia. Los autores concluyeron que el pelaje de los animales juega un papel fundamental en la transferencia de calor y humedad de la superficie de la piel al entorno circundante y el control de la TC.

2.3. Métodos de monitoreo de la temperatura corporal

El monitoreo de la TC en el ganado lechero se ha convertido en un objetivo importante, tanto para científicos como para productores (Dias-Batista et al., 2021). Por lo anterior, se han desarrollado varias técnicas de medición de la TC en los últimos años, entre las que se incluyen aquellas medidas tanto por métodos invasivos (interna) como no invasivos (externa).

2.3.1. Métodos invasivos

La temperatura interna del cuerpo es similar a la temperatura de los órganos principales como el cerebro, corazón y vísceras (Sellier et al., 2014). Para obtener la temperatura interna del cuerpo generalmente las mediciones involucran procesos invasivos como alguna técnica quirúrgica u otros métodos que involucran algún tipo de contacto con el animal, situación que puede afectar el bienestar animal. Bitman et al. (1984) utilizaron métodos de monitoreo automáticos a través de termistores digitales, mientras que otros autores utilizaron transmisores con unidades de radiotelemetría (Lefcourt y Adams, 1998; Aizahal et al., 2009); estos métodos consisten en la implantación quirúrgica de estos dispositivos en diferentes regiones anatómicas (es decir, subcutáneamente; flanco izquierdo, cavidad peritoneal y ubre). No obstante, el canal auditivo, rumen, vagina y recto son los lugares más comunes para medir la TC en ganado bovino (George et al., 2014; Boehmer et al., 2015; Godyń et al., 2019).

La temperatura timpánica (TT) mide la radiación de calor del canal auditivo y también se utiliza como un equivalente confiable de la temperatura interna, ya que la sangre que fluye a través de los vasos de la membrana timpánica proviene de la rama de la arteria carótida que también irriga al hipotálamo (Chue et al., 2012). Por lo tanto, la TT puede servir como un referente confiable de la TC y del bienestar térmico de un animal. Bergen y Kennedy (2000) monitorearon simultáneamente la temperatura vaginal (TV) y la TT colocando termistores en ambas regiones anatómicas en ganado bovino productor de carne, encontrando que las mediciones se correlacionaron de manera importante ($r= 0.77$), y que la TV fue ligeramente mayor que la TT (38.8 vs 38.6 °C); asimismo, Mader et al. (2010) demostraron que la TT es elevada en animales expuesto a TA altas durante horas de la noche. Además, Ybañez et al. (2015) reportaron también una correlación moderada ($r=0.67$) entre la TT y TR en bovinos.

Otro método invasivo lo constituyen los bolos inteligentes (Liang et al., 2013), bolos transmisores de temperatura (Boehmer et al., 2015) o bolos ruminales (Ipema et al., 2008); estos bolos se introducen oralmente y tienen sensores que al llegar al retículo o rumen permiten un monitoreo automático (Adams et al., 2013; Liang et al., 2013; Prendiville et al., 2002). Los bolos sirven para medir de manera constante la TC en ganado bovino (Prendiville et al., 2002) y se introducen vía oral en los animales, son fáciles de usar y requieren mínima mano de obra (Adams et al., 2013). Se han realizado varios estudios para medir la TC utilizando esta tecnología invasiva, uno de ellos es el conducido por Boehmer et al. (2015), quienes encontraron una correlación alta ($r=0.63$) entre temperatura del rumen y la TR en vacas de raza Angus. Similarmente, Bewley et al. (2008) reportaron una correlación de 0.64 entre la temperatura reticular y la TR en ganado de carne; pero Ammer et al. (2016) obtuvieron solamente una correlación moderada ($r= 0.41$) entre temperatura reticular y rectal en ganado lechero Holstein. Cabe mencionar que algunos autores señalan que la determinación de la TC a partir de la temperatura ruminal medida por bolos resulta una medición no muy precisa, ya que puede verse alterada por la fermentación y por el consumo de agua y/o alimento (Iwasaki et al., 2019). Bewley et al. (2008) determinaron que el consumo de agua fría sostenido y

en grandes cantidades puede generar una disminución de 9.2 °C en la temperatura ruminal. Además, la posición del dispositivo (horizontal o vertical) y la distancia del animal al centro de control pueden generar pérdidas en la transmisión de la señal, generando datos erróneos (Ipema et al., 2008).

La TC del ganado también ha sido monitoreada insertando microprocesadores vaginalmente (Burdick et al., 2011; Burfeind et al., 2011; Kendall et al., 2006; Dikmen et al., 2008; Kendall et al., 2009). Andrade et al. (2017) y Kaufman et al. (2018) observaron que la TV está fuertemente correlacionada con la TR ($r= 0.74$), aunque la TV es 0.6 °C más elevada; sin embargo, otros estudios realizados en ganado lechero han reportado una asociación más fuerte ($0.81 \leq r \leq 0.97$) entre TR y TV (Burfeind et al., 2011; Burdick et al., 2012; Vickers et al., 2010). Diversos estudios señalan que la TV puede ser un mejor indicador de cambios en la temperatura central del cuerpo en comparación con la rectal, ya que su gran vascularización permite un mayor flujo sanguíneo (Vickers et al., 2010; Sellier et al., 2014; Burdick et al., 2012). Esto es importante porque la temperatura del tracto reproductivo tiene implicaciones directas en los parámetros reproductivos, ya que un aumento de sólo 0.5 °C en la temperatura uterina puede disminuir la tasa de preñez entre 6.9 y 12.8 % (Collier et al., 2006). En este sentido, un monitoreo continuo de la TV puede ser muy útil para establecer programas reproductivos. La TV también ha servido para identificar problemas reproductivos (Polsky et al., 2017) y detección de estro en ganado (Sakatani et al., 2016). Debido a la capacidad de recopilar lecturas de TV en serie con el tiempo utilizando registros de datos permanentes, este parámetro puede prestarse a un mayor uso en la evaluación de la investigación sobre EC en el ganado. Sin embargo, la principal limitación de la TV es que sólo puede ser utilizada en hembras (Reuter et al., 2010).

En general, la inserción de un termómetro en el recto es el método más utilizado para evaluar la TC y ha servido como un indicador fisiológico, de salud y de bienestar tanto en humanos como animales (Godyń et al., 2019). Pero también es un indicador de parámetros productivos y reproductivos, ya que se ha documentado que la TR se correlaciona negativamente ($r= -0.59$) con el consumo

de materia seca (De Andrade et al., 2017) y producción de leche, llegándose a reducir hasta en 10 % por un incremento de 0.5 °C en la TR. El aumento de la TR por encima de 39 °C también disminuye linealmente la tasa de concepción en ganado Holstein (Polsky et al., 2019). Adicionalmente, la TR ha presentado un valor de heredabilidad en ganado lechero entre bajo y medio cuyas estimaciones oscilan entre 0.11 y 0.17 (Luo et al., 2021). Se ha reportado que la TR óptima en bovinos es entre 38.0 y 39.2 °C (Ammer et al., 2016). Sin embargo, una variación de $\pm 1^{\circ}\text{C}$ en la TR es suficiente para reducir la productividad en el ganado (Kadzere et al., 2002; McManus et al., 2009). Normalmente las vacas lecheras tienden a aumentar su TR cuando se presenta una TA que excede la parte superior de su zona termoneutral ($>25^{\circ}\text{C}$); se ha observado que vaquillas lecheras aumentaron su TR 1.2 °C por encima de lo normal cuando se presentó una TA de 33 °C con valores de HR superiores a 80% (Purwanto et al., 1993; Nonaka et al., 2012). La TR se incrementó en 0.95 °C cuando el ITH incrementó de 70 a 88 unidades. Otros estudios han reportado una TR por encima de 39° C en vaquillas en EC cuando el ITH supera 83 unidades (Correa et al., 2014). La TR es el método más común para estimar la TC en explotaciones pecuarias, pero el uso de un termómetro manual es un método invasivo y estresante para el animal, por lo que se considera que los resultados de esas mediciones pueden ser sesgadas, ya que implica en muchas ocasiones conducir al ganado de los corrales hacia la zona de enfermería y sujetarlos para llevar a cabo la medición (Hoffman et al., 2012).

2.3.2. Métodos no invasivos

La reciente preocupación por el bienestar animal ha alentado a los investigadores a optar por metodologías no invasivas que conduzcan a reducir el estrés causado al animal durante el proceso de experimentación. Por lo que a continuación se detallan uno de estos métodos.

2.4. Termografía infrarroja

Se ha demostrado que la tecnología conocida como termografía infrarroja (TIR) puede ser una herramienta útil para evaluar la TC sin comprometer el bienestar animal (Stewart et al., 2005). Hoy en día, la cámara infrarroja se puede aplicar en estudios tanto de humanos como de animales, ya que es un dispositivo que permite captar una imagen y después cuantificar en ella su temperatura (Knížková et al., 2007). La termografía detecta la luz infrarroja emitida por el cuerpo y muestra los cambios relacionados con la TC con respecto a la variación del flujo sanguíneo (Stewart et al., 2010).

La luz infrarroja fue descubierta en 1800 por el astrónomo William Herschel después de realizar varios experimentos que llevaron al descubrimiento de la radiación térmica, más tarde llamada luz o radiación infrarroja (Ricca, 2013). Todo objeto en el planeta genera calor en la parte infrarroja del espectro de luz, la intensidad y la distribución del espectro de la que dependen la temperatura de la masa y de las propiedades de radiación de su capa superficial. El uso del escaneo termográfico (cámara termográfica) es capaz de detectar el grado de radiación, incluso pequeños cambios de temperatura que pueden ser monitoreado con precisión (McManus et al., 2016). Los datos obtenidos por el escaneo son procesados por software específicos los cuales muestran termogramas que proporcionan un detallado análisis del campo de temperatura (Zeng et al., 2020). El hecho de que la radiación es una función de la temperatura de la superficie del objeto hace posible que la cámara calcule y muestre esta temperatura (Poikalainen et al., 2012). Sin embargo, la radiación medida por la cámara no sólo depende de la temperatura del objeto, ya que también es una función de la emisividad de la radiación que se origina en los alrededores y es reflejada por el objeto (Usamentiaga et al., 2104).

La mayor parte de la energía radiante se emite con una longitud de onda entre 7 y 14 μm , y se denomina radiación infrarroja. El principio de la TIR es que todo objeto o cuerpo con una temperatura superior a 0 $^{\circ}\text{K}$, equivalente a 273.15 $^{\circ}\text{C}$, emite radiación térmica al ambiente en todas las longitudes de onda del espectro

infrarrojo (Poikalainen et al., 2012). La radiación es capturada y registrada por una cámara especial, la cual crea una imagen térmica en color, y cada color corresponde a una temperatura. La imagen termográfica presenta una escala de colores que varía de frío (verde y azul) hasta caliente (amarillo, naranja, rojo y blanco; Castells et al., 2019), como se observa en la Figura 2, observándose las temperaturas que emiten los cuerpos superiores 0 absoluto (i.e., temperatura $> 0^{\circ}\text{K}$) formando un espectro electromagnético.

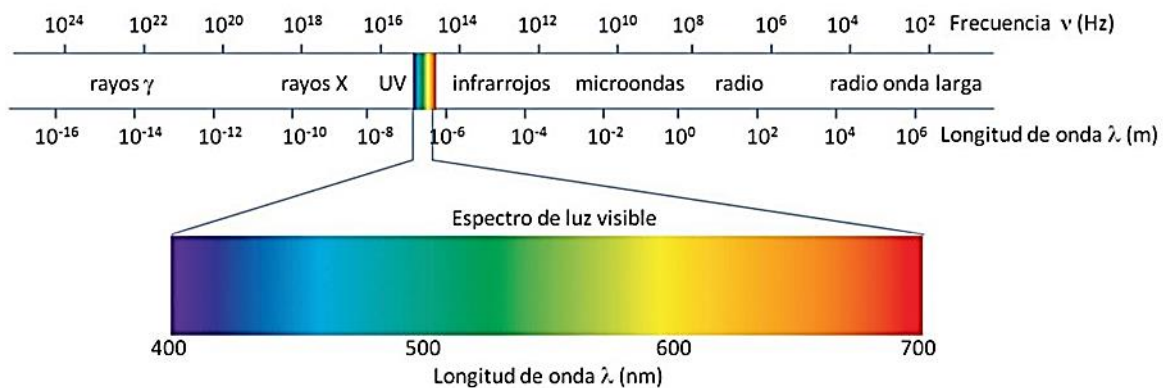


Figura 2. Escala de colores y el espectro electromagnético.

La termografía por los infrarrojos es capaz de producir una imagen visible de la luz infrarroja pero invisibles para los ojos humanos emitida por objetos o cuerpos de acuerdo a su condición térmica (Knížková et al., 2007). Una cuestión clave con respecto a la evaluación de la temperatura de la superficie es el coeficiente de emisividad (ϵ) del material a medir. La emisividad es la capacidad de cuerpo para emitir infrarrojos, hay que destacar que solamente los cuerpos negros tienen una emisividad perfecta $\epsilon=1$ (Usamentiaga et al., 2104). El conocimiento de la emisividad de la superficie es vital para obtener mediciones de temperatura precisas utilizando TIR, pero pocos estudios han intentado medir la emisividad del pelaje de los mamíferos (McGowan et al., 2018). En años anteriores algunos investigadores han establecido un rango de 0.95 – 1.00 (Hammel, 1956), sin embargo, más recientes

estudios indican valores de emisividad que oscilan entre 0.86 y 0.98 (Montanholi et al., 2009; Weschenfelder et al., 2013; Talukder et al., 2014; Abudabos et al., 2013; Martins et al., 2013; Alsaad et al., 2014; Paim et al., 2012; Ferreira et al., 2011). Como la condición de la superficie afecta la emisividad, es poco probable que el único valor recomendado de 0.98 es aplicable para todas las especies de mamíferos o, de hecho, para diferentes sitios en el cuerpo de un individuo, ya que la estructura del pelaje puede variar. En una investigación relacionada con la crianza de animales, hubo varias suposiciones para establecer el coeficiente de emisividad de la temperatura de la superficie (Cuadro 1).

Cuadro 1. Coeficientes de emisividad recomendados por especie en animales domésticos.

Especie	Emisividad (ϵ)
<i>Bos tarus</i> (vaca)	0.95
<i>Sus scrofa domesticus</i> (cerdo)	0.89
<i>Capra aegagrus hircus</i> (cabra)	0.89
<i>Equus ferus caballus</i> (caballo)	0.90
<i>Gallus gallus domesticus</i> (pollos)	0.95

Adaptado de McGowan et al. (2018).

Además de los antecedentes, los equipos de termografía infrarrojo están acompañados de un manual que establece algunas sugerencias de emisividad que normalmente van de 0.95 y 0.98 para los mamíferos. Por tanto, la mayoría de las cámaras utilizada para las investigaciones permiten grabar la imagen exactamente en la misma banda. En los animales, el 40-60 % de la pérdida de calor se produce como resultado de la radiación térmica y cae dentro del rango del factor de emisión de 0.97-0.98 (Poikalainen et al., 2012).

2.4.1. Termografía infrarroja en producción animal

La TIR tiene numerosas aplicaciones, no sólo en la industria sino también en medicina humana y veterinaria. Esta tecnología comenzó a utilizarse en equinos con fines veterinarios desde mediados de la década 60's; posteriormente, a mediados de la década de los 80's, se utilizó en animales de granja, específicamente en bovinos, sin reportarse ningún efecto desfavorable en el bienestar animal debido a que no implica ningún tipo de contacto con el animal (Ammer et al., 2017; McManus et al., 2016). A lo largo de los años, la TIR ha sido ampliamente utilizada en la producción ganadera para registrar las variaciones de temperatura superficial en los animales emitidas como radiación infrarroja (McManus et al., 2016; Salles et al., 2016; Sathiyabarathi et al., 2016; Zhang et al., 2019). La TIR puede detectar pequeñas variaciones de temperaturas en varios sitios del cuerpo de terneros; nariz (0.35 °C), oreja (0.40 °C), costado (0.19 °C) y dorsal (0.18 °C) (Schaefer et al., 2004).

La TIR es un método de teledetección no invasivo utilizado para medir los cambios en la transferencia de calor y el flujo sanguíneo a través de la detección de pequeños cambios en la TC (Nääs et al., 2014). Por lo tanto, cada región del cuerpo animal emite diferentes cantidades de radiación infrarroja de acuerdo al nivel de inervación de vasos sanguíneos. Las imágenes obtenidas permiten la observación directa de la distribución de la temperatura en una superficie (Knížková et al., 2007). Por tanto, la TIR se puede aplicar en prácticamente todas las especies pecuarias (Cuadro 2), sin embargo, se deben considerar las diferencias morfológicas, metabólicas y fisiológicas de cada una de ellas (McManus et al., 2016; Godyń et al., 2019).

Cuadro 2. Aplicaciones de la termografía infrarroja en diferentes especies de animales domésticos.

Animal	Regiones	Aplicaciones	Autor
Ganado de carne	Frente, costillas, flanco, grupa, pies y cara	Eficiencia alimenticia	Martello et al., 2016
Ganado de carne	Ojo, costillas, pierna	Eficiencia alimenticia	Montanholi et al., 2009
Ganado de carne	Ojo	Salud	Schaefer et al., 2007
Ganado de carne	Ojo, cara y región digital	Selección animal	Schaefer et al., 2018
Ganado lechero	Ubre	Mastitis	Digiovani et al., 2016
Ganado lechero	Flanco, grupa, zona trasera y pie	Estrés por calor	Montanholi et al., 2008
Ganado lechero	Ojo	Estrés por calor	Stewart et al., 2007
Ganado lechero	Ubre	Salud	Sathiyabarathi et al., 2018
Ganado lechero	Pata	Dermatitis digital	Alsaad et al., 2014
Búfalo	Ojo, vulva y hocico	Estro	Ruediger et al., 2018
Búfalo	Testículos	Calidad del semen	Yadav et al., 2019
Vaquillas	Flanco, ojo y partes de las patas	Termorregulación	Salles et al., 2016
Ovino	Ojo	Termorregulación	Sutherland et al., 2020
Ovino	Oculares, dorsales, ventrales, hombro, anca, patas delanteras, patas traseras y costillas	Termorregulación	Pulido-Rodríguez et al., 2021
Ovino	Anca, flanco anterior y posterior	Estrés por calor	Paim et al., 2012

Cabra	Vulva	Detección de estro	Façanha et al., 2018
Cerdo	Oreja, hombro y tercio caudal de la ubre	Estrés por calor	Soerensen et al., 2014
Cerdo	Vulva	Estro	Sykes et al., 2012
Cerdo	Dorsal y región ocular	Estrés por calor	Weschenfelder et al., 2013
Caballo	Ojo	Estrés por calor	Valera et al., 2012

El cuerpo de un animal genera calor que se disipa a través de la piel y ésta obtiene su temperatura de la circulación periférica local y del metabolismo de los tejidos (Zeng et al., 2020). El patrón de circulación y el flujo sanguíneo van a expresar el patrón térmico, que es la base para la interpretación termográfica (McManus et al., 2016; Stewart et al., 2017), técnica que detecta variaciones de temperatura, identificando el daño potencial antes de que haya manifestaciones clínicas aparentes. Así, el cuerpo del animal siempre está generando calor el cual se disipa a través de la piel por radiación, convección y conducción (Correa et al., 2022). Por esta razón, la temperatura de la piel es generalmente 5 °C menor que el resto del cuerpo (Martello et al., 2016), aunque depende de la región anatómica, como se muestra en el Cuadro 3.

Cuadro 3. Medias de temperatura en diferentes regiones anatómicas en ganado lechero registradas con termografía infrarroja

Etapa del animal	Región anatómica	Media (°C)	Autores
Vaca lactante	Ojo	28.60	Poikalainen et al., 2012
	Ubre	30.70	
	Flanco	27.90	
	Boca	27.70	
Vaquilla	Ojo	36.30	Peng et al., 2019
	Oído	35.20	
	Flanco	35.28	
	Frente	32.80	
	Ubre	36.08	
	Anca	35.19	
Vaquilla	Ojo	36.80	Salles et al., 2016
	Frente	28.40	
	Flanco	30.10	

El metabolismo de los tejidos que aporta temperatura a la piel es normalmente constante, por consiguiente, las variaciones en la temperatura de la piel son frecuentemente debidas a los cambios en la perfusión local de los tejidos (McManus et al., 2018). Normalmente, las venas presentan mayor temperatura que las arterias debido a que están drenando áreas metabólicamente muy activas; las venas superficiales incrementan en mayor grado la temperatura de la piel que las arterias superficiales, y el drenaje venoso de los tejidos u órganos con una elevada tasa de metabolismo incrementan más la temperatura que el drenaje venoso de tejidos normales. Por tanto, el patrón de circulación y el flujo sanguíneo van a manifestar el patrón térmico (Figura 3), que es la base para la interpretación termográfica (Mota-Rojas et al., 2022).

El ojo y la ubre se caracterizan por poseer temperaturas más altas que otras regiones del cuerpo (Poikalainen et al., 2012; Hoffmann et al., 2013; Soerensen y Pedersen, 2015; Peng et al., 2019), ya que son áreas menos aisladas y con una buena perfusión de sangre, por lo tanto, una mayor emisión de energía infrarroja; por eso se les conoce como “ventanas térmicas” (Klir y Heath, 1992). Además, estas áreas del cuerpo no son tan afectadas por la TA como otras regiones (Soerensen y Pedersen, 2015). Hoffmann et al. (2013) utilizaron un video de cámara infrarroja para registrar la temperatura de las vacas, y los resultados mostraron que el ojo tuvo una temperatura más alta en comparación con la temperatura del hombro (36.98 °C vs 34.91 °C).

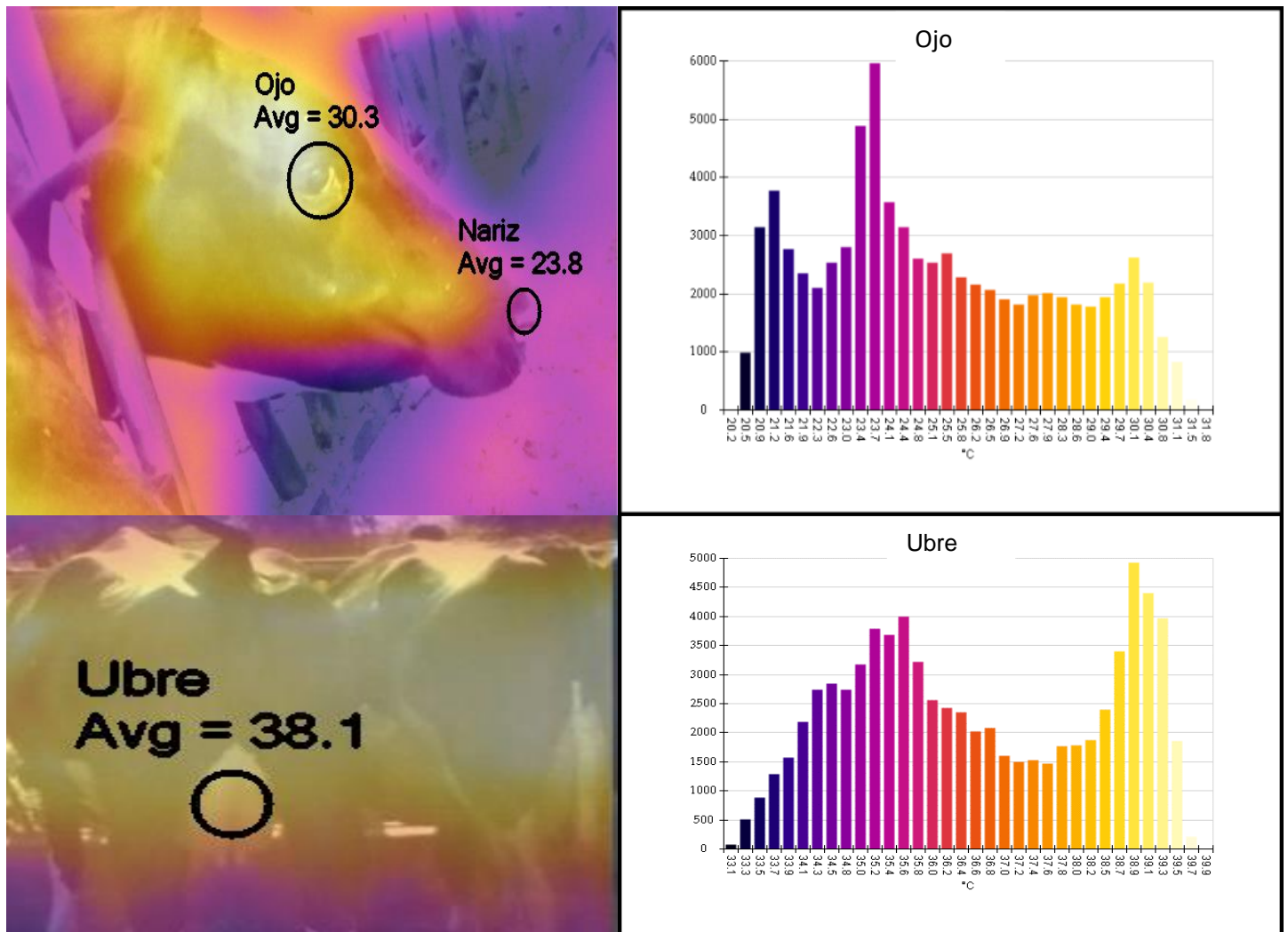


Figura 3. Representación gráfica de la temperatura del ojo y de la ubre en vacas Holstein en una zona árida.

Igualmente, Salles et al. (2016) reportan que la temperatura del ojo (36.88 °C) es más alta comparado con las demás regiones del cuerpo. Similarmente, Poikalainen et al. (2012) indicaron que la temperatura del ojo y de la ubre son los más altos de todas partes analizadas. La temperatura de la ubre se ha registrado en diversos estudios para detección de mastitis (Wanderley et al., 2020; Nakagawa et al., 2016; Daltro et al., 2017), y algunos de estos estudios se realizaron usando una infección experimental de cuartos individuales de la ubre con bacterias

patógenas. Sathiyabarathi et al. (2016) encontraron diferencias significativas entre la temperatura de la ubre en cuartos sanos y afectados por mastitis, ya que observaron un aumento de 1.51 °C en la temperatura de la ubre con mastitis subclínica en comparación con ubre sanas. En ese estudio, la temperatura media de la superficie de la piel de la ubre del cuarto sin mastitis fue 37.2 °C. Por otro lado, Zaninelli et al. (2018) encontraron una correlación significativa y positiva entre la temperatura de la superficie de la piel de la ubre y el recuento de células somáticas en leche de vacas lactantes, registrando un aumento de aproximadamente 1.6 °C en los cuartos mamarios afectados. En estos dos últimos estudios se concluye que la TIR fue efectiva en la detección de animales con mastitis subclínica. Por su parte, Golzarian et al. (2017) observaron una diferencia de temperatura de la ubre entre vacas sanas y enfermas de 0.44 °C, lo que demuestra que la TIR fue capaz de detectar pequeñas diferencias en la temperatura de la ubre, siendo extremadamente útil en el diagnóstico de mastitis.

Otros problemas de salud también han podido ser identificados con el uso de la TIR. Schaefer et al. (2012) probaron la posibilidad de usar mediciones termográficas en la región ocular para asociarlas a la detección de infecciones respiratorias bovinas; los resultados indicaron un aumento de 0.9 °C en la temperatura orbital en terneros con enfermedad respiratoria bovina en comparación a los sanos. Harris-Bridge et al. (2018) pudieron detectar problemas de dermatitis (cojeras vs sanas) en vacas lecheras usando la TIR, mientras que Alsaad et al. (2014) mostraron que la temperatura superficial de la capa y pezuña en vacas lecheras con dermatitis digital fue de 2.56 y 2.49 °C más elevada comparada con las que estaban sanas. El uso de TIR como método de diagnóstico por imágenes ha sido probado para algunas enfermedades respiratorias, como el adenocarcinoma nasal en ovinos. La imagen TIR de la fosa nasal de un animal sano presenta colores azul y verde, y en animales enfermos las imágenes termográficas mostraron colores rojo y blanco en la región lesionada de la nariz (Castells et al., 2019). La laminitis es una de las principales enfermedades que afectan a los bovinos, especialmente en lugares con alta humedad y zonas lluviosas, pero el seguimiento y diagnóstico de esta enfermedad en el campo es muy laborioso y difícil de llevar a cabo,

especialmente en hatos grandes donde es necesario examinar visualmente cada pezuña de los animales. En este sentido, Byrne et al. (2019) evaluaron el uso de la TIR para detectar cojeras en ovejas, encontrando una diferencia en temperatura de 8.5°C entre pezuñas sanas e infectadas.

Actualmente las técnicas tradicionales de detección de celos presentan algunas limitaciones en los hatos lecheros, comprometiendo directamente la eficiencia reproductiva y la longevidad de los animales (Pérez-Marqués et al., 2019). La TIR se ha aplicado como una herramienta para facilitar la detección del celo y la ovulación. Vicentini et al. (2020) demostraron que la TIR es una herramienta útil para detectar variación de la temperatura durante las fases de proestro y estro en novillas, y que las regiones alrededor de la vulva exhibieron la temperatura más alta. Adicionalmente, Pérez-Marqués et al. (2019) identificaron que la temperatura en varias regiones del cuerpo en las vacas durante el estro pasa de 0.5 a 1.20 °C en un periodo de 24 h, antes de la ovulación; los autores concluyeron que la TIR puede ser útil en las actividades reproductivas en el ganado lechero. Talukder et al. (2014) reportaron resultados similares, observaron un incremento de 1.5 °C en la temperatura de la superficie de la vulva de las vacas 24 h antes del inicio de la ovulación y una regresión a la normalidad 48 h después de la ovulación; la TIR fue capaz de identificar el 83% de las vacas en estro. Según Ruediger et al. (2018), la capacidad de detectar el estro o la ovulación puede ser debido al aumento de la vascularización del tejido, así como del flujo sanguíneo de la superficie de la vulva en las hembras en celo. También en otras especies domésticas se ha informado que la TIR ha sido una herramienta útil para identificar la variación de temperatura en la superficie de la vulva de hembras en celo. Façanha et al. (2018) identificaron un aumento en la temperatura en la región de la vulva por medio de TIR en cabras en celo. Asimismo, otros autores utilizaron cambio de temperatura de la vulva y cadera para identificar el estro en la cerda y orientar la producción (Sykes et al., 2012). Por lo tanto, la TIR tiene un enorme potencial para monitorear animales en reproducción, con el objetivo de mejorar las tasas de fertilidad del rebaño.

Adicionalmente la TIR se ha utilizado como método para evaluar la calidad del semen a través del análisis de la temperatura de la superficie de los testículos. Yadav et al. (2019) informaron que la TIR de la superficie escrotal en búfalos fue positivamente correlacionado con la motilidad de la masa espermática ($r= 0.29$) y la concentración espermática ($r= 0.20$). Ahirwar et al. (2018) demostraron que, a mayor temperatura y humedad en el ambiente, mayor es la temperatura superficial de los testículos medida con TIR en búfalos, en consecuencia, mayor la tasa de espermatozoides anormales; los autores concluyen que el uso de la TIR para medir la temperatura escrotal fue suficiente para identificar las variables ambientales que afectan la calidad del semen. De acuerdo con Chacur (2017), eso se debe a la correlación positiva entre temperatura escrotal y calidad del semen. No obstante, la calidad del semen en búfalos puede ser asociada con el grosor de la cobertura testicular, lo que puede comprometer el calor testicular.

Cabe mencionar que se ha demostrado que la TIR puede ser una alternativa para evaluar la asociación entre la temperatura de la superficie corporal y algunos procesos fisiológicos relacionados con la eficiencia alimenticia, especialmente en granjas comerciales (Montanholi et al., 2009). Leão et al. (2018) identificaron que la temperatura infrarroja del ojo fue más alta ($0.5\text{ }^{\circ}\text{C}$) en terneros con alta eficiencia alimenticia. Montanholi et al. (2009) registraron correlaciones moderadas entre la temperatura de ojos, cara y patas con el consumo de alimento residual, consumo de materia seca y el aumento de peso en bovinos de carne adultos, lo que indica que su temperatura infrarroja puede ser útil para facilitar la evaluación de la eficiencia alimenticia en bovinos de carne adultos. Schaefer et al. (2018) encontraron un aumento de $0.37\text{ }^{\circ}\text{C}$ en la temperatura de la cara de vacas lecheras con positivos consumos de alimento residual (1.93 kg/d), en comparación con los animales con consumo de alimento residual negativo (-1.76 kg/d). Los autores concluyeron que la TIR es una forma rápida y no invasiva para medir la eficiencia metabólica en ganado lechero debido a la correlación ($r=0.39$) entre el consumo residual de alimentos y la temperatura de la cara.

En los últimos años, la TIR se ha aplicado a diferentes objetivos en producción animal como la evaluación del estrés térmico a través de la temperatura corporal, la termorregulación y la calidad del semen, entre otros. La temperatura infrarroja del cuerpo se puede utilizar como indicador de estrés en un entorno determinado (Salles et al., 2016). Según Mitchell (2013), cuando un animal está bajo EC, su termorregulación involucra mecanismos homeostáticos que aumentarán la transferencia de calor desde el núcleo a la periferia del cuerpo. Esto ocurre debido al cambio en el flujo de sangre desde el núcleo a la superficie de la piel y/o un aumento de la frecuencia respiratoria, y en la periferia del cuerpo (piel) se produce una vasodilatación que regula la liberación de calor hacia el ambiente.

McManus et al. (2015) demostraron que los corderos menos eficientes para regular su TC tuvieron mayor TIR y menor peso en canal. Estos autores concluyeron que las canales más ligeras podrían estar asociados con la reducción de la ingesta de alimentos, comprometiendo la productividad de los animales. Silva et al. (2014) evaluaron la adaptación de las razas lecheras caprinas Parda Alpina y Anglo-Nubiana en una región árida, encontrando que el cuello, anca, muslo y flanco presentaron una FR y temperatura medida con TIR más altas en la raza Parda Alpina, por lo que se le consideró menos adaptada al clima seco de la zona de estudio. En otro estudio se utilizó la TIR para registrar una reacción fisiológica a través del cambio en la temperatura del ojo como respuesta al estrés y al miedo durante la manipulación.

Cabe mencionar que la TIR es un método prometedor para varios objetivos en la ganadería producción. Por su carácter no invasivo y procedimientos seguros, la TIR proporciona una forma de obtener datos en diferentes regiones anatómicas sin estrés (Chacur et al. al., 2016; Castells et al., 2019). Las correlaciones moderadas entre las variaciones térmicas normales y anormales, variaciones térmicas de procesos inflamatorios y otros datos clínicos, muestran que la TIR todavía tiene mucho que aportar para la producción ganadera. Por lo tanto, algunas regiones del cuerpo con alta vascularización muestran temperaturas más altas que otras, como es el caso del ojo y la ubre (Peng et al., 2019). Debido a la variación de

temperatura de la piel, es difícil señalar una región exacta que corresponda a la temperatura central del cuerpo.

2.5. Predicción de la TR por medio de la TIR

Como se muestra en el Cuadro 4, varios autores han propuesto esquemas de modelación buscando las regiones anatómicas que se relacionen con la TR para obtener una predicción lo más precisa posible usando termografía infrarroja. Chung et al. (2010) informaron que la TR en cerdos podía predecirse a partir de la temperatura de la piel en el abdomen central, dorso central y la región perianal. En vacas lecheras lactantes, Martello et al. (2010) encontraron que las temperaturas de la piel en la base interna de la cola, la vulva y las regiones auriculares eran predictores aceptables de la TR. Por su parte, Vicente-Pérez et al. (2016) en ovejas de pelo gestantes, indicaron que la temperatura del vientre y cabeza eran medidas adecuadas para predecir la TR. En otro estudio, Daltro et al. (2017) señalaron que la temperatura de la región lateral de la ubre fue la mejor para predecir la TR en vacas lactantes.

Cuadro 4. Algunas ecuaciones de regresión para predecir la temperatura rectal usando termografía infrarroja en animales domésticos.

Ecuaciones	Especie	R ²	Referencia
$TR = 29.24 + 0.40 TSC_{\text{promedio}}$	Ganado lechero	0.44	Martello et al., 2010
$TR = -26.071 + 0.295 \times T_{UL} + 0.449 \times T_{UP} + 1.935 \times T_{AI} - 0.023 \times T_{AI}^2 - 0.065 \times T_{\text{pata}}$	Ganado lechero	0.70	Daltro et al., 2017
$TR = 35.87 + 0.028 TSC$	Ganado de carne	0.51	Sousa et al., 2018
$TR = 33.87 + 0.15 \times T_{\text{cabeza}}$	Ovinos	0.55	Vicente-Pérez et al., 2019
$TR = 28.07 + 0.30 \times T_{\text{abdomen}}$	Cerdo	0.34	Chung et al., 2010

TR: temperatura rectal; UL: ubre lateral; UP: ubre posterior; AI: área izquierda; TSC: temperatura de la superficie corporal.

Es recomendable explorar el método TIR para facilitar el seguimiento y el control en los cambios de la TC de los animales. Aunque la TIR tiene algunas limitaciones, se puede utilizar en estudio termográfico de animales para aumentar la precisión de la medición de temperatura, ya que los estudios han demostrado que varios factores pueden afectar su precisión, como pueden ser la configuración de la cámara (Knížková et al., 2007), limpieza de la cámara, período de alimentación, hora del día, pelaje o plumaje, color del pelaje, presencia de radiación solar, época del año, genotipo, período de ordeño, temperatura y humedad ambiental, nivel de estrés del animal y etapa reproductiva. Según Polat et al. (2010), la identificación y el control de estos factores es necesario cuando se utiliza la TIR (Church et al., 2014). La elaboración de protocolos específicos para cada especie animal, regiones anatómicas y características fenotípicas permitirá reducir errores y aumentar la precisión de la medición de la temperatura en estudios futuros.

La mayoría de los estudios en los que se trata de predecir la TR utilizando la TIR han utilizado modelos de regresión lineal múltiple, con valores de coeficiente de determinación que van de 40 a 60% en condiciones a la intemperie. Sin embargo, un estudio donde se indujo artificialmente la condición de EC en vacas lecheras, esta medida de ajuste aumentó a 70% (Daltro et al., 2017). No obstante, la ecuación resultante incluyó 5 variables independientes, siendo una de ellas de tipo cuadrática, lo que confiere aspectos no prácticos al posible uso en campo de esta ecuación. Es importante reiterar que la predicción de la TR en producción animal, específicamente usando la tecnología TIR, tiene como objetivo sustituir una técnica invasiva por una no invasiva, pero sin perder precisión en la medición de la TR. Esto es, contar con una tecnología de fácil uso, confiable y sin causar un estrés adicional a los animales, para poder utilizarla con fines prácticos en el manejo diario de la explotación, ya sea con fines productivos, reproductivos o de salud (Mota-Rojas et al., 2021).

Dado que no existen estudios en los que el modelo animal sea la vaquilla de reemplazo, el presente estudio se centró en predecir la TR en vaquillas antes de ser

inseminadas, tomando en consideración el color de su pelaje para mejorar el ajuste de las ecuaciones propuestas por medio de regresión múltiple.

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CAPÍTULO III. ARTÍCULOS

3.1. Prediction of rectal temperature in Holstein heifers using infrared thermography, respiration frequency and climatic variables

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Nomenclature (abbreviations):

RT: Rectal temperature

BST: Body surface temperature

RF: Respiration frequency

HS: Heat stress

r: Coefficient of correlation

R²: Coefficient of determination

AIC=Akaike information criteria

BT: Body temperature

IRT: Infrared temperature

NOM: Norma Oficial Mexicana (Official Mexican Standards)

AT: Ambient temperature

RH: Relative humidity

THI: Temperature-humidity index

FH: Forehead

RMSE: Root mean square error

CV: Coefficient of variation

bpm: breaths per minute

MLRE: Multiple linear regression equations

DW: Durbin – Watson

DFFITS: Difference in fits

DFBETAS: Difference in betas

Q – Q: Quantile - quantile

PRESS: Predicted residual error sum of squares

Abstract

The objective of this study was to develop an equation to predict rectal temperature (RT) using body surface temperatures (BST), physiological and climatic variables in pubertal Holstein heifers in an arid region. Two hundred Holstein heifers were used from July to September during two consecutive summers (2019 and 2020). Respiratory frequency (RF) was used as a physiological variable and ambient temperature, relative humidity and temperature-humidity index as climatic variables. For the body surface temperatures, infrared thermography was used considering the following anatomical regions: shoulder, belly, rump, leg, neck, head, forehead, nose, loin, leg, vulva, eye and flank, lateral area (right side). Initially, a Pearson correlation analysis examined the relationship among variables, and then multiple linear regression analysis was used to develop the prediction equation. Physiological parameters RT and RF were highly correlated with each other ($r = 0.73$; $P < 0.0001$), while all BST presented from low to moderate correlations with RT and RF. BST forehead temperature (FH) showed the highest ($r = 0.58$) correlation with RT. The equation $RT = 35.55 + 0.033 (RF) + 0.030 (FH) + e_i$ is considered the best regression equation model to predict RT in Holstein heifers in arid zones. This decision was made on the indicators $R^2 = 60\%$, $RMSE = 0.25$, and $AIC = 0.25$, which were considered adequate variability indicators.

Key words: Holstein cattle, Infrared images, Regression models, Body temperature, Arid environment

Introduction

Recently have emerged many controversies about the evaluation of body core temperature in cattle. Broadly, two methods can be used to assess body temperature (BT): invasive and non-invasive. In this sense, invasive methods involve handling of animals which can alter their behavior and physiology, while non-invasive methods imply minimum disturbance of animals while taken measurements. Invasive methods can affect animal well-being causing an increment in stress levels due to the contact with appliances and/or the human presence for animal immobilization (Nääs and Garcia, 2014; Zhang et al., 2019). In this regard, consumers are paying more attention to the rearing conditions of the animals, especially when management procedures involve pain and suffering (Godyn et al., 2013). Other disadvantage of invasive methods is the increased in BT due to the manipulation of the animal, which may disturb its physiology, conducting to a scenario of stress-induced hyperthermia. Consequently the use of non-invasive methods takes great relevance by detecting small changes in BT of the animal, with no effect on animal welfare because it involves just slight or no contact with the animal (McManus et al., 2016; Ammer et al., 2017). Furthermore, the physiological parameter BT represents a key indicator of the presence of diseases in dairy cows as well as heat stress (Stokes et al., 2012). In addition, BT can be measured at different locations in the animal's body such as rectum, ear, or mouth (George et al., 2014), or inserting devices inside the rumen, vagina, intestines, or other internal organs (Boehmer et al., 2015; Ammer et al., 2017). However, the most common method used to obtain BT is inserting a manual thermometer into the rectum to register rectal temperature, which is considered an invasive method.

Rectal temperature (RT) is the physiological response most used not only to monitor changes in BT, but also to assess health status and to identify any type of stress in animals (Sejian et al., 2018). Nonetheless, this measurement could overestimate the actual value of RT because involves the restraint of the animal, resulting in a biased measure since this procedure may produce an extra stress that can increase the production of metabolic heat (Vicente-Pérez et al., 2016). As a result, there are non-invasive methods that do not involve physical contact with the

animal, so they do not interfere with the precision of the BT value neither with the animal well-being. Recent studies have shown that infrared thermography (IRT) is an alternative method to evaluate BT without compromising animal welfare, as it is safe, precise and an undisturbed visual technique (McManus et al., 2016; Salles et al., 2016). The IRT is an imaging technique that functions through a thermal imaging device that detects the infrared light radiated by the body and converts it into temperature, showing changes related to BT with respect to blood flow variation (Stewart et al., 2007). Some studies have reported variations less than one degree Celsius in some superficial regions of the body (Sathiyabarathi et al., 2016). Therefore, some regions of the animal's body with high vascularization show higher temperatures than others, such as the case of the eye and udder (Peng et al., 2019).

Due to the temperature variation of the skin, it is difficult to point out an exact region that corresponds to the body core temperature. On this regard, several authors have used equations searching for the best anatomical regions to predict RT using these types of non-invasive measures in different domestic species. In lactating dairy cows, Martello et al. (2010) found that skin temperatures of the internal base of the tail, vulva, and auricular regions were acceptable predictors of RT. Likewise, Vicente-Pérez et al. (2016) indicated that belly and head temperature were suitable measures to predict RT in pregnant ewes. In other study, Daltro et al. (2017) found that the lateral region of the udder was the best region to predict the RT in lactating dairy cows using IRT. Chung et al. (2010) reported that RT in pigs could be predicted from skin temperature using the central abdomen, central dorsum and perianal region. In addition to the IRT technology, there are other non-invasive measures that can be used to predict RT; this is the case of respiratory frequency (RF). Collier and Gebremedhin (2015), Vicente-Pérez et al. (2016), and Li et al. (2020) reported a strong, moderate correlation and very strong between RT and RF in ruminants, respectively. Moreover, Li et al. (2020) estimated that an increment of 0.1°C in RT caused 4.8 more breaths per minute (bpm) in dairy cows.

Nevertheless, most of the studies about prediction of RT in dairy cattle using non-invasive methods have been developed in lactating cows (Daltro et al., 2017), and do not consider further physiological stages such as younger cattle. Also, several of these studies were conducted under tropical or subtropical conditions, lacking information from arid regions (Martello et al., 2010). Pubertal heifers represent the replacement of cows that are discarded in dairy herds, thus depicting the future milk production in the herd. So it is essential to assess health and thermal condition of heifers in arid environments, which are characterized by extreme climatic conditions, with very high ambient temperatures in summer that compromise the thermoregulatory capacity of the animals. Therefore, the objective of this study was to develop an equation to predict rectal temperature using respiration frequency, climatic variables and body surface temperatures in pubertal Holstein heifers reared in a desert ecosystem. The relationship among physiological and climatic variables with body surface temperatures was also examined.

Materials and Methods

All procedures and data collection of this study were carried out according to the local guidelines, officially approved for the care of animals in Mexico (NOM-051-ZOO-1995: humane care of animals during mobilization).

Location of the study and climate

The study was conducted in a commercial dairy heifers herd located in the Mexicali Valley, Baja California state, in northwestern México (latitude 30° 52' N; longitude 114° 42' W), at 12 m above sea level. This region belongs to the Sonoran Desert ecosystem, so the climate is dry and arid, with extreme temperatures above 50°C during summer and below 0°C during winter, with very low rainfall averaging just 85 mm per year (INEGI, 2016).

Calf and heifer management and diet

Heifers used in this study came from two different dairies established in the mountain region of the Baja California peninsula. The heifers were born in both dairy herds

and spent some time after weaning there, and later they are sent to the heifer herd in the Mexicali valley at an age of 4 to 6 months. Once calves arrived to the Mexicali valley, they were separated in pens according to their size and receive a diet based on forage. Calves received 2 more diets (based on sorghum silage, alfalfa hay, and rolled wheat) until they reach an age of 12 months; at this point they were monitored to detect heifers in estrus. When a heifer showed estrous signs, she was palpated to confirm her reproductive status and along with an adequate height and body condition score, they were artificially inseminated. Heifers received until 4 inseminations before the use of a bull to be naturally bred. Pregnant heifers stayed at the heifer herd when they reached seven months of pregnancy, and they are returned to their respective dairy herd in the mountain region for parturition and be part of the milk production line.

Animals, duration of the study and diet

The study included 200 Holstein heifers with 12-14 months of age and average weight of 297.0 ± 12 kg; body condition score was measured following the methodology proposed by Ferguson et al. (1994) and was 3.5 ± 0.1 points. The experimental period comprised from July to September in the years of 2019 (n=100) and 2020 (n=100). The data was obtained from six days of each month during the morning (07:00 to 09:00 h). Both years, heifers were kept in the same two adjacent corrals which were provided with two waterers, a feed-bunk line and shade. The dimensions of the corrals were 45.0 x 33.20 m. Heifers were fed a diet for her age and physiological stage, and consisted of 52% sorghum silage, 10% ground wheat straw, 13% alfalfa hay, 10% mixed alfalfa hay, 10% rolled wheat, 3% distillers grains, and 2% of a mineral mixed. The chemical composition of the diet included 91.0% dry matter, 18.9% crude protein, 7.3% ash, 15.4% neutral detergent fiber (NDF), and 9.4% acid detergent fiber (ADF). All heifers were examined and no heifer showed signs of disease during the study.

Climatic variables

The climatic information included air temperature (AT; °C) and relative humidity (RH; %), data collected from a nearby climatic station located in the study area, which

belongs to the Meteorological Monitoring Network of the National Water Commission (CONAGUA) of Baja California. Both AT and RH were collected every 15 min and were used to calculate the temperature-humidity index (THI; units) using the following formula proposed by Hahn (1999):

$$\text{THI} = (0.81 * \text{AT}) + (\text{RH} / 100) * (\text{AT} - 14.4) + 46.4$$

Data collection and experimental procedure

Initially, feed was served and heifers were trapped closing the lock heads of the corrals. Without disturbing the heifers, rectal temperature (°C) was measured using a digital thermometer (Nsf DeltaTrak, Mod. 11062, Pleasanton, CA, USA) for veterinary use, which was inserted in the rectum of each animal at approximately 20 cm depth until temperature value was stabilized (2-3 minutes approximately). This measurement was collected avoiding fecal residues in the rectum and sticking the thermometer to the wall of the rectum. This digital probe lab thermometer has an operation range of 0 -50°C, accuracy of ± 0.5 °C, display resolution of 0.1 °C, and a probe length of 99 mm. Secondly and almost at the same time, body surface temperatures (BST) were recorded including the following anatomical regions (Figure 1A) on each heifer: shoulder, belly, rump, leg, neck, head, forehead, nose, loin, leg, vulva, eye, flank, and lateral area (right side). These data were obtained using an infrared thermographic camera (Fluke Ti400, Everett, WA, USA), with an emissivity coefficient of 0.98 (which is the recommendation of the camera company for biological tissues). The pictures were taken at a distance of 1 to 1.5 m from the animal and without the incidence of solar radiation. Also, heifers must be standing and free of mud, humidity, or any other material that may interfere with the BST to be considered a valid picture. Since no rain was registered during the sampling and no cooling system was used besides the shades in the corrals, heifers were mostly free of mud or feces in the body. Four photos of each animal were taken: 1) in front of the heifer to include their head, 2) close to the head to include the eyes, 3) a lateral picture of the right side area (opposite to the rumen location), and 4) at the back of the body to include the vulva. After photos were taken, they were downloaded in a laptop using the software Fluke SmartView® 3.9 to detect and register each BST.

To register the temperature and depending on the part of the body, different geometric shapes were used (Figure 1B); i.e., a circle was used for the ocular area, nose, belly and vulva; for the head, a line was drawn following the contour of this body part; a rectangle for the shoulder, leg, loin, rump, ear, neck, forehead and lateral area and for the flank a triangle was used. In order to collect the final temperature of every anatomical site, the average temperature of the geometric shape was considered. Heifers were unlocked and then other person, but always the same, evaluated respiration frequency (RF) by counting the number of movements of the flank for 30 seconds with the support of a manual counter and a chronometer; this result was multiplied by two to determine RF in breaths per minute (bpm). Sampling time was between 1 to 2 hours in the morning, from 06:00 to 08:00 h.

Statistical analyses

A total of 1,242 observations were collected, which included for each heifer: BST of every anatomical region, respiration frequency and rectal temperature. Descriptive statistics (means, standard deviation, coefficient of variation and minimum and maximum values, respectively) and Pearson correlation analysis were developed initially using PROC MEANS and PROC CORR from SAS (version 9.4, SAS Institute Inc., Cary N.C., USA). Correlation coefficients were classified according to its value in strong ($r > 0.70$), moderate ($0.40 \leq r \leq 0.70$), and weak ($r < 0.40$), and were declared significant when $P < 0.05$. Since RT was the variable to predict, it was considered the dependent variable in the regression models. The BST, respiratory frequency and climatic variables (AT, RH and THI) were considered independent variables. All variables were checked for normal distribution through the Shapiro-Wilk test using PROC UNIVARIATE of SAS. To predict RT, a multiple linear regression (MLR) analysis was performed and the stepwise selection method was used for the determination of the final equation using PROC REG. This method constructed a regression model by adding or removing potential predictor variables in sequence and examining the statistical significance of each explanatory variable after each iteration was performed (Sen and Srivastava, 1990). Assumptions of the MLR analysis were verified and were, normal distribution (Shapiro-Wilk test and

normality plots), homogeneity of variance (residual plots), multicollinearity (variance inflation factors and tolerance), and autocorrelation (Durbin-Watson [DW] test). To select the best model, the following statistical indicators were used: adjusted R^2 , significance of the explanatory variables (F-test), CV, RMSE, and influence diagnosis through the use of the popular measures of influence Cook's D, DFFITS and DFBETAS (Myers, 1990). To assess the model's predictive ability, the statistical PRESS (prediction error of sum of squares) and AIC were estimated. Finally, the cross-validation method was used to validate the performance of the selected model as follows: randomly, the data set was divided into two parts: 70% and 30%, where 70% of the data was used as training set and use 30% of the data set to test the model selected with 70% of the data set. These procedures were repeated 3 times and the results of the training model should be similar to the original model to confirm the fitting performance of the model. Significance was declared at $P < 0.05$.

Results

Climatic and physiological variables

Descriptive statistics for the climatic, physiological and thermographic variables during the study period are observed in Table 1. Average values for AT, RH and THI during the experiment were 29.3 °C, 43.09 %, and 76.6 units, respectively. The climatic variable RH showed the highest variation in relation to its average, with a CV higher than 10%. In the physiological variables, RF presented the higher variation with a CV higher than 10%. From the thermographic variables, the temperature of ear and nose showed the higher variation with a CV higher than 13%. Conversely, the lowest variability was detected in THI, RT, and neck temperature ($CV \leq 7\%$).

Infrared thermography measurements

According to the summary statistic of thermography measurements, a moderate variation in some body surface regions was observed (Table 1). The highest BST registered was in the eye (36.25 °C), followed by the belly and vulva (≥ 35.8 °C);

while the lowest BST was registered in the nose (28.36 °C), followed by the ear and forehead (≤ 31.7 °C). Based on CV results, the nose temperature exhibited the greatest variation, followed by the ear and forehead ($\geq 12\%$). In contrast, the BST with less variability were the neck, rump and eye. Although this variability of BST areas, certain body regions observed some similarities such as head, forehead, and ear (between 31.4 and 31.8 °C), as well as neck, leg, rump, flank, belly, shoulder and vulva (between 35.4 and 35.8 °C). Finally, a similarity in temperature was registered between loin and lateral area (34.78 and 34.30 °C).

Pearson's correlations

Pearson's correlation coefficients between physiological, climatic and thermographic variables are shown in Table 2. In general, all variables were positively correlated with RT and RF ($P < 0.01$). In addition, the physiological parameters RT and RF were strongly correlated with each other ($r = 0.73$; $P < 0.01$), while all BST showed from weak to moderate correlations with RT and RF. There was a moderate and highly significant correlation ($r \geq 0.50$; $P < 0.01$) between RT with forehead, head, nose, ear, and lateral area. Likewise, RF showed moderate correlation just with forehead ($r = 0.54$; $P < 0.01$). Regarding to the other BST, correlations between 0.30 and 0.49 with RT and RF, but highly significant ($P < 0.01$) were observed. The AT presented moderate to strong correlations ($r \geq 0.50$; $P < 0.01$) with all BST variables, being the lowest of this classification with eye ($r = 0.47$; $P < 0.01$). All BST variables showed weak and non-significant correlations with RH, and even some were close to zero (neck, loin, rump, flank, and shoulder) or negative (eye, leg, and lateral area). Finally, the THI had strong and moderate correlations ($r \geq 0.50$; $P < 0.01$) with almost all BST variables, being the lowest with eye ($r = 0.42$; $P < 0.01$).

Multiple linear regression equations (MLRE's)

The resultant equations to predict RT using BST, RF, and climatic variables are illustrated in Table 3. After several runs with the data using the stepwise procedure, three equations were considered. The first equation included just one predictor variable, RF, which resulted in a moderate coefficient of determination ($R^2 = 0.53$); however, when the variable forehead (FH) was added in the next step, the R^2

increased 7% ($R^2 = 0.60$). The difference was not only reflected in R^2 , but also in lower values of other indicators such as CV, RMSE, PRESS Residual and AIC (0.70, 0.27, 91.6 and -3237.92 vs 0.65, 0.25, 79.46 and -3415.23 respectively). In the next step, the climatic variable RH was added, which had a low impact on the coefficient of determination ($R^2 = 0.61$), but slightly lower values of CV, RMSE and PRESS Residual (0.64, 0.24 and 76.58). Then, according to these results along with the other indicators, the second equation was selected as the best fit to predict RT. In addition to the indicators previously mentioned, assumptions analysis was carried out to check for normality, homoscedasticity and multicollinearity of the final equation. The DW was 1.50, and according to this result there was no autocorrelation in the residuals (Table 3). Multicollinearity was also tested and an estimate value for VIF of <10 (Table 4) confirmed that there was no perfect exact association among predictor variables. The mean values of TOL were in the range from 0.4 to 1.0 (Table 4), which confirmed the absence of multicollinearity in the models. Figure 2 shows the diagnostic plots of the residuals. The comparison between observed and predicted values is shown in a), which assumed a precise relationship between the response and independent variable, and also suggests a reasonable fit of the model to the data with a positive linear relationship. The plots b) and c) indicate a reasonable spread of the data implying approximately constant variance. Likewise, the plot residual vs predicted values demonstrated that equation 2 is acceptable to predict RT. The Q-Q plot is illustrated in d), which demonstrated that it does not deviate much from a straight line, indicating that the normal distribution assumption is satisfied; the histogram of residuals in e) support this statement since appears to be very consistent with normality. The influential diagnostics revealed that only 3 observations were outside the absolute values established for the three diagnostic measures tested (DFFITS, DFBETAS, and Cook's distance), but when the model was re-ran without these 3 observations, no significant change on the model indicators was noted, so the model was declared without influential observations. The cross-validation technique was used for model validation purposes. The results of R^2 , RMSE, and CV for the MLRE model during the training and testing stages are also indicated in Table 3. The values of the

parameters were compared for the model's training and testing data sets, respectively. The MLRE that achieved the highest accuracy in predicting RT was the equation 2, even more, the values of the parameters in the model and the test were very similar (training test: $R^2 = 0.60$, $CV = 0.65$, and $RMSE = 0.25$ vs training test: $R^2 = 0.59$, $CV = 0.63$, and $RMSE = 0.25$; Table 3).

Discussion

Climatic and physiological variables

The environmental conditions observed during the experimental period were considered to be moderately harmful for heifers, since they were not very intense. Collier and Gebremedhin (2015) reported the ideal AT for dairy cattle is between 13 to 20 °C; however, also the RH should be considered as an additional factor associated with the intensity of HS. Berman (2006) reported that the efficiency of thermoregulatory mechanisms for evaporation decreased in dairy cattle at AT 32 °C and RH 45%. These standards coincide with the maximum values of our study; but considering the average AT, no presence of heat stress was observed. However, when AT is combined with RH, a THI of 77 units was obtained, which indicates a moderate HS according to the THI classification (72 – 77 units) stated by Armstrong (1994). Also, it has been pointed out that in lactating cows, for each unit of increase in THI above 69 units, a reduction of 0.41 kg/cow/d is likely to be observed (Bernabucci et al., 2014). However, heifers begin to experience signs of hyperthermia with higher values because lactating cows are more susceptible to high environmental temperatures.

The average of the physiological constants obtained in the present study was within the normal range for dairy cattle. Silanikove (2000) and Seijan et al. (2018) reported that the normal range of RT is between 38.0 and 39.3°C in heifers, and from 38.0°C to 39.0°C in mature cows. The RT is also affected by the physiological state, since lactating cows appears to be more influenced by elevated ambient temperature than non-lactating and non-pregnant cows (39.2 vs 38.5 °C, respectively) (Lee et al., 2015; De Andrade et al., 2017). The reason is because

lactating cows produce great amounts of metabolic heat as a result of the high quantities of feed consumed motivated for their milk production, mechanism that raises body temperature due to the presence of several physiological processes such as fermentation and digestion (Fabris et al., 2019). A small increase in this parameter indicate failure in the mechanisms of heat release to the environment (Avendaño-Reyes et al., 2014). Most authors agree that HS occurs when RT exceeds a predefined threshold and considering a single instance of a temperature above the threshold value as an indication of illness (Collier et al., 2017). Another physiological variable that can be associated to the animal's discomfort is RF. According to Silanikove (2000), the normal range for RF in dairy cattle is around 40 to 60 bpm. An increase in RF of cows is a typical mechanism indicating greater sensitivity to high environmental temperature; in fact, RF contributes with an 18% of the metabolic heat production in ruminants (NRC, 1981). Fabris et al. (2019) declared that RT and RF are the best physiological variables to assess the tolerance of animals to heat.

Thermographic variables

The infrared thermography camera captures the infrared radiation emitted from the cow and then uses this information to create thermal images also called thermograms. The largest part of radiant energy is emitted with a wavelength of 7–14 μm , and is referred to as infrared radiation, which is emitted by anybody whose temperature is greater than 0 Kelvin, i.e. -273.15 degrees Celsius (absolute zero). The ITR detects changes related to the body temperature with respect to the variation of the blood flow, so the variability of the BST follows different patterns depending on the body region (Martello et al., 2015).

Taking into account coat temperatures of the 14 anatomical regions considered in this study, the range was 7.9 °C between the lowest and the highest temperature values. This variation was lower compared to the study conducted by Salles et al. (2016), who reported 8.5 °C. Likewise, others have reported lower variability (5 °C) than the previous report (Martello et al., 2015). According to several studies, the range of variability of the BST is around of 4 and 10 °C, depending on

the species (Caja et al., 2014; Freitas et al., 2018; Peng et al., 2019; Wanderley et al., 2020). Therefore, the variations among BST have probably occurred because of the distribution of blood flow, which in turn is closely related to the vascularity and surface area of the anatomical region of interest. Consequently, Salles et al. (2016) stated that the differences between studies may be explained by differences in the thermal environment where cattle were evaluated, or even the genotype (*Bos taurus* vs *Bos indicus*), and productive status (lactating cows vs non-lactating cows) could be involved. Thus, to make any inference about BST, it is necessary to contemplate variability as an important factor.

An additional factor that could increase the variability of BST in the present study is the fact that the data included two consecutive years, so individual heifers used for BST were different per year, but always Holstein heifers of similar age and weight. Climatic variables were similar between the two years, so this variable was deleted from the model. Other studies contemplate animals of different genotype, diverse environments, different times, and even several regions for the evaluation. Compared with other anatomical regions, the eye presented the highest BST and the lowest variation, results that agree with other studies (Stewart et al., 2008; Martello et al., 2015; Peng et al., 2019). As a result of this low variability and closeness to RT, several authors considered eye temperature as the best for assessing body temperature. Stewart et al. (2008) considered the eye temperature as a stress indicator in animal, allowing diagnosis of some diseases in some species.

Regarding to the other BST's, it is questionable their capacity to estimate BT since most of them are influenced by other factors. For instance, the infrared images of the flank and belly were easy to obtain, which was the advantage for using IRT to monitor them. However, due to its close distance to the rumen, it probably reflects the rumen temperature. According to the biomechanics of the BST, the skin that covers the muscles (leg, loin, or shoulder) is subject to increases in temperature during muscular activity (Yarnell et al., 2014). In general, the parts of the body that function as thermoregulatory mechanism such as the nose and extremities (feet) have normally lower temperature than the rest of the body. The animal's body is

always generating heat which is dissipated through the skin by radiation, convection, or conduction. For this reason, the temperature of the skin is generally 5 °C cooler than the rest of the body. Other studies involving cows and calves kept in facilities with a controlled environment reported differences of 3 to 5 °C among the body surface temperatures determined by IRT (Hoffmann and Schmidt, 2012). Thus, BST has been more sensitive to thermal environment than RT; this reason could help to explain the inconsistency found by researchers. In addition, physical characteristics associated to the subject (i.e. coat length, color, and shape) also affect BST output. Taking in account the variation among BST's, it is necessary to consider the temperature of the part that is more associated with RT to assess BT in a non-invasive approach.

Pearson's correlation

Coefficients of correlation demonstrated that all BST measured were positively associated with RF and RT, and also a strong relationship was observed between RT and RF; in hot environments, these physiological parameters are considered the more reliable variables to assess heat tolerance of cattle. In fact, the first evident physiological parameter that shows that animals are heat-stressed is a change in RF (Sejian et al., 2018), afterwards an increase in RT occurs. Li et al. (2020) estimated an increase of 0.1°C in RT for each 4.8 bpm of RF in dairy cattle. Consequently, a physiologic change that occurs in bovines in response to HS is the result of the temperature variation in the body surface. Among the body regions studied, the forehead temperature showed the highest ($r = 0.58$) correlation with RT. In agreement with our study, other authors have reported similar correlation between forehead and TR. For instance, Peng et al. (2019) reported a correlation of 0.55 and Martello et al. (2015) of 0.60 between these two variables. Likewise, Salles et al. (2016) indicated that head and forehead are the most promising regions for the estimation of body temperature in cattle. The relationship between forehead and RT observed in this study could be explained by its proximity to the brain, which comprises the central nervous system and is responsible of body temperature regulation (Weschenfelder et al., 2013). Moreover, some organs are considered as

corporal heat sources since they are described as high metabolic rate promoters (brain, heart, liver, kidneys). Kessel et al. (2010) considered the regions of the head as indicators of core temperature because of its contiguity to the brain. Sellier et al. (2014) mentioned that internal temperature of the body is similar to the temperature of the main organs such as the brain, heart and viscera. This may explain why correlations of different parts of the head showed the highest associations with RT (head, forehead, nose, and ear) in the present study, except for the eye. However, other studies have obtained intermediate correlation values between eye and RT such as 0.38 (Peng et al., 2019) and 0.43 (Martello et al., 2015). According to this evidence, the eye is probably not a precise anatomical region to predict body temperature.

In contrast to our findings, Tan et al. (2016) reported that ocular temperature was a good indicator of core temperature in humans observing a high correlation between the two variables ($r > 0.80$). Additionally, Johnson et al. (2011) found that thermographic eye temperature was feasible as an index of body temperature in horses. In another study, Schaefer et al. (2012) assessed the possibility of using IRT measurements of the eye region for automated detection of bovine respiratory disease with satisfactory results. This inconsistency in results involves several factors, such as weather conditions, species used, and even the selected area for temperature measurement inside the eye (Salles et al., 2016; Peng et al., 2019). The eye is the first part of the body to react to any type of distraction, so when the technician is trying to take the image, the animal captures the movement and blinks right away, sending signals of stress to the brain. When the animal's sympathetic nervous system is activated due to this type of stress, heart rate increases and blood flow is redistributed. The eye and its surrounding skin tissue provides an image that may reflect the sympathetic-vagal balance of the animal (Stewart et al., 2007), so when the animal become stressed, there is an increase in blood flow in the eyes; therefore, the temperature of the eye increases, even though the internal body temperature is still low. Based on the results of this study and others on eye temperature using IRT technology, we can hypothesize that the temperature of the eye increases abruptly by the reflex while the temperature of the body increases

gradually. It is important to mention that IRT is performed at different sites since the use of a single surface temperature for the identification of thermal zones is questionable due to differences in vasomotor activity among different body regions.

Additionally, it should be noted that only forehead temperature was moderately correlated with RF, and a similar result was found by Martello et al. (2010) who reported a correlation of 0.54 between RF and head temperature in dairy cattle. Based on the evidence of our study, the BST's, especially forehead, have an acceptable association with RF and RT, the most important physiological variables for monitoring animal welfare.

Multiple linear regression equations (MLRE)

Based on the MLRE results, some BST and RF were sufficient to explain the variation in RT of Holstein heifers. Three models were considered: a) The first one with only one variable (RF) that explained 53% of the total variation, so this equation had a moderate coefficient of determination; b) In the second model, the BST of forehead was introduced to the model, accomplishing an increment of 7% in the adjustment (60%), which was considered an important increment for the model. Additionally, there was a decrease in CV and obtained the lowest RMSE; so this model with two variables is may be the most suitable because it had the lowest RMSE value. This information indicates a lower variability than the first model, since the RMSE is considered as criteria to check the veracity of the model; c) When a third equation was proposed including a third variable in the model (RH), an increase of about 1% was noticed (61%), a very limited benefit for model consideration purposes. According to Montgomery et al. (2006), it is not necessary to add variables in the MLRE when a little gain of percentage in R^2 is observed. One of the very important indicators is PRESS statistic, which suggests how good a regression model is to predict new data, so the lower the PRESS statistic the better. When the variable forehead was added to the second model the value of PRESS statistic decreases from 91.64 to 79.46; in this case the first equation is excluded, since the model with two predictors is better than the one with just one predictor. The main objective of the regression analysis is to find the function that best explains the

relationship between the dependent and the independent variables, so in this case the third model was excluded similar to the first model.

Therefore, $RT = 35.55 + 0.033 (\text{respiration frequency}) + 0.030 (\text{forehead temperature}) + e_i$ is considered the best regression equation model to predict RT in Holstein heifers in arid zones. This model explained a higher proportion of the variation in RT ($R^2 = 60\%$) and had the lowest variability indicators. This model presented no multicollinearity in the predictors, neither autocorrelation in the residuals. In addition, plot analysis of the residuals also allowed verifying the adequacy of the model. The distributional assumption was satisfied, as well as the homoscedasticity and the normal distribution of the residuals.

Besides, the model was validated through the cross-validation method. The estimated model and the prediction model were similar in their parameters which confirm the effectiveness of the model. Additionally, the predictors selected in the model were highly significant, indicating that all the predictor variables have a significant relationship with the response variable. Even though no previous study were found using Holstein heifers in arid regions, the adjustment obtained in the present study represented an improvement compared to other studies trying to predict RT and carried out in dairy cattle. An equation developed by Martello et al. (2010) in dairy cattle showed a moderate adjustment to predict RT through BST with coefficients of determinations ranging from 43 to 51 %. However, using 49 lactating dairy cows from 3 genotypes (Girolando, Gir, and Holstein) in a tropical environment to predict RT, Daltro et al. (2017) reported a coefficient of determination of 70% in a multiple regression equation that included the predictor variables lateral region of the udder, posterior region of the udder, and left area of the animal (linear and quadratic); the data came from measures of 3 consecutive days and no information about the precision or validation of such prediction model was provided. The discrepancy among results could be due to the use of type and number of predictor variables, genotype and physiological stage of the cows, facilities, and climatic conditions.

Conclusion

Body surface temperatures in heifers were positively correlated with rectal temperature and respiration frequency, which suggests that increases in body temperature is associated with the increase in these two important livestock physiological variables. Thermal imaging in combination with respiration frequency explained 60% of the variability of rectal temperature, given a multiple regression model with a satisfactory variability. This equation was validated and the result indicated a consistent equation. The surface temperature of the forehead was the most promising region for the estimation of body temperature in Holstein heifers. Therefore, infrared thermography could be used as a non-invasive technique to assess thermal condition in Holstein heifers in arid zones.

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Conflict of interest

All authors declare no conflict of interest to disclose.

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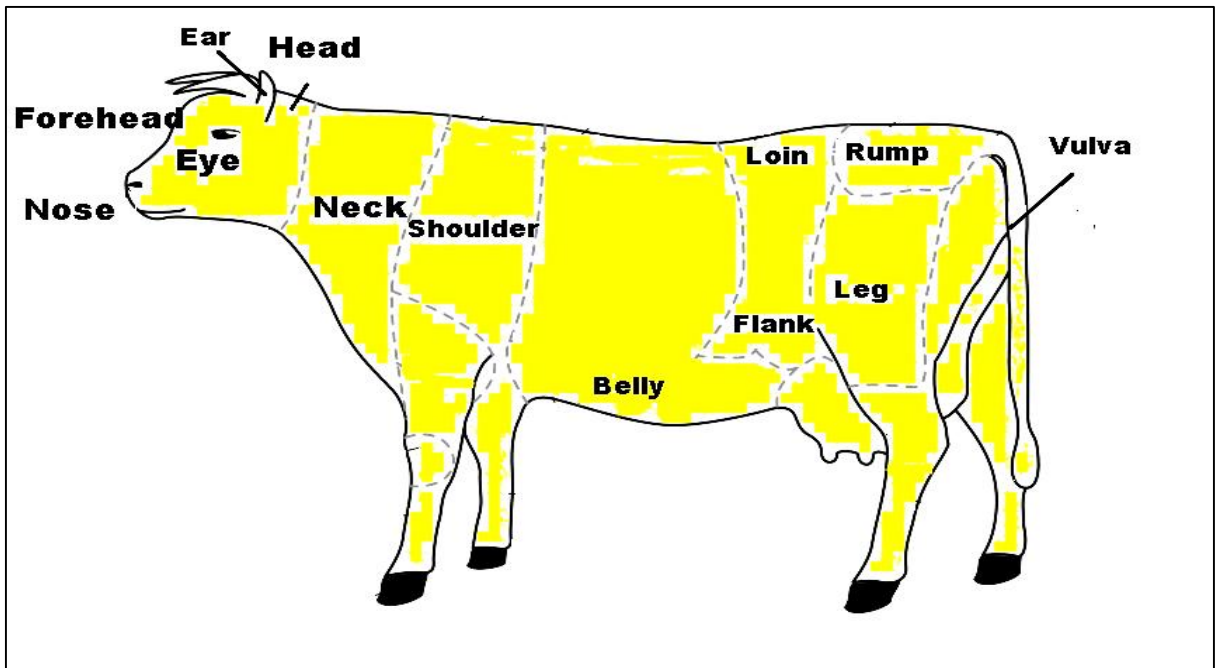


Figure 1 A. Cattle anatomical regions where the images were taken.

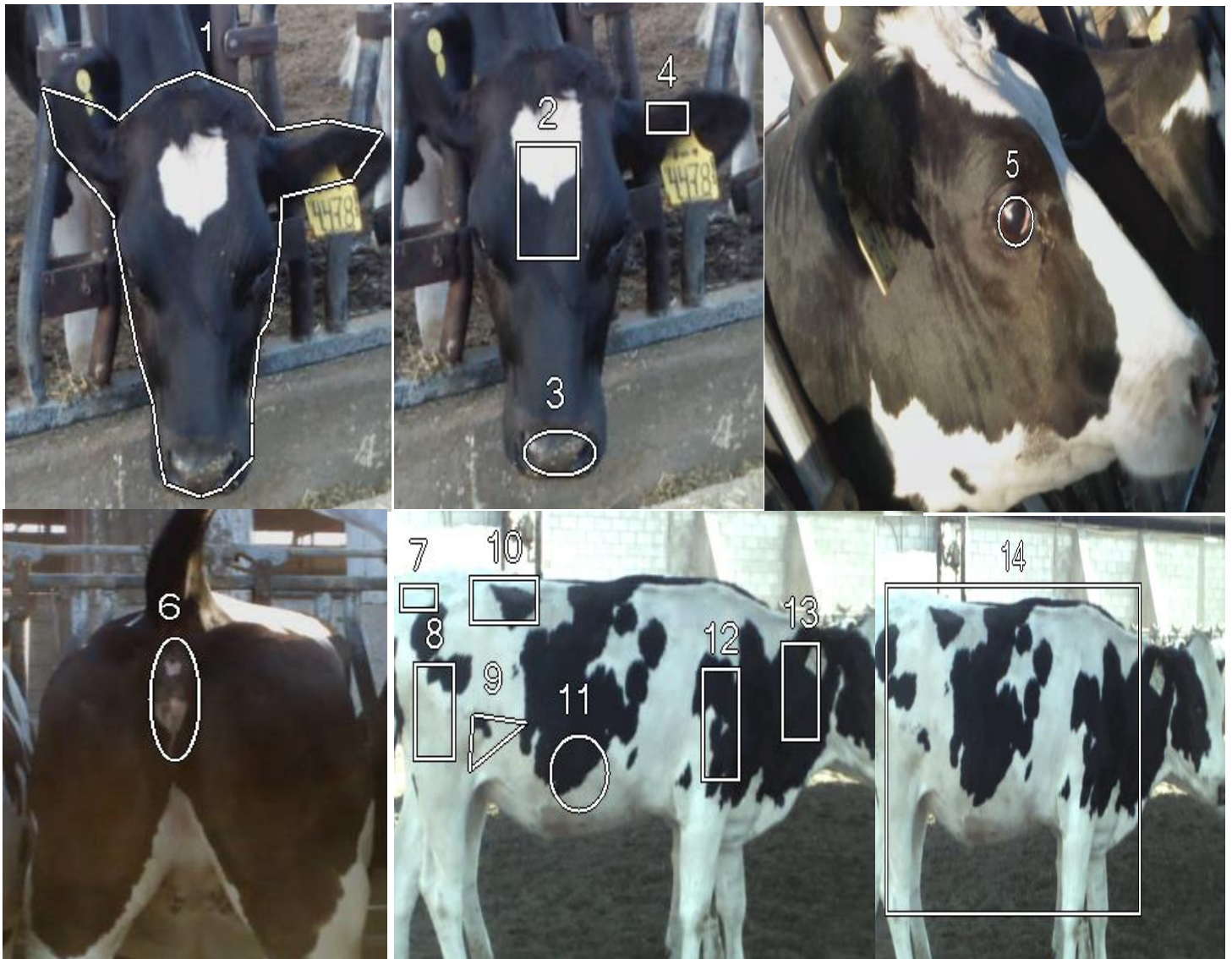


Figure 1B. Illustrative infrared thermography images off each anatomical region where the infrared thermography images were obtained using different shapes: Head (1); Forehead (2), Nose (3), Ear (4), Eye (5), Vulva (6), Rump (7), Leg (8), Flank (9), Loin (10), Belly (11), Shoulder (12), Neck (13), Lateral area (14).

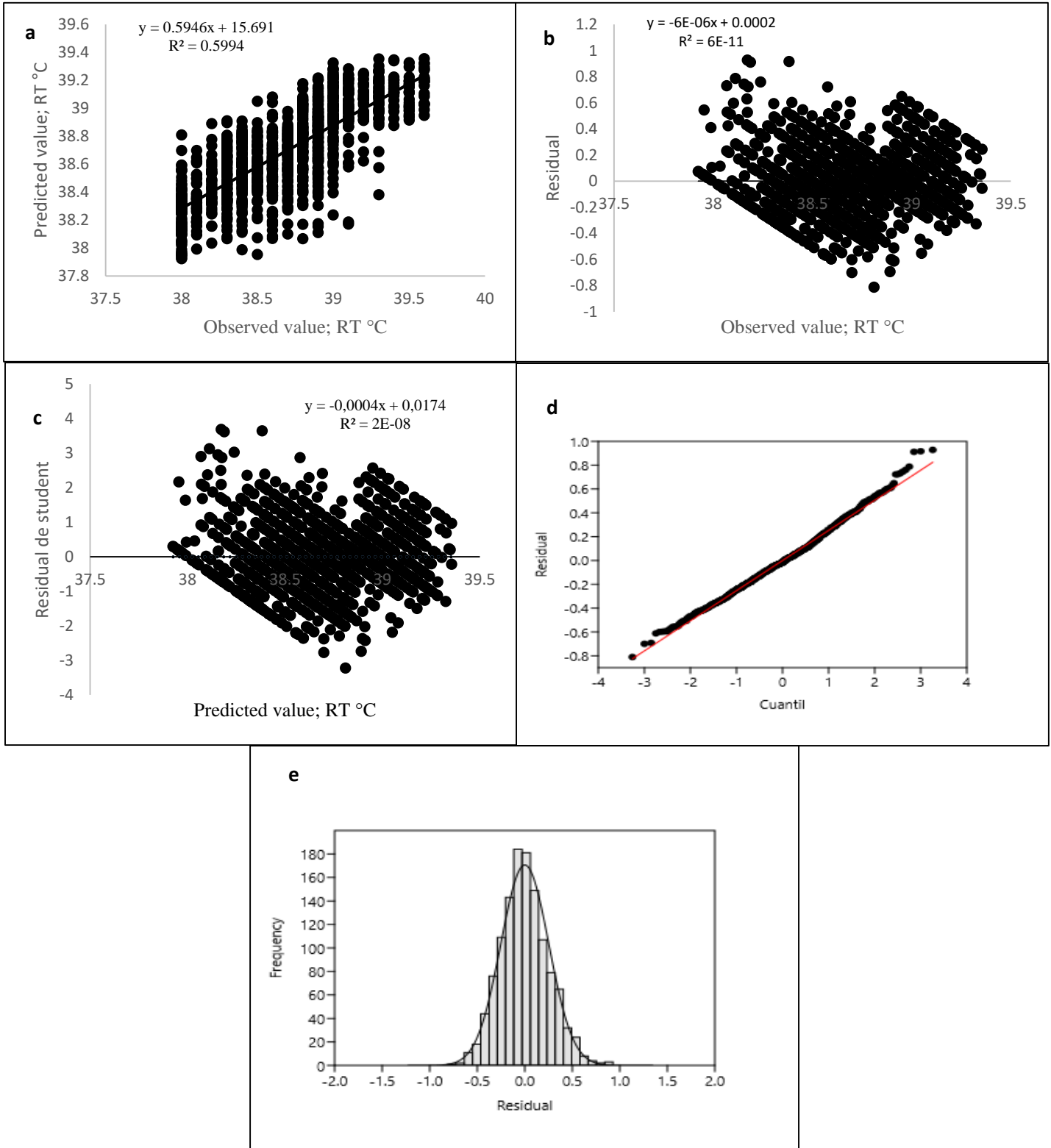


Figure 2. Verification of the assumptions of the model by analysis of the residuals for the final equation

Table 4. Descriptive statistics of climatic, physiological and thermographic variables of Holstein heifers.

Variables	Mean	SD	CV	Mínimum	Máximo
Climatic					
AT (°C)	29.30	2.57	8.79	24.64	32.89
RH (%)	43.09	4.41	10.24	35.00	50.00
THI (units)	76.58	3.49	4.56	71.48	81.18
Physiological					
RF (bpm)	66.87	7.08	10.59	44.00	82.60
RT (°C)	38.70	0.39	1.02	38.00	39.60
Thermographic (°C)					
Head	31.78	3.45	10.85	26.00	36.99
Forehead	31.70	3.82	12.04	23.58	39.89
Nose	28.36	4.00	14.09	20.10	34.95
Eye	36.25	2.16	7.06	30.00	39.99
Ear	31.47	4.20	13.35	21.28	38.09
Neck	35.61	2.20	6.18	29.40	40.40
Loin	34.78	3.23	9.30	27.10	42.00
Leg	35.46	3.14	8.86	26.30	42.40
Rump	35.74	2.48	6.94	27.83	41.70
Flank	35.77	2.32	7.48	29.07	42.40
Belly	35.83	2.74	7.65	27.12	41.90
Shoulder	35.75	2.68	7.48	28.50	42.40
Vulva	35.80	2.55	7.12	30.00	41.90
Lateral area	34.30	3.29	9.59	25.00	39.93

RH: Relative humidity; AT: Air temperature; THI: Temperature-humidity index; RT: Rectal temperature; RF: Respiration frequency; SD: Standard deviation; CV: Coefficient of variation.

Table 5. Pearson's correlations among physiological, thermographic and environmental variables of Holstein heifers.

Variable	RT	RF	AT	RH	THI
RT (°C)	1.00**	0.73**	0.45**	0.021 ^{ns}	0.42**
RF (bpm)	0.73**	1.00**	0.44**	0.19**	0.44**
THI (units)	0.42**	0.44**	0.98**	0.45**	1.00**
Thermographic (°C)					
Head	0.57**	0.48**	0.78**	0.21**	0.76**
Forehead	0.58**	0.54**	0.72**	0.11**	0.69**
Nose	0.56**	0.48**	0.77**	0.20**	0.76**
Eye	0.38**	0.36**	0.47**	-0.10**	0.42**
Ear	0.55**	0.48**	0.78**	0.21**	0.77**
Neck	0.50**	0.42**	0.72**	0.05 ^{ns}	0.69**
Loin	0.41**	0.35**	0.68**	0.02 ^{ns}	0.64**
Leg	0.49**	0.39**	0.67**	-0.03 ^{ns}	0.62**
Rump	0.46**	0.40**	0.54**	0.067*	0.52**
Flank	0.45**	0.37**	0.66**	0.03 ^{ns}	0.63**
Belly	0.36**	0.33**	0.53**	0.12**	0.52**
Shoulder	0.46**	0.38**	0.66**	0.03 ^{ns}	0.63**
Vulva	0.48**	0.42**	0.69**	0.14**	0.67**
Lateral area	0.53**	0.42**	0.61**	-0.06 ^{ns}	0.57**

RT: Rectal temperature; RF: Respiration frequency; AT: Ambient temperature; RH: Relative humidity; THI: Temperature-humidity index; ** Significant at P<0.01; *Significant at P<0.05; ns: Non-significant

Table 6. Multiple linear regression equations to predict rectal temperature using climatic and physiological variables and results from cross validation method.

Equations	N	R ² (%)	R ² _{adj} (%)	CV	RMSE	AIC	PRESS	DW	P-value
1) RT = 35.97+ 0.04 (RF)	1242	53.15	53.11	0.70	0.27	- 3237.92	91.64	1.33	<.0001
2) RT = 35.55+0.033 (RF)+ 0.030 (FH)	1242	59.95	59.88	0.65	0.25	- 3415.23	79.46	1.50	<.0001
3) RT = 35.94+0.034 (RF)+0.030 (FH) - 0.011 (RH)	1242	60.98	60.89	0.64	0.24	- 3461.09	76.88	1.56	<.0001
Cross validation results									
1) RT=36.03 + 0.040 (RF)	869	52.17	52.11	0.60	0.27	- 2258.32	64.66	1.33	<.0001
2) RT=35.59+ 0.032 (RF) + 0.030 (FH)	869	58.98	58.89	0.63	0.25	- 2389.87	55.59	1.50	<.0001
3) RT=36+ 0.033 (RF) + 0.031 (FH) - 0.012 (RH)	869	60.76	60.62	0.64	0.24	- 2426.36	53.31	1.56	<.0001

R²: Coefficient of determination, R²_{adj}: Adjusted coefficient of determination; AIC: Akaike Information Criterion; RMSE: Root mean square error; CV: Coefficient of variation; RT: rectal temperature, RF: Respiratory frequency, FH: Forehead temperature; DW: Durbin-Watson test; PRESS: Predicted residual error sum of squares.

Table 4. Parameter estimators of the independent variables included in the final equation Holstein heifers

Items	Estimator s	SE	Partial R²	VIF	Tol	P- value
Intercept	35.55	0.0745	-	-	-	<.0001
RF	0.0330	0.0011	0.5315	1.30782	0.76463	<.0001
FH	0.0301	0.0021	0.0680	1.30782	0.76463	<.0001

RF: Respiration frequency; FH: Forehead temperature; Tol: Tolerance; SE: Standard error; VIF: Variance inflation factor.

Holstein heifers in desert climate: effect of coat color on physiological variables and prediction of rectal temperature

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Abstract

Two hundred Holstein heifers were divided by hair coat color in black ($n_1=60$), white ($n_2=62$), and mixed ($n_3=78$) to accomplish two objectives: 1) to compare physiological variables using an analysis of variance, and 2) to construct regression equations to predict rectal temperature. In each heifer, rectal temperature (RT), respiration frequency (RF), and body surface temperatures (obtained with infrared thermography in eye, nose, forehead, head, neck, ear, shoulder, flank, belly, leg, loin, rump, and vulva) were measured. Black heifers had more RF and RT ($P < 0.01$) than mixed and white coat heifers; white heifers had similar RT than mixed color heifers, but they exhibited less RF ($P < 0.05$). In general, black and mixed coat color heifers had higher BST ($P < 0.01$) than white heifers in the majority of the anatomical regions measured. For black coat heifers, the best regression model to predict RT included three predictor variables: $[RT=35.59 - 0.013(RH) + 0.045(RF) + 0.019(T_{Ear}); R^2=71\%]$. For white coat heifers, the best model included two predictor variables: $[RT=35.29 + 0.035(RF) + 0.033(T_{Forehead}); R^2=71\%]$; and for mixed coat color heifers the best model included two predictor variables: $[RT=35.07 + 0.022 (RF) + 0.038 (T_{Head}); R^2=44\%]$. Heifers with dark hair coat color showed higher physiological constants than white heifers; the prediction of rectal temperature was more precise in heifers with well-defined hair coat color. Physiological and climatic variables, along with infrared thermography, represent an appropriate combination to predict rectal temperature in Holstein heifers with predominant white or black hair coat color.

Key words: Holstein cattle, Hair coat color, Infrared thermography, Respiration frequency, Arid environment

Introduction

Climate change is causing a significant increase in ambient temperature around the world, and these temperatures are exceeding the upper critical temperature of cattle in summer, leading to the physio-pathological condition called heat stress (Theusme et al., 2021; Rojas-Downing et al., 2017). Heat stress (HS) is one of the main causes of economic losses and welfare concerns for the global dairy cattle industry in the 21st century (St-Pierre et al., 2003). In this regard, genetic and phenotypic selection for heat tolerance in dairy cattle have a great potential to deal with climate change, as epigenetic effects of heat stress can persist in future generations of progenies (Weller et al., 2021). Rectal temperature (RT) is a physiological constant considered a suitable indicator of heat tolerance and health status. Practically, there are two drawbacks to obtain RT in cattle. First, the traditional method is inserting a manual thermometer into the rectum, activity considered invasive because it involves considerably restraint of the animal, producing an extra stress that may lead to an overestimation of the RT (Godyn et al., 2019). And second, using non-invasive methods (temperature probes, telemetry, microchip implants, etc.) have not shown really accurate estimations of RT. This is because many factors (i.e., environmental conditions, breed, diet consumed, type of production system, coat color, thickness of the adipose tissue) may affect the final output (Munsell et al., 2006; Landgraf et al., 2014).

Infrared thermography (IRT) is a technology that began to be used in equines for veterinary purposes since the middle 1960's. Later in the mid 1980's was used in farm animals, specifically in cattle, with no report of any unfavorable effect on animal welfare because it does not involve any type of contact with the animal (McManus et al., 2016; Ammer et al., 2017; Racewicz et al., 2018). Therefore, in the following years this non-invasive technology was used to monitor clinical status such as fever, diarrhea, lameness, and mastitis in cattle (Stewart et al., 2017; Alsaad et al., 2015) as well as to detect early signs of bovine respiratory disease in growing cattle and feed efficiency in feedlots (Montanholi et al., 2015; Schaefer et al., 2012). Even more, some researchers have dedicated efforts to predict RT using this non-invasive technology considering the temperature of different body regions in the animal (Daltro et al., 2017; Peng et al., 2019).

In fact, some of these studies have found moderate variability in skin temperature (4 to 7.8 °C; Salles et al., 2016) and have also tried to find regions of the body that best predict the RT; examples are the equations obtained by Chung et al. (2010), Martello et al. (2010), Alsaad et al. (2015), and Daltro et al. (2017). However, these equations do not take into account the characteristic coat color of the animal.

The relationship between hair coat color and productive parameters in dairy cattle began to be documented in the early 1990's (Becerril et al., 1993). Later on, there was interest in classifying the cows according to hair color as a potential trait for adaptation to warm climates (King et al., 1988). According to more recent studies, coat color can provide information about how some cattle breeds are more likely to adapt to a particular environment, since this characteristic is directly influenced by the environment (Anzures-Olvera et al., 2019). Authors have reported that white coat color animals absorb between 40 to 50% less solar radiation than black coat color animals do (Isola et al., 2020). Hillman et al. (2013) exposed black and white Holstein cows to the direct sunlight during 90 minutes under hot conditions, reporting that white cows increased RT at a rate of 0.8 °C per hour, while black cows at a rate of 1.3 °C per hour. In addition, surface temperature of black cows increased in average 4.8°C, and white cows just 0.7°C. These results were explained by the fact that white cows absorbed 66% and black cows 89% of the solar radiation. Warm climate regions are characterized by experimenting elevated ambient temperature, relative humidity, and solar radiation, which generally exceed the limit of the thermoneutral zone for lactating dairy cows, whereby cows activate a sequence of thermoregulation mechanisms (i.e., body temperature, respiratory rate, heart rate, and sweating) that favor a homoeothermic status against the thermal challenge. Nevertheless, the thermoregulatory response of dairy cattle to HS also varies with physiological status, age, breed, diet, and coat color. Considering the effect that coat color exerts on the responses of Holstein cows under HS, we hypothesized that the equations to predict RT using ITR may vary according to this trait; so it is advisable to construct equations including the coat color of the animals. So far, these equations have been calculated using lactating cows, so there is a necessity of using other type of animals, such as younger cattle or heifers. Therefore, the present study has two objectives: 1) to evaluate the effect of coat color on physiological variables measured with infrared thermography in Holstein

heifers; and 2) to predict rectal temperature using those physiological variables according the coat color of Holstein heifers.

Materials and Methods

Location of the study

The study was performed in a private heifer farm situated in the Mexicali valley, Baja California, in the northwestern region of México, at geographic coordinates 30° 52' N and 114° 42' W. During summer of this desert region, temperatures exceed 45 °C and average relative humidity ranges between 20 and 50%, especially from June to September. Therefore, the climate is arid, hot and dry; with very low rainfall yearly average (85 mm) and a solar radiation that reaches 1700 WS (INEGI, 2016).

Facilities, animal's management, diets, and study variables collection

Livestock facilities, calf and heifer management, diets offered to animals, as well as collections of physiological and climatic variables are described in detail on Theusme et al. (2022). Briefly, two hundred non-pregnant Holstein heifers (BW 297±12 kg; BCS 3.5±1 units, and age between 12 and 14 months) were selected to be physiologically monitored during two consecutive summers. Physiological variables measured in cows were respiratory frequency (RF), rectal temperature (RT), and body surface temperatures (BST). The RF was measured visually counting the number of movements of the flank. The RT was measured inserting a digital thermometer (Nsf DeltaTrak, Mod. 11,062, Pleasanton, CA, USA) into the rectum, and finally BST was obtained using an infrared thermographic camera (Fluke Ti400, Everett, WA, USA) in the following anatomical sites: eye, nose, front head, head, neck, ear, shoulder, flank, belly, leg, loin, rump, and vulva. All sampling procedures were performed during the morning from 06:00 to 08:00 h, during 6 days of each month (July to September) of summer. The total number of observations collected was 1,242. Climatic data elements were ambient temperature (AT) and relative humidity (RH) were acquired from the state government officials (CONAGUA of Baja

California). With this data, the temperature-humidity index (THI) was estimated according to the formula proposed by Hahn (1999).

Coat color classification

Coat color was defined as the percentage of black and white colors in the body surface as shown in Figure 1. This trait was determined by visual observation directly in the pens and confirmed reviewing the pictures of each heifer following the procedure cited by Becerril & Wilcox (1992). In addition, heifers were photographed to better classify the coat color. A black coat color was considered when the heifers had $\geq 85\%$ of black coat color in their body (60 heifers). A white coat color when the heifer had $\geq 85\%$ of white coat color in their body (62 heifers). A mixed coat color when the heifer had approximately 50% of black coat color and 50% of white coat color (78 heifers). Thus, from the total data obtained (N=1, 242), it was divided into three groups according to this classification: black ($n_1=533$; 43%); white ($n_2=376$; 30%); and mixed ($n_3=333$; 27%).

Statistical analyses

An analysis of variance was performed to determine the effects of coat color on the physiological characteristics of heifers using PROC MIXED from SAS software (version 9.4, SAS Institute Inc., Cary N.C., USA). Significance was considered ($P < 0.05$). Pearson correlation analysis was developed within each coat color group using PROC CORR from SAS (version 9.4, SAS Institute Inc., Cary N.C., USA). The magnitude and direction of the correlations were classified according to its value in high ($r > 0.70$), modest ($0.35 \leq r \leq 0.70$), and low ($r < 0.35$). Also within each coat color of the heifers, multiple regression models were performed in order to predict RT as it was considered the dependent variable. The independent variables were BST measures, RF, and the three climatic variables (AT, RH, and THI). Before running the model, a series of analyzes were carried out to verify that the data follows a normal distribution in order to continue with the regression analysis using PROC UNIVARIATE of SAS. After verification that all data followed a normal distribution within each coat color group, a multiple linear regression (MLR) analysis was performed; the stepwise selection method was used for the determination of the final equation using PROC REG and PROC GLM to predict RT. This method builds regression models by adding or removing predictor variables in sequence

and examining the statistical significance of each explanatory variable after repeating mathematical assessment of the variable inclusion in the model (Sen and Srivastava, 1990). Assumptions of the regression model verified were: normal distribution with Shapiro-Wilk test and normality plots, homogeneity of variance using residual plots, multicollinearity checking variance inflation factors (VIF) and tolerance, and autocorrelation with the Durbin-Watson test (DW). The following statistical indicators were assessed to select the final model for each coat color group: adjusted R^2 , significance of the explanatory variables (F-test), CV, root mean square error (RMSE) and influence diagnosis through the use of the Cook's distance (Cook's D), differences in fits (DFFITS) and difference in betas (DFBETAS). The model's predictive ability was assessed thorough the estimation of statistical PRESS residual (prediction error of sum of squares). In order to validate the robustness of the selected model, the cross-validation method was applied randomly dividing the data set into two subsets: 70% of the data used as training model and the remaining 30% for testing purposes. If the regression indicators are close to those obtained with the original model, the selected model is declared the final model and can be used for prediction purposes. Significance was declared at ($P < 0.05$).

Results

Physiological and BST variables according to hair coat color

Averages and standard errors of physiological and thermal imaging variables of Holstein heifers according to their hair coat color are shown in Table 1. The influence of coat color on physiological parameters was evident as black heifers presented more RF and RT ($P < 0.01$) than mixed and white coat color heifers; even though white heifers had similar RT than mixed color heifers, they obtained less RF ($P < 0.05$) than mixed heifers. For the thermal imaging variables, black color heifers had higher BST ($P < 0.01$) in flank than mixed and white coat color heifers, with no differences between these last two groups. Black and mixed coat color heifers had higher BST ($P < 0.01$) than white heifers in ear, eye, head, forehead, rump, and vulva, with no differences between the first two color groups. Mixed coat color heifers had higher BST ($P < 0.01$) than black and white coat color heifers in nose and leg, with no difference between these last two groups. The BST of neck, lateral area, belly, and loin was higher ($P < 0.01$) in mixed coat color heifers than

in black coat color heifers; white coat heifers had the lowest BST compared to mixed and black color heifers in the same anatomical regions. Finally, white coat color heifers had higher BST ($P < 0.01$) just in shoulder compared to black and mixed coat color heifers; mixed coat color heifers had the lowest BST ($P < 0.01$) in shoulder.

Pearson's correlation coefficients of variables according to hair coat color

The Pearson's correlation coefficients among physiological and climatic variables with thermographic variables according to hair coat color are presented in Table 2. In general, the RT and RF were highly correlated ($P < 0.01$) in the three coat color groups, being very similar in the coefficients (black $r = 0.84$; white $r = 0.82$, and mixed $r = 0.78$). All correlations between RT and RF with BST variables in the black ($0.50 \leq r \leq 0.81$; $P < 0.01$) and white ($0.36 \leq r \leq 0.81$; $P < 0.01$) coat color groups were from modest to high, meanwhile for the mixed coat color group were from low to moderate ($0.25 \leq r \leq 0.64$; $P < 0.01$). Climatic variables AT and THI had high correlation ($r > 0.70$; $P < 0.01$) with RF and RT in black coat color heifers, while in white coat color heifers were from moderate to high ($0.64 \leq r \leq 0.75$; $P < 0.01$); in the mixed coat color heifers group those correlations were moderate ($0.50 \leq r \leq 0.59$; $P < 0.01$). The correlations among BST of black coat color heifers with AT and THI were from 0.51 to 0.86, which were considered from moderate to high associations ($P < 0.01$). This correlation pattern was a little wider in white coat color heifers ($0.40 \leq r \leq 0.84$; $P < 0.01$); but for mixed coat color heifers was more variable, being from low to moderate correlation ($0.10 \leq r \leq 0.59$; $P < 0.05$). In general, the climatic variables RH presented low correlation coefficients with all the physiological and BST variables, ranging from -0.21 to 0.30, and the majority of them were non-significant ($P > 0.05$).

Prediction of RT with MLR according to hair coat color

The equations built from MLR to estimate RT are shown in Table 3. Multicollinearity and heteroscedasticity among all predictor variables were tested. Influence diagnostic analysis resulted in the detection of just one outlier in the equation used for the black coat heifers, and that observation was deleted from the analysis. The remaining equations did not show any observation considered as outlier. Three models were proposed for each of the three coat color groups. In every single equation constructed, RF was the first predictor variable selected, except for the third equation proposed for black coat color heifers. In the black

coat color heifers, the first predictor variable and more important was RF (first equation), then RF and ear temperature (second equation), and finally the third equation was composed by relative humidity, RF, and ear temperature. For the mixed coat color heifers, RF was the first and more important predictor variable (first equation), then RF and head temperature (second equation), and finally RF, head temperature, and lateral area temperature (third equation). For the group of heifers with white coat color, again RF was the first variable to enter the equations (first equation), then in the second equation was RF and front head temperature, and the third equation was comprised by RF, front head temperature, and rump temperature. The p-value of the nine models proposed to predict RT was highly significant ($P < 0.01$).

For the three coat color groups, the three equations were ranked by the adjusted R^2 and the second criterion was the variation measures. It can be noted that adjustment increases and variation measures decrease as more variables are included in the model. For the group of black coat color heifers, the equation with RF explained 66.4% of the variation in RT; when ear temperature is included in combination with RF, this explanation increases in 2.2% (68.6%), and to 70.8% when the equation is comprised by relative humidity, RF and ear temperature (Table 3). For the group of mixed coat color heifers, the equation with RF explained 36.7% of the variation in RT; when the head temperature was added to the equation, the R^2 increased to 44.1% and to 45.8% when the third variable, temperature of lateral area entered to the equation. For the last coat color group of heifers, white, only RF explained 64.9% of the variation in RT; temperature of front head increased this R^2 to 70.4%, and the inclusion of rump temperature as the third predictor variable in the equation increased the adjustment to 71.9%.

Discussion

Physiological and BST variables according to hair coat color

The color of the coat, as well as the hair number and density, can influence physiological variables and the surface temperature of the animals when they are exposed to solar radiation in geographical areas with warm climates (Hansen, 1990; Becerril et al., 1993). In the present study, the black coat heifers had higher body temperature and respirations

per minute than the other two groups, white and mixed coat colors, which agrees with the results obtained by Anzures-Olvera et al. (2019) and Isola et al. (2020). Even more, RT of black coat color heifers was 0.5 °C warmer than the white coat color heifers, which was higher than the result reported by Anzures-Olvera et al. (2019), who found that black coat color cows in mid-lactation registered a rectal temperature 0.1 °C higher than white coat color cows. While Anzures-Olvera (2019) did not find a difference in RF in black and white coat color cows, we found a difference of almost 3.7 bpm in black coat heifers over white coat heifers and 2.5 bpm over mixed coat color heifers. The increased in RT and RF observed in black coat heifers supports the statement that animals with black coat have less ability to maintain thermal balance in the hot environment with high solar radiation during summer season (Maia et al., 2003). In this sense, Hillman et al. (2013) reported that black coat Holstein cows increase body temperature above 1.3°C compared to white coat cows (0.7°C) when they were exposed to direct sunlight; this result was attributed to the higher absorption of solar radiation of black coat cows (89%) compared to white coat cows (66%). Likewise, Stewart et al. (1953) indicated that solar radiation can have a major impact on thermal balance on black coat ruminants because white hair color may absorb only 40% solar radiation, meanwhile black hair color until 90%, consequently heat flux in the skin is greater for dark animals. The RT for mixed and white coat color heifers was similar, suggesting similar thermoregulatory capacity of these two groups of heifers. Coat color is one of the main distinguishing characteristics of breeds of cattle in the world, and the proportion of black spots ($h^2=0.75$; Maia et al., 2003) and the percentage of white color ($h^2=0.71$ to 0.79; Becerril et al., 1994) have been determined as highly heritable traits in Holstein cows.

Regarding the thermography measures, heifers classified as mixed coat color (approximately 50% black and 50% white) presented more anatomic regions (forehead, nose, ear, neck, leg, loin, and lateral area) with higher temperature than black or white coat heifers. Black coat heifers exhibited higher temperatures in head and flank, and white heifers just in shoulder. These results suggest that black and mixed coat color heifers had better thermoregulatory capacity compared to white-coated heifers. Isola et al. (2020) found that red and white Holstein heifers had higher superficial temperatures than black and white Holsteins under tropical conditions. Anzures-Olvera et al. (2019) reported lack

of association between coat color and skin temperature, which may be due to the presence of a cooling system used during summer in that study. In general, the estimated BSTs of the present study were below the body temperature of heifers (from 38.4 to 38.9 °C), which indicates that heifers were not in heat stress status. In this respect, Hansen (2004) stated that a surface body temperature greater than 36 °C indicates the presence of heat stress in cattle. According to the summary of the results of the physiological variables and thermography of the heifers in this study, it is important to take into account the phenotypic characteristics for any prediction model, because physiological variables behave differently according to the coat colors.

Pearson's correlations according to hair coat color

Pearson's correlation was a pre-analysis performed before running the regression model to assess the degree of association of the variables that will enter the regression model. This analysis was performed by the hair coat color of the heifers. Consistent with other studies, the correlation coefficient analysis showed a strong and positive relationship between RT and RF in the three hair coat colors (Vicente-Pérez et al., 2016). Also, this analysis demonstrated that all parts of the BST measured were positively associated with RT and RF for each coat color, where the black coat color had the highest association with physiological variables compared to the other two phenotypes. Unexpectedly, only the black coat heifers presented high correlation between eye temperature and RT, contrary to white and mixed coat color heifers, where correlations were from moderate to low ($r \leq 0.40$). Other studies where hair coat was not categorized by color, eye temperature observed moderate correlation with RT, such as Martello et al. (2015), Peng et al. (2019) and Theusme et al. (2022), who reported correlations around between 0.40 and 0.47.

In this study, temperatures of head and forehead were the most correlated variables with RT and RF for the three coat color groups. However, for the black coat color, head was the variable with the highest correlation with RT, while for white and mixed groups was forehead temperature. In spite of that, some anatomical regions did not correlate the same for the three phenotype groups with RT, such as the eye and vulva temperatures in black coat color heifers. However, temperature of the head area can provide very important information about the body temperature due to its proximity to the brain; some studies

reported that temperature internal organs such as the brain could be similar to body temperature (Kessel et al., 2010; Sellier et al., 2014). On the other hand, our results show a strong relationship between physiological and the climatic variables AT and THI, except with RH. This result can be because in desert climates, AT contributes most to the degree of heat stress than RH does because AT reaches very high values during summer, and the humidity is generally low in these ecosystems. Although some studies mention that heifers are not as vulnerable to high temperatures as lactating cows (Wang et al., 2020; Ji et al., 2020), it is important that milk producers consider this characteristics in their selection criterion. For many years, coat color was considered to have only esthetic worth, and for that reason, most breed livestock were formed using color patterns as trademark. Later on, some researchers began to show interest in this topic and started to notice the relationship between hair coat color and production performance (Finch et al., 1984). Holstein cows exposed to direct solar radiation that were predominately white were better at regulating body temperature and in maintaining milk yield than predominately black cows (Hansen, 1990). In another study with Holsteins (King et al., 1988), predominately-white hair coat cows were associated with better reproduction parameters in hot climate. Certainly, there is not much information about the association of RT and BST in livestock according to hair coat color. The relationship between coat color and thermoregulation in animals depends also on environmental factors and the individual ability of the animal to absorb solar radiation (Silva et al., 2001). Coat color is a characteristic that can play an important role in the bovine's ability to resist the detrimental effects of heat stress, and is associated with high incidence of direct solar radiation. The zone of study (Baja California) is characterized by extreme climatic conditions during summer since temperature may reach more than 50 °C, and solar radiation until 1000 WS (Theusme et al., 2021). Therefore, characteristics like coat color are important to include in the mathematical models built to make any inference or prediction on dairy cattle (Lees et al., 2018).

Regression models to predict RT according to hair coat color

The multiple regression equations to predict RT according to hair coat color are shown in Table 3. Three equations for each coat color are proposed for this prediction, and it is important to note that RF is a variable common in all resultant equations. This result is in

line with the degree of association between RT and RF, which is considered the highest coefficient of correlation estimated in this study. In the equation for each of the three coat color heifers, it is noted that as more predictor variables enter the model, the higher adjustment is observed (R^2) along with a reduction in variability indicators (MSE, CV, PRESS). In general, this is a common pattern in multiple regression analysis (Myers, 1990).

For black coat color heifers, an increase of more than 2% is observed in the model from one to two predictor variables, and from two to three predictor variables; the model with 3 predictor variables, $RT = 35.59 - 0.013(\text{relative humidity}) + 0.045(\text{respiration frequency}) + 0.019(\text{ear temperature}) + e_i$, was selected as the best to predict RT. In this model, a coefficient of determination of almost 71% was obtained. For white coat color heifers, the first model including RF reached a R^2 of 65%; when the variable temperature of forehead entered the model, the R^2 increase to 70%, and when a third variable was included the R^2 rose just 1.5% ($R^2=71.4\%$). Considering this last increased very low, the best equation to predict RT was the second one which included two predictor variables: $RT = 35.29 + 0.035(\text{respiration frequency}) + 0.033(\text{forehead temperature}) + e_i$. The lowest adjustments were observed for the mixed coat color heifers (~ 50:50 black and white); again, the predictor variable that most explained RT was RF, reaching a R^2 of 37%. When the second variable entered the model, the R^2 increase to 44%, and with a third predictor variable the adjustment reached 45.8%. So the second model was selected as the best to predict RT which was: $RT = 35.07 + 0.022(\text{respiration frequency}) + 0.038(\text{head temperature}) + e_i$. In all cases, all predictor variables in the selected models were highly significant and variability indicators decreased as predictor variables entered the model.

Construction of equations to predict RT using multiple regression analysis in dairy cattle does not overcome 70% of adjustment. Theusme et al. (2022) used multiple regression analysis to predict RT in Holstein heifers using thermography, climatic and physiological variables, and reached a R^2 of 60% with respiration frequency and forehead temperature as predictor variables. However, using lactating cows in a tropical environment, Daltro et al. (2017) reported in equation with a R^2 of 70% including the variables lateral region of the udder, posterior region of the udder, and left area of the cow; this last variable in its form linear and quadratic. Furthermore, Martello et al. (2010) elaborated equations to

predict RT based on simple linear regression analysis; respiratory frequency was the variable that most explained RT and the estimated R^2 was 51%. Results obtained in the present study showed that for heifers with well-defined hair coat color (i.e., mostly white or black), the use of physiological and climatic variables, along with temperature of different anatomical regions of the animal body registered with infrared thermography, have the potential to be used as indicators of body temperature. This information can be used to identify possible thermal stress in Holstein prepubertal heifers. For heifers with hair coat color mixed, that is black and white coat color in similar proportions, this technique did not achieve good results since the adjustment was insufficient ($R^2 < 50\%$). More studies are needed to confirm the association of these variables, especially in arid zones with high solar radiation.

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Data availability. Not applicable.

Declarations

Ethical standards. All animal used in this research were managed according to the procedures and data collection indicated in the local guidelines and officially endorsed for the care of animals in Mexico (NOM-051-ZOO-1995: humane care of animals during management and mobilization).

Conflict of interests. The authors declare that there are no competing interests.

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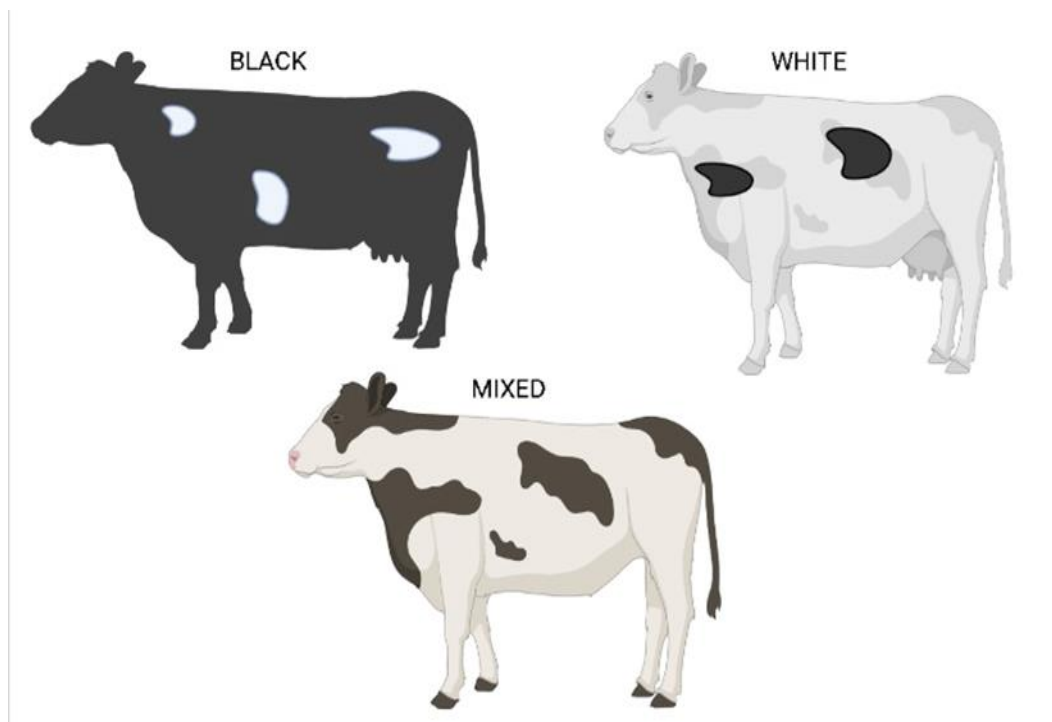


Figure 1. Hair coat color classification according to the amount of black and white colors in the body.

Table 9 Respiratory frequency and rectal and body surface temperatures of Holstein heifers according to the hair coat color (black, white, or mixed [50 % black and 50 % white]).

Variables	Black	White	Mixed	SEM
Respiratory frequency (rpm)	71.73 ^a	68.01 ^b	69.29 ^c	0.26
Rectal temperature (°C)	38.91 ^a	38.40 ^b	38.44 ^b	0.02
Body surface temperatures (°C)				
Head	32.27 ^a	31.50 ^b	32.12 ^a	0.17
Forehead	32.40 ^a	31.40 ^b	32.50 ^b	0.18
Nose	28.19 ^a	28.04 ^a	29.00 ^b	0.11
Eye	36.51 ^a	35.75 ^b	36.37 ^a	0.12
Ear	31.34 ^a	31.25 ^a	31.89 ^b	0.20
Neck	35.55 ^a	35.36 ^a	35.95 ^b	0.10
Loin	34.89 ^a	34.21 ^b	35.24 ^c	0.15
Leg	35.52 ^a	34.82 ^b	36.09 ^c	0.15
Rump	35.84 ^a	35.41 ^b	35.94 ^a	0.12
Flank	36.01 ^a	35.52 ^b	35.67 ^a	0.11
Shoulder	35.87 ^a	36.03 ^b	35.31 ^a	0.13
Belly	35.88 ^a	35.42 ^b	36.21 ^b	0.13
Vulva	35.90 ^a	35.51 ^b	35.89 ^a	0.13
Lateral area	34.12 ^a	33.70 ^a	35.29 ^c	0.16

Different superscripts (a, b, c) within row indicate differences at $P < 0.05$.

SEM= Standard error of the mean.

Table 10 Correlation coefficients of the body surface temperatures with rectal temperature, respiratory frequency, and climatic variables according to the hair coat color (black, white, or mixed [50 % black and 50 % white]) in Holstein heifers.

	Black					White					Mixed				
	RT	RF	AT	RH	THI	RT	RF	AT	RH	THI	RT	RF	AT	RH	THI
RT	---	0.84 **	0.80 **	0.17 **	0.81 **		0.82 **	0.73 **	0.17 **	0.75 **		0.78 **	0.51 **	0.20 **	0.55 **
RF	---	---	0.70 **	0.20 **	0.75 **			0.64 **	0.18 **	0.67 **			0.50 **	0.30 **	0.59 **
T _{Head}	0.81 **	0.80 **	0.85 **	0.24 **	0.86 **	0.80 **	0.74 **	0.84 **	0.15 **	0.84 **	0.64 **	0.64 **	0.61 **	0.27 **	0.65 **
T _{Forehead}	0.81 **	0.81 **	0.81 **	0.17 **	0.81 **	0.81 **	0.76 **	0.84 **	0.09 ns	0.82 **	0.59 **	0.59 **	0.55 **	0.13 *	0.54 **
T _{Nose}	0.75 **	0.72 **	0.78 **	0.26 **	0.80 **	0.72 **	0.64 **	0.80 **	0.16 **	0.80 **	0.55 **	0.54 **	0.56 **	0.23 **	0.58 **
T _{Eye}	0.70 **	0.58 **	0.59 **	- 0.12 *	0.51 **	0.39 **	0.36 **	0.48 **	- 0.21 **	0.40 **	0.30 **	0.30 **	0.19 **	0.07 ns	0.25 **
T _{Ear}	0.75 **	0.70 **	0.79 **	0.26 **	0.82 **	0.71 **	0.65 **	0.79 **	0.16 *	0.79 **	0.57 **	0.57 **	0.59 **	0.26 **	0.62 **
T _{Neck}	0.70 **	0.66 **	0.75 **	0.05 ⁿ s	0.71 **	0.65 **	0.55 **	0.75 **	0.02 ⁿ s	0.71 **	0.52 **	0.50 **	0.59 **	0.18 ns	0.59 **

T _{Loin}	0.67 **	0.61 **	0.74 **	0.02 ⁿ s	0.68 **	0.64 **	0.53 **	0.77 **	0.04 ⁿ s	0.73 **	0.26 **	0.26 **	0.43 **	0.09 ns	0.38 **
T _{Leg}	0.65 **	0.60 **	0.74 **	- 0.04 ⁿ s	0.66 **	0.64 **	0.54 **	0.74 **	- 0.05 ⁿ s	0.69 **	0.34 **	0.34 **	0.49 **	0.08 ns	0.45 **
T _{Rump}	0.56 **	0.55 **	0.56 **	0.05 ns	0.54 **	0.57 **	0.52 **	0.56 **	- 0.01 ⁿ s	0.54 **	0.34 **	0.34 **	0.43 **	0.21 **	0.46 **
T _{Flank}	0.54 **	0.50 **	0.53 **	0.08 ns	0.51 **	0.50 **	0.48 **	0.57 **	0.05 ns	0.56 **	0.25 **	0.33 **	0.42 **	0.28 **	0.21 **
T _{Belly}	0.54 **	0.59 **	0.71 **	0.06 ns	0.67 **	0.63 **	0.52 **	0.72 **	- 0.02 ⁿ s	0.68 **	0.31 **	0.31 **	0.46 **	0.11 *	0.11 *
T _{Shoulder}	0.66 **	0.64 **	0.70 **	0.04 ⁿ s	0.66 **	0.61 **	0.52 **	0.72 **	0.08 ns	0.67 **	0.33 **	0.33 **	0.51 **	0.15 **	0.15 **
T _{Vulva}	0.66 **	0.60 **	0.71 **	0.15 **	0.70 **	0.75 **	0.54 **	0.72 **	0.08 ns	0.71 **	0.40 **	0.42 **	0.51 **	0.24 **	0.25 **
T _{LA}	0.62 **	0.62 **	0.68 **	- 0.05 ⁿ s	0.61 **	0.53 **	0.68 **	0.68 **	- 0.12 *	0.61 **	0.33 **	0.33 **	0.49 **	0.10 *	0.10 *

RT= Rectal temperature, RF= Respiration frequency, AT= Air temperature, RH= Relative humidity, THI= Temperature-humidity index, T= Coat temperature, T_{LA}= Lateral area temperature.

** P < 0.01, ns P > 0.05.

Table 11 Equations developed to predict rectal temperature (RT) in Holstein heifers according to the hair coat color using physiological and climatic variables as predictors.

Prediction equations	R ²	R ² _{adj}	MSE	CV	PRESS	Cp	DW	P-value
Black heifers (n=532)								
1) RT= 35.28 + 0.049(RF)	0.66	0.66	0.05	0.56	25.50	87.1	1.42	<0.0001
2) RT= 35.11 + 0.045(RF) + 0.016(T _{Ear})	0.69	0.69	0.04	0.54	23.86	47.6	1.49	<0.0001
3) RT= 35.59 – 0.013(RH) + 0.045(RF) + 0.019(T _{Ear})	0.71	0.71	0.04	0.53	22.24	8.8	1.61	<0.0001
White heifers (n=377)								
1) RT= 35.49 + 0.048(RF)	0.65	0.65	0.60	0.66	24.82	110.8	1.59	<0.0001
2) RT= 35.29 + 0.035(RF) + 0.033(T _{Forehead})	0.71	0.70	0.05	0.61	20.99	35.9	1.70	<0.0001
3) RT= 34.65 + 0.035(RF) + 0.022(T _{Forehead}) + 0.029(T _{Rump})	0.72	0.72	0.05	0.59	20.02	16.8	1.79	<0.0001
Mixed heifers (n=332)								
1) RT= 36.76 + 0.03(RF)	0.37	0.37	0.09	0.77	29.92	124.2	1.09	<0.0001
2) RT= 35.07 + 0.022(RF) + 0.038(T _{Head})	0.44	0.44	0.08	0.73	26.52	72.5	1.14	<0.0001
3) RT= 35.61 + 0.021(RF) + 0.029(T _{Head}) + 0.023(T _{LA})	0.46	0.46	0.08	0.72	25.81	61.5	1.17	<0.0001

R²= Coefficient of determination, R²_{adj}= Adjusted coefficient of determination, MSE= Mean square error, CV= Coefficient of variation, Cp= Mallow's statistics, DW= Durbin-Watson test, RF= Respiratory frequency, RH= Relative humidity, T=temperature, T_{LA}= Temperature of lateral area

CAPÍTULO IV. CONCLUSIONES GENERALES

La mayoría de las temperaturas de la superficie corporal registradas con termografía infrarroja de vaquillas Holstein se correlacionaron positiva y moderadamente ($0.40 < r < 0.75$; $P < 0.05$) con la temperatura rectal y con la frecuencia respiratoria, ya sea incluyendo o no el color del pelaje, lo que sugiere que el aumento de la temperatura corporal se asocia con el aumento de estas dos importantes variables fisiológicas de ganado lechero.

En general, la temperatura de la frente medida con termografía infrarroja, en combinación con la frecuencia respiratoria, explicaron 60% de la variación en temperatura rectal, ecuación que fue validada y proporcionó un modelo de regresión múltiple con ajuste satisfactorio ($R^2=60\%$). Sin embargo, al considerar el color del pelaje, la frecuencia respiratoria junto con la humedad relativa y la temperatura de la oreja, la ecuación de predicción logró un aumento en el ajuste de 11% ($R^2=71\%$) en vaquillas blancas, mientras que en vaquillas negras se logró este mismo ajuste, pero considerando la frecuencia respiratoria y la temperatura de la oreja. La ecuación para vaquillas de color de pelaje mixto (~ 50% color blanco y 50% color negro) tuvo un ajuste más bajo ($R^2=46\%$) e incluyó frecuencia respiratoria y temperaturas de cabeza y zona lateral derecha. Las vaquillas de color de pelaje negro presentaron mayor ($P < 0.05$) frecuencia respiratoria y temperatura rectal que vaquillas de color blanco y las de color mixto. En general, las vaquillas de color negro o mixto tuvieron mayores ($P < 0.01$) temperaturas de la superficie del cuerpo que las vaquillas mayormente blancas en su pelaje.

Estos resultados sugieren que la temperatura rectal puede ser predicha utilizando un método no invasivo de medición de temperatura corporal junto con variables climáticas y variables fisiológicas en vaquillas Holstein. La precisión de esta predicción puede mejorar al considerar el color del pelaje del animal, principalmente en vaquillas con color definido (> 85% de color blanco o negro). Por lo tanto, la termografía infrarroja representa una tecnología que puede sustituir a la medición tradicional invasiva consistente en el uso de un termómetro que se inserta en el recto del animal, para lo cual tuvo que ser inmovilizado. Este estudio también significa una aportación al Bienestar Animal al proponer el uso de una tecnología moderna para realizar una medición de una constante fisiológica asociada al grado de estrés calórico que sufre un animal, lo cual también se puede asociar a múltiples complicaciones de salud que se presentan en explotaciones lecheras Holstein en zonas cálidas.