UNIVERSIDAD AUTÓNOMA METROPOLITANA

MAESTRÍA EN CIENCIAS AGROPECUARIAS

Termoestabilidad del búfalo de agua durante la movilización y caracterización fisicoquímica de diferentes cortes cárnicos en comparación con el bovino

T E S I S

(Idónea Comunicación de Resultados)

Que para obtener el grado de

Maestra en Ciencias Agropecuarias

PRESENTA

M.V.Z. Daniela Rodríguez González

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Dr. Daniel Mota Rojas

Co-Directora:

Dra. Isabel Guerrero Legarreta

Asesora:

Dra. Rosy Gabriela Cruz Monterrosa

Ciudad de México, noviembre, 2023

AGRADECIMIENTOS

A mi Comité Tutoral

Agradezco infinitamente a cada miembro de mi comité, Dr. Mota, Dra. Isabel y Dra. Rosy, gracias infinitas por cada asesoría, cada minuto de información, dedicación y paciencia que me regalaron, son sin dudarlo el mejor Comité que pude pedir, son profesores brillantes y sumamente generosos, gracias por dar oportunidad a aquel que busca aprender de ustedes, son el ejemplo de que siempre es bueno hacer equipos para sobresalir en la vida tanto personal como académica, Dr. Mota, gracias por creer en mí, su guía y todos sus consejos, los llevaré grabados para siempre en la memoria en mi futuro profesional.

A mi familia

Agradezco a mi familia por apoyarme siempre y en cualquier proceso, ustedes han sido mi ejemplo, mi motor y mi lugar seguro, sé lo afortunada que soy por contar con ustedes y todo el cariño que han puesto en cada aprendizaje que me han dado, hoy me permiten tomar las mejores decisiones para mi futuro… Mamá, tú eres mi mayor ejemplo, la mujer más fuerte y brillante que conozco, gracias por demostrármelo cada día y por estar siempre al pendiente de todos nosotros, por favor no nos faltes jamás. Papá, tú me enseñas a ser yo misma y me preparas para el mundo con tus anécdotas (contadas en voz bajita), gracias por decirme de tu manera tan particular lo mucho que me amas, es un verdadero honor ser tu xocoyota, me apoyas en todo y jamás me juzgas, no tienes idea de cómo lo valoro. Hermanos (Alfo, Lui y Paco), ustedes son brillantes en cada trabajo que desempeñan y cada tarea que se proponen, me han brindado justo lo que una hermana menor necesita sin siquiera esforzarse, y, chiquis (sobrinos), ustedes son preciosos desde que nacieron y no tienen idea de la fortuna que tienen por estar con estas personas que, sin importar lo que suceda, siempre los amaremos y estaremos con ustedes en cada decisión y paso que den, gracias por sonreírme siempre, son mi mayor alegría.

A mis amigos

Esto no habría sido posible sin la familia que uno escoge, que, sin saber algo de los bufalitos, calidad de carne o bienestar animal, siempre me escucharon atentos, hablando con honestidad y apoyando mis ideas respecto a cada cosa que he querido hacer, abrazándome, amándome y acompañándome, repitiéndome que todo saldría bien, gracias

por impulsarme, son maravillosos, los amo muchísimo y siempre me sentiré afortunada y orgullosa del privilegio de acompañarnos en nuestras vidas y nuestros sueños… quédense siempre.

Agradezco a la empresa ganadera “Búfalo Ranch” y a Andrés Facundo por todas las facilidades, el apoyo y la confianza brindada en este proceso, dándome la oportunidad de monitorear movilizaciones animales.

Gracias al jurado designado por La Comisión Académica de la Maestría en Ciencias Agropecuarias, de la Universidad Autónoma Metropolitana por sus valiosas sugerencias.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por la beca otorgada para los estudios de maestría en ciencias, con el número de registro 802442, que comprendió del periodo de 01 de agosto de 2021 al 31 de Julio de 2023.

Daniela Rodríguez González

Ciudad de México, 16 de noviembre del 2023.

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ÍNDICE GENERAL

ÍNDICE DE FIGURAS

ÍNDICE DE TABLAS

ABREVIATURAS

ACTH: hormona adrenocorticótropa

SADER: Secretaría de Agricultura y Desarrollo Rural

IRT: termografía infrarroja

VC: Viajes cortos

VL: Viajes largos

F1: Fase 1- Potrero

F2: Fase 2- Arreo

F3: Fase 3- Estabulación previa al embarque

F4: Fase 4- Manga de manejo

F5: Fase 5- Embarque

F6: Fase 6- Pre transporte

F7: Fase 7- Post transporte

CRA: Capacidad de retención de agua

pH: Potencial de hidrógeno

POA: Región preóptica del hipotálamo

TRPV: Receptor de potencial transitorio vaniloide

LPBd: Núcleo parabraquial lateral

RVMM: Médula ventromedial rostral

SNS: Sistema nervioso simpático

SNA: Sistema nervioso autónomo

HPA: Hipotálamo pituitario suprarrenal

TA: Temperatura ambiente

HR: Humedad relativa

NaCl: Cloruro de sodio

M: Moles

RESUMEN

Evaluar el bienestar de los búfalos durante el transporte es clave para la obtención y comercialización de productos cárnicos de alta calidad, por ello se requiere una evaluación integral que ayude al reconocimiento de factores estresantes y evaluaciones efectivas requieren del reconocimiento de factores estresantes que activan mecanismos fisiológicos que pueden tener repercusiones en la salud y el desempeño productivo de las especies. Por lo anterior el objetivo de esta tesis fue evaluar el efecto del arreo, embarque y duración del traslado de búfalos de agua en la respuesta de la microcirculación dérmica, así como, estimar las características fisicoquímicas de tres diferentes cortes cárnicos en comparación con el bovino criado en trópico húmedo mexicano. Para lograr este objetivo en 2 fases; ante y post mortem. La primera fase se subdividió se en dos trabajos centrales, el primero se basó en la monitorización de la temperatura superficial mediante termografía infrarroja (IRT) de búfalos de agua transportados en viajes cortos con el objetivo de caracterizar el comportamiento térmico durante las etapas de movilización y, para el segundo trabajo se realizó la monitorización de búfalos de agua transportados en viajes cortos (VC) vs largos (VL) para analizar el factor tiempo sobre el comportamiento térmico.

Para el primer trabajo de la fase ante mortem se monitorizaron 624 búfalos de agua durante 12 VC (duración promedio = 2 h ± 20 min) centrándose en 11 regiones del cuerpo y la cara (carúncula lagrimal, periocular), área nasal (ventana térmica de las fosas nasales), conducto auditivo, párpado inferior, zona parietofrontal y regiones torácica, abdominal, apendicular, dorsal y lumbar. Los registros se realizaron durante siete fases: potrero (F1), arreo (F2), estabulación (F3), manga de manejo (F4), embarque (F5) y pre (F6) y post-transporte (F7). Se capturaron 48,048 lecturas de las 11 ventanas térmicas. Los resultados mostraron que las temperaturas de la superficie de las ventanas aumentaron hasta 5°C durante F2, F3, F5, F6 y F7, en comparación con F1 y F4 ($p<0,0001$). También se observaron diferencias de al menos 1°C entre ventanas en las zonas frontal-parietal, corporal lateral y periférica ($p<0,0001$). Respecto al segundo trabajo (VC vs VL) de la fase ante mortem se monitorizaron 1516 búfalos de agua durante 15 VC (783 animales, duración promedio del viaje= 50.33 min ± 5.48 min) y 14 VL (733 animales, duración promedio del viaje=13.31 h ± 47.32 min). La monitorización se realizó sobre las mismas regiones corporales y faciales que el primer documento, y durante las mismas fases del transporte que el primer documento, capturándose un total de 60291 lecturas de las 11 ventanas térmicas. Los resultados indicaron que la temperatura de todas las ventanas fue significativamente mayor durante el VC (+3°C) con respecto al VL ($p<0.001$). Para ambos grupos la región frontal-parietal tuvo un aumento significativo de 10°C durante la F7 ($p<0.001$). Así mismo, las fases de arreo, manga de manejo, embarque, pre y post transporte presentaron una mayor respuesta térmica, posiblemente influenciada por la interacción humano-animal. Finalmente, en ambos trabajos se encontró una fuerte correlación positiva ($r=0,9$, $p<0,0001$) entre las ventanas. Para la fase post mortem se realizó la caracterización y análisis de las características fisicoquímicas de cortes 3 cárnicos (New York, Pierna y Rib Eye) de búfalos de agua y bovinos del género *Bos*, así como un análisis hedónico para evaluar el grado de aceptación de ambas especies. De manera general se observaron valores más elevados de pH (dentro de los valores reportados para este especie) en los cortes bufalinos, afectando características como color, terneza y capacidad de retención de agua (CRA) ($p<0.05$), pese a estos valores variados entre especies no se presentaron afectaciones significativas respecto al nivel de aceptación de la carne de búfalo vs bovino del género *Bos*, lo anterior genera bases para desmitificar la no aceptación de la carne de búfalo por presentar características físicas y sensoriales poco atractivas para el consumidor final. En conclusión, los cortes de búfalo de agua presentaron una mayor fuerza de corte (Kgf), a*, pH, y CRA (%), así mismo, los procesos de cocción impactaron en las características fisicoquímicas estudiadas, sin embargo, estas diferencias no representan un impacto sobre el nivel de aceptación del consumidor final (excepto aroma para carne de bovino).

Palabras clave; Calidad de carne, búfalo de agua, IRT, movilización animal, bienestar animal.

ABSTRACT

Assessing the buffalo welfare during transportation plays a crucial role in acquiring and marketing high- quality, therefore a comprehensive evaluation is required to help recognize stressors and evaluations assessments necessitate the identification of stressors that trigger physiological processes with potential implications for the health and productivity of the species. Therefore, the aim of this thesis was to evaluate the effect of herding, loading and duration of transport of water buffaloes on the response of dermal microcirculation, as well as to estimate the physicochemical characteristics of three different cuts of meat in comparison with the raised bovine in the humid Mexican tropics. To achieve this goal in 2 phases; ante and post mortem. The first phase was subdivided into two main stages. The initial stage involved the use of infrared thermography (IRT) to monitor the surface temperatures of water buffalo during short-distance journeys, with the objective of characterizing their thermal responses during the various phases of transportation. In the second stage, water buffalo transported on short journeys (SJ) were compared with those transported on long journeys (LJ) to investigate the influence of time on thermal behavior. For the first stage of the ante mortem phase, 624 water buffaloes were monitored during 12 SJ (average duration = 2 h ± 20 min) focusing on 11 regions of the body and face (lacrimal caruncle, periocular), nasal area (thermal window of nostrils), auditory canal, lower eyelid, parietofrontal area and thoracic, abdominal, appendicular, dorsal and lumbar regions.

The records were carried out during seven phases: paddock (P1), herding (P2), corral (P3), handling chute (P4), loading (P5) and pre (P6) and post-transport (P7). 48,048 readings were captured from the 11 thermal windows. The results showed that window surface temperatures increased by up to 5°C during P2, P3, P5, P6 and P7, compared to P1 and P4 ($p<0.0001$). Differences of at least 1°C were also observed between windows in the frontal-parietal, lateral body and peripheral areas ($p<0.0001$). Regarding the second stage (SJ vs LJ) of the ante mortem phase, 1516 water buffaloes were monitored during 15 SJ (783 animals, average journey duration = 50.33 min ± 5.48 min) and 14 LJ (733 animals, average journey duration = 13.31 h ± 47.32 min). Monitoring was carried out on the same body and facial regions as the first stage, and during the same transport phases as the first stage, capturing a total of 60,291 readings from the 11 thermal windows. The results indicated that the temperature of all windows was significantly higher during SJ (+3°C) compared to LJ ($p<0.001$). For both groups, the frontal-parietal region had a significant increase of 10°C during P7 ($p<0.001$). Likewise, the herding, handling, loading, pre- and post-transport phases presented a greater thermal response, possibly influenced by human-animal interaction. Finally, in both stages a strong positive c correlation (r=0.9, p<0.0001) was found between the windows.

In the post-mortem phase, the characterization and analysis of the physicochemical characteristics of 3 meat cuts (New York, Shank and Rib Eye) of water buffaloes and bovines of the *Bos* genus were carried out, as well as a hedonic analysis to evaluate the degree of acceptance of both species. In general, higher pH values (within the values reported for this species) were observed in the buffalo cuts, affecting characteristics such as color, tenderness and water retention capacity (WHC) (p<0.05), despite these varied values between species, there were no effects significant with respect to the level of acceptance of buffalo meat vs bovine meat of the *Bos* genus, the above generates bases to demystify the non-acceptance of buffalo meat due to its physical and sensory characteristics that are unattractive to the final consumer. In conclusion, water buffalo cuts presented greater cutting force (Kgf), a*, pH, and WHC (%), likewise, the cooking processes impacted the physicochemical characteristics studied, however, these differences did not represent an impact on the level of acceptance of the final consumer (except aroma for beef).

Key words: Meat quality, water buffalo, IRT, animal mobilization, animal welfare.

1. INTRODUCCIÓN

La producción de búfalos de agua a nivel mundial se ve enfocado en producir leche y carne, en el primer caso representa más del 15% de la producción mundial y su producción cárnica aporta más del 4% del total global (IDF, 2020), resaltando el continente asiático y particularmente la India con un 80% de la población total de búfalos (Mota-Rojas et al., 2019). El búfalo de agua es catalogado como la sexta especie productiva más abundante en el mundo, por debajo de los inventarios avícola, bovino convencional del género *Bos,* porcino, ovino y caprino (Young et al., 2019).

En México se estima una población de 60 mil búfalos de agua, distribuidos en todo el país y presentes principalmente en áreas del trópico húmedo, teniendo un crecimiento importante debido a sus características positivas tanto económica como agroecológicamente (Bertoni, 2019; Bertoni et al., 2021), pese a ello no se cuenta con legislación específica para esta especie, aunque sí ha sido señalada como un vector y hospedero de enfermedades en campaña sanitaria y de importancia para México, presentando inconsistencias legislativas y limitaciones comerciales y gubernamentales, además de no proporcionar a los productores, manejadores y procesadores información referente a la aplicación de buenas prácticas pecuarias específicas para el búfalo de agua (Rodríguez-González et al., 2022a; Rodríguez-González et al., 2022b), pudiendo generar un impacto negativo en el grado de bienestar durante su crianza, desarrollo, movilización, finalización y matanza (DOF, 1995, 1996, 2012, 2018).

Resultando de suma importancia el conocimiento de las condiciones óptimas productivas y el alcance que esta especie, así como los factores generadores de estrés y dolor de acuerdo con ambiente en el cual se desenvuelve, siendo el transporte uno de los escenarios más estresantes durante la vida productiva (Schwartzkopf-Genswein et al., 2016). Esta actividad es comúnmente observada en animales próximos a ser sacrificados o aquellos que pasarán el último ciclo productivo de finalización y engorda en potreros especializados (Chandra & Das, 2001; José-Pérez et al., 2022; Rodríguez-González et al., 2022c).

Durante este proceso los búfalos son expuestos a factores estresantes como movimiento, ruido, condiciones climáticas extremas, hacinamiento, manipulación excesiva e inadecuada, lesiones, restricción de alimento y bebida, modificaciones en estructuras jerárquicas (Alam et al., 2010a; Bethancourt-Garcia et al., 2019; Gallo & Huertas, 2016; Marai & Haeeb, 2010; Strappini et al., 2012; Valkova et al., 2021). De esta forma, factores logísticos como descanso inadecuado (José-Pérez et al., 2022), uso excesivo de palos, arreadores eléctricos o herramientas físicas por parte de los operadores (Alam et al., 2010b) y tiempo de traslado (Gupta et al., 2007) destacan como los principales aspectos que pueden generar problemas agudos físicos y psicológicos sobre los búfalos movilizados. Con respecto a la duración del traslado, este factor es considerado un determinante en el bienestar de los semovientes debido a que la suma de factores estresantes durante la movilización se ve exponenciada por la duración del mismo (Damtew et al., 2018), y se ha señalado que a mayor duración del traslado se tiene una disminución del peso vivo y agotamiento físico (Cueto, 2020), sin embargo y para el búfalo de agua no existen investigaciones comparativas respecto al tiempo del traslado y su impacto sobre valores fisiológicos (Rodríguez-González et al., 2023a).

Así mismo, los cambios fisiológicos que se generan durante el transporte van desde la deshidratación, hiporexia, daño tisular, inhalación de humo, estrés físico, térmico y psicológico (Murata et al., 2004; Van Engen & Coetzee, 2018; Wong et al., 2003, 2004), y pueden resultar en la inhibición del sistema inmune por la exposición prolongada a estos estímulos adversos (Mackenzie et al., 1997). En este sentido, se han reportado indicadores conductuales, patológicos y fisiológicos con el objetivo de evaluar el nivel de estrés que perciben los animales durante el transporte, entre ellos el aumento de cortisol sanguíneo, proteínas séricas y los niveles de glicemia posterior a 16 h de transporte, así como la pérdida de peso yendo de 7.9% hasta 10.5% (Cueto, 2020; Liotta et al., 2007), aspecto relacionado con la pérdida de energía debido a la glucogenólisis muscular y hepática (Averós et al., 2008).

De igual forma otro indicador fisiológico que se ha sugerido es el incremento de la temperatura corporal, el cual puede ayudar a reconocer procesos de estrés y relacionarse con el estado de salud de los animales (Idris et al., 2021; Rodríguez-González et al., 2023b). Sumado a lo anterior el búfalo de agua se ha señalado como una especie con especial susceptibilidad al estrés térmico debido a un ineficiente sistema de termorregulación (Bertoni et al., 2020a; Mota-Rojas et al., 2021a), por lo anterior se recomienda su monitorización térmica evitando generar mayor estrés en los animales ante una excesiva manipulación, por ello se recomienda el uso de tecnologías no invasivas como IRT para conocer y monitorizar los factores que pudieran tener mayor impacto y la respuesta sobre búfalos de agua movilizados.

De este modo, en caso de generarse hiperemia o estrés durante el proceso ante mortem se observan efectos post mortem que impactan de manera negativa en características fisicoquímicas cárnicas que afectan la comercialización de los productos cárnicos de búfalos en México (Rodríguez-González et al., 2023b), en donde, inicialmente es necesario el conocimiento de las características fisicoquímicas de estos para su divulgación, comercialización y desarrollo de mercados dirigidos e informados (Rodríguez-González et al., 2022c), Sin embargo y pese a el crecimiento visto de esta especie y sus múltiples propiedades beneficiosas, no se cuenta con una caracterización de cortes de búfalos producidos en México, en este sentido, de presentarse modificaciones en los parámetros de la carne cuando se tienen malas prácticas durante su desarrollo y finalización se desconocería su impacto específico sobre valores como pH, textura, CRA, color, sabor y olor, entre otros (Napolitano et al., 2020; Gallo et al., 2018; Gallo y Huertas, 2016; José-Pérez et al., 2022; Muñoz et al., 2012; Rodríguez-González et al., 2022). Igualmente, no se cuenta con la diferenciación de parámetros cárnicos respecto a bovinos del género *Bos* producidos en las mismas condiciones de sistema de doble propósito en el trópico húmedo mexicano (área de mayor concentración de ganado bufalino) (Rodríguez-González et al., 2022c).

Por lo anterior, el objetivo del presente estudio fue evaluar la temperatura superficial de diferentes regiones corporales y cráneo- faciales en búfalos de agua durante eventos previos a la movilización (desde la estancia en potrero hasta el embarque) y posterior a su movilización por un periodo corto, además de observar si estos cambios se comportan de manera similar en transportes cortos vs largos, evaluando a su vez, el nivel de correlación entre ventanas centrales y periféricas, sumado a ello, también se tuvo el interés de caracterizar y comparar las propiedades fisicoquímicas de tres diferentes cortes cárnicos de búfalo de agua y carne de bovino (cruda y cocida) criado en el trópico húmedo mexicano y su nivel de aceptación de ambas especies mediante un análisis hedónico de la carne.

2. MARCO TEÓRICO

Artículo de revisión intitulado:

2.1. CAPÍTULO I

Aspectos críticos de la legislación y su impacto en el bienestar de los búfalos de agua durante el transporte

Publicado en la revista Journal of Animal Behaviour and Biometeorology, indizada a Web of Science y Scopus, con un factor de impacto de 2.1.

Rodríguez-González, D.; Napolitano, F.; Guerrero-Legarreta, I.G.; Mora-Medina, P.; Ghezzi, M.; José-Pérez, N.; Domínguez-Oliva, A.; Mota-Rojas, D. Critical aspects of legislation and their impact on the welfare of water buffaloes during transport*. J. Anim. Behav. Biometeorol.* 2022, 10;2231, 1–13, http://dx.doi.org/10.31893/jabb.22032

JOURNAL OF ANIMAL BEHAVIOUR AND BIOMETEOROLOGY

REVIEW ARTICLE
Published Online: September 12, 2022
https://doi.org/10.31893/jabb.22032

Critical aspects of legislation and their impact on the welfare of water buffaloes during transport

Daniela Rodríguez-González[a] | Fabio Napolitano[b] | Isabel Guerrero Legarreta[c] |
Patricia Mora-Medina[d] | Marcelo Daniel Ghezzi[e] | Nancy José-Pérez[f] |
Adriana Domínguez-Oliva[a] | Daniel Mota-Rojas[f*]

[a]Master's Program in Agricultural Sciences "Maestria en Ciencias Agropecuarias", Xochimilco Campus, Universidad Autónoma Metropolitana, Mexico City 04960, Mexico.
[b]Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, 85100, Potenza, Italy.
[c]Emeritus Professor Universidad Autónoma Metropolitana-Iztapalapa, (UAM-I), Deparment of Biotechnology: Food Science, Mexico City, Mexico.
[d]Department of Livestock Sciences, Universidad Nacional Autónoma de México (UNAM), FESC. Mexico.
[e]Animal Welfare Area, Faculty of Veterinary Sciences (FCV). Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Argentina.
[f]Neurophysiology, Behavior and Animal Welfare Assessment, DPAA, Universidad Autónoma Metropolitana (UAM), Mexico City, Mexico.

*Corresponding author: dmota@correo.xoc.uam.mx

Abstract Transport is considered a stressful period for livestock that can cause economic and biological losses if inadequate handling procedures exist. Several manuals and guides include recommendations for common domestic species based on scientific evidence. Still, the legislative freedom of each country means that they are not mandatory worldwide, nor do they cover all species, such as the water buffalo (*Bubalus bubalis*). This review aims to analyze the legal strictures currently applicable to the transport of this species. Critical aspects of the legislation that address the pre-transport, transportation, and post-transport stages are discussed, focusing on Asian and Latin American countries and the European Union. Areas of opportunity to improve the norms and laws that impact public policies, civil aspects, and water buffalo welfare in production systems are elucidated.

Keywords: animal welfare, *Bubalus bubalis*, legislation, meat quality, transport, water buffalo

1. Introduction

Worldwide, the handling and agricultural practices involving the water buffalo (*Bubalus bubalis*) have intensified over the past 20 years (Cornish et al 2016) due to the increase in inventories of this species, which now number over 204 million head and have a strategic role in the economy (Borghese et al 2022; Di Stasio and Brugiapaglia 2021). This growth entails social, cultural, and political changes regarding this species (Webster 2001). However, the generation of norms to regulate the welfare of these animals during transport has not evolved at the same pace as this growth (Rojas et al 2005).

Generating norms to ensure compliance, guide inspections, and evaluations, and stipulate sanctions entail adjustments and responsibilities for diverse sectors of society, such as ranchers, shippers, slaughterhouse workers, and processors. This usually involves a complex, prolonged process (Velde et al 2002) that is often delayed or postponed due to vested interests that leave legal voids, which can allow poor practices to persist along the production chain (White 2013; Jacques 2014). Poor handling practices can endanger the physical and mental health of livestock and reduce levels of animal welfare (AW) in general by fostering disease development, high incidences of injuries, fear, anxiety, and chronic or unnecessary stress that can directly affect meat quality and the quality of the death of animals destined for human consumption (Mota-Rojas et al 2005; Mota-Rojas et al 2010a,b; Mota-Rojas et al 2021a,b). Individual countries, of course, are free to legislate following their system of governance and the demands and priorities of industry, consumers, political actors, and intermediaries by generating guidelines and regulatory documents for livestock handling (Rushen and Passillé 2010). The problems just mentioned, coupled with scarce scientific knowledge and a null or deficient regulation of procedures, result in significant biological and economic losses in the raising, transport, and slaughter of water buffaloes (Alarcón-Rojo et al 2021; Guerrero-Legarreta et al 2020; Mota-Rojas et al 2021a; Napolitano et al 2020b; OIE 2021; Schipp and Sheridan 2013).

These circumstances have generated diverse efforts to promote the application of good practices in water buffalo transport by analyzing the economic and biological repercussions of procedures that are not performed adequately. Those works are reference recommendations issued by international organizations like the World Organization for Animal Health (WOAH) and voluntary practices related to AW during the production and transport of livestock. A second important document is the Terrestrial Animal Health Code (TAHC), which is based on scientific findings that promote the maintenance of the good welfare of buffaloes during land and maritime transport (OIE 2021).

Received: July 13, 2022 | Accepted: August 30, 2022

Because these practices are voluntary degrees of application, they depend on various factors. In European countries, for example, where consumers consider AW a factor of great importance, there is a movement toward making compliance with the norms proposed to prevent poor agricultural practices (Lundmark et al 2014, 2018) and rules for the commercialization of products and byproducts obligatory (Maciel 2015). This movement seeks to relate AW to the quality and safety of final food products by recognizing that their physicochemical and microbiological characteristics are affected by handling practices and transport conditions and that this impacts indices of consumer acceptance (Cruz-Monterrosa et al 2020). While this evidences the weight that civil society and consumers can bring to bear, the application of good practices to promote AW is also influenced by industry and its needs. In this regard, countries like Mexico have enacted laws to foment AW to promote good sanitary conditions on farms and ranches to ensure the quality and safety of their products. This legislation includes recommendations for several optional practices whose implementation could allow producers and processors to access better markets (Rojas et al 2005; DOF 2018). Although these policies are not obligatory, producers and processors may begin to apply them due to their commercial and economic interests.

Other means of promoting AW operate in countries where legislative action is lacking since the growth of commerce in products of animal origin has engendered diverse strategies in the private sector regarding AW (Veissier et al 2011; Lundmark et al 2014; Ghezzi 2018). One goal is to negotiate commercial accords to increase profits. Agreements of this kind stipulate specific import/export conditions for consortiums and companies in competitive markets that require their application and proof of compliance with AW protocols (Ghezzi 2018; Romero and Sánchez 2011). Another aim is to ensure that supervision, verification, and compliance assessments are documented in mandatory certifications. Advances are generating significant changes in infrastructure, capacitation, and methodologies, but despite the increase in the commercial importance of water buffaloes and growing insistence on AW, especially in the ante-mortem period of animals destined for slaughter legal voids persist.

Given these circumstances, the objective of this review is to compile, describe, and analyze existing legislation applicable to the transport of water buffaloes by addressing the norms and guidelines adopted in various Latin American countries and other areas of the world to compare and contrast the requirements for the welfare of this species during transport, as well as their application, aims, and impact concerning the critical points of transport that produce the greatest impact on AW.

2. Legislation and the importance of good welfare during the transport of water buffaloes

The WOAH is the international organization responsible for issuing guidelines to promote good AW. Its recommendations reflect the joint efforts of scholars, citizens with policy influence, concerned organisms, and scientific principles whose shared objective is to attend to the ethical responsibility of the five freedoms to foment higher yields and lower economic losses by addressing aspects like environmental conditions, handling, and infrastructure to minimize the risk of trauma, fear, pain, and stress in all livestock species, including in nations where water buffaloes are raised as important productive animals (Napolitano et al 2022).

The CSAT covers supervising the planning, loading, transport, and unloading of livestock and the participation of competent personnel. Specific topics addressed include training handlers, professional competence, optimal trip times, selecting adequate vehicles and maintaining them in optimal conditions for each species, and preparing for emergency conditions that may occur during transport, such as severe injury, illness, animals' inability to move or remain standing, or death. More generally, it recommends measures to minimize the stress that can arise and intensify during transport, prepare for adverse environmental conditions, ensure that the buffaloes have sufficient space in the vehicle, avoid mixing animals from different production units, select compatible groups, and avoid brusque contact and the use of herding tools to move the animals (Figure 1). Finally, the WOAH and CSAT work to promote and improve legislation and government policies related to AW worldwide to reduce animal cruelty and poor welfare by emphasizing the importance of adequate inspections before, during, and after transport and suggesting the application of sanctions for non-compliance. They sustain that improving the laws that govern AW will drive progress and socioeconomic development (Otter et al 2012; Peters et al 2015).

Maintaining the good welfare of livestock destined for human consumption during transport requires adequate nutrition and suitable environments while preventing negative physical impacts and conditions that may later affect their behavior (Mellor 2017). Though these goals are fundamental elements of the ethical and humane treatment of livestock (Deters and Hansen 2020), they are not always achieved during trips because the buffaloes may be exposed to stressors like heat, cold, poor air quality, vibrations, and noise (Omran and Hamdon 2018) that affect not only their health but also increase the risk of injuries, such as skin lacerations and abrasions, among others (Alam et al 2020), of respiratory and digestive pathologies like oxidative or heat stress, or even death (Bertoni et al 2020a; Broom 2019; Deters and Hansen 2020; Nielsen et al 2020).

In addition, these animals may suffer physiological alterations that affect their thermoregulatory mechanisms (since this species has low heat-dissipating capacity) due to such anatomical characteristics as their scarce hair, thicker epidermis than bovine cattle, and black coloration with large amounts of melanin that absorbs heat from the exterior (Barboza 2011; Bertoni et al 2020). Buffaloes have physiological responses –for example, regulating their metabolic rate using energy produced by cell metabolism–

that can eliminate some excess heat by irradiation (Casas-Alvarado et al 2020; Napolitano et al 2020; Mota-Rojas et al 2021b,c,d,e) to regulate their body temperature as long as external conditions are not extreme and physical resources like natural or artificial shade are available, though it is difficult to provide these resources during transport (Bertoni et al 2020b). The impact of the animals' thermal responses can be observed by infrared thermography (Mota-Rojas et al 2021d; Mota-Rojas et al 2022), a useful technique for analyzing and modifying routine practices performed during herding and transport that can produce harmful physiological alterations like heat stress (Figure 2).

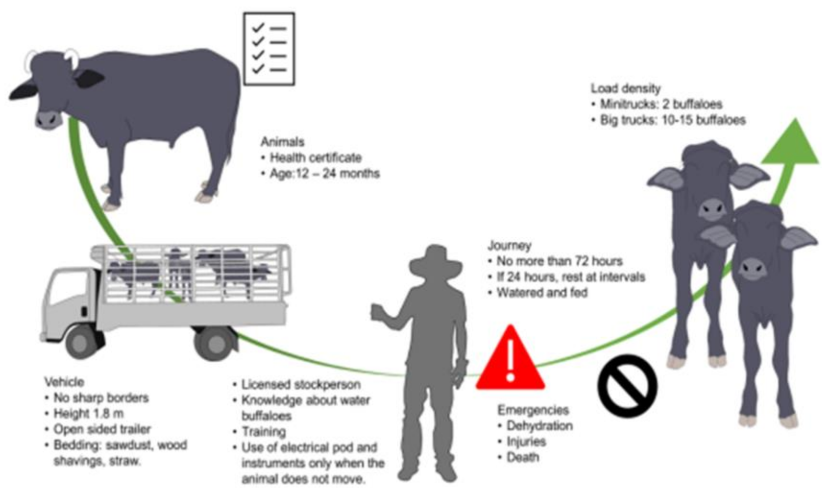


Figure 1 Recommendations for water buffalo transport. The manuals that promote the appropriate handling of water buffalo during transport in some countries include such elements as verifying the health status of the animals before loading and inspecting the physical characteristics of the vehicle. Recommended load densities and trip lengths are also highlighted, as is the importance of stockpeople trained in the proper use of herding instruments. Attending to these aspects will contribute to the welfare of buffaloes during transport and the quality of their meat and byproducts.

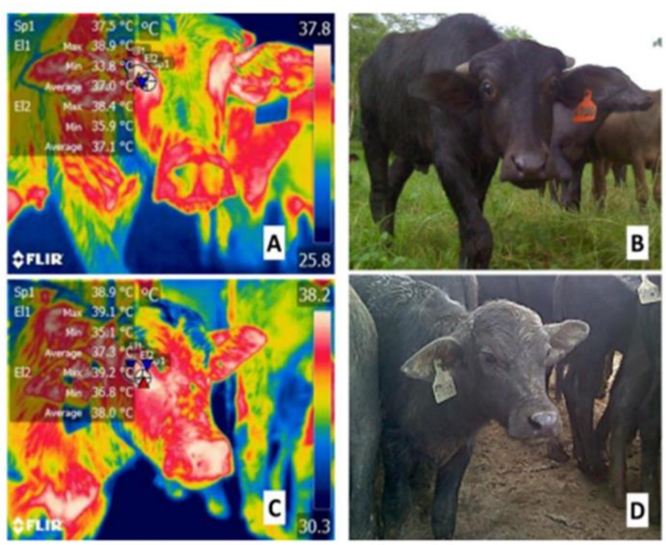


Figure 2 Routine handling of buffalo calves before and after herding and its effect on dermal microcirculation observed by infrared thermography. A, B. Radiometric and digital images of a buffalo in the paddock before transport (corralling). C, D. Radiometric and digital images of a buffalo upon arrival at the holding pen. The average surface temperature of the periocular region (El1) increased by 0.3 °C, while the lacrimal gland (Sp1) and lacrimal caruncle (El2) show average increases of 1.4 °C and 0.8 °C, respectively.

3. Impact of AW on public policies, production, and society

In recent years, AW has established its place in public policy, regulations, and legislative calendars propelled by the specifications imposed by importing countries, private companies, society in general, producers, and processors, all of whom have a shared interest in improving the quality of final products. Practices based on scientific data have been implemented to improve livestock's sanitary and health conditions under the supervision of professionals in various related fields (Jacques 2014; Alonso et al 2020). The growing interest in ensuring the welfare of livestock species has led to the standardization and certification of production units and transport and slaughtering processes (Cockram et al 2020). Internationally, chapter 7.3 of the CSAT contains specifications and requirements for the land transport of animals. It also mentions the capacitation that handlers of each species should receive, adequate methods for moving different animals, the ideal characteristics of trips, vehicles, and containers, and the documentation that must accompany the transported animals (WOAH 2021).

Although we include water buffaloes among the domesticated animals to which that chapter applies, to date, there are no specific, universal guidelines for this species. Despite this, the CSAT has served as the basis for elaborating official documents on the transport of water buffaloes in various countries, including some in Asia that have the largest buffalo populations in the world: 109.8 million in India, 40 million in Pakistan, and 27.3 million in China (Di Stasio and Brugiapaglia 2021). Those countries have passed laws to foster the welfare of water buffaloes, often with a clear religious orientation. In India, for example, a Prevention of Cruelty to Animals Act (#56) was published in 1960. Its goal was to prevent unnecessary pain and stress in animals. It outlines the features of adequate transport and stipulates sanctions for striking, injuring, or inflicting lesions on the in-transport animal, recommends not overloading vehicles and installing physical divisions when necessary as long as they do not cause pain or discomfort (Figure 3). Finally, it names the officials responsible for its application and enforcement (India 1960).

Figure 3 Physical adaptations of vehicles used to transport water buffaloes. A. A truck with the recommended load density reduces the risk of falls and injuries due to the space provided per animal It is equipped with physical divisions to transport animals of different ages, sizes, or origins. B. Vehicles with a roof design provide ventilation but do not open any means of escape.

In 1890, Pakistan ratified a Law for the Prevention of Cruelty to Animals. The original act was modified most recently in 2018 to increase the sanctions and administrative fines applied to those who affect the welfare of livestock, including water buffaloes (PAWS 2018). In 1950, Pakistan enacted a Cattle Slaughter Control act in Karachi, the nation's largest city. That act states that animals must be slaughtered in abattoirs that provide adequate lairage times and effectuate inspections by trained personnel to verify that all animals are in optimal conditions post-transport. Finally, it stipulates monetary sanctions and prison sentences for those who fail to comply (Karachi 1950).

In China, controversy surrounds the long distances that livestock must travel from production units to slaughterhouses and the conditions in which this is carried out. Diverse actors in Chinese society, including the Chinese Normalization Association, the Chinese Association of Veterinary Medicine, and government sectors, are actively working to regulate the practices that impact AW during transport. However, none of the legislative proposals presented to date have been ratified, so there are no specific laws there. In the absence of effective supervision, harmful practices persist (Li et al 2018).

Australia, in contrast, has made supervision mandatory. The water buffalo is classified among the meat and milk protein producer species and is deemed important because of its high efficiency in exploiting forage of medium-to-low quality and the properties of its meat and byproducts. Additionally, buffaloes are valued because they help reduce forest fires due to their natural habitat in swampy areas. In some areas, annual licenses must be obtained to regulate and ensure the correct handling of this species, supported by guidelines published by the federal government that outline the good handling practices for these animals, including during transport (Lemcke 2015a).

The principal water buffalo-producing countries in Latin America are Colombia, Brazil, Argentina, and Venezuela (Patiño 2011; Napolitano et al 2020a; Guerrero-Legarreta et al 2020), followed by Mexico and Chile. While norms for the production and welfare of buffaloes exist, there are marked differences in their respective systems of governance and, hence, divergent levels of concern for AW that open perspectives and areas of opportunity in each. Specific key characteristics of the water buffalo have proven advantageous for production in those nations: the ability to adapt to distinct climatic conditions, the potential for increases in growth and yield rates, potential profitability, contributions to environmental sustainability, and the optimal, attractive nutritional and sensory characteristics of final products (Álvarez-Macías et al 2020; Bertoni et al 2020; Guerrero-Legarreta et al 2020; Joele et al 2017; Mora-Medina et al 2018; Mota-Rojas et al 2020; Sabia et al 2018).

Unfortunately, most of these nations have legal vacuums in their social, commercial, and political policies that impact buffalo production systems. In Venezuela, for example, the only legal referent is the Law for the Protection of Free and Captive Domestic Fauna, which establishes norms for protection, control, and welfare that apply to all animals, including those destined for human consumption. However, that law (in force since 2010) does not specifically mention water buffaloes' transport. It only stipulates that transport conditions must ensure good welfare and prevent, insofar as possible, states of metabolic stress, pain, abuse, and cruelty. Finally, it provides detailed descriptions of acts of cruelty and lists the sanctions that can be imposed when livestock is not handled under optimal conditions (Venezuela 2010).

A detailed reading of this law reveals that it does not mention, or elaborate on, concepts, practices, or methods related to ensuring AW during pre-slaughter transport, nor any projects or proposals for norms, laws, decrees, or regulations that might suggest a tangible interest in effectuating change. This reflects not only voids in political infrastructure, deficient policies, problems in the existing legal framework, and issues of food safety, but also the alimentary, health, and economic crises that the country is experiencing (Page et al 2019; Doocy et al 2019; Pielago 2020). It is clear that passing laws on AW during the transport of water buffaloes is not a priority of the government or most Venezuelans even though it is the second-largest producing country in America (Nava-Trujillo et al 2020).

Colombia presents a contrast, for it has made strides in establishing AW as the means for generating positive actions in agricultural products designed to improve health, food quality, and food safety parameters through regulations, decrees, resolutions, and manuals that optimize the application, vigilance, inspection, and development of measures similar to those supported by the WOAH and FAO. This approach generates production and processing conditions that international markets find attractive due to adopting some of the CSAT's recommendations, recent legislation and guidelines, and international trade agreements. Colombia´s concern for health, food safety, food quality, and ensuring AW during transport has caught the attention of foreign countries and positively impacted society as a whole and, specifically, the companies involved in buffalo production (MADR 2020; OIE 2021).

Brazilian legislation on maintaining AW during the transport of water buffaloes presents a different panorama from that of Colombia. In 1934, the government emitted decree 24.548 that cited the need to organize sanitary conditions to ensure that people would have access to final products that were safe and of good quality. It established measures, such as inspections at key junctures of the production chain, to prevent the spread of disease, and provided guidelines on times and logistics (Matias et al 2019). Diverse political actors, entrepreneurs, and policies recognize Brazil's deficiencies and areas of opportunity. The country's legislature has targeted these through proposals for laws that would ensure adequate environments for maintaining good AW and guidelines that would indirectly impact this domain. The measures proposed are based on scientific findings and international recommendations for each production species. Unfortunately, none of those proposals or measures have been enacted into law due to vested interests and the priorities of the government in power (Henrique et al 2017).

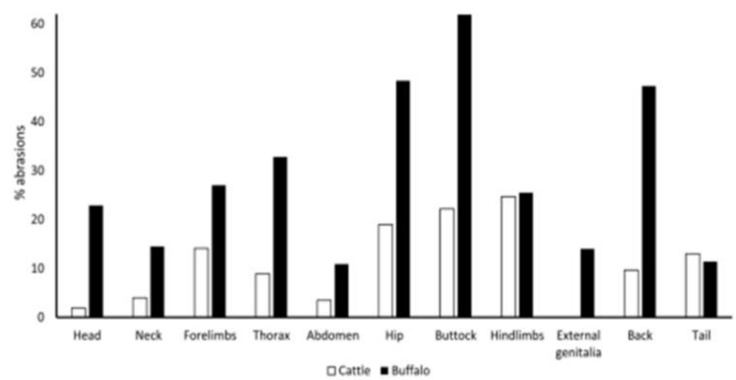
4. Documentation and preparation of animals before transport

The documentation required includes transport permits and certificates of health and vehicular disinfection, among others. The objectives are to prevent the spread of disease and ensure the traceability of the source animals and final products. Several countries, Mexico among them, have cattle identification systems that include the water buffalo. However, the Mexican system has incongruencies because the buffalo is classified as a bovine breed, so it is not differentiated from conventional cattle. This impedes effective traceability though zoosanitary certificates are mandatory for in-country transport. The fact that this species is not identified specifically in existing legislation causes problems during transport (DOF 2018).

Planning is crucial to transporting farm animals and ensuring AW throughout the process. Preparing water buffaloes for transport requires considering numerous factors, such as age, size, and place of origin. Ideally, shippers will move animals with similar characteristics to minimize the potential for injuries (Figure 4) that can impact the meat quality (Guerrero-Legarreta et al 2020). In this regard, it is preferable to transport young animals aged 12-18 months rather than ones aged three years or more (Di Stasio and Brugiapaglia 2021). Following established good practices during loading and unloading (Figure 4a) is also important.

Figure 4 Animal handling during loading. A. Animals on the loading ramp with no apparent separation by size. A young buffalo is being moved with larger ones. This increases the potential for abrasions. B. Loading of buffaloes of similar size and age carried out by trained personnel without physical instruments to avoid injuries on the trip.

On this issue, the guides in some countries –China, for example– recommend transporting and sacrificing animals aged 24 months to obtain adequate yields and meat quality (Li et al 2018). Concerning vehicle design, one study reported that 95.3% of 192 water buffaloes suffered abrasions during transport, as Figure 5 describes in detail based on Alam et al (2010a). It is important to note that water buffaloes may be more susceptible to injury than bovines, possibly due to differences in body structure, a slower pace of walking that often provokes the use of sticks or prods, the style of herding, and flaws in vehicle design that increase friction and impacts (Alam et al 2010a,b).



Adapted from Alam et al., 2010

Figure 5 Comparison of abrasions on various body regions of cattle (Hariana, exotic and local to Bangladesh) *vs.* water buffaloes due to transport. Note the greater number of injuries in the buffaloes than cattle on the head (1.9 *vs.* 22.9%), neck (4 *vs.* 14.5%), forelimbs (14.1 *vs.* 27%), thorax (8.9 *vs.* 32.8%), abdomen (3.5 *vs.* 10.9%), hip (19 *vs.* 48.4%), rump (22.2 *vs.* 61.9%), hind limbs (24.7 *vs.* 25.5%) and back (9.7 *vs.* 47.3%).

Legislation in the European Union (EU) stipulates only shippers previously authorized to transport animals can be hired. Shippers must ensure that AW will not be affected by planning for the various phases of trips and considering climatic and road conditions, among other aspects. They must also designate the personnel in charge who, among other responsibilities, must provide the competent authorities with all required information and documentation on the preparations for and the execution and completion of each trip. For especially long trips, they must fill out, sign, and seal planning forms that identify the starting point and destination and include the shipper's declaration and any reports of anomalies found in the animals sampled. Obviously, this cannot occur if the load is not inspected and approved by those authorities (Union 2019).

5. Feeding and lairage periods at the abattoir

Food consumption before and during trips is a matter of debate because of the scarce scientific evidence currently available on the effects of fasting in water buffaloes. Studies of Hereford and Angus steers show that prolonged fasting (>24h) increases dehydration while fasting for 12-13h produces changes in water consumption (Clariget et al 2021). In other studies, mestizo steers showed the transfer of fatty acids from adipose tissue after 48h of fasting (Herdt 2020; Ortolani et al 2020). These findings suggest that cattle's adequate fasting time should not exceed 12h. Studies of Aberdeen Angus × Nellore bovines indicate that waiting times of 12h in the abattoir allowed the animals to adapt to the new environment, but that after 12h, cortisol tended to increase from 8.7 to 12.5 mcg dL -1 (Moura et al 2021), indicating that fasting and lairage times at the abattoir should not exceed 12h. Handling strategies must take these issues into account.

Legislation like Mexico's official norm, "NOM-051-ZOO-1995, Humane treatment during animal transport, does not stipulate maximum fasting times or optimal lairage periods for cattle, nor does it mention the water buffalo in its specifications. It does, however, state that transport times for bovines should not exceed 18h without rest periods and opportunities to drink water and that longer trips (>24h) must include rest periods at least every 18h and the provision of food, though it fails to recommend the types or amounts of food that should be made available (DOF 1995).

Brazil's laws emphasize preventing disease spread among livestock, including water buffaloes. They establish, for instance, that trip times must not exceed 72 h and that when transport requires more than 24 h, vehicles must make rest stops at specific sites to prevent conditions that may cause sanitary problems. However, those laws do not establish norms for the installations, supervision, and training of handlers, nor do they present guidelines for herding, loading, transport, and unloading practices that could help minimize travel-induced stress (Rojas et al 2005; Henrique et al 2017).

Legislation in Chile mentions that food, water, and rest must be provided in relation to the needs of each species at least every 8 h on trips that exceed 24 h and that this must take place at sites authorized by the Department of Agriculture. When, for whatever reason, rest cannot be provided, the vehicle must be equipped to allow the provision of food and water (Ministerio de Agricultura de Chile 2013).

Similar guidelines are applied in countries like Australia, which specifies that trips should not exceed 30h without unloading the animals and offering them food and water (Lemcke 2017). Other recommendations are that the buffaloes be given long hay instead of pelleted feed in the rest zones to promote ruminal stability and that they are allowed to remain in the zone for at least 12h before renewing the trip (Lemcke 2017).

In the case of the EU, Regulation 1/2005 states that trip duration for cattle should not exceed 8 h and that on longer trips, one hour of rest must be allowed after 14h of travel to provide water and food. After that, the trip can continue for another 14h. When transport ends and the animals have been unloaded, they must be given food and water and allowed to rest for 24h (European Union 2019).

6. Transport

Australia's Department of Primary Industry and Resources stipulates that the trailers used to transport buffaloes must be free of protrusions or sharp structures that could cause injuries (Figure 6). The recommended height of walls is 1.8 m. Ideally, trailers will be of the open-sided type to facilitate loading (Lemcke 2017). Section 14 of the Non-Indigenous Animal Act stipulates that all personnel responsible for handling buffaloes must be licensed (Sheehan 2012).

Figure 6 Characteristics that increase or decrease injuries during transport. A. Buffaloes are transported in a tubular structure that allows adequate ventilation with walls free of sharp edges and non-slip flooring to prevent falls and injury. B. Animals of different sizes are about to be transported. This requires physical divisions in the vehicle.

In India, buffalo meat represents 30% of all meat production, making it one of the leading producers countries in the world. The Federation of the Indian Chamber of Commerce and Industry (FICCI) recommends that the vehicles used to transport buffaloes be small trucks with the capacity for two animals or large trailers that can hold 10-15. Data show that 97% of producers in India report that transport is their largest production cost, in part because each animal must have a health certificate issued by a qualified veterinarian that identifies its precedence and quality (Singh et al 2012).

Returning to Latin America, most current regulations fail to distinguish between bovine cattle and water buffaloes. Chapter VII of Colombia's Law 84 (1989) on the protection of animals stipulates principles and procedures for transport designed to prevent cruelty, fatigue, fasting, dehydration, and injuries and to avoid adverse environmental conditions. Decree 1500 (2007) specifies the vehicles and documentation required for each trip. It emphasizes the need for continuous personnel training while underscoring the importance of compliance with these measures because of their impact on food safety. It further states that the vehicles' design must be appropriate for the species transported, has mechanisms of physical division to reduce overcrowding, aggressions, and injuries, and satisfy all published sanitary dispositions (Colombia 2020).

Chile's Law 19162, with regulation 2401, indicates the means of transport that can be used to ship live animals to slaughterhouses and the characteristics they must have, such as divisions when animals of different ages and physical conditions will be loaded. Vehicles must have smooth, perforated walls free of sharp protuberances or be equipped with mechanical ventilators to maintain comfortable temperatures and prevent heat stress. Floors must be made of non-slip materials to prevent injuries and ensure the safety of the animals during loading, transport, and unloading (Figure 4) (Ministerio de Agricultura de Chile 1992). A law passed in 2012 binds shippers to modernize the vehicles used to transport cattle (Strappini 2012), while Decree 30 (2013) prohibits shipping cattle in conditions that cause unnecessary pain or suffering. In Chile, one especially observes strict adherence to the parameters established in the CSAT, including the selection of vehicle characteristics in accordance with the species to be loaded, trip times, and adequate thermoregulation conditions (Ministerio de Agricultura de Chile 2013).

EU Regulation (CE) 1/2005, "Relative to the protection of animals during transport and related operations that modify directives 64/432/CEE and 93/119/CE and Regulation (CE) 1255/97", stipulates the parameters for transport for cattle, sheep, goats, and pigs during long trips. These include roofed vehicles, bedding adequate for each species, the number of animals, trip duration, climatic conditions, and means of ensuring the absorption of urine and feces (European Union 2019). In contrast to other regulations, this one stipulates that the food provided must be sufficient and free of contamination and that the feeding equipment must be designed and installed so that food cannot fall. Moreover, this equipment must be fastened down when the vehicle is in movement. Finally, all food must be stored separately from the animals.

The characteristics of vehicles can also contribute to improving AW about climatic conditions, as demonstrated in a recent study of sheep (Dorper × Mongolia), where a closed truck reduced the incidence of cold stress. That report affirmed that consuming food before transport improved the animals' capacity to resist cold temperatures over short distances (Carnovale et al 2021). Research of this kind suggests that key topics for future studies will be the effects of cold and potential measures to minimize those effects on long trips, especially concerning their impact on the water buffalo.

At the opposite end of the temperature scale, the high temperatures that occur in some months of the year increase animals' susceptibility to heat stress during transport, especially on long trips. This can be aggravated by overcrowding which can trigger anxiety and, on occasions, death (Bhatt et al 2021; Bachelard 2022). Considering this danger, EU Regulation 1/2005 stipulates that vehicles must maintain a temperature between 5 and 30 °C throughout the trajectory with a tolerance of +/-5 °C depending on the exterior temperature (European Union 2019).

7. Load density

Australia's Manual on the River Buffalo, published by the Northern Territory Government (NTG) (Lemcke 2017), and based on the Australian Animal Welfare Standards and Guidelines for the Land Transport of Livestock (Animal Health Australia 2012), states that when the load density of buffaloes is low, the animals must have sufficient space to lie down. The recommendation for long trips is to provide bedding made of sawdust, wood shavings, or straw (Lemcke 2015b). In contrast, if the density is high, the buffaloes will have to remain standing, so it is important to avoid space conditions that would allow them to lie on the floor since they could be trampled by other animals and suffer severe injuries (Lemcke 2017).

Another case of observations on load densities comes from Mexico, where regulations specify the height of the vehicle's roof or tubular structure and the space that should be allowed for each animal. Those parameters, however, fail to differentiate between species with or without horns and make no mention of modifications due to the age or weight of the animals. In addition, they are only applicable to bovines, as the reference utilized by medical officials at zoosanitary inspection and verification points shows (DOF 1995, 2015). Figure 3 presents an example of the load densities stipulated there.

In this regard, Act 56 for the Prevention of Animal Cruelty in India only states that loading densities should not be excessive to ensure that the animals have some degree of comfort and to lessen the risk of injuries that could affect their carcasses when processed. The Act fails, however, to specify numbers for optimal load densities for buffaloes (India 1960).

8. Capacitation and the human-animal relationship

Studies in Chile have documented that the guillotine doors injure 75.1% of bovines at slaughterhouses due to the inadequate capacitation of handlers. In addition, 49.2% were moved to utilize electric prods (Muñoz et al 2012). This raises concern because the use of such equipment should not exceed 20% (Grandin 2012). Electric prods can cause hematomas during loading and unloading and in the stunning chamber. This is a clear case of poor human-animal interaction (Alam et al 2010a) that occurs in Chile even though current norms stipulate that all handlers involved in transport and slaughtering must demonstrate their competence based on training received on correct handling procedures and AW. Alternatively, these activities must be performed by agricultural professionals or technicians (Ministerio de Agricultura de Chile 1992, 2013).

EU regulation 1/2005, meanwhile, establishes that training is required for all persons involved in handling animals during all stages of transport. Authorized organizations must give the training to teach the personnel to carry out their labors without violent acts or measures that cause fear, physical harm, or suffering (European Union 2019). Article 7.3.8 of the aforementioned CSAT recommends that electric prods be used only on animals that refuse to move when there is sufficient space and adequate conditions for them to do so. It further states that prods must not be applied to the hindquarters of large ruminants, nor to the ears, mouth, eyes, abdomen, or anogenital region. Using authorized instruments (flags, plastic bags, panels, banderillas) should be encouraged only to stimulate the animals to move in the correct direction. This specification is mentioned in current legislation in Mexico that, in addition, indicates the obligation to train all personnel involved in handling livestock and stresses the need to foment the correct utilization of the tools and infrastructure available in loading and reception areas, such as ramps with a maximum slope of 25° (DOF 1995) (Figure 7). These measures, however, are not obligatory there.

Figure 7 Loading and unloading ramps used in Mexico for buffalo transport. A. Trailer and uneven ramp. Though it has slots and an adequate slope, this ramp is not aligned correctly with the trailer, a factor that may cause injuries to the buffaloes' hind limbs. B. Type of trailers used to transport buffaloes in Mexico.

In Colombia, Resolution 253 (2020) preceded the publication of the Manual of Conditions of Animal Welfare. That document mentions that blunt, sharp, or electric instruments should not be used to force livestock if they can cause any type of injury. Instead, it recommends using flags and rattles that promote handling free of stress, pain, fear, and suffering (Colombia 2020).

The capacitation strategies adopted in countries like Australia –available in online courses sponsored by the government– include educating personnel on the differences between buffaloes and conventional cattle. Key differences include the more docile temperament of buffaloes and the fact that they learn more quickly under frequent, careful handling. Scientific findings of this kind emphasize that it is recommendable to move buffaloes calmly without challenging them, though this is often necessary with cattle (*Bos taurus* or *indicus*) (Lemcke 2015a).

9. Procedures for emergency situations

Documents and guidelines elaborated by the government of Australia's Northern Territory (e.g., No. J65, Transportation to the Abattoir, Australia, 2012; Lemcke 2017) recommend that in the event of dehydration, post-transport buffaloes should have their heads sprayed gently with water before unloading and then be moved to a shaded area. There should also be supervision in the lairage pens before slaughter to ensure that the animals do not drink water in excess to prevent premature mortality (Lemcke 2015a).

Colombia's manual on AW also considers that the personnel involved in planning and organizing transport procedures should anticipate and plan for situations of emergency and prepare contingency measures in the event, for example, of natural disasters, circumstances that require providing food and water, or when signs of disease appear (Colombia 2020; Virviescas-Sanabria 2020).

Guidelines in Mexico specify the conditions under which livestock should not be transported to a slaughterhouse. Those conditions include disease, limiting injuries, fatigue, and females close to following, among others. The objective is to avoid transport problems that could put the animals' AW at risk. Legislation in Mexico further stipulates that planning for emergencies should include providing the vehicle with ramps, specific spaces for injured animals, handling animals that die *en route*, and equipment that facilitates efficient unloading. It also stresses the importance of humanely slaughtering animals that have suffered life-threatening injuries (DOF 1995, 2015).

Legislation in Chile specifies the steps to be taken in cases of emergency, emphasizing that the personnel responsible for transport at each cattle production unit must have plans that anticipate emergency situations by identifying the types of possible incidents, the corrective actions to be taken in each case, and the person responsible for ensuring that those actions are taken. It also outlines –in the event that this becomes necessary– a slaughtering method that prevents unnecessary suffering in animals that become ill or are severely injured (e.g., fractures) such that transporting them becomes difficult (Ministerio de Agricultura de Chile 2013).

10. Future perspectives

While the publication of decrees, regulations, laws, norms and other measures have produced positive changes that favor animals, research by NGOs and institutions in the EU and its member states, together with other scientific work, show that much remains to be done to achieve satisfactory protection of livestock, since existing laws, norms, and guidelines are inadequate or their enforcement deficient (Bachelard 2022). In the case of the water buffalo, our review found (i) that this species is rarely identified specifically in the documents discussed, and (ii) where it is included, no specific measures are indicated, despite its productive value and economic importance in numerous regions. These findings highlight the need to include the water buffalo in national norms and regulations to improve the welfare of this species due to its significant physical differences compared to conventional cattle (José-Pérez et al 2022).

Modifications to legislation in the future should stipulate maximum trip times for each species, specify adequate ages for transport, outline strategies for improving inspection procedures, establish vehicle specifications that consider dimensions and physical characteristics, address potential meteorological conditions, and recommend rest periods and the provision of food and water on long trips.

Public and social demands regarding commercializing products of animal origin and practices focused on AW have intensified in recent years; indeed, consumers and industries increasingly see enhancing livestock welfare as a requirement and a non-negotiable issue for acquiring products. An important objective in some geographic areas is to identify opportunities to improve existing legislation following technical and scientific recommendations emitted by international organisms. However, this may upset established distributions of responsibilities and functions. Unfortunately, advances in this direction are not occurring in many of the most important water buffalo-producing and transporting nations. For these two activities, it is essential to promote procedures based on scientific research that establishes optimal conditions for their development and AW as a means, as well, to reduce economic losses worldwide.

The greater availability of information has generated social and political changes such that the scientific community and international organisms have emerged as fundamental entities for modifying laws and guidelines. In addition to providing tools and strategies, they raise awareness that improving AW will generate greater economic profits and significant ethical advances for society as a whole through an emphasis on the traceability of products and improved food safety.

As Broom (2019) argues, traceability is a fundamental aspect of animal transport that must be developed rigorously wherever livestock is raised, including water buffaloes. Traceability is a method for supervising and controlling key elements of livestock production, from the animals' birth to their slaughter, including monitoring the handling procedures applied from the farm to the animals' final destination. It is a procedure that inspects and verifies the health conditions and welfare of the animals transported with strict observance of laws and norms. In Italy, where the production of buffalo milk for elaborating mozzarella cheese is an important industry, a ministerial decree in 2014 made traceability obligatory for all milk production units (Cappelli et al 2021). However, to date, no similar system exists for animals destined for meat.

11. Final considerations

Currently, no global regulations specify standards for the transport of water buffaloes. However, the CSAT, which recognizes the buffalo as a domesticated species, does contain recommendations for preventing or reducing injuries, pain, suffering, and stress during transport. In countries where the populations and production of domesticated water buffaloes grow each year –Pakistan and India, for example– only a few of the points covered in the CSAT are respected; for instance, load densities, rest times, and establishments authorized for sacrifice. China has elaborated legislative proposals related to AW but has not been enacted. Legislation in Australia, meanwhile, is tailored to the specific needs and conditions of different regions. While Australia does not have such large water buffalo populations as India or Pakistan, it has norms and regulations for livestock transport. It stipulates that causing unnecessary injury, pain, or stress are punishable crimes.

In Latin America, Colombia, Chile, and Argentina follow some of the WOAH's recommendations, and Argentina has elaborated an AW manual that covers the production, transport, and sacrifice of water buffaloes. In contrast, Venezuela only pays lip service to the welfare of buffalo populations, for it has no statutes that refer specifically to the transport of this species. Conditions in Mexico are similar because the water buffalo is not mentioned in legislation to prevent the inadequate handling of livestock. This omission opens legal voids because it is impossible to differentiate each species' products or to effectively supervise the areas of health, production, and commercialization.

Understanding the degrees of application of legislation in the various water buffalo-producing countries demands evaluating regional social and political conditions. All these nations manifest some interest in implementing measures to inspect, supervise, sanction, and certify the practices employed in water buffalo production. Most have developed legislative proposals, decrees, accords, manuals, and norms with recommendations for existing production units. But ratification and enactment processes often take a great deal of time, months or even years.

Fomenting a culture focused on the quality of products for human consumption, voluntary certifications, and the auditing of good agricultural practices in which civil society plays a fundamental role are strategies that can help close existing legal voids related to the water buffalo.

In summary, providing better conditions for domesticated buffalo species during transport requires developing, modifying, and applying laws and regulations based on solid scientific research through the participation of scholars, public organisms, private companies, and the social sector. Achieving these goals requires political changes in each country that, hopefully, will lead to the consolidation of laws for the welfare of the water buffalo.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This research did not receive any financial support.

References

Alam MR, Gregory NG, Jabbar MA, et al (2010a) Skin injuries identified in cattle and water buffaloes at livestock markets in Bangladesh. Vet Rec 167:415-419.

Alam MR, Gregory NG, Uddin MS, et al (2010b) Frequency of nose and tail injuries in cattle and water buffalo at livestock markets in Bangladesh. Anim Welf 19:295-300.

Alam MR, Islam MJ, Amin A, et al (2020) Animal-based welfare assessment of cattle and water buffalo in Bangladeshi slaughterhouses. J Appl Anim Welf Sci 23:219-230.

Alarcón-Rojo A, Mota-Rojas D, García-Galicia I, et al (2021) Dark cutting in large in water buffalo: Effect of management and environmental factors. Agro Product 13:93-98.

Alonso ME, González-Montaña JR, Lomillos JM (2020) Consumers' Concerns and Perceptions of Farm Animal Welfare. Animals 10:385.

Álvarez-Macías A, Mota-Rojas D, Bertoni A, Dávalos-Flores JL (2020) Opciones de desarrollo de los sistemas de producción de búfalos de agua de doble propósito en el trópico húmedo latinoamericano. In: Napolitano F, Mota-Rojas D, Guerrero-Legarreta I, Orihuela A (eds) El búfalo de agua en Latinoamérica, hallazgos recientes., 3ra ed. BM Editores, México, pp 43-74.

Animal Health Australia (2012) Australian Animal Welfare Standards and Guidelines- Land Transport of Livestock. Animal Health Australia (AHA). Canberra, pp 1-115.

Bachelard N (2022) Animal transport as regulated in Europe: a work in progress as viewed by an NGO. Anim Front 12:16-24.

Barboza-Jiménez G (2011) Bondades ecológicas del búfalo de agua: camino hacia la certificación. Tecnol en Marcha 24:82-88.

Bertoni A, Mota- Rojas D, Napolitano F, et al (2020a) Ventanas térmicas en el búfalo de agua: aspectos prácticos para la valoración reproductiva. In: Napolitano F, Mota- Rojas D, Guerrero- Legarreta J, Orihuela A (eds) El búfalo de agua en Latinoamérica, hallazgos recientes, 3ra ed. BM Editores, Mexico City, pp 690-719.

Bertoni A, Mota-Rojas D, Álvarez-Macias A, et al (2020b) Scientific findings related to changes in vascular microcirculation using infrared thermography in the river buffalo. J Anim Behav Biometeorol 8:288-297.

Bertoni A, Napolitano F, Mota-Rojas D, et al (2020c) Similarities and Differences between River Buffaloes and Cattle: Health, Physiological, Behavioral and Productivity Aspects. J Buffalo Sci 9:92-109.

Bhatt N, Singh N, Mishra A, et al (2021) A detailed review of transportation stress in livestock and its mitigation techniques. Int J Livest Res. https://doi.org/10.5455/ijlr.20201109102902

Borghese A, Chiariotti A, Lucia V (2022) Biotechnological applications in buffalo research. Springer Singapore, Singapore.

Broom DM (2019) Welfare of Transported Animals: Welfare Assessment and factors affecting welfare. In: Grandin T (ed) Livestock Handling and Transport, 5 Th. CAB International. Oxfordshire, Oxford, pp 12-29.

Cappelli G, Di Vuolo G, Gerini O, et al (2021) Italian Tracing System for Water Buffalo Milk and Processed Milk Products. Animals 11:1737.

Carnovale F, Xiao J, Shi B, et al (2021) The effects of vehicle type, transport duration and pre-transport feeding on the welfare of sheep transported in low temperatures. Animals 11:1659.

Casas-Alvarado A, Mota-Rojas D, Hernández-Ávalos I, et al (2020) Advances in infrared thermography: Surgical aspects, vascular changes, and pain monitoring in veterinary medicine. J Therm Biol 92:102664.

Clariget J, Banchero G, Luzardo S, et al (2021) Effect of pre-slaughter fasting duration on physiology, carcass and meat quality in beef cattle finished on pastures or feedlot. Res Vet Sci 136:158-165.

Cockram MS, Dulal KJ, Stryhn H, Revie CW (2020) Rearing and handling injuries in broiler chickens and risk factors for wing injuries during loading. Can J Anim Sci 100:402-410.

Council Regulation (EC) No 1/2005 of 22 December 2004 (2019) on the protection of animals during transport and related operations and amending. https://eur-lex.europa.eu/legal-content/en/ALL. Accessed on: July 01, 2022.

Cornish A, Raubenheimer D, McGreevy P (2016) What we know about the public's level of concern for farm animal welfare in food production in developed countries. Animals 6:74.

Cruz-Monterrosa RG, Mota-Rojas D, Ramírez-Bibriesca E, et al (2020a) Scientific findings on the quality of river buffalo meat and prospects for future studies. J Buffalo Sci 9:170-180.

Cruz-Monterrosa RG, Mota-Rojas D, El-aziz AHA, et al (2020b) Calidad de la carne de búfalo de agua: análisis nutricional, sensorial e inocuidad, conservación, empacado y autenticidad. In: Napolitano F, Mota-Rojas D, Guerrero-Legarreta I, Orihuela A (eds) El búfalo de agua en Latinoamérica, hallazgos recientes, 3ra ed. BM Editores, México, pp 1279-1319

Deters EL, Hansen SL (2020) Invited Review: Linking road transportation with oxidative stress in cattle and other species. Appl Anim Sci 36:183-200.

Di Stasio L, Brugiapaglia A (2021) Current knowledge on river buffalo meat: A critical analysis. Animals 11:2111.

DOF (2018) Ley federal de sanidad animal. DOF la Fed 1-55

DOF (1995) NOM-051-ZOO-1995- Trato humanitario en la movilización de animales. http://publico.senasica.gob.mx/?doc=531 Accessed on: July 01, 2022.

DOF (2015) NOM-033-SAG/ZOO-2014 Métodos para dar muerte a animales domésticos y silvestres. https://www.gob.mx/profepa/documentos/norma-oficial-mexicana-nom-033-sag-zoo-2014-metodos-para-dar-muerte-a-los-animales-domesticos-y-silvestres Accessed on: July 01, 2022.

Doocy S, Ververs M-T, Spiegel P, Beyrer C (2019) The food security and nutrition crisis in Venezuela. Soc Sci Med 226:63-68.

Gallo C (2008) Transporte e bem-estar animal. Ciênc vet tróp 11:11

Gallo CB, Huertas SM (2016) Main animal welfare problems in ruminant livestock during preslaughter operations: a South American view. Animal 10:357-364.

Ghezzi MD (2018) ¿Cómo medimos el bienestar animal? Los porcicultores y su entorno. https://bmeditores.mx/porcicultura/como-medimos-el-bienestar-animal/ Accessed on: July 01, 2022.

González-Lozano M, Mota-Rojas D, Orihuela A, et al (2020) Review: Behavioral, physiological, and reproductive performance of buffalo cows during eutocic and dystocic parturitions. Appl Anim Sci 36:407-422.

Gonzalez-Rivas PA, Chauhan SS, Ha M, et al (2020) Effects of heat stress on animal physiology, metabolism, and meat quality: A review. Meat Sci 162:108025.

Grandin T (2012) Developing measures to audit welfare of cattle and pigs at slaughter. Anim Welf 21:351-356.

Guerrero-Legarreta I, Napolitano F, Cruz-Monterrosa R, et al (2020) River buffalo meat production and quality: sustainability, productivity, nutritional and sensory properties. J Buffalo Sci 9: 159-169.

Henrique A, Regis DP, Cornelli G (2017) Situación jurídica de los animales y propuestas de modificación en el Congreso de la Nación Brasileña. 25:191-198.

Herdt TH (2020) Utilización postabsortiva de los nutrientes. In: Klein BG (ed) Cunningham fisiología veterinaria, 6.ª ed. Elsevier, Barcelona, pp 361-377.

India G of (1960) The prevention of cruelty to animals act 1960. In: Gov Cent Sect 59 1960. https://www.indiacode.nic.in/bitstream/123456789/11237/1/the_prevention_of_cruelty_to_animals_act%2C_1960.pdf Accessed on: July 01, 2022

Jacques S (2014) Science and animal welfare in France and European Union: Rules, constraints, achievements. Meat Sci 98:484-489.

Jeleníková J, Pipek P, Staruch L (2008) The influence of ante-mortem treatment on relationship between pH and tenderness of beef. Meat Sci 80:870-874.

Joele MR, Lourenço LF, Lourenço Júnior JB, et al (2017) Meat quality of buffaloes finished in traditional or silvopastoral system in the Brazilian Eastern Amazon. J Sci Food Agric 97:1740-1745.

José-Pérez N, Mota-Rojas D, Ghezzi M, et al (2022) Effects of transport on water buffaloes (*Bubalus bubalis*): factors associated with the frequency of skin injuries and meat quality. J Anim Behav Biometeorol 10:2216.

Karachi G (1950) Karachi cattle slaughter control Act. http://faolex.fao.org/docs/pdf/pak116012.pdf Accessed on: July 01, 2022.

Lemcke B (2015a) Water buffalo handling: property to abattoir. North Territ Gov 1-4.

Lemcke B (2017) The Australian water buffalo manual. Department of primary industry and resources. Northern Territory Government: 1-147.

Lemcke B (2015b) Water buffalo handling: Property to abattoir, transportation to the abattoir. In: North. Territ. Gov.

Li X, Zito S, Sinclair M, Phillips CJC (2018) Perception of animal welfare issues during Chinese transport and slaughter of livestock by a sample of stakeholders in the industry. PLoS One 13:e0197028.

Lundmark F, Berg C, Röcklinsberg H (2018) Private animal welfare standards—opportunities and risks. Animals 8:4.

Lundmark F, Berg C, Schmid O, et al (2014) Intentions and values in animal welfare legislation and standards. J Agric Environ Ethics 27:991-1017.

Maciel CT (2015) Public morals in private hands? A study into the evolving path of farm animal welfare governance. Dissertation, Wageningen University.

Matheny G, Leahy C (2007) Farm-animal welfare,legislation, and trade. https://scholarship.law.duke.edu/cgi/viewcontent.cgi?article=1415&context=lcp Accessed on: July 01, 2022.

Matias BF, Okano W, Pértile SFN, et al (2019) Economic losses due to hematoma in bovine carcasses from the north central mesoregion of Paraná. Acta Vet Bras 13:114-119.

Mellor D (2017) Operational details of the five domains model and its key applications to the assessment and management of animal welfare. Animals 7:60.

Ministerio de Agricultura de Chile (1992) Ley 19162, Sistema obligatorio de clasificación de ganado, tipificación y nomenclatura de sus carnes y regula funcionamiento de mataderos, frigoríficos y establecimientos de la industria de la carne. https://derechoanimal.info/sites/default/files/doc-law/Ley%20de%20la%20carne.pdf Accessed on: July 01, 2022.

Ministerio de Agricultura de Chile (2013) Decreto 30. Reglamento sobre protección del ganado durante el transporte. https://derechoanimal.info/sites/default/files/doc-law/Reglamento%20de%20protecci%2B%EF%BF%BDn%20del%20ganado%20durante%20el%20transporte.pdf Accessed on: July 01, 2022.

Ministerio de agricultura y desarrollo rural (2020) Manual de condiciones de bienestar animal propias de cada una de las especies de producción del sector agropecuario; bovina, bufalina, aves de corral y animales acuáticos. In: Minist Agric y Desarrrollo Rural. Colombia, pp 1-35.

Mora-Medina P, Napolitano F, Mota-Rojas D, et al (2018) Imprinting, sucking and allosucking behaviors in buffalo calves. J Buffalo Sci 7:49-57.

Mota-Rojas D, Becerril-Herrera M, Gay JFR, Lemus FC, Alonso SML, Ramírez NR (2005) Calidad de la carne, salud pública e inocuidad alimentaria. México. Primera edición. Universidad Autónoma Metropolitana Serie Académicos CBS No. 52. México D.F. 353 pp.

Mota-Rojas D. Guerrero Legarreta I, Trujillo O.M.E, (2010a). Bienestar animal y calidad de la carne. Editorial BM Editores. México. p.361.

Mota-Rojas D, Alarcón-Rojo AD, Vázquez GG, Guerrero Legarreta I (2010b). Músculo oscuro firme y seco en bovinos, mecanismos involucrados. En: Bienestar animal y calidad de la carne. Primera edición- BM Editores. México D.F. p. 271-286.

Mota-Rojas D, Broom DM, Orihuela A, et al (2020) Effects of human-animal relationship on animal productivity and welfare. J Anim Behav Biometeorol 8:196-205.

Mota-Rojas D, Ghezzi MD, Napolitano F, et al (2021a) Quality of death in the river buffalo (*Bubalus bubalis*). J Anim Behav Biometeorol 9:1-10.

Mota-Rojas D, Napolitano F, Strappini A, et al (2021b) Pain at the slaughterhouse in ruminants with a focus on the neurobiology of sensitisation. Animals 11:1085.

Mota-Rojas D, Wang D, Titto CG, et al (2021c) Pathophysiology of fever and application of infrared thermography (IRT) in the detection of sick domestic animals: recent advances. Animals 11:2316.

Mota-Rojas D, Pereira AMF.; Wang D, et al (2021d) Clinical applications and factors involved in validating thermal windows used in infrared thermography in cattle and river buffalo to assess health and productivity. Animals, 11, 2247.

Mota-Rojas D, Napolitano F, Braghieri A, et al (2021e) Thermal Biology in River Buffalo in the Humid Tropics: Neurophysiological and Behavioral Responses Assessed by Infrared Thermography. J Anim Behav Biometeorol 2021, 9.

Mota-Rojas D, Wang D, Titto CG, et al (2022) Neonatal infrared thermography images in the hypothermic ruminant model: Anatomical-morphological-physiological aspects and mechanisms for thermoregulation. Front Vet Sci 9:963205.

Moura SV de, Silveira IDB, Ferreira OGL, et al (2021) Lairage periods on temperament score and meat quality of beef cattle. Pesqui Agropecuária Bras 56:1-8. https://doi.org/10.1590/s1678-3921.pab2021.v56.02349.

Muñoz D, Strappini A, Gallo C (2012) Indicadores de bienestar animal para detectar problemas en el cajón de insensibilización de bovinos. Arch Med Vet 44:297-302.

Napolitano F, Bragaglio A, Sabia E, et al (2020a) The human–animal relationship in dairy animals. J Dairy Res 87:47-52.

Napolitano F, Mota-Rojas D, Guerrero-Legarreta I, Orihuela A (2020b) El búfalo de agua en Latinoamérica, hallazgos recientes., 3ra ed. BM Editores, México. https://www.lifescienceglobal.com/journals/journal-of-buffalo-science/97-abstract/jbs/4550-el-bufalo-de-agua-en-latinoamerica-hallazgos-recientes

Napolitano F., Mota-Rojas D., Guerrero-Legarreta I, Orihuela A, Strappini A, Pereira A, Martínez-Burnes J (2022). El búfalo de agua en las Américas, enfoques prácticos y experimentales. 4th. edición. B.M. Editores, Ciudad de México, 2100 p.

Nava-Trujillo H, Valeris-Chacin R, Quintero-Moreno A, Escalona-Muñoz J (2020) Milk yield at first lactation, parity, and season of calving affect the reproductive performance of water buffalo cows. Anim Prod Sci 60:1073.

Nielsen SS, Alvarez J, Bicout DJ, et al (2020) Welfare of cattle at slaughter. EFSA J 18:e06275.

OIE (2021) Transporte de animales por vía terrestre. Código sanitario para los animales terrestres 1-16.

Omran F, Hamdon H (2018) Transportation, adaptive and productive performance of transported buffalo herd in new environment. J Anim Poult Prod 9:191-196.

Ortolani E, Maruta C, Barrêto Júnior R, et al (2020) Metabolic profile of steers subjected to normal feeding, fasting, and re-feeding conditions. Vet Sci 7:95.

Otter, O'Sullivan, Ross (2012) Laying the foundations for an international animal protection regime. J Anim Ethics 2:53.

Page KR, Doocy S, Reyna Ganteaume F, et al (2019) Venezuela's public health crisis: a regional emergency. Lancet 393:1254-1260.

Patiño EM (2011) Producción y calidad de la leche bubalina. Tecnol en Marcha 24:25-35.

PAWS (2018) The prevention of cruelty to animals. In: Pakistan Anim Welf Soc Pakistan, Govermet.

Pérez-Linares C, Sánchez-López E, Ríos-Rincón FG, et al (2013) Factores de manejo pre y post sacrificio asociados a la presencia de carne DFD en ganado bovino durante la epoca cálida. Rev Mex Ciencias Pecu 4:149-160.

Peters MDP, Silveira IDB, Fischer V (2015) Impact of subclinical and clinical mastitis on sensitivity to pain of dairy cows. Animal 9:2024-2028.

Pielago BS (2020) Uncovering the 5 Major Causes of the Food Crisis in Venezuela. Glocality 3:1-12.

Rojas H, Stuardo L, Benavides D (2005) Políticas y prácticas de bienestar animal en los países de América: estudio preliminar. Rev sci tech Off int Epiz 24:549-565.

Romero M, Sánchez J (2011) Implicaciones de la inclusión del bienestar animal en la legislación sanitaria Colombiana. Rev Colomb Ciencias Pecu 24:83-91.

Rushen J, Passillé AM de (2010) The importance of good stockmanship and its benefits to animals. In: Grandin T (ed) Improving animal welfare: a practical approach. CAB International, Wallingford, UK, pp 50-63.

Sabia E, Napolitano F, Claps S, et al (2018) Environmental impact of dairy buffalo heifers kept on pasture or in confinement. Agric Syst 159:42-49.

Schipp MA, Sheridan AD (2013) Applying the OIE terrestrial animal health code to the welfare of animals exported from Australia. Rev Sci Tech l'OIE 32:669-683.

Sheehan J (2012) Husbandry guidelines for water buffalo. In: West Sydney Inst TAFE.

Singh GS, Kumar AH, Ruchira, et al (2012) The Indian buffalo- meat vale chain. FICCI , New Delhi, pp 1-94.

Strappini A (2012) Problemas y errores comunes encontrados en Chile durante el manejo del Ganado. In: Mota-Rojas D, Huertas S, Guerrero-Legarreta I, Trujillo ME (eds) Bienestar animal: Productividad y calidad de la carne, 2.ª ed. Elsevier, Spain, pp 331-341.

Veissier I, Jensen KK, Botreau R, Sandøe P (2011) Highlighting ethical decisions underlying the scoring of animal welfare in the Welfare Quality® scheme. Anim Welf 20:89-101.

Velde H Te, Aarts N, Woerkum C Van (2002) Dealing with ambivalence: Farmers' and consumers' perceptions of animal welfare in livestock breeding. J ofAgricultural Environ Ethics 15:203-219.

Venezuela G oficial de la RB de (2010) Ley para la protección de la fauna doméstica libre y en cautiverio. In: Gac Of la República Boliv Venez.

Virviescas Sanabria JF (2020) Determinación de los efectos del transporte en las canales procesadas en la planta ecológica de beneficio animal Río Frío SAS. Universidad cooperativa de Colombia facultad de medicina veterinaria y zootecnia.

Webster AJF (2001) Farm animal welfare: the five freedoms and the free market. Vet J 161:229-237.

White S (2013) Into the void: international law and the protection of animal welfare. Glob Policy 4:391-398.

Artículo de revisión intitulado:

2.2. CAPÍTULO II

Manejo y aspectos fisiológicos del búfalo de agua en un sistema doble propósito en el trópico húmedo mexicano

Publicado en la revista *Animals*, misma que se encuentra indizada al JCR, Scopus y PubMed, y tiene un factor de impacto de 3.0.

Rodríguez-González, D.; Minervino, A.H.H.; Orihuela, A.; Bertoni, A.; Morales-Canela, D.A.; Álvarez-Macías, A.; José-Pérez, N.; Domínguez-Oliva, A.; Mota-Rojas, D. Handling and physiological aspects of the dual-purpose water buffalo production system in the mexican humid tropics. *Animals* 2022, 12(608), 1-29. https://doi.org/10.3390/ani12050608

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Review

Handling and Physiological Aspects of the Dual-Purpose Water Buffalo Production System in the Mexican Humid Tropics

Daniela Rodríguez-González [1], Antonio Humberto Hamad Minervino [2,*], Agustín Orihuela [3], Aldo Bertoni [4], Diego Armando Morales-Canela [4,5], Adolfo Álvarez-Macías [4], Nancy José-Pérez [4], Adriana Domínguez-Oliva [4] and Daniel Mota-Rojas [4,*]

[1] Master's Program in Agricultural and Livestock Sciences [Maestría en Ciencias Agropecuarias], Xochimilco Campus, Universidad Autónoma Metropolitana (UAM), Mexico City 04960, Mexico; drg.mvz02@gmail.com
[2] Laboratory of Animal Health, LARSANA, Federal University of Western Pará, UFOPA, Rua Vera Paz, s/n, Santarém 68040-255, PA, Brazil
[3] Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Cuernavaca 62209, Mexico; aorihuela@uaem.mx
[4] Neurophysiology, Behavior, and Animal Welfare Assessment, Department of Animal Production and Agriculture (DPAA), Xochimilco Campus, Universidad Autónoma Metropolitana (UAM), Mexico City 04960, Mexico; aldo_bm@hotmail.com (A.B.); damoralesc@outlook.com (D.A.M.-C.); aalvarez@correo.xoc.uam.mx (A.Á.-M.); nancy.jopz@gmail.com (N.J.-P.); mvz.freena@gmail.com (A.D.-O.)
[5] Holistic Management, Savory Institute, Agriculture Science, Universidad EARTH, San José 4442-1000, Costa Rica
* Correspondence: ah.minervino@gmail.com (A.H.H.M.); dmota100@yahoo.com.mx (D.M.-R.)

Simple Summary: Buffalo is a domesticated large ruminant that can be raised for beef, dairy, and work. In some systems, these animals can be raised with a dual purpose (beef and dairy). The present review describes the characteristics of the dual-purpose water buffalo production system in Mexico's humid wetlands. This article provides extensive information on the water buffalo and includes comparisons with other species to note similarities and differences. The aim is to describe the buffalo handling procedures used in this system, particularly during breeding, milking, confinement, and mobilization, relating them to the neurological processes involved and analyzing the productive results. Understanding these processes will allow us to obtain a more precise vision of the advantages that this species can offer, and the possible implications of the development of this type of livestock under tropical conditions.

Abstract: The purpose of this paper is to describe the characteristics of the dual-purpose water buffalo production based on the Mexican production system as a model in tropical wetlands. It includes a broad literature review emphasizing the most recent and specialized publications examining key findings to improve our understanding in the performance of the buffalo species (*Bubalus bubalis*). The complementary topics addressed include reproductive management, parturition, the dam–calf bond, milking routines, and models of confinement and management, in addition to aspects related to milk commercialization. This article summarizes the advances made to date in this production system and its current margins for improvement. The development of dual-purpose water buffalo production systems in Mexico's tropical wetlands is a relatively recent phenomenon that has progressed and improved due to herd management. Buffaloes are an interesting alternative for dual purpose systems that offer several advantages. The lower milk production of this species compared to cattle is its main limitation. However, the properties of their milk allow one to obtain an added value and make this type of farms competitive. In synthesis, consolidating buffalo production in Mexico's tropical wetlands will require broadening our knowledge of this species, and perfecting the most appropriate handling procedures. The activities of government agencies and processing enterprises will play vital roles in achieving the integral modernization of this potentially important economic activity.

Keywords: allosuckling; *Bubalus bubalis*; calving; imprinting; Mexico wetlands

Citation: Rodríguez-González, D.; Minervino, A.H.H.; Orihuela, A.; Bertoni, A.; Morales-Canela, D.A.; Álvarez-Macías, A.; José-Pérez, N.; Domínguez-Oliva, A.; Mota-Rojas, D. Handling and Physiological Aspects of the Dual-Purpose Water Buffalo Production System in the Mexican Humid Tropics. *Animals* **2022**, *12*, 608. https://doi.org/10.3390/ani12050608

Academic Editor: Marina Von Keyserlingk

Received: 23 November 2021
Accepted: 14 February 2022
Published: 28 February 2022

1. Introduction

In recent years, the FAO has maintained that one of the principal challenges facing global society is alimentary insecurity, given the high levels of poverty and the difficulties that existing agricultural and food systems encounter in guaranteeing food supplies and their adequate distribution [1]. The FAO estimates that by the year 2050, the world's population will be 9.73 billion, which means that food production must increase by 49%. It also stresses that this is not just a future problem, but also a current one, as about two billion people have micronutrient deficiencies, and almost 800 million suffer from chronic hunger.

In response to global food insecurity, the expansion of water buffalo could be considered because they can thrive in many regions with low agricultural potential. Despite recent controversies surrounding the raising of large ruminants, the physiological and technical characteristics of the water buffalo have stimulated the growth of its populations worldwide. This has been achieved specifically due to its rustic nature and productive efficiency in regions with low agricultural potential, such as wetlands, areas of difficult access, and regions with medium- and low-quality grasses. In these kind of habitats, the adequate management of this species can contribute to a process of progressive regeneration of pasture [1–5].

The water buffalo is ranked as the sixth most abundant productive species on the planet, surpassed only by inventories of poultry, conventional bovines (genus *Bos*), swine, sheep, and goats [6]. In 2018, the FAO reported a population of 206 million water buffaloes worldwide, distributed in 48 countries, concentrated mainly in Asia, with roughly 97% of the total population, especially India, China, and Pakistan. The remaining water buffalo are distributed Africa (1.7%, mostly in Egypt, the Americas (1.2%), Europe (0.2%), and a small herd in Oceania (0.07%) [2]. Other important data presented by the FAO include the fact that the growth of water buffalo populations around the world in the 2008–2018 period was 11%, 6% greater than that of *Bos* cattle in the same period [1].

Unverified reports on Mexico estimate a water buffalo population of 45,000 individuals, distributed primarily in tropical wetland areas where a dual-purpose buffalo production system (DPBPS) has been adopted, obtaining meat and dairy products simultaneously. To date, only a few documents have characterized these enterprises in the tropical areas [7–10]. The equal priority that female and male animals require in dual-purpose production units requires complementary forms of management, especially for processes such as breeding.

Considering the above, the aim of this review article is to describe and characterize the key technical and commercial processes and practices of dual-purpose water buffalo production systems in Mexico's tropical wetlands, with an emphasis on reproductive, physiological, and behavioral aspects, among others, that are strategic for the efficient development of these systems. The broader objective is to advance towards the goal of achieving efficient, sustainable production models.

2. Materials and Methods

This study was based on a broad literature review that focused on the most recent publications examining information that contributes to a better understanding of the performance of this animal species.

To visualize and analyze the processes employed on water buffalo ranches, we made periodic visits to production units and programmed meetings with key actors, including owners and administrators, coupled with an update of the scientific literature available. The visits were performed for three months, starting in August, and ending in October 2021; from 04:30 h to 19:00 h. We utilized technological tools to map study areas by means of infrared thermographic evaluation and the observation of the operations conducted. To assess such topics as production flows, pasturing zones, the mobilization and transport of animals, and how handlers identify sick animals or females during calving, a drone was flown daily over the study area (DJI Phantom Drone 4 pro V2.0, DJI, Shenzhen, China) at an altitude of up to 60 m (Figure 1). Regarding the thermographic images, an infrared camera was used (FLIR Thermal TM E95, FLIR Systems Inc., Wilsonville, OR, USA) with an emissivity of 0.95 and IR resolution 464 × 348. Our characterizations of the production processes in each zone include descriptions of important physiological and neurobiological processes of the water buffalo during the reproductive phase of the estrus and the use of artificial insemination, and explaining the neurobiological processes involved in calving, imprinting, milking, and allonursing. Our goal was to obtain a broad panorama of the physiological particularities of female buffaloes as a species and, where possible, identify aspects that require improvement. Finally, we elaborated a map of routine processes and elaborated a descriptive discussion of neurobiological processes. These activities required a broad bibliographic review and consulting and selecting scientific articles available in several databases, including ScienceDirect, Web of Science, Scopus, and PubMed, using the following keywords: "water buffalo", "river buffalo", "domestic buffalo", "breeding", "dystocia", "parturition", "productivity management". The information chosen for analysis was recent and, preferably, of high impact.

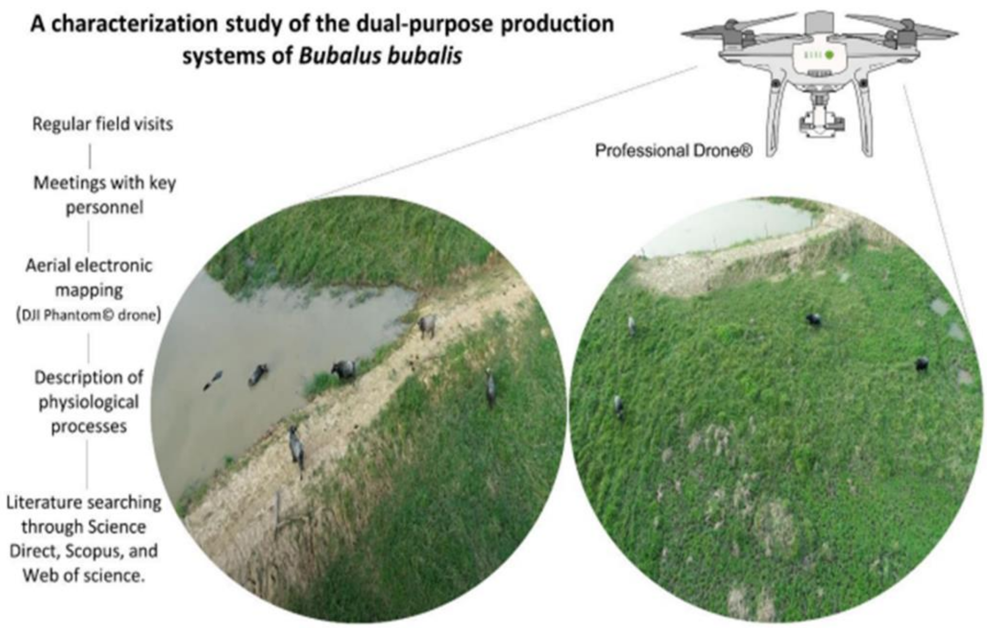

Figure 1. Methodology to obtain the data and describe the dynamic process in the dual-purpose production system of water buffaloes (17°38′54.0″ N 94°36′05.0″ W). Signs of estrus and parturition are facilitated by this method. The data obtained through regular field visits and aerial mapping with a DJI Phantom 4 Pro V2.0, DJI, Shenzhen, China, drone were compared to recently published scientific references.

3. Reproductive Management of Female Buffaloes

3.1. Genetic Selection

The genetic selection of water buffaloes is based on traditional criteria such as levels of milk production and docility. Animal docility, milk production, and maternal ability traits are phenotypic characteristics selected in the farms by producers. This means that both qualitative and quantitative characteristics are considered in efforts to optimize the entire system, and this process entails evaluating traits associated with high productivity, such as an adequate body structure that permits ingesting and then transforming food to build muscle, and the development of a mammary system capable of synthetizing large amounts of milk [10,11]. Another feature commonly assessed is the conformation of the legs and hooves, as they play an essential role in buffalo health [12], allowing them to move through pasturelands for forage access. In synthesis, these varied selection criteria all play key roles in the level of productivity of dual-purpose systems in genetic and reproductive management. In the case of males used as stallions, the selected animals must be from artificial insemination with a birth weight above the average, and should have desirable behavioral aspects such as docility and meekness. In females, these behavioral characteristics are also considered, in addition to a record of the parents and phenotypic characteristics such as mammary gland development and kilograms of milk produced by lactation, information retrieved from production records (Figure 2).

Breeders consider essential selection criteria in efforts to improve the selection of dams and bulls on their ranches. The latter are selected from catalogues that stress the genetic value that they can pass on to their descendants. This genetic material is normally utilized through artificial insemination, in some cases when estrus is detected, but more often at fixed times. Studies have detected that the Buffalypso breed is the one most often utilized in DPBPS, although breeds with greater milk-producing potential, such as Mediterranean Italian and Murrah, are being introduced gradually. This has entailed implementing assisted reproduction technologies, as we describe in the following section [8,13,14].

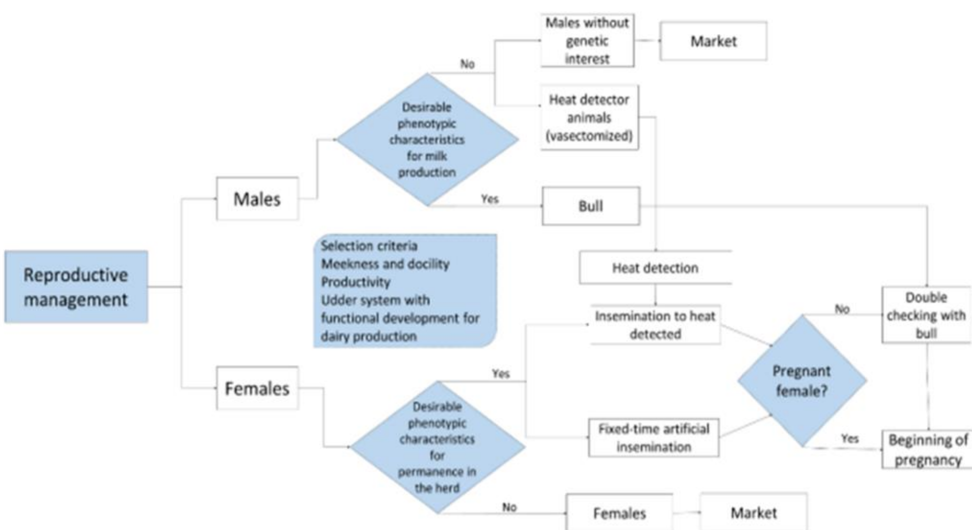

Figure 2. Reproductive management in the dual-purpose production system of water buffalo housed in the humid Mexican tropics.

3.2. Estrous Detection

Identifying estrus in female buffaloes is especially complicated because these animals manifest the signs commonly seen in cows including vulvar edema, frequent urination, and vaginal secretions with very low intensity [13]. For this reason, breeders have opted to use a vasectomized male to identify females that are viable for insemination. Female buffaloes have a small follicular size that generates low concentrations of estradiol. Research has documented that 3.4% of female buffaloes manifest estrous behavior and that over 60% have what is called "silent estrus" [8,15]; thus, some ranches have implemented fixed-time reproductive protocols that foster genetic improvement by responding to the physiological characteristics of female buffaloes if no male capable of identifying estrus is available (Figure 3). In addition to this, non-invasive tools such as infrared thermography have been used in the detection of estrus due to the changes presented in the vulva prior to ovulation, which could be an efficient and safe indicator of estrus [16–19].

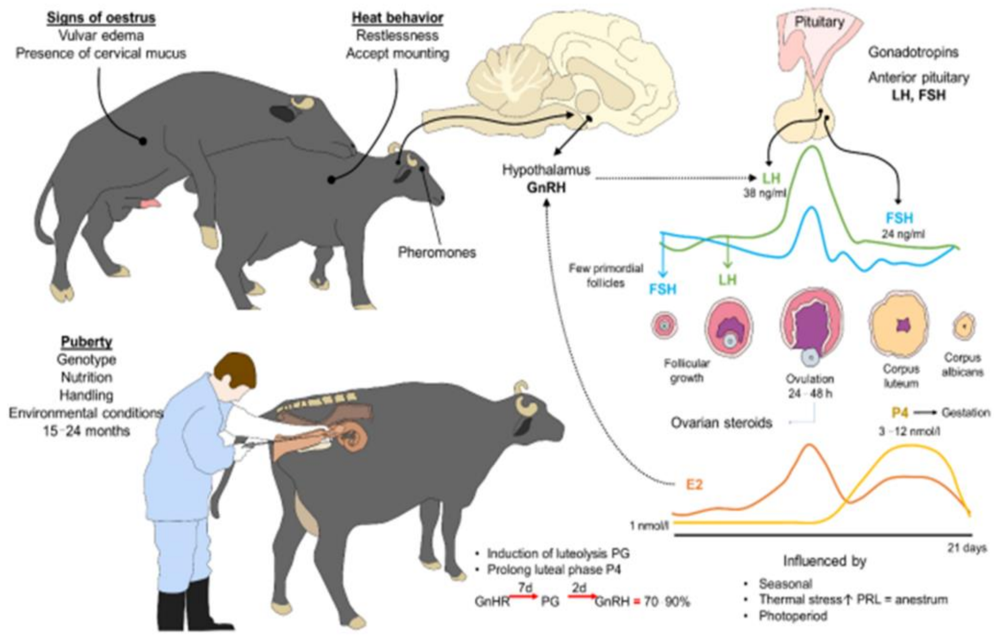

Figure 3. Neuroendocrinology of the estrus cycle, estrous detection, and artificial insemination technique. During estrus, multiple signs and behaviors may be present in female buffaloes. Concentrations of LH and FSH, two gonadotropins secreted by the anterior pituitary through the action of the hypothalamus, are the main hormones involved. When LH and FSH levels are sufficiently high, ovulation takes place (lasting 24–48 h in buffaloes) with the consequent secretion of E2. Estrogen levels decrease gradually while progesterone levels increase to restart the estrous cycle. Heat detection based on signs, behaviors, or hormone levels is an essential aspect of artificial insemination programs, where one main goal is to avoid influences by season or other factors. E2: 17β-estradiol; FSH: follicle-stimulating hormone; GnRH: gonadotropin releasing hormone; LH: luteinizing hormone; P4: progesterone; PG: prostaglandins; PRL: prolactin.

3.3. Artificial Insemination

Artificial insemination has been widely incorporated into buffalo production systems in Mexican tropics, so the females selected to remain with the group and that are viable for reproduction are subjected to methods of assisted reproduction. Once a vasectomized male identifies the females that display natural estrus, they are inseminated artificially at a fixed time, while the others are included in a protocol that consists of applying a hormonal treatment prior to insemination. In both cases, the females that do not become pregnant are served directly by a bull to improve pregnancy rates. This makes it possible to scale the birth of calves throughout the year, keeping milk production relatively stable [14]. Thus, we believe that the joint implementation of reproductive biotechnologies facilitates advancing genetically towards the goals of achieving productive transcendence and maintaining controlled estrus cycles (Figure 3). Other reproductive technologies, such as embryo transfer (ET) or sexed semen, are not utilized; the first due to sanitary restrictions on their importation, and the second because of high costs.

4. Calving

Calving begins physiologically at least ten days prior to parturition, which occurs after 300–329 days of gestation (mean range of 310–315 days) [20–22]. For calving, cows are taken to a secure area for continuous supervision and, if necessary, timely obstetric intervention. Once installed in the maternity paddock, the female buffaloes are evaluated daily to ensure that calving is detected promptly. The personnel in charge verify the condition of the udders, the frequency of urination, and postural changes such as repeatedly lying down and standing up, all of which indicate that calving is imminent [23].

The area set aside for calving and the onset of maternity must have certain, basic physical and biological characteristics, including native forage such as Camalote (*Paspalum fasciculatum*) and Azuche (*Hymenachne amplexicaulis*), which are the primary forages for this species in the region [24,25]. The dams' daily diet is often supplemented with 50 g of minerals [26]. This area must also have tree species that provide the shade that female buffaloes require for thermoregulation [27]. Other recommendations in the calving area are to supply drinking water in troughs and avoid deep bodies of water to eliminate the possibility of drownings. When the cow is about to give birth, monitoring can be complemented using a drone, a technological tool that does not interfere with the animals or cause the stress that can arise from human presence. The objective is to identify any cow that presents symptoms of dystocia during parturition and immediately call for veterinary attention, though this is rarely required [28].

When brought to a satisfactory conclusion, parturition is considered successful thanks, in part, to the constant supervision of the females that are close to delivery. Although the frequency of dystocia is low in buffaloes, it may occur [29]. This condition not only threatens the welfare of both dam and offspring, but also means economic losses for producers, especially if they have invested significant economic resources in implementing reproductive protocols such as artificial insemination.

The following section describes the calving process from a physiological perspective, including both normal (eutocic) deliveries and deviations that can occur. The latter are considered dystocic deliveries and require veterinary assistance.

4.1. Eutocic Births

According to the literature, labor in buffaloes begins with the onset of regular uterine contractions accompanied by the progressive dilatation of the uterine neck. This typically occurs in three stages: dilatation of the cervix, expulsion of the fetus, and expulsion of the fetal membranes [23,28,30].

The first stage usually lasts 1–2 h, possibly longer in primiparous females [31]. In this stage, the buffaloes show a structural change in the dilatation of the uterine neck, triggering the onset of contractions of the myometrium. Then, the fetus adopts the position for expulsion [32]. The cow may seem restless and her heart rate and respiratory frequency may increase [30,32].

Ensuring the availability of natural shade in the birthing area where the prepartum female buffaloes are held could have a positive effect on the parturition process, reducing the incidence of thermal stress and the probability of suffering placental hormonal imbalances that could negatively affect the birth weight of her calf and interfere with the passive immunity acquired by the calf when ingesting colostrum [33,34]. Mudgal [35] suggested that supplementing the diet of multiparous female Murrah buffaloes with vitamins A and E had positive effects on increasing the protein and total solid content of their colostrum during the first 2 to 3 d postpartum, respectively, compared to a group of females that did not receive supplementation.

It is important to underline the importance of electronically monitoring the females during calving with a drone to detect signs of the approaching delivery. Telltale signs include separation from the group, a continuous standing posture, frequent tail movements and vocalizations. In addition, recent studies have documented other observable signs, such as sagging of the sacrosciatic and sacroiliac ligaments 12–24 and 24–72 h prepartum, respectively. The relaxation of these pelvic ligaments results in an apparent raising of the base of the tail. In some cases, it might be possible to observe edematization of the vulva with the presence of crystalline mucus around 72–96 h prepartum. In addition, 24–36 h before calving, the vulva appears extremely flaccid and the cow's teats look distended. Then, 1.8 d before delivery, the mammary veins appear tense [36]. Additional signs may appear because of pain. These include nervousness, reduced appetite, and postural changes (lying down/standing up and vice versa). Cows also tend to look at their flanks constantly, keep their heads raised, scratch the ground, and raise their tails during contractions. Handlers may also observe lateral tail movement, arching of the back, and flexions of the hocks accompanied by restlessness [36] (Figure 4). Watery diarrhea has been reported as another sign of approaching delivery [36].

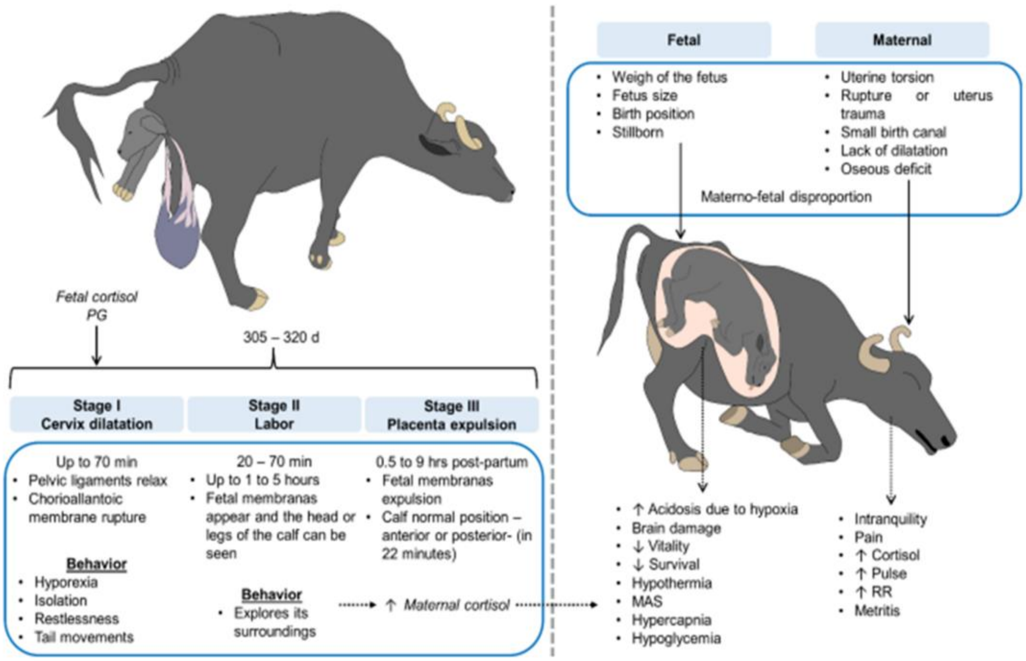

Figure 4. Stages of parturition and maternal and fetal causes of dystocia in the water buffalo. MAS: meconium aspiration syndrome; PGF2 alfa: prostaglandin F2 alpha; RR: respiratory rate.

The second stage, expulsion of the fetus, is marked by strong uterine and abdominal contractions and cervical dilatation [30]. Other signs are the rupture of the allantochorion, the release of liquid through the vulva, the appearance of the amnion in the vulva, its breakage and, finally, the expulsion of the calf [30]. This stage generally lasts 30–60 min [31].

The third phase occurs when the dam expels the fetal membranes. It usually lasts 4–5 h after the expulsion of the calf [31]. During this stage, the intensity of the uterine contractions diminishes, lessening the dam's physical exertions and paving the way for the shedding of the chorionic villosities from the maternal crypts [30,32], Finally, placentophagia occurs, an observed behavior that consists of the consumption of placental components by the buffalo [37].

A study by Deka et al. [38] evaluated the behavior of water buffaloes during the three stages of calving. They determined that in the first stage, 100% of females showed nervousness, reduced food and water consumption, displayed postural changes (standing or in repose), tail-raising, abdominal exertion, arching of the back, vaginal secretion, and frequent urination. For stage 2, they found that the buffaloes showed signs akin to those observed in stage 1, except for tail movements (in 100% of cases). The behavior of the buffaloes in stage 3 was characterized mainly by licking the neonate and renewed interest in food and water, with occasional manifestations of abdominal effort [38].

4.2. Dystocic Calving

The importance of understanding the three stages of parturition in female buffalo, including the associated times, behaviors, and signs, lies in recognizing when labor is prolonged, ensuring that the cow expels the fetus in a normal position, or opportunely determining when some unexpected event occurs that could indicate an anomaly in the calving process. Dystocia has severe consequences for both dam and fetus (neonate) that may include uterine infections, placental retention, excessive expulsion time, decreased milk production and, in extreme cases, increased mortality and morbidity of dam and calf [39].

The occurrence of dystocic births in DPBPS is uncommon. In the isolated cases that do occur, handlers are limited to help the female by means of manual traction manipulations to aid in expelling the fetus. Surgical techniques and pharmacological therapies are not routine obstetric practices because veterinarians are rarely called in to assist in deliveries involving female buffaloes.

In contrast to cattle (*Bos taurus* and *B. indicus*), female buffaloes show a low predisposition towards dystocic births due to key anatomical differences, such as their pelvic and genital structure. The iliac area of buffaloes is larger than in cattle, and the fifth sacral vertebra is separated, allowing greater freedom of movement and a wider birth canal that facilitates expulsion of the fetus [40,41]. The morphology of the female buffalo's reproductive tract includes longer, wider vaginal lips, and a small vaginal canal that dilatates easily to help complete labor in a shorter time than in cattle [40]. For example, some studies have documented that 5% of the total of dystocic births attended in cows are attributed to their narrow pelvic canal [42], in sharp contrast to the low percentage reported in buffaloes (1.6%) (Figure 3). The pelvic measure is used to assess in cattle if the canal is narrow. The pelvic area obtained before breeding divided by the estimated calf birth weight is considered a reliable indicator of dystocia [43].

Although the DPBPS reports low incidences of dystocic births in female buffaloes in Mexico's tropical wetlands, retrospective studies in India—the principal water buffalo-producing country in the world—reported the incidence of such births in buffaloes at 80.3%, which is a similar rate to what has been observed in cattle (78.89%) [42]. This could be due to different obstetric management in Asia, or a different genetic line that could predispose buffalo females to developing dystocia, such as a weaker broad ligament, a factor that can be found in newly born buffalo calves [44]

In mammals in general, complications during birth are attributed to either maternal or fetal causes. These conditions are examined separately in the following sections.

4.2.1. Maternal

Maternal conditions, such as uterine torsion, narrowing of the birth canal, and incomplete cervical dilatation are the main causes of complications in female buffaloes [45]. Numerous studies have reported that approximately 53.6% of dystocic births in female buffaloes can be attributed to such maternal factors [40,46]. In one experiment with 142 Murrah buffaloes, 59.2% of the dystocic births were due to maternal causes, with the main etiology being uterine torsion (83.3%) [47]. These findings coincide with the results of a study by Jeengar et al. [42], in which for 55.7% of the 51 buffaloes that had dystocic births, dystocia was caused by uterine torsion. While figures for the survival of the dams in those cases were high (90%), up to 70% of the newborns died as a result of the dam's condition [48]. Clearly, this is a topic that water buffalo breeders must address.

Uterine torsion occurs when the uterus twists (90–120 degrees) along its longitudinal axis. The point of torsion is classified as post- or pre-cervical, with the former being more frequent (98.4%) [49]. Most of the females that present this condition do so during the final stage of gestation (5–8 mo) [50]. Uterine torsion occurs more often in multiparous than primiparous females (81.7 vs. 18.3) [49]. Some of the signs associated with this pathology are hyperoxia, constipation, colic, and straining [48]. One reason why uterine torsion is the main etiology of dystocic births in female buffaloes has to do with the anatomy of *Bubalus bubalis* dams. These animals have a wide, but weak, ligament with few, underdeveloped, muscle fibers [51]. Because this wide ligament is not fixed to the dorsal or right walls and lacks muscle folds, the ligament is prone to twisting [50]. Regarding this condition, a study of 20 buffaloes by Ali et al. [49] estimated that 96% of uterine torsions were towards the right (clockwise), due to the characteristics of this ligament compared to the one on the left side where the rumen is located [50]. Other elements associated with uterine torsion are low amounts of fetal fluids, reduced tone and size of the uterus at the end of gestation that increase fetal movement, cases in which the uterus is not anchored appropriately and so can twist on its own axis, the female buffalo's pendulous abdomen, and wallowing behavior, though the role of the latter factor has not yet been objectively demonstrated [50].

Maternal causes of dystocia, especially uterine torsion, have numerous effects that run from a high incidence of stillbirths to organic imbalances and alterations in the dam [23,28]. In this vein, in a study of 28 female buffaloes that presented dystocia, their biochemical and mineral profiles showed significant increases in plasma iron, copper, zinc, calcium, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase concentrations [52]. Likewise, hematological parameters such as high levels of urea, nitrogen, and phosphorus, accompanied by decreased mean corpuscular hemoglobin, derive from insufficient irrigation that can affect renal and liver function [49]. Creatinine, progesterone, cortisol, tumor necrosis factor (TNF-α), interleukin 6 (IL-6), and glucose concentrations also increase in response to the stress caused by prolonged births and pain [48]. Research on dystocic female buffaloes has verified that plasma cortisol concentrations may almost double in animals with dystocic vs. eutocic births (64.3 +/− 10.1 ng/mL vs. 39.3 +/− 2.0 ng/mL, respectively) [53], and that this can have lethal consequences for dams and offspring.

Other consequences are adherence to abdominal tissues, edema, intense uterine contractions, increases in creatine phosphokinase (CK), and placental retention [50]. If the fetal membranes have not been expelled after 12 h (normal time = 0.5–8 h postpartum), there is a risk that the dam may develop metritis, endometritis, or pyometra [28].

4.2.2. Fetal Factors

One of the most common causes of dystocic births is maternal–fetal disproportion [41]. Srinivas et al. [47] reported that 40.8% of dystocic births in female Murrah buffaloes are due to fetal factors, including oversized fetuses and abnormalities such as deformities, congenital and acquired diseases (e.g., hydrocephaly, ascites, anasarca, hydrothorax), and inadequate positioning in the birth canal. On the topic of fetal size, Kumar et al. [54] reported a dystocia incidence of 40.8%, and found that 22.4% of these cases were due to an oversized fetus, a condition that requires fetotomy (if the fetus is dead inside the uterus) or caesarean section. In some cases, a size disproportion can cause pathologies or deformities in the fetus. For example, though rare, fetal ascites—due to insufficient drainage of the peritoneal fluid or reduced excretion of urine [41]—and congenital diseases such as hydrocephaly (present in India in 1.5 of every 1000 births) increase the size of the fetus' body or head, preventing it from passing through the birth canal [55]. A similar phenomenon has been detected in cases of deformities (monstrous births). Though the latter events are extremely rare, several cases have been identified by Gahlod et al. [56] and by the *Instituto de Educación e Investigación Veterinaria* [57].

The position that the calf adopts during parturition has a significant influence on whether the birth will be eutocic or dystocic. Dystocic births occurred mostly with an anterior (86.7% of cases) presentation rather than with a posterior (13.3%) one; deviated limbs (57.8%) were more frequent than deviations of the head (42.2%), regardless of the dystocic presentation [47]. Severe lateral torsions of the head and neck carry a high risk of fetal mortality [58]. Abnormal positions and difficult expulsions affect both the newborn and dam, especially in production units where births are overseen.

Intervention during a dystocic birth consists of manually pulling the fetus out of the birth canal; but if this movement is not executed with great care, or if an incorrect technique is used, severe perineal lesions may occur, including infection and necrotization, and surgical intervention might even be required [59].

Consequences for the neonate depend largely on the time between what began as a normal birth and the identification of a condition with dystocic features. The longer this period is, the more severe the repercussions for the calf's health are. Studies have documented that the second stage of a eutocic birth lasts around 20–70 min. When this time is exceeded, the risk of fetal acidosis (caused by hypoxia or anoxia) increases. This condition can trigger organ failure and even death of the newborn [60]. Other problems identified are hypercapnia, hypothermia, hypoglycemia, meconium aspiration syndrome, and ruptured umbilical cord. All these factors can predispose calves to other pathologies or low vitality during the first hours of life [28]. In general, dystocic births, whatever their cause, are accompanied by biochemical, physiological, and behavioral alterations, such as restlessness, pawing the floor, or arching the back due to pain [61]. The importance of monitoring parturitions ensures the early detection of abnormalities during labor, so that strategies can be implemented immediately to prevent more severe consequences for both dam and calf.

5. Dam–Offspring Bond

During the expulsion of the fetus, handlers in the calving area generally watch over movements of the limbs and the thoracic region to verify adequate respiration. If a lack of movement is detected in the calf, the observers must enter the paddocks and approach carefully to determine if it requires assistance or, in the worst case, is stillborn. Observers should also ensure that the dam stays close to the calf and begins calf–cow interaction by licking the offspring to remove the fetal membranes. If the dam moves away from a calf that is still unable to stand or suckle, handlers must attempt to facilitate the formation of the bond between the dam and the newborn. If the dam continues to reject her offspring, the neonate will need to be integrated into a process of communal rearing where it will be fed by surrogates, a method that has been seen to be highly feasible among water buffaloes. Keeping the dam and calf together, followed by the onset of suckling—including colostrum ingestion—are especially important processes that are intimately related to the survival and development of these animals (Figure 5).

Neurophysiological Mechanisms between Dam and Offspring Activated by Sight, Licking, Smell, and Vocalizations

At birth, the dam is the calf's first social contact and main source of learning. At her side, the newborn memorizes the behavioral patterns necessary for its survival and other important information concerning its physical and social environment [62]. Establishing the dam–calf bond after parturition is, therefore, a transcendental step, especially in production systems based on free pasturing [63] due to the risk of losing those calves in extensive pasturelands [64,65]. The formation of the dam–calf bond involves learning processes that permit a clear acquisition of information and the establishment of preferences through specific sensory stimuli (sight, touch, hearing, smell, taste) [62]. In the case of buffaloes, sight and smell are the most active senses [65], as they consist of a series of sensory communication channels that consolidate mutual recognition and have a lasting influence that resists eventual temporary separations [64] (Figure 5).

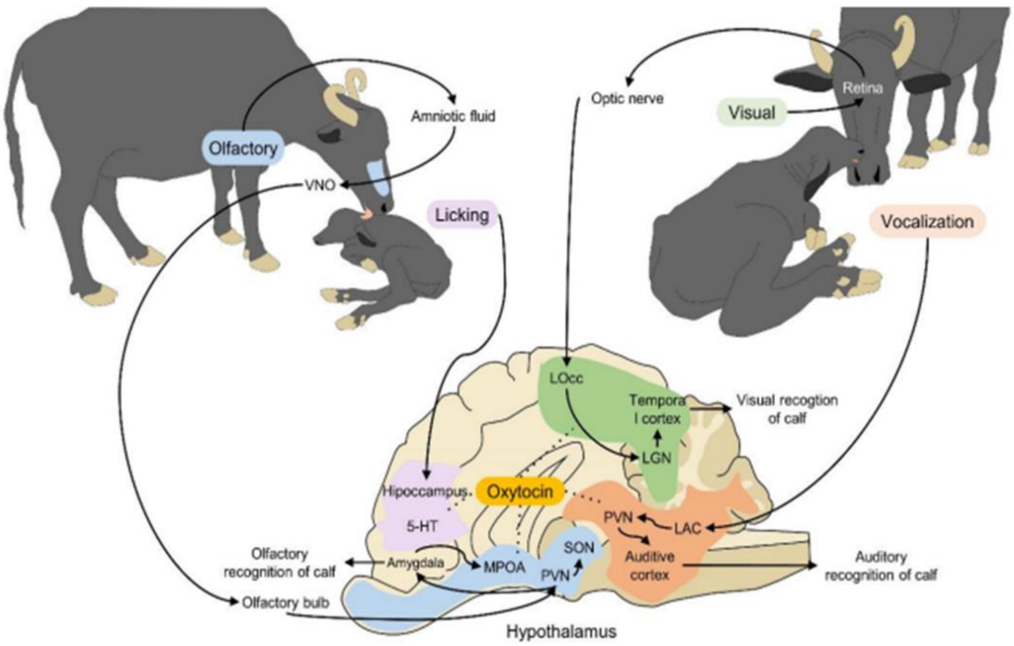

Figure 5. Olfactory, auditory, visual, and tactile recognition of calves in the water buffalo (*Bubalus bubalis*). Various structures and brain nuclei are activated when a calf is born. First, the amniotic fluid activates the VNO. The VNO, through the olfactory bulb, projects the stimuli to the PVN, SON, MPOA, and amygdala. Licking the fetal membranes off the newborn activates areas of the hypothalamus that release 5-HT and oxytocin. Visual recognition of the calf is achieved through the projection of the optic nerve to the Locc, LGN, and temporal cortex. Vocalizations contribute to recognition with the LAC, PVN, and auditory cortex all playing important roles. All these stimuli participate in the secretion of oxytocin, the main neurotransmitter involved in maternal behavior. 5-HT: serotonin; LAC: left auditory cortex; LGN: lateral geniculate nucleus; Locc: occipital lobe; MPOA: medial preoptic area; OXT: oxytocin; PVN: paraventricular nucleus; SON: supraoptic nucleus: VMH: ventromedial nucleus of the hypothalamus; VNO: vomeronasal organ.

During stage 2 of parturition, imprinting occurs, which starts when the product begins its passage through the cervical-vaginal portion of the birth canal [23]. Stimulated by the transit of the fetus, mechanoreceptors in the uterus send information through the spinal cord to hypothalamic structures as the paraventricular and supraoptic nuclei, which stimulate the release of oxytocin from the posterior hypophysis [65]. Oxytocin's dual action generates simultaneous responses, generating contractions of the birth canal, and acting as a neurotransmitter in the dam's olfactory bulb that aids in the secretion of dopamine, which triggers the process of mutual recognition between dam and calf [64].

In water buffaloes, the most common pattern of maternal behavior in the first 6 h after birth is licking [66]. Around this time, the calf makes its first attempts to stand up, seek the udder, and suckle to receive the nutritional and immunological benefits that the dam's milk provides. The dam facilitates access to her teats by arching her back and flexing her hind limbs. Allosuckling is often seen in multiparous female buffaloes, accepting calves from different biological dams. This behavior, considered altruistic, is examined in detail in subsequent sections.

Calves and dams can perceive a series of signals that respond to auditory, visual, olfactory, and tactile stimuli. The olfactory pathway is thought to be the most direct one between the environment and brain, and actively participates during imprinting. As with taste, smell is classified as a chemical sense that has the capacity to capture volatile and non-volatile chemical substances by means of receptors coupled with G proteins [67]. The sense of smell is integrated with two pathways: the main olfactory epithelium and the vomeronasal organ (VNO). The first detects odorific chemical substances suspended in the air, associated with functions such as alimentation and predator detection. The second identifies non-volatile chemical substances, such pheromones, more commonly related to sexual behavior [65,67]. Both pathways convey signals to the olfactory bulb, which evaluates the odorific configuration detected and sends signals to the aforementioned hypothalamic structures—paraventricular (PVN) and supraoptic nuclei (SON)—which in turn contain neuroendocrine cells that synthetize oxytocin [62]. These signals also reach limbic system structures. The hippocampus stores the information, and the medial portion of the amygdala (MPOA) generates emotional responses linked to the stimuli, including maternal recognition, attachment to the calf, and reproductive functions [64].

Visual imprinting begins with preliminary recognition which occurs thanks to the newborn calf's ability to follow a moving object or being that offers protection and sustenance. Visual stimuli are projected through the optic nerve to the occipital lobe (LOcc). The lateral geniculate nucleus (LGN) receives the visual information perceived and sends the signal over the geniculate-striatal pathway to the visual cortex, which decodifies it and transforms it into visual characteristics [64]. Normally, the definitive recognition of calves occurs through olfactory stimuli, with visual communication playing a complementary role [65].

The auditory communication pathway permits acoustic recognition that stimulates the care of the offspring based on the emission and recognition of auditory patterns. This pathway operates bidirectionally between the dam and calf: on one side, an emitter that conveys differentiated vocalizations, and on the other, a receptor that captures clearly recognizable auditory patterns. This link can also transcend temporary separations [65]. The vocalizations emitted are projected to the auditory thalamus, which conveys the signals to the PVN and auditory cortex where the bidirectional auditory recognition of the emission takes place [64].

All the communication pathways that generate and maintain the dam–calf bond are related to structures that release oxytocin and positive (satisfactory) emotional responses. The oxytocinergic and dopaminergic systems are the biological systems involved in maternal recognition and care. Of prime importance here is the release of oxytocin to activate dopamine pathways [65].

6. Colostrum

Colostrum Ingestion in the Dual-Purpose System

The mixture of milk secretions, immunoglobulins, and other serum proteins that accumulate in the dam's mammary gland during the prepartum period are defined as colostrum [68]. There is a consensus that the adequate management of colostrum plays a key role in determining neonatal health and survival [69]. Compared to cow colostrum, buffalo colostrum contains higher concentrations of fat (8.04% vs. 9.59%), lactose, ash, vitamin E (234 IU/100 mL vs. 342 IU/100 mL), total solids (24.19% vs. 26.67%), phosphorus (53 vs. 58 mg/100 g), and IGF-1 [70]. Analyses of the colostrum of female Murrah buffaloes have determined that the predominant white blood cells are macrophages, followed by lymphocytes and neutrophils. Phagocytic activity at birth was measured at 23%, but decreased drastically to 14% on day 5 postpartum [71].

During the development of the dam–calf bond in production units in the tropical wetlands of Mexico, the dam and its calf remain together for one day, to permit the calf to suckle for the first time. Subsequently, both are guided to the maternity area, where they stay for an average of 12 days, considering the adequate motor development of the calf. After this period, the female is incorporated into the milking process, and the calf is used to stimulate milk ejection during the milking (Figure 6).

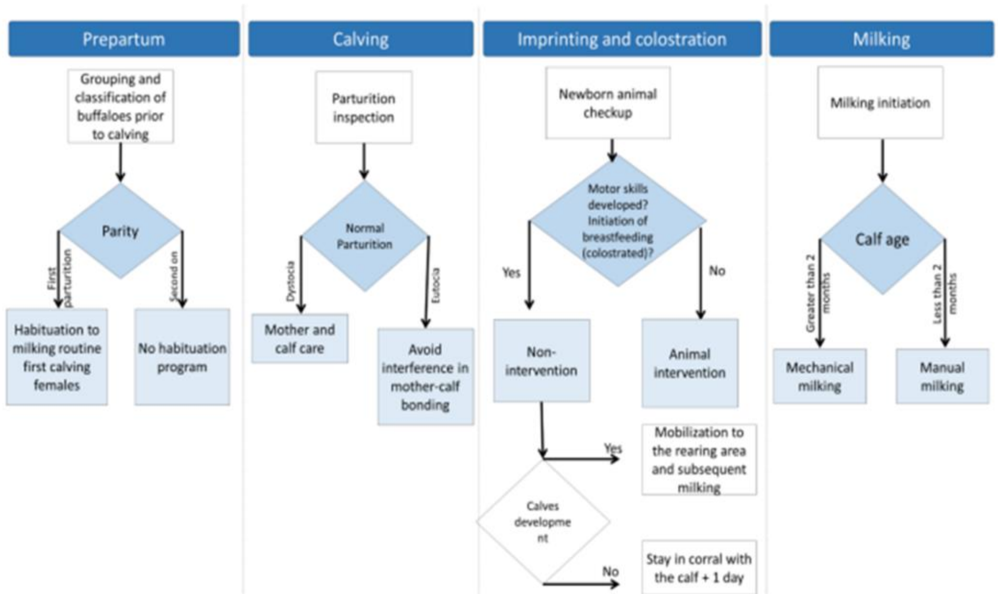

Figure 6. Flow diagram of the prepartum, calving, imprinting, colostrum, and milking processes in the dual-purpose buffalo production system in Mexico's tropical wetlands.

This process generates two transcendental effects. First, it allows a high number of calves to benefit by ingesting colostrum from different dams through allosuckling. Second, it favors the conditioning of the cow for the milking period, whether manual or mechanical. Colostrum ingestion is important because it confers passive immunity by transferring antibodies and leucocytes of maternal origin [72,73] to the calf during the first hours post-birth. It is important to consider that the absorption of immunoglobulins ends within 36 h postpartum. During this interval, only the abomasum is active, so colostrum ingestion not only exerts effects on the calf's immune system but also supplies valuable nutritional compounds, such as vitamins, fats, and proteins [72,74]. The practice of allonursing among water buffaloes also has two main repercussions; it can compensate for any nutritional deficiency in the calves, but it might increase the risk of the transmission of pathogens [62].

What is clear, despite this potential risk, is that allonursing favors greater colostrum ingestion during the first days of life, which is important in light of the reduction in fats, proteins, and dry substance [74], as well as in antioxidant parameters (Trolox equivalent antioxidant capacity, total phenolics, reducing power, and DPPH radical scavenging activity) that occurs postpartum [75]. This has been demonstrated by analyses of colostrum collected from female Murrah buffaloes on days 1 to 5 postpartum, which show that levels of the immunoglobulins IgA and IgM (3.22 mg/mL and 5.22 mg/mL, respectively) decrease during these 5 d. Moreover, somatic cell counts are elevated (500,000/mL) at the moment of birth, but diminish markedly by day 2 and then more gradually up to day 5 (180,000/mL) [71].

It is important to emphasize that the simple act of allowing calves to ingest their dams' milk for a period of 90 days (one month more than is normally allowed in production units in Mexico's tropical wetlands) positively impacts weight gain (0.506 vs. 0.438 kg/day). In addition, allowing calves to suckle reduces the time spent on crossed suckling and licking themselves and other objects, compared to calves that are weaned immediately after birth [76] (Figure 7).

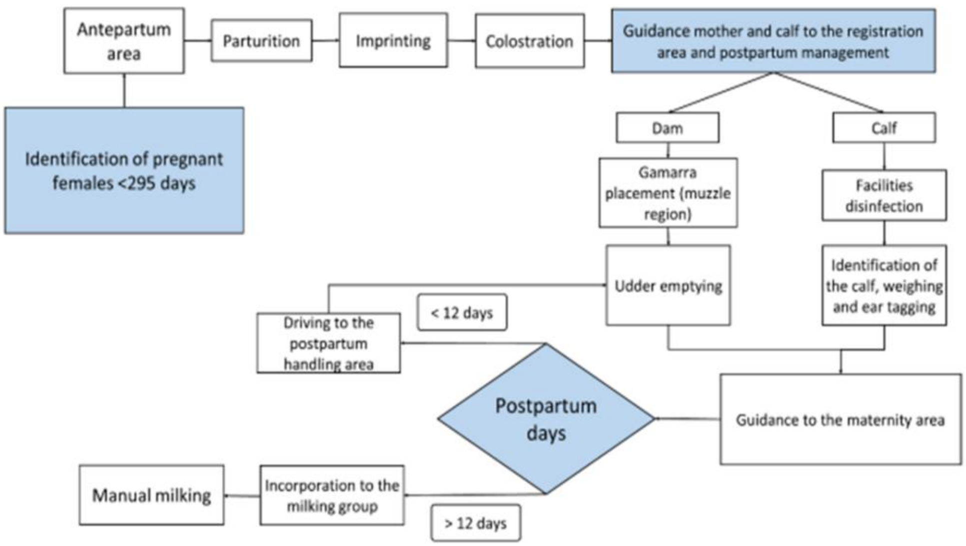

Figure 7. Criteria for handling in the calf and dam.

7. Milking Handling

Lactation in female buffaloes in the study area begins shortly after birth and lasts an average of 240 days, but it is largely influenced by several factors such as breed, genetic background, season and period of calving, health status, and environmental factors [2]. This coincides with the values published by Hernández et al. [77], Vázquez-Luna et al. [78], and Gutiérrez et al. [79] that found 240–270 days periods. The Italian Mediterranean breed, registered in the Buffalo Genealogical Book, has an average lactation period of 270 days [2]. Once the milk-obtaining process begins, managing the milking program comes to play a crucial role, although it can foster negative behaviors in females triggered by external factors such as the inadequate maintenance of milking machinery and inappropriate practices by operators [23,80,81]. This process requires a series of decisions concerning the conditions of dams and calves, beginning with the choice between manual and mechanical milking (Figures 8 and 9) and possible modifications in the movement or location of animals in certain areas (Figure 7). Breeders must also decide between applying exogenous oxytocin to the females during mechanical milking or using their calves to provide the somatosensory stimulation required for milk ejection.

Because most of the milk of the female buffalo is stored in the alveolar compartment (95%), it can be ejected through the action of oxytocin on myoepithelial cells [9,82,83]. Milk ejection is induced by a reflex triggered by stimulating the teat that activates dermal receptors which send signals to the spinal cord and hypothalamus for the release of oxytocin into the bloodstream. This reduces the interalveolar pressure through the contraction of the myoepithelial cells that surround the alveolus, allowing the milk to flow towards the cistern of the udder for subsequent ejection. Oxytocin also aids in reestablishing normal mammary blood flow [84,85] (Figure 10). If the calves are not used to stimulate milk ejection during mechanical milking (Figure 8), then exogenous oxytocin must be administered to ensure milk ejection.

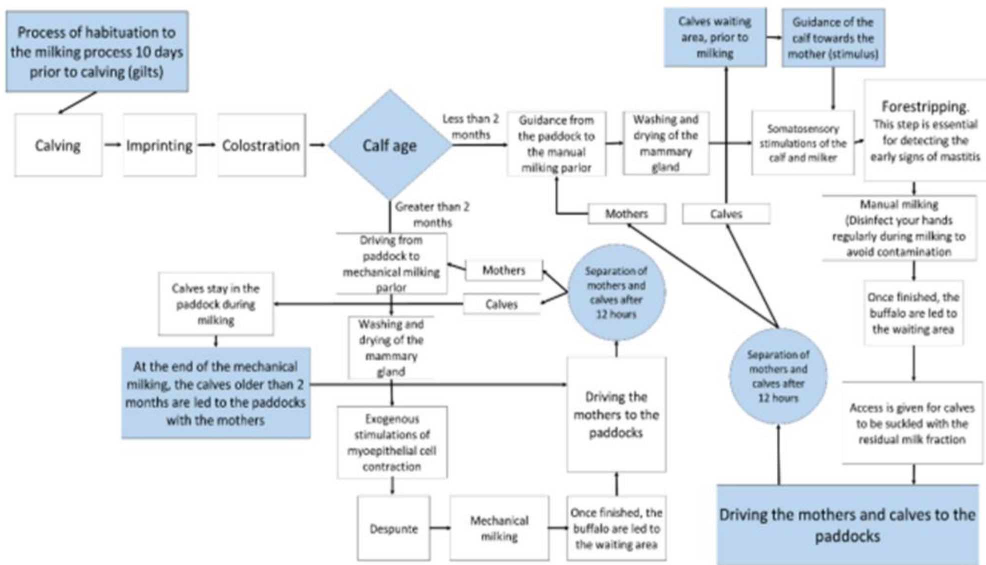

Figure 8. Flow chart of the characteristic decisions required in the manual and mechanical milking processes of water buffaloes.

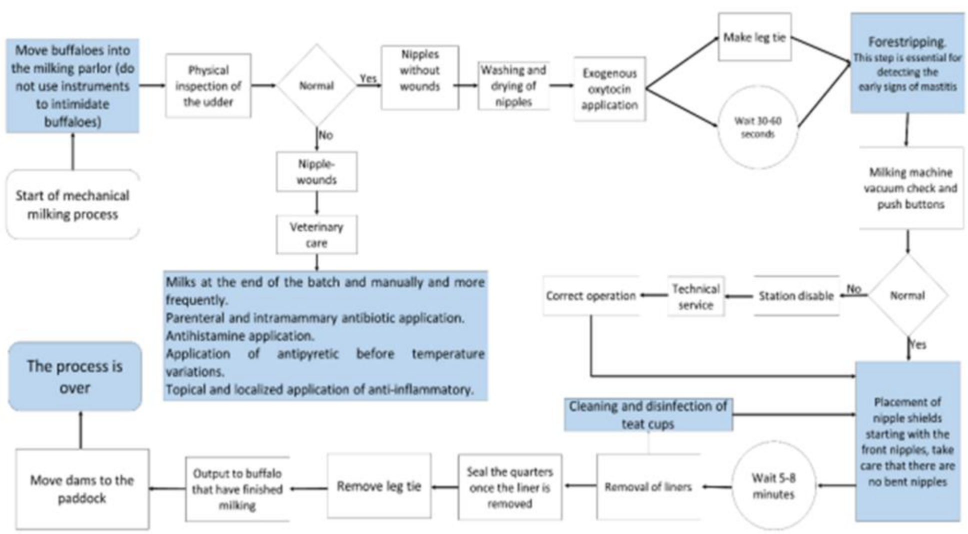

Figure 9. Flow chart of the characteristics of the mechanical milking process. In the dual-purpose production system, the presence of clinical or subclinical mastitis is low; however, when it occurs, veterinary attention is necessary.

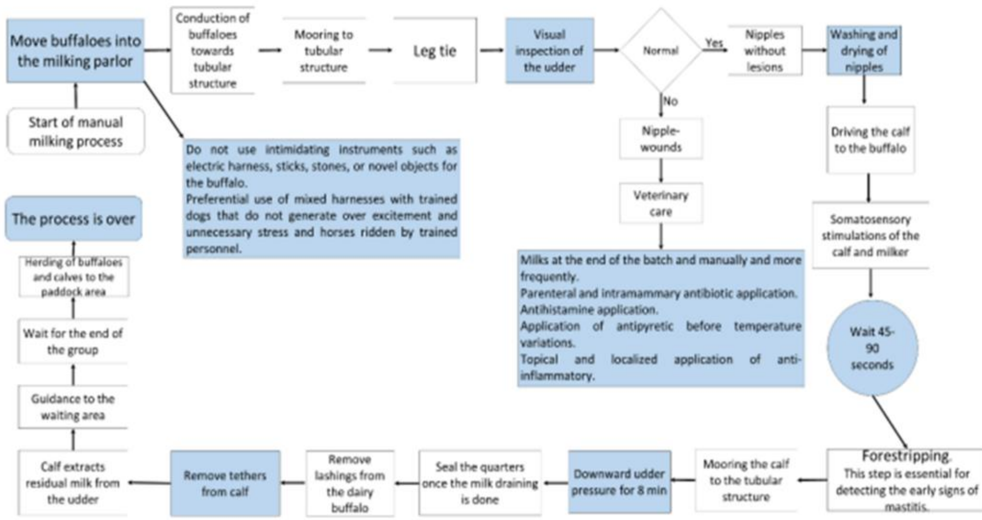

Figure 10. Flow chart of the characteristics of manual milking. In the dual-purpose production system, the presence of clinical or subclinical mastitis is low; however, if it occurs, veterinary care is necessary.

On this topic, Murtaza et al. [86] found that 30-UI of exogenous oxytocin in female Nili-Ravi buffaloes increased milk production when compared to the group of untreated dams. Similarly, Espinosa et al. [82] compared milk production using calves and down-stimulation and the application of 20 UI of oxytocin. The highest values found correspond to the latter group.

Though studies of female buffaloes reveal productive advantages of using exogenous oxytocin, Faraz et al. [87] found that daily applications of oxytocin can affect the mineral content of the milk produced. They recommend suspending the regular use of this hormone because of its impact on consumers. Other observations show that daily oxytocin administration affects females' reproductive indicators, leading to prolonged periods of anestrus due to slow uterine involution [88,89].

8. Productivity during Lactation

Studies of the DPBPS show an average milk production of 5.4 L/day during lactation periods that last around 240 days, for a total of 1300 L/cow. It is important to note that a portion of this milk production is destined for calves, since their presence during manual milking constitutes a series of visual, olfactory, and sensory stimuli for the milk ejection (Figure 11), so these values refer exclusively to the milk destined for the market. Reports from Brazil indicate that milk production values range from 1500 to 4500 L per lactation, varying according to the degree of intensification of different production units. It is important to analyze cost–benefit ratios here to verify whether investing in intensive systems is justified.

Productivity levels of lactation are dictated by genetics, environmental, and alimentary factors, management systems, number of lactations, seasonality, and animal welfare. The transition period is especially important due to the impact of the neuroendocrine changes that occur during and after parturition and during lactogenesis on the health, welfare, and productivity of female buffaloes [90–92]. Zicarelli [93] concluded that over the past 10 years, milk production by female buffaloes has been fostered worldwide due to the characteristics of these animals allowing them to develop in a resilient and efficient manner, and their capacity to potentiate sustainable production models [94]. This aspect takes on great importance when we consider the high nutritional value of buffalo products, which could respond well to consumption habits associated with good nutrition and health [14].

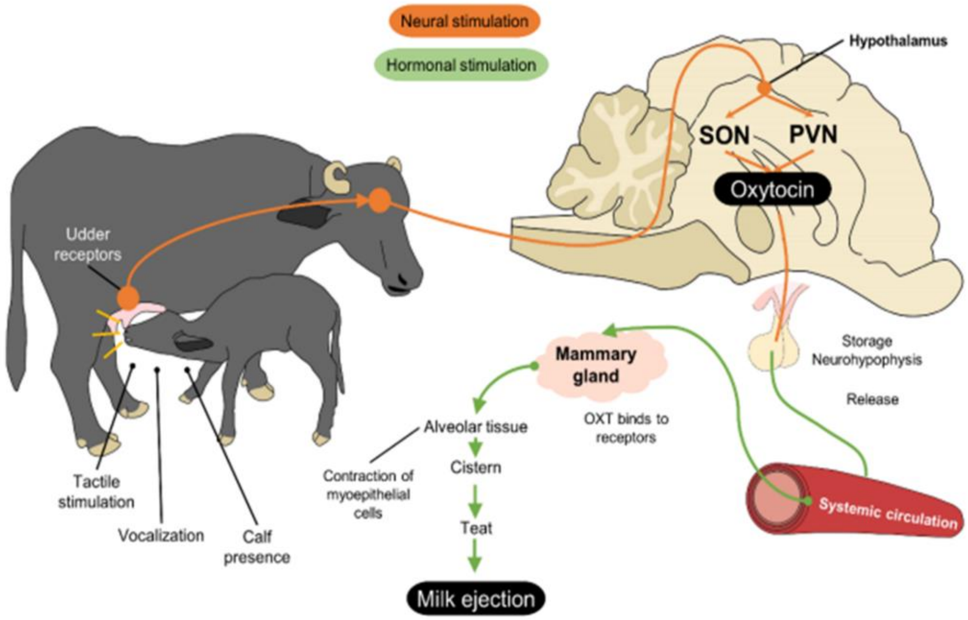

Figure 11. The role of oxytocin (OXT) and suckling behavior. Buffaloes require the presence of the calf and tactile, auditory, and olfactory stimuli for the neural and hormonal stimulation of milk release. The neural pathway begins in receptors located in the udder that are stimulated by the newborn. This stimulus is carried to brain nuclei in the hypothalamus, specifically the SON and PVN, which are responsible for producing OXT. Subsequently, the OXT produced is stored in the neurohypophysis, ready to be secreted into the bloodstream when maternal stimuli are perceived. Once in the mammary gland, OXT binds to specialized receptors in the alveolar tissue, causing contraction of the myoepithelial cells. This allows the milk to pass from the alveolar tissue into the cistern and teat. Finally, milk is ejected in a cycle in which the calf plays a key role essential. PVN: paraventricular nucleus of the hypothalamus; SON: supraoptic nucleus of the hypothalamus.

9. The Importance of Allosuckling for Offspring and Dams

As mentioned above, allonursing/allosuckling (communal nursing) refers to the practice in which a female allows (accepts) calves that are not her biological progeny to feed from her udder while she cares for and protects them [95]. This behavior is seldom seen in ungulates, but female buffaloes have shown the capacity to passively accept calves from other biological dams [96], although in a study of 35 female buffaloes with their calves conducted by Mandella-Oliveira et al. [97], it was found that events of nonfilial nursing are not common in this breed after observing daily frequencies of just 0.61–0.67, compared to filial feeding (0.61–1.06).

Several theories have been proposed to explain why these dams allow non-filial calves to suckle and, in the other direction, why calves seek to obtain milk from other females in the herd [62]. Some theories hold that allonursing reflects the female buffalo's maternal instincts and that factors such as kinship, reciprocity, parental care, social benefits, milk-dumping, and misdirected care may be involved [98,99]. Other proposals center on the newborn's efforts to satisfy its requirements, emphasizing phenomena such as milk theft, compensation, immunological benefits, and better nutrition [99].

Water buffaloes are seasonal polyestrous animals. Females present estrus most often in the months with the shortest days, which means that births are concentrated in one period of the year, producing groups of newborn calves that might facilitate allosuckling/allonursing. The closeness characteristic of water buffalo herds in terms of the number of animals and the genetic kinship among them is another theory posited to explain why females permit allosuckling by the calves of dams with which they share consanguinity [100]. A study of 34 female buffaloes and 31 calves of Murrah and mixed breeds analyzed the effect of consanguinity on 570 attempts to perform allosuckling, where 351 were successful. In those cases, four to eight calves were observed to feed from non-filial dams, and 13 were involved in the communal care of the newborns. These results suggest that the calves that attempted to feed from females considered their dams' sisters or half-sisters had less success ($\overline{x}$ = 0.457) than those that had no kinship ($\overline{x}$ = 0.563) [95], so that kinship may not be of special importance in this type of behavior in this species.

Something similar occurs regarding the reciprocity theory, which highlights allosuckling as an act of communal rearing [101]. However, other studies report that up to 85% of the buffalo dams that acceded to care for non-filial calves did so regardless of whether or not their own calves had been fed by herd mates [95]. This acceptance of community maternal care is manifested mainly by young or primiparous females, perhaps with the goal of improving their maternal abilities, or due to their inexperience [102]. In support of this, one experiment found that 97% of 30 buffaloes that participated in allonursing were young females with limited maternal experience [95]. In the case of high milk production cows, allonursing has been explained as a way to evacuate the milk that remains after their own calves finish feeding [103]. In this regard, Oliveira et al. [104] found that the females that fed both their own and non-filial calves had greater daily milk production and total milk production than the ones that did not participate in this practice. They further determined that dams with male calves produced larger amounts of milk and were more prone to accepting allosuckling.

With respect to the sex of the newborns, a study of 29 female Murrah-Mediterranean buffaloes recorded that most cases of allosuckling occurred between November and February, during the first 4 months of extra-uterine life, and that they had an average maximum duration of approximately 7 min/day. In another finding, male calves attempted to feed from non-filial dams more often than females (1.52 vs. 1.37 times per day). Their results led those authors to suggest that allonursing in buffaloes occurs as a way to (i) increase fitness in the herd, and (ii) ensure the survival of newborns [105].

Another advantage that calves may obtain with allosuckling is improved nutrition. In this approach, the calf's sex and birth order are factors that have been related to the incidence of allosuckling and that influence weight gain. A study by da Costa et al. [106] found that male calves had higher weights after longer periods of care, and calves born towards the end of the season had to compete harder to feed from their own dams because they had older calves. Those authors recommend separating young animals into age groups, and having handlers identify the females that are prone to allonursing to safeguard the growth and development of all calves. Not surprisingly, the calves of dams with low milk production are most prone to suckling from other females [95]. It seems clear that calves learn to identify the dams that accept both their own calves and non-filial offspring and tend to approach them for milk [107]. It is important to keep in mind that allosuckling may provide immunological benefits to the calf if it obtains a broader range of antibodies and immunoglobulins (IgA, IgG) [108], though it may entail a risk of infection for the dam (e.g., paratuberculosis) [23,109].

The currently available evidence indicates that water buffaloes, unlike dairy cattle selected specifically for production, do not let down milk in a conditioned manner when stimuli such as milking pails or machines are present [110]. Rather, they need their own calf to provide the visual, olfactory, and auditory stimulation required to generate the neurohormonal control of milk release by oxytocin [95]. For this reason, milk theft is one of the theories most often proposed for species such as buffaloes that have only one offspring at a time, and for which providing milk to non-filial calves entails enormous energy expenditures without bringing any benefits for the dam or her own newborn [111].

Interested in allonursing, Napolitano et al. [112] evaluated water buffalo calves whose dams did not produce sufficient milk, considering that those newborns suffered hunger. It was found that these kinds of calves were motivated to steal milk from other females to compensate their lack of food (the basis of another theory) [105]. Allonursing in general is less common in animals that bear only one offspring because it is easier for those females to identify an alien calf [101]. In water buffaloes, allonursing is usually seen while the dam is feeding her own calf because at that time a non-filial calf may be able to approach and suckle to disguise its presence [113]. As a result of these studies, allonursing in water buffaloes is not thought to constitute a factor of natural or artificial selection of great significance [95]. However, when it happens, it may have benefits for both the dam and the newborn, such as increased milk production or better nutrition, though its implementation and the risks involved depend largely on the goals of the production system and the sanitary and innocuity measures applied [99] (Figure 12).

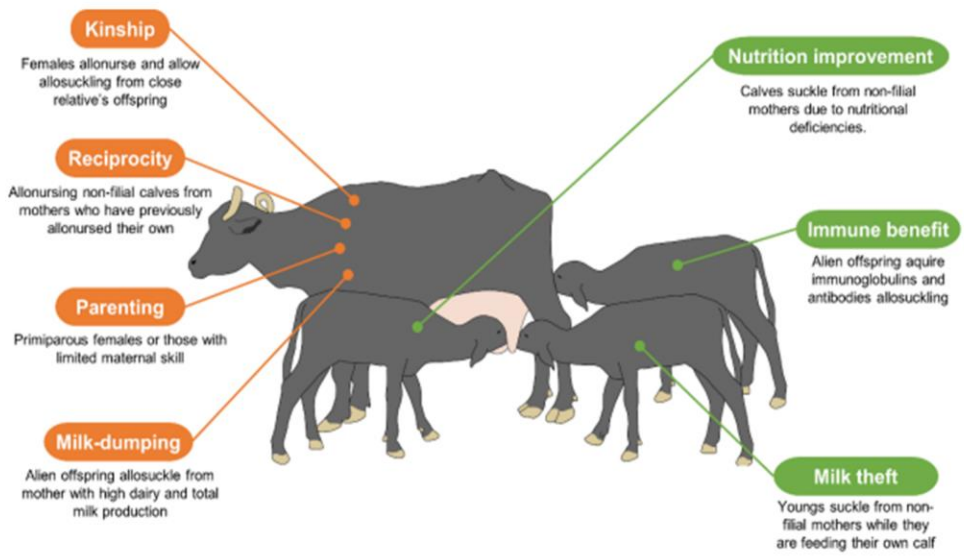

Figure 12. Main hypotheses regarding allosuckling in water buffaloes (*Bubalus bubalis*).

10. Confinement Systems and Herding

Since the mid-1990s, the growing human population and its demand for foods have induced the formation of organizations, systems, and groups dedicated to generating strategies to satisfy food needs [114,115]. This has brought about important changes in traditional, extensive livestock production systems by moving them towards more intensive models based on confinement. This has impacted several livestock species, including the water buffalo, because it can increase yields to respond to the growing demand for final products. The process of reconversion towards confinement in buffalo production units involves developing more controlled environments and implementing stricter programs of genetic selection and nutritional enhancement [114]. These changes, however, may have consequences for the welfare of the animals due to greater human–buffalo contact, modified feeding regimes, and restrictions on the amount of space available per animal. Observations in production units that have taken this direction have identified both physiological and behavioral responses [66] that need to be analyzed to quantify their impact [80].

The early calf–dam bond established in this species is essential for the calf's survival and welfare [9,14,63], and must be respected in confinement-based production systems to prevent the economic losses that will surely occur if this is not done.

Breeders who are conscious of the significance of this bond have developed various housing procedures for calves at weaning, from pasture cells with or without the presence of the dam, to total confinement. The latter strategy requires independent areas inside the production unit to facilitate the movement of buffalo steers using one of various herding techniques available to drive them to the fattening area.

Mobilization by Mixed Herding

The movement of calves inside production units can require various techniques and may or may not include the use of physical tools or auditory equipment to induce movement. These methods include one called mixed herding, which involves personnel on horseback and dogs [116]. This form of herding tends to be used when travel distances are great and/or using motorized transport is not feasible. In Mexico's tropical wetlands, mixed herding is often employed in buffalo production systems to move females and their calves, after birth, to different areas for milking or confinement, or into paddocks. In the latter, the animals are moved to areas of pastureland (called paddocks) until weaning. Weaning usually occurs when calves reach a weight of approximately 240–270 kg (Figure 13).

Figure 13. Areas of dual-purpose buffalo production systems. (**A**) Potrero destined for prepartum and delivery days. (**B**) Calf weighing area. (**C**) Manual milking with the presence of calf. (**D**) Grazing in the pasture of the dam in the company of the calf. (**E**). Herding of calves on horseback. (**F**) Confinement of calves after grazing with the dam. (**G**) Specific paddocks for steers where the characteristics that allow their natural behavior of thermoregulation are provided. (**H**) Loading of finished animals or intermediate fattening.

In extensive or semi-extensive systems, moving the buffaloes in different stages of their development is essential. Experience shows that herding methods influence daily milk production and have repercussions on the weight gain of calves as a consequence of stressful factors such as handling and the aggressive, even painful, use of tools [117].

Some studies report that using dogs is another source of stress during operations that constitute poor animal handling [117–119]. Kuhl [120] described the use of dogs and horses as a critical point that provoked stress in the animals as they moved, and caused injuries due to poor training in their use. Huertas [121], in turn, recommended not using instruments—or unknown, poorly trained handlers—to force buffaloes to move in order to prevent threats, nervousness, and excitation.

Observations in Mexico show that animals herded by handlers on horseback presented lower cortisol levels, which meant a 15.3-times lower likelihood of producing tough, dark, dry meat, compared to the animals that were herded by handlers on foot. This was attributed to the time required in each case, in part because the buffaloes put up less resistance when herded by mounted handlers. Another factor identified was the effect of poorly trained personnel whose actions affected the welfare of the animal and triggered more acute states of alert [122]. Mixed herding, therefore, is functional and suitable, and is likely one of the best options for moving animals and for displacements over long distances, where goals include preventing stress, fear, and any deterioration of animal welfare during such mobilizations.

11. Handling during the Loading, Transport, and Unloading of Buffalo

Turning to the male buffalos, most of these animals are sold after weaning or at the intermediate fattening stage, depending on the availability of forage at each production unit and prices in markets: if prices are low and good forage is available, the animals may be retained to gain weight while awaiting price rebounds that will benefit producers. This is feasible thanks to the flexibility that distinguishes dual-purpose production systems [25].

After weaning, the next stage of production, often called intermediate-fattening, stage involves transporting weaned animals weighing about 270 kg, and/or aged about one year, to specific finishing areas. This stage is usually carried out at a distinct location, so the buffaloes are subjected to handling activities such as loading, transport, and unloading. These management practices require herding and mobilization techniques and procedures that involve vehicles and unloading in the fattening area. Before this process begins, the animals are housed in paddocks. As in the case of the calves and female milk producers described above, steers are also normally herded using the mixed method that facilitates their movement over middle to long distances, thus avoiding both loss of time and lesions caused using foreign objects. After herding, the animals enter a lairage corral or a tubular-shaped handling chute in single file and enter the transport vehicle by climbing a stationary concrete ramp designed to facilitate their ascent. Upon arrival at the fattening and finishing area, similar processes are followed to unload the animals down through stationary ramps that connect to pathways to the reception corrals. Finally, they are released into new sites, often characterized by pasturing systems or semi-extensive feedlots with areas of confinement to shorten finishing times. The exact conditions vary from one production unit to another.

The animals destined for meat rather than milk production are transported to a different site at least once during their lifetime [123]. Currently, buffaloes can be transported by railroad, ship, airplane, or truck to areas dedicated to fattening, breeding, or slaughter, but available evidence suggests that confinement in a moving vehicle is a stressful event for these animals [124]. Especially important here are issues such as the amount of space allowed per animal, load density, driving styles, road conditions, vibrations, strange sounds, and prolonged trip times that can result in lesions, damaged meat in the canal, and deteriorated meat quality, among other problems [116,125–127].

For these reasons, transport is closely associated with reductions in the animals' immune system, the impact of which is mediated, preferentially, by the hypothalamic-adrenocortical axis and the consequent secretion of corticosteroid hormones [128]. Associated disorders were studied by El-Deeb and El-Bahr [129] in a group of 50 water buffalo calves divided into two groups. One group was subjected to transport, while the other was not. Hematological and biochemical parameters were analyzed after unloading post-transport. Their results show increases in the serum concentrations of acute stage proteins—haptoglobin, serum amyloid A, and fibrinogen—in the transported calves [129].

While precise transport conditions can vary depending on producers' objectives, transporting an animal without companions from its group can generate signs of stress. For this reason, the recommendation is to transport animals with a congener or, if this is not feasible, implement alternatives that reduce the isolation effect, such as placing mirrors inside the vehicle [128]. The transport of water buffaloes in the study area is usually by trucks equipped with tubular structures that can carry up to 60 animals, or smaller vehicles adapted to carry smaller loads of just 1–3 animals per trip.

There is scarce information on the incidence of lesions and economic affectations caused by the transport of buffaloes, but in Bangladesh, Alam et al. [126] studied a sample of 560 *Bos* genus bovines and water buffaloes. They determined that 89% of all the study animals had at least one lesion, but that 99% of the buffaloes presented evident cutaneous lesions, compared to 84% of the cattle [126].

Following the same scheme, scores for hematomas in cattle transported at high densities (0.89 m^2/animal) have shown a strong relation when compared to animals transported at medium loading densities (1.16 m^2/animal). Results such as these reveal the need to evaluate—and determine—suitable loading densities in accordance with species, the groups of animals to be transported (i.e., calves, feeders, finished cattle, culls, and so on), the characteristics of the vehicles, and the prevailing climatic conditions during transport [123].

Another study assessed tail and muzzle lesions in buffaloes and *Bos* genus bovines during handling. The authors determined that 54% of the water buffaloes suffered injuries due to the tearing or chafing of the nostrils caused by the use of ropes looped through perforations [130]. Tail lesions were found in 39% of the *Bos* genus bovines and buffaloes. The predominant injury (98%) was bent tails [130].

The unloading process used depends on the characteristics and infrastructure in the reception area of the production unit, but it is common to observe similar facilities, consisting of stationary concrete ramps and tubular chutes.

12. Handling, Processing, and Commercialization of Milk

In Mexico, few water buffalo products are fully differentiated from other products due to the absence of established norms to promote schemes of product tracing and the lack of valorization products and their derivatives. In the case of milk, current selling prices range from 9 to 10 peso/L, around 20% higher than the price of bovine milk. This is because buyers value it for its high yields in the elaboration of cheeses and other byproducts.

While water buffalo producers in Mexico consider this activity profitable, they know that this depends on three principal factors: daily weight gain, liters produced per lactation, and the number of calves born per year. Obviously, the production costs of milk, meat, and derived dairy products such as cheese, among others, are fundamental in determining the levels of profitability of production units. In this regard, production in pasture systems is essential because of its advantages for reducing production costs [78].

The commercialization of water buffalo products has increased gradually in Mexico, but obstacles to distribution and access to more demanding markets continue to exist because their quality, hygiene, safety, and nutritional value are not guaranteed. Due to these circumstances, the commercial channels available to producers are mostly popular markets. There, sanitary controls are less strict and transactions are dominated by intermediaries and regional-level processors that tend to offer prices lower than those of industrial and organic markets [10,131].

There are, however, countries that have a broad development of products made from buffalo milk that have high added value. In Italy, for example, the market demand for and the value of buffalo milk are higher, sometimes tripling the selling price of products of *Bos* genus origin, because of its high value for mozzarella cheese production. Studies show that the yield in cheese elaborated with milk from Mediterranean buffaloes is 25.5%, compared to just 12.5% for dairy cattle [93].

Circumstances in Bangladesh are quite unique, as producers raise the Murrah, Nili-Ravi, Surti, and Jafarabadi breeds, constituting an important economic, social, and cultural resource, even though these are considered the least favorable breeds for milk production due to the poor practices applied and low prices for milk, worsened by the fact that byproducts are usually distributed through informal commercial channels [90,132].

To stimulate the economy of water buffalo producers, it is necessary to value the benefits of water buffalo milk. This will require the opening and recognition of a differentiated market where strategies can be applied combining productive aspects (including measures to ensure hygiene and innocuity) with marketing initiatives to inform and convince consumers. In the degree that this is implemented, it will be possible to follow the route traced in Italy by publicizing the properties and benefits of mozzarella cheese made from water buffalo milk to increase sales. To give one example, exports of mozzarella cheese to Japan, South Korea, and the United States increased by 16.4% between 2016 and 2017 [133]. This case highlights the potential that exists, but also the need to induce changes in the management of productions units and the chain of commercialization of water buffalo products.

13. Conclusions

The development of dual-purpose water buffalo production systems in Mexico's tropical wetlands is a relatively recent phenomenon that has progressed and improved due to herd management that combines the accumulated experience of ranchers with innovations generated at the national and, above all, international levels. However, as this review shows, there are many areas that need to be addressed for improvement, including the abandonment of offspring, routine practices in animal management, and a deep understanding of behavioral and physiological patterns in water buffalo, among others. Buffaloes are an interesting alternative for dual purpose systems that offer several advantages: They move in groups, facilitating their adaptation to rotating pasture systems. Their rusticity makes them feasible to exploit flood-prone areas and fields with low-to-medium quality grasses. Moreover, water buffaloes in Mexico have shown a significant resistance to many of the plagues and diseases that severely affect conventional cattle.

Ranchers are gradually adopting better buffalo reproductive practices. Advances in artificial insemination have been made in Mexico, but it is still difficult to obtain high-quality semen or sexed semen. Embryo transfer is not practiced due to the lack of the material required in the region.

Colostrum and cow–calf relationships are highly valued, as ranchers understand that the presence of the calf and its consumption of part of the milk produced by its dam stimulate milk ejection and represents health benefits for both. However, the introduction of mechanical milking in some production units has modified this routine and the calf's role has been taken over by the administration of oxytocin, though this change affects the dam–calf bond and the full exploitation of high-quality colostrum.

In contrast, some producers have begun to exploit allonursing during calf-rearing, though it is not clear how the best use could be made of this practice.

The lower milk production of this species compared to cattle is its main limitation. However, the properties of buffalo milk allow one to obtain an added value and make this type of farms competitive. This, however, could be potentiated by establishing specific norms for buffalo milk including commercial differentiation and campaigns designed to inform consumers by emphasizing the value and quality of buffalo milk, meat, and byproducts.

In synthesis, consolidating buffalo production in Mexico's tropical wetlands will require broadening our knowledge of this species, and perfecting the most appropriate handling procedures. Only in this way will it be possible to raise the efficiency of the dual-purpose production system and improve the standard of living of primary producers.

The activities of government agencies and processing enterprises will play vital roles in achieving the integral modernization of this potentially important economic activity.

Author Contributions: Conceptualization, D.M.-R., A.B., A.H.H.M., A.O.; investigation, D.M.-R., A.B., A.H.H.M., A.O., D.A.M.-C., A.Á.-M., D.R.-G., N.J.-P. and A.D.-O.; writing—original draft preparation, D.M.-R., A.O. and A.H.H.M.; writing—review and editing, D.M.-R., A.O., A.B., D.A.M.-C. and A.H.H.M. and project administration, D.M.-R., A.O. and A.H.H.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Food and Agriculture Organization of the United Nations (FAO). *The Future of Food and Agriculture Trends and Challenges*; FAO: Rome, Italy, 2018; pp. 1–52.
2. Minervino, A.H.H.; Zava, M.; Vecchio, D.; Borghese, A. *Bubalus bubalis*: A short story. *Front. Vet. Sci.* **2020**, *7*, 1–15. [CrossRef] [PubMed]
3. Álvarez-Macías, A.; Mota-Rojas, D.; Bertoni, A.; Dávalos-Flores, J.L. Opciones de desarrollo de los sistemas de producción de búfalos de agua de doble propósito en el trópico húmedo latinoamericano. In *El Búfalo de Agua en Latinoamérica, Hallazgos Recientes*; Napolitano, F., Mota-Rojas, D., Guerrero-Legarreta, I., Orihuela, A., Eds.; BM Editores: Mexico City, Mexico, 2020; pp. 43–74.
4. Barboza-Jiménez, G.; Barboza, J.G. Bondades ecológicas del búfalo de agua: Camino hacia la certificación. *Tecnol. Marcha* **2011**, *24*, 82–88.
5. Mota-Rojas, D.; Braghieri, A.; Álvarez-Macías, A.; Serrapica, F.; Ramírez-Bribiesca, E.; Cruz-Monterrosa, R.; Masucci, F.; Mora-Medina, P.; Napolitano, F. The use of draught animals in rural labour. *Animals* **2021**, *11*, 2683. [CrossRef] [PubMed]
6. Young, R.; Lefevre, L.; Bush, S.J.; Joshi, A.; Singh, S.H.; Jadhav, S.K.; Dhanikachalam, V.; Lisowski, Z.M.; Iamartino, D.; Summers, K.M.; et al. A gene expression atlas of the domestic water buffalo (*Bubalus bubalis*). *Front. Genet.* **2019**, *10*, 1–14. [CrossRef] [PubMed]
7. Crudeli, G.A.; Konrad, J.L.; Patiño, E.M. *Reproducción en Búfalas*; Mongolia Ediciones: Buenos Aires, Argentina, 2016; Volume 1, p. 272.
8. Bertoni, A.; Napolitano, F.; Mota-Rojas, D.; Sabia, E.; Álvarez-Macías, A.; Mora-Medina, P.; Morales-Canela, A.; Berdugo-Gutiérrez, J.; Guerrero- Legarreta, I. Similarities and differences between river buffaloes and cattle: Health, physiological, behavioral and productivity aspects. *J. Buffalo Sci.* **2020**, *9*, 92–109. [CrossRef]
9. Napolitano, F.; Mota-Rojas, D.; Guerrero-Legarreta, I.; Orihuela, A. *El Búfalo de Agua en Latinoamérica, Hallazgos Recientes*, 3rd ed.; BM Editores: Mexico City, Mexico, 2020; pp. 1–1492. Available online: https://www.lifescienceglobal.com/journals/journal-of-buffalo-science/97-abstract/jbs/4550-el-bufalo-de-agua-en-latinoamerica-hallazgos-recientes (accessed on 14 September 2021).
10. Napolitano, F.; Mota-Rojas, D.; Álvarez-Macías, A.; Braghieri, A.; Mora-Medina, P.; Bertoni, A.; Cruz-Monterrosa, R.; De Rosa, G. Factores productivos y su incidencia en el bienestar de la búfala lechera en sistemas de producción extensivos e intensivos. *Soc. Rural Prod. Med. Amb.* **2020**, *20*, 155–173.
11. Napolitano, F.; Mota-Rojas, D.; De Rosa, G.; Alvarez Macias, A.G.; Braghieri, A.; Bertoni, A.; Flores, K.; Gómez, J.; López, G.; Guerrero-Legarreta, I.; et al. Advances and perspectives in research on buffalo milk production and mozzarella cheese. *Agro Product.* **2021**, *14*, 1–9. [CrossRef]
12. Associazione Nazionale Allevatori Specie Bufalina (ANASB). Indice di Selezione Aggregato per la Bufala Mediterranea Italiana. Available online: https://www.anasb.it/attivita-anasb/indice-di-selezione-ibmi/ (accessed on 4 February 2022).
13. Bertoni, A.; Mota-Rojas, D.; Álvarez-Macias, A.; Mora-Medina, P.; Guerrero-Legarreta, I.; Morales-Canela, A.; Gómez-Prado, J.; José-Pérez, N.; Martínez-Burnes, J. Scientific findings related to changes in vascular microcirculation using infrared thermography in the river buffalo. *J Anim. Behav. Biometeorol.* **2020**, *8*, 288–297. [CrossRef]
14. Bertoni, A.; Álvarez-Macías, A.; Mota-Rojas, D. Desempeño productivo de los búfalos y sus opciones de desarrollo en las regiones tropicales. *SRPMA* **2019**, *19*, 59–80.
15. Neglia, G.; de Nicola, D.; Esposito, L.; Salzano, A.; D'Occhio, M.J.; Fatone, G. Reproductive management in buffalo by artificial insemination. *Theriogenology* **2020**, *150*, 166–172. [CrossRef]
16. Mota-Rojas, D.; Titto, C.G.; Orihuela, A.; Martínez-Burnes, J.; Gómez-Prado, J.; Torres-Bernal, F.; Flores-Padilla, K.; Carvajal-de la Fuente, V.; Wang, D. Physiological and behavioral mechanisms of thermoregulation in mammals. *Animals* **2021**, *11*, 1733. [CrossRef] [PubMed]

17. Mota-Rojas, D.; Pereira, A.M.F.; Wang, D.; Martínez-Burnes, J.; Ghezzi, M.; Hernández-Avalos, I.; Lendez, P.; Mora-Medina, P.; Casas, A.; Olmos-Hernández, A.; et al. Clinical applications and factors Involved in validating thermal windows used in infrared thermography in cattle and river buffalo to assess health and productivity. *Animals* **2021**, *11*, 2247. [CrossRef]
18. Mota-Rojas, D.; Napolitano, F.; Braghieri, A.; Guerrero-Legarreta, I.; Bertoni, A.; Martínez-Burnes, J.; Cruz-Monterrosa, R.; Gómez, J.; Ramírez-Bribiesca, E.; Barrios-García, H.; et al. Thermal biology in river buffalo in the humid tropics: Neurophysiological and behavioral responses assessed by infrared thermography. *J. Anim. Behav. Biometeorol.* **2021**, *9*, 2103. [CrossRef]
19. Mota-Rojas, D.; Wang, D.; Titto, C.G.; Gómez-Prado, J.; Carvajal-de la Fuente, V.; Ghezzi, M.; Boscato-Funes, L.; Barrios-García, H.; Torres-Bernal, F.; Casas-Alvarado, A.; et al. Pathophysiology of fever and application of infrared thermography (IRT) in the detection of sick domestic animals: Recent advances. *Animals* **2021**, *11*, 2316. [CrossRef] [PubMed]
20. Jainudeen, M.R. Buffalo husbandry. Asia. In *Encyclopedia of Dairy Sciences*; Academic Press: Cambridge, MA, USA, 2002; pp. 186–193.
21. Nascimento, V.A.; Dias, M.; Machado, T.M.M. Reproductive and productive performance of water buffaloes in central plateau of Brazil. *Ital. J. Anim. Sci.* **2007**, *6*, 640–642. [CrossRef]
22. Chase, C.; Lutz, K.; McKenzie, E.; Tibary, A. *Blackwell's Five-Minute Veterinary Consult: Ruminant*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, USA, 2017; p. 876.
23. Mota-Rojas, D.; De Rosa, G.; Mora-Medina, P.; Braghieri, A.; Guerrero-Legarreta, I.; Napolitano, F.; Mota Rojas, D. Dairy buffalo behaviour and welfare from calving to milking. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* **2019**, *14*, 1–9. [CrossRef]
24. Enríquez, Q.J.F.; Hernández, G.A.; Quero, C.A.R.; Martínez, M.D. *Producción y Manejo de Gramíneas Tropicales para Pastoreo en Zonas Inundables*; SAGARPA: Mexico City, Mexico, 2015; pp. 1–75.
25. Bertoni, A.; Álvarez-Macías, A.; Mota-Rojas, D.; Dávalos, J.L.; Minervino, A.H.H. Dual-purpose water buffalo production systems in tropical latin america: Bases for a sustainable model. *Animals* **2021**, *11*, 2910. [CrossRef]
26. Bülbül, T. Energy and nutrient requirements of buffaloes. *Kocatepe. Vet. J.* **2010**, *3*, 55–64.
27. Galloso-Hernández, M.A.; Soca-Pérez, M.; Dublin, D.; Alvarez-Díaz, C.A.; Iglesias-Gómez, J.; Díaz-Gaona, C.; Rodríguez-Estévez, V. Thermoregulatory and feeding behavior under different management and heat stress conditions in heifer water buffalo (*Bubalus bubalis*) in the tropics. *Animals* **2021**, *11*, 1162. [CrossRef]
28. González-Lozano, M.; Mota-Rojas, D.; Orihuela, A.; Martínez-Burnes, J.; Di Francia, A.; Braghieri, A.; Berdugo-Gutiérrez, J.; Mora-Medina, P.; Ramírez-Necoechea, R.; Napolitano, F. Review: Behavioral, physiological, and reproductive performance of buffalo cows during eutocic and dystocic parturitions. *Appl. Anim. Sci.* **2020**, *36*, 407–422. [CrossRef]
29. Mota-Rojas, D.; Martínez-Burnes, J.; Napolitano, F.; Domínguez-Muñoz, M.; Guerrero-Legarreta, I.; Mora-Medina, P.; Ramírez-Necoechea, R.; Lezama-García, K.; González-Lozano, M. Dystocia: Factors affecting parturition in domestic animals. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* **2020**, *15*, 1–16. [CrossRef]
30. Jainudeen, M.R.; Hafez, E.S.E. Gestation, prenatal, physiology, and parturition. In *Reproduction in Farm Animals*; Hafez, E., Hafez, B., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2016; pp. 140–155.
31. Jainudeen, M.R.; Hafez, E.S.E. Cattle and buffalo. In *Reproduction in Farm Animals*; Hafez, E., Hafez, B., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2016; pp. 157–171.
32. Taverne, M.; Noakes, D.E. Parturition and the care of parturient animals and the newborn. In *Veterinary Reproduction and Obstetrics*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 115–147.
33. Tao, S.; Dahl, G.E.; Laporta, J.; Bernard, J.K.; Orellana Rivas, R.M.; Marins, T.N. Physiology symposium: Effects of heat stress during late gestation on the dam and its calf. *J. Anim. Sci.* **2019**, *97*, 2245–2257. [CrossRef]
34. Mota-Rojas, D.; Habeeb, A.A.; Napolitano, F.; Sarubbi, J.; Ghezzi, M.D.; Ceriani, M.C.; Cuibus, A.; Martínez-Burnes, J.; Braghieri, A.; Lendez, P.A.; et al. Bienestar del búfalo de agua, bovino europeo y bovino índico: Aspectos medioambientales, fisiológicos y conductuales en respuesta a la sombra natural y artificial. In *El Búfalo de Agua en Latinoamérica. Hallazgos Recientes*; Napolitano, F., Mota-Rojas, D., Guerrero-Legarreta, I., Orihuela, A., Eds.; B.M. Editores: Mexico City, Mexico, 2020; pp. 959–1015.
35. Mudgal, V.; Bharadwaj, A.; Verma, A.K. Vitamins supplementation affecting colostrum composition in Murrah buffaloes. *Indian J. Anim. Res.* **2021**, *55*, 900–904. [CrossRef]
36. Das, G.; Kumar, A.; Dangi, S.; Khan, F. Parturition and puerperium in the buffalo. In *Bubaline Theriogenology*; Purohit, G., Ed.; International Veterinary Information Service: Ithaca, NY, USA, 2013; pp. 238–251.
37. Mota-Rojas, D.; Orihuela, A.; Strappini, A.; Villanueva-García, D.; Napolitano, F.; Mora-Medina, P.; Barrios-García, H.B.; Herrera, Y.; Lavalle, E.; Martínez-Burnes, J. Consumption of maternal placenta in humans and nonhuman mammals: Beneficial and adverse effects. *Animals* **2020**, *10*, 2398. [CrossRef] [PubMed]
38. Deka, R.; Nath, K.C.; Bhuyan, M.; Bhuyan, D.; Das, G.C.; Dutta, L.; Borpujari, D. Parturition behavior of Swamp buffalo cows (*Bubalus bubalis*) under organized system of rearing. *Biol. Rhythm. Res.* **2021**, *52*, 444–453. [CrossRef]
39. Kebede, A.; Mohammed, A.; Tadesse, W.; Abera, D. Review on economic impacts of dystocia in dairy farm and its management and prevention methods. *Nat. Sci.* **2017**, *15*, 32–42. [CrossRef]
40. Purohit, G.N.; Barolia, Y.; Shekhar, C.; Kumar, P. Maternal dystocia in cows and buffaloes: A Review. *Open J. Anim. Sci.* **2011**, *1*, 41–53. [CrossRef]
41. Ahuja, A.K.; Dogra, P.; Kumar, S.; Dhindsa, S.S.; Singh, H. A case of dystocia due to fetal ascites in murrah buffalo. *Int. J. Environ. Agric. Biotechnol.* **2017**, *2*, 1767–1769. [CrossRef]

42. Jeengar, K.; Purohit, G.N.; Mehta, J.S.; Choudhary, V.; Nirwan, L.K. A retrospective study on incidence of dystocia in cattle and buffaloes at referral center. *Theriogenol. Insight Int. J. Reprod. Anim.* **2015**, *5*, 41. [CrossRef]
43. Deutscher, G.H. Pelvic measurements for reducing calving difficulty. In *Beef Cattle Handbook*; Iowa Beef Center, Ed.; University of Wisconsin-Extersion: Iowa, NY, USA, 1914; pp. 1–5.
44. Nanda, A.; Brar, P.; Prabhakar, S. Enhancing reproductive performance in dairy buffalo: Major constraints and achievements. *Biosci. Proc.* **2019**, *61*, 27–36. [CrossRef]
45. Kamalakar, G.; Mahesh, R.; Prithvidhar Reddy, Y.V.; Devi Prasad, V. Surgical management of dystocia due to narrow pelvis in a she buffalo-a case report. *Buffalo Bull.* **2016**, *35*, 503–505.
46. Purohit, G.N.; Kumar, P.; Solanki, K.; Shekher, C.; Yadav, S.P. Perspectives of fetal dystocia in cattle and buffalo. *Vet. Sci. Dev.* **2012**, *2*, 8. [CrossRef]
47. Srinivas, M.; Sreenu, M.; Rani, N.L.; Naidu, K.S.; Prasad, V.D. Studies on dystocia in graded Murrah buffaloes: A retrospective study. *Buffalo Bull.* **2007**, *26*, 40–45.
48. Mahmoud, E.A.; Essawi, W.M.; Neamat-Allah, A.N.F. Influence of uterine torsion in water buffaloes (*Bubalus bubalis*) with insights into the hematological and biochemical prognostic values regarding to manual correction. *Trop. Anim. Health Prod.* **2020**, *52*, 3165–3171. [CrossRef] [PubMed]
49. Ali, A.; Derar, R.; Hussein, H.A.; Abd Ellah, M.R.; Abdel-Razek, A.K. Clinical, hematological, and biochemical findings of uterine torsion in buffaloes (*Bubalus bubalis*). *Anim. Reprod. Sci.* **2011**, *126*, 168–172. [CrossRef]
50. Purohit, G.N.; Gaur, M. Uterine torsion in buffaloes: A critical analysis. *Buffalo Bull.* **2014**, *33*, 263–278.
51. Satish, M.G.; Nirwan, S.S.; Chahar, S.K.; Kumar, D. A retrospected studies on the incidence, type of uterine torsion in surti buffaloes (*Bubalus bubalis*). *J. Entomol. Zool. Stud.* **2018**, *14*, 1113–1117. [CrossRef]
52. Singh, A.; Brar, P.; Singla, V.; Gandotra, V.; Nayyar, S.; Jindal, R. Effect of handling different types of dystocia on minerals and biochemical profiles in dairy buffaloes. *Vet. Pract.* **2009**, *10*, 116–120.
53. Singh, P.K.; Kamboj, M.; Chandra, S.; Singh, R.K. Effect of calf suckling dummy calf used and weaning on milk ejection stimuli and milk yield of Murrah buffaloes (*Bubalus bubalis*). *J. Pharmacogn. Phytochem.* **2017**, *6*, 1012–1015.
54. Kumar, P.; Sharma, A.; Vasishta, N.; Khan, S.; Barman, P. Dystocia due to relative oversized fetus and fetal maldisposition in a buffalo. *Vet. World* **2011**, *4*, 569. [CrossRef]
55. Tejpal, A.K.O.; Singh, D.; Tripathi, A.; Talluri, T.R.; Chaudhary, A.K. Dystocia in Buffalo due to hydrocephalic fetus: A case report. *Buffalo Bull.* **2017**, *36*, 695–698.
56. Gahlod, B.M.; Animal, M.; Akhare, S.B.; Animal, M.; Sheetal, S.K. Dystocia due to monocephalic thoracopagus tetrabrachius tetrapus monster in Nagpuri Buffalo- A rare case. *Int. J. Sci. Environ. Tecnol.* **2017**, *4*, 2400–2404.
57. Wani, J.M. Dystocia in murrah buffalo with fetal limb anomaly: A case report. *Theriogenol. Insight -Int. J. Reprod. All Anim.* **2018**, *8*, 91–93. [CrossRef]
58. Das, G.K.; Dutt, R.; Kumar, R.; Deori, S.; Shanker, U. Dystocia due to fetal ascites in a Buffalo. *Indian Vet. J.* **2009**, *87*, 59–60.
59. Abdullah, F.; Adamu, L.; Hashim, N.; Osman, A.; Haron, A.; Yimer, N.; Awang, D.; Roslim, N. Surgical Management of a Second Degree Perineal Laceration in a Buffalo Sequel to Dystocia. *Int. J. Livest. Res.* **2014**, *4*, 146. [CrossRef]
60. Nasr, M.A.F. The impact of crossbreeding Egyptian and Italian buffalo on milk yield and composition under subtropical environmental conditions. *J. Dairy Res.* **2016**, *83*, 196–201. [CrossRef] [PubMed]
61. Derar, D.R.I.; Abdel-Rahman, M.A. A comparative study on behavioral, physiological and adrenal changes in blood cortisol of buffalo during actual labour. *Buffalo Bull.* **2012**, *31*, 129–135.
62. Mora-Medina, P.; Napolitano, F.; Mota-Rojas, D.; Berdugo-Gutiérrez, J.; Ruiz-Buitrago, J.; Guerrero-Legarreta, I. Imprinting, sucking and allosucking behaviors in buffalo calves. *J. Buffalo Sci.* **2018**, *7*, 49–57. [CrossRef]
63. Bertoni, A.; Álvarez-Macías, A.; Morales-Canela, A.; Orozco-Corrales, C.; Mota-Rojas, D. Los sistemas de producción de búfalos en el tropico húmedo de América Latina:Un acercamiento desde el enfoque agroecológico. In *El Búfalo de Agua en las Américas*; Guerrero-Legarreta, I., Napolitano, F., Mota-Rojas, D., Orihuela, A., Eds.; BM Editores: Mexico City, Mexico, 2019; pp. 226–263.
64. Orihuela, A.; Mota-Rojas, D.; Strappini, A.; Serrapica, F.; Braghieri, A.; Mora-Medina, P.; Napolitano, F. Neurophysiological mechanisms of Cow–Calf bonding in buffalo and other farm animals. *Animals* **2021**, *11*, 1968. [CrossRef]
65. Orihuela, A.; Mota-Rojas, D.; Ungerfeld, R.; Strappini, A.; Mora-Medina, P.; de Rosa, G.; Braghieri, A.; Napolitano, F. La impronta en la búfala y otros animales de granja: Macanismo neurofisiológicos. In *El Búfalo de Agua en Latinomerica: Hallazgos Recientes*; Napolitano, F., Mota-Rojas, D., Guerrero-Legarreta, I., Orihuela, A., Eds.; BM Editores: Mexico City, Mexico, 2020; pp. 592–648.
66. Lanzoni, L.; Chincarini, M.; Giammarco, M.; Fusaro, I.; Gloria, A.; Contri, A.; Ferri, N.; Vignola, G. Maternal and neonatal behaviour in italian mediterranean buffaloes. *Animals* **2021**, *11*, 1584. [CrossRef]
67. Mota-Rojas, D.; Orihuela, A.; Napolitano, F.; Mora-Medina, P.; Orozco-Gregorio, H.; Alonso-Spilsbury, M. Olfaction in animal behaviour and welfare. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* **2018**, *13*, 1–13. [CrossRef]
68. Foley, J.A.; Otterby, D.E. Availability, storage, treatment, composition, and feeding value of surplus colostrum: A review. *J. Dairy Sci.* **1978**, *61*, 1033–1060. [CrossRef]
69. Godden, S. Colostrum management for dairy calves. *Vet. Clin. N. Am. Food Anim. Pract.* **2008**, *24*, 19–39. [CrossRef]
70. Abd El-Fattah, A.M.; Abd Rabo, F.H.; EL-Dieb, S.M.; El-Kashef, H.A. Changes in composition of colostrum of Egyptian buffaloes and Holstein cows. *BMC Vet. Res.* **2012**, *8*, 19. [CrossRef] [PubMed]

71. Dang, A.K.; Kapila, S.; Purohit, M.; Singh, C. Changes in colostrum of Murrah buffaloes after calving. *Trop. Anim. Health Prod.* **2009**, *41*, 1213–1217. [CrossRef] [PubMed]
72. Martin, C.C.; de Oliveira, S.M.F.N.; Costa, J.F.d.R.; Baccili, C.C.; Silva, B.T.; Hurley, D.J.; Gomes, V. Influence of feeding fresh colostrum from the dam or frozen colostrum from a pool on indicator gut microbes and the inflammatory response in neonatal calves. *Res. Vet. Sci.* **2021**, *135*, 355–365. [CrossRef] [PubMed]
73. Stockler, J.; Chamorro, M.F. Colostrum. In *Bovine Reproduction*; Wiley: Hoboken, NJ, USA, 2021; pp. 924–944.
74. Coroian, A.; Erler, S.; Matea, C.T.; Mireșan, V.; Răducu, C.; Bele, C.; Coroian, C.O. Seasonal changes of buffalo colostrum: Physicochemical parameters, fatty acids andcholesterol variation. *Chem. Cent J.* **2013**, *7*, 40. [CrossRef]
75. Qureshi, T.M.; Yaseen, M.; Nadeem, M.; Murtaza, M.A.; Munir, M. Physico–chemical composition and antioxidant potential of buffalo colostrum, transition milk, and mature milk. *J. Food Process. Preserv.* **2020**, *44*, e14763. [CrossRef]
76. Singh, P.K.; Kamboj, M.L.; Chandra, S.; Kumar, A.; Kumar, N. Influence of weaning on growth, health and behaviour of Buffalo (*Bubalus bubalis*) calves. *Indian J. Anim. Res.* **2019**, *53*, 680–684. [CrossRef]
77. Hernández-Herrera, G.; Lara-Rodríguez, D.A.; Vázquez-Luna, D.; Ácar-Martínez, N.; Fernández-Figueroa, J.A.; Velásquez-Silvestre, M.G. Water buffalo (*Bubalus bubalis*): An approach to sustainable management in southern Veracruz, Mexico. *Agro Product.* **2018**, *11*, 27–32.
78. Vázquez-Luna, D. Búfalo de agua (*Bubalus bubalis*): Un acercamiento al manejo sustentable en el sur de Veracruz, Mexico. *Agro Product.* **2018**, *11*, 27–32. [CrossRef]
79. Gutiérrez-Valencia, A.; Hurtado-Lugo, N.; Cerón-Muñoz, M. Estimativas de factores de corrección para duración de la lactancia, edad y época de parto en búfalas de la Costa Atlántica Colombiana. *Livest. Res. Rural Dev.* **2006**, *18*, 19–23.
80. De Rosa, G.; Grasso, F.; Braghieri, A.; Bilancione, A.; Di Francia, A.; Napolitano, F. Behavior and milk production of buffalo cows as affected by housing system. *J. Dairy Sci.* **2009**, *92*, 907–912. [CrossRef] [PubMed]
81. Rosa, D.; Napolitano, F.; Saltalamacchia, F.; Bilancione, A.; Sabia, E.; Grasso, F.; Bordi, A. The effect of rearing system on behavioural and immune responses of buffalo heifers. *Ital. J. Anim. Sci.* **2007**, *6*, 1260–1263. [CrossRef]
82. Espinosa, Y.; Ponce, P.; Capdevila, J. Efecto de la estimulación con bucerro, oxitocina y manual sobre los indicarores de ordeño en búfalas. *Rev. Salud Anim.* **2011**, *33*, 90–96.
83. Bidarimath, M.; Aggarwal, A. Studies on cisternal and alveolar fractions & Its composition and mammary health of Murrah buffaloes administered oxytocin. *Trop. Anim. Health Prod.* **2007**, *39*, 433–438. [CrossRef] [PubMed]
84. Lollivier, V.; Guinard-Flament, J.; Ollivier-Bousquet, M.; Marnet, P.-G. Oxytocin and milk removal: Two important sources of variation in milk production and milk quality during and between milkings. *Reprod. Nutr. Dev.* **2002**, *42*, 173–186. [CrossRef]
85. Park, Y.W.; Marnet, P.-G.; Yart, L.; Haenlein, G.F.W. Mammary secretion and lactation. In *Milk and Dairy Products in Human Nutrition*; John Wiley & Sons: Oxford, UK, 2013; pp. 31–45.
86. Murtaza, S.; Sattar, A.; Ahmad, N.; Ijaz, M.; Akhtar, M.; Shahzad, M. Consequence of exogenous administration of oxytocin on reproductive and productive parameters during postpartum involution period in newly calved Nili-Ravi buffaloes. *Pak. J. Zool.* **2019**, *4*, 1–16. [CrossRef]
87. Faraz, A.; Tauqir, N.A.; Waheed, A.; Hameed, A. Effect of exogenous oxytocin administration on the performance of lactating Nili Ravi buffalo. *Irian J. Appl. Anim. Sci.* **2021**, *11*, 517–525.
88. Kumar, V.S.; Kumar, R.P.; Harikrishna, C.H.; Rani, M.S. Effect of heat stress on production and reproduction performance of buffaloes-A review. *Pharma. Innov.* **2018**, *7*, 629–633.
89. García, H.B.-; Alva-Pérez, J.; Corona-González, B.; Álvarez, D.O.; Martinez-burnes, J. 'Hallazgos recientes del proceso salud-enfermedad en búfalo de agua (Bubalus bubalis)' Enfermedades bacterianas y parasitarias. In *El Búfalo de Agua en Latinoamérica, Hallazgos Recientes*; Napolitano, F., Mota-Rojas, D., Guerrero-Legarreta, I., Orihuela, A., Eds.; BM Editores: Mexico City, Mexico, 2020; pp. 863–921.
90. Rahman, S.M.R.; Islam, M.N.; Harun-ur-Rashid, M.; Sarker, N.R.; Siddiki, M.S.R.; Islam, M.Z.; Islam, M.A. Buffalo milk yield, quality, and marketing in different agro-climatic districts of bangladesh. *J. Buffalo Sci.* **2019**, *8*, 62–67. [CrossRef]
91. Gabr, A.E.; Ebrahim, S.; El-Hawary, A.; Fathala, M.; EL-Gohary, E.; Ahmed, S.E.; Abdel-Latif, M. Upgrading milk productivity of primiparous buffaloes using glycogenic precursors; implications on milk production and blood biochemical parameters. *Zagazig Vet. J.* **2017**, *45*, 92–103. [CrossRef]
92. Patbandha, T.K.; Raviikala, K.; Maharana, B.R.; Marandi, S.; Ahlawat, A.R.; Gajbhiye, P.U. Effect of season and stage of lactation on milk components of Jaffrabadi. *Bioscan* **2015**, *10*, 635–638.
93. Zicarelli, L. Current trends in buffalo milk production. *J. Buffalo Sci.* **2020**, *9*, 121–132. [CrossRef]
94. Acosta-Alba, I.; Lopéz-Ridaura, S.; van der Werf, H.M.G.; Leterme, P.; Corson, M.S. Exploring sustainable farming scenarios at a regional scale: An application to dairy farms in Brittany. *J. Clean. Prod.* **2012**, *28*, 160–167. [CrossRef]
95. Murphey, R.M.; Paranhos da Costa, M.J.R.; Da Silva, R.G.; de Souza, R.C. Allonursing in river buffalo, *Bubalus bubalis*: Nepotism, incompetence, or thievery? *Anim. Behav.* **1995**, *49*, 1611–1616. [CrossRef]
96. Martínez, J.G. La evolución y la conservación de la biodiversidad. In *Evolución: La Base de la Biología.*; Soler, M., Ed.; Proyecto Sur de Ediciones, S.L.: Madrid, Spain, 2012; pp. 407–416.
97. Madella-Oliveira, A.d.F.; Bastos, R.; Quirino, C.R. Comportamentos de amamentação e mamada em bubalinos, suas relações com período de lactação, idade e sexo dos bezerros. *Rev. Ceres* **2010**, *57*, 211–217. [CrossRef]

98. Brandlová, K.; Bartoš, L.; Haberová, T. Camel calves as opportunistic milk thefts? the first description of allosuckling in domestic bactrian camel (*Camelus bactrianus*). *PLoS ONE* **2013**, *8*, e53052. [CrossRef] [PubMed]
99. Mota-Rojas, D.; Marcet-Rius, M.; Freitas-de-Melo, A.; Muns, R.; Mora-Medina, P.; Domínguez-Oliva, A.; Orihuela, A. Allonursing in wild and farm animals: Biological and physiological foundations and explanatory hypotheses. *Animals* **2021**, *11*, 3092. [CrossRef] [PubMed]
100. Packer, C.; Lewis, S.; Pusey, A. A comparative analysis of non-offspring nursing. *Anim. Behav.* **1992**, *43*, 265–281. [CrossRef]
101. Roulin, A. Why do lactating females nurse alien offspring? A review of hypotheses and empirical evidence. *Anim. Behav.* **2002**, *63*, 201–208. [CrossRef]
102. Víchová, J.; Bartoš, L. Allosuckling in cattle: Gain or compensation? *Appl. Anim. Behav. Sci.* **2005**, *94*, 223. [CrossRef]
103. Engelhardt, S.C.; Weladji, R.B.; Holand, Ø.; de Rioja, C.M.; Ehmann, R.K.; Nieminen, M. Allosuckling in reindeer (Rangifer tarandus): Milk-theft, mismothering or kin selection? *Behav. Process.* **2014**, *107*, 133–141. [CrossRef]
104. Oliveira, A.D.F.M.; Quirino, C.R.; Bastos, R. Effect of nursing behaviour, sex of the calf, and parity order on milk production of buffaloes. *Rev. Colomb. Cienc. Pecu.* **2017**, *30*, 30–38. [CrossRef]
105. Andriolo, A.; Schmidek, W. Suckling behaviour in water buffalo (*Bubalus bubalis*). *Rev. Etol.* **2001**, *3*, 129–136.
106. Paranhos Da Costa, M.J.R.; Andriolo, A.; Simplício De Oliveira, J.F.; Schmidek, W.R. Suckling and allosuckling in river buffalo calves and its relation with weight gain. *Appl. Anim. Behav. Sci.* **2000**, *66*, 1–10. [CrossRef]
107. Murphey, R.M.; Paranhos da Costa, M.J.R.; de Souza Lima, L.O.; de Moura Duarte, F.A. Communal suckling in water buffalo (*Bubalus bubalis*). *Appl. Anim. Behav. Sci.* **1991**, *28*, 341–352. [CrossRef]
108. Roulin, A.; Heeb, P. The immunological function of allosuckling. *Ecol. Lett.* **1999**, *2*, 319–324. [CrossRef]
109. Dalto, A.C.; Bandarra, P.M.; Pavarini, S.P.; Boabaid, F.M.; de Bitencourt, A.P.G.; Gomes, M.P.; Chies, J.; Driemeier, D.; da Cruz, C.E.F. Clinical and pathological insights into Johne's disease in buffaloes. *Trop. Anim. Health Prod.* **2012**, *44*, 1899–1904. [CrossRef] [PubMed]
110. Murphey, R.M.; Moura Duarte, F.A. Calf control by voice command in a Brazilian dairy. *Appl. Anim. Ethol.* **1983**, *11*, 7–18. [CrossRef]
111. MacLeod, K.J.; Lukas, D. Revisiting non-offspring nursing: Allonursing evolves when the costs are low. *Biol. Lett.* **2014**, *10*, 1–4. [CrossRef]
112. Napolitano, F.; Pacelli, C.; Grasso, F.; Braghieri, A.; De Rosa, G. The behaviour and welfare of buffaloes (*Bubalus bubalis*) in modern dairy enterprises. *Animal* **2013**, *7*, 1704–1713. [CrossRef] [PubMed]
113. Greenberg, G.; Partridge, T.Y. Comparative psychology, a new perspective for the 21st century: Response to criticism. *Dev. Psychobiol.* **2004**, *44*, 31–36. [CrossRef]
114. Mora-Medina, P.; Berdugo-Gutiérrez, J.A.; Mota-Rojas, D.; Ruiz-Buitrago, J.D.; Nava-Adame, J.; Guerrero-Legarreta, I. Behaviour and Welfare of Dairy Buffaloes: Pasture or Confinement? *J. Buffalo Sci.* **2018**, *7*, 43–48. [CrossRef]
115. Mota-Rojas, D.; Broom, D.M.; Orihuela, A.; Velarde, A.; Napolitano, F.; Alonso-Spilsbury, M. Effects of human-animal relationship on animal productivity and welfare. *J. Anim. Behav. Biometeorol.* **2020**, *8*, 196–205. [CrossRef]
116. Napolitano, F.; Strappini, A.; Ramírez-Bribiesca, E.; Alarcón-Rojo, A.D.; Cruz-Monterrosa, R.; Guerrero Legarreta, I.; Rayas-Amor, A.A.; García-Galicia, I.A.; Ghezzi, M.D.; Becerril-Herrera, M.; et al. Estresores previos a la muerte y su efecto en la calidad de la carne del búfalo de agua. In *El Búfalo de Agua en Latinoamérica, Hallazgos Recientes*; Napolitano, F., Mota-Rojas, D., Guerrero-Legarreta, I., Orihuela, A., Eds.; BM Editores: Mexico City, Mexico, 2020; pp. 1178–1233.
117. Ghoneim, E.; Omar, S.; El-Dahshan, E. Measuring welfare of egyptian buffaloes in different management systems. *J Anim. Poult. Prod.* **2018**, *9*, 407–414. [CrossRef]
118. Mendonça, F.S.; Vaz, R.Z.; Cardoso, F.F.; Restle, J.; Vaz, F.N.; Pascoal, L.L.; Reimann, F.A.; Boligon, A.A. Pre-slaughtering factors related to bruises on cattle carcasses. *Anim. Prod. Sci.* **2018**, *58*, 385. [CrossRef]
119. Pérez-Valverde, Y.C. Condiciones Del Transporte Hacia el Matadero que Afectan las Características Ante Mortem y Post Mortem en el Ganado Bovino. Ph.D. Thesis, Universidad Científica del sur, Lima, Peru, 2021.
120. Kuhl, F.N. Prácticas de Manejo na Fase Ante Mortem de Bovinos para Identificação de Pontos Críticos à Qualidade da Carne. Master's Thesis, 1157 Universidad Federal de Pelotas, Pelotas, Brasil, 2013.
121. Huertas, S. Buenas practicas de manejo durante el embarque y transporte a la planta de sacrificio. In *Bienestar Animal: Productividad y Calidad de la Carne*; Mota-Rojas, D., Guerrero-Legarreta, I., Trujillo-Ortega, M.E., Eds.; BM Editores: Mexico City, Mexico, 2009; pp. 73–84.
122. Pérez-Linares, C.; Sánchez-López, E.; Ríos-Rincón, F.G.; Olivas-Valdéz, J.A.; Figueroa-Saavedra, F.; Barreras-Serrano, A. Factores de manejo pre y post sacrificio asociados a la presencia de carne DFD en ganado bovino durante la epoca cálida. *Rev. Mex. Cienc. Pecu.* **2013**, *4*, 149–160.
123. Schwartzkopf-Genswein, K.; Ahola, J.; Edwards-Callaway, L.; Hale, D.; Paterson, J. Symposium Paper: Transportation issues affecting cattle well-being and considerations for the future 1 1Presented at the Cattle Transportation Symposium sponsored by the Beef Checkoff Program, National Cattlemen's Beef Association, and Colorado State U. *Prof. Anim. Sci.* **2016**, *32*, 707–716. [CrossRef]
124. Alarcón-Rojo, A.; Mota-Rojas, D.; García-Galicia, I.; Ramírez-Bribiesca, E.; Olmos-Hernández, A.; Guerrero-Legarreta, I. Dark cutting in large ruminants: Effect of management and environmental factors. *Agro Product.* **2021**, *13*, 93–98. [CrossRef]
125. Tarrant, P.V. Transportation of cattle by road. *Appl. Anim. Behav. Sci.* **1990**, *28*, 153–170. [CrossRef]

126. Alam, M.R.; Gregory, N.G.; Jabbar, M.A.; Uddin, M.S.; Kibria, A.S.M.G.; Silva-Fletcher, A. Skin injuries identified in cattle and water buffaloes at livestock markets in Bangladesh. *Vet. Rec.* **2010**, *167*, 415–419. [CrossRef] [PubMed]
127. Mota-Rojas, D.; Ghezzi, M.D.; Napolitano, F.; Rosmini, M.R.; Guerrero-Legarreta, I.; Martínez-Burnes, J.; Lezama-García, K.; Miranda-Cortés, A.; de la Vega, L.T.; Mora-Medina, P.; et al. Quality of death in the river buffalo (*Bubalus bubalis*). *J. Anim. Behav. Biometeorol.* **2021**, *9*, 2115. [CrossRef]
128. Adenkola, A.Y.; Ayo, J.O. Physiological and behavioural responses of livestock to road transportation stress: A review. *Afr. J. Biotechnol.* **2010**, *9*, 4845–4856. [CrossRef]
129. EL-Deeb, W.M.; El-Bahr, S.M. Acute-phase proteins and oxidative stress biomarkers in water buffalo calves subjected to transportation stress. *Comp. Clin. Path.* **2014**, *23*, 577–582. [CrossRef]
130. Alam, M.R.; Gregory, N.G.; Uddin, M.S.; Jabbar, M.A.; Chowdhury, S.; Debnath, N.C. Frequency of nose and tail injuries in cattle and water buffalo at livestock markets in Bangladesh. *Anim. Welf.* **2010**, *19*, 295–300.
131. Bertoni, A.; Martínez, G.M.; Napolitano, F.; Álvarez-Macías, A.; Ghezzi, M.D.; Mora-Medina, P.; Braghieri, A.; Guerrero-Legarreta, I.; Cruz-Monterrosa, R.; Daniel, M.-R.; et al. Ventajas y desventajas de los sistemas de ordeño manual y mecánico: Productividad, bienestar animal y rentabilidad. In *El Búfalo de Agua en Latinoamérica, Hallazgos Recientes*; Napolitano, F., Mota-Rojas, D., Guerrero-Legarreta, I., Orihuela, A., Eds.; BM Editores: Mexico City, Mexico, 2020; pp. 772–807.
132. Rahman, S.M.R.; Islam, M.N.; Harun-ur-Rashid, M.D.; Siddiki, M.S.R.; Islam, M.A. Dairy buffalo production under intensive system in semi arid area of Bangladesh. *Buffalo Bull.* **2019**, *38*, 83–98.
133. Alinovi, M.; Mucchetti, G.; Wiking, L.; Corredig, M. Freezing as a solution to preserve the quality of dairy products: The case of milk, curds and cheese. *Crit. Rev. Food. Sci. Nutr.* **2020**, *61*, 3340–3360. [CrossRef]

2.3. El búfalo de agua como productor de carne

La producción cárnica proveniente del búfalo de agua se ha incrementado en diversos países durante los últimos años debido a sus propiedades y su capacidad de adaptarse a diferentes condiciones climáticas y presentar una mayor digestibilidad en el consumo de pastos de mala calidad con un crecimiento más rápido siendo una especie versátil y útil en una producción ganadera sostenible. Al igual que con otras especies sus características dependen de la dieta proporcionada, edad a la matanza, raza y prácticas aplicadas durante la movilización previa a la muerte (Guerrero-Legarreta et al., 2020).

Este carne roja se ha mostrado como una alternativa más saludable que la proveniente de bovino del género *Bos* por los niveles de proteína y grasas poliinsaturadas, resultando en mejores oportunidades de éxito (Cruz-Monterrosa et al., 2020; Guerrero-Legarreta et al., 2020). Igualmente, presenta características organolépticas satisfactorias, un mayor contenido de hierro y menor contenido de grasas, calorías y colesterol en comparación con los vacunos convencionales brindando efectos positivos en los consumidores comparándose con el ganado *Bos* (Marrone et al., 2020) (Tabla 1).

Tabla 1. Características nutricionales de bovinos y búfalos en 100 g de carne

	Agua	**MS**	**Proteína**	**Grasa**	**Minerales**	**Carbohidratos**
Búfalo	74.9	23.33	23.4	1.1	1.06	0.52
Bovino del género *Bos*	75.0	24.64	20.4	1.8	1.15	0.42

Modificado de (Crudeli et al., 2001; Rey & Povea, 2012).

La calidad de la carne se describe por diversas características como color, atributo crítico asociado con frescura, terneza más aceptable que la proveniente de animales cebuinos; jugosidad, sabor y palatabilidad además de sus cualidades nutricionales, fisicoquímicas y sanitarias (Cruz-Monterrosa et al., 2020; Guerrero-Legarreta et al., 2020, 2022; Jaspal et al., 2021).

Además de los valores previamente mencionados la carne de búfalo contiene propiedades funcionales para el procesamiento de productos cárnicos de interés en el mercado como salchichas y hamburguesas mismas que pueden obtener un valor diferenciado por los valores nutricionales que la distinguen (Cruz-Monterrosa et al., 2020).

2.4. Efecto del transporte sobre el BA

2.4.1. Importancia del BA durante la movilización

Se ha demostrado que el transporte previo a la muerte es un factor genera estrés en el búfalo de agua, modificando constantes fisiológicas y generando respuestas en los animales y su conducta, impactando directamente en la calidad de la carne y disminuyendo las ganancias económicas del productor (Matias et al., 2019), lo anterior está presente en diversas especies desde el embarque, Dalla et al. (2019) encontraron que una inclinación de la rampa de embarque mayor a 20 grados generaba 4 veces más probabilidades de muerte en cerdos comparadas con una rampa menor a 20 grados además de 1.56 veces más probabilidades de morir por cada hora adicional en el transporte y 1.32 veces más probabilidades de morir en animales con problemas al caminar, artritis y hernias. Para la movilización de aves de engorda a rastro, se encontró que la alta densidad de las jaulas, seguido por la mezcla de sexos y la hora del embarque (diurnas) son los principales factores que favorecen la presencia de lesiones en alas (Cockram et al., 2020).

2.4.2. Repercusión del horario, densidad de carga y duración del transporte sobre el Bienestar animal (BA).

La afectación por efecto del horario de movilización sobre el BA se observa de acuerdo a las condiciones ambientales como temperatura y humedad y su intensidad, añadido a su duración (efecto multiplicativo) pueden presentarse cambios en valores fisiológicos, conductuales, químicos, endócrinos y de rendimiento del búfalo de agua, por lo anterior se ha señalado que se tiene un mayor impacto cuando la movilización es realizada en los horarios que van entre las 11:00 y las 18:00 horas (González et al., 2012), además de procurar que los vehículos tengan suficiente ventilación y se encuentren cubiertos para generar un microclima favorecedor para el mantenimiento del BA.

Cuando las características antes mencionadas no son respetadas puede iniciarse un proceso de estrés por calor previo a la matanza, lo cual genera respuestas metabólicas y fisiológicas en el búfalo de agua generando el aumento de la frecuencia cardiaca y respiratoria, al igual que la temperatura corporal, reducción del flujo sanguíneo en tracto gastrointestinal y vasodilatación a piel para una apropiada termorregulación y a extremidades ante una posible respuesta de huida y para el mantenimiento de una postura durante la movilización, además de la utilización de energía mediante reservas corporales por medio de la glucogenólisis muscular (Cruz-Monterrosa et al., 2020; Guerrero-Legarreta et al., 2020)

Por otro lado, la densidad de carga es definida como el espacio concedido a un animal durante su movilización y es considerado como uno de los aspectos que más peso genera sobre el BA, esto debido a que el proporcionar mayor espacio provocará que el búfalo de agua realice un mayor esfuerzo por mantener el equilibrio, fatiga, estrés y caídas, y en el caso de brindar menor espacio se observará una sopreposición de los animales, fatiga, estrés y una elevada presencia de hematomas y lesiones (Ejemplificación en Figura 1), así mismo, deben ser considerados factores como presencia de cuernos (aumento de 7% del espacio respecto a sus contrapartes descornadas) (Panel & Ahaw, 2011; Warren et al., 2010).

Figura 1. Sopreposición de búfalos de agua movilizados que puede generar fatiga, estrés y una elevada presencia de hematomas y lesiones.

Otro factor que explica la presencia de lesiones y animales caídos se encuentra relacionada con el manejo previo, durante y post transporte, teniendo relación con calidad de la interacción humano-animal (Mota-Rojas et al., 2020b) (Figura 2), así como, la duración del transporte, además, su aumento está asociado con disminución del peso, agotamiento físico, entre otros, la duración es definida como el tiempo que pasa desde que el primer animal a movilizar aborda hasta que el último baja del vehículo (Cueto, 2020).

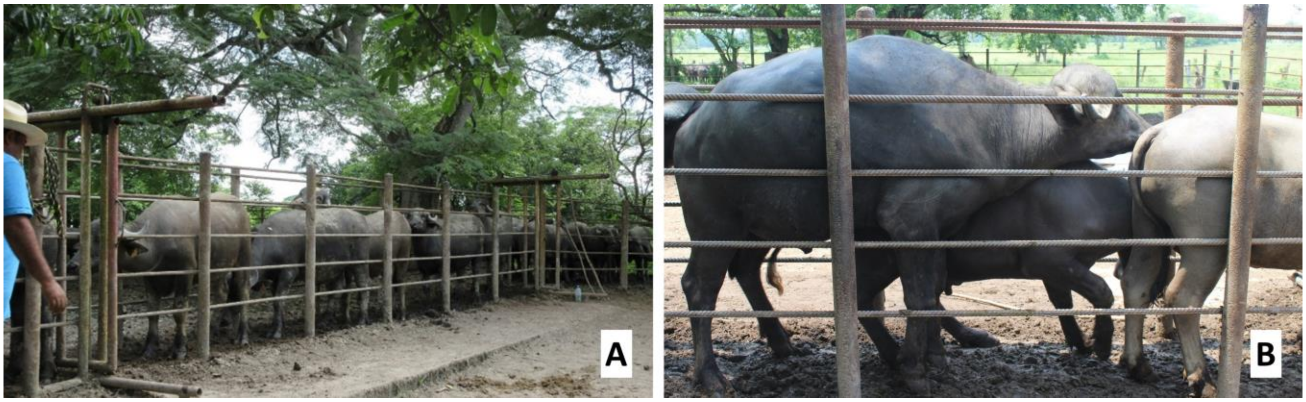

Figura 2. Manejo durante la manga de manejo previo al embarque. A. Adecuada interacción en donde se evita la sobreposición de animales y la generación de lesiones. B. Inadecuado ingreso de búfalos en la manga de manejo con sopreposición de animales que coloca a los mismos en situaciones de riesgo.

En la evidencia científica relacionada con el BA durante la movilización la duración del mismo en un factor constante a considerar ya que si existe algún estímulo percibido como amenaza para el bienestar que genere un conjunto de cambios y respuestas conductuales y fisiológicas este estímulo estresante se potencializará conforme la duración del transporte sea mayor (Dalla Costa et al., 2019; Sommavilla et al., 2017).

2.5. Impacto del BA sobre los productos finales

2.5.1. Pérdidas económicas por lesiones y contusiones generadas durante el transporte.

Se ha encontrado que el no considerar factores que propician un deficiente BA impacta de forma negativa los valores finales, por ejemplo, un escenario estudiado por las mermas generadas son las prácticas aplicadas durante el transporte y manejo previo a la matanza en donde los daños afectan los rendimientos de la cadena productiva debido a afectaciones en la canal, disminución del peso vivo, cambios en la calidad e incluso inocuidad del producto final (José-Pérez et al., 2022; Mota-Rojas et al., 2023; Rodríguez-González et al., 2023c).

Ante la presencia de contusiones, lesiones o hematomas es necesaria la eliminación de carne dañada ya que esta no cumple con estándares de calidad y el no hacerlo representaría un riesgo a la inocuidad del producto ya que las áreas con hematomas o lesiones sufren un proceso de descomposición más rápido si estas no son retiradas se generará un ambiente favorable para la proliferación de bacterias (Cruz-Monterrosa et al., 2017).

Las lesiones y contusiones generadas durante ese proceso propician la pérdida de kg a nivel mundial de carne de acuerdo con el grado y extensión, Huertas et al. (2018) en Uruguay reportaron 1.6 kg de carne promedio retirados por animal debido a lesiones, en México, Carrasco- García et al. (2020) indicaron una persistencia de 81% de lesiones en canal. Algunos estudios han cuantificado las pérdidas económicas, USA estimó en 2011 que sólo en bovinos se tienen pérdidas por 10 dólares por animal procesado en rastro debido al desecho de áreas lesionadas en canales y la disminución del peso vivo por factores como densidades y tiempos de movilización mal aplicados, además indicó en 2016 que por hematomas encontrados en las canales se pierden 5.55 millones de dólares anualmente (National Beef Quality Audit, 2011; 2016).

Por lo anterior, es importante resaltar que el impacto económico negativo debido al transporte y a las malas prácticas de manejo es considerable, por lo que la implementación de estrategias para mejorar el BA de los animales durante el transporte puede tener un impacto económico para los participantes en la cadena de producción de carne ya que está demostrado que el grado de lesiones y afectaciones en calidad de carne son un reflejo y fuente de información respecto al bienestar que presentan los animales durante la movilización y matanza.

2.5.2. BA y su relación con características fisicoquímicas de la carne

Las afectaciones al bienestar del búfalo de agua durante la movilización se verán retribuidas en la modificación de los valores físicos, organolépticos y microbiológicos del producto final, condicionando la calidad deseada e, inclusive, atentando contra su inocuidad por presencia y frecuencia de lesiones, contusiones, encogimiento, ayuno prolongado y estrés causando decomisos y retiros de producto en mercado disminuyendo vida de anaquel por alteraciones en los valores de pH, color, CRA, textura y olor, la modificación de estos valores puede tener como resultado la presencia de cortes oscuros (Gallo et al., 2018; Gallo & Huertas, 2016; Muñoz et al., 2012).

Este corte es generado principalmente por el sometimiento a estrés crónico previo a la matanza (en movilización y tiempo de reposo) lo cual genera el uso de glucógeno muscular previo a la muerte en preparación de un gasto energético amplio ante un entorno desafiante teniendo como consecuencia la presencia deficiente de ácido láctico debilitando la caída de pH durante la conversión de músculo a carne presentando valores superiores a 6.0 a las 24h posteriores a la muerte (Alarcón-Rojo et al., 2021; Fabio et al., 2020).

Este pH alto, superior a 6.5, genera una mayor absorción de luz debido al incremento de CRA propiciando un color más oscuro (Chulayo et al., 2016; Ijaz et al., 2020a) incrementando la fuerza de corte (Jeleníková et al., 2008), los valores presentes en un corte oscuro están íntimamente relacionados con una mayor actividad y proliferación bacteriana (Motaghifar et al., 2021) modificando una de las características más importantes que debe mantener cualquier producto alimenticio, su inocuidad, además de generar cambios fisicoquímicos relativos a descomposición y disminuir la vida de anaquel (Cruz-Monterrosa et al., 2020).

2.6. Conversión de músculo a carne

La conversión de músculo a carne es el resultado de una compleja cascada de cambios energéticos, bioquímicos y físicos producidos en el músculo durante el periodo post mortem, posterior a la exanguinación y anoxia en el animal, en donde el músculo continúa sintetizando y utilizando ATP en un intento por mantener la homeostasis celular (Matarneh et al., 2017; Rodríguez-González et al., 2021).

Este proceso está dividido en 3 etapas, pre-rigor, rigor y post- rigor. En la primera etapa se observa el agotamiento del oxígeno, entonces el glucógeno y los compuestos de fosfato de alta energía presentes en músculo son metabolizados anaeróbicamente con el objetivo de producir ATP, sin embargo este proceso resulta menos eficiente que cuando se tiene la presencia de oxígeno, como resultado la tasa de hidrólisis de ATP excede su generación y desencadena el inicio de la rigidez de la muerte (rigor mortis) generando que el músculo pierda gradualmente la capacidad de generar ATP y agotándose eventualmente estas sustancias, promoviendo la desnaturalización de proteínas (Díaz-Luis et al., 2020; Ortega-Torres & Ariza-Botero, 2012; Ouali et al., 2006).

En ausencia de ATP la miosina se une irreversiblemente a actina lo que lleva a la finalización del rigor mortis, dando paso a la fase de post- rigor con la pérdida de excitabilidad y extensibilidad muscular, finalizando el rigor mortis de 1 a 12 horas, durante el envejecimiento realiza la degradación proteolítica de las proteínas del citoesqueleto provocando la pérdida de la integridad estructural del músculo y por tanto una disminución de la tensión muscular, comenzando la etapa de maduración de la carne por las enzimas proteolíticas endógenas que actúan sobre la estructura muscular (England et al., 2013; Longo et al., 2015; Matarneh et al., 2017).

Ante el cambio metabólico que reduce la producción de ATP y da lugar al fosfato inorgánico que estimula la degradación de glucosa a piruvato, la cual debido a la ausencia de oxígeno genera la formación y acumulación de ácido láctico generando la caída del pH acidificando el producto (Ouali et al., 2006).

La tasa y la extensión del metabolismo post mortem influyen significativamente en el desarrollo de los atributos de la calidad e inocuidad de la carne siendo influenciados por factores previos a la matanza de los animales con repercusiones en la vida de anaquel (Cruz-Monterrosa et al., 2020; Gonzalez-Rivas et al., 2020; Guerrero-Legarreta et al., 2020; Joele et al., 2017; Turan et al., 2021).

2.6.1. Factores predisponentes para la presencia de características no deseadas en carne; corte oscuro

El no brindar las condiciones óptimas durante la movilización de los animales propicia pérdidas económicas por los decomisos de carne de acuerdo con grado, extensión y profundidad de las lesiones. Ante la presencia de estas afectaciones es necesaria la eliminación de carne dañada ya que esta no cumple con estándares de calidad y el no hacerlo representaría un riesgo a la inocuidad del producto ya que las áreas sufren un proceso de descomposición más rápido. Si estas no son retiradas se generará un ambiente favorable para la proliferación de bacterias generando el desarrollo de olor desagradable de la carne (Motaghifar et al., 2021; Cruz-Monterrosa et al., 2020; Pircher et al., 2007).

Además, al generar estrés en los animales vivos previo a su muerte es común animales vivos previo a su muerte es común la presencia cortes oscuros, por la disminución hasta concentraciones mínimas de las reservas de glucógeno y la síntesis y liberación de lactato en donde los valores de pH (mayor a 6.0 a las 24 horas post mortem) presentando variaciones no deseadas sobre textura, capacidad de retención de agua y vida de anaquel, jugosidad, entre otros. (Cruz-Monterrosa et al., 2020; Fabio et al., 2020; Guerrero-Legarreta et al., 2020).

El proceso que genera esta característica indeseable en carne tiene su origen en la metodología previa a la matanza, que, en caso de ser un ambiente que propicia el estrés como una movilización inadecuada, con generación de lesiones físicas, con condiciones climáticas adversas y una duración y tipo de viaje complicado además de no considerar tiempos de reposo posterior al arribo de los animales en rastro, ayuno prolongado,

deshidratación, inadecuado aturdimiento o desangrado, entre otros, generan una glucólisis muscular antes de la exanguinación del semoviente (Alarcón-Rojo et al., 2021; Cruz-Monterrosa et al., 2020; Mota-Rojas et al., 2020; Mota-Rojas et al., 2021).
Además de tener influencia sobre la caída de pH, el ácido láctico retarda el crecimiento bacteriano propiciando una menor contaminación de la canal durante su procesamiento, empaquetado y almacenamiento (Cruz-Monterrosa et al., 2020; Fabio Napolitano et al., 2020).

2.7. Métodos para medir el BA

2.7.1. Métodos invasivos y no invasivos para la medición de BA

El estrés es definido como una respuesta ante un estímulo percibido como amenaza para el BA que propicia cambios conductuales, físicos, productivos, sanitarios y fisiológicos preparando al organismo para una respuesta o acción. El estrés durante el transporte puede ser medido de forma invasiva o no invasiva, en la primera se considera la toma de muestras sanguíneas o aquellas que requieran una interacción física con el animal a estudiar para observar los niveles de cortisol, catecolaminas, hemoconcentración, vasopresina, β-endorfinas, CRH, ACTH, lactato, glucógeno, entre otros,. Por el contrario para la medición del estrés de manera no invasiva se tiene la evaluación de cambios conductuales en el búfalo de agua, el uso de IRT en diversas y específicas ventanas térmicas, medición de frecuencia cardiaca y respiratoria, valores de rendimiento de canal, entre otros.

Pese a los valores y aporte generado por métodos invasivos, estos normalmente requieren de mayor tiempo y manejo animal, provocando con frecuencia respuestas de estrés que se buscan evitar cuando se realiza la medición del BA (Cuthbertson et al., 2020), en este sentido, existen métodos no invasivos como la evaluación de cambios de conductas, incidencia de enfermedades, disminución en valores productivos, IRT y en el caso de movilización una vez que se obtienen las canales es común el registro de lesiones que indican que el bienestar se perdió durante este proceso (Church et al., 2014; Ghezzi, 2018; OMSA, 2021).

2.7.2. IRT

La IRT es un método no invasivo, confiable, útil y de alta sensibilidad para la detección y cuantificación de temperatura irradiada por un objeto o un cuerpo, en este último se identifican áreas anatómicas que por su tipo de vascularización ofrecen información respecto al estado de bienestar o disconfort en el búfalo de agua mediante el análisis de los cambios suscitados entre actividades y durante ellas (Mota-Rojas et al., 2021b; Zhang et al., 2020), pudiendo ser utilizado como herramienta para estimar el estado fisiológico del ganado, brindando un mapeo de la temperatura presente en la superficie y sus variaciones dependerán del escenario y ambiente en el cual se encuentre (Casas-Alvarado et al., 2020; Cook, 2021).

El búfalo de agua es un animal homeotermo y produce respuestas ante estímulos externos para la regulación de su temperatura corporal (Mota-Rojas et al., 2021a), sumado a su deficiente sudoración, color de piel, y folículos limitados brindando una mayor receptividad a la radiación solar, pese a todas estas características ha demostrado ser un animal adaptable, rústico y con altos niveles de productividad (Álvarez-Macías et al., 2020).

En la actualidad el desarrollo de tecnologías implica mejoras en procesos productivos y eficiencia de los recursos disponibles (Zhang et al., 2020), la importancia de la IRT radica en los múltiples usos que se han aplicado, tales como descripción de procesos inflamatorios, BA en las diferentes etapas productivas y cambios durante cada fase reproductiva (Bertoni et al., 2020b).

En este sentido se ha promovido su uso para la observación, explicación y análisis de procesos inflamatorios debido a la hiperpermeabilidad de vasos sanguíneos, contracción del músculo liso, dilatación de vasos sanguíneos y producción de leucocitos que eventualmente aumentarán el metabolismo local del cuerpo del animal y por tanto aumentarán la temperatura, (Hovinen & Pyörälä, 2011), resultando en diversos estudios que identificaron los cambios térmicos ante enfermedades como onfalitis, mastitis, laminitis, enfermedades respiratorias y procesos inflamatorios originados por lesiones físicas (Zhang et al., 2020).

Otra aplicación ha sido la evaluación del BA durante el transporte mismo que se encuentra relacionado con impactos negativos cuando este es pobre por los cambios generados en la calidad de producto final con características no deseadas por el consumidor y una menor vida de anaquel (Mota-Rojas et al., 2021a; Rodríguez-González et al., 2023b) mismos que serán aplicados en este estudio para valorar los cambios en la microcirculación dérmica y su relación con el grado de bienestar en búfalos de agua del trópico húmedo mexicano.

2.8. Termorregulación en el búfalo de agua

El sistema de termorregulación en el búfalo de agua ha sido señalado como ineficiente frente a calor extremo debido a las características anatómicas que este presenta, además de lo aparente como su color de piel oscuro con un mayor nivel de melanina que provoca que de acuerdo con la temperatura ambiental la interna se eleve con facilidad se ha demostrado que presenta menor proporción de folículos pilosos (entre 135 y 145/ cm^2) respecto al bovino convencional (3000/cm^2) que pudiera brindarle protección a condiciones extremas además de complicar la disipación de calor en el búfalo de agua (Bertoni et al., 2020; Mota-Rojas et al., 2021, 2021b).

Sin embargo, se ha reportado también que los búfalos cuentan con glándulas sudoríparas de mayor diámetro, compensando la densidad disminuida y brindándole una mayor capacidad de termorregular (Crudeli et al., 2016). En este sentido, son generados diversos cambios fisiológicos y de comportamiento con el objetivo de alcanzar el confort térmico, tales como la dilatación de vasos sanguíneos para propiciar la disipación de calor, realización de comportamientos que contribuyen a la pérdida de calor con rapidez, en este sentido, los termorreceptores detectan aumento en la temperatura y desencadenan cambios fisiológicos y de comportamiento para evitar efectos negativos en el animal como estrés térmico (Bertoni et al., 2020; Gonzalez-Rivas et al., 2020; Marai et al., 2009; Mota-Rojas et al., 2021).

2.8.1. Neuromodulación hipotalámica de la termorregulación

La neuromodulación hipotalámica al termorregular se basa en una serie de respuestas autónomas a estímulos p.e. elevadas temperaturas en donde se involucran estructuras como fibras colinérgicas simpáticas en la sudoración y fibras adrenérgicas para la piloerección, vasoconstricción y vasodilatación siendo la piel el órgano central regulador debido a su capacidad de detección y transmisión de estímulos, en este sentido, tanto las respuestas fisiológicas como conductuales se rigen por señales térmicas dirigidas a médula espinal, posteriormente a la región preóptica (POA) del hipotálamo en donde de acuerdo al estímulo se generan diversas respuestas con ayuda de circuitos neuronales (Casas-Alvarado et al., 2020; Mota-Rojas et al., 2021).

Dentro de la percepción de temperatura se encuentra involucrado el receptor de potencial transitorio vaniloide (TRPV), en donde el TRPV1 se desempeña como integrador molecular de estímulos físicos y químicos en nociceptores periféricos que destaca en la participación de la percepción del dolor y la hipersensibilidad térmica en procesos de inflamación expresado en cerebro, en donde se incluye la participación de TPRV3 y TPRV4, expresados en queratinocitos en dermis (Vásquez et al., 2013).

Cuando se realiza la sensibilización de receptores en piel son generados impulsos nerviosos de médula espinal hacia el asta dorsal en donde se activan las neuronas sensibles al calor, para transmitir información a la subregión dorsal del núcleo parabraquial lateral (LPBd) del tronco encefálico para emitir una proyección glutamatérgica hacia el POA del hipotálamo a través de vías eferentes (Mota-Rojas et al., 2021; Wang, et al., 2021a).
El seguimiento de esta respuesta comienza con la transmisión desde el POA hasta la médula ventromedial rostral (RVMM) que transmite la señal a las células de la columna intermediolateral (IML) en médula espinal, desencadenando transmisión de información a los nervios que inervan las glándulas sudoríparas periféricas con la liberación de acetilcolina propiciando la sudoración (Mota-Rojas et al., 2021).
Cuando la respuesta es la vasodilatación se inicia la transmisión de impulsos nerviosos a las neuronas IML hacia fibras eferentes simpáticas que inervan vasos sanguíneos cutáneos que desencadenan la generación de disminución de temperatura por convección y conducción para la disipación del calor mediante vasodilatación cutánea, inhibiendo también las neuronas del área tegumental ventral (VTA) para evitar una vasoconstricción y conservación del calor (Casas-Alvarado et al., 2020).

2.8.2. Ventanas térmicas en el búfalo de agua

En el búfalo de agua han sido señaladas algunas ventanas térmicas ubicadas en áreas faciales, dorsales, caudales y laterales, conocimiento de relevancia para la obtención de datos térmicos y toma de decisiones al observar alteraciones los semovientes ante temperaturas y porcentajes de humedad altos, situación común ya que el 99% de los animales de esta especie habitan en regiones tropicales y subtropicales, pudiendo generar signos de incomodidad térmica, problemas reproductivos y productivos (Liu et al., 2020).
Se define como ventana térmica a superficies corporales cuyas características permiten la pérdida o ganancia de temperatura con facilidad debido al cambio de diámetro de los vasos sanguíneos, esto debido a una alta densidad de capilares sanguíneos superficiales, ausencia de pelo o una menor cantidad comparándose con el resto del cuerpo y mayor presencia de anastomosis arteriovenosas facilitando la pérdida de calor localizada (Andrade, 2015; Tattersall, 2016; Verduzco-Mendoza et al., 2021).
En este sentido se han descrito diversas ventanas en animales no humanos con estas características, tales como orejas en elefantes con una alta vascularización y con una excelente superficie blanco con el uso de IRT, nasales en cerdos, ventanas podales en

aves de producción, picos en tucanes, o ventanas corporales en animales como cerdos, entre otros (Cilulko et al., 2013; Soerensen et al., 2014; Soerensen & Pedersen, 2015).

Las condiciones previamente descritas son observadas en la región periocular (*Regio orbitalis*), en la región auricular (*Regio auricularis*) gracias a que ambas presentan una alta densidad de vasos sanguíneos cercanos a la superficie de la dermis, generando datos observados mediante IRT que son afectados por la vasoconstricción y vasodilatación de cada zona (Mota-Rojas et al., 2021). En el primer caso la región periocular ha sido descrita como una ventana de alta susceptibilidad, con la señalización de algunos factores negativos como velocidad del viento, radiación solar directa y humedad que limitan su validez en trabajos de IRT, esta zona se encuentra inervada por capilares de las arterias facial e infraorbitaria y contienen fibras nerviosas simpáticas del nervio facial, región susceptible a estímulos estresantes y con fibras sensibles a epinefrina y norepinefrina promoviendo vasoconstricción de capilares y disminuyendo el calor, fungiendo así como mecanismo termorregulador local, en este sentido, la relación existente entre la actividad del sistema nervioso autónomo (SNA) a los cambios de temperatura de esta región proporcionan características en esta ventana térmica (Giro et al., 2019).

Además, se han descrito las ventanas faciales como indicativas de confort térmico, en condiciones experimentales y comparativas de animales sometidos a condiciones de estrés térmico se encontró que la temperatura de la carúncula lagrimal ocular aumenta hasta 5°C en comparativa con animales del grupo control, del mismo modo se ha realizado investigación respecto al impacto del uso de sombras en búfalos de agua en donde encontraron una correlación positiva entre la temperatura de la región orbital respecto a la temperatura rectal (Athaíde et al., 2020).

Sin embargo, se han reportado correlación entre temperatura rectal y temperaturas observadas en ventana facial, en zonas como región orbital, nasal y carúncula lagrimal, encontrando una r^2 de 0.38, 0.28, 0.27 respectivamente con una especificidad de 32%, estos documentos sugieren que estas regiones no son de peso para inferir respecto al estado térmico de un animal además de mencionar diversos factores que pudieran conducir a interpretaciones inadecuadas (Lowe et al., 2020).

Con respecto a la región nasal (*Regio nasalis*) se ha observado como una ventana térmica debido a la irrigación proporcionada por la arteria maxilar y los capilares superficiales abundantes en esta zona anatómica (Strutzke et al., 2019), justificándose su uso para la determinación de estados de salud y confort térmico, vinculándose al desempeño productivo.

Desafortunadamente en esta especie no existen suficientes documentos que validen las ventanas térmicas y las zonas específicas de mayor relevancia en ellas, por ello en este documento se realizará la observación y análisis de la región auricular, periocular, carúncula lagrimal, párpado y región nasal.
Además de las regiones previamente mencionadas la región pectoral (*Regio pectoris*) se ha sugerido para evaluar rendimientos y eficiencias productivas considerándose como una ventana con un gran potencial en grandes rumiantes (Marques da Silva, 2019).

2.9. Estrés

El estrés es definido como una respuesta inconsciente a un estímulo percibido como amenaza para el bienestar que genera un conjunto de cambios y respuestas conductuales, autonómicas, metabólicas, hormonales, inmunológicas y neuroendocrinas que preparan al animal para una respuesta o acción (Cruz-Monterrosa et al., 2020; Hernández-Avalos et al., 2021; Mota-Rojas et al., 2020a; OMSA, 2021).
El sistema nervioso central percibe dicho estímulo y activa el eje hipotálamo-pituitario-suprarrenal (HPA) y el sistema nervioso simpático (SNS) junto con el aumento de las concentraciones de la hormona adrenocorticotrópica (ACTH) y cortisol (Kinlein et al., 2015). En este sentido, el estrés presente en animales impacta de forma directa en el grado de bienestar, definido como el estado físico y mental que mantiene un animal de acuerdo a las condiciones en las que este vive y muere (OMSA, 2021), así, en la producción de búfalo de agua, al igual que otras especies, se cuentan con pasos a seguir; crianza, desarrollo, engorda y finalización, figurando como etapa final la matanza del animal para la obtención de carne y proteínas de alta calidad, este paso puede realizarse tanto en la unidad de producción como en un matadero (más común) (Nielsen et al., 2020), fases en donde resulta inevitable la movilización del ganado con la observación de acciones definidas como los escenarios más estresantes en la vida productiva de un animal (Yerpes et al., 2021).
De esta manera, el tipo de manejo y las prácticas aplicadas pueden modificar aspectos fisiológicos, de comportamiento, de desempeño, bioquímicos, endócrinos, entre otros (Strappini, 2012; Bozzo et al., 2018; Cannas et al., 2018; Gonzalez-Rivas et al., 2020).
Como se ha mencionado con anterioridad el búfalo de agua es comúnmente producido en condiciones climáticas con elevada temperatura y humedad, sin embargo, cuando estas bio-condiciones se encuentran en rangos muy elevados que no permiten una termo neutralidad el semoviente induce cambios y alteraciones fisiológicos, bioquímicos y de comportamiento en donde, debido a sus características anatómicas es propenso a la

generación de estrés por calor, mismo que puede impactar en su desempeño productivo dentro de la unidad de producción y mermar las ganancias en el productor cuando no se brindan las condiciones para contrarrestarlo debido a que las condiciones de alojamiento son un indicador directo del bienestar en búfalos de agua (Bertoni et al., 2020; Lendez et al., 2021).

2.9.1. Hiperemia inducida por estrés

El búfalo de agua es un animal endotérmico, ello significa que tiene la habilidad fisiológica de controlar su temperatura corporal mediante un proceso llamado termorregulación, esto mediante la regulación de su tasa metabólica con energía producida por el metabolismo celular que puede eliminarse mediante la irradiación de calor por el animal (Casas-Alvarado et al., 2020; Mota-Rojas et al., 2021b; Napolitano et al., 2020).

En este sentido, se puede regular su temperatura mientras las condiciones externas no sean extremas y se cumplan con herramientas físicas como sombras naturales y artificiales, cuando esto no se cumple puede presentarse una deficiencia en la pérdida de calor y el búfalo entra en un estrés por calor (Bertoni et al., 2020a), aunado a ello se ha reportado que el búfalo de agua presenta un sistema termorregulador ineficiente relacionado a características anatómicas como una menor cantidad de pelo y un grosor de epidermis mayor en comparativa con el bovino convencional, además de tener un color negro con mayor melanina que absorbe el calor externo (Barboza, 2011; Bertoni et al., 2020).

Una vez que se inicia un proceso de estrés calórico se generan diversas respuestas metabólicas y fisiológicas en el búfalo de agua por vía SNA o por el eje HPA, en la primera se genera el aumento de la frecuencia cardiaca y respiratoria, al igual que la temperatura corporal, reducción del flujo sanguíneo en tracto gastrointestinal y vasodilatación a piel para una apropiada termorregulación y a extremidades ante una posible respuesta de huida y para el mantenimiento de una postura durante un proceso de movilización, además de la utilización de energía mediante reservas corporales por medio de la glucogenólisis muscular. En el mismo sentido, mediante la respuesta del eje HPA acrecentando la liberación a torrente de glucocorticoides que generan pérdida de calor por medio de vasodilatación e incrementar la degradación proteica y lipídica aumentando la disminución de peso (González-Lozano et al., 2020; Gonzalez-Rivas et al., 2020; Pérez-Linares et al., 2013; Law et al., 2007).

Debido a estas características pueden generarse pérdidas biológicas y económicas asociadas a una productividad deteriorada, menores tasas de crecimiento, mayor susceptibilidad a enfermedades debido a la afectación de la expresión génica de citoquinas y receptores (Abdelnour et al., 2019; Lendez et al., 2021), además se ha reportado que la hiperemia por estrés impacta también en parámetros reproductivos, disminuyendo porcentajes de fertilidad en la relación de búfalas gestantes (Di Palo et al., 2007) y afecta la espermatogénesis y la maduración espermática, además de generar anomalías en la morfología en estas células cuando el estrés por calor es crónico (Gonçalves et al., 2021). Observando estas marcadas repercusiones se han generado diversas estrategias para proporcionar a los semovientes espacios que les permitan la expresión de conductas para disipar el calor y evitar estrés, tal es el caso del uso de charcas en unidades productivas además del conocimiento termorregulatorio del búfalo de agua (Napolitano et al., 2020).

3. JUSTIFICACIÓN

Durante la producción, el búfalo de agua atraviesa diversas etapas como lo son el destete y la engorda previa a la finalización, en cada de uno de ellos existe la posibilidad de realizar procesos de movilización dentro y fuera de la unidad de producción, aplicando metodologías, logística y herramientas que propicien estresores acumulativos, propiciando factores adversos que afectan el nivel de bienestar de los búfalos.

En México no se cuenta con legislación vigente y aplicable en la movilización del búfalo de agua, lo cual propicia la aplicación de malas prácticas durante su traslado (Rodríguez-González et al., 2022). Con lo anterior, la valoración del estrés durante el transporte es necesaria para analizar los puntos críticos de control y observar los efectos de estos factores estresantes sobre parámetros fisiológicos, entre los cuales, la temperatura ha sido analizada en procesos fisiopatológicos como inflamación, cicatrización, procesos mórbidos, quirúrgicos y prácticas habituales como el transporte.

Con lo anterior queda claro que, el transporte puede generar lesiones y cambios en parámetros fisiológicos en los búfalos de agua mermando su nivel BA su desempeño productivo, afectando con ello las características fisicoquímicas de la carne. En tal situación, el uso de IRT resulta una herramienta atractiva debido a que detecta variaciones en la temperatura superficial de diversas ventanas térmica y sus respectivos cambios en la microcirculación dérmica además de ser una herramienta no invasiva, justificando su uso innovador en el búfalo de agua transportado en el trópico húmedo mexicano.

Sumado a ello, se ha reportado en otras especies domésticas los efectos adversos del transporte y malas prácticas sobre la temperatura corporal y características fisicoquímicas en carne, sin embargo; estas últimas características no se encuentran descritas en esta especie criada en México, lo anterior, sumado a una nula descripción e inclusión del búfalo de agua en la normativa mexicana ha propiciado una comercialización con bastas áreas de oportunidad, además del crecimiento y divulgación de mitos alrededor de la carne de búfalo, o su venta sin regulación específica y comercialización como carne de res, por ello surge la necesidad de caracterizar y comparar las propiedades fisicoquímicas de la carne de búfalo de agua y el bovino del género *Bos*, además de realizar un análisis hedónico para medir el grado de aceptación cárnico de las especies previamente mencionadas.

4. PREGUNTAS DE INVESTIGACIÓN

1. ¿Cuál es el efecto de la movilización del búfalo de agua desde el arreo en potrero, corral pre-embarque, embarque y la duración del transporte corto en la respuesta térmica de diferentes regiones corporales?

2. ¿Cuál es el efecto de la movilización del búfalo de agua desde el arreo en potrero, corral pre-embarque, embarque y la duración del transporte largo en la respuesta térmica de diferentes regiones corporales?

3. ¿Cuáles serán las modificaciones en las propiedades fisicoquímicas de tres diferentes cortes cárnicos de búfalo de agua y bovino (cruda y cocida) criado en el trópico húmedo mexicano?

4. ¿Cuál será el nivel de aceptación de la carne de búfalo de agua y bovino criado en el trópico húmedo mexicano mediante un análisis hedónico?

5. HIPÓTESIS

Las mayores alteraciones en la microcirculación dérmica probablemente se presenten en búfalos transportados en periodos cortos, respecto a búfalos transportados por periodos largos. En otras especies domésticas, se ha reportado que el transporte corto es más estresante y no permite a los animales habituarse al ruido, vibraciones y corrientes de aire entre otros factores adversos inherentes al transporte. Por otra parte, las ventanas térmicas periféricas (dorsal, lumbar, nasal y apendicular) probablemente presenten más desajustes en los cambios microcirculatorios que las ventanas térmicas centrales, ya que estas últimas posiblemente tengan mayor estabilidad térmica. Respecto a las características fisicoquímicas, los valores más bajos de pH, CRA y textura se presentarán en las muestras de bovino convencional y los valores más altos de color y Aw se presentarán en las muestras de búfalo de agua.

6. OBJETIVO GENERAL

Evaluar el efecto del arreo, embarque y duración del traslado de búfalos de agua en la respuesta de la microcirculación dérmica en diferentes ventanas térmicas, así como, estimar las modificaciones fisicoquímicas de tres diferentes cortes cárnicos en comparación con el bovino criado en trópico húmedo mexicano.

7. OBJETIVOS ESPECÍFICOS

1. Evaluar el efecto de la movilización del búfalo de agua desde el arreo en potrero, manejo, embarque y transporte con una duración corta, en la respuesta térmica evaluada con infrarrojos en diferentes regiones corporales.

2. Evaluar el efecto de la movilización del búfalo de agua desde el arreo en potrero, manejo, embarque y transporte con una duración larga, en la respuesta térmica evaluada con infrarrojos en diferentes regiones corporales.

3. Caracterizar y comparar las propiedades fisicoquímicas de tres diferentes cortes cárnicos de búfalo de agua y carne de bovino (cruda y cocida) criado en el trópico húmedo mexicano.

4. Evaluar el nivel de aceptación de la carne de búfalo de agua y bovino criado en el trópico húmedo mexicano.

8. MATERIALES Y MÉTODOS

8.1. Tipo de estudio

Este fue un estudio experimental comparativo-prospectivo. Respecto a las tomas térmicas y el análisis fisicoquímico de la carne fueron realizadas por una sola evaluadora entrenada en un ensayo no ciego. Para el análisis hedónico se realizó una prueba de preferencia cuantitativa con paneles de consumidores no entrenados.

8.2. Localización y aprobación ética

El presente estudio se dividió en fase ante mortem (transporte corto y transporte corto vs largo) y post mortem, en el primer caso se realizó en las instalaciones de la empresa ganadera "Búfalo Ranch", para la fase post mortem, los análisis tanto fisicoquímicos como hedónico se realizaron en la Universidad Autónoma Metropolitana, Unidad Lerma, para mayor referencia se especifica lo siguiente:

Localización para el Transporte corto (Fase ante mortem 1)

El estudio se realizó en el estado de Veracruz al sur- sureste de México, de junio de 2021 a agosto de 2022. La unidad de producción se encuentra en una zona de clima tropical húmedo, con temperatura media de 31 ± 2°C, humedad relativa de 86 %, una elevación de 20 m.s.n.m. y una precipitación anual de 1500-2000 mm (INEGI, 2021).

Localización para el Transporte corto vs largo (Fase ante mortem 2)

El viaje corto (SJ) de los búfalos de agua se realizó dentro del sur-sureste de México (Estado de Veracruz de Ignacio de la Llave) desde junio de 2021 hasta agosto de 2022, la unidad de producción en donde los animales fueron embarcados para ambos grupos tuvo una temperatura promedio de 31 ± 2°C. , humedad relativa del 86%, elevación de 20 m.s.n.m. y precipitación anual de 1500-2000 mm, catalogándose dicha zona como de clima tropical húmedo, en el caso de viajes largos, los búfalos fueron monitorizados en la fase de post- transporte en la unidad de producción de destino, misma que tuvo una temperatura promedio de 13.18 ± 1.42°C, humedad relativa de 63%, una altitud de 2807 m.s.n.m. y precipitación anual de 1679 mm, catalogando dicha zona de clima cálido y templado (INEGI, 2021).

Localización para el análisis hedónico y fisicoquímico de la carne (Fase post mortem)

Los análisis fisicoquímicos y hedónicos se realizaron en las instalaciones de la Universidad Autónoma Metropolitana Unidad Lerma, localizada en Avenida de las Garzas 10, 52005 Lerma de Villada, Estado de México, México.

Aprobación ética

Este estudio se derivó de la aceptación del protocolo experimental de la Comisión Científica de la Maestría en Ciencias "Maestría en Ciencias Agropecuarias" mediante el oficio CAMCA.11.21 de la Universidad Autónoma Metropolitana, Ciudad de México, México.

Además, los animales utilizados en este estudio fueron manejados sin el uso de herramientas físicas que puedan causar lesiones y estrés, de acuerdo con los lineamientos de la Norma Oficial Mexicana NOM-051-ZOO-1995 que establece especificaciones técnicas para el manejo humanitario en la movilización de animales publicado por el Departamento de Agricultura, Desarrollo Rural, Pesca y Alimentación. Por último, se resalta la importancia de que para este estudio los animales no fueron tocados por motivo de monitorización de cambios térmicos, ya que la IRT es una técnica no invasiva.

8.3. Sujetos de estudio y distribución de grupos

Transporte corto

Para este estudio se seleccionaron 624 búfalos Buffalypso machos destinados a engorde. El peso medio de los animales fue de ~220-240 kg. Fueron transportados en 12 viajes cortos que recorrieron 110 km con una duración media de 2 horas ± 20 minutos y una velocidad media de 55 km/h.

Los búfalos se dividieron para los 12 viajes de la siguiente manera: 53, 51, 49, 56, 53, 50, 53, 52, 54, 51, 52 y 50. Antes de cada viaje, se los alojó en el mismo potrero y se arrearon suavemente (sin uso de utensilios físicos ni gritos por parte de los manipuladores).

Transporte corto vs largo

Para este estudio, las movilizaciones se dividieron en dos grupos según el tiempo de transporte, quedando de la siguiente forma: Viajes cortos (VC) con una duración media de 50,33± 5,48 minutos y Viajes largos (VL) con una duración media de 13:31 horas ± 47.32 minutos, seleccionándose 783 (VC) y 733 (VL) búfalos Buffalypso machos destinados a engorde, para ambos casos el peso promedio de los animales fue de 245±19.36 kg. Dentro del VC se realizaron 15 viajes en los que, de acuerdo con la capacidad del primer piso del camión se distribuyeron de la siguiente manera: 41, 56, 46, 50, 48, 51, 57, 58, 53, 49, 52, 54, 56, 40 y 47. Para el VL se monitorizaron en 14 viajes cuya distribución animal fue 53, 52, 55, 53, 54, 53, 51, 52, 53, 52, 53, 51, 51 y 50.

Para el VC la distancia recorrida fue de 27.5 km, lo anterior en promedio de duración= 50.33 min ± 5.48 min a una velocidad media de 55 km/h, esta velocidad fue igual a la del VL, sin embargo, la distancia recorrida fue de 732 km, lo cual afectó el tiempo de cada viaje, resultando en una duración promedio= 13:31 horas ± 47.32 min. Para todos los grupos los búfalos de agua fueron arreados suavemente desde el potrero sin el uso de herramientas físicas que pudieran causar lesiones y dolor en los animales o gritos por parte de los manejadores.

Selección y procesamiento de cortes cárnicos

Los cortes cárnicos seleccionados fueron Rib eye (*Pectoralis profundus*), New York (*Longissimus dorsi*) y Pulpa de pierna (*Biceps femoris*) (Temizkan et al., 2019) obtenidos de 8 bovinos del género *Bos* y 8 búfalos de agua procedentes de unidades de producción ubicadas en el sureste de México, zona de la República Mexicana caracterizada por un clima húmedo subtropical o Cfa de acuerdo con la clasificación Köppen-Geiger.

8.4. Alojamiento previo a la carga y tipo de vehículo

Características del vehículo utilizado

Respecto a las características del vehículo utilizado para transportar a los búfalos de agua este presenta unas dimensiones de 15,24 m largo × 2,59 m ancho × 4,6 m alto, el camión contó con dos pisos y puertas laterales corredizas y una puerta principal en la parte trasera con sistema de guillotina, las paredes eran de acero galvanizado y aluminio, con aberturas para ventilación, además presentó un piso antideslizante, un techo de plástico reforzado y fibra de vidrio (Figura 3).

Figura 3. Vehículo utilizado para movilizar a los búfalos de agua.

Para el transporte corto. La topografía de las vías se clasificó en no pavimentadas y pavimentadas. Los primeros tenían especificaciones de vías clase E, según la clasificación de la Secretaría de Gobernación de México (31), con una pendiente máxima del 13%.

La velocidad máxima media fue de 25 km/h. Se tomaron caminos de tierra para salir de la unidad de producción (10 km) y entrar al sitio de recepción (12,5 km). La longitud total del viaje en los caminos no pavimentados fue de 22,5 km con una sobreelevación máxima del 10%, curvas verticales de 4 m/% y un ancho de camino de 6 m. Los otros 87,5 km se recorrieron sobre una vía asfaltada con una pendiente transversal máxima del 7%, una velocidad máxima de 55 km/h, curvas verticales de 5 m/% y un ancho de vía de 8 m.

En el transporte corto vs largo, la topografía de las vías recorridas tanto para VC como VL fue clasificada como no pavimentada o pavimentada, lo anterior debido a las especificaciones descritas por de la Secretaría de Gobernación de México (SEGOB 2004), para las vías no pavimentadas la clasificación de carreteras es E y presentó una pendiente máxima del 13%, durante esta vía se alcanzó una velocidad máxima de 25 km/h, recorriendo 10 km de caminos de terracería tanto para el VC y VL a la salida de la unidad de producción de origen y 6.3 km para el VC y 13.2 km para el VL a la entrada de la unidad de producción de recepción.

Por otra parte, para la vía pavimentada se recorrió una distancia de 11.2 km en el SJ y 718.8 km para el LJ, este tipo de vía presenta una pendiente transversal máxima del 7% y debido a las características de esta vía se alcanzó una velocidad máxima de 55 km/h, en ambos casos (SJ y LJ) se observarán curvas verticales de 5 m/%, y un ancho de vía de 8 m.

8.5. Materiales e insumos físicos

8.5.1. Material Físico

- o Palillo de madera redondo doble punta
- o Plato de papel de 9 pulgadas

8.5.2. Material de laboratorio

- o Guantes de látex estériles para exploración (Ambiderm ®, México)
- o Cubrebocas quirúrgico de tres pliegos con filtro antibacterial (Ambiderm ®, México)
- o Termómetro metaltex modelo termocarne con estructura de acero inoxidable

8.5.3. Equipo de laboratorio

- o Cámara termográfica (FLIR Thermal TM E60, FLIR Systems ®, USA)
- o Cámara fotográfica Canon EOS Rebel Digital Slr Con Ef-s 18-55m
- o Medidor de actividad de agua modelo HBD5-MS2100Wa
- o Potenciómetro de penetración portátil HANNA (Model-HI99163, USA)
- o Colorímetro portátil Hunter Lab (CR-410, Konica-Minolta, Inc. Japan)
- o Equipo de medición de textura TAX.T2 (Texture Technologies, Nueva York) acoplado al software TexturePro CT V1.9 Build 35®
- o Agitador magnético de laboratorio SH-3 de placa calefactora

8.5.4. Formato de registro

- o Formato de monitorización para la IRT
- o Formato de identificación de características fisicoquímicas
- o Formato de análisis hedónico.

8.5.5. Material químico

o NaCl al 0.6 M

8.6. Diseño experimental

8.6.1. Fase ante mortem; monitorización térmica

Los protocolos experimentales se llevaron a cabo con apego estricto a la Norma Oficial Mexicana NOM-051-ZOO-1995 que establece especificaciones técnicas para el manejo humanitario en la movilización de animales publicado por el Departamento de Agricultura, Desarrollo Rural, Pesca y Alimentación.

Los animales fueron monitorizados de una misma unidad de producción y fueron divididos de manera aleatoria de acuerdo con peso vivo previo al transporte. Cada grupo atravesó las mismas fases de monitorización como se especifica en la figura 4.

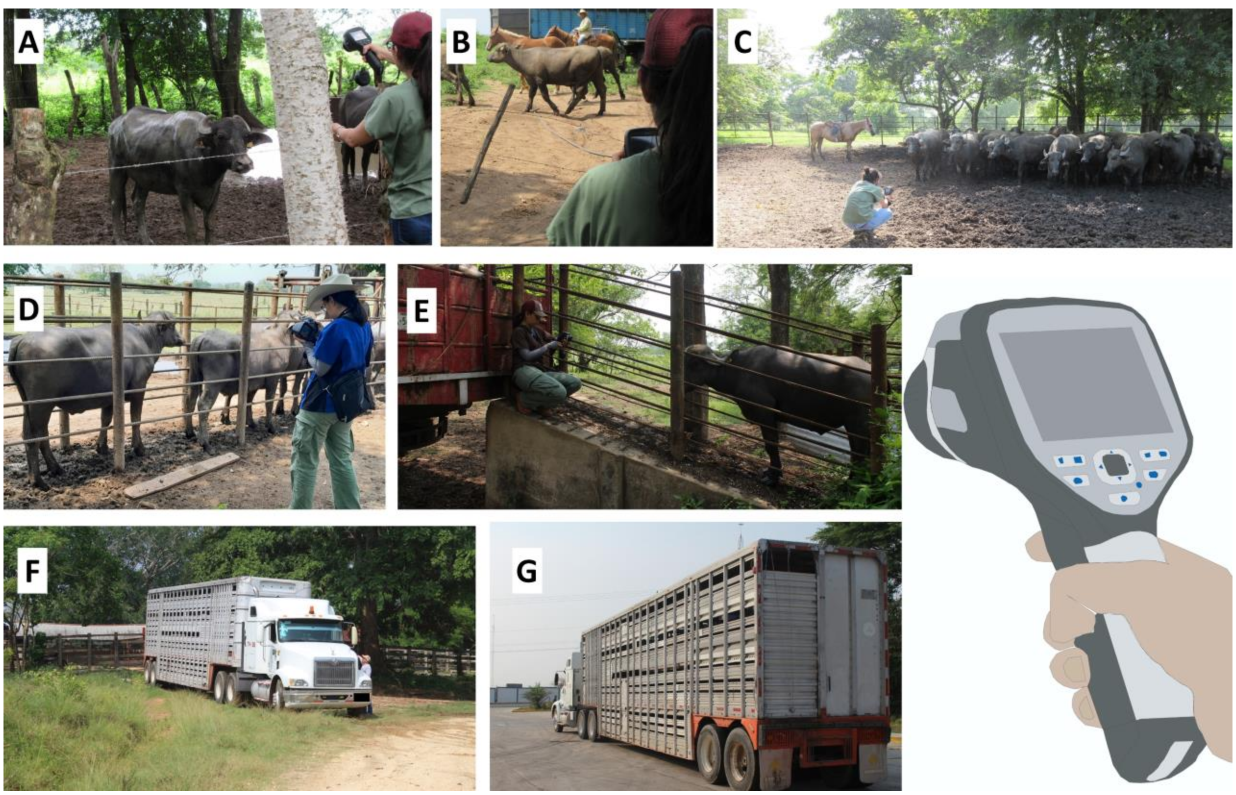

Figura 4. Áreas donde se llevaron a cabo las fases de evaluación. A. El potrero de la unidad de producción; monitorización basal (F1). B. Después del arreo por vaqueros a caballo (F2). C. Después de pasar la noche en el corral de espera, antes de entrar a la manga de manejo (F3). D. En manga de manejo (F4). E. Acceso al vehículo por rampa de carga (F5). F y G. El vehículo antes del transporte (F6) y al llegar al lugar de recepción (F7).

Las características de alojamiento y vehículo fueron las mismas para la monitorización de transporte corto y el corto vs largo (Tabla 2).

Tabla 2. Características de alojamiento para cada fase ante mortem.

Fase	Descripción	Características
F1	Potrero	Esto considerando el monitorización basal, los animales se alojaron en potreros cuyo sistema de alimentación se basa en pastos y aportan 50g de suplementos minerales por animal, en cada potrero se registró la presencia de pastos nativos (*Paspalum fasciculatum*) (materia seca = 14.7%, 56.7% fibra detergente neutra, 38,8% fibra detergente ácida y 6,2% proteína cruda (Rodríguez; & Romero, 2016).
F2	Arreo con vaqueros a caballo	Para esta fase se seleccionan animales con el peso deseado para ser movilizados, los manejadores escuchan a los búfalos con ayuda de caballos y sin el uso de herramientas físicas que podrían causar lesiones. Para todos los viajes, esta fase tuvo una duración promedio de 25-35 minutos.
F3	Sistema de corrales	Una vez que los animales ingresaron al corral permanecieron durante 12 horas consecutivas (incluyendo la noche previa al pesaje, manejo y traslado de estos). El corral tenía una superficie de 630 m2 y piso de tierra, este sistema se encontraba delimitado por estructuras metálicas con 1,6 m de altura y dos puertas, de las cuales una conectaba al conducto de manipulación (F4), no se les brindó alimentos sólidos durante las últimas 8h que permanecieron en el corral y tuvieron acceso ad libitum al agua.
F4	Manga de manejo	Las dimensiones del conducto de manipulación consistían en 1.6 m 1 m ancho × 1.6 m alto × 3.5 m largo para un pasillo, mismo que, estaba conectado a una báscula de peso individual y consecutivamente a una rampa de carga, debido al pasaje individual de los búfalos esta fase tuvo una duración promedio de 50 min.
F5	Embarque	Los búfalos de cada grupo fueron monitorizados por 30-50 min durante su paso sobre una rampa de carga con ranuras antideslizantes con una pendiente de 20°.
F6	Pre transporte	Los búfalos de agua fueron monitorizados durante el tiempo que pasaron en el vehículo antes del transporte, esta fase se demostró desde que todos los animales habían sido cargados en el vehículo.
F7	Post transporte	La monitorización térmica se realizó cuando los animales aún se encontraban dentro del vehículo a la sombra, posteriormente llegada al lugar de recepción.

8.6.2. Fase post mortem; análisis cárnico

Todos los cortes cárnicos fueron adquiridos al alto vacío bajo una temperatura de congelación de -18 °C. De manera general, se utilizaron 3 cortes diferentes (ncorte=3) de una muestra poblacional de 8 bovinos del género *Bos* (nbovino=8) y 8 búfalos de agua (nbúfalo=8) por tanto, se obtuvieron un total de 48 muestras (Figura 5) de las cuales se analizaron las propiedades fisicoquímicas de pH, color, Aw, CRA y textura por triplicado en crudo y en cocido, para ello y previo a su análisis en crudo, fueron descongeladas 24 h previas a su uso bajo una refrigeración con temperatura constante de 4°C.

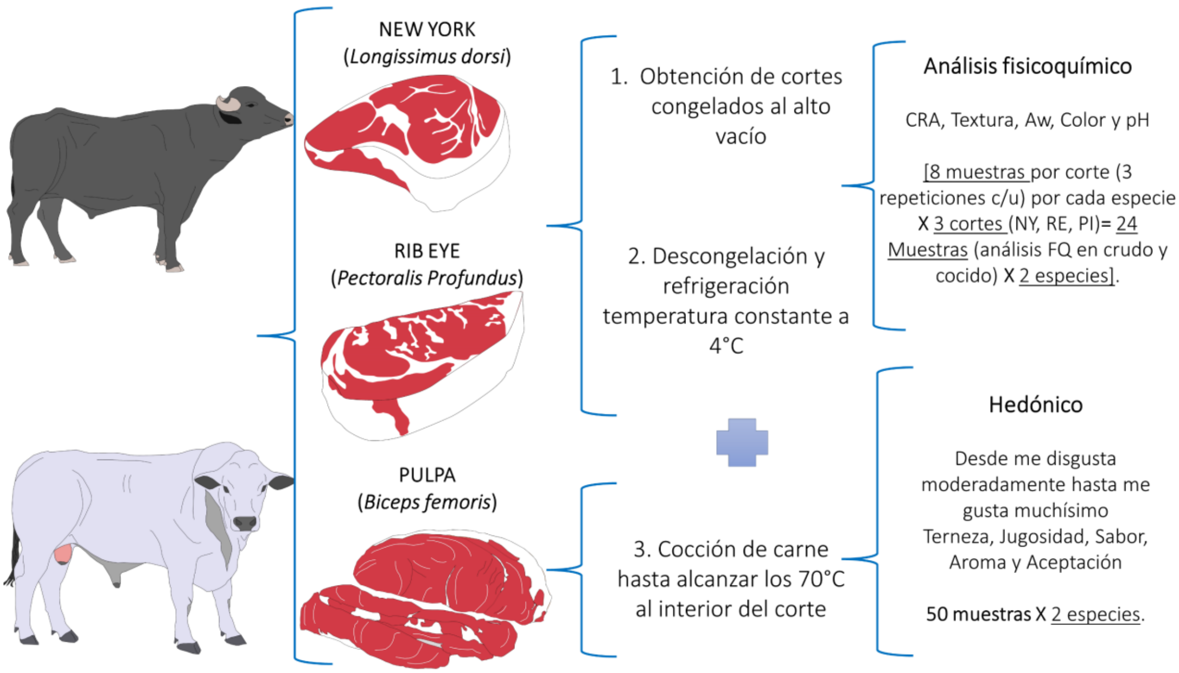

Figura 5. Selección y muestreo de diferentes tipos de cortes para la evaluación de las propiedades fisicoquímicas de carne de búfalo de agua y bovino.

8.7. Descripción de las variables de estudio para la fase ante mortem; Monitorización con IRT

Se utilizó una cámara infrarroja FLIR® Thermal TM E60 (FLIR Systems, EE. UU.) (Figura 6) con una resolución IR de 320×240 píxeles, sensibilidad térmica <0,045°C, precisión ± 2°C o 2% y emisividad de 0.95, se almacenaron todas las imágenes digitales y radiométricas, en formato JPG para su posterior análisis mediante el software FLIR (Tools Systems, EE. UU.) para obtener las lecturas de temperatura máxima, mínima y promedio, las cuales luego se ingresaron a una base de datos.

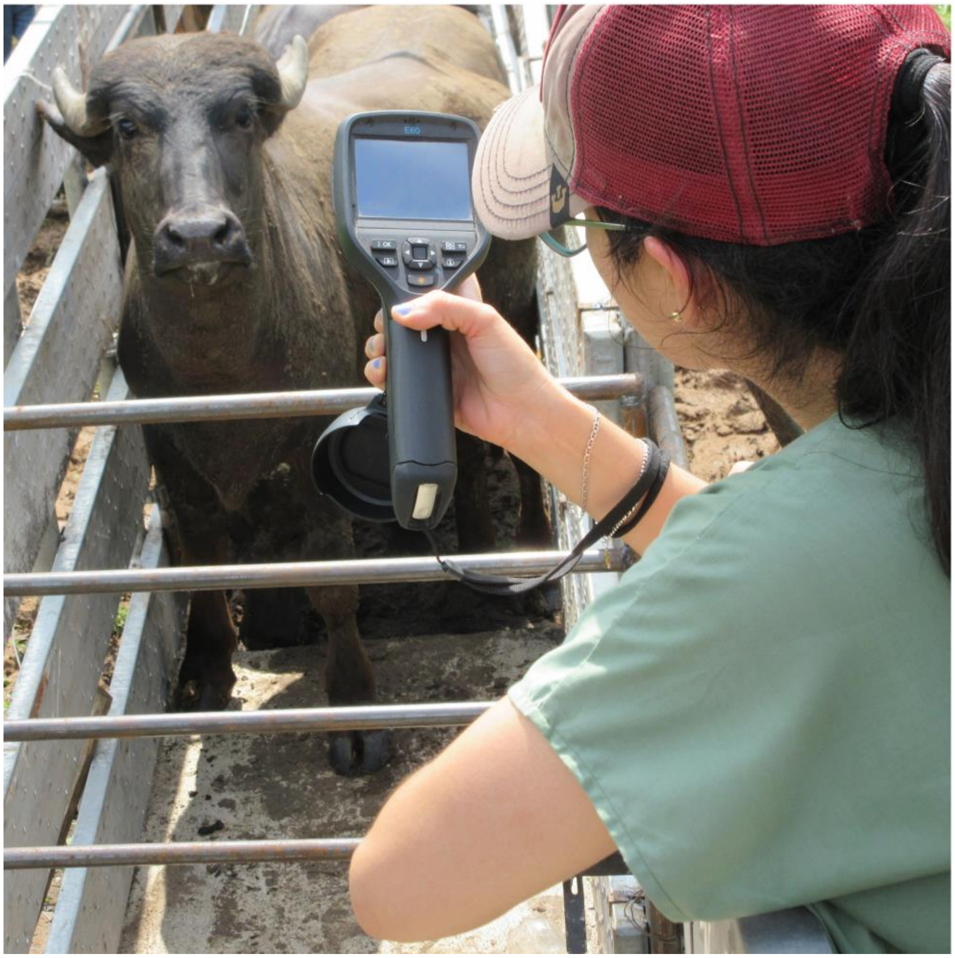

Figura 6. Cámara infrarroja FLIR® Thermal TM E60 (FLIR Systems, EE. UU.) utilizada durante las movilizaciones de búfalos de agua.

Todas las imágenes radiométricas fueron tomadas a una distancia de 1-1,5 m de los búfalos enfocándose en las regiones faciales (*Regiones faciei*) que incluían la región orbitaria (*Regio orbitalis*) con la fijación de puntos en las ventanas térmicas carúncula lagrimal (1), área periocular (2) y párpado inferior (*Regio palpebralis inferior*) (3), región nasal (*Regio nasalis*) con especial atención a las fosas nasales (4), región del cráneo (Regiones cranii) con la fijación de puntos en la región auricular (*Regio auricularis*) con especial atención al canal auditivo (5) y región frontal-parietal (*Regio frontalis-parietalis*) (6) (Figura 7).

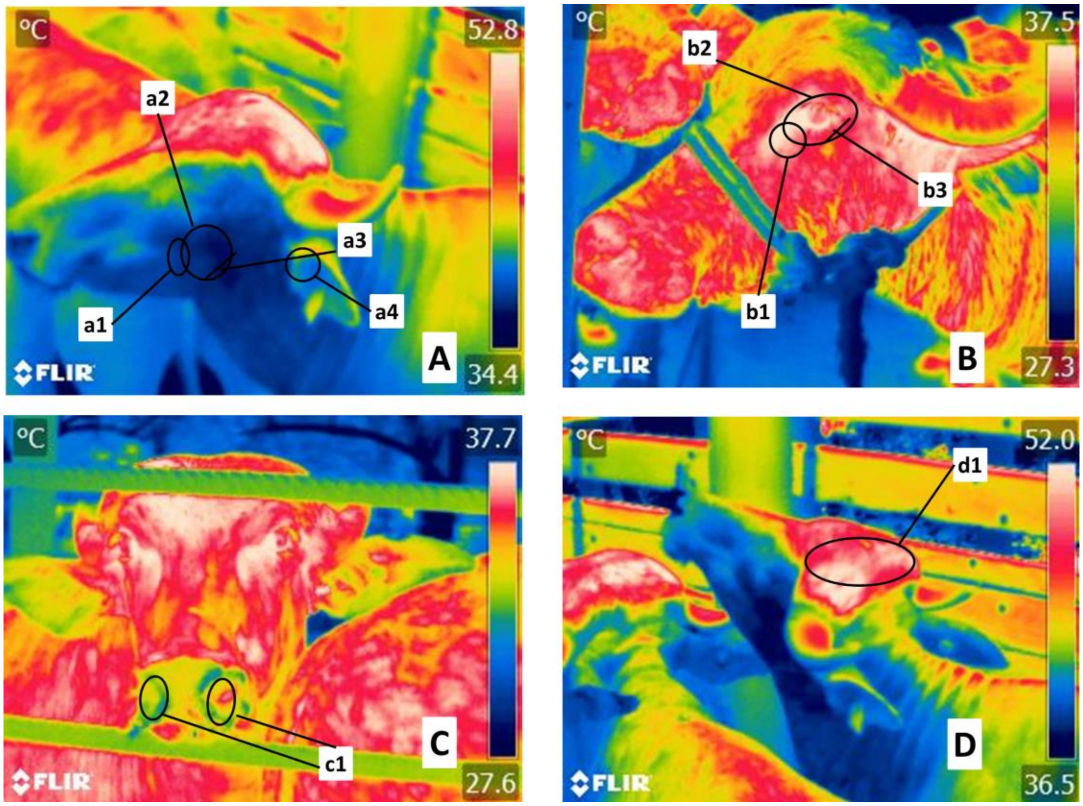

Figura 7. Delimitación de ventanas térmicas en las regiones de cabeza (*Regiones capitis*). A y B. Ventanas térmicas oculares en la región orbital (*Regio orbitalis*) y la región auricular (*Regio auricularis*). Los registros en esta zona se centraron en la carúncula lagrimal, delimitada como círculo a1 y b1, ventana térmica de la carúncula lagrimal consideraron la comisura medial de los párpados hasta medio centímetro hacia el cráneo. Esta área refleja la radiación del flujo sanguíneo de la arteria infraorbitaria inervada por las ramas simpáticas del nervio facial. La ventana térmica periocular, marcada por los círculos a2 y b2, se extiende por la periferia de los párpados superior e inferior, irrigada por las arterias supraorbitaria e infraorbitaria. La ventana térmica del párpado inferior, definida por una línea de aproximadamente 3 cm de largo (a3 y b3), se utilizó para evaluar la radiación de la mucosa conjuntival y fue irrigada por la arteria infraorbitaria. La ventana térmica del conducto auditivo enmarcada por el círculo a4 se utilizó para evaluar la cresta del antihélix y el pedúnculo lateral de la hélice, irrigados por la media y las arterias auriculares lateral y central. C y D. Ventana térmica de las fosas nasales: óvalos c1 y c1 se utilizó para evaluar la radiación de la arteria maxilar. Ventana térmica de la región frontal-parietal (*Regio frontalis-parietalis*). Delineado por un óvalo (d1) que abarca los límites de la región fronto-parietal (*Regio frontalis*), la región cornual (*Regio cornualis*) y la región parietal (*Regio parietalis*), zona irrigada por las arterias cornuales y supraorbitarias.

Respecto a las ventanas térmicas de la región del tronco (*Truncus regionis*) fueron delimitadas las regiones torácica (7) y abdominal (8), y las regiones del miembro pélvico (*Regiones membri pelvini*) (9) con dos regiones anatómicas indicadas la región femoral (*Región femoral*) y la región del tarso (*Regio tarsi*) (Figura 8).

Así mismo, en la figura 8 se señalan las últimas 2 ventanas térmicas consideradas en este estudio, estas se enfocaron en regiones de la columna vertebral (*Columna vertebralis*) con la región vertebral torácica (*Regio vertebralis thoracis*) (10) y la región lumbar (*Regio lumbar*) (11).

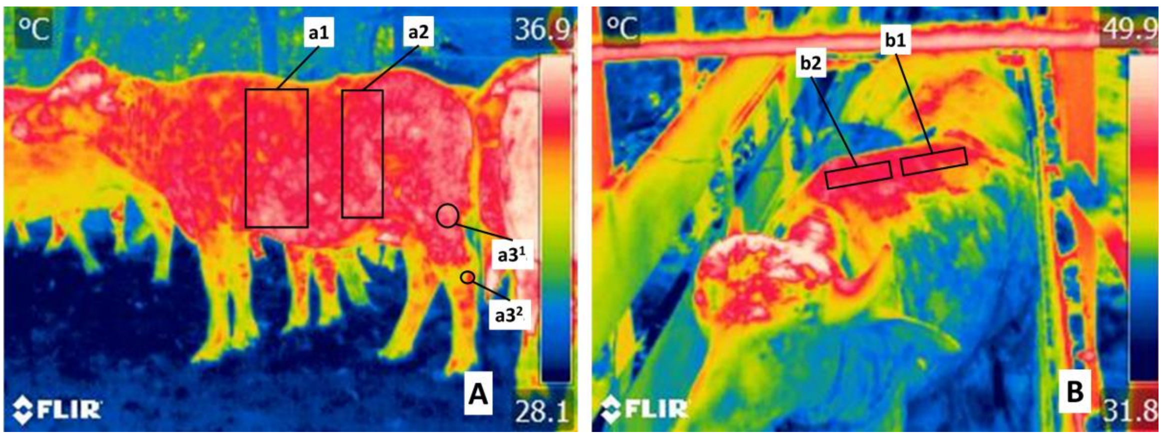

Figura 8. Delimitación de las ventanas térmicas del en la región del corporales (*Truncus regionis*). **A**. La ventana térmica de la región torácica en la región costal (*Regio costalis*) se muestra mediante un rectángulo (a1) trazado desde el arco costal de 1° a 12°. La región abdominal craneal (*Regio abdominis cranialis*) delimitada por un rectángulo (a2), incluye los músculos oblicuo abdominal y recto abdominal irrigados por la arteria epigástrica superficial (*A. epigastrica cranialis superficiales*), y también la ventana térmica de las regiones de la pelvis. Las extremidades (*Regiones membri pelvini*) estaban marcadas por dos círculos en la *Regio femoris* (a3^1) y en la *Regio tarsi* (a3^2). Incluye la región femoral, el músculo femoral y la región del tarso, proyección lateral de la región del tendón calcáneo común (*Regio tendinis calcanei communis*), regiones irrigadas por la arteria femoral y ramas de la arteria safena. **B.** Delimitación de las regiones de las ventanas térmicas de la columna vertebral (*Columna vertebralis*). La ventana térmica de la región vertebral torácica (*Regio vertebralis thoracis*) está marcada con un rectángulo. Incluye las proyecciones izquierda (a1) y derecha (b1) de las vértebras torácicas y el ancho de las apófisis transversas de las vértebras torácicas 1ª-12ª. Esta zona está irrigada por la rama espinal (*Ramus spinalis*) procedente de las ramas dorsales de la arteria aorta abdominal, en la región vertebral torácica y por la

ventana térmica de la región vertebral lumbar (*Regio vertebralis lumbalis*) (b2) proyecciones de las vértebras lumbares por encima de la apófisis transversa de las vértebras lumbares 1ª –7ª. Esto permite evaluar la radiación de la rama espinal (*Ramus spinalis*) de las ramas dorsales de la arteria aorta abdominal en la región vertebral lumbar.

Todas las imágenes digitales y radiométricas se almacenaron en formato JPG para su posterior análisis mediante el software FLIR (Tools Systems, USA®) para obtener las lecturas de temperatura máxima, mínima y promedio, las cuales luego se ingresaron a una base de datos.

8.7.1. Transporte corto

De acuerdo con las 7 fases experimentales, las lecturas del IRT se tomaron de la siguiente manera: F1: antes de que los búfalos fueran arreados por vaqueros a caballo. Los registros se realizaron mientras permanecían en reposo durante una hora a la sombra, entre las 17:00 y las 18:00 h; F2: dentro del corral de espera el día anterior al transporte; F3: a las 08:00 h, previo a guiar a los animales hacia la rampa de manipulación para ser pesados y cargados; F4: mientras los búfalos se encontraban en la rampa de manipulación esperando ser pesados; F5: durante la carga mientras los animales subían la rampa y entraban al camión; F6: durante 40 min después de cargar todos los búfalos en el camión pero con el motor apagado; y F7: durante 40 minutos inmediatamente después de concluir el VC, pero con el motor del camión aún encendido.

En este mismo grupo se realizó la monitorización de la temperatura ambiente (TA) y la humedad relativa (HR) en diferentes momentos, de acuerdo con la fase experimental. F1: La TA y la HR oscilaron entre 23-25°C y 81-90%, respectivamente (17:00-18:00 h). F2: Los valores de TA y HR estuvieron entre 21-25°C y 81-88%, respectivamente (18:00-18:30 h). F3: La TA y la HR fluctuaron alrededor de 20-23°C y 81-86%, respectivamente (08:00 h). F4: TA y HR registraron valores de 22-25°C y 81-88%, respectivamente (09:00 h). P5: los valores fueron 22-27°C y 81-88% a las 10:00 h. F6 y F7: los datos de TA y HR oscilaron entre 22-28°C y 81-89% a las 11:00 h y 23-30°C y 83-94% a las 14:00-15:00 h.

8.7.2. Transporte corto vs largo

Respecto al transporte corto vs largo los valores de TA y HR se observan en la tabla 3 así como, las especificaciones de la toma de capturas térmicas de acuerdo con las siete fases experimentales.

Tabla 3. Horarios, descripción y características ambientales (TA y HR) de las fases de monitorización de la etapa de transporte corto vs largo.

Fases	Descripción del momento de capturas térmicas	Horarios	Temperatura ambiente (TA) y Humedad relativa (HR)
F1	Previo arreo por los vaqueros, mientras los búfalos de agua descansaban durante una hora bajo la sombra.	Entre 17:00 y 18:00	TA- 23 to 25°C HR- 81-90%
F2	Dentro del corral el día previo al transporte.	Entre 18:00 y 18:30	TA- 21-25°C HR- 81-88%
F3	Antes de guiar a los animales hacia la rampa de manipulación para ser pesados y cargados.	08:00	TA- 20-23°C HR- 81-86%
F4	Mientras los búfalos estaban en la manga de manejo esperando ser pesados	09:00	TA- 22-25°C HR- 81-88%
F5	Durante la carga, mientras los animales subían por la rampa y entraban al camión.	10:00	TA- 22-27°C HR- 81-88%
F6	Después de que todos los búfalos fueron cargados en el camión, con el motor apagado.	11:00	TA- 22-28°C HR- 81-89%
F7	Inmediatamente después de concluir el corto viaje, pero con el motor del camión aún encendido	SJ- 11:50± 5.48 minutos LJ- 00:31± 47.32 minutos	TA- 23-30°C HR- 83-94% TA-10.5-15.7°C HR- 84-46%

8.8. Descripción de las variables de estudio para la fase post mortem; Análisis fisicoquímico de los cortes cárnicos de búfalo de agua y cortes cárnicos de bovino convencional.

Se realizó el análisis fisicoquímico en crudo de las 48 muestras por triplicado para cada característica, respecto al análisis de las muestras en cocido se aplicó el protocolo de cocción en donde se calentó cada corte en un baño de agua a 58-59°C durante 1.5 a 1.7 h hasta que esta alcanzara una temperatura interna de 70°C (Figura 9), lo anterior se observó con ayuda de un termómetro metaltex modelo termocarne con estructura de acero inoxidable, altura de 12 cm y lente de vidrio de laboratorio durable. Una vez alcanzada esta temperatura cada corte fue colocado en un recipiente debidamente identificado para analizar las mismas características fisicoquímicas observadas en los cortes en crudo, mismas que se describen a continuación.

Figura 9. Toma de valores fisicoquímicos; A. pH en cortes crudos; B. Color (L*, a* y b*); C. Medición de temperatura 70°C al interior del corte previo a realizar medidas a cortes cocidos.

8.8.1. CRA

Respecto a la CRA se aplicó el método reportado por Pérez y Ponce (2013), el cual consiste en la determinación del volumen de una solución de NaCl al 0.6 M retenido por una muestra homogenizada de carne.

8.8.2. Aw

La obtención de los datos para la Aw se utilizó un equipo de medición portátil de actividad de agua para alimentos Modelo HBD5-MS2100Wa del fabricante Graigar, de acuerdo con la técnica descrita por Sánchez-Hernández et al. (2017) (Figura 10).

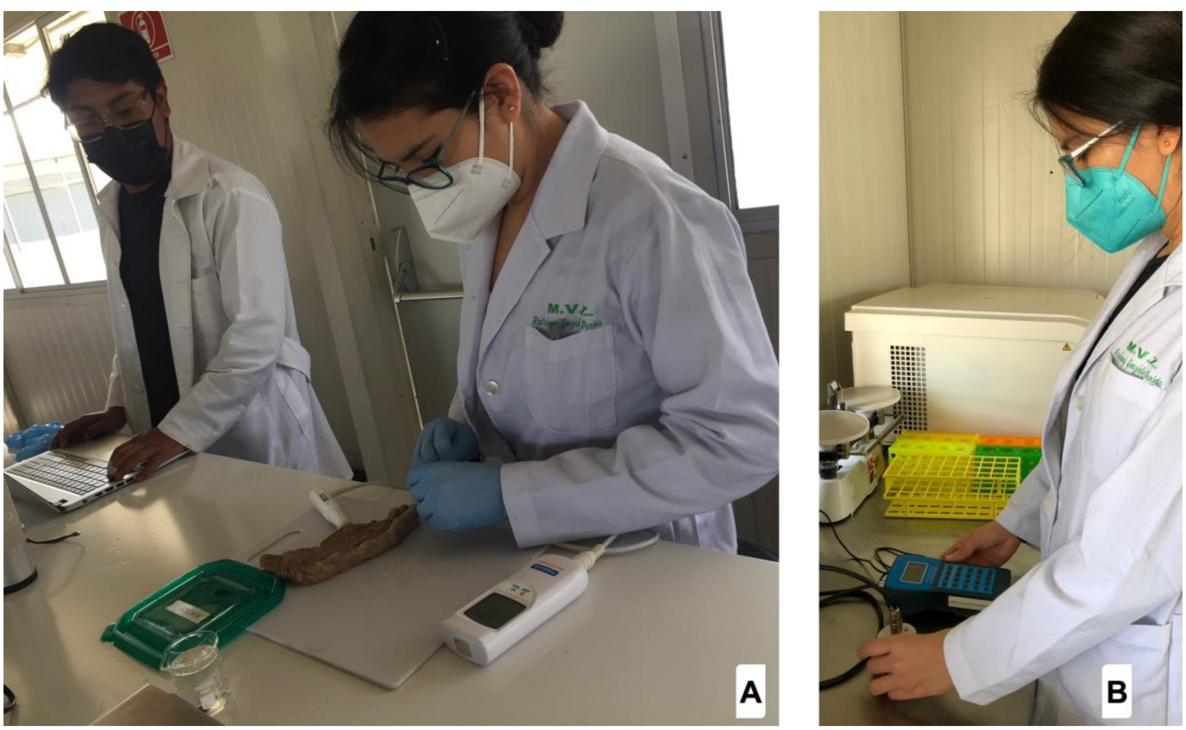

Figura 10. Toma de valores de pH (A) a cortes cocidos y medición de Aw a cortes cocidos.

8.8.3. pH

Para la evaluación de pH fue utilizado un potenciómetro de penetración portátil HANNA (Model-HI99163, Estados Unidos), mismo que fue lavado con agua desionizada entre cada muestra, de esta forma el electrodo se introdujo moderadamente (12 mm) en la superficie de cada muestra para su evaluación por triplicado de manera consecutiva en cortes, tanto en crudo (Figura 9) como en cocido (Figura 10) (Reséndiz-Cruz et al., 2022).

8.8.4. Color

Para el análisis de color se utilizó un colorímetro portátil Hunter Lab (CR-410, Konica-Minolta, Inc. Japón) mismo que se colocó en la superficie de cada muestra buscando cubrir el área del lente por completo, previamente este fue calibrado con las coordenadas de referencia; L*=94,7, a*=0,3130 y b*=0,3191, lo anterior con el objetivo de medir las variables de luminosidad (L*), rojo-verde (a*) y amarillo-azul (b*) (Ma et al., 2020), esta metodología también se aplicó por triplicado tanto en carne cruda como cocida para las muestras de cada corte (New york, rib eye, pulpa de pierna) tanto de búfalo de agua como de bovino convencional (Figura 9 y 11).

Para la medición de las características L*, a* y b* en cocido se permitió el enfriamiento de la carne posterior a su proceso de cocción con el fin de evitar vapor de agua que pudiera afectar las mediciones.

8.8.5. Textura

Para la característica de textura de utilizó un equipo de medición de textura TAX.T2 (Texture Technologies, Nueva York) acoplado al software TexturePro CT V1.9 Build 35®, para ello se obtuvieron muestras de carne de 1x1x4 cm^3 tanto en crudo como en cocido para los tres diferentes cortes cárnicos de ambas especies de interés, evaluando la fuerza de corte (reportado en Kg fuerza) con una navaja Warner-Bratzler y una sonda TA10 (Barrios Castillo, 2016; Pérez & Ponce, 2013) (Figura 11).

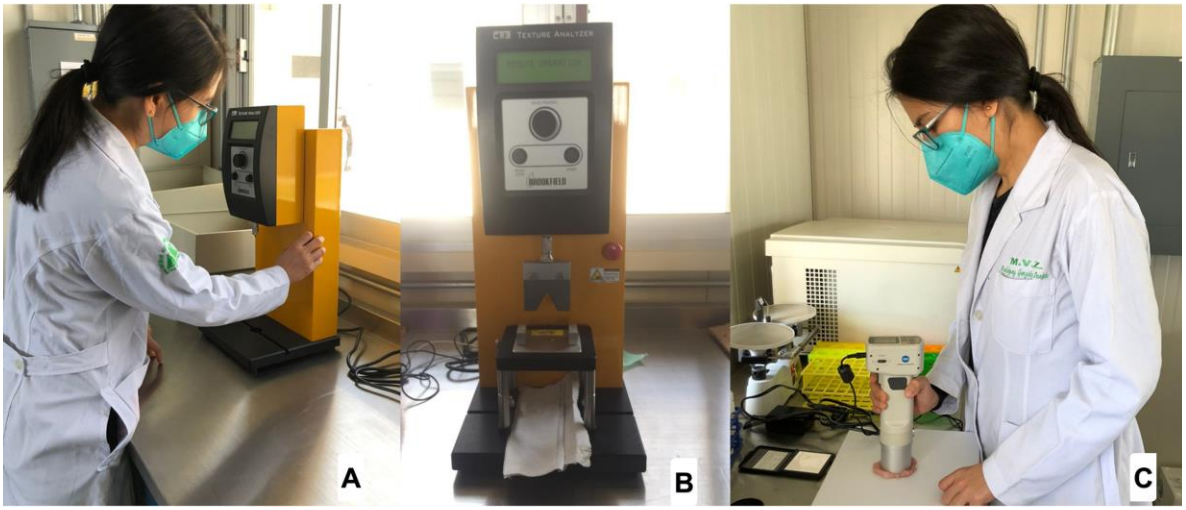

Figura 11. Equipo utilizado para obtener valores de textura en cortes tanto crudos como cocidos (A y B); C. Toma de valores de color (L*, a* y b*) de cortes cárnicos.

8.9. Análisis hedónico

Con el objetivo de determinar el nivel de aceptación se realizó una evaluación hedónica en donde se consideraron características como; terneza, jugosidad, sabor, aroma y aceptación en donde se informó a los participantes (n=50) que consumirían muestras de carne de bovino del género *Bos* y Búfalo de agua (*Bubalus bubalis*), con el objetivo de degustarlas y evaluarlas sin previo conocimiento de que tipo de carne calificarían.

Para ello se sometieron a cocción cortes de Rib Eye de ambas especies a baño de agua con ayuda de un agitador magnético de laboratorio SH-3 de placa calefactora a temperatura constante hasta que cada corte alcanzara los 70°C al interior de este (uso de termómetro metaltex modelo termocarne), para posteriormente seccionar en muestras de 1x1x3 cm^2 y colocarlas en charolas divididas e identificadas mediante claves y posteriormente calificar desde "Me disgusta moderadamente" hasta "Me gusta muchísimo" (Fabela Morón et al., 2020).

Los jueces dieron su consentimiento por escrito e informado antes del comienzo del estudio y las pruebas hedónicas se realizados en el laboratorio de Biociencia y Biotecnología Agroalimentaria del Departamento de Ciencias de la Alimentación de la Universidad Autónoma Metropolitana, unidad Lerma, en donde, a los 50 panelistas no entrenados (24 hombres, 26 mujeres) de entre 20 y 31 años y, una vez que se obtuvieron las respuestas de los panelistas se realizó una ponderación de la siguiente manera:

1. Me disgusta moderamente

2. Me disgusta poco
3. No me gusta ni me disgusta
4. Me gusta poco
5. Me gusta moderadamente
6. Me gusta mucho
7. Me gusta muchísimo

En esta prueba se contempló seleccionar a los panelistas mediante las siguientes respuestas que nos otorgaban de las preguntas realizadas antes de la prueba hedónica: ¿Consumió bebidas o comida una hora antes de la siguiente evaluación?, ¿Consumió bebidas alcohólicas antes de las 24 horas de la siguiente evaluación? ¿Tiene alguna sintomatología de enfermedades respiratorias o gastroentéricas?
Posteriormente se les impartió instrucciones cuando se les presento el formato de evaluación de cada producto, donde observaron las muestras debidamente identificadas y con los códigos que correspondía, además proporcionándoles Galletas Habaneras® que corresponde a un sabor neutro, un vaso de agua para cada cambio de muestra y servilleta. Otra consideración importante mencionada a la par de las instrucciones antes descritas fue evitar ruidos o algún comentario entre los participantes durante la evaluación, tampoco se puede voltear o generar gestos faciales ni sonidos durante o el término de la evaluación.

8.10. Análisis estadístico

8.10.1. Análisis estadístico fase ante mortem; transporte corto.

Para transporte corto se utilizó el paquete estadístico GraphPad Prism (ver. 9.4.1). En primer lugar, se obtuvieron estadísticas descriptivas para cada ventana térmica (periocular, carúncula lagrimal, párpado inferior, canal auditivo, fosas nasales, área parietofrontal y área torácica, abdominal, apendicular, lumbar y dorsal) y evento (F1-F7). Los resultados se expresaron como media ± error estándar (EE). Para el análisis de normalidad se aplicó la prueba de Shapiro-Wilk.

Los eventos y ventanas térmicas se consideraron como variables independientes, la temperatura superficial se tomó como variable dependiente. Se realizó un análisis de varianza (ANOVA) para evaluar el efecto de estas variables bajo un modelo lineal mixto, utilizando el siguiente modelo estadístico:

$Y_{ijak} = \mu + \tau i + \tau j + \tau i \tau j + \beta k + e_{ijk}$

Donde:

Y = variable de respuesta (temperatura de la superficie)

τi= efecto de la ventana térmica

τj= efecto del evento

$\tau i \tau j$= efecto de interacción

β= efecto aleatorio (animal)

μ= media poblacional

e= error

8.10.2. Análisis estadístico fase ante mortem; transporte largo vs corto

Para todos los análisis se utilizó el paquete estadístico GraphPad Prism (ver. 10). En primer lugar, se obtuvieron estadísticas descriptivas para cada ventana térmica (periocular, carúncula lagrimal, párpado inferior, canal auditivo, fosas nasales, área parietofrontal y área torácica, abdominal, apendicular, lumbar y dorsal) y evento (F1-F7). Los resultados se expresan como media ± error estándar (EE). Para el análisis de normalidad se utilizó la prueba de Shapiro-Wilk.

Los eventos y ventanas térmicas se consideraron como variables independientes y la temperatura superficial como variable dependiente. Se realizó un análisis de varianza (ANOVA) para evaluar el efecto de estas variables en un modelo lineal mixto utilizando el siguiente modelo estadístico:

$$Y_{ijak} = \mu + \tau a + \tau i + \tau j + \tau k + \tau a \tau i \tau j \tau k + \beta k + e_{aijk}$$

Donde:

Y = variable de respuesta (temperatura de la superficie)

Ta= efecto del transporte

τi= efecto de la ventana térmica

τj= efecto del evento

τk=efecto del tiempo de transporte

$\tau i \tau j \tau k$= efecto de interacción

β= efecto aleatorio (animal)

μ= media poblacional

e= error

Las diferencias entre medias se analizaron mediante una prueba de Tukey post-hoc. El nivel de significación se fijó en $p<0.05$. El análisis de correlación se realizó utilizando el coeficiente de correlación de Pearson.

8.10.3. Análisis estadístico fase post mortem; análisis fisicoquímico de cortes cárnicos de búfalo de agua y cortes cárnicos de bovino *Bos.*

Los resultados obtenidos del análisis de pH, color, CRA, Aw, y textura para los 3 tipos de cortes y las dos especies animales se capturaron en el "Formato de identificación de características fisicoquímicas", posteriormente fue analizado con un diseño estadístico completamente al azar ANOVA y comparación Tukey post- hoc con el programa SPSS, considerando el nivel de significancia en $P<0.05$.

8.10.4. Análisis estadístico fase post mortem; análisis hedónico de carne de búfalo de agua y carne de bovino *Bos*.

Se solicitó a cada panelista colocar sus respuestas en el documento físico "Formato de análisis hedónico", posteriormente, dichas respuestas fueron capturadas en una base de datos de Excel para facilitar el ingreso de valores el programa SPSS realizando una prueba de U Mann-Whitney y comparación Tukey post- hoc, considerando el nivel de significancia en $P<0.05$.

9. RESULTADOS

Resultados de la fase ante mortem; Transporte corto

Artículo experimental original intitulado:

9.1. CAPÍTULO III

Evaluación de cambios térmicos en búfalo de agua movilizado desde el potrero y transportado en viajes cortos

Publicado en la revista Frontiers in Veterinary Science, misma que se encuentra indexada al JCR con un factor de impacto de 3.2

Así mismo, este artículo fue seleccionado e incluido en el libro digital "Behavior and Heat Stress" publicado por la editorial Frontiers in Veterinary Science en su sección de Reseach Topics editado por los doctores Cristiane Gonçalves Titto, Messy Hannear De Andrade Pantoja, Cihan Çakmakçı, Fabio Luis Henrique and Priscila dos Santos Silva.

Rodríguez-González, D.; Guerrero-Legarreta, I.; Cruz Monterrosa, R.G.; Napolitano, F.; Gonçalves-Titto, C.; El-Aziz, A.H.A.; Hernández- Ávalos, I.; Casas-Alvarado, A.; Oliva-Domínguez, A.; Mota-Rojas, D. Assessment of thermal changes in water buffalo mobilized from the paddock and transported by short journeys. *Front. Vet. Sci.* **2023**. https://doi.org/10.3389/fvets.2023.1184577

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 12 May 2023
DOI 10.3389/fvets.2023.1184577

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OPEN ACCESS

EDITED BY
Severiano Silva,
Universidade de Trás-os-Montes e Alto,
Portugal

REVIEWED BY
Mariana Almeida,
University of Trás-os-Montes and Alto Douro,
Portugal
Marcelo Ghezzi,
Universidad Nacional del Centro de Buenos
Aires, Argentina

*CORRESPONDENCE
Daniel Mota-Rojas
✉ dmota100@yahoo.com.mx

†Deceased

RECEIVED 12 March 2023
ACCEPTED 24 April 2023
PUBLISHED 12 May 2023

CITATION
Rodríguez-González D, Guerrero Legarreta I, Cruz-Monterrosa RG, Napolitano F, Titto CG, Abd El-Aziz AH, Hernández-Avalos I, Casas-Alvarado A, Domínguez-Oliva A and Mota-Rojas D (2023) Assessment of thermal changes in water buffalo mobilized from the paddock and transported by short journeys. *Front. Vet. Sci.* 10:1184577.
doi: 10.3389/fvets.2023.1184577

Assessment of thermal changes in water buffalo mobilized from the paddock and transported by short journeys

Daniela Rodríguez-González[1], Isabel Guerrero Legarreta[2], Rosy G. Cruz-Monterrosa[3], Fabio Napolitano[4†], Cristiane Gonçalves Titto[5], Ayman H. Abd El-Aziz[6], Ismael Hernández-Avalos[7], Alejandro Casas-Alvarado[8], Adriana Domínguez-Oliva[8] and Daniel Mota-Rojas[8]*

[1]Master's Program in Agricultural and Livestock Sciences [Maestría en Ciencias Agropecuarias], Universidad Autónoma Metropolitana (UAM), Xochimilco Campus, Mexico City, Mexico, [2]Department of Biotechnology: Food Science, Universidad Autónoma Metropolitana, Iztapalapa Campus (UAM-I), Mexico City, Mexico, [3]Department of Food Science, Universidad Autónoma Metropolitana (UAM-L), Campus Lerma, Lerma City, Mexico, [4]Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Potenza, Italy, [5]Laboratório de Biometeorologia e Etologia, Faculdade de Zootecnia e Engenharia de Alimentos, FZEA-USP, Universidade de São Paulo, Pirassununga, Brazil, [6]Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt, [7]Department of Biological Science, FESC, Universidad Nacional Autónoma de México (UNAM), Cuautitlán Izcalli, Mexico, [8]Neurophysiology, Behavior and Animal Welfare Assessment, DPAA, Universidad Autónoma Metropolitana (UAM), Xochimilco Campus, Mexico City, Mexico

Evaluating the welfare of buffaloes during transport is key to obtaining and commercializing high-quality meat products; however, effective assessments require recognizing several stressors that activate physiological mechanisms that can have repercussions on the health and productive performance of species. The aim of this study was to evaluate the surface temperatures of different body and head regions in this species during events prior, and posterior, to transport for short periods; that is, from paddock to loading. The second goal was to determine the level of correlation between thermal windows. This study used infrared thermography (IRT) to evaluate the surface temperature of 624 water buffaloes (Buffalypso breed) during 12 short trips (average duration=2 h±20 min) by focusing on 11 regions of the body (*Regio corporis*), in the head regions (*Regiones capitis*) the face regions (*Regiones faciei*), Orbital region (*Regio orbitalis*) with special attention to structures such as the lacrimal caruncle, periocular area and lower eyelid (*Regio palpebralis inferior*); nasal region (*Regio nasalis*) with special attention to nostril thermal window; and regions of the skull (*Regiones cranii*) such as auricular region (*Regio auricularis*) with special attention to auditory canal and frontal–parietal region (*Regio frontalis-parietalis*) and trunk region (*Truncus regionis*) such as thoracic and abdominal regions, regions of the vertebral column (*Columna vertebralis*) with the thoracic vertebral region (*Regio vertebralis thoracis*) and lumbar region (*Regio lumbalis*); and regions of the pelvis limb (*Regiones membri pelvini*). Recordings were made during seven phases: paddock (P1), herding (P2), corral (P3), chute handling (P4), shipping (P5), pre- (P6), and post-transport (P7). A total of 48,048 readings were obtained from 11 thermal windows. The results showed that the surface temperatures of the windows increased by as much as 5°C during P2, P3, P5, P6, and P7 compared to P1 and P4 ($p<0.0001$). Differences of at least 1°C were also observed between thermal windows in the craniofacial, lateral corporal, and peripheral zones ($p<0.0001$). Finally, a strong positive correlation ($r=0.9$, $p<0.0001$) was found between the thermal windows.

These findings lead to the conclusion that the surface temperature of the craniofacial and corporal regions of buffaloes transported for short periods varied in relation to the phase of mobilization (from paddock to post-transport), likely as a response to stressful factors, since herding and loading increased the thermal values in each window. The second conclusion is that there are strong positive correlations between central and peripheral thermal windows.

KEYWORDS

Bubalus bubalis, infrared thermography, animal welfare, transport, herding method

1. Introduction

Animal transport is an integral element in the process of breeding and fattening buffaloes (1–3) to obtain and commercialize meat products (4–7). However, transport is considered one of the most stressful procedures for farm animals because it exposes them to environmental factors, drivers' aptitude, road conditions, and trip duration, all of which can affect their welfare by provoking injuries or negative mental states such as fear and anxiety, due to the triggering of physiological and behavioral mechanisms that can modify muscular metabolism and muscle-to-meat conversion, and affect the amount, quality, and innocuity of the final products (8–11). Obviously, this means economic losses for the producers.

In light of the negative effects of mobilization-induced stress, diverse behavioral, pathological, and physiological indicators are being used to evaluate the levels of animal welfare during this phase, where observable physiological changes include dehydration, hyporexia, tissue damage, smoke inhalation, and physical, thermal, and psychological stress (12–15), which can inhibit the immune system under long exposure to adverse environments (16). Endocrine and metabolic alterations, increased plasma cortisol and serum protein concentrations, and glycemia levels have been documented after 16 h of transport, while loss of live weight (from 7.9 to 10.5%) (17, 18) has been associated with energy deficits caused by muscular and hepatic glycogenolysis (19). Another physiological indicator suggested for monitoring is body temperature, as increases can aid in recognizing stressful processes and may be related to the overall health condition of animals (20). Regarding behavioral changes in buffaloes, reports have emphasized that transport conditions increase the frequency of aggressive and dominant behaviors (21, 22), urination (28%), defecation, and urination and defecation (72%), as well as attempts to jump from vehicles (12%) (7) which results in falls and injuries that affect both the animals and the quality of the final product.

Water buffaloes have shown a greater predisposition to transport-induced stress due to certain morphophysiological characteristics that make them susceptible to thermal stress, a very common consequence of transport. Buffaloes have fewer sweat glands and a lower proportion in relation to body weight than other livestock species (394/cm^2), low hair density (100–200/cm^2), and hide with more pigmentation that impedes adequate thermal exchange (23, 24). Evaluating body temperature in buffaloes is a parameter that permits the determination of the degree of thermal stress. The "gold standard" for monitoring this factor was obtained via invasive methods, like measuring rectal and vaginal temperatures (25). However, these techniques can generate stress in animals due to the handling requirements. For this reason, non-invasive technologies such as IRT have been proposed as tools to evaluate surface temperatures in these animals (26, 27).

IRT enables evaluation of the amount of heat that a body radiates, a phenomenon related to changes in peripheral blood flow through activation of the autonomic nervous system (ANS) in the face of diverse stressors (28). Because transport entails a certain degree of stress that has not been widely studied in water buffaloes, the aim of this study was to evaluate the surface temperatures of different body and craniofacial regions in this species during events prior, and posterior, to transport for short periods; that is, from paddock to loading. The second goal was to determine the level of correlation between central (abdominal and thoracic region) and peripheral (limb and nasal) thermal windows.

2. Materials and methods

2.1. Study location

The study was conducted in the state of Veracruz in south-southeast Mexico, from June 2021 to August 2022. The production unit is in a zone with a tropical humid climate, with a mean temperature of 31 ± 2°C, relative humidity of 86%, an elevation of 20 m.a.s.l., and annual rainfall of 1,500–2,000 mm (29).

2.2. The animals and the distribution of the phases

For this study, 624 male Buffalypso buffaloes destined for fattening were selected. The mean weight of the animals was 230 ± 21.78 kg. They were transported on 12 short trips covering 110 km with a mean duration of 2 h ± 20 min and an average velocity of 55 km/h.

The buffaloes were divided for the 12 trips according to the capacity of the first floor of the truck as follows: 53, 51, 49, 56, 53, 50, 53, 52, 54, 51, 52, and 50. Before each trip, they were housed in the same paddock and herded gently (no use of physical utensils or shouting by handlers).

2.3. Housing prior to loading and vehicle type

To carry out the experimental protocol, the transport process was divided into seven phases (Figure 1):

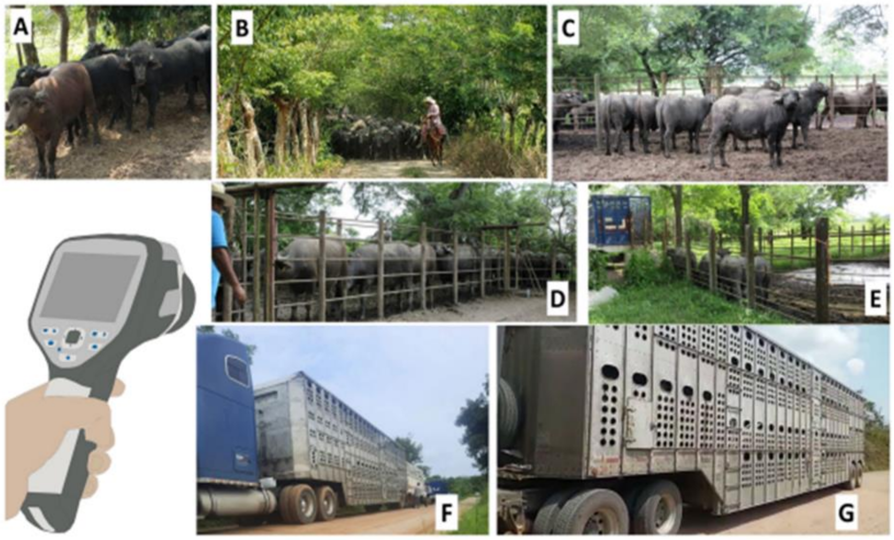

FIGURE 1
Areas where the evaluation phases were carried out. **(A)** The paddock of the production unit; basal sampling (P1). **(B)** After herding by men on horseback (P2). **(C)** After spending the night in the cattle corral system, prior to entering the handling chute (P3). **(D)** Handling chute (P4). **(E)** Entering the vehicle via a loading ramp (P5). **(F,G)** The vehicle prior to transport (P6) and upon arrival at the reception site (P7).

Phase 1 (P1). Paddock, basal monitoring: The animals were housed in paddocks with natural shade until they reached a weight of 208–251 kg. Alimentation consisted of native grasses (*Paspalum fasciculatum*) (percentage of dry matter = 14.7% with 56.7% neutral detergent fiber, 38.8% acid detergent fiber, 6.2% raw protein (30), and 50 g/animal of mineral complements).

Phase 2 (P2). Herding by handlers on horseback: Once the steers reached the desired weight, they were herded gently by men on the horseback without physical utensils or electric prods. For all trips, herding from the paddock to the corral system (P3) required an average of 25–35 min. This was always performed at 18:00. The animals remained in the cattle corral system for 12 h (including the night before transport).

Phase 3 (P3). Cattle corral system: The pen had a surface area of 630 m^2 and a dirt floor. It was framed by 1.6 m high tubular structures with an entrance gate and an exit gate that led to the handling chute. The buffaloes remained there for 8 h without solid food but with *ad libitum* access to water.

Phase 4 (P4): Handling chute: The chute consisted of a hallway (1 m wide × 1.6 m high × 3.5 m long), framed by tubular structures, and connected to an individual weight scale. This hallway led to a loading ramp. The average duration was 50 min.

Phase 5 (P5). Loading: During an interval of approximately 30–50 min, the buffaloes were led up with a concrete loading ramp made of anti-slip grooving. Slope was 20°.

Phase 6 (P6). Pre-transport: The time spent in the vehicle before transport, counted from the moment when all the animals had been loaded into the truck.

Phase 7 (P7). Post-transport: The time when the animals were still inside the vehicle, immediately upon arrival at the reception site, after transport over dirt and paved roads.

The topography of the roads was classified as unpaved or paved. The former had the specifications of Class E roads, according to the classification of Mexico's Department of State (*Secretaría de Gobernación*) (31), with a maximum slope of 13%.

The mean maximum velocity was 25 km/h. Dirt roads were used to exit the production unit (10 km) and enter the reception site (12.5 km). The total trip length on the unpaved roads was 22.5 km with a maximum overelevation of 10%, vertical curves of 4 m/%, and road width of 6 m. The other 87.5 km were covered on a paved road with a maximum transversal slope of 7%, a maximum speed of 55 km/h, vertical curves of 5 m/%, and a road width of 8 m.

The vehicle was a Wilson Trailers Silver Star (dimensions: 15.24 m long × 2.59 m wide × 4.6 m high) with a load capacity of 22,500 kg. The truck was designed especially for animal transport with two floors, sliding side doors, a rear guillotine door, a non-slip floor, a reinforced plastic roof, and fiberglass. The walls were made of galvanized steel and aluminum, with openings for ventilation during the trips.

2.4. Monitoring by infrared thermography

Thermographic monitoring was performed using a FLIR® Thermal TM E60 infrared camera (FLIR Systems, United States) with an IR resolution of 320 × 240 pixels, thermal sensitivity <0.045°C, and precision ±2°C or 2%. All the radiometric images were taken at a distance of 1–1.5 m from the buffaloes, focusing on one of the following regions: facial regions (*Regiones faciei*) and skull region (*Regiones cranii*), trunk region (*Truncus regionis*) left or right, or regions of the vertebral column (*Columna vertebralis*), and regions of the pelvis limb (*Regiones membri pelvini*). Eleven body thermal windows in the head (*Regiones capitis*) and the trunk (*Truncus*

10.3389/fvets.2023.1184577

regionis) were selected and delimited using FLIR tools software. The facial regions (*Regiones faciei*) included the orbital region (*Regio orbitalis*) with special attention to structures such as the lacrimal caruncle, periocular area and lower eyelid (*Regio palpebralis inferior*); nasal region (*Regio nasalis*) with special attention to nostril thermal window and regions of the skull (*Regiones cranii*) such as auricular region (*Regio auricularis*) with special attention to auditory canal and frontal–parietal region (*Regio frontalis-parietalis*) (Figure 2).

The trunk region (*Truncus regionis*) thoracic region and abdominal region, thermal window of the trunk, and regions of the pelvis limb (*Regiones membri pelvini*) with two anatomical regions indicated the femoral region (*Region femoris*) and the tarsus region (*Regio tarsi*), thermal windows of the regions of the pelvis limb (*Regiones membri pelvini*) are shown in Figure 3.

Figure 4 shows the regions of the vertebral column (*Columna vertebralis*) with the thoracic vertebral region (*Regio vertebralis thoracis*) and lumbar region (*Regio lumbalis*).

2.5. Recording and reading of the IRT

In accordance with the seven experimental phases, IRT readings were recorded as follows: P1, prior to the buffaloes being herded by men on horseback. Recordings were made while they were at rest for 1 h under shade, between 17:00 and 18:00 h; P2, inside the corral on the day prior to transport; P3, at 08:00 h, prior to guiding the animals into the handling chute to be weighed and loaded; P4, while the buffaloes were in the handling chute waiting to be weighed; P5, during loading as the animals walked up the ramp and entered the truck; P6, for 40 min after all the buffaloes were loaded into the truck but with the motor shut off, and P7, for 40 min immediately after concluding the short trip, but with the truck's motor still turned on.

All digital and radiometric images were stored in JPG format for later analysis using FLIR software (Tools Systems, United States) to obtain the maximum, minimum, and average temperature readings, which were then entered into a database.

The ambient temperature (AT) and relative humidity (RH) were monitored at different times, in accordance with the experimental phase. P1: AT and RH ranged from 23 to 25°C and 81–90%, respectively (17:00–18:00 h). P2: AT and RH values were 21–25°C and 81–88%, respectively (18:00–18:30 h). P3: AT and RH fluctuated around 20–23°C and 81–86%, respectively (08:00 h). P4: AT and RH were 22–25°C and 81–88%, respectively (09:00 h). P5: values were 22–27°C and 81–88% at 10:00 h. P6 and P7: data for AT and RH ranged from 22–28°C and 81–89% at 11:00 h and 23–30°C and 83–94% at 14:00–15:00 h.

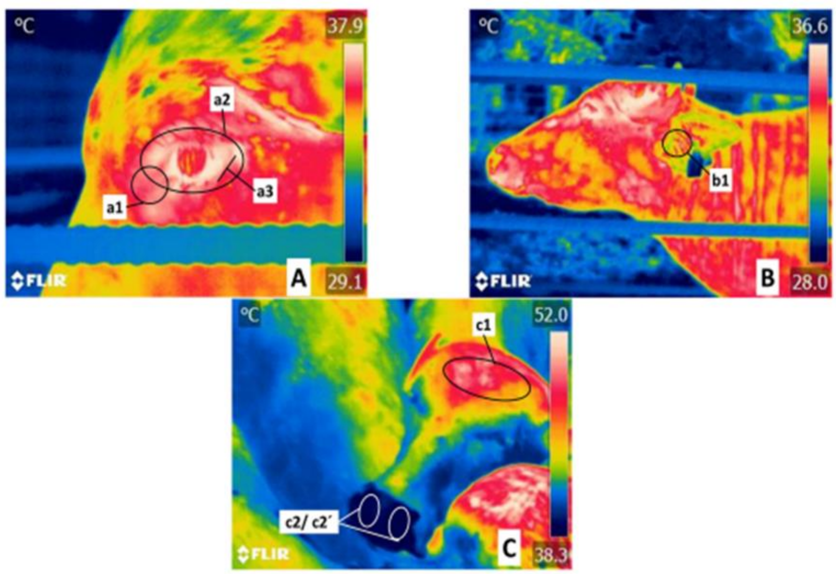

FIGURE 2
Delimitation of thermal windows in the head regions (*Regiones capitis*). **(A)** Ocular thermal windows in Orbital region (*Regio orbitalis*). Recordings in this zone centered on the lacrimal caruncle, delimited as circle a1, and considered the medial commissure of the eyelids up to half a centimeter toward the cranium. This area reflects the radiation of blood flow from the infraorbital artery innervated by the sympathetic branches of the facial nerve. The periocular thermal window, marked by circle a2, spans the periphery of the upper and lower eyelids, irrigated by both the supraorbital and infraorbital arteries. The lower eyelid thermal window, defined by a line approximately 3 cm long (a3), was used to evaluate radiation from the conjunctival mucosa and was irrigated by the infraorbital artery. **(B)** Regions of the skull (*Regiones cranii*), thermal window of the auricular pavilion in the auricular region (*Regio auricularis*). This area is framed by circle b1 in the region of the auditory canal, the antihelix ridge, and the lateral peduncle of the helix, irrigated by the media and the lateral and central auricular arteries. **(C)** Thermal window of the frontal-parietal region (*Regio frontalis-parietalis*). Outlined by an oval (c1) that encompasses the frontal-parietal region (*Regio frontalis*), cornual region (*Regio cornualis*) limits, and parietal region (*Regio parietalis*), a zone irrigated by cornual and supraorbital arteries. *Regio naris* thermal window in the frontal region (*Regio frontalis*): ovals c2 and c2´. It was used to evaluate the radiation from the maxillary artery.

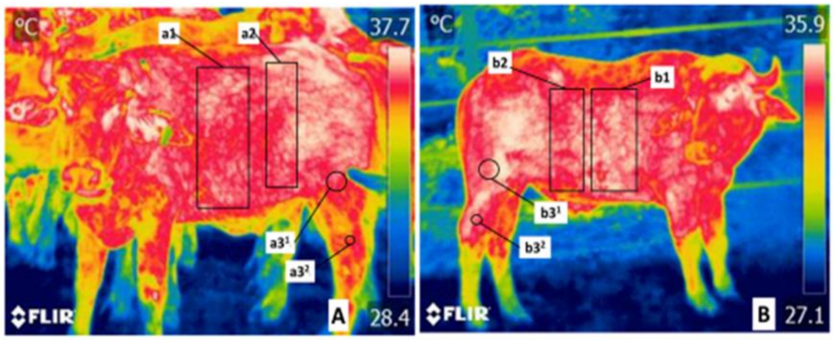

FIGURE 3
Delimitation of the trunk thermal windows in the trunk region (*Truncus regionis*). **(A)** The thermal window of the thoracic region in costal region (*Regio costalis*) is shown by a rectangle (a1, b1) traced from the costal arch from 1° to 12°. The cranial abdominal region (*Regio abdominis cranialis*) delimited by a rectangle (a2, b2), includes the oblique abdominal and rectus abdominis muscles irrigated by the surface epigastric artery (*A. epigastrica cranialis superficiales*). **(B)** The thermal window of the regions of the pelvis limb (*Regiones membri pelvini*) were marked by two circles at the *Regio femoris* ($a3^1$, $b3^1$), and at the *Regio tarsi* ($a3^2$, $b3^2$). It includes the femoral region the femoral muscle and the tarsus region lateral projection of the region of the common calcaneal tendon (*Regio tendinis calcanei communis*), regions irrigated by the femoral artery, and branches of the saphenous artery.

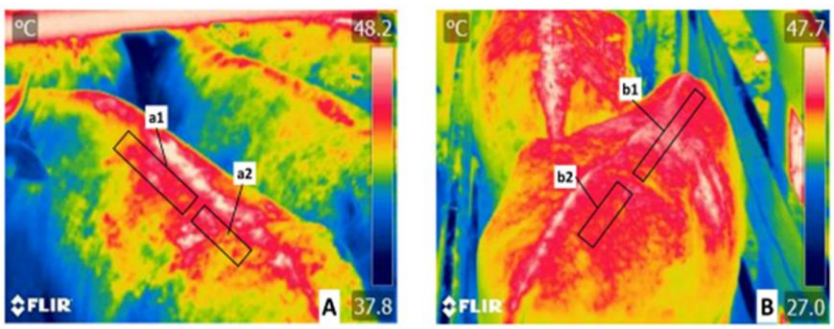

FIGURE 4
Delimitation of the regions of the vertebral column (*Columna vertebralis*) thermal windows. **(A)** The thermal window of the thoracic vertebral region (*Regio vertebralis thoracis*) is marked with a rectangle. It includes the left (a1) and right (b1) projections from the thoracic vertebrae and the thoracic vertebrae and the with of the transverse processes from thoracic vertebrae 1ª–12ª. This area is irrigated by spinal branch (*Ramus spinalis*) from the dorsal branches of the abdominal aorta artery, in the thoracic vertebral region. **(B)** The thermal window of the lumbar vertebral region (*Regio vertebralis lumbalis*). This rectangle encompasses the left (a2) and right (b2) projections from the lumbar vertebrae above the transverse processes of lumbar vertebrae 1ª–7ª. This permits the evaluation of radiation from the spinal branch (*Ramus spinalis*) from the dorsal branches of the abdominal aorta artery, in the lumbar vertebral region.

2.6. Statistical analysis

The GraphPad Prism statistical package (ver. 9.4.1) was used for all analyses. First, descriptive statistics were obtained for each thermal window (periocular, lacrimal caruncle, lower eyelid, auditory canal, nostrils, parieto-frontal area, and the thoracic, abdominal, appendicular, lumbar, and dorsal areas) and event (P1–P7). Results are expressed as mean ± standard error (SE). The Shapiro-Wilk test was used for normality analysis.

The events and thermal windows were considered as independent variables, and the surface temperature was considered as the dependent variable. Analysis of variance (ANOVA) was performed to evaluate the effect of these variables in a linear mixed model using the following statistical model:

$$Y_{ijak} = \mu + \tau i + \tau j + \tau i \tau j + \beta k + e_{ijk}$$

where:
Y = response variable (surface temperature).
τi = effect of the thermal window.
τj = effect of the event.
$\tau i \tau j$ = interaction effect.
β = random effect (animal).

μ = population mean.

e = error.

Differences between means were analyzed using a *post-hoc* Tukey test. The level of significance was set at $p < 0.05$. Correlation analysis was conducted using the Pearson's correlation coeficient.

2.7. Ethics statement

The experimental protocol was approved by the Scientific Commission of the Master in Science (CAMCA) "Maestría en Ciencias Agropecuarias" of the Faculty of Veterinary Medicine and Animal Husbandry, Universidad Autónoma Metropolitana, Mexico City, Mexico. The animals used in this study were handled gently, without the use of physical utensils that could cause stress, in accordance with the guidelines of the Official Mexican Standards NOM-062-ZOO-1999, which establish technical specifications for the production, care, and ethical use of animals during experimentation; and NOM-051-ZOO which establish technical specifications for humanitarian handling in the mobilization of animals published by the Department of Agriculture, Rural Development, Fisheries and Alimentation. It is also important to consider that the animals were not touched or stressed, since infrared thermography is a non-invasive technique.

3. Results

During the seven phases analyzed, we obtained 48,048 surface temperature readings from 11 thermal windows of 624 water buffaloes transported on 12 short trips. Table 1 shows the mean temperatures of the thermal windows in the head regions (*Regiones capitis*) and their corresponding standard errors.

Observations showed the thermal windows considered in the orbital region (*Regio orbitalis*) such as the lacrimal caruncle, periocular region, and lower eyelid thermal windows had significant average increases of 4.3°C, 4.8°C, and 3.7°C, respectively, during P2, P3, and P4, compared to P1 ($p < 0.0001$). Readings for the Regions of the skull (*Cranii regions*), the auditory canal thermal window showed increases of 1.3°C, 0.8°C, and 1.3°C in those windows compared to P5 ($p < 0.0001$), while the comparison of P3 to P2 and P4 revealed a significant reduction of 1.3° ($p < 0.0001$). Observations during P6 and P7 showed a gradual increase in surface temperature compared with P5 ($p < 0.0001$).

Readings for the auditory canal window showed an increase of 7.7°C from P1 to P2 ($p < 0.0001$), but the auricular temperature decreased by 2.6°C from P2 to P3 ($p < 0.0001$). Later, it increased by 1°C and 0.8°C during P4 and P5, respectively, compared to P2 ($p < 0.0001$). Recordings for P6 and P7 showed significant increases of 2.7°C and 2.5°C compared to P4 and P5 ($p < 0.0001$).

For the thermal windows the nasal region (*Regio nasalis*), the nostrils and frontal-parietal region, the study found that temperatures in P1 were 4.7°C and 11.6°C lower, respectively, than those in P2 ($p < 0.0001$), but those temperatures decreased by 1.3°C and 6.4°C, respectively, in P3 ($p < 0.0001$). In P4 and P5, the temperatures increased by 0.5°C and 0.8°C, respectively ($p < 0.0001$). This increase was maintained in P6 (2°C) and P7 (2.6°C), compared to P5 ($p < 0.0001$). Readings from the thermal window of frontal- parietal region showed increases of 8.4°C in P4, 4.5°C in P5 ($p < 0.0001$), and 8.6°C in P7 compared to P5 and P6 ($p < 0.0001$).

Considering the different phases of this study and the thermal responses in the windows described, during P1, temperatures in the orbital region (*Regio orbitalis*) such as the lacrimal caruncle, periocular, and lower eyelid were significantly 2°C higher than in the auditory canal, nostrils, and thermal window of frontal-parietal region ($p < 0.0001$). During P2, the parieto-frontal window presented the highest temperature among all windows ($p < 0.0001$). The orbital region (*Regio orbitalis*) such as the lacrimal caruncle, periocular, and auditory canal had temperatures 0.7°C higher than those of the lower eyelid ($p < 0.0001$), but the latter was 1.9°C higher than the nostrils window ($p < 0.0001$).

Observations from P3 showed that the temperature of the periocular window was 0.3°C higher than that of the lacrimal caruncle ($p < 0.0001$), and 1°C higher than that of the lower eyelid ($p < 0.0001$). Similarly, the temperature of the lower eyelid was 1.2°C higher than that in the nostril window ($p < 0.0001$). For P4, P5, P6, and P7, the thermal window of frontal–parietal region showed the highest temperatures in all regions ($p < 0.0001$), followed in the orbital region (*Regio orbitalis*) by the lacrimal caruncle, periocular region, and lower eyelid region ($p < 0.0001$).

The temperatures of the thermal windows of the lateral region of the trunk are presented in Table 2. In P1, the thermal window of the thoracic region presented a lower temperature than the other phases but was 8.6°C lower in P2 ($p < 0.0001$). Readings for phases P3, P4, and P5 showed reductions of 5.4°C, 3°C, and 3.3°C, respectively, compared to P2 ($p < 0.0001$), but increases of 3.4°C in P6 and 4.9°C in P7 were determined ($p < 0.0001$). The thermal window of the abdominal region recorded a temperature increase of 6.8°C in P2 compared to P1 ($p < 0.0001$). With respect to P3, P4, and P5, we observed decreases of 4°C, 1.7°C, and 2.8°C, respectively, compared to P2 ($p < 0.0001$), whereas P6 and P7 registered increases of 4.6°C and 5.3°C, respectively ($p < 0.0001$). Temperature readings for the thermal window of the pelvic limb in P1 were the lowest of all events ($p < 0.0001$), but the temperatures recorded in P2 and P6 were significantly higher than those in P3, P4, and P5 by 2.7, 0.2, and 2.3°C, respectively ($p < 0.0001$).

For P1 and P6, we found that the thermal window had values 0.5 and 0.4°C above those of the thermal window of the thoracic region ($p < 0.0001$). Readings for the latter, compared to the limbs regions, showed increases of 0.9 and 3.7°C, respectively ($p < 0.0001$). During P2 and P7, the study found that the thermal window of the thoracic region was 1.3 and 0.4°C higher than the thermal window of the abdominal region ($p < 0.0001$) while the latter was 1.6 and 3.7°C higher than the thermal window of the pelvic limb regions ($p < 0.0001$).

Table 3 presents the thermal values recorded in the thermal windows of the regions of the vertebral column. The reading for the thermal window of the thoracic vertebral region (*Regio vertebralis thoracis*) in P1 was lower than in all other events ($p < 0.0001$). The temperature of this window was 6°C and 6.6°C higher in P2 and P4 than in P3 ($p < 0.0001$) but decreased by 2.1°C and 1.9°C in P5 and P6, respectively ($p < 0.0001$). Later, the surface temperature of this thermal window increased by 4.3°C compared to P6 ($p < 0.0001$). Observations of the thermal window of the lumbar region (*Regio lumbalis*) showed that P6 had the highest temperature of all thermal windows ($p < 0.0001$), although in P1, it obtained the lowest value ($p < 0.0001$). In P2 and P4, this zone registered temperatures of 6.4°C and 6.7°C, respectively, higher than in P3 ($p < 0.0001$). Finally, the surface

10.3389/fvets.2023.1184577

TABLE 1 Mean±standard error (SE) temperatures for the thermal windows of the head regions (*Regiones capitis*) of 624 water buffaloes in the 7 phases of transport for short periods.

Thermal window		Phase						
		P1. Paddock	P2. Herding	P3. Corral	P4. Handling chute	P5. Loading	P6. Pre-transport	P7. Post-transport
Facial region (*Regions faciei*)	**Orbital region** (*Regio orbitalis*) Lacrimal caruncle	$33.9 \pm 0.03^{e,1}$	$38.9 \pm 0.06^{c,2}$	$37.4 \pm 0.02^{e,2}$	$38.7 \pm 0.03^{c,3}$	$38.3 \pm 0.03^{d,3}$	$40.2 \pm 0.04^{b,2}$	$41.1 \pm 0.09^{a,4}$
	Periocular region	$33.7 \pm 0.03^{f,2}$	$39.0 \pm 0.04^{c,2}$	$37.7 \pm 0.02^{e,1}$	$39.0 \pm 0.03^{c,2}$	$38.5 \pm 0.03^{d,2}$	$40.0 \pm 0.03^{b,2,3}$	$41.5 \pm 0.09^{a,2}$
	Lower eyelid	$33.9 \pm 0.04^{f,1,2}$	$38.3 \pm 0.02^{c,3}$	$36.4 \pm 0.06^{e,3}$	$38.1 \pm 0.04^{c,4}$	$37.7 \pm 0.02^{d,4}$	$40.3 \pm 0.08^{b,2}$	$41.3 \pm 0.09^{a,3}$
	Nasal region (*Regio nasalis*) Nostrils	$31.7 \pm 0.04^{g,3}$	$36.4 \pm 0.04^{c,4}$	$35.1 \pm 0.03^{f,5}$	$35.6 \pm 0.02^{e,6}$	$35.9 \pm 0.03^{d,6}$	$37.3 \pm 0.03^{b,4}$	$38.5 \pm 0.03^{a,6}$
Regions of the skull (*Cranii regions*)	Auricular pavilion	$31.2 \pm 0.05^{f,3}$	$38.9 \pm 0.03^{b,2}$	$36.3 \pm 0.05^{e,4}$	$37.3 \pm 0.03^{c,5}$	$37.1 \pm 0.02^{d,5}$	$39.8 \pm 0.03^{a,3}$	$39.7 \pm 0.04^{a,5}$
	Frontal-parietal region	$31.5 \pm 0.06^{g,3}$	$43.1 \pm 0.08^{c,1}$	$36.7 \pm 0.03^{f,3}$	$45.1 \pm 0.07^{b,4}$	$41.2 \pm 0.06^{e,1}$	$41.1 \pm 0.09^{e,1}$	$49.7 \pm 0.15^{a,1}$

Different literals a, b, c, d, e, f, g indicate statistically significant differences among the temperatures of the animals according to the phase.
Different numbers 1, 2, 3, 4, 5, 6 indicate statistically significant differences between the temperatures of the thermal windows.

TABLE 2 Mean±standard error (SE) of the temperatures in the thermal windows of the lateral region of the trunk of 624 water buffaloes transported during seven phases of short trips.

Thermal window		Phase						
		P1. Paddock	P2. Herding	P3. Corral	P4. Handling chute	P5. Loading	P6. Pre-transport	P7. Post-transport
Lateral region of the trunk	Thoracic region	$33.0 \pm 0.03^{e,2}$	$41.6 \pm 0.06^{b,1}$	$36.2 \pm 0.02^{d,1}$	$38.6 \pm 0.03^{c,1}$	$38.3 \pm 0.07^{c,1}$	$41.7 \pm 0.06^{b,2}$	$43.2 \pm 0.08^{a,1}$
	Abdominal region	$33.5 \pm 0.02^{g,1}$	$40.3 \pm 0.05^{c,2}$	$36.3 \pm 0.03^{f,1}$	$38.6 \pm 0.03^{d,1}$	$37.5 \pm 0.05^{e,2}$	$42.1 \pm 0.07^{b,1}$	$42.8 \pm 0.08^{a,2}$
Limbs region	Pelvic limb	$32.1 \pm 0.04^{f,3}$	$38.7 \pm 0.02^{b,3}$	$35.9 \pm 0.03^{e,2}$	$38.4 \pm 0.03^{c,1}$	$36.3 \pm 0.02^{d,3}$	$38.6 \pm 0.04^{b,3}$	$39.1 \pm 0.04^{a,3}$

Different literals a, b, c, d, e, f, g indicate statistically significant differences among the temperatures of the animals according to the phase.
Different numbers 1, 2, 3 indicate statistically significant differences between the windows.

TABLE 3 Mean±standard error (SE) temperatures for the thermal windows of the trunk region (*Truncus regionis*) left or right of 624 water buffaloes during 7 phases of short periods of transport.

Thermal window	Phase						
	P1. Paddock	P2. Herding	P3. Corral	P4. Handling chute	P5. Loading	P6. Pre-transport	P7. Post-transport
Thoracic vertebral region	$32.1 \pm 0.05^{f,2}$	$41.6 \pm 0.09^{b,1}$	$35.6 \pm 0.04^{e,1}$	$42.2 \pm 0.06^{b,1}$	$40.1 \pm 0.10^{d,1}$	$40.8 \pm 0.08^{c,2}$	$45.1 \pm 0.11^{a,2}$
Lumbar vertebral region	$33.4 \pm 0.04^{g,1}$	$42.0 \pm 0.08^{c,1}$	$35.6 \pm 0.04^{f,1}$	$42.3 \pm 0.03^{b,1}$	$40.4 \pm 0.13^{e,1}$	$41.4 \pm 0.07^{d,1}$	$44.6 \pm 0.07^{a,1}$

Different literals a, b, c, d, e, f, g indicate statistically significant differences between the temperatures of the animals according to the phase.
Different numbers 1, 2 indicate statistically significant differences between the thermal windows.

temperature decreased in P5 and P6 by 1.9°C and 1°C, respectively, compared to P4 ($p<0.0001$).

Regarding statistically significant differences between the thermal windows of the thoracic vertebral region and lumbar region, the findings showed that in P1 and P6, the temperatures of the thermal window of the lumbar region were 1.3 and 0.6°C higher, respectively, than in the thermal window of the thoracic vertebral region ($p<0.0001$). At P7, the temperature of the thermal window of the thoracic vertebral region was 0.5°C higher than that in the thermal window of the lumbar region ($p<0.0001$).

Table 4 shows the level of correlation observed between the 11 thermal windows. Overall, the correlations among the different thermal windows were positive, strong, and statistically significant ($p<0.0001$).

4. Discussion

The results obtained for the buffaloes transported for short periods showed that the surface temperatures of thermal windows of the head and trunk increased significantly in P2, P4, P6, and P7, that is, during the events that required handling ($p<0.0001$). These responses may be associated with the loss of thermal stability in the buffaloes, a species whose optimal ambient for achieving thermoneutrality is in the range of 13–18°C (32, 33).

Although adequate temperatures have been reported for water buffaloes, there is limited literature on the thermal responses of these species during transport. For conventional bovines, a study on the transport by land and sea of 481 animals reported a significant association between increases in such biomarkers as glucose, creatine kinase, and lactate levels with IRT temperature readings (34). A study of 120 pigs transported for 40 min reported temperature increases in the orbital region and behind the ear, which correlated positively with increased salivary cortisol concentrations ($r=0.49$ and $r=0.50$, respectively) (35). Studies of this type in other livestock species subjected to transport suggest that surface temperatures can be useful indicators of physiological changes triggered in response to transport-induced stress (36, 37), which may be reflected as central and peripheral nervous system reply with activation of vasomotor modulation hyperthermia (38).

In our study, the largest surface temperature increases in the buffaloes were recorded in the post-transport phase ($p<0.0001$). This can be explained by the activation of Autonomic nervous system (ANS) and its sympathetic branch, which generates catecholamine production as a short-term response (24). Catecholamines act on metabolically active organs such as brown adipose tissue to initiate thermogenesis, thus increasing heat irradiation (39, 40). Activation of the hypothalamic-pituitary-adrenal axis (HPA) causes the secretion of glucocorticoids (e.g., cortisol) that promote gluconeogenesis and lipolysis (41, 42). In this regard, studies on ruminants have reported increases in plasma cortisol by up to 10-fold after 30 and 45 min, 1 h, 2 h, and up to 4 h post-transport (43–47). Mitchell et al. (48), cited in Ali-Gholi and Daryoush (49), stated that sympathetic, adrenal, and medullar reactions are related to physiological responses during transport, while hypothalamus, hypophysis, and suprarenal reactions are responsible for cortisol secretion under adverse environmental conditions. Therefore, this physiological response is responsible for heat production during the

TABLE 4 Correlations between the thermal windows of 624 water buffaloes during all seven phases of short periods of transport.

	Lacrimal caruncle	Periocular region	Lower eyelid	Auricular pavilion	Nostrils	Frontal-parietal region	Thoracic region	Abdominal region	Limbs region	Lumbar region	Thoracic vertebral region
Lacrimal caruncle	1*	0.96*	0.97*	0.94*	0.95*	0.95*	0.94*	0.94*	0.95*	0.95*	0.93*
Periocular region		1*	0.97*	0.99*	0.99*	0.98*	0.97*	0.96*	0.99*	0.97*	0.95*
Lower eyelid			1*	0.96*	0.97*	0.98*	0.97*	0.97*	0.96*	0.98*	0.97*
Auricular pavilion				1*	0.99*	0.96*	0.96*	0.94*	1*	0.96*	0.95*
Nostrils					1*	0.98*	0.98*	0.97*	0.99*	0.98*	0.97*
Frontal-parietal region						1*	1*	1*	0.96*	0.99*	0.99*
Thoracic region							1*	1*	0.96*	0.99	0.99*
Abdominal region								1*	0.95*	0.99*	0.98*
Limbs region									1*	0.97*	0.96*
Lumbar region										1*	0.99*
Thoracic vertebral region											1*

*$p<0.0001$; $^{ns}p>0.05$.

perception of stressful factors under conditions of transport, handling, and environmental challenges.

This explanation may aid in understanding the report by Sakakibara et al. (50), who found a weak negative correlation between blood cortisol concentrations and surface temperatures ($r = -0.209$) in five bovines transported for 8 h. Robertson et al. (51) observed that activation of the ANS leads to catecholamine secretion (52) that can generate physiological effects like tachycardia, tachypnea, and hyperthermia (53, 54). This could be related to the level of stress experienced by the animals, as Hagenmaier et al. observed (55) in 80 calves transported under two levels of handling. Animals under intensive handling, with the use of electric prods and minimal trotting during loading, had higher lactate, epinephrine, norepinephrine, cortisol, and glucose levels but lower levels of blood pH and bicarbonate, with excess bases, compared to the animals that received minimal handling during transport. This means that the external factors which increase the stress response of animals during transport (e.g., higher surface temperatures) include social interaction with handlers and the training that stockpeople receive to mobilize animals of different species, two factors that are fundamental for preventing intense stress responses, even during short trips like those in the present study (2 h ± 20 min). Some authors have reported that even when the trip time is maintained below 12 h, some species are unable to become habituated immediately to the intrinsic and individual factors that cause stress (56, 57).

Another important condition observed during animal transport that needs to be emphasized is neophobia, that is, fear of new situations. This may be another factor involved in stress responses prior to transporting water buffaloes (58, 59). This fear involves activation of the basolateral region of the amygdala, which coordinates responses by the cerebral cortex and hypothalamus, the physiological center that modulates heart and respiratory rates, and body temperature during events that cause fear or anxiety (60, 61).

With respect to the temperature differences detected among the different thermal windows, the minimal increase of 1°C among the facial, skull and nasal regions in comparison with lateral corporal trunk limbs region, and lateral region of the trunk ($p < 0.0001$) can be explained by the extensive distribution of capillaries and arteriovenous anastomoses that allow heat exchange with the environment (62). When mechanisms that preserve thermoneutrality are activated, heat loss by evaporation (as water vapor), convection, or conduction is responsible for the changes in the amount of heat irradiated through the skin (38, 63). Specifically, the thermal windows of head and periocular, and frontal-parietal regions showed a difference of 4°C ($p < 0.0001$), possibly due to distribution of the blood vessels in those areas. For example, the head regions is were irrigated by the facial artery and its branches in the infraorbital artery, which carries blood to the lower eyelid and lacrimal caruncle (24, 64), whereas the frontal-parietal region is supplied by branches (*A. transversa faciei*, *A. auricularis rostralis*, *A. palpebralis inferior lateralis*, *A. palpebralis superior lateralis*) of the superficial temporal artery (*A. temporalis superficialis*) (64). This distribution of blood vessels could confer a thermoregulatory advantage to water buffaloes by serving as a pathway for heat loss. Taylor (65) found that circulation around the horns allowed Toggenburg goats to serve as a pathway for thermoregulation. This could explain the increase in the temperature of the frontal-parietal region recorded in our study.

The thermal windows in the trunk regions, such as the abdominal or thoracic regions, had temperatures 2°C higher than those in the limbs region ($p < 0.0001$). This is related to the presence of vital metabolically active organs, such as the heart and liver, which contribute to raising body temperature as animals try to compensate through peripheral vasomotor changes in the structures of the limbs (66). Temperatures in these regions are produced by blood flow from the metatarsal dorsal and saphenous arteries (64). Changes in the limbs temperatures in dogs and horses have been associated with levels of muscle activity that increase blood flow at the local level (67, 68); however, the specific muscular response was not evaluated in our study.

The thermal windows of the nostrils were the one that presented the lowest temperatures of all nasal region (*Regio nasalis*) ($p < 0.0001$). Although this area has a high density of surface blood vessels from the maxillary artery and vein (24), elimination of water vapor during the respiratory cycle and tachypnea that occurs during stress are two factors that may influence nostril temperatures, leading to a greater loss of water vapor and heat (28). Finally, it is important to mention that a strong, positive, and significant correlation was observed among the thermal windows ($r = 0.9$, $p < 0.0001$). This means that as the temperature of one body region increases, those of other regions also increase because of the greater radiation of surface heat in the different thermal windows (69, 70).

In a similar manner, Napolitano et al. (71) the surface temperature of 109 buffalo calves was evaluated with the aim of recognizing variations in various thermal windows (both regions of the body and head) with respect to their birth weight, finding that the head region registers higher values (except the thermal window of the nostril) and with less variation with respect to pelvic limbs in all groups studied.

The results of the present study confirm the usefulness of the IRT technique for evaluating large ruminants, for example, to identify changes in the surface temperatures of buffaloes subjected to short periods of transport. However, it is important to emphasize that one of the limitations of this study is that we did not evaluate other response variables, such as physiological (e.g., body temperature) and endocrine parameters (e.g., cortisol concentrations), to relate the temperature increases detected to other factors. Likewise, another limitation of the present study could be the lack of monitoring the cleanliness of the animals to prevent that soiling, fecal matter, sweat, or urine could alter the thermal readings. The inclusion of physiological indicators during the pre- and post-transport phases could provide a clearer picture of the stress experienced by water buffaloes during transport. We consider this an important field for future explorations that will assess the degree of relationship between the surface temperatures of thermal windows and physiological indicators that are modified by the effects of transport.

5. Conclusion

According to the surface temperature values of water buffaloes transported for a short period, transport and the practices applied during this activity affect the temperature of various body and head regions. The responses of each region depend largely on the phase in which they are evaluated (from the paddock to post-transport). Herding and loading were identified as the moments when the buffaloes had the most marked stress responses, which increased the recorded thermal values. The strong positive correlations between the central and peripheral windows can help understand the importance of good handling of buffalo species during transport, with the goal of minimizing the potential stress they may experience and physiological responses that can impact their welfare.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the animal study because the animals used in this study were handled gently, without the use of physical utensils that could cause stress, in accordance with the guidelines of the Official Mexican Standards NOM-062-ZOO-1999, which establish technical specifications for the production, care, and ethical use of animals during experimentation; and NOM-051-ZOO which establish technical specifications for humanitarian handling in the mobilization of animals published by the Department of Agriculture, Rural Development, Fisheries and Alimentation. It is also important to consider that written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Grandin T, Shivley C. How farm animals react and perceive stressful situations such as handling, restraint, and transport. *Animals*. (2015) 5:1233–51. doi: 10.3390/ani5040409

2. Rodríguez-González D, Minervino AHH, Orihuela A, Bertoni A, Morales-Canela DA, Álvarez-Macías A, et al. Handling and physiological aspects of the dual-purpose water buffalo production system in the mexican humid tropics. *Animals*. (2022) 12:608. doi: 10.3390/ani12050608

3. José-Pérez N, Mota-Rojas D, Ghezzi MD, Rosmini MR, Mora-Medina P, Bertoni A, et al. Effects of transport on water buffaloes (*Bubalus bubalis*): factors associated with the frequency of skin injuries and meat quality. *J Anim Behav Biometeorol*. (2022) 10:1–9. doi: 10.31893/jabb.22016

4. Gupta S, Earley B, Crowe MA. Effect of 12-hour road transportation on physiological, immunological and haematological parameters in bulls housed at different space allowances. *Vet J*. (2007) 173:605–16. doi: 10.1016/j.tvjl.2006.03.002

5. Sporer KRB, Xiao L, Tempelman RJ, Burton JL, Earley B, Crowe MA. Transportation stress alters the circulating steroid environment and neutrophil gene expression in beef bulls. *Vet Immunol Immunopathol*. (2008) 121:300–20. doi: 10.1016/j.vetimm.2007.10.010

6. Adenkola AY, Ayo JO. Physiological and behavioural responses of livestock to road transportation stress: a review. *Afr J Biotechnol*. (2010) 9:4845–56.

7. Chandra BS, Das N. The handling and short-haul road transportation of spent buffaloes in relation to bruising and animal welfare. *Trop Anim Health Prod*. (2001) 33:155–63. doi: 10.1023/A:1005242815540

8. Bhatt N, Singh N, Mishra A, Kandpal D, Rajneesh R, Jamwal S. A detailed review of transportation stress in livestock and its mitigation techniques. *Int J Livest Res*. (2021) 11:1. doi: 10.5455/ijlr.20201109102902

9. Napolitano F, Orihuela A, AHH M, Strappini AC, Bertoni A, Braghhieri A, et al. Zootechnical management of dual-purpose water buffalo systems in the Mexican humid tropics In: F Napolitano, D Mota-Rojas, A Orihuela, A Braghieri, D Hufana-Duran and AC Strappini et al., editors. *El búfalo de agua en las Américas: Comportamiento y productividad*. 4th ed. Mexico City: B.M. Editores (2022). 69–110.

10. Alarcón-Rojo A, Mota-Rojas D, García-Galicia I, Ramírez-Bribiesca E, Olmos-Hernández A, Guerrero-Legarreta I. Dark cutting in large ruminants: effect of management and environmental factors. *Agro Product*. (2021) 13:93–8. doi: 10.32854/agrop.v13i12.1927

11. Cruz-Monterrosa RG, Mota-Rojas D, Ramírez-Bibriesca E, Mora-Medina P, Guerrero-Legarreta I. Scientific findings on the quality of river buffalo meat and prospects for future studies. *J Buffalo Sci*. (2020) 9:170–80. doi: 10.6000/1927-520X.2020.09.18

12. Van Engen NK, Coetzee JF. Effects of transportation on cattle health and production: a review. *Anim Health Res Rev*. (2018) 19:142–54. doi: 10.1017/S1466252318000075

13. Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet J*. (2004) 168:28–40. doi: 10.1016/S1090-0233(03)00119-9

14. Wong SS, Sun NN, Keith I, Kweon C-B, Foster DE, Schauer JJ, et al. Tachykinin substance P signaling involved in diesel exhaust-induced bronchopulmonary neurogenic inflammation in rats. *Arch Toxicol*. (2003) 77:638–50. doi: 10.1007/s00204-003-0485-4

15. Wong SS, Sun NN, Lantz RC, Witten ML. Substance P and neutral endopeptidase in development of acute respiratory distress syndrome following fire smoke inhalation. *Am J Physiol Cell Mol Physiol*. (2004) 287:L859–66. doi: 10.1152/ajplung.00388.2003

16. Mackenzie A, Drennan M, Rowan T, Dixon J, Carter S. Effect of transportation and weaning on humoral immune responses of calves. *Res Vet Sci*. (1997) 63:227–30. doi: 10.1016/S0034-5288(97)90025-4

17. Liotta L, Costa LN, Chiofalo B, Ravarotto L, Chiofalo V. Effect of lairage duration on some blood constituents and beef quality in bulls after long journey. *Ital J Anim Sci*. (2007) 6:375–84. doi: 10.4081/ijas.2007.375

18. Cueto SL. Factores que influyen en la respuesta productiva y bienestar animal durante el transporte de bovinos. *Universidad Autónoma de Chapingo* (2020). Available at: https://repositorio.chapingo.edu.mx/bitstream/handle/20.500.12098/501/dcig-lcsi_20.pdf?sequence=2 (Accessed August 28, 2022).

19. Averós X, Martín S, Riu M, Serratosa J, Gosálvez LF. Stress response of extensively reared young bulls being transported to growing-finishing farms under Spanish summer commercial conditions. *Livest Sci*. (2008) 119:174–82. doi: 10.1016/j.livsci.2008.04.002

20. Idris M, Uddin J, Sullivan M, McNeill DM, Phillips CJC. Non-invasive physiological indicators of heat stress in cattle. *Animals*. (2021) 11:71. doi: 10.3390/ani11010071

21. Strappini AC, Frankena K, Metz JHM, Gallo C, Kemp B. Characteristics of bruises in carcasses of cows sourced from farms or from livestock markets. *Animal*. (2012) 6:502–9. doi: 10.1017/S1751731111001698

22. Napolitano F, Strappini A, Ramírez-Bribiesca E, Alarcón-Rojo AD, Cruz-Monterrosa R, Guerrero Legarreta I, et al. Estresores previos a la muerte y su efecto en la calidad de la carne del búfalo de agua In: F Napolitano, D Mota-Rojas and IOA Guerrero-Legarreta, editors. *El búfalo de agua en Latinoamérica, hallazgos recientes*. México: BM Editores (2020). 1178–233. Available at: https://www.lifescienceglobal.com/journals/journal-of-buffalo-science/97-abstract/jbs/4550-el-bufalo-de-agua-en-latinoamerica-hallargos-recientes (Accessed December 20, 2022).

23. Bertoni A, Napolitano F, Mota-Rojas D, Sabia E, Álvarez-Macías A, Mora-Medina P, et al. Similarities and differences between river buffaloes and cattle: health, physiological, behavioral and productivity aspects. *J Buffalo Sci.* (2020) 9:92–109. doi: 10.6000/1927-520X.2020.09.12

24. Mota-Rojas D, Pereira MFA, Wang D, Martínez-Burnes J, Ghezzi M, Hernández-Avalos I, et al. Clinical applications and factors involved in validating thermal windows used in infrared thermography in cattle and river Buffalo to assess health and productivity. *Animals.* (2021) 11:2247. doi: 10.3390/ani11082247

25. Vickers LA, Burfeind O, von Keyserlingk MAG, Veira DM, Weary DM, Heuwieser W. Technical note: comparison of rectal and vaginal temperatures in lactating dairy cows. *J Dairy Sci.* (2010) 93:5246–51. doi: 10.3168/jds.2010-3388

26. Mota-Rojas D, Wang D, Titto CG, Gómez-Prado J, Carvajal-de la Fuente V, Ghezzi M, et al. Pathophysiology of fever and application of infrared thermography (irt) in the detection of sick domestic animals: recent advances. *Animals.* (2021) 11:2316. doi: 10.3390/ani11082316

27. Mota-Rojas D, Wang D-H, Titto CG, Martínez-Burnes J, Villanueva-García D, Lezama K, et al. Neonatal infrared thermography images in the hypothermic ruminant model: anatomical-morphological-physiological aspects and mechanisms for thermoregulation. *Front Vet Sci.* (2022) 9:963205. doi: 10.3389/fvets.2022.963205

28. Lowe G, Sutherland M, Waas J, Schaefer A, Cox N, Stewart M. Infrared thermography—a non-invasive method of measuring respiration rate in calves. *Animals.* (2019) 9:535. doi: 10.3390/ani9080535

29. INEGI. *Clima Veracruz de Ignacio de la Llave. Inf por Entid* (2021). Available at: https://cuentame.inegi.org.mx/monografias/informacion/ver/territorio/clima.aspx?tema=me&e=30 (Accessed August 21, 2022).

30. Rodríguez A, Romero E. Evaluación del efecto de diferentes dosis de la fertilización orgánica en la calidad nutricional del pasto camelote (*Paspalum fasciculatum* willd). *Tlatemoani Rev Acad Invest.* (2016) 1:1–14.

31. SEGOB. Norma Oficial Mexicana PROY-NOM-034-SCT2-2003, Señalamiento horizontal y vertical de carreteras y vialidades urbanas. *DOF.* (2004):2–7.

32. De RF, Scaramuzzi RJ. Heat stress and seasonal effects on reproduction in the dairy cow—a review. *Theriogenology.* (2003) 60:1139–51. doi: 10.1016/S0093-691X(03)00126-2

33. Lan Q, Cao Z, Yang X, Gu Z. Physiological and proteomic responses of dairy buffalo to heat stress induced by different altitudes. *Meta.* (2022) 12:909. doi: 10.3390/metabo12100909

34. Cuthbertson H, Tarr G, Loudon K, Lomax S, White P, McGreevy P, et al. Using infrared thermography on farm of origin to predict meat quality and physiological response in cattle (*Bos Taurus*) exposed to transport and marketing. *Meat Sci.* (2020) 169:108173. doi: 10.1016/j.meatsci.2020.108173

35. Rocha LM, Devillers N, Maldague X, Kabemba FZ, Fleuret J, Guay F, et al. Validation of anatomical sites for the measurement of infrared body surface temperature variation in response to handling and transport. *Animals.* (2019) 9:425. doi: 10.3390/ani9070425

36. Alam M, Hasanuzzaman M, Hassan MM, Rakib TM, Hossain ME, Rashid MH, et al. Assessment of transport stress on cattle travelling a long distance (≈648 km), from Jessore (Indian border) to Chittagong, Bangladesh. *Vet Rec Open.* (2018) 5:e000248–10. doi: 10.1136/vetreco-2017-000248

37. Hong H, Lee E, Lee IH, Lee S-R. Effects of transport stress on physiological responses and milk production in lactating dairy cows. *Asian Aust J Anim Sci.* (2019) 32:442–51. doi: 10.5713/ajas.18.0108

38. Mota-Rojas D, Titto CG, Orihuela A, Martínez-Burnes J, Gómez-Prado J, Torres-Bernal F, et al. Physiological and behavioral mechanisms of thermoregulation in mammals. *Animals.* (2021) 11:1733. doi: 10.3390/ani11061733

39. Kataoka N, Hioki H, Kaneko T, Nakamura K. Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown adipose tissue thermogenesis and hyperthermia. *Cell Metab.* (2014) 20:346–58. doi: 10.1016/j.cmet.2014.05.018

40. Nakamura K. Neural circuit for psychological stress-induced hyperthermia. *Temperature.* (2015) 2:352–61. doi: 10.1080/23328940.2015.1070944

41. Oka T. Stress-induced hyperthermia and hypothermia. *Handb Clin Neurol.* (2018) 157:599–621. doi: 10.1016/B978-0-444-64074-1.00035-5

42. Wang L, Liu F, Luo Y, Zhu L, Li G. Effect of acute heat stress on adrenocorticotropic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. *Biomed Reports.* (2015) 3:425–9. doi: 10.3892/br.2015.445

43. Agnes F, Sartorelli P, Abdi B, Locatelli A. Effect of transport loading or noise on blood biochemical variables in calves. *Am J Vet Res.* (1990) 51:1679–81.

44. Mehdid A, Martí-De Olives A, Fernández N, Rodríguez M, Peris C. Effect of stress on somatic cell count and milk yield and composition in goats. *Res Vet Sci.* (2019) 125:61–70. doi: 10.1016/j.rvsc.2019.05.015

45. Al-Qarawi AA, Ali BH. Isolation stress in desert sheep and goats and the influence of pretreatment with xylazine or sodium betaine. *Vet Res Commun.* (2005) 29:81–90. doi: 10.1023/B:VERC.0000046741.97331.29

46. Sanhouri AA, Jones RS, Dobson H. Effects of xylazine on the stress response to transport in male goats. *Br Vet J.* (1992) 148:119–28. doi: 10.1016/0007-1935(92)90103-8

47. Sartorelli P, Dominoni S, Agnes F. Influence of duration of simulated transport on plasma stress markers in the calf. *J Vet Med Ser A.* (1992) 39:401–3. doi: 10.1111/j.1439-0442.1992.tb00198.x

48. Mitchell G, Hattingh J, Ganhao M. Stress in cattle assessed after handling, after transport and after slaughter. *Vet Rec.* (1988) 123:201–5. doi: 10.1136/vr.123.8.201

49. Ali-Gholi R, Siamak AR, Daryoush M. Influence of the short-term road transport stress on blood parameters in cows. *Med Weter.* (2007) 63:1311–5.

50. Sakakibara S, Arai S, Hukui Y, Sawada H. Fluctuation of body surface temperature in calves during transportation using infrared thermography. *J Japan Vet Med Assoc.* (2014) 67:125–8. doi: 10.12935/jvma.67.125

51. Robertson JK, Mastromonaco G, Burness G. Evidence that stress-induced changes in surface temperature serve a thermoregulatory function. *J Exp Biol.* (2020) 223:jeb213421. doi: 10.1242/jeb.213421

52. Krakoff LR. Glucocorticoid excess syndromes causing hypertension. *Cardiol Clin.* (1988) 6:537–45. doi: 10.1016/S0733-8651(18)30475-2

53. Oka T, Oka K, Hori T. Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosom Med.* (2001) 63:476–86. doi: 10.1097/00006842-200105000-00018

54. Sapolsky RM. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev.* (2000) 21:55–89. doi: 10.1210/er.21.1.55

55. Hagenmaier JA, Reinhardt CD, Bartle SJ, Henningson JN, Ritter MJ, Calvo-Lorenzo MS, et al. Effect of handling intensity at the time of transport for slaughter on physiological response and carcass characteristics in beef cattle fed ractopamine hydrochloride. *J Anim Sci.* (2017) 95:1963. doi: 10.2527/jas2016.0821

56. Gallo C, Lizondo G, Knowles TG. Effects of journey and lairage time on steers transported to slaughter in Chile. *Vet Rec.* (2003) 152:361–4. doi: 10.1136/vr.152.12.361

57. Valadez Noriega M, de la Lama GC. Implications, trends, and prospects for long-distance transport in cattle. Review. *Rev Mex Ciencias Pecu.* (2020) 11:517–38. doi: 10.22319/rmcp.v11i2.4767

58. Flores-Peinado S, Mota-Rojas D, Guerrero-Legarreta I, Mora-Medina P, Cruz-Monterrosa R, Gómez-Prado J, et al. Physiological responses of pigs to preslaughter handling: infrared and thermal imaging applications. *Int J Vet Sci Med.* (2020) 8:71–84. doi: 10.1080/23144599.2020.1821574

59. Stewart M, Webster JR, Verkerk GA, Schaefer AL, Colyn JJ, Stafford KJ. Non-invasive measurement of stress in dairy cows using infrared thermography. *Physiol Behav.* (2007) 92:520–5. doi: 10.1016/j.physbeh.2007.04.034

60. Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron.* (2005) 48:175–87. doi: 10.1016/j.neuron.2005.09.025

61. Angilletta MJ, Youngblood JP, Neel LK, VandenBrooks JM. The neuroscience of adaptive thermoregulation. *Neurosci Lett.* (2019) 692:127–36. doi: 10.1016/j.neulet.2018.10.046

62. Tattersall GJ. Infrared thermography: a non-invasive window into thermal physiology. *Comp Biochem Physiol Part A Mol Integr Physiol.* (2016) 202:78–98. doi: 10.1016/j.cbpa.2016.02.022

63. Romanovsky AA. Skin temperature: its role in thermoregulation. *Acta Physiol.* (2014) 210:498–507. doi: 10.1111/apha.12231

64. International Committee Veterinary Gross Anatomical Nomenclature. *Nomina Anatómica veterinaria.* (2017), Available at: https://uia.org/s/or/en/1100039338 (Accesed November 30, 2022).

65. Taylor CR. The vascularity and possible thermoregulatory function of the horns in goats. *Physiol Zool.* (1966) 39:127–39. doi: 10.1086/physzool.39.2.30152426

66. Isola JVV, Menegazzi G, Busanello M, dos Santos SB, Agner HSS, Sarubbi J. Differences in body temperature between black-and-white and red-and-white Holstein cows reared on a hot climate using infrared thermography. *J Therm Biol.* (2020) 94:102775. doi: 10.1016/j.jtherbio.2020.102775

67. Casas-Alvarado A, Martínez-Burnes J, Mora-Medina P, Hernández-Avalos I, Domínguez-Oliva A, Lezama-García K, et al. Thermal and circulatory changes in diverse body regions in dogs and cats evaluated by infrared thermography. *Animals.* (2022) 12:789. doi: 10.3390/ani12060789

68. Mota-Rojas D, Martínez-Burnes J, Casas-Alvarado A, Gómez-Prado J, Hernández-Ávalos I, Domínguez-Oliva A, et al. Clinical usefulness of infrared thermography to detect sick animals: frequent and current cases. *CABI Rev.* (2022) 2022:1–17. doi: 10.1079/cabireviews202217040

69. Brcko CC, da SJAR, Martorano LG, Vilela RA, Nahúm B d S, AGM S, et al. Infrared thermography to assess thermoregulatory reactions of female buffaloes in a humid tropical environment. *Front Vet Sci.* (2020) 7:180. doi: 10.3389/fvets.2020.00180

70. Mota-Rojas D, Napolitano F, Braghieri A, Guerrero-Legarreta I, Bertoni A, Martínez-Burnes J, et al. Thermal biology in river buffalo in the humid tropics: neurophysiological and behavioral responses assessed by infrared thermography. *J Anim Behav Biometeorol.* (2021) 9:1–12. doi: 10.31893/jabb.21003

71. Napolitano F, Bragaglio A, Braghieri A, el-Aziz AHA, Titto CG, Villanueva-García D, et al. The effect of birth weight and time of day on the thermal response of newborn water buffalo calves. *Front Vet Sci.* (2023) 10:1084092. doi: 10.3389/fvets.2023.1084092

Resultados de la fase ante mortem; Transporte largo vs corto

Artículo experimental original intitulado:

9.2. CAPÍTULO IV

Balance térmico en búfalos de agua machos transportados en viajes cortos y largos

Publicado el 20 de octubre de 2023 en la revista *Animals*, misma que se encuentra indexada al JCR con un factor de impacto de 3, volumen 13, número 20, páginas 1-18.

Así mismo, este artículo fue seleccionado e incluido en el Special Issue "Water Buffalo Welfare, Strategies to Improve Health, Behavior, Productivity, and Food Quality and Safety" publicado por la editorial Animals en su sección de Reseach Topics editado por el doctor Daniel Mota Rojas.

Rodríguez-González, D., Legarreta, I. G., Chay-Canul, A., Hernández-Ávalos, I., Napolitano, F., García-Herrera, R., Pereira, A. M., Domínguez-Oliva, A., Casas-Alvarado, A., & Mota-Rojas, D. Thermal balance in water buffaloes transported by long journeys. *Animals*, **2023.** 13(20), 1–18. https://doi.org/https://doi.org/10.3390/ani13203274

animals

Article

Thermal Balance in Male Water Buffaloes Transported by Long and Short Journeys

Daniela Rodríguez-González [1], Isabel Guerrero Legarreta [2], Alfonso Chay-Canul [3], Ismael Hernández-Avalos [4], Fabio Napolitano [5,†], Ricardo García-Herrera [3], Alfredo M. F. Pereira [6], Adriana Domínguez-Oliva [7], Alejandro Casas-Alvarado [7], Brenda Reyes-Sotelo [7] and Daniel Mota-Rojas [7,*]

1 Master in Science Program [Maestría en Ciencias Agropecuarias], Universidad Autónoma Metropolitana (UAM), Xochimilco Campus, Mexico City 04960, Mexico
2 Department of Biotechnology: Food Science, Universidad Autónoma Metropolitana, Iztapalapa Campus (UAM-I), Mexico City 09340, Mexico
3 División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Villahermosa 86040, Mexico
4 Department of Biological Science, FESC, Universidad Nacional Autónoma de México (UNAM), Cuautitlán 04510, Mexico
5 Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, 85100 Potenza, Italy
6 Mediterranean Institute for Agriculture, Environment and Development (MED), Institute for Advanced Studies and Research, Universidade de Évora, 7006-554 Évora, Portugal
7 Neurophysiology, Behavior, and Animal Welfare Assessment, Department of Animal Production and Agriculture (DPAA), Universidad Autónoma Metropolitana (UAM) Xochimilco Campus, Mexico City 04960, Mexico
* Correspondence: dmota100@yahoo.com.mx
† In honor of his memory.

Citation: Rodríguez-González, D.; Guerrero Legarreta, I.; Chay-Canul, A.; Hernández-Avalos, I.; Napolitano, F.; García-Herrera, R.; Pereira, A.M.F.; Domínguez-Oliva, A.; Casas-Alvarado, A.; Reyes-Sotelo, B.; et al. Thermal Balance in Male Water Buffaloes Transported by Long and Short Journeys. *Animals* **2023**, *13*, 3274. https://doi.org/10.3390/ani13203274

Academic Editor: Umberto Bernabucci

Received: 24 September 2023
Revised: 14 October 2023
Accepted: 17 October 2023
Published: 20 October 2023

Simple Summary: Livestock transport is a stressor with potential physiological, psychological, and financial consequences that can alter animal welfare, particularly in water buffalo, a species that has certain anatomical characteristics that make them susceptible to heat stress. Stress-induced hyperthermia is frequently observed in mobilized livestock, making it a parameter that could help to evaluate this event. Infrared thermography (IRT) is a tool that non-invasively assesses the thermal state of animals. Therefore, this study aimed to evaluate the surface temperature of water buffaloes monitored from the pasture to post-transport on short (SJs) and long journeys (LJs). When considering both groups, the highest temperatures were observed in the frontal-parietal region, while the lowest temperatures were registered in the nostrils. Moreover, a strong correlation was observed in all thermal windows. It is concluded that IRT can be used to accurately assess the thermal changes in buffaloes during transport.

Abstract: Transport is a stressor that can cause physiological and metabolic imbalances in livestock, resulting in stress-induced hyperthermia. In water buffaloes, studies regarding the thermal state of animals during mobilization are scarce. Therefore, this study aimed to compare the thermal response of 1516 water buffaloes using infrared thermography (IRT) during 15 short trips (783 animals, 60,291 records, average duration = 50.33 min ± 5.48 min) and 14 long trips (733 animals, 56,441 records, average duration = 13.31 h ± 47.32 min). The surface temperature was assessed in 11 regions (periocular, lacrimal caruncle, nasal, lower eyelid, auricular, frontal-parietal, pelvic limb, torso, abdominal, lumbar, and thoracic) during seven phases from pasture to post-transport. It was found that the surface temperature of the periocular, lacrimal caruncle, nasal, auricular, frontal-parietal, pelvic limb, torso, abdominal, lumbar, and thoracic regions was significantly higher during SJs (+3 °C) when compared to LJs ($p < 0.0001$). In particular, the frontal-parietal region had a significant increase of 10 °C during the post-transport phase ($p < 0.0001$) in both groups, recording the highest temperatures during this phase. Likewise, a strong positive significant correlation between the different regions was found ($r = 0.90$, $p < 0.0001$). It is worth mentioning that the herding, loading, pre-, and post-transport phases were the ones where the greatest thermal response was recorded, possibly due to the influence of human interaction. Finally, a strong positive correlation (r above 0.9, $p > 0.001$) between the periocular, lacrimal caruncle, pinna, and pelvic limb was found. According to the results, SJ could be considered a stressful event that hinders thermal generation, contrarily to LJ.

Keywords: water buffalo; infrared thermography; journey time; thermostability; stress-induced hyperthermia

1. Introduction

Animal transport is an almost mandatory event for livestock. It is recognized as a stressor due to several elements such as the type of vehicle, load density, type of road, vibrations, and the presence of unknown animals [1]. The frequency of injuries during transport is another issue that is more prevalent in water buffalo than in *Bos cattle* [2]. This might lead to the activation of the hypothalamic-pituitary-adrenal (HPA) axis [3–6]. The activation of this axis causes physiological alterations such as tachycardia, changes in the respiratory pattern, and metabolic modifications that can impact animal health and meat quality, making it necessary to evaluate the stress level that buffaloes might perceive during transport [3–5,7–10]. For example, increases in cortisol, lactate, serum protein, and hyperglycemia have been reported in cattle mobilized in larger distances of up to 16 h [11,12], together with behavioral alterations such as the increase in defecation, falls, and aggression [13,14]. As mentioned, the distance or duration of the journey is a critical factor that can hinder the health and mental state of animals when transported, causing weight loss and physical exhaustion [12], particularly when the type of vehicle and load density are not adequate for the species [15].

Currently, stress-related biomarkers are used to assess livestock welfare during transport such as blood or salivary cortisol levels. Nonetheless, it might not be easy to implement routine evaluations using these markers. As an alternative, infrared thermography (IRT) has been suggested to evaluate the surface thermal response of animals associated with stress. According to some studies, when an animal perceives a stressor, the activation of the HPA axis promotes catabolic metabolism and, consequently, an increase in body temperature [16,17]. The increase in core temperature can be reflected as a greater amount of radiated heat, an element that is detected through IRT. In water buffaloes, it has been reported that regions such as the ocular, pelvic limb, and frontal-parietal show increases between 3 and 5 °C after transport. This could be due to the limited thermoregulatory efficiency of water buffaloes due to the scarce presence of sweat glands, which makes them susceptible to heat stress or stress-induced hyperthermia [18].

Currently, there are no comparative studies on water buffalo assessing the effect of short and long journeys on the thermal state of buffaloes. Therefore, this study aimed to compare short (SJs) and long journeys (LJs) on the thermal response of the central and peripheral body regions of male water buffaloes, using IRT. It was hypothesized that LJs would record the highest temperature in central and peripheral regions, contrary to SJs.

2. Materials and Methods

2.1. Study Location

Short journeys (SJs) of water buffaloes were performed in south-southeast Mexico from June 2021 to August 2022. The average ambient temperature of the production unit where the animals were shipped was 31 ± 2 °C, with a relative humidity of 86%, 20 m.a.s.l., and annual rainfall of 1500–2000 mm (tropical humid climate). For long journeys (LJs), the buffaloes were monitored in the post-transport phase in the production unit of destination, having an average ambient temperature of 13.18 ± 1.42 °C, relative humidity of 63%, an elevation of 2807 m.a.s.l., and annual rainfall of 1679 mm (warm and temperate climate) [19].

2.2. Animals

SJs and LJs were classified according to their duration, where SJs had a mean duration of 50.33 ± 5.48 min and LJs had an average duration of 13.31 h ± 47.32 min. A total of 783 and 733 male buffaloes Buffalypso were included in SJs and LJs, respectively. For SJs, the animals were mobilized in 15 journeys, distributing the animals according to trucks' the capacity of the first floor as follows: 41, 56, 46, 50, 48, 51, 57, 58, 53, 49, 52, 54, 56, 40, and 47. For LJs, the distribution during the 14 journeys was 53, 52, 55, 53, 54, 53, 51, 52, 53, 52, 53, 51, 51, and 50. For both groups, the inclusion criterion was that the selected animals had to be Buffalypso males with an approximate weight of 245 ± 19.36 kg, close to being mobilized to fattening and fattening units. On the contrary, clinically ill animals were considered an exclusion criterion.

All animals were from the same farm mentioned in subtopic 2.1. Regarding the distance traveled according to the groups, it was 27.5 km for SJs at an average speed of 55 km/h, while it was 732 km for LJs, with the same average speed. For both groups, water buffaloes were gently handled from the paddock without using physical tools or shouting that could cause injuries or pain.

2.3. Road Characteristics and Vehicle Type

The topography of the traveled roads for both SJs and LJs was classified as unpaved or paved according to the specifications described by Mexico's Internal Ministry [20]. For unpaved roads, the classification was E and presented a maximum slope of 13%. During this route, a maximum speed of 25 km/h was reached, traveling 10 km of dirt roads for both SJs and LJs at the exit of the original production unit 6.3 km for SJs, and 13.2 km for LJs at the entrance of the reception production unit. On the other hand, for the paved road, a distance of 11.2 km was traveled in SJs and 718.8 km for LJs. This type of road had a maximum transversal slope of 7%; due to the characteristics of this road, a maximum speed of 55 km/h was reached. Vertical curves of 5m/% and a road of 8 m width was present in SJs and LJs.

Regarding the characteristics of the vehicle used, the truck had the following dimensions: 15.24 m long × 2.59 m wide × 4.6 m high. The truck had two floors of sliding side doors and a main door in the rear with a guillotine system. The walls were made of galvanized steel and aluminum, with ventilation openings, a non-slip floor, and reinforced plastic roof, and fiberglass.

2.4. Experimental Phases

The experimental protocol was divided in seven phases, described in Table 1.

2.5. Monitoring by Infrared Thermography

An infrared camera FLIR® Thermal TM E60 (FLIR Systems, Wilsonville, OR, USA) was used. The camera had an IR resolution of 320 × 240 pixels, thermal sensitivity of <0.045 °C, and precision ± 2 °C or 2%. The thermal images were taken at an approximate distance of 1–1.5 m from the animals. After thermal imaging, the images were saved as JPG files to obtain the maximum, minimum, and average values using FLIR software (Version 6.4. FLIR Systems, USA). Six regions were evaluated: facial regions (*Regiones faciei*): the orbital region (*Regio orbitalis*) delimiting the lacrimal caruncle (1), periocular area (2), lower eyelid (*Regio palpebralis inferior*) (3), nasal region (*Regio nasalis*) (4). The skull regions (*Regiones cranii*) were the auricular (*Regio auricularis*) (5) and frontal-parietal region (*Regio frontalis-parietalis*) (6) (Figure 1).

Table 1. Description of the characteristics of each evaluation phases in both SJs and LJs.

Phases	Description	Characteristics
P1	Paddock	Considered as basal monitoring. The animals were housed in paddocks whose feeding system is based on pastures, providing 50 g of mineral supplements per animal. In each paddock, the presence of native grasses (*Paspalum fasciculatum*) (dry matter = 14.7%, 56.7% neutral detergent fiber, 38.8% acid detergent fiber and 6.2% raw protein [21].
P2	Herding on horseback	Animals with and appropriate body weight were selected. Handlers moved the buffaloes with the help of horses and without the use of physical tools that could cause injuries. For both SJs and LJs, the average duration of this phase was between 25 and 35 min.
P3	Corral system	Once the animals entered the pen, they remained for 12 consecutive hours (including the night before weighing, handling, and transport). The pen surface area was 630 m2 with a dirt floor. It was delimited by metal structures with 1.6 m height and two doors, of which one connected to the handling chute (P4). The animal did not receive solid food during the last 8 h but had water ad libitum.
P4	Handling chute	The handling chute hallway dimensions were 1.6 m 1 m wide × 1.6 m height × 3.5 m long. It was connected to an individual weight scale and a loading ramp. The individual passing of animals through the ramp had an average duration of 50 min.
P5	Loading	Buffaloes were monitored for 30–50 min during their passage on a loading ramp with anti-slip grooving with a slope of 20°.
P6	Pre transport	Monitorization was performed after the animal was loaded into the truck, inside the vehicle.
P7	Post transport	Thermal monitoring was carried out when the animals were still inside the vehicle, in the shade, after arriving at the reception site.

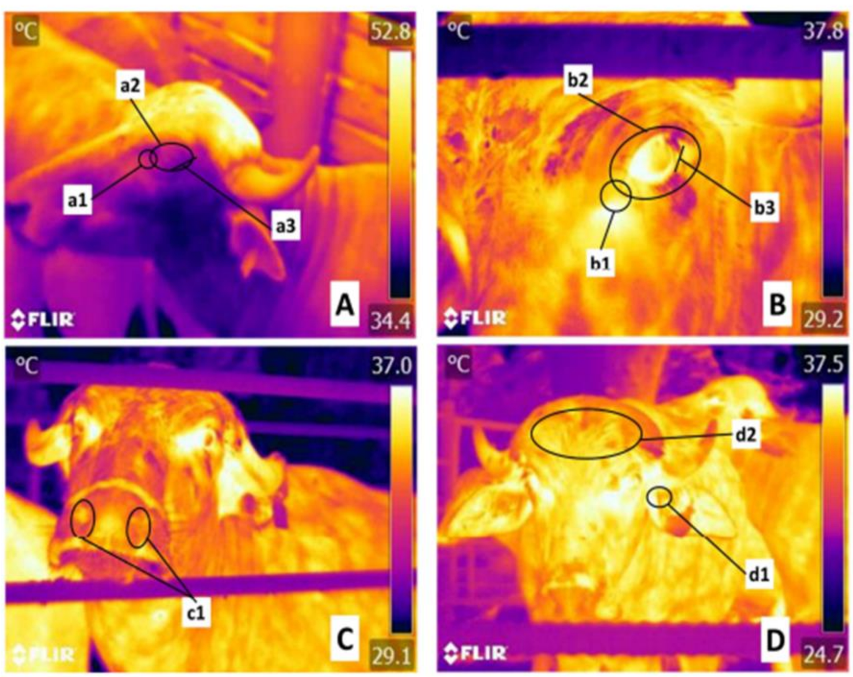

Figure 1. Thermal windows assessed in the head. (**A**,**B**). In the orbital region (*Regio orbitalis*), the lacrimal caruncle was delimited with a circle (**a1**,**b1**), from the medial commissure of the eyelids up to

half a centimeter toward the cranium. The periocular thermal window (circles (**a2**,**b2**)) extends above the upper and lower eyelid. The lower eyelid (**a3**,**b3**) was defined by a line of approximately 3 cm, from which the conjunctival irrigation can be assessed. In the skull region (*Regiones cranii*) (**C**,**D**), nostril, auricular, and frontal-parietal windows were delimited. The nostrils (**c1**) were marked by ovals. For the auricular evaluation (*Regio auricularis*), a circle was used to mark the auditory canal (**d1**). Lastly, the frontal-parietal region (*Regio frontalis*) was delimited by a circle (**d2**), a zone irrigated by cornual and supraorbital arteries.

The trunk region (*Truncus regionis*), the thoracic (7) and abdominal (8) regions, and regions of the pelvis limb (*Regiones membri pelvini*) (9) were delimited with two anatomical regions indicating the femoral region (*Region femoris*) and the tarsus region (*Regio tarsi*) (Figure 2A). Figure 2B shows the last 2 thermal windows considered in this study: the thoracic vertebral region (*Regio vertebralis thoracis*) (10) and lumbar region (*Regio lumbalis*) (11).

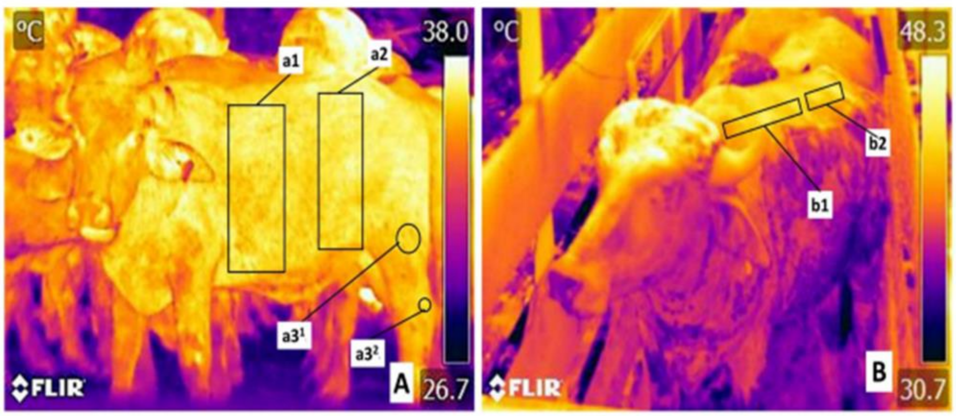

Figure 2. Evaluated thermal windows in the trunk region (*Truncus regionis*). (**A**) The coastal region (*Regio costalis*) was marked by a rectangle (**a1**) in the costal arch from 1° to 12° costal space. The cranial abdominal region (*Regio abdominis cranialis)* was delimited by a rectangle (**a2**) where the oblique abdominal and rectus abdominis muscles are present. The thermal window for the pelvic limbs (*Regiones membri pelvini*) was defined by two circles at the *Regio femoris* (**a3^1**) and at the *Regio tarsi* (**a3^2**), including the femoral muscle and the tarsus region. (**B**) Vertebral column (*Columna vertebralis*) thermal windows. The thoracic vertebral region (*Regio vertebralis thoracis*) is marked with a rectangle (**b1**), covering the thoracic vertebrae (1a to 12a) and the width of the transverse processes. The lumbar vertebral region (*Regio vertebralis lumbalis*) (**b2**) was delimited by a rectangle above the transverse processes of lumbar vertebrae L1–L7.

Table 2 summarizes the experimental phases, its characteristics, hours, and ambient elements such as temperature and relative humidity.

2.6. Statistical Analysis

Statistical analyses were performed with GraphPad Prism (ver. 10.0.2; San Diego, CA, USA). Descriptive statistics were obtained for each thermal window (periocular, lacrimal caruncle, lower eyelid, auditory canal, nostrils, frontal-parietal area, and the thoracic, abdominal, appendicular, lumbar, and dorsal areas) and event (P1–P7). Values are expressed as mean ± standard error (SE). The Shapiro–Wilk test was used for normality analysis.

Table 2. Specifications of each experimental phase (hours, ambient temperature, and relative humidity) for both SJ and LJ.

Phases	Description	Hours	Ambient Temperature (AT) and Relative Humidity (RH)
P1 Paddock	Prior to the buffaloes being herded by men on horseback, while they were at rest for one hour under shade	Between 17:00 and 18:00	AT-23–25 °C RH-81–90%
P2 Herding	Inside the corral on the day prior to transport	Between 18:00 and 18:30	AT-21–25 °C RH-81–88%
P3 Corral	Before entering the handling chute to be weighed and loaded	08:00	AT-20–23 °C RH-81–86%
P4 Chute	While the buffaloes were inside the handling chute	09:00	AT-22–25 °C RH-81–88%
P5 Loading	During loading, with the buffaloes walking up the ramp	10:00	AT-22–27 °C RH-81–88%
P6 Pre transport	After loading the truck, with the motor shut off	11:00	AT-22–28 °C RH-81–89%
P7 Post transport	Immediately after reaching the destination for both short and long trips, with the truck's motor still turned on	SJ-11:50 ± 5.48 LJ-00:31 ± 47.32	AT-23–30 °C RH-83–94% AT-10.5–15.7 °C RH-84–46%

The independent variables were the events and thermal windows, while the surface temperature was considered as the dependent variable. An analysis of variance (ANOVA) in a linear mixed model was used as follows:

$$Y_{ijak} = \mu + \tau a + \tau i + \tau j + \tau k + \tau a \tau i \tau j \tau k + \beta k + e_{aijk}$$

where:

Y = response variable (surface temperature)
τa = effect of the transport
τi = effect of the thermal window
τj = effect of the event
τk = effect of transport duration
τiτjτk = interaction effect
β = random effect (animal)
μ = population mean
e = error

To calculate the sample size, the G *Power program (ver. 3.1) was used considering an error probability of 0.05, a confidence level of 95%, a power (1-error probability) of 0.95 and a correction between repeated measures of 0.5 (total sample size = 700 animals per group) for two experimental groups with fixed effects, main effects and interactions of eleven thermal windows and seven event measurements.

A post-hoc Tukey test analyzed the differences between means. The level of significance was set at $p < 0.05$. Correlation analysis was conducted using the Pearson's correlation coefficient.

2.7. Ethics Statement

The Scientific Commission of the Master's in Science (CAMCA.11.21) "Maestría en Ciencias Agropecuarias" of the Faculty of Veterinary Medicine and Animal Husbandry, Universidad Autónoma Metropolitana, Mexico City, Mexico approved the methodology of the present research.

Animal ethical handling was performed following the Official Mexican Standards NOM-051-ZOO [22]. The animals included in the present study were not touched or stressed, since infrared thermography is a non-invasive technique.

3. Results

During the seven phases, 116,732 surface temperature readings were obtained from 11 thermal windows of 783 water buffaloes transported on SJs (50.33 ± 5.48 min) and 733 water buffaloes transported on JLs (13:31 h ± 47.32 min). Figure 3 shows the mean ± SE temperatures of the thermal windows in the head region (*Regiones capitis*).

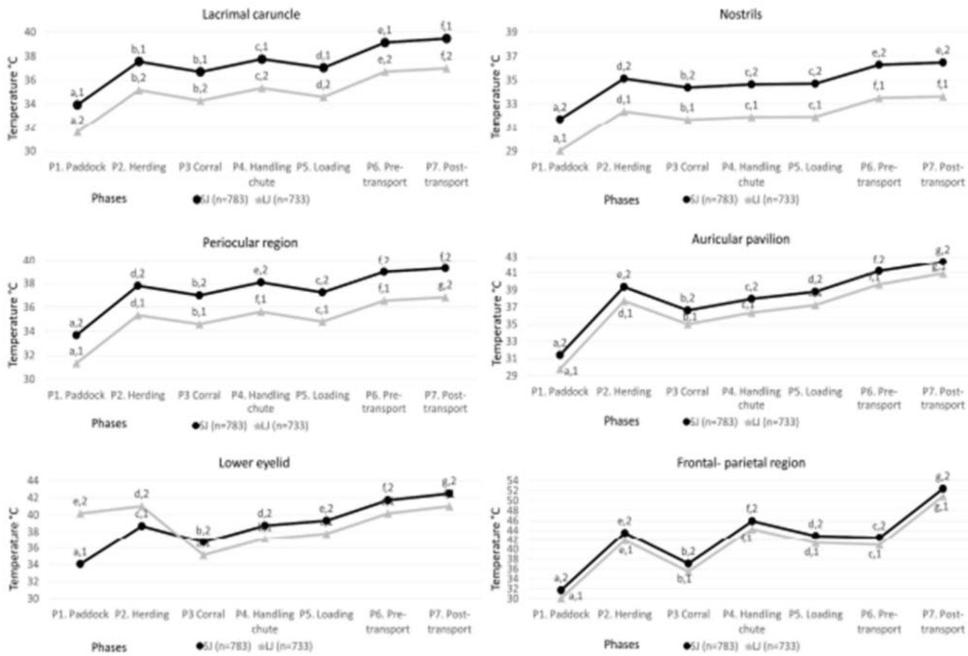

Figure 3. Mean ± standard error (SE) temperatures for the thermal windows of the head region (*Regiones capitis*) of SJ and LJ water buffaloes in the seven phases. Different literals [a, b, c, d, e, f, g] indicate statistically significant differences among the temperatures of the animals according to the phase (p value < 0.001). Different numbers [1,2] indicate statistically significant differences between short and long journeys of the thermal windows (p value < 0.001).

The results obtained for the lacrimal caruncle in the SJ group show that the lowest temperature was recorded during P1, being 5.53 °C lower than P7, the phase with the highest temperature ($p < 0.001$). P2, P3, P4, and P5 did not have significant differences, with a maximum variation of 1.06 °C between P3 and P4 ($p < 0.001$). When comparing SJs and LJs, regardless of the phase, the highest temperatures were observed in SJs ($p < 0.001$), reporting the highest differences during P7 of up to 2.49 °C ($p < 0.001$).

For the periocular region, greater differences were observed in SJs. The lowest temperature was registered during P1 with a maximum variation of 5.59 °C in comparison to P7 ($p < 0.001$. This last phase was statistically similar to P6 with a minimum difference of 0.3 °C ($p < 0.001$). Regarding the LJ group, a maximum variation was observed between P1 and P7 (of up to 5.5 °C, $p < 0.001$), followed by the phases where human–animal interaction was present (P6, P4, P2, and P5).

Regarding the lower eyelid, SJs had the lowest temperature during P1, differing by 8.45 °C when compared with P7, followed by P6, P5, P4, and P2 (7.67, 5.25, 4.69, and 4.63 °C, respectively, $p < 0.001$). Regarding the LJ group, the lowest temperature was observed in P3 with a difference of 5.88° when compared with P7 and P2 ($p < 0.001$), followed by P1 and P6, a difference of 5.02; both were without numerical and statistical differences ($p > 0.001$).

When comparing both groups, the greatest variation was observed in LJ buffaloes during P1, differing by 6.11 °C from SJ ($p < 0.001$).

For the nostril window in SJs, the greatest differences were observed during P1 and P7, with a statistically significant difference of 4.76 °C ($p < 0.001$). In contrast, similar values were maintained during P4 and P5 with minimum differences of 0.06 °C ($p > 0.001$). The highest temperatures were recorded in the phases where human interaction was present. For the LJ group, the greatest temperature variation was observed between P1 and P7 (4.61 °C, $p < 0.001$). In contrast to SJs, no significant differences were observed in the phases that had human interaction; this increase was only observed in phases 2, 6, and 7, (+3.33, +2.65, and +4.61 °C, respectively, $p < 0.001$). Regarding the differences between groups, the greatest numerical increase was observed in P7 in SJs, being 3.1 °C higher than LJ.

For the region of the skull (*Cranii regions*), specifically in the auricular thermal window, SJs had a marked difference and variation between phases. P1 presented the lowest temperature when compared with P7, recording a difference of 10.97 °C ($p < 0.001$), followed by P6, P2, and P5 with differences of 9.81, 7.95, and 7.38 °C, respectively ($p < 0.001$). As observed in the facial thermal windows (except the lower eyelid), a temperature drop in the LJ group was observed ($p < 0.001$). For the LJ group, there were differences in temperature of up to 13.16 °C when comparing P1 and P7 ($p < 0.001$). Regarding the differences found between groups, during P7, SJs differed by 1.45 °C from LJ animals.

Regarding the frontal-parietal region of the SJ group, marked differences were observed in all phases. P1 and P7 had differences of up to 20.7 °C, followed by phases with greater human handling (P4, P2, P5, and P6) with a temperature increase of 14.19, 11.78, 11.15, and 10.79 °C, respectively ($p < 0.001$). Regarding the LJ group, as in SJs, the lowest values were observed in P1; however, the variation was greater when compared with P7, with a difference of 20.86 °C ($p < 0.001$).

Figure 4 shows the temperatures regarding the results of the lateral region of the trunk divided into the thoracic and abdominal windows and the region of the extremities (pelvic limb).

Regarding the thoracic thermal window of the SJ group, significant differences were obtained in all phases of transport ($p < 0.001$), with the highest values in P7, P6, and P2, with differences of 12.67, 9.9, and 8.76 °C compared to P1 ($p < 0.001$) (lower values). Likewise, temperatures differed between P3 P2, and P4, where the animals had greater handling and movement by the operators (5.35 and 2.56 °C, respectively, $p < 0.001$). For the LJ group, the greatest variation between phases was also observed between P1 and P7, with an increase of 12.81 °C ($p < 0.001$) and an increase of 2.83 °C between pre- and post-transport ($p < 0.001$). Regarding the differences between groups (SJ and LJ), higher temperatures were observed in SJ buffaloes, with minimum differences of 0.97 °C ($p < 0.001$) in P7 and a maximum of 1.11 °C ($p < 0.001$) in P1 and P4.

For the abdominal thermal window, it was observed that SJ animals had the highest temperature during P7 and P6 with an increase of 11.89 and 8.85 °C, respectively, compared to P1 ($p < 0.001$). In phases where human–animal interaction was present, an increase of 2.52 °C was reported. In the LJ group, a different trend was observed from the rest of the thermal windows because the highest temperature was reported in P6 (13.01 °C higher than P1, $p < 0.001$). Regarding the comparison between SJ and LJ, differences were observed. P5 and P6 of LJ animals were higher than in SJ with differences of 1.01 °C ($p < 0.001$) and 2.84 °C ($p < 0.001$), respectively.

Regarding the pelvic limb of the SJ group, a similar trend was observed with the highest temperature recorded in P6, with an increase of 5.55 °C vs. P1 ($p < 0.001$), followed by P4, P2, and P7 increases of 5.48, 5.34, and 5.06 °C ($p < 0.001$). When comparing SJ and LJ, higher temperatures were observed in SJ regardless of the phase ($p < 0.001$), and higher variations were reported than in the lateral region of the trunk, with an average difference of 1.19 °C vs. 2.76 °C in the region of the extremities.

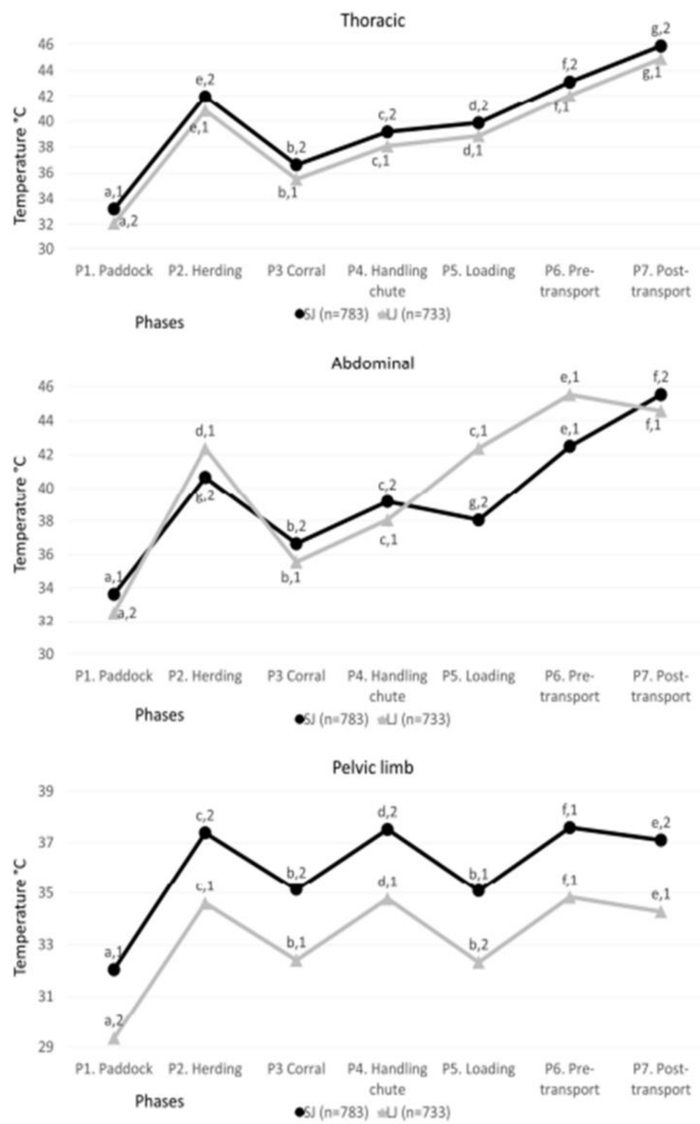

Figure 4. Mean ± standard error (SE) of the temperatures in the thermal windows of the lateral region of the trunk of SJ (short journey) and LJ (long journey) water buffaloes in the seven phases. Different literals [a, b, c, d, e, f, g] indicate statistically significant differences among the temperatures of the animals according to the phase (p value < 0.001). Different numbers [1,2] indicate statistically significant differences between short and long journeys of the thermal windows (p value < 0.001).

The thermal results obtained from the thoracic and lumbar vertebral region windows are seen in Figure 5.

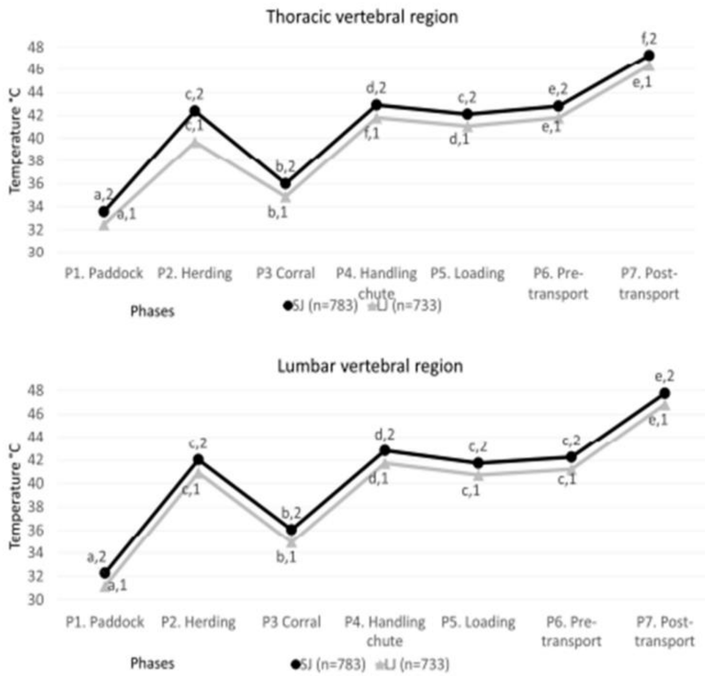

Figure 5. Mean ± standard error (SE) temperatures for the thermal windows of the trunk region (*Truncus regionis*) left or right of SJ (short journey) and LJ (long journey) water buffaloes in the seven phases. Different literals [a, b, c, d, e, f,] indicates statistically significant differences among the temperatures of the animals according to the phase (*p* value < 0.001). Different numbers [1,2] indicate statistically significant differences between short and long journeys of the thermal windows (*p* value < 0.001).

For the thermal window of the thoracic vertebral region in the SJ group, a marked difference was observed between P1 and P7, with an increase of 13.72 °C ($p < 0.001$). Likewise, in P4 and P6, the values were statistically different, but they presented low numerical differences with a thermal drop of 0.1 °C ($p < 0.001$). In the LJ group, there were differences between all phases, recording the lowest temperature during P1, being 7.23 °C lower than P2 ($p < 0.001$), while P3 had a reduction of 3.36 °C when compared with P2 ($p < 0.001$) and increases of 2.11, 1.38, and 2.09 for P4, P5, and P6, respectively ($p < 0.001$). Regarding the comparison between groups, the lowest temperatures were observed in the LJ group with minimum numerical variations of 0.95 °C in P7 and a maximum of 2.67 °C in P2 ($p < 0.001$).

The values for the lumbar vertebral region showed that, for the SJ group, the temperature readings of P1 were the lowest ($p < 0.001$) with a variation of 9.74 °C compared with P2 ($p < 0.001$). P7 increased by 15.49 °C compared to P1 ($p < 0.001$). Regarding the LJ group, a marked difference of 15.68 °C was also observed in P7 with respect to P1 ($p < 0.001$). This trend was similar to the SJ group, with a variation at discharge from P1 to P2 of 9.76 °C ($p < 0.001$) and statistical similarity in P2 with P5 and P6 ($p < 0.001$). When making the comparison between groups, significant increases were observed in SJ ($p < 0.001$) with numerical variations from 0.93 °C in P7 ($p < 0.001$) to 1.12 °C in P1 ($p < 0.001$).

Table 3 reports the degree of correlation presented between the 11 thermal windows, having a positive and statistically significant correlation in all cases ($p < 0.001$).

Table 3. Correlations between the thermal windows of SJ (short journey) and LJ (long journey) water buffaloes in the seven phases.

	Group	Lacrimal Caruncle	Periocular Region	Lower Eyelid	Auricular Pavilion	Nostrils	Frontal-Parietal Region	Thoracic Region	Abdominal Region	Limbs Region	Lumbar Region	Thoracic Vertebral Region
Lacrimal caruncle	SJ (n = 7383) LJ (n = 733)	0.935 * 0.935 *										
Periocular region	SJ (n = 7383) LJ (n = 733)	0.963 * 0.989 *	1 * 0.999 *									
Lower eyelid	SJ (n = 7383) LJ (n = 733)	0.971 * 0.976 *	0.998 * 0.974 *	1 * 0.999 *								
Auricular pavilion	SJ (n = 7383) LJ (n = 733)	0.941 * 0.811 *	0.997 * 0.839 *	0.974 * 0.801 *	1 * 0.999 *							
Nostrils	SJ (n = 7383) LJ (n = 733)	0.962 * 0.989 *	0.999 * 0.989 *	0.992 * 0.999 *	0.999 * 0.984 *	1 * 0.999 *						
Frontal-parietal region	SJ (n = 7383) LJ (n = 733)	0.957 * 0.976 *	0.999 * 0.977 *	0.991 * 0.990 *	0.981 * 0.964 *	0.999 * 0.971 *	1 * 0.999 *					
Thoracic region	SJ (n = 7383) LJ (n = 733)	0.954 * 0.981 *	0.988 * 0.977 *	0.995 * 0.993 *	0.987 * 0.965 *	0.999 * 0.974 *	0.999 * 0.999 *	1 * 0.999 *				
Abdominal region	SJ (n = 7383) LJ (n = 733)	0.952 * 0.967 *	0.989 * 0.953 *	0.987 * 0.989 *	0.963 * 0.948 *	0.986 * 0.986 *	0.999 * 0.988 *	1 * 0.999 *	1 * 0.999 *			
Limbs region	SJ (n = 7383) LJ (n = 733)	0.952 * 0.996 *	0.999 * 0.999 *	0.988 * 0.999 *	0.999 * 0.999 *	0.999 * 0.976 *	0.987 * 0.999 *	0.988 * 0.999 *	0.975 * 0.982 *	1 * 0.999 *		
Lumbar region	SJ (n = 7383) LJ (n = 733)	0.957 * 0.982 *	0.999 * 0.975 *	0.988 * 0.999 *	0.986 * 0.978 *	0.999 * 0.987 *	0.999 * 1 *	0.999 * 0.987 *	0.999 * 0.994 *	0.999 * 0.976 *	1 * 0.999 *	
Thoracic vertebral region	SJ (n = 7383) LJ (n = 733)	0.945 * 0.975 *	0.982 * 0.974 *	0.999 * 0.999 *	0.975 * 0.963 *	0.983 * 0.978 *	0.999 * 0.999 *	1 * 0.999 *	0.999 * 0.999 *	0.985 * 0.978 *	1 * 0.999 *	1 * 0.999 *

* $p < 0.0001$.

4. Discussion

In general, it was observed that the surface temperature in the periocular, lacrimal caruncle, nasal, lower eyelid, auricular region, frontal-parietal, pelvic limb, torso, abdominal, lumbar, and thoracic regions was significantly higher (3 °C) during the herding, loading, handing chute, pre- and post-transport phases in SJs compared to LJs ($p < 0.001$). These findings show that animals might have presented a stress-mediated response. Moreover, shorter trips possibly increase the thermal response compared to longer ones [23].

The possible explanation for this is that SJs might initiate an acute stress response that can lead to the activation of the HPA axis and the secretion of glucocorticoids, triggering lipolysis and gluconeogenesis, which can generate problems in the quality of the meat due to the increase in pH [24,25] due to depletion of muscle glycogen; in the living animal with the increase in body temperature [26], it activates adaptive and compensatory mechanisms for restoring homeothermy and homeostasis [27]. Moreover, catecholamines are also released, increasing the metabolic activity of the heart, resulting in stress-induced hyperthermia during the first 10–15 min after exposure to the stressor [28,29] in preparation for the possibility of rapid energy expenditure [30]. In this regard, the secretion and action of catecholamines and the stress induced by hyperthermia also have effects on muscle metabolism and membrane integrity, generating the appearance of undesirable characteristics in meat [31] and the decrease in live weight and the modification of physicochemical characteristics that affect both the quality and safety of the final cut [32]; the above is mainly associated with the depletion of muscle glycogen reserves and the accumulation of ante-mortem lactic acid, increasing post-mortem pH values [33], cooking loss, meat hardness [34], and ageing potential [35].

This coincides with what was reported by Burdick et al. [36], who found that the highest rectal temperature of Brahman bulls was recorded within the first 30 min after transport, while the lowest temperature occurred at 6 h and 40 min after transport. In this sense, SJs might not give enough time for the animals to habituate, culminating in an acute stress response.

For LJs, the buffaloes might be able to habituate to transport and its related stressors based on what has been indicated in previous studies [37,38]. This coincides with what was observed by Lei et al. [39], where they found that 15 to 17 h transport of 20 Arouquesa calves had an initial increase of 3 °C in periocular temperature and a subsequent decrease of 2 °C. The authors concluded that the animals habituated to the transport, although the increase in temperature represents an important metabolic cost, which can be difficult to maintain over a long period as represented by the LJ group. According to the findings of the present study and already published studies, the proposed hypothesis can be rejected.

Another element related to the thermal response observed in LJ animals would be the secretion of catecholamines, which leads to physiological effects such as tachycardia and vasoconstriction [40]. Therefore, a decrease in heat radiation may occur, associated with an increased sympathetic response as has been observed in cattle under conditions associated with acute pain [41–43]. Thus, it is possible to suggest that although the surface temperature was 5 °C lower in the animals from the LJ group, the thermal response may be associated with a chronic event compared to the animals in the SJ group. It should be noted that this consensus is difficult to affirm due to the lack of evidence because other factors such as the decrease in visceral activity due to prolonged fasting on long-distance trips and decreased body temperature could also influence the thermal response [44,45].

On the other hand, it was observed that the surface temperature in both SJs and LJs, the herding, handling chute, and pre-and post-transport phases significantly increased by at least 3 °C compared to the rest of the phases ($p < 0.001$). The explanation for these findings is that in these phases, interaction with humans is involved [46], which can often be negative due to the shouting, hitting, or the use of sticks and electric prods that generate pain and fear in the animal, increasing the acute stress response [47]. It is mentioned that mobilization and human–animal interaction without prior training are stressful for livestock, as they involve both physical and psychological aversive stimuli [3].

These results coincide with those reported by Rodríguez-González et al. [9], where they observed that the surface temperature in 624 water buffaloes mobilized in SJs (average duration = 2 h ± 20 min) had an average increase of 5 °C during the phases, where some type of handling was present. Then, it is possible to reaffirm that these interactions can cause acute fear and a physiological response associated with this handling, limiting the productivity, performance, and animal welfare [48].

From a neurobiological perspective, fear is processed by brain regions such as the somatosensory cortex, hippocampus, thalamus, and amygdala, which in coordination with the latter structure process the fear response (e.g., tachypnea, tachycardia, or hyperthermia) [49–51]. This response may explain the increase in surface temperature in the evaluated thermal windows due to the activation of HPA [52] and the subsequent secretion of endogenous glucocorticoids [50]. A possible additional explanation is neophobia, a trait present in water buffaloes, which possibly makes them susceptible to fear of unknown environments such as transport [53]. In this sense, it has been reported that dairy cattle may experience more fear in unfamiliar milking parlors and stocks [54].

The same response associated with fear may explain the differences found between the thermal windows evaluated in the present study. Perhaps the clearest example could be observed in the nasal window, where the thermal response serves to evaluate the physiological parameter of the respiratory frequency [54,55]. Considering that tachypnea is one of the physiological responses associated with stress, this can lead to an increase in radiated heat [56].

Regarding the differences found between groups in the facial region, greater imbalances were present in the SJ group between P1 and P7 in the lower eyelid window. This greater variation was also present in the rest of the thermal windows of the facial region for this group. The changes in the temperature of the facial region may be related to parasympathetic activity, the vasodilation response, which generates a lowers cardiac output and blood pressure and the consequent increase in temperature [39]. In particular, the wide distribution of capillaries and arteriovenous anastomoses in these thermal windows facilitates the exchange of body heat with the environment [57].

In another way, the lowest values were observed in the nasal window of the LJ group during P1 (29.03 ± 0.04 °C, $p > 0.001$). The above may be due to the nature of the anatomical region itself, which presents a high superficial vascularization from the maxillary vein and artery [58], in addition to the humidity given by the water vapor eliminated during respiration, which is affected by the stress generated during tachypnea [54]. Other authors also describe changes in temperature coming from the nasal region as a reflection of the activity of the autonomic nervous system, which can be used to detect an increase in the level of arousal (with a decrease in temperature) due to increased sympathetic activity and decreased blood flow in peripheral vessels [59,60].

Regarding the regions of the skull and their respective thermal windows, a greater variation was observed between groups. This region receives irrigation by the corneal artery, the superficial temporal artery (*A. temporalis superficialis*), and its branches (*A. transversa faciei, A. auricularis rostralis, A. palpebralis inferior lateralis, A. palpebralis superior lateralis*) [61]. In this sense, Badakhshan et al. [62] evaluated the temperature of Jersey cattle in different anatomical regions and its correlation coefficient to the rectal temperature, respiration rate, and heart rate. The authors observed that forehead values had a strong, positive, and significant correlation (r = 0.57, r = 0.88, and r = 0.70 respectively, $p > 0.01$). It has also been specified that, in bovid species raised in arid areas, the horn has thinner keratin sheaths than in temperate climates to facilitate heat loss [63,64]. Algra et al. [65] supported the role of horns for thermoregulation in dairy cattle, monitoring dehorned cows using IRT. These animals presented higher eye temperatures than horned cows.

Regarding the body regions specified in Figure 4, a similar trend was observed in the thoracic thermal window and the pelvic limb, with higher thermal variations in the pelvic limb. On the contrary, the thoracic thermal window presented smaller variations with minimums of 0.97° and maximums of 1.11 °C in phases 7 and 1–4, respectively

($p < 0.001$). With the exception of the abdominal thermal window, LJ group reported the highest temperatures without considering the phase or the window. The behavior of the thoracic and abdominal regions may be due to the presence of metabolically active organs. Furthermore, it has been established that the relationship between the environmental and surface temperature of animals is usually closer to that of the thorax, where the variation in blood is minimal [66], due to the irrigation of the caudal aorta artery (*A. aorta abdominalis*) towards abdominal organs and the cranial, median, and caudal quadrants [67].

Conversely, the lowest surface temperatures for all phases were observed in the pelvic limb and, in this same window, a greater variation was observed between groups ($p > 0.001$). This was also observed in a recent study conducted on 109 newborn water buffalo calves, who found the lowest temperature values in the pelvic limb compared to thermal windows of the facial region (lacrimal caruncle, periocular region, and lacrimal gland) [68]. The above could be explained due to the reduction in blood flow due to vasoconstriction and closure of arteriovenous anastomoses of extremities during stressful processes such as transport [69], prioritizing blood supply to the thoracic and abdominal area.

Finally, Table 3 shows the level of correlation between both central and peripheral windows being positive, strong, and significant (r above 0.9, $p > 0.001$). This means that, with an increase in body temperature, the surface temperature of the animals might also increase [9,16,70]. The above could be due to the effect of glucocorticoids that trigger the degradation of lipids and glycogen, affecting the temperature of both central and peripheral windows, with a greater increase in those thermal windows close to metabolically active organs [26,71].

The analysis carried out in this study represents novel information regarding the thermal behavior and the effect of the time factor during the transport of water buffaloes monitored by IRT. However, it is necessary to make visible the limitations of this article by not evaluating other physical variables such as rectal temperature, heart, and respiratory rate, as well as blood concentrations of cortisol, glucose, lactate, and pH values, or biomarkers related to dehydration processes (osmolarity, albumin, and hematocrit) and other biomarkers such as catecholamines, alpha amylase, IL, TNF alpha, and creatine kinase, among others, as well as the evaluation of facial expressions, behavior, and emotions, which opens a range of future research regarding the effect of transportation and the handling that this implies on physiological and thermal indicators in water buffalo.

Although the present study compared the thermal response before and after short and long journeys, a limitation of this study and a field of research for future studies is the consideration of the thermal response of buffaloes during the journey. Furthermore, changing climatic conditions between a short and a long journey, such as ambient temperature or relative humidity, need to be considered when interpreting infrared imaging. This is relevant because ambient temperature affects animals' surface temperature [72]. Nonetheless, although environmental factors might have some influence on thermoregulation, the findings of the present study show that transport is a potential stressor that alters the thermal response of buffaloes [73,74]. Further research should adopt and consider these variables to comprehend the influence of said variables in the possible stress-mediated thermal response of water buffaloes.

5. Conclusions

In conclusion, short journeys increase the thermal response in water buffaloes compared to long journeys monitored by IRT, and this could be associated with acute stress, refuting the proposed hypothesis. This could be because, during LJ, water buffaloes can become accustomed to stressors. Likewise, it is concluded that the phases that involve human–animal interaction generate an increase in the surface temperature for all the thermal windows evaluated, reaffirming the importance of adequate management during the transportation process to avoid negative effects and surface temperatures of the mobilized buffaloes.

This study is of utmost importance as it is a pioneer in the characterization and analysis of the thermal behavior of water buffalo during long journeys.

Author Contributions: All authors contributed to the conceptualization, writing, and reading. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The procedures of the present study were approved by the Scientific Commission of the Master's in Science (CAMCA.11.21) "Maestría en Ciencias Agropecuarias" of the School of Veterinary Medicine and Animal Husbandry, Universidad Autónoma Metropolitana, Mexico City, Mexico. The animals used in this study were handled gently, without the use of physical tools that could cause injuries and stress, in accordance with the guidelines of the Official Mexican Standards NOM-051-ZOO. The animals included in the present study were not touched or stressed, since infrared thermography is a non-invasive technique.

Informed Consent Statement: Informed consent was obtained from the owner of all animals, who also authorized and oversaw the gentle treatment of the animals monitored in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pascual-Alonso, M.; Lama, G.C.M.l.; Aguayo-Ulloa, L.; Villarroel, M.; Mitchell, M.; María, A.G. Thermophysiological, haematological, biochemical and behavioural stress responses of sheep transported on road. *J. Anim. Physiol. Anim. Nutr.* **2016**, *101*, 541–551.
2. Chandra, B.S.; Das, N. Behaviour of indian river buffaloes (Bubalus bubalis) during short- ultrasonographic findings of urinary retention caused by a vaginal stricture following dystocia in a ewe. *Vet. Rec.* **2001**, *148*, 314.
3. Gupta, S.; Earley, B.; Crowe, M.A. Effect of 12-hour road transportation on physiological, immunological and haematological parameters in bulls housed at different space allowances. *Vet. J.* **2007**, *173*, 605–616. [CrossRef] [PubMed]
4. Mendonça, F.S.; Vaz, R.Z.; Cardoso, F.F.; Restle, J.; Vaz, F.N.; Pascoal, L.L.; Reimann, F.A.; Boligon, A.A. Pre-slaughtering factors related to bruises on cattle carcasses. *Anim. Prod. Sci.* **2018**, *58*, 385. [CrossRef]
5. Guerrero-Legarreta, I.; Napolitano, F.; Cruz-Monterrosa, R.; Mota-Rojas, D.; Mora-Medina, P.; Ramírez-Bribiesca, E.; Bertoni, A.; Berdugo-Gutiérrez, J.; Braghieri, A. River buffalo meat production and quality: Sustainability, productivity, nutritional and sensory properties. *J. Buffalo Sci.* **2020**, *9*, 159–169. [CrossRef]
6. Nielsen, S.S.; Alvarez, J.; Bicout, D.J.; Calistri, P.; Canali, E.; Drewe, J.A.; Garin-Bastuji, B.; Gonzales Rojas, J.L.; Gortázar Schmidt, C.; Michel, V.; et al. Welfare of cattle during transport. *EFSA J.* **2022**, *20*, e07442. [CrossRef]
7. Mota-Rojas, D.; Martínez-Burnes, J.; Casas-Alvarado, A.; Gómez-Prado, J.; Hernández-Ávalos, I.; Domínguez-Oliva, A.; Lezama-García, K.; Jacome-Romero, J.; Rodríguez-González, D.; Pereira, A.M.F. Clinical usefulness of infrared thermography to detect sick animals: Frequent and current cases. *CABI Rev.* **2022**, *17*, 1–17. [CrossRef]
8. Idris, M.; Uddin, J.; Sullivan, M.; McNeill, D.M.; Phillips, C.J.C. Non-invasive physiological indicators of heat stress in cattle. *Animals* **2021**, *11*, 71. [CrossRef]
9. Rodríguez-González, D.; Guerrero-Legarreta, I.; Cruz Monterrosa, R.G.; Napolitano, F.; Gonçalves-Titto, C.; El-Aziz, A.H.A.; Hernández-Ávalos, I.; Casas-Alvarado, A.; Oliva-Domínguez, A.; Mota-Rojas, D. Assessment of thermal changes in water buffalo mobilized from the paddock and transported by short journeys. *Front. Vet. Sci.* **2023**, *10*, 1184577. [CrossRef]
10. Cruz-Monterrosa, R.; Mota-Rojas, D.; Ramírez-Bibriesca, E.; Mora-Medina, P.; Guerrero-Legarreta, I. Scientific findings on the quality of river buffalo meat and prospects for future studies. *J. Buffalo Sci.* **2020**, *9*, 170–180. [CrossRef]
11. Chulayo, A.Y.; Bradley, G.; Muchenje, V. Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, hspa1a and how they relate with meat quality in cattle. *Meat Sci.* **2016**, *117*, 89–96. [CrossRef] [PubMed]
12. Liotta, L.; Costa, L.N.; Chiofalo, B.; Ravarotto, L.; Chiofalo, V. Effect of lairage duration on some blood constituents and beef quality in bulls after long journey. *Ital. J. Anim. Sci.* **2007**, *6*, 375–384. [CrossRef]
13. Alam, M.R.; Gregory, N.G.; Jabbar, M.A.; Uddin, M.S.; Kibria, A.S.M.G.; Silva-Fletcher, A. Skin injuries identified in cattle and water buffaloes at livestock markets in Bangladesh. *Vet. Rec.* **2010**, *167*, 415–419. [CrossRef] [PubMed]
14. Alam, M.R.; Gregory, N.G.; Uddin, M.S.; Jabbar, M.A.; Chowdhury, S.; Debnath, N.C. Frequency of nose and tail injuries in cattle and water buffalo at livestock markets in Bangladesh. *Anim. Welf.* **2010**, *19*, 295–300.
15. Mota-Rojas, D.; Napolitano, F.; Braghieri, A.; Guerrero-Legarreta, I.; Bertoni, A.; Martínez-Burnes, J.; Cruz-Monterrosa, R.; Gómez, J.; Ramírez-Bribiesca, E.; Barrios-García, H.; et al. Thermal biology in river buffalo in the humid tropics: Neurophysiological and behavioral responses assessed by infrared thermography. *J. Anim. Behav. Biometeorol.* **2021**, *9*, 2103. [CrossRef]
16. Robertson, J.K.; Mastromonaco, G.; Burness, G. Evidence that stress-induced changes in surface temperature serve a thermoregulatory function. *J. Exp. Biol.* **2020**, *223*, jeb213421. [CrossRef]

17. Mota-Rojas, D.; Titto, C.G.; de Mira Geraldo, A.; Martínez-Burnes, J.; Gómez, J.; Hernández-Ávalos, I.; Casas, A.; Domínguez, A.; José, N.; Bertoni, A.; et al. Efficacy and function of feathers, hair, and glabrous skin in the thermoregulation strategies of domestic animals. *Animals* **2021**, *11*, 3472. [CrossRef]
18. Damtew, A.; Erega, Y.; Hussen, E.; Tsegaye, S.; Desiye, M. The effect of long distance transportation stress on cattle: A review. *Biomed. J. Sci. Tech. Res.* **2018**, *3*, 1–5. [CrossRef]
19. INEGI Clima Veracruz de Ignacio de La Llave. Available online: https://cuentame.inegi.org.mx/monografias/informacion/ver/territorio/clima.aspx?tema=me&e=30 (accessed on 5 January 2023).
20. SEGOB Norma Oficial Mexicana. *Señalamiento Horizontal y Vertical de Carreteras y Vialidades Urbanas*; PROY-NOM-034-SCT2-2003; Diario Oficial de la Federación: Mexico City, Mexico, 2004; pp. 2–7.
21. Rodríguez, A.; Romero, E. Evaluación del efecto de diferentes dosis de la fertilización orgánica en la calidad nutricional del pasto camelote (Paspalum fasciculatum willd). *Tlatemoani Rev. Académica Investig.* **2016**, *1*, 1–14.
22. DOF. *Trato Humanitario en la Movilización de Animales*; NOM-051-ZOO-1995; Diario Oficial de la Federación: Mexico City, Mexico, 1995; pp. 1–23.
23. Peeters, E.; Deprez, K.; Beckers, F.; De Baerdemaeker, J.; Auben, A.E.; Geers, R. Effect of driver and driving style on the stress responses of pigs during a short journey by trailer. *Anim. Welf.* **2008**, *17*, 189–196.
24. Bozzo, G.; Barrasso, R.; Marchetti, P.; Roma, R.; Samoilis, G.; Tantillo, G.; Ceci, E. Analysis of stress indicators for evaluation of animal welfare and meat quality in traditional and jewish slaughtering. *Animals* **2018**, *8*, 43. [CrossRef] [PubMed]
25. Mounier, L.; Dubroeucq, H.; Andanson, S.; Veissier, I. Variations in meat pH of beef bulls in relation to conditions of transfer to slaughter and previous history of the animals. *J. Anim. Sci.* **2006**, *84*, 1567–1576. [CrossRef] [PubMed]
26. Wang, L.; Liu, F.; Luo, Y.; Zhu, L.; Li, G. Effect of acute heat stress on adrenocorticotropic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. *Biomed. Rep.* **2015**, *3*, 425–429. [CrossRef]
27. Rebez, E.B.; Sejian, V.; Silpa, M.V.; Dunshea, F.R. Heat stress and histopathological changes of vital organs: A novel approach to assess climate resilience in farm animals. *Sustainability* **2023**, *15*, 1242. [CrossRef]
28. Oka, T. Stress-Induced Hyperthermia and Hypothermia. In *Handbook of Clinical Neurology*; Elsevier: Amsterdam, The Netherlands, 2018; Volume 157, pp. 599–621. ISBN 9780444640741.
29. Olivier, B.; van Bogaert, M.; van Oorschot, R.; Oosting, R.; Groenink, L. *Stress-Induced Hyperthermia*; Elsevier B.V.: Amsterdam, The Netherlands, 2005; Volume 15, ISBN 0444518231.
30. Peaston, R.T.; Weinkove, C. Measurement of catecholamines and their metabolites. *Ann. Clin. Biochem.* **2004**, *41*, 17–38. [CrossRef]
31. Ghassemi Nejad, J.; Kim, B.-W.; Lee, B.-H.; Sung, K.-I. Coat and hair color: Hair cortisol and serotonin levels in lactating holstein cows under heat stress conditions. *Anim. Sci. J.* **2017**, *88*, 190–194. [CrossRef]
32. Archana, P.R.; Sejian, V.; Ruban, W.; Bagath, M.; Krishnan, G.; Aleena, J.; Manjunathareddy, G.B.; Beena, V.; Bhatta, R. Comparative assessment of heat stress induced changes in carcass traits, plasma leptin profile and skeletal muscle myostatin and HSP70 gene expression patterns between indigenous osmanabadi and salem black goat breeds. *Meat Sci.* **2018**, *141*, 66–80. [CrossRef]
33. Hagenmaier, J.A.; Reinhardt, C.D.; Bartle, S.J.; Henningson, J.N.; Ritter, M.J.; Calvo-Lorenzo, M.S.; Vogel, G.J.; Guthrie, C.A.; Siemens, M.G.; Thomson, D.U. Effect of handling intensity at the time of transport for slaughter on physiological response and carcass characteristics in beef cattle fed ractopamine hydrochloride. *J. Anim. Sci.* **2017**, *95*, 1963. [CrossRef]
34. Chulayo, A.Y.; Muchenje, V. The effects of pre-slaughter stress and season on the activity of plasma creatine kinase and mutton quality from different sheep breeds slaughtered at a smallholder abattoir. *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 1762–1772. [CrossRef]
35. Hultgren, J.; Segerkvist, K.A.; Berg, C.; Karlsson, A.H.; Öhgren, C.; Algers, B. Preslaughter stress and beef quality in relation to slaughter transport of cattle. *Livest. Sci.* **2022**, *264*, 105073. [CrossRef]
36. Burdick, N.C.; Carroll, J.A.; Hulbert, L.E.; Dailey, J.W.; Willard, S.T.; Vann, R.C.; Welsh, T.H.; Randel, R.D. Relationships between temperament and transportation with rectal temperature and serum concentrations of cortisol and epinephrine in bulls. *Livest. Sci.* **2010**, *129*, 166–172. [CrossRef]
37. Gallo, C.; Lizondo, G.; Knowles, T.G. Effects of journey and lairage time on steers transported to slaughter in Chile. *Vet. Rec.* **2003**, *152*, 361–364. [CrossRef] [PubMed]
38. Valadez Noriega, M.; de la Lama, G.C. Implications, trends, and prospects for long-distance transport in cattle. Review. *Rev. Mex. Cienc. Pecu.* **2020**, *11*, 517–538. [CrossRef]
39. Lei, M.C.; Félix, L.; Cardoso, R.; Monteiro, S.M.; Silva, S.; Venâncio, C. Non-invasive biomarkers in saliva and eye infrared thermography to assess the stress response of calves during transport. *Animals* **2023**, *13*, 2311. [CrossRef] [PubMed]
40. Borell, E.H. The biology of stress and its application to livestock housing and transportation assessment. *J. Anim. Sci.* **2001**, *79*, E260. [CrossRef]
41. Stewart, M.; Schaefer, A.; Haley, D.; Colyn, J.; Cook, N.; Stafford, K.; Webster, J. Infrared thermography as a non-invasive method for detecting fear-related responses of cattle to handling procedures. *Anim. Welf.* **2008**, *17*, 387–393. [CrossRef]
42. Stewart, M.; Stookey, J.M.; Stafford, K.J.; Tucker, C.B.; Rogers, A.R.; Dowling, S.K.; Verkerk, G.A.; Schaefer, A.L.; Webster, J.R. Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. *J. Dairy Sci.* **2009**, *92*, 1512–1519. [CrossRef]
43. Stewart, M.; Verkerk, G.A.; Stafford, K.J.; Schaefer, A.L.; Webster, J.R. Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *J. Dairy Sci.* **2010**, *93*, 3602–3609. [CrossRef]

44. Knizkova, I.; Kunc, P.; Gurdil, G.; Pinar, Y.; Selvi, K. Applications of infrared thermography in animal production. *Anadolu Tarım Bilim. Derg.* **2007**, *22*, 329–336.
45. Schaefer, A.L.; Jones, S.D.M.; Tong, A.K.W.; Vincent, B.C. Infrared thermography of pigs with known genotypes for stress susceptibility in relation to pork quality. *Can. J. Anim. Sci.* **1989**, *69*, 491–495. [CrossRef]
46. Schmitz, L.; Ebinghaus, A.; Ivemeyer, S.; Domas, L.; Knierim, U. Validity aspects of behavioural measures to assess cows' responsiveness towards humans. *Appl. Anim. Behav. Sci.* **2020**, *228*, 105011. [CrossRef]
47. Mota-Rojas, D.; Broom, D.M.; Orihuela, A.; Velarde, A.; Napolitano, F.; Alonso-Spilsbury, M. Effects of human-animal relationship on animal productivity and welfare. *J. Anim. Behav. Biometeorol.* **2020**, *8*, 196–205. [CrossRef]
48. Hemsworth, P. Human–animal interactions in livestock production. *Appl. Anim. Behav. Sci.* **2003**, *81*, 185–198. [CrossRef]
49. Lees, A.M.; Sejian, V.; Wallage, A.L.; Steel, C.C.; Mader, T.L.; Lees, J.C.; Gaughan, J.B. The impact of heat load on cattle. *Animals* **2019**, *9*, 322. [CrossRef]
50. Sapolsky, R.M. How do glucocorticoids influence stress responses? integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **2000**, *21*, 55–89. [CrossRef]
51. Hernández-Avalos, I.; Mota-Rojas, D.; Mendoza-Flores, J.E.; Casas-Alvarado, A.; Flores-Padilla, K.; Miranda-Cortes, A.E.; Torres-Bernal, F.; Gómez-Prado, J.; Mora-Medina, P. Nociceptive pain and anxiety in equines: Physiological and behavioral alterations. *Vet. World* **2021**, *14*, 2984–2995. [CrossRef]
52. Nakamura, K. Neural circuit for psychological stress-induced hyperthermia. *Temperature* **2015**, *2*, 352–361. [CrossRef]
53. Grandin, T.; Shivley, C. How farm animals react and perceive stressful situations such as handling, restraint, and transport. *Animals* **2015**, *5*, 1233–1251. [CrossRef]
54. Lowe, G.; Sutherland, M.; Waas, J.; Schaefer, A.; Cox, N.; Stewart, M. Infrared thermography—A non-invasive method of measuring respiration rate in calves. *Animals* **2019**, *9*, 535. [CrossRef]
55. Stewart, M.; Wilson, M.T.; Schaefer, A.L.; Huddart, F.; Sutherland, M.A. The Use of Infrared Thermography and accelerometers for remote monitoring of dairy cow health and welfare. *J. Dairy Sci.* **2017**, *100*, 3893–3901. [CrossRef]
56. Thomson, D.U.; Loneragan, G.H.; Henningson, J.N.; Ensley, S.; Bawa, B. Special report of finished feedlot cattle following transportation. *J. Am. Vet. Med. Assoc.* **2015**, *247*, 66–72. [CrossRef] [PubMed]
57. Tattersall, G.J. Infrared thermography: A non-invasive window into thermal physiology. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2016**, *202*, 78–98. [CrossRef]
58. Mota-Rojas, D.; Pereira, A.M.F.; Wang, D.; Martínez-Burnes, J.; Ghezzi, M.; Hernández-Avalos, I.; Lendez, P.; Mora-Medina, P.; Casas, A.; Olmos-Hernández, A.; et al. Clinical applications and factors involved in validating thermal windows used in infrared thermography in cattle and river buffalo to assess health and productivity. *Animals* **2021**, *11*, 2247. [CrossRef] [PubMed]
59. Diaz-Piedra, C.; Gomez-Milan, E.; Di Stasi, L.L. Nasal skin temperature reveals changes in arousal levels due to time on task: An experimental thermal infrared imaging study. *Appl. Ergon.* **2019**, *81*, 102870. [CrossRef] [PubMed]
60. Matsuno, S.; Mizuno, T.; Asano, H.; Mito, K.; Itakura, N. Estimating autonomic nerve activity using variance of thermal face images. *Artif. Life Robot.* **2018**, *23*, 367–372. [CrossRef]
61. International Committee Veterinary Gross Anatomical Nomenclature Nomina Anatómica Veterinaria. 2017, 160. Available online: https://www.wava-amav.org/index.html (accessed on 2 February 2023).
62. Badakhshan, Y.; Mohammadabadi, M.R. Thermoregulatory mechanisms of jersey adult cattle and calves based on different body sites temperature. *Iran. J. Appl. Anim. Sci.* **2015**, *5*, 793–798.
63. Picard, K.; Thomas, D.W.; Festa-Bianchet, M.; Belleville, F.; Laneville, A. Differences in the thermal conductance of tropical and temperate bovid horns. *Ecoscience* **1999**, *6*, 148–158. [CrossRef]
64. Cain, J.W.; Krausman, P.R.; Rosenstock, S.S.; Turner, J.C. Mechanisms of thermoregulation and water balance in desert ungulates. *Wildl. Soc. Bull.* **2006**, *34*, 570–581. [CrossRef]
65. Algra, M.; de Keijzer, L.; Arndt, S.S.; van Eerdenburg, F.J.C.M.; Goerlich, V.C. Evaluation of the thermal response of the horns in dairy cattle. *Animals* **2023**, *13*, 500. [CrossRef]
66. Upadhyay, R.C.; Singh, A.K.; Devi, R.; Kumar, Y.; Kumar, P. Physiological changes and blood flow in murrah buffaloes during summer and winter season. *J. Buffalo Sci.* **2014**, *3*, 63–69. [CrossRef]
67. Mota-Rojas, D.; Pereira, A.M.F.; Martínez-Burnes, J.; Domínguez-Oliva, A.; Mora-Medina, P.; Casas-Alvarado, A.; Rios-Sandoval, J.; de Mira Geraldo, A.; Wang, D. Thermal imaging to assess the health status in wildlife animals under human care: Limitations and perspectives. *Animals* **2022**, *12*, 3558. [CrossRef] [PubMed]
68. Napolitano, F.; Bragaglio, A.; Braghieri, A.; El-Aziz, A.H.A.; Titto, C.G.; Villanueva-García, D.; Mora-Medina, P.; Pereira, A.M.F.; Hernández-Avalos, I.; José-Pérez, N.; et al. The effect of birth weight and time of day on the thermal response of newborn water buffalo calves. *Front. Vet. Sci.* **2023**, *10*, 1084092. [CrossRef] [PubMed]
69. Ewart, S. Termorregulación. In *Cunningham Fisiología Veterinaria*; Klein, B.G., Ed.; Elsevier: Barcelona, Spain, 2020; pp. 596–607.
70. Brcko, C.C.; da Silva, J.A.R.; Martorano, L.G.; Vilela, R.A.; de Nahúm, B.S.; Silva, A.G.M.; Barbosa, A.V.C.; Bezerra, A.S.; Lourenço Júnior, J. de B. Infrared thermography to assess thermoregulatory reactions of female buffaloes in a humid tropical environment. *Front. Vet. Sci.* **2020**, *7*, 180. [CrossRef] [PubMed]
71. Gonzalez-Rivas, P.A.; Chauhan, S.S.; Ha, M.; Fegan, N.; Dunshea, F.R.; Warner, R.D. Effects of heat stress on animal physiology, metabolism, and meat quality: A review. *Meat Sci.* **2020**, *162*, 108025. [CrossRef] [PubMed]

72. Soerensen, D.D.; Pedersen, L.J. Infrared skin temperature measurements for monitoring health in pigs: A review. *Acta Vet. Scand.* **2015**, *57*, 5. [CrossRef]
73. Arduini, A.; Redaelli, V.; Luzi, F.; Dall'Olio, S.; Pace, V.; Nanni Costa, L. Relationship between deck level, body surface temperature and carcass damages in italian heavy pigs after short journeys at different unloading environmental conditions. *Animals* **2017**, *7*, 10. [CrossRef]
74. Carnovale, F.; Xiao, J.; Shi, B.; Kaart, T.; Arney, D.; Phillips, C.J.C. The effects of vehicle type, transport duration and pre-transport feeding on the welfare of sheep transported in low temperatures. *Animals* **2021**, *11*, 1659. [CrossRef]

9.3. Resultados de la fase post mortem; Análisis fisicoquímico y hedónico de cortes cárnicos de búfalo de agua y bovino del género Bos.

9.3.1. Análisis Fisicoquímico

9.3.1.1. Color

Los tres cortes cárnicos de las especies de búfalo de agua y bovino convencional del género *Bos* fueron analizados tanto en crudo, obteniendo así 1,008 registros en la base de datos, que contemplaron los valores de L*, a*, b* contemplados en la tabla 4 con su correspondiente error estándar para cada parámetro**.**

Tabla 4. Variables fisicoquímicas de color en diferentes cortes de carne de bovino y búfalo de agua

	Bovino							Búfalo						
Variable	NY		PI		RE			NY		PI		RE		
	Cocido	Crudo	Cocido	Crudo	Cocido	Crudo	EEM	Cocido	Crudo	Cocido	Crudo	Cocido	Crudo	EEM
L	51.66^{a}	44.61^{cd}	50.00^{ab}	41.47^{ef}	48.48^{b}	36.31^{g}	0.34	47.68^{b}	39.70^{f}	47.82^{b}	42.72^{de}	47.48^{bc}	35.45^{g}	0.65
a	6.30^{d}	21.35^{a}	8.13^{c}	20.84^{a}	7.42^{cd}	17.72^{b}	0.28	8.13^{c}	18.48^{b}	7.98^{c}	20.84^{a}	8.69^{c}	17.97^{b}	0.20
b	11.89^{b}	8.43^{d}	13.12^{a}	7.13^{ef}	12.33^{ab}	5.97^{fg}	0.18	10.52^{c}	7.58^{de}	12.51^{ab}	7.13^{ef}	12.39^{ab}	5.91^{g}	0.24

NY= New York; PI= Pierna; RE= Rib eye; L= Luminosidad; a= coordenadas rojo/verde; b= coordenadas amarillo/azul; EEM= error estándar media;

$^{a\text{-}g}$ Letras diferentes, indican diferencia significativa p <0.05.

En los cortes de bovino, la L (luminosidad) del NY cocido no tuvo diferencia significativa (P>0.05) al PI cocido (51.6 vs 50.0, P>0.05), pero el RE cocido disminuyó 3.18 unidades en comparación el L del NY cocido (P<0.05). En el caso de los cortes crudos, la L de NY fue 3.14 y 8.3 unidades mayor (P <0.05) que la L de PI y RE crudo, respectivamente. En el caso la L en cortes cocido de búfalo, no hubo diferencia significativa (P>0.05) entre los tres tipos de cortes, promediando el valor de L en 47.66 (P>0.05). La L de PI crudo fue de 3.02 y 10.27 unidades mayor (P <0.05) que de NY y RE, también hubo diferencia significativa entre NY y RE crudo (39.7 vs 35.45, P <0.05).

Las diferencias de L entre los cortes de bovino y búfalo muestran que el NY cocido tuvo el mayor valor (51.66) fue diferente con todos los cortes de búfalo (P <0.05). Los cortes de L crudo entre bovino y búfalo de los cortes de PI y RE presentaron similitud estadística (P>0.05), mientras el L de NY fue mayor (44.61 vs 39.70, P <0.05) en bovino. De manera general los valores de L (luminosidad) fueron más elevados en los cortes de bovino (tanto en crudo como cocido), indicando que estos cortes son mas claros y los de búfalo de agua más oscuros.

Respecto a los valores de a* (contribución del rojo) para los cortes de bovino NY cocido fue estadísticamente similar al valor de RE cocido (6.3 vs 7.42, $p>0.05$), sin embargo, el valor encontrado para a* de PI cocido fue 1.83 más elevado con diferencia significativa ($p<0.05$) con respecto a NY cocido, no teniendo a su vez diferencia significativa con RE cocido (8.13 vs 7.42 $p>0.05$). Para los cortes crudos de bovino, el valor de a* fue similar para NY y PI (21.35 vs 20.84 $p>0.05$), sin embargo, se observó una disminución significativa en el valor de a* RE ($p<0.05$) con respecto a a* de NY y PI crudo de 3.63 y 3.12 respectivamente.

Para los cortes bufalinos no se observaron diferencias significativas en a* entre los tres diferentes cortes en cocido, promediando un valor de 8.26 ($p>0.05$). Respecto al valor de a* en NY crudo se observa un aumento de 2.36 unidades con respecto a a* en PI crudo ($p<0.05$), por el contrario, se observa una disminución de 0.51 unidades con respecto a a* en RE crudo ($p<0.05$).

Las diferencias de a* entre los cortes de bovino y búfalo muestran que el NY cocido (6.30) es diferente a los valores de a* con respecto a todos los cortes cocidos de búfalo ($p<0.05$), al igual que para el valor de a* de PI cocido, sin embargo, se observa similitud estadística entre a* de NY cocido de bovino y RE de esta misma especie ($p>0.05$). De manera general los valores de a* fueron más elevados en los cortes de bovino (a excepción de NY cocido y RE tanto en crudo como cocido) indicando que estos cortes son más rojizos que los de búfalo de agua.

Respecto al valor de b* (contribución del azul) en cortes bovinos cocidos PI presentó diferencias significativas y fue más elevado que NY por 1.23 unidades ($p<0.05$), por el contrario, para b* RE cocido los valores fueron similares con respecto a NY (12.33 vs 13.12 respectivamente, $p>0.05$) y PI cocidos (12.33 vs 11.89 respectivamente, $p>0.05$). Respecto a los cortes crudos de bovino b* todos los cortes presentaron diferencias significativas ($p<0.05$), presentándose los valores más bajos en el corte RE crudo (5.97), seguido de PI (7.13) y NY crudo (8.43).

Para los cortes cocidos de búfalo con respecto a la característica b* se observaron valores similares para PI cocida y RE cocido con un incremento mínimo de 0.12 en PI, sin embargo, se observó una diminución de 1.99 de PI a NY cocido de búfalo ($p<0.05$) y 1.87 de RE a NY cocido de búfalo ($p<0.05$). En el caso de los cortes crudos la característica de b* para NY de búfalo presentó similitud con PI crudo (7.58 vs 7.13, $p<0.05$), sin embargo, se observó una disminución de 1.67 unidades en b* RE crudo con respecto a b* NY crudo de búfalo ($p>0.05$) y 1.22 en relación con b* PI crudo de búfalo($p<0.05$).

Las diferencias de b* entre cortes de bovino y búfalo solo se observaron en el corte NY cocido, este presentó un valor más elevado (1.37 unidades), siendo diferente con respecto a NY cocido de búfalo ($p<0.05$), en el caso de b* en PI de bovino fue similar al valor de la misma característica para el búfalo (13.12 vs 12.51, $p>0.05$) y, se observó el mismo patrón en b* de RE de bovino y búfalo, siendo ligeramente más alta (0.06) en la segunda especie ($p>0.05$). Respecto a valores en crudo se observaron similitudes en NY y RE crudo de ambas especies ($p>0.05$), el mismo valor en bovinos y búfalos para el corte de PI crudo ($p>0.05$). De manera general los valores de b* fueron más elevados en los cortes de bovino (a excepción de RE cocido), indicando que estos cortes son más pálidos con respecto a los de búfalo de agua.

9.1.2. pH, Aw, Textura y CRA

Para las variables de pH, Aw, textura y CRA se obtuvieron los valores especificados en la tabla 5, al respecto, en los cortes de bovino, el pH de NY cocido fue similar al de PI y RE cocido (6.08, 6.15 y 6.15 $p>0.05$), en el caso de pH de los mismos cortes crudos se observaron diferencias significativas ($p<0.05$), siendo el pH de PI cruda 0.07 más elevada que RE crudo y 0.33 que NY. Respecto al pH en los cortes cocidos de búfalo se encontró similitud entre el pH de NY y PI cocidos (6.23 vs 6.34, $p>0.05$), además, se observó una disminución de pH en el corte RE de 0.04 con respecto a NY y 0.15 con respecto a PI cocida ($p>0.05$). Para los cortes bufalinos en crudo se observó también similitudes en los valores de pH de NY (6.01) con un aumento de 0.14 unidades en PI cruda ($p>0.05$) y 0.15 unidades en RE crudo ($p>0.05$).

Tabla 5. Variables fisicoquímicas en diferentes cortes de carne de bovino y búfalo de agua (pH, Aw, textura y CRA).

Variable	Bovino							Búfalo						
	NY		PI		RE			NY		PI		RE		
	Cocido	Crudo	Cocido	Crudo	Cocido	Crudo	EEM	Cocido	Crudo	Cocido	Crudo	Cocido	Crudo	EEM
pH	6.08b^{cd}	5.74^{e}	6.15^{bcd}	6.07^{cd}	6.15^{bcd}	6.00^{d}	0.02	6.23^{ab}	6.01^{d}	6.34^{a}	6.15^{bcd}	6.19^{abc}	6.16^{bcd}	0.03
Aw	0.92^{b}	0.93^{ab}	0.93^{ab}	0.95^{a}	0.93^{ab}	0.93^{b}	0.01	0.92^{b}	0.92^{b}	0.92^{b}	0.93^{ab}	0.92^{b}	0.92^{b}	0.004
Textura	4.31^{c}	3.91^{e}	5.14^{a}	4.26^{cd}	4.74^{b}	3.94^{e}	0.03	4.99^{a}	3.68^{f}	5.14^{a}	4.10^{d}	5.02^{a}	3.73^{f}	0.02
CRA	23.63^{c}	28.66^{a}	21.09^{e}	26.51^{b}	23.47^{cd}	28.59^{a}	0.05	23.35^{d}	28.84^{a}	21.11^{e}	26.28^{b}	23.67^{c}	28.79^{a}	0.05

NY = New York; PI= Pierna; RE= Rib eye, Aw= Actividad de agua; CRA= Capacidad de retención de agua; EEM= error estándar media.

$^{a\text{-}g}$Letras diferentes, indican diferencia significativa $P<0.05$.

Respecto a los resultados obtenidos para pH entre cortes bovinos y bufalinos se observan diferencias en NY cocido para estas especies con un incremento de 0.15 unidades en el NY de búfalo con respecto al valor del mismo corte de bovino ($p<0.05$), también se encontraron valores más elevados en el pH de PI cocida de búfalo con respecto a PI cocida de bovino (6.34 vs 6.15, $p<0.05$), sin embargo, en el caso de RE cocido se observó similitud entre especies con un pH de 6.15 en RE cocido de bovino y 6.19 en RE cocido de búfalo ($p>0.05$). Con respecto al pH de NY en crudo se observó una marcada disminución de esta característica en el bovino con respecto al búfalo (6.01 vs 5.74, $p<0.05$), por el contrario, se obtuvieron valores más elevados en pH de PI de búfalo cruda con respecto a PI de bovino cruda, con una baja de 0.08 ($p>0.05$), por último, también se obtuvo similitud estadística ($p>0.05$) en RE cruda de búfalo y bovino, siendo más elevado el valor de la primera especie (6.16 vs 6.00).

Para los valores de Aw en cortes cocidos de bovino se observó similitud en NY con respecto a PI y RE (0.92, 0.93 y 0.93, $p>0.05$). Los valores previamente mencionados fueron similares con respecto a los cortes bovinos crudos, encontrando similitud estadística en Aw de NY, PI y RE crudo (0.93, 0.95 y 0.93, $p>0.05$), sin embargo, los valores de Aw en PI y RE crudos fueron estadísticamente diferentes con la diminución de 0.02 unidades en RE con respecto a PI crudos de bovinos ($p<0.05$). Respecto a Aw de cortes bufalinos cocidos se observó el mismo valor de 0.92 en NY, PI y RE cocidos de búfalo ($p>0.05$), este comportamiento fue similar en Aw de NY, PI y RE crudos de búfalo, donde, únicamente la Aw en PI presentó un valor de 0.93 vs 0.92 para el resto de los cortes en crudo ($p>0.05$). No se observaron diferencias significativas entre especies por corte para Aw.

En los cortes de bovino, la textura de NY cocido fue diferente a PI cocido (4.13 vs 5.14, $p<0.05$), mismo caso para RE cocido de bovino (4.13 vs 4.74, $p<0.05$), así mismo, esta diferencia se observó entre los cortes PI y RE cocidos de bovino (5.14 vs 4.74, $p<0.05$). Respecto a estos cortes en crudo y para esta especie se observó un incremento en la textura de PI de bovino en crudo de 0.35 unidades con respecto a NY de bovino en crudo ($p<0.05$) y de 0.32 con respecto a RE ($p<0.05$), sin embargo, los valores encontrados para textura en NY y RE de bovino en crudo fueron similares (3.91 vs 3.94, $p>0.05$). Respecto a los cortes bufalinos se observó similitud en la textura (Kg fuerza) de NY, PI y RE cocidos (4.99, 5.14 y 5.02, $p>0.05$), mismo caso para los valores de textura de NY y RE de búfalo en crudo (3.68 vs 3.73, $p>0.05$), sin embargo, la textura de NY y RE en crudo fue estadísticamente diferente con respecto al valor de PI en crudo ($p<0.05$) con aumentos de 0.42 con respecto a NY y 0.37 con respecto a RE crudo de búfalo ($p<0.05$).

Entre especies se observó un incremento de 0.68 unidades en NY cocido de búfalo con respecto a este mismo corte de bovino ($p<0.05$), al igual que la textura de RE cocido con 0.28 unidades en RE cocido de búfalo con respecto a RE cocido de bovino ($p<0.05$), sin embargo, para el corte PI se obtuvo el mismo valor en ambas especies (5.14, $p>0.05$). Esta tendencia también se visualizó en los cortes en crudo, con similitud en PI cruda de bovino y búfalo (4.26 vs 4.10, $p>0.05$) y diferencias en NY crudo de búfalo (3.68) y NY crudo de bovino (3.91) $p<0.05$, al igual que los valores de textura en RE crudo con un incremento de 0.21 unidades en RE crudo de bovino con respecto a este mismo corte pero proveniente de búfalo.

Por último, en los cortes de bovino cocidos la CRA presentó diferencias en NY con respecto a PI, con una disminución de 2.64 unidades, siendo mayor el valor del primer corte ($p<0.05$), con respecto a la CRA de RE cocido de bovino este presentó similitud con NY ($p>0.05$) y diferencias con PI (23.47 vs 21.09, $p<0.05$). Respecto a los cortes crudos NY y RE presentaron similitud en los valores de CRA ($p>0.05$), pero una disminución significativa de 2.51 unidades en NY con respecto a PI y 2.08 unidades en RE con respecto a PI ($p<0.05$).

En el caso de los cortes cocidos de búfalo se observaron diferencias para todos los cortes estudiados, presentando mayor CRA el RE que NY (23.67 vs 23.35, $p<0.05$), y el menor valor de CRA en PI con una baja de 2.56 unidades con respecto a RE ($p<0.05$) y 2.24 unidades con respecto a la CRA de NY ($p<0.05$). Del mismo modo, se observaron diferencias en la CRA presente en NY crudo con respecto a PI crudo (28.84 vs 26.28, $p<0.05$), mismo caso que la diferencia entre PI de búfalo y RE de búfalo crudo (26.28 vs 28.79, $p<0.05$). Respecto a las diferencias encontradas sobre CRA entre especies únicamente se observó esta diferencia en la CRA de NY cocido, siendo más elevada en bovinos que en búfalos por 0.28 unidades ($p<0.05$), para el resto de los cortes tanto en crudo como en cocido se encontraron valores similares de CRA entre especies ($p>0.05$).

9.3.3. Análisis hedónico

Una vez realizado el análisis fisicoquímico, se seleccionaron cortes RE de ambas especies y se sometieron a cocción para realizar el análisis hedónico, se obtuvieron 500 respuestas, las cuales se presentan en la tabla 6 para las características de textura, jugosidad, sabor, aroma y aceptación.

Tabla 6. Valores medios de las características de textura, jugosidad, sabor, aroma y aceptación obtenidos mediante una evaluación hedónica para carne de búfalo de agua y carne bovina convencional.

Variable	Especie	n	Rango promedio	P value
Textura	Búfalo de agua	50	51.48	0.730
	Bovino del género *Bos*	50	49.52	
Jugosidad	Búfalo de agua	50	54.74	0.134
	Bovino del género *Bos*	50	46.26	
Sabor	Búfalo de agua	50	47.06	0.226
	Bovino del género *Bos*	50	53.94	
Aroma	Búfalo de agua	50	44.62*	0.039
	Bovino del género *Bos*	50	56.38	
Aceptación	Búfalo de agua	50	49.97	0.852
	Bovino del género *Bos*	50	51.03	

El análisis de rango realizada con la prueba de U de Mann-Whitney muestra diferencia significativa * $p<0.05$.

Respecto al análisis hedónico, sólo en aroma hubo diferencias significativas entre especies ($p>0.05$), con mayor nivel de agrado en el RE de bovinos del género *Bos* con 11.76 unidades sobre el corte de RE de búfalo de agua.

Además, en general no se observaron diferencias significativas entre especies en cuanto al grado de aceptación, jugosidad, ternura y sabor de los panelistas. En este sentido, los valores de textura y jugosidad presentaron un mayor nivel de agrado en la carne de búfalo RE con diferencias de 1,96 y 8,48 unidades sobre la carne de bovino del género *Bos* respectivamente.

Por el contrario, el RE del género *Bos* presentó mayor nivel de aceptación, aroma y sabor respecto a la carne de búfalo con diferencias de 1.06, 11.76 y 6.88 unidades respectivamente.

10. DISCUSIÓN

Dentro del proceso de obtención de productos cárnicos, la movilización es señalada como un factor estresante potencial con afectaciones fisiológicas, conductuales y emocionales sobre los semovientes (Flores-Peinado et al., 2020). Siendo relevante la monitorización, evaluación e identificación del comportamiento y las variaciones fisiológicas generadas de acuerdo a las características de cada especie productiva y las condiciones del transporte para, posteriormente, identificar puntos críticos de control y generar estrategias que minimicen los efectos negativos sobre los animales en pie y sobre los productos y subproductos para los cuales fueron criados en unidades de producción (Rodríguez-González et al., 2022c).

En este sentido, y, particularmente en el búfalo de agua se ha señalado como un animal susceptible al estrés inducido por calor, por lo cual la IRT ha sido indicada como una herramienta efectiva que evalúa el estado y los cambios en la temperatura superficial de los animales (Mota-Rojas et al., 2021a; Napolitano et al., 2023; Teja et al., 2023; Weschenfelder et al., 2013). Pese a ello, no existían documentos científicos que monitorizaran y caracterizaran el comportamiento térmico mediante IRT del búfalo de agua durante el transporte ni el efecto del tiempo de la movilización sobre el balance térmico en esta especie.

10.1. Fase ante mortem; etapa 1 “Evaluación de cambios térmicos en búfalos de agua movilizados desde el potrero y transportados en trayectos cortos”.

Los resultados obtenidos para los búfalos transportados por períodos cortos mostraron que las temperaturas superficiales de las ventanas térmicas de la faciales y el corporales aumentaron significativamente en las fases 2, 4, 6 y 7, es decir, durante los eventos que requirieron manipulación ($p<0.0001$). Estas respuestas pueden estar asociadas a la pérdida de estabilidad térmica en los búfalos, especie cuyo ambiente óptimo para alcanzar la termoneutralidad está en el rango de 13-18°C (Lan et al., 2022; Rensis & Scaramuzzi, 2003).

Aunque se han reportado temperaturas adecuadas para los búfalos de agua, existe literatura limitada sobre las respuestas térmicas de estas especies durante el transporte. Por otra parte, para los bovinos del género *Bos*, un estudio sobre el transporte terrestre y marítimo de 481 animales informó una asociación significativa entre los aumentos en biomarcadores como los niveles de glucosa, creatina quinasa y lactato con las lecturas de temperatura IRT (Cuthbertson et al., 2020). Así mismo, un estudio de realizado con 120 cerdos transportados durante 40 minutos informó aumentos de temperatura en la región orbital y detrás de la oreja, lo que se correlacionó positiva y moderadamente con un aumento de las concentraciones de cortisol en la saliva (r= 0,49 y r=0,50, respectivamente) (Rocha et al., 2019). De este modo, estudios en otras especies de ganado sometidas a transporte sugieren que las temperaturas superficiales pueden ser indicadores útiles de los cambios fisiológicos desencadenados en respuesta al estrés inducido por el transporte (Alam et al., 2018; Hong et al., 2019), que puede reflejarse como respuesta del sistema nervioso central y periférico con activación de la hipertermia de modulación vasomotora (Mota-Rojas et al., 2021d).

En este estudio, los mayores aumentos de temperatura superficial en los búfalos se registraron en la fase post transporte (p<0,0001). Esto puede explicarse por la activación del SNA y su rama simpática, que genera la producción de catecolaminas como respuesta a corto plazo (Mota-Rojas et al., 2021b). Las catecolaminas actúan sobre órganos metabólicamente activos, como el tejido adiposo marrón, para iniciar la termogénesis, aumentando así la irradiación de calor (Kataoka et al., 2014; Nakamura, 2015). La activación del eje HPA provoca la secreción de glucocorticoides (p. ej., cortisol) que promueven la gluconeogénesis y la lipólisis (Oka, 2018; Wang et al., 2015). En este sentido, estudios en rumiantes han reportado aumentos del cortisol plasmático de hasta 10 veces después de 30 y 45 minutos, 1h, 2h y hasta 4h post-transporte (Agnes et al., 1990; Al-Qarawi & Ali, 2005; Mehdid et al., 2019; Sanhouri et al., 1992; Sartorelli et al., 1992).

Mitchell et al. (1988), op. cit., en Ali- Gholi y Daryoush (2007), afirmaron que las reacciones simpáticas, suprarrenales y medulares están relacionadas con respuestas fisiológicas durante el transporte, mientras que el hipotálamo, la hipófisis y las reacciones suprarrenales son responsables de la secreción de cortisol en condiciones ambientales adversas. Por lo tanto, esta respuesta fisiológica es responsable de la producción de calor durante la percepción de factores estresantes en condiciones de transporte, manipulación y desafíos ambientales (Figura 12).

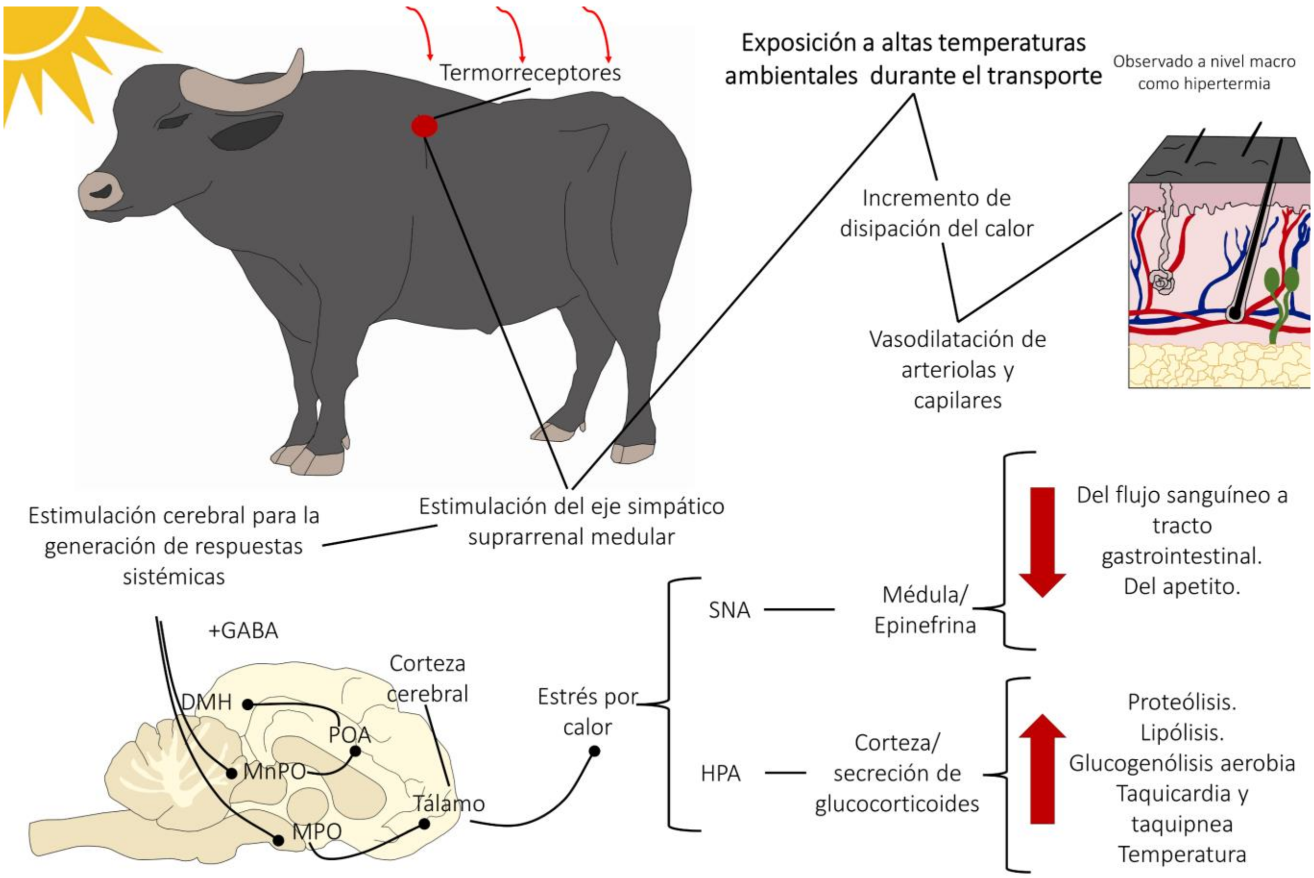

Figura 12. Vía termorreguladora de los búfalos de agua durante inducción de estrés por calor. Los termorreceptores periféricos procesan y transmiten información sobre el ambiente térmico externo. A través de una conexión entre la médula espinal (DRG y DH), el LPBd y las estructuras supraespinales (principalmente POA, MPO y MnPO), el organismo desencadena diferentes respuestas para aumentar la disipación de calor. Por ejemplo, la vasodilatación de las arteriolas y capilares dérmicos aumenta la pérdida de calor en los búfalos de agua expuestos al estrés por calor. De manera similar, otros mecanismos compensatorios, como los aumentos de la frecuencia respiratoria y cardíaca, sirven para restaurar la termoneutralidad. DH: asta dorsal; DMH: hipotálamo dorsomedial; HPA: hipotalámico-pituitario-suprarrenal; IML: intermediolateral; MnPO: núcleo preóptico mediano; MPO: área preóptica medial; POA: área preóptica; SNA: sistema nervioso autónomo; ↑: aumento.

Esta explicación puede ayudar a comprender el informe de Sakakibara et al. (2014), quienes encontraron una débil correlación negativa entre las concentraciones de cortisol en sangre y las temperaturas superficiales (r=-0,209) en cinco bovinos transportados durante 8h. Robertson et al. (2020) observó que la activación de la secreción de catecolaminas

(Krakoff, 1988) que pueden generar efectos fisiológicos como taquicardia, taquipnea e hipertermia (Oka et al., 2001; Sapolsky, 2000).

Esto podría estar relacionado con el nivel de estrés que experimentan los animales, como Hagenmaier et al. (2017) observaron a 80 terneros transportados bajo dos niveles de manejo, encontrando que, los animales sometidos a manejo intensivo, con uso de picanas eléctricas y trote mínimo durante la carga, tuvieron niveles más altos de lactato, epinefrina, norepinefrina, cortisol y glucosa pero niveles más bajos de pH sanguíneo y bicarbonato, con exceso de bases, en comparación con los animales que recibieron mínima manipulación durante el transporte. Esto significa que los factores externos que aumentan la respuesta al estrés de los animales durante el transporte (por ejemplo, temperaturas superficiales más altas), incluyendo la interacción social con los cuidadores y el entrenamiento que reciben los ganaderos para movilizar animales de diferentes especies, son dos factores fundamentales para prevenir respuestas intensas al estrés, incluso durante viajes cortos como los del presente estudio (2 h ± 20 minutos). Algunos autores han informado que incluso cuando el tiempo de viaje se mantiene por debajo de las 12 h, algunas especies no logran habituarse inmediatamente a los factores intrínsecos e individuales que causan estrés (Gallo et al., 2003; Valadez Noriega & de la Lama, 2020).

Otra condición importante que se observa durante el transporte de animales y que es necesario destacar es la neofobia, es decir, el miedo a situaciones nuevas. Este puede ser otro factor implicado en las respuestas al estrés antes del transporte de búfalos de agua (Flores-Peinado et al., 2020; Stewart et al., 2007). Este miedo implica la activación de la región basolateral de la amígdala, encargada de coordinar las respuestas de la corteza cerebral y el hipotálamo, centro fisiológico que modula la frecuencia cardíaca y respiratoria y la temperatura corporal durante eventos que causan miedo o ansiedad (Angilletta et al., 2019; Phelps & LeDoux, 2005).

Respecto a las diferencias de temperatura detectadas entre las diferentes ventanas térmicas, se destaca el mínimo incremento de 1°C entre las regiones facial, craneal y nasal en comparación con la región corporal lateral del miembro femoral y la región lateral abdominal y torácica ($p<0,0001$), ello puede explicarse por la extensa distribución de capilares y anastomosis arteriovenosas que permiten el intercambio de calor con el ambiente (Tattersall, 2016). Cuando se activan mecanismos que preservan la termoneutralidad, la pérdida de calor por evaporación (como vapor de agua), convección o conducción es responsable de los cambios en la cantidad de calor irradiado a través de la piel (Mota-Rojas et al., 2021d; Romanovsky, 2014). Específicamente, las ventanas térmicas de la cabeza, las regiones periocular y frontal- parietal mostraron una diferencia de 4°C ($p<0,0001$), posiblemente debido a la distribución de los vasos sanguíneos en esas áreas. Por ejemplo, las regiones de la cabeza están irrigadas por la arteria facial y sus ramas en la arteria infraorbitaria, que lleva sangre al párpado inferior y a la carúncula lagrimal (International Committee Veterinary Gross Anatomical Nomenclature, 2017; Mota-Rojas et al., 2021d), mientras que la región frontal-parietal está irrigada por ramas (*A. transversa faciei, A. auricularis rostralis, A. palpebralis inferior lateralis, A. palpebralis superior lateralis*) de la arteria temporal superficial (*A. temporalis superficialis*) (International Committee Veterinary Gross Anatomical Nomenclature, 2017). Esta distribución de vasos sanguíneos podría conferir una ventaja termorreguladora a los búfalos de agua al servir como vía para la pérdida de calor (Figura 13). Taylor (1966) describió que la circulación alrededor de los cuernos de las cabras Toggenburg podía servir como vía para la termorregulación. Esto podría explicar el aumento de temperatura de la región frontal-parietal registrado en nuestro estudio.

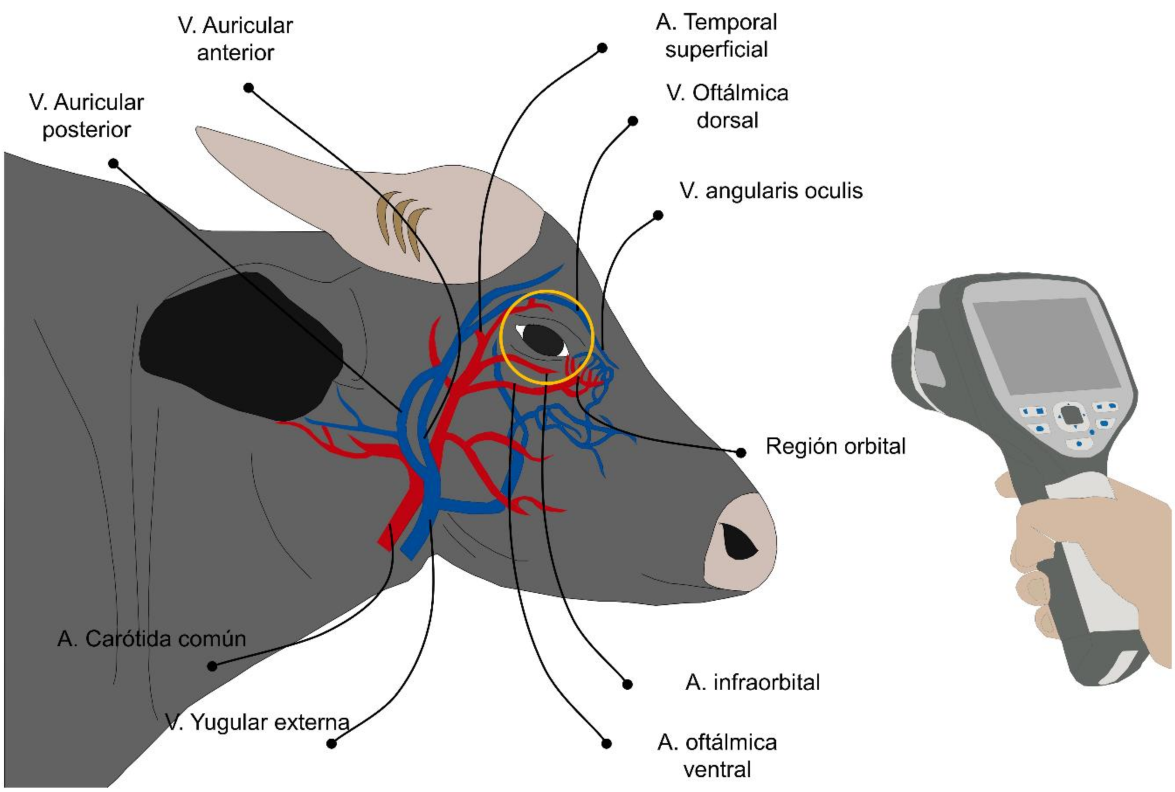

Figura 13. Distribución arterio venosa presente la región de cabeza (*Regiones capitis*) del búfalo de agua (*Bubalus bubalis*). Esta ventana térmica está delimitada por un círculo o

cuadrado trazado desde la región medial del ojo 3 o 4 mm hacia la zona rostral de la comisura o canto palpebral medial en la porción central del círculo alrededor de la glándula lagrimal. Esta zona se caracteriza por una alta densidad de capilares de las arterias maxilar e infraorbitaria que están inervados por fibras simpáticas. Cuando se estimulan, estas fibras provocan una neuro secreción de epinefrina y norepinefrina que desencadena vasoconstricción y la consiguiente disminución de la tasa de intercambio de calor, como ocurre en condiciones de estrés o nocicepción.

Las ventanas térmicas en las regiones del corporales, como la abdominal o torácica, tuvieron temperaturas 2°C superiores a las de la región de las extremidades ($p<0,0001$). Esto está relacionado con la presencia de órganos vitales metabólicamente activos, como el corazón y el hígado, que contribuyen a elevar la temperatura corporal mientras los animales intentan compensar mediante cambios vasomotores periféricos en las estructuras de las extremidades (Isola et al., 2020). Las temperaturas en estas regiones son producidas por el flujo sanguíneo de las arterias metatarsiana dorsal y safena (International Committee Veterinary Gross Anatomical Nomenclature, 2017). Los cambios en la temperatura de las extremidades en perros y caballos se han asociado con niveles de actividad muscular que aumentan el flujo sanguíneo a nivel local (Casas-Alvarado et al., 2022; Mota-Rojas et al., 2022); sin embargo, en nuestro estudio no se evaluó la respuesta muscular específica.

Las ventanas térmicas de las fosas nasales fueron las que presentaron temperaturas más bajas de toda la región nasal (*Regio nasalis*) ($p<0,0001$). Aunque esta área tiene una alta densidad de vasos sanguíneos superficiales de la arteria y vena maxilares (Mota-Rojas et al., 2021b), la eliminación del vapor de agua durante el ciclo respiratorio y la taquipnea que se produce durante el estrés son dos factores que pueden influir en la temperatura de las fosas nasales, provocando una mayor pérdida de vapor de agua y calor (Lowe et al., 2019). Finalmente, es importante mencionar que se observó una correlación fuerte, positiva y significativa entre las ventanas térmicas ($r=0,9$, $p<0,0001$). Esto significa que a medida que aumenta la temperatura de una región del cuerpo, la de otras regiones también aumenta debido a la mayor radiación de calor superficial en las diferentes ventanas térmicas (Brcko et al., 2020; Mota-Rojas et al., 2021a).

De manera similar, Napolitano et al. (2023) evaluaron la temperatura superficial de 109 bucerros con el objetivo de reconocer variaciones en diversas ventanas térmicas (regiones corporales y craneofaciales) con respecto a su peso al nacer, encontrándose que la región facial registra valores más altos (excepto la ventana térmica de la fosa nasal) y con menor variación respecto a los miembros pélvicos en todos los grupos estudiados.

Los resultados del presente estudio confirman la utilidad de la técnica IRT para evaluar a grandes rumiantes, identificando cambios en la temperatura superficial de búfalos sometidos a periodos de transporte cortos. Sin embargo, es importante resaltar que una de las limitaciones de este estudio es que no se evaluaron otras variables de respuesta, como parámetros fisiológicos (p. ej., temperatura corporal) y endocrinos (p. ej., concentraciones de cortisol), para relacionar el aumento de temperatura detectado a otros factores. La inclusión de indicadores fisiológicos durante las fases previas y posteriores al transporte podría proporcionar una imagen más clara del estrés que experimentan los búfalos de agua durante el transporte.

10.2. Fase ante mortem; etapa 2 "Balance térmico en búfalos de agua transportados en viajes largos y cortos".

En general, se observó que la temperatura superficial en las regiones periocular, carúncula lagrimal, nasal, párpado inferior, región auricular, frontal-parietal, miembro pélvico, torso, abdomen, lumbar y torácica fue significativamente mayor (3°C) durante las fases de potrero, carga, rampa de entrega, pre y post transporte en el VC en comparación con el VL ($p<0,001$). Estos hallazgos muestran que los animales podrían haber presentado una respuesta mediada por el estrés. Además, los viajes más cortos posiblemente aumenten la respuesta térmica en comparación con los más largos (Peeters et al., 2008).

La posible explicación a esto es que el VC podría iniciar una respuesta de estrés agudo que puede conducir a la activación del eje HPA y la secreción de glucocorticoides, desencadenando lipólisis y gluconeogénesis, lo que puede generar problemas en la calidad de la carne debido al aumento de pH (Bozzo et al., 2018; Mounier et al., 2006). El agotamiento del glucógeno muscular en el animal vivo con el aumento de la temperatura corporal (Wang et al., 2015). Con el aumento de la temperatura corporal se activan mecanismos compensatorios y adaptativos para restaurar la homeotermia y homeostasis (Rebez et al., 2023). Además, también se liberan catecolaminas, lo que aumenta la actividad metabólica del corazón, lo que produce hipertermia inducida por el estrés durante los primeros 10 a 15 minutos después de la exposición al factor estresante (Oka, 2018; Olivier et al., 2005) en preparación para la posibilidad de un rápido gasto de energía (Peaston & Weinkove, 2004).

En este sentido, la secreción y acción de las catecolaminas así como, el estrés inducido por la hipertermia tienen efectos sobre el metabolismo muscular y la integridad de las membranas, generando la aparición de características indeseables en la carne (Ghassemi Nejad et al., 2017), la disminución del peso vivo y la modificación de características fisicoquímicas que afectan tanto a la calidad como a la seguridad del corte final (Archana et al., 2018), lo anterior se asocia principalmente con el agotamiento de las reservas de glucógeno muscular y la acumulación de ácido láctico ante mortem, aumentando los valores de pH post mortem (Hagenmaier et al., 2017), pérdida por cocción, dureza de la carne (Chulayo & Muchenje, 2013) y potencial de envejecimiento (Hultgren et al., 2022).

Esto coincide con lo reportado por Burdick et al. (2010), quienes encontraron que la temperatura rectal más alta de los toros Brahman se registró dentro de los primeros 30 minutos después del transporte, mientras que la temperatura más baja ocurrió a las 6 horas y 40 minutos después del transporte. En este sentido, es posible que VC no de suficiente tiempo a los animales para que se habitúen, lo que culminará en una respuesta de estrés agudo.

Para VL, los búfalos podrían acostumbrarse al transporte y sus factores estresantes relacionados según lo que se ha indicado en los estudios (Gallo et al., 2003; Valadez Noriega & de la Lama, 2020). Esto coincide con lo observado por Lei et al. (2023), donde encontraron que el transporte de 15 a 17 h de 20 terneros Arouquesa tuvo un aumento inicial de 3°C en la temperatura periocular y una disminución posterior de 2°C. Los autores concluyeron que los animales se habitúan al transporte, aunque el aumento de temperatura representa un coste metabólico importante, que puede ser difícil de mantener durante un largo período como lo representa el VL.

Otro elemento relacionado con la respuesta térmica observada en animales VL sería la secreción de catecolaminas que conduce a efectos fisiológicos como taquicardia y vasoconstricción (Borell, 2001). Por lo tanto, puede ocurrir una disminución en la radiación de calor, asociada con una mayor respuesta simpática, como se ha observado en ganado en condiciones asociadas con dolor agudo (Stewart et al., 2008, 2009, 2010). Por lo tanto, es posible sugerir que, aunque la temperatura de la superficie fue 5°C menor en los animales del grupo VL, la respuesta térmica puede estar asociada con un evento crónico en comparación con los animales del grupo VC. Cabe señalar que este consenso es difícil de afirmar por la falta de evidencia porque otros factores como la disminución de la actividad visceral por ayunos prolongados en viajes de larga distancia y la disminución de la temperatura corporal también podrían influir en la respuesta térmica (Knizkova et al., 2007; Schaefer et al., 1989).

Por otro lado, se observó que la temperatura superficial tanto en VC como en VL, las fases de arreo, rampa de manipulación y pre y post transporte aumentaron significativamente en al menos 3°C en comparación con el resto de las fases (p< 0,001). La explicación a estos hallazgos es que en estas fases interviene la interacción con los humanos (Schmitz et al., 2020), que muchas veces puede ser negativa debido a los gritos, golpes o el uso de palos y picanas eléctricas que generan dolor y miedo en el animal, aumentando la respuesta al estrés agudo (Mota-Rojas et al., 2020a). Se menciona que la movilización y la interacción humano-animal sin entrenamiento previo son estresantes para el ganado, ya que involucran estímulos aversivos tanto físicos como psicológicos (Gupta et al., 2007). Estos resultados coinciden con los reportados por Rodríguez-González et al. (2023), donde observaron que la temperatura superficial en 624 búfalos de agua movilizados en VC (duración promedio = 2 h ± 20 min) tuvo un aumento promedio de 5°C durante las fases donde estuvo presente algún tipo de manejo. Entonces, es posible reafirmar que estas interacciones pueden provocar miedo agudo y una respuesta fisiológica asociada a este manejo, limitando la productividad, el rendimiento y el bienestar de los animales (Hemsworth, 2003).

Desde una perspectiva neurobiológica, el miedo es procesado por regiones del cerebro como la corteza somatosensorial, el hipocampo, el tálamo y la amígdala, que en coordinación con esta última estructura procesan la respuesta de miedo (p. ej., taquipnea, taquicardia o hipertermia) (Hernández-Avalos et al., 2021b; Lees et al., 2019; Sapolsky, 2000). Esta respuesta puede explicar el aumento de la temperatura superficial en las ventanas térmicas evaluadas debido a la activación de HPA (Nakamura, 2015) y la posterior secreción de glucocorticoides endógenos (Sapolsky, 2000) (Figura 12). Una posible explicación adicional es la neofobia, un rasgo presente en los búfalos de agua, que posiblemente los hace susceptibles al miedo a entornos desconocidos como el transporte (Grandin & Shivley, 2015). En este sentido, se ha informado que el ganado lechero puede experimentar más miedo en salas de ordeño y ganado desconocidos (Lowe et al., 2019).

La misma respuesta asociada al miedo puede explicar las diferencias encontradas entre las ventanas térmicas evaluadas en el presente estudio. Quizás el ejemplo más claro se pueda observar en la ventana nasal, donde la respuesta térmica sirve para evaluar el parámetro fisiológico de la frecuencia respiratoria (Lowe et al., 2019; Stewart et al., 2017). Teniendo en cuenta que la taquipnea es una de las respuestas fisiológicas asociadas al estrés, esto puede provocar un aumento del calor irradiado (Thomson et al., 2015) (Figura 14).

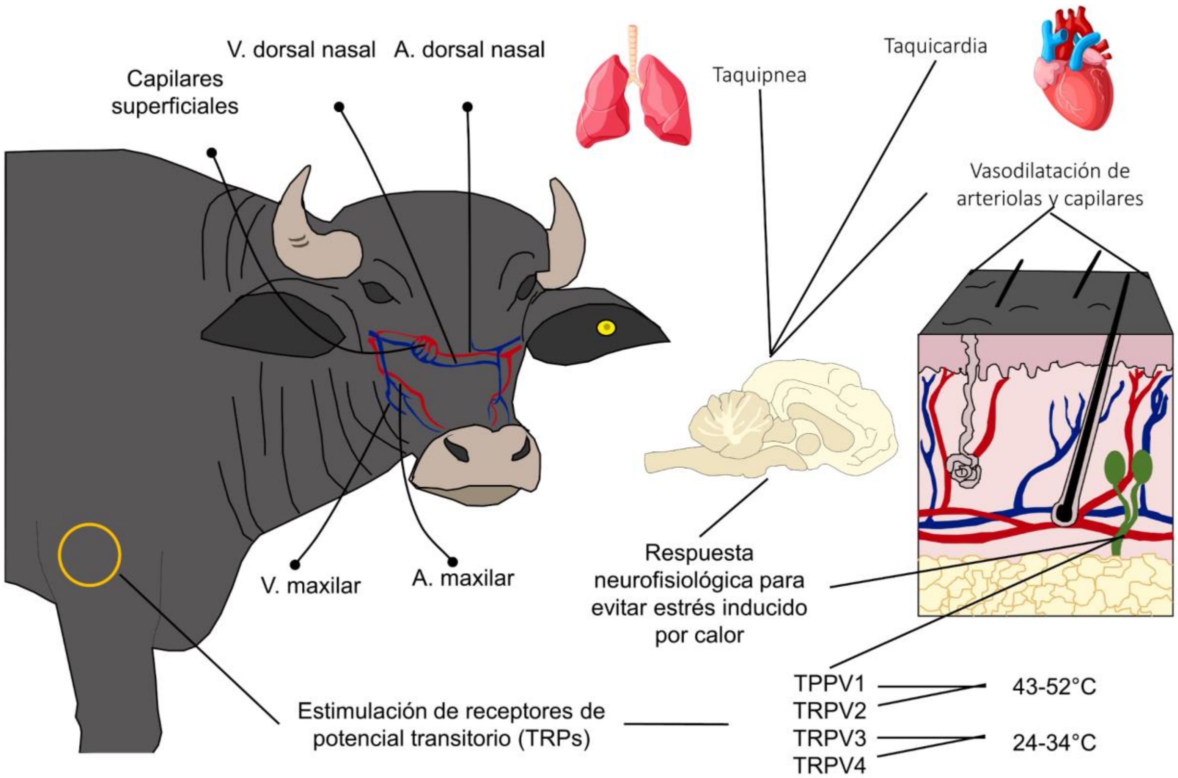

Figura 14. Distribución arterio venosa presente la región nasal del búfalo de agua (Bubalus bubalis). Ante la estimulación de receptores de potencial transitorio (TRPs) específicos para altas temperaturas se realiza la respuesta neurofisiológica para evitar o hacer frente al estrés inducido por calor. Esta región permite evaluaciones de dos condiciones: cambios en la microcirculación de los capilares superficiales de la arteria maxilar y, la eliminación de vapor de agua durante el proceso respiratorio que permite evaluar, a distancia, la frecuencia respiratoria observando cambios en el patrón térmico a nivel central de las fosas nasales.

En cuanto a las diferencias encontradas entre grupos en la región facial, se presentaron mayores desequilibrios en el grupo VC entre F1 y F7 en la ventana del párpado inferior. Esta mayor variación también estuvo presente en el resto de las ventanas térmicas de la región facial para este grupo. Los cambios en la temperatura de la región facial pueden estar relacionados con la actividad parasimpática, la respuesta de vasodilatación, que genera una disminución del gasto cardíaco y de la presión arterial y el consecuente aumento de la temperatura (Lei et al., 2023). En particular, la amplia distribución de capilares y anastomosis arteriovenosas en estas ventanas térmicas facilita el intercambio de calor corporal con el medio ambiente (Tattersall, 2016).

Por otra parte, los valores más bajos se observaron en la ventana nasal del grupo VL durante F1 (29,03±0,04°C, p<0,001). Lo anterior puede deberse a la naturaleza de la propia región anatómica, la cual presenta una alta vascularización superficial proveniente de la vena y arteria maxilar (Mota-Rojas et al., 2021b), además de la humedad dada por el vapor de agua eliminado durante la respiración, que se ve afectado por el estrés generado durante la taquipnea (Lowe et al., 2019) (Figura 14). Otros autores también describen los cambios de temperatura provenientes de la región nasal como un reflejo de la actividad del SNA, que puede usarse para detectar un aumento en el nivel de excitación (con una disminución de la temperatura) debido al aumento del sistema simpático, actividad y disminución del flujo sanguíneo en los vasos periféricos (Diaz-Piedra et al., 2019; Matsuno et al., 2018).

En cuanto a las regiones del cráneo y sus respectivas ventanas térmicas, se observó una mayor variación entre grupos. Esta región recibe irrigación por la arteria corneal, la arteria temporal superficial (*A. temporalis superficialis*) y sus ramas (*A. transversa faciei, A. auricularis rostralis, A. palpebralis inferior lateralis, A. palpebralis su-perior lateralis*) (International Committee Veterinary Gross Anatomical Nomenclature, 2017). En este sentido, Badakhshan et al. (2015) evaluaron la temperatura del ganado Jersey en diferentes regiones anatómicas y su coeficiente de correlación con la temperatura rectal, la frecuencia respiratoria y la frecuencia cardíaca. Los autores observaron que los valores de la frente tuvieron una correlación fuerte, positiva y significativa (r= 0,57, r= 0,88 y r=0,70 respectivamente, p<0,01). También se ha especificado que, en especies de bóvidos criados en zonas áridas, el cuerno tiene vainas de queratina más delgadas que en climas templados para facilitar la pérdida de calor (Cain et al., 2006; Picard et al., 1999). Algra et al. (2023) apoyaron el papel de los cuernos para la termorregulación en el ganado lechero, monitorizando a las vacas descornadas mediante IRT. Estos animales presentaban una temperatura ocular más alta que las vacas con cuernos.

Respecto a las regiones del cuerpo especificadas, se observó una tendencia similar en la ventana térmica torácica y el miembro pélvico, con mayores variaciones térmicas en el miembro pélvico. Por el contrario, la ventana térmica torácica presentó menores variaciones con mínimas de 0,97° y máximas de 1,11°C en las fases 7 y 1-4 respectivamente (p<0,001). Con excepción de la ventana térmica abdominal, VL reportó las temperaturas más altas sin considerar la fase ni la ventana. El comportamiento de las regiones torácica y abdominal puede deberse a la presencia de órganos metabólicamente activos. Además, se ha establecido que la relación entre la temperatura ambiental y superficial de los animales suele ser más cercana a la del tórax, donde la variación de la sangre es mínima (Upadhyay et al., 2014), debido a la irrigación de la arteria aorta caudal (*A .aorta abdominalis*) hacia los órganos abdominales y los cuadrantes craneal, mediano y caudal (Mota-Rojas et al., 2022a).

Por el contrario, las temperaturas superficiales más bajas para todas las fases se observaron en el miembro pélvico y, en esta misma ventana, se observó una mayor variación entre grupos ($p<0{,}001$). Esto también se observó en un estudio reciente realizado en 109 terneros recién nacidos de búfalo de agua, que encontraron los valores de temperatura más bajos en el miembro pélvico en comparación con las ventanas térmicas de la región facial (carúncula lagrimal, región periocular y glándula lagrimal) (Napolitano et al., 2023). Lo anterior podría explicarse por la reducción del flujo sanguíneo por vasoconstricción y cierre de anastomosis arteriovenosas de extremidades durante procesos estresantes como el transporte (Ewart, 2020), priorizando el aporte sanguíneo a la zona torácica y abdominal.

Finalmente, el nivel de correlación entre las ventanas central y periférica fue positivo, fuerte y significativo (r superior a 0,9, $p<0{,}001$). Esto significa que, con un aumento de la temperatura corporal, la temperatura superficial de los animales también podría aumentar (Brcko et al., 2020; Mota-Rojas et al., 2021a; Rodríguez-González et al., 2023b). Lo anterior podría deberse al efecto de los glucocorticoides que desencadenan la degradación de lípidos y glucógeno, afectando la temperatura de las ventanas tanto centrales como periféricas, con mayor aumento en aquellas ventanas térmicas cercanas a órganos metabólicamente activos (Gonzalez-Rivas et al., 2020b; Wang et al., 2015).

10.3. Fase post mortem; “Caracterización fisicoquímica de diferentes cortes cárnicos de búfalo de agua en comparación con el bovino”.

Esta última fase tuvo como objetivo la caracterización y comparación de las propiedades fisicoquímicas de diferentes cortes cárnicos de búfalo de agua y carne de bovino criado en el trópico húmedo mexicano, además de evaluar el grado de aceptación de ambas especies por medio de un análisis hedónico. Lo anterior debido a que, el búfalo de agua ha sido una opción atractiva para unidades de producción mexicanas cuyas características ambientales y orográficas complican el desarrollo de especies “convencionales” como el bovino del género *Bos* con enfoque a producción cárnica (Rodríguez-González et al., 2022c). Pese a lo anterior, se continua la percepción pública de que la carne de búfalo presenta características asociadas a una menor calidad que la carne de vacuno (Neath et al., 2007). Por lo anterior la realización de la presente investigación es sumamente importante para la generación de información científica de utilidad práctica respecto a las características de calidad presentes en la carne de búfalo y vacuna, además del conocimiento del nivel de aceptación por el consumidor.

10.3.1. Color

El color es descrito como el principal atributo que el consumidor percibe y juzga a simple vista para adquirir un producto, este se ve influenciado por características genéticas, condiciones ante y post mortem, procesos químicos musculares, procesamiento, almacenamiento, exhibición y preparación final para el consumo (Ahmed et al., 2017; Mancini & Hunt, 2005).

Para este estudio se presentaron los valores más bajos para L* y b* en los cortes crudos de RE, siendo el más bajo el RE de búfalo de agua con 35.45 y 5.91 unidades respectivamente, por el contrario, los valores más elevados para ambas características en cortes crudos se presentaron en los cortes de NY de bovino del género *Bos* (L*=44.61 y b*=8.43). Al comparar entre especies, los valores más altos de L* y b* se presentaron siempre en los cortes provenientes de bovino del género *Bos* con diferencias significativas entre cortes ($p<0.05$), presentando también los valores más elevados en cortes que atravesaron un proceso de cocción. Respecto a los valores de a* se observaron comportamientos similares entre especies para cortes tanto en crudo como en cocido, estas coordenadas, discrepando de lo observado con algunos autores que encontraron un aumento significativo ($p<0.05$) en a* en cortes provenientes de búfalos de agua, lo anterior podría deberse a un mayor contenido de proteína soluble sarcoplásmica, contribuyendo a observar una carne de búfalo más roja (Hassan et al., 2018).

Este comportamiento también fue reportado por Hassan et al. (2018) quienes evaluaron las características de L* (Luminosidad), a* (rojo-verde) y b* (amarillo-azul) en muestras provenientes del músculo *Longissimus dorsi* de carne de res brasileña y búfalo indio, reportando valores de L* más elevados en la carne de res con respecto a la carne de búfalo (40.08 y 37.09 unidades respectivamente, $p<0.05$), así mismo, para las coordenadas de b* se presentaron valores más altos en la carne de res con 11.19 vs 10.66 unidades (carne de búfalo), contrario al valor de a* el cual fue 8.25% más elevado en la carne de búfalo ($p<0.05$). Así mismo, Failla et al. (2007) registraron valores de L* más altos en la carne de búfalo, contrario a los valores de a* y b* en donde, los resultados fueron más bajos en la carne de búfalo con respecto a la carne vacuna.

Por otra parte, la modificación para de las coordenadas analizadas en este estudio sobre los cortes cárnicos se debe a la desnaturalización proteica con la pérdida de la estructura helicoidal de las proteínas (Liu & Chen, 2001). En este sentido, los componentes que controlan las características de dureza (proteínas miofibrilares, elastina, tejido conectivo y colágeno) son modificadas estructuralmente debido al proceso de cocción, provocando la contracción de las fibras de la carne, solubilización del tejido conectivo y la destrucción de membranas celulares (Tornberg, 2005). Además, proteínas como mioglobinas (pigmento primario responsable del color en la carne) se interconvierten y degradan mediante reacciones de oxigenación, oxidación y reducción influyendo en el color de la carne **(Figura 15)** (Lorenzo et al., 2015).

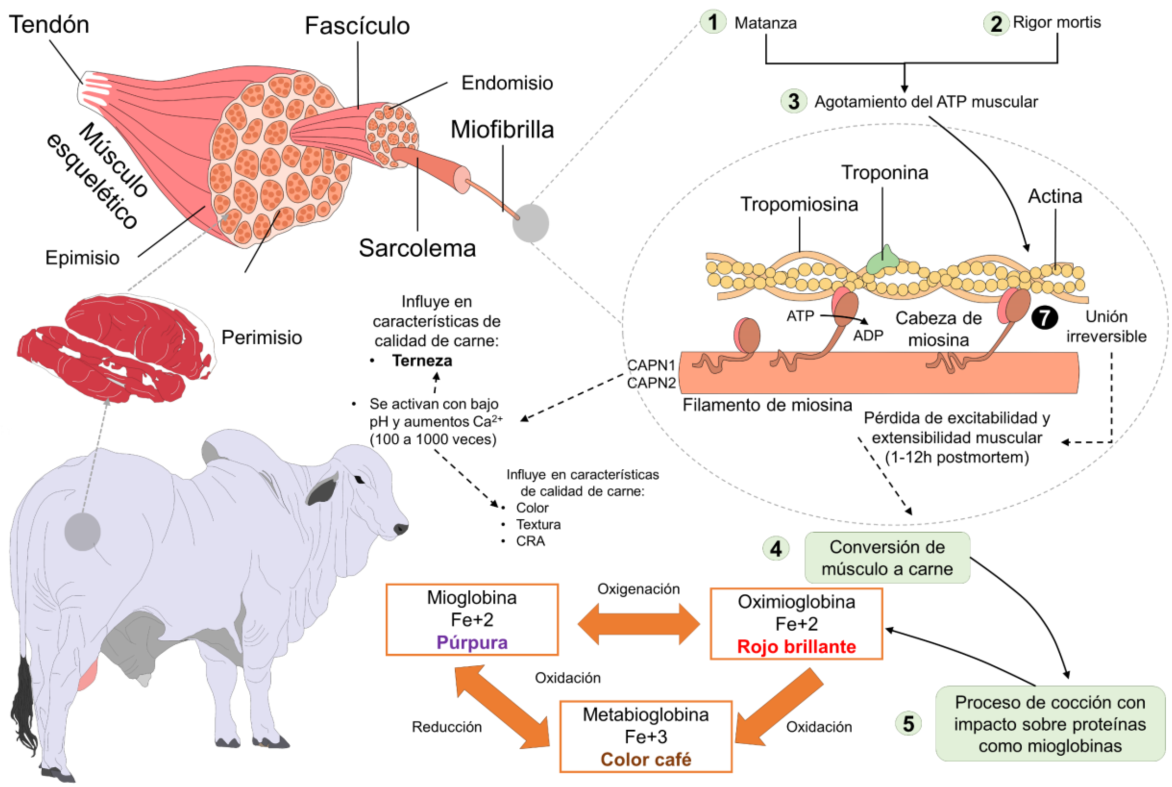

Figura 15. Conversión de músculo a carne y cambios sobre mioglobinas ante procesos de cocción. Una vez que se lleva a cabo el proceso de sacrificio y exanguinación de los búfalos de agua, este mismo evento y la síntesis y utilización muscular del ATP genera un efecto

de anoxia tisular. La reducción en la concentración de O2 muscular induce al metabolismo anaeróbico del glucógeno para producir ATP. No obstante, debido a que la capacidad de este mecanismo es limitada y la hidrólisis de ATP supera la producción de este, se presenta el efecto del rigor mortis, un efecto que también se favorece con el agotamiento de las reservas de ATP muscular y la unión irreversible de la miosina y actina. La pérdida de la excitabilidad y extensibilidad muscular, un proceso que puede durar entre 1 y 12 horas dependiendo de la especie, da lugar a la conversión de músculo a carne. Un efecto que ocurre a la par con dicho evento es la acidificación de la carne por un aumento en las concentraciones de lactato, lo cual influye en las características de calidad de la carne. Asimismo, la acción de las calpaínas en los filamentos de miosina, las cuales se activan con pH bajos y aumentos en las concentraciones de Ca2+, participan en la maduración de la carne y pueden alterar la terneza de esta. ADP: adenosín difosfato; ATP: adenosín trifosfato; Ca2+: calcio; CAPN1: calpaína 1; CAPN2: calpaína 2.

Así, la cocción genera un proceso de oxidación de los grupos hemo del pigmento, observándose que, ante un aumento de la temperatura se reduce la intención de DeoxyMb y OxyMb (color rojizo) de intensidad máxima y un aumento de MetMb (rojo parduzco) y SulfMb (verdoso), lo anterior explica la disminución de los valores de a* (color rojo), y el incremento de los valores de L* (los filetes cocidos eran más claros) y b* (los filetes más amarillos) de todos los cortes después del proceso de cocción ($p<0,05$) (García-Segovia et al., 2007).

10.3.2. pH, Aw, Textura y CRA

De manera general, los valores más elevados de pH se observaron en los cortes de búfalo de agua (tanto en crudo como cocido), lo anterior concuerda con lo especificado por autores como (Ijaz et al., 2020b; Naveena & Kiran, 2014), y, pese a que los valores en crudo fueron de los 6.01 a los 6.16 en el búfalo de agua *vs* 5.74- 6.07 en el bovino del género Bos estos datos no representan un riesgo a la calidad o inocuidad del producto.

La importancia del conocimiento de los valores de pH radica en su influencia sobre posibles variaciones en características como color, terneza, sabor, capacidad de retención de agua y vida útil (Nuraini et al., 2019) debido a las concentraciones de ácido láctico durante el proceso de conversión de músculo a carne y la capacidad de crecimiento bacteriano que limita o permite una menor contaminación de la canal durante el procesamiento y almacenamiento de cortes comerciales (Figura 15) (Cruz-Monterrosa et al., 2020).

Particularmente en el búfalo de agua se han reportado valores de van de los 5,4 a los 5, 7 (Kandeepan et al., 2013) en muestras de carne fresca (Naveena & Kiran, 2014) y de 6.15 a 6.26 en cortes cárnicos en punto fijo de venta que ya atravesaron un proceso de enfriamiento (Ijaz et al., 2020b). Por otra parte, en el bovino convencional los valores de pH en cortes a venta van de los 5.74- 6.07 (Figura 16) (Ijaz et al., 2020b).

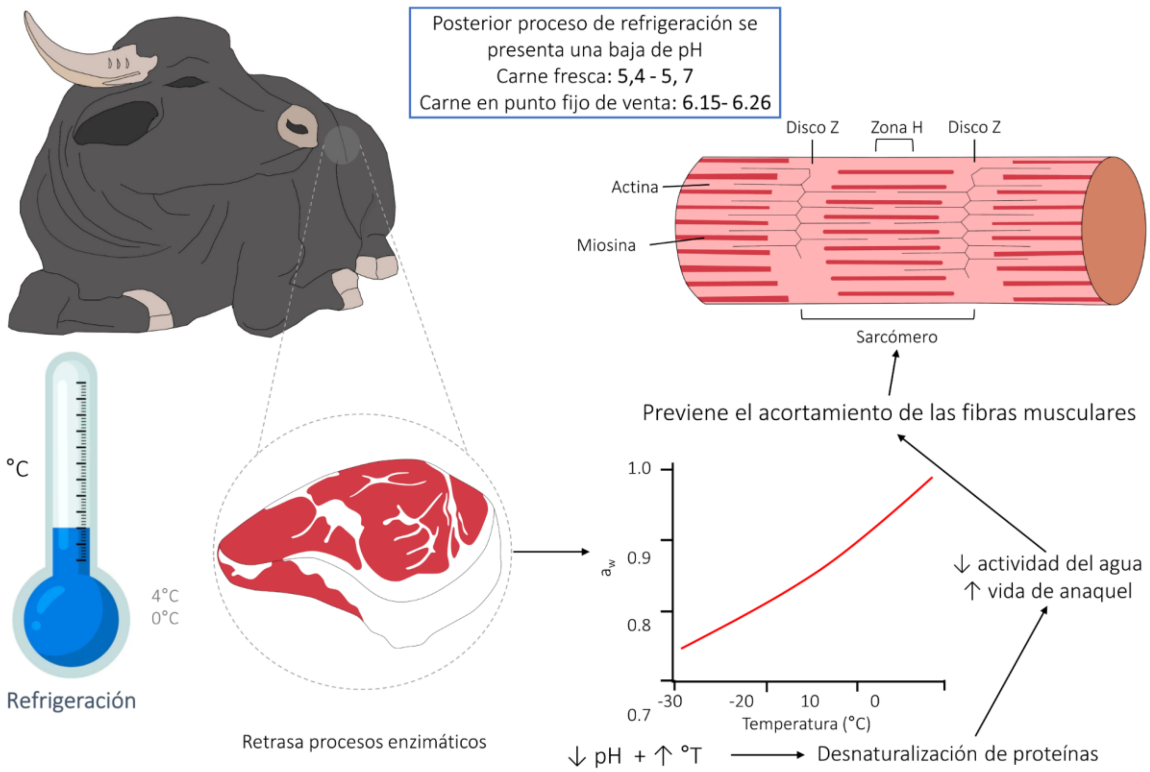

Figura 16. Influencia de la temperatura en la calidad de la carne de búfalo. La función de la refrigeración de la carne (entre 0 a 4°C) es prevenir el acortamiento de las fibras musculares, particularmente miosina, favoreciendo la terneza de la carne. De igual forma, mantener la carne en refrigeración a bajas temperaturas disminuye la actividad del agua de la carne, aumentando su vida de anaquel y previniendo el crecimiento de microorganismos.

Así mismo, Neath et al. (2007) determinaron el efecto de las variaciones de pH post mortem sobre la textura de músculos *Longissimus thoracis* y *semimembranosus* provenientes de 5 hembras mestizas de búfalo de agua Murrah y 5 hembras mestizas de ganado Brahman encontrando que la disminución del pH post mortem de la carne bufalina es significativamente más lenta ($p<0.05$) que la carne de ganado Brahman y que, los valores de pH son más elevados en el búfalo de agua, sin embargo, este estudio también demostró una terneza superior en la carne de búfalo en comparación con carne de vacuno Brahman.

De esta forma, los valores de pH se ven influenciados por características genéticas y ambientales, en donde, el proceso de movilización o manejo pre mortem juega un rol de suma importancia (Alarcón-Rojo et al., 2021), así mismo, el manejo dado a las canales y los cortes post mortem tiene efectos sobre la transformación enzimática de músculo a carne (Aksoy et al., 2021) modificando a su vez valores fisicoquímicos con efectos en características de importancia para la toma de decisión del comprador final (Andrade et al., 2021).

Para la variable de Aw los valores en los cortes de búfalo de agua se mantuvieron en 0.92 sin considerar cortes o procesos de cocción, teniendo una diferencia significativa ($p<0.05$) únicamente en el corte crudo de pierna, por el contrario, al realizar una comparativa entre especies se observan valores más elevados en los cortes vacuno, con valores de 0.93 y 0.95 (solo para pierna cruda).

La actividad de agua es una variable de importancia al ser el principal parámetro a observar en la estabilidad de los alimentos y su capacidad de modulación de la respuesta microbiana (Tapia et al., 2020), esta variable se encuentra influenciada por factores como temperatura ambiental, pH y presencia de microorganismos patógenos en el ambiente en el cual se mantiene el producto cárnico (Chirife & Fontana, 2007). En este sentido, los valores reportados en este documento se encuentran dentro de los parámetros observados para productos cárnicos de búfalo (Malik & Sharma, 2010) y bovino Bos (Kinsella et al., 2006).

Respecto a la textura, de manera general, los valores más bajos de textura de cortes crudos se observaron en los provenientes de búfalo de agua (mínimo de 3.68 unidades en NY crudo de búfalo), y esto se observó a la inversa cuando los cortes fueron sometidos a un proceso de cocción (máximo de 5.02 en RE cocido de búfalo). La textura es un criterio asociado a calidad de carne, afectada por diversos factores y observándose como una cualidad representada por propiedades estructurares de las proteínas del músculo esquelético (Ahmed et al., 2017), esta depende de la cantidad y calidad del tejido conectivo y especialmente, en la carne de animales jóvenes, de la estructura de su sistema miofibrilar, la naturaleza y origen del corte, las condiciones de instauración del rigor mortis, pH, CRA y procesos post mortem como aplicación de enfriamiento y maduración (Andrade et al., 2021; Kandeepan et al., 2013).

Autores como Belew et al. (2003) agruparon y categorizaron características de dureza de acuerdo a los valores de fuerza obtenido con Warner-Bratzler Shear (WBS) de la siguiente manera; WBS <3.2 como muy blando, WBS<3.9 blando, WBS<4.6 intermedio y WBS>4.6 duro, mencionando que la fuerza de corte mantiene una correlación negativa con la sensibilidad (Nuraini et al., 2019), siguiendo esta clasificación los valores de fuerza de corte obtenidos en esta caracterización se ubican en blando para todos los cortes de búfalo de agua (3.68 a 4.10) y los de bovino *Bos* (3.91 a 4.26). Sin embargo, al atravesar procesos de cocción la clasificación en ambas especies se modifica a intermedio a duro dependiendo el corte analizado.

Este incremento en los valores de dureza con respecto al proceso de cocción se atribuye al proceso de desnaturalización del colágeno intramuscular y los cambios en la estructura miofibrilar, solubilizando el tejido conectivo, desnaturalizando las proteínas miofibrilares y endurecimiento de la carne (García-Segovia et al., 2007).

Contrario a lo observado en este documento, Hassan et al. (2018) compararon las características fisicoquímicas y los atributos sensoriales de carnes de res y búfalo, al respecto reportaron una fuerza de corte (Kgf) 35% mayor en la carne de búfalo ($p<0.05$), lo anterior se ha asociado con una menor solubilidad del colágeno, en combinación con el porcentaje de tejido conectivo presente en la carne de búfalo, y la solubilidad de proteínas sarcoplásmicas y miofibrilares contribuye a los niveles de dureza en esta carne (Naveena et al., 2004).

Finalmente, para la característica de CRA de manera general se observaron valores ligeramente más elevados en los cortes de búfalo de agua (0.18% para NY y 0.20% para RE, $P>0.05$), a excepción de Pi cruda que presentaron valores más elevados en los bovinos del género *Bos*, para ambas especies los valores fluctuaron en valores mínimos de 21.09% (pierna cocida de bovino *Bos*) y máximos de 28.84% (NY crudo de búfalo de agua). De esta forma, los valores encontrados son similares a los mencionados por (Kiran et al., 2016) con porcentajes de 31.33% para CRA para *Longissimus dorsi* en búfalos de agua jóvenes y 31% en bovinos *Bos* (Naveena & Kiran, 2014).

Así mismo, se observó siempre una disminución de los valores de CRA posterior al proceso de cocción. Lo anterior se debe a que se inducen cambios estructurales que minimizan la capacidad de retención de agua de la carne debido a que, desde los 60 a 70°C la red de tejido conectivo y fibras musculares son contraídas longitudinalmente incrementando el grado de contracción de acuerdo con la temperatura a la cual es sometida la carne

(Puolanne & Halonen, 2010), ocasionando presión y el vacío extracelular por la presión ejercida por el tejido conectivo y, minimizando los porcentajes de CRA (Tornberg, 2005).

10.3.3. Análisis hedónico

Los atributos fisicoquímicos de la carne como el pH, color (L*, a* y b*), la capacidad de retención de agua, actividad de agua y textura se encuentran relacionados con las características organolépticas que dictan el nivel de aceptación o agrado de un producto para el consumidor final, así mismo, otros factores estructurales de la carne influyen en la expresión de aromas percibidos durante la masticación (Baune et al., 2023).

Con lo anterior, la realización de un análisis hedónico fue necesario en este estudio para observar el grado de aceptación entre especies (bovinos género *Bos* y Búfalos de agua) con respecto a características como textura, jugosidad, sabor, aroma y aceptación en general. Contrario a esto, Hassan et al. (2018) evaluaron mediante un análisis sensorial apariencia, terneza, jugosidad, sabor y aceptabilidad y, a diferencia de este documento las variables de apariencia, terneza y jugosidad de cortes vacunos (Brahman) fue superior ($p<0.05$).

Únicamente se observaron diferencias significativas ($p<0.05$) para aroma, teniendo un mayor nivel de agrado sobre el RE de bovinos del género *Bos*. Esta característica se encuentra influenciada por componentes aromáticos obtenidos a través de precursores no volátiles durante la cocción permitiendo la oxidación y degradación lipídica, la interacción peptídica, proteica y de aminoácidos y ribonucleótidos y la consecuente degradación térmica de tiaminas (Khan et al., 2015).

Para el resto de las variables (textura, jugosidad, sabor y aceptación) no se presentaron diferencias significativas ($p>0.05$), sin embargo, es relevante la mención de una mayor preferencia de la carne de búfalo de agua para las variables terneza y jugosidad. Con respecto a la terneza esta influenciada por las características estructurales de las proteínas musculares y, tanto terneza como jugosidad son variables influenciadas por el porcentaje de grasa intra muscular, el cual, se ha reportado que es menor en el caso del búfalo de agua (Rey & Povea, 2012), así mismo, factores como porcentaje de pérdida por cocción minimizan la preferencia por cortes bufalinos con respecto a los cortes vacunos.

11. CONCLUSIONES

Respecto a la fase ante mortem (etapa 1) se concluye que, de acuerdo con los valores de temperatura superficial de los búfalos de agua transportados por un periodo corto, el transporte y las prácticas aplicadas durante esta actividad se observan afectaciones a la temperatura de diversas regiones corporales y craneofaciales. Las respuestas de cada región dependen en gran medida de la fase en la que se evalúan (desde el potrero hasta el post transporte). El arreo y el embarque fueron identificados como las fases en que los búfalos presentan respuestas de estrés inducido por calor más marcadas, incrementando los valores térmicos registrados. Las fuertes correlaciones positivas entre las ventanas central y periférica pueden ayudar a comprender la importancia de brindar un buen manejo a las especies de búfalos durante el transporte, con el objetivo de minimizar el estrés potencial que pueden experimentar y las respuestas fisiológicas que pueden afectar su bienestar.

Siguiendo con la fase ante mortem (etapa 2) se concluyó que los viajes cortos aumentan la respuesta térmica en los búfalos de agua en comparación con los viajes largos monitorizados por IRT, y esto podría estar asociado con estrés térmico agudo, refutando la hipótesis propuesta en esta etapa. Lo anterior podría deberse a que, durante los VL, los búfalos de agua pueden habituarse a los factores estresantes. Asimismo, se concluye que las fases que involucran la interacción humano-animal generan un aumento en la temperatura superficial para todas las ventanas térmicas evaluadas, reafirmando la importancia de un manejo adecuado durante el proceso de transporte para evitar efectos negativos y temperaturas superficiales de los búfalos movilizados.

Respecto a la fase post mortem (etapa 3) se concluyó que, los cortes provenientes de búfalo de agua (*Bubalus bubalis*) presentaron una mayor fuerza de corte, a*, pH, y CRA, además, los procesos de cocción y la naturaleza de cada corte impactaron en todas las características fisicoquímicas estudiadas, sin embargo, estas diferencias no representan un impacto negativo significativo sobre el nivel de aceptación del consumidor final, niveles similares entre especies, exceptuando el aroma, característica que presentó mayor aceptación para los cortes provenientes de bovino del género Bos. Para todos los estudios, tanto ante como post mortem se resalta la importancia de su realización, publicación y difusión debido a que son pioneros en el análisis del comportamiento térmico del búfalo de agua durante el transporte y la comparación e identificación de características fisicoquímicas y nivel de aceptación de carne proveniente de búfalo de agua y bovino del género Bos.

12. APLICACIONES PRÁCTICAS

El desarrollo de los sistemas doble propósito de búfalos de agua en el trópico húmedo mexicano es relativamente reciente y progresivamente se ha mejorado debido a una gestión informada y basada en objetivos acorde a la especie, cambiando la experiencia acumulada de los ganaderos y las innovaciones que se han generado en el plano nacional y, sobre todo, en el internacional. Sin embargo, como se ha mostrado a lo largo de este trabajo, existen amplios márgenes de mejora que sería importante atender de manera paulatina.

El presente estudio demuestra que el búfalo de agua genera una respuesta térmica que puede tener origen en la aplicación de prácticas durante la movilización, interacción humano animal y la exposición a ambientes nuevos y aversivos para los animales a transportar, proceso necesario en una unidad de producción ganadera, y lo anterior se refleja en el aumento de la temperatura superficial para las diferentes ventanas térmicas analizadas durante las diferentes fases, además de que el comportamiento térmico es diferente según la ventana estudiada. Estas condiciones pueden generar efectos fisiológicos por lo que es necesario la monitorización de temperatura, siendo la IRT una opción asertiva porque su uso no implica la generación de más estrés al búfalo al ser una herramienta no invasiva.

Las opciones de mejora pasan por abandonar poco a poco las prácticas y rutinas que los ganaderos aprendieron a partir de los sistemas de doble propósito de los bovinos convencionales y comprender con mayor detalle los patrones de conducta y la fisiología de los búfalos de agua. En efecto, los búfalos tienen el hábito de moverse en forma agrupada y, son animales más lentos con diferencias anatómicas y conductuales que deberían ser informadas a los operadores, vaqueros, ganaderos y transportistas, ya que, es durante las fases de mayor interacción humano animal cuando se observa un mayor incremento de temperatura.

Por otra parte, la información contenida en esta tesis es útil para justificar la aplicación de logística planificada tanto en viajes cortos como largos con el objetivo de minimizar el traslado de búfalos durante horarios y condiciones climáticas que incrementen el desarrollo de procesos de estrés inducido por calor durante el transporte.

El segundo enfoque abordado (etapa post mortem) tiene grandes aplicaciones para la difusión de información científica tanto a productores como consumidores con respecto a las características presentes en la carne de búfalo de agua y bovino convencional del género Bos, y contrarresta la idea de que no se tiene una aceptación por aspecto, olor y sabor de la carne de búfalo en México. Lo anterior puede ser aplicado en asociaciones o empresas para el crecimiento productivo y comercial de productos de origen bufalino.

Así mismo, la información publicada con respecto a los artículos de revisión tiene aplicaciones a nivel legislativo para la observación de oportunidades y áreas de mejora en gobiernos y para la identificación y conocimiento del manejo llevado en diversas unidades productivas, así como la identificación y conocimiento de la fisiología, anatomía y comportamiento presente en el búfalo de agua para el perfeccionamiento de un manejo más apropiado que permita incrementar la eficiencia de este sistema de doble propósito y, en segunda instancia, mejorar el nivel de vida de los productores primarios.

13. PERSPECTIVAS

El análisis realizado en este estudio representa información novedosa sobre el comportamiento térmico y el efecto del factor tiempo durante el transporte de búfalos de agua monitorizados por IRT. Sin embargo, es necesario visibilizar las limitaciones, perspectivas y futuras investigaciones relacionadas con este enfoque, por ejemplo, al no evaluar otras variables físicas como la temperatura rectal, la frecuencia cardíaca y respiratoria, así como las concentraciones sanguíneas de cortisol, glucosa, lactato y valores de pH, o biomarcadores relacionados con procesos de deshidratación (osmolaridad, albúmina y hematocrito) y otros biomarcadores como catecolaminas, alfa amilasa, IL, TNF alfa y creatina quinasa, entre otros, así como la evaluación de las expresiones faciales, el comportamiento y las emociones, puede abrirse un abanico de futuras investigaciones sobre el efecto del transporte y el manejo que este implica sobre los indicadores fisiológicos y térmicos en búfalos de agua.

Por otra parte, sería relevante considerar a futuro las condiciones climáticas cambiantes entre un viaje corto y uno largo, como la temperatura ambiente y la humedad relativa (Rodríguez-González, Guerrero Legarreta, Chay-Canul, et al., 2023). Esto es relevante porque la temperatura ambiente afecta la temperatura de la superficie de los animales (Soerensen & Pedersen, 2015). No obstante, aunque los factores ambientales podrían tener cierta influencia en la termorregulación, los hallazgos del presente estudio muestran que el transporte es un factor estresante potencial que altera la respuesta térmica de los búfalos (2017; Carnovale et al., 2021). Investigaciones adicionales deberían adoptar y considerar estas variables para comprender la influencia de dichas variables en la posible respuesta térmica mediada por el estrés de los búfalos de agua.

Respecto al análisis y caracterización de cortes cárnicos resulta importante mencionar que esta tesis no se realizaron estudios complementarios que podrían brindar una explicación más profunda respecto al porque se prefirieron ciertas características en el análisis hedónico para la carne de búfalo o para la carne de res, considerando la obtención de perfiles proteicos, nutricional y perfiles de ácidos grasos, así como, realizar estudios comparativos respecto a sistemas productivos o la aplicación de programas de finalización específicos y su efecto sobre características fisicoquímicas y organolépticas del producto final.

14. REFERENCIAS

Abdelnour, S. A., Abd El-Hack, M. E., Khafaga, A. F., Arif, M., Taha, A. E., & Noreldin, A. E. (2019). Stress biomarkers and proteomics alteration to thermal stress in ruminants: A review. *Journal of Thermal Biology*, *79*, 120–134. https://doi.org/10.1016/j.jtherbio.2018.12.013

Agnes, F., Sartorelli, P., Abdi, B., & Locatelli, A. (1990). Effect of transport loading or noise on blood biochemical variables in calves. *American Journal of Veterinary Research*, *51*, 1679–1681.

Ahmed, I., Lin, H., Zou, L., Brody, A. L., Li, Z., Qazi, I. M., Pavase, T. R., & Lv, L. (2017). A comprehensive review on the application of active packaging technologies to muscle foods. *Food Control*, *82*, 163–178. https://doi.org/10.1016/j.foodcont.2017.06.009

Aksoy, Y., Şahin, A., Ulutaş, Z., & Uğurlutepe, E. (2021). The effect of different slaughter weights on some meat quality traits of musculus longissimus dorsi thoracis of male Anatolian buffaloes. *Tropical Animal Health and Production*, *53*(1), 1–9. https://doi.org/10.1007/S11250-021-02571-Z/FIGURES/1

Al-Qarawi, A. A., & Ali, B. H. (2005). Isolation stress in desert sheep and goats and the influence of pretreatment with xylazine or sodium betaine. *Veterinary Research Communications*, *29*(1), 81–90. https://doi.org/10.1023/B:VERC.0000046741.97331.29

Alam, M., Hasanuzzaman, M., Hassan, M. M., Rakib, T. M., Hossain, M. E., Rashid, M. H., Sayeed, M. A., Philips, L. B., & Hoque, M. A. (2018). Assessment of transport stress on cattle travelling a long distance (≈648 km), from Jessore (Indian border) to Chittagong, Bangladesh. *Veterinary Record Open*, *5*(1), 1–10. https://doi.org/10.1136/vetreco-2017-000248

Alam, M. R., Gregory, N. G., Jabbar, M. A., Uddin, M. S., Kibria, A. S. M. G., & Silva-Fletcher, A. (2010a). Skin injuries identified in cattle and water buffaloes at livestock markets in Bangladesh. *Veterinary Record*, *167*(11), 415–419. https://doi.org/10.1136/vr.c3301

Alam, M. R., Gregory, N. G., Uddin, M. S., Jabbar, M. A., Chowdhury, S., & Debnath, N. C. (2010b). Frequency of nose and tail injuries in cattle and water buffalo at livestock markets in Bangladesh. *Animal Welfare*, *19*(3), 295–300.

Alarcón-Rojo, A., Mota-Rojas, D., García-Galicia, I., Ramírez-Bribiesca, E., Olmos-Hernández, A., & Guerrero-Legarreta, I. (2021). Dark cutting in large ruminants: Effect of management and environmental factors. *Agro Productividad*, *13*(12), 93–98. https://doi.org/10.32854/agrop.v13i12.1927

Algra, M., de Keijzer, L., Arndt, S. S., van Eerdenburg, F. J. C. M., & Goerlich, V. C. (2023). Evaluation of the thermal response of the horns in dairy cattle. *Animals*, *13*(3), 500. https://doi.org/10.3390/ani13030500

Ali-Gholi, R., Siamak, A. R., & Daryoush, M. (2007). Influence of the short-term road transport stress on blood parameters in cows. *Medycyna Weterynaryjna*, *63*(11), 1311–1315.

Álvarez-Macías, A., Mota-Rojas, D., Bertoni, A., & Dávalos-Flores, J. L. (2020). Opciones de desarrollo de los sistemas de producción de búfalos de agua de doble propósito en el trópico húmedo latinoamericano. In F. Napolitano, D. Mota-Rojas, I. Guerrero-Legarreta, & A. Orihuela (Eds.), *El búfalo de agua en Latinoamérica, hallazgos recientes.* (3rd ed., pp. 43–74). BM Editores.

Andrade, B. F., Castro, M. M. de, Rodrigues, L. M., Torres Filho, R. de A., Fontes, P. R., Ramos, E. M., & Ramos, A. de L. S. (2021). Rigor development and meat quality of murrah buffalo from different production systems. *Research, Society and Development*, *10*(6), e42810615814. https://doi.org/10.33448/rsd-v10i6.15814

Andrade, D. V. (2015). Thermal windows and heat exchange. *Temperature*, *2*(4), 451. https://doi.org/10.1080/23328940.2015.1040945

Angilletta, M. J., Youngblood, J. P., Neel, L. K., & VandenBrooks, J. M. (2019). The neuroscience of adaptive thermoregulation. *Neuroscience Letters*, *692*, 127–136. https://doi.org/10.1016/j.neulet.2018.10.046

Archana, P. R., Sejian, V., Ruban, W., Bagath, M., Krishnan, G., Aleena, J., Manjunathareddy, G. B., Beena, V., & Bhatta, R. (2018). Comparative assessment of heat stress induced changes in carcass traits, plasma leptin profile and skeletal muscle myostatin and HSP70 gene expression patterns between indigenous Osmanabadi and Salem Black goat breeds. *Meat Science*, *141*, 66–80. https://doi.org/10.1016/j.meatsci.2018.03.015

Arduini, A., Redaelli, V., Luzi, F., Dall'Olio, S., Pace, V., & Nanni Costa, L. (2017). Relationship between deck level, body surface temperature and carcass damages in Italian heavy pigs after short journeys at different unloading environmental conditions. *Animals*, *7*(12), 10. https://doi.org/10.3390/ani7020010

Athaíde, L. G., Joset, W. C. L., de Almeida, J. C. F., Pantoja, M. H. de A., Noronha, R. de P. P., Bezerra, A. S., Barbosa, A. V. C., Martorano, L. G., da Silva, J. A. R., & Lourenço Júnior, J. de B. (2020). Thermoregulatory and behavioral responses of buffaloes with and without direct sun exposure during abnormal environmental condition in Marajó Island, Pará, Brazil. *Frontiers in Veterinary Science*, *7*(November), 1–10. https://doi.org/10.3389/fvets.2020.522551

Averós, X., Martín, S., Riu, M., Serratosa, J., & Gosálvez, L. F. (2008). Stress response of extensively reared young bulls being transported to growing-finishing farms under Spanish summer commercial conditions. *Livestock Science*, *119*(1–3), 174–182. https://doi.org/10.1016/j.livsci.2008.04.002

Badakhshan, Y., & Mohammadabadi, M. R. (2015). Thermoregulatory mechanisms of jersey adult cattle and calves based on different body sites temperature. *Iranian Journal of Applied Animal Science*, *5*(4), 793–798.

Barboza-Jiménez, G. (2011). Bondades ecológicas del búfalo de agua: camino hacia la certificación. *Tecnología En Marcha*, *24*(5), 82–88.

Barrios Castillo, V. (2016). Effect of storage in formation biogenic amines in rabbit meat with different packaging [Colegio de postgraduados]. In *Colegio de postgraduados*. http://www.biblio.colpos.mx:8080/jspui/bitstream/handle/10521/256/Sanchez_Borja_M_DC_Fitosanidad_2010.pdf?sequence=1

Baune, M.-C., Broucke, K., Ebert, S., Gibis, M., Weiss, J., Enneking, U., Profeta, A., Terjung, N., & Heinz, V. (2023). Meat hybrids–An assessment of sensorial aspects, consumer acceptance, and nutritional properties. *Frontiers in Nutrition*, *10*(February). https://doi.org/10.3389/fnut.2023.1101479

Belew, J. B., Brooks, J. C., McKenna, D. R., & Savell, J. W. (2003). Warner–Bratzler shear evaluations of 40 bovine muscles. *Meat Science*, *64*(4), 507–512. https://doi.org/10.1016/S0309-1740(02)00242-5

Bertoni, A., Álvarez-Macías, A., Dávalos-Flores, J. L., & Mota-Rojas, D. (2020). Productividad de los búfalos de agua y sus expectativas de desarrollo en zonas tropicales. In F. Napolitano, D. Mota-Rojas, I. Guerrero-Legarreta, & A. Orihuela (Eds.), *El búfalo de agua en Latinoamérica, hallazgos recientes.* (3 ed, pp. 167–207). BM Editores.

Bertoni, A., Álvarez-Macías, A., Morales-Canela, A., Orozco-Corrales, C., & Mota-Rojas, D. (2019). Los sistemas de producción de búfalos en el tropico húmedo de América Latina:Un acercamiento desde el enfoque agroecológico. In I. Guerrero-Legarreta, F. Napolitano, D. Mota-Rojas, & A. Orihuela (Eds.), *El Búfalo de Agua en las Américas* (2nd ed., pp. 226–263.). BM Editores.

Bertoni, A., Álvarez-Macías, A., & Mota-Rojas, D. (2019). Desempeño productivo de los búfalos y sus opciones de desarrollo en las regiones tropicales. *Sociedades Rurales Producción y Medio Ambiente*, *19*(38), 59–80. https://www.researchgate.net/profile/Daniel-Mota-Rojas/publication/346220938_Desempeno_productivo_de_los_bufalos_y_sus_opciones_de_desarrollo_en_las_regiones_tropicales/links/5fbceae6299bf104cf6f54f3/Desempeno-productivo-de-los-bufalos-y-sus-opciones-de-d

Bertoni, A., Álvarez-Macías, A., Mota-Rojas, D., Dávalos, J. L., & Minervino, A. H. H. (2021). Dual-purpose water buffalo production systems in tropical Latin America: Bases for a sustainable model. *Animals*, *11*(10), 2910. https://doi.org/10.3390/ani11102910

Bertoni, A., Mota-Rojas, D., Álvarez-Macias, A., Mora-Medina, P., Guerrero-Legarreta, I., Morales-Canela, A., Gómez-Prado, J., José-Pérez, N., & Martínez-Burnes, J. (2020b). Scientific findings related to changes in vascular microcirculation using infrared thermography in the river buffalo. *Journal of Animal Behaviour and Biometeorology*, *8*(4), 288–297. https://doi.org/10.31893/jabb.20038

Bertoni, A., Napolitano, F., Mota-Rojas, D., Sabia, E., Álvarez-Macías, A., Mora-Medina, P., Morales-Canela, A., Berdugo-Gutiérrez, J., & Guerrero- Legarreta, I. (2020a). Similarities and differences between river buffaloes and cattle: Health, physiological, behavioral and productivity aspects. *Journal of Buffalo Science*, *9*(September), 92–109. https://doi.org/10.6000/1927-520X.2020.09.12

Bethancourt-Garcia, J. A., Vaz, R. Z., Vaz, F. N., Silva, W. B., Pascoal, L. L., Mendonça, F. S., Vara, C. C. da, Nuñez, A. J. C., & Restle, J. (2019). Pre-slaughter factors affecting the incidence of severe bruising in cattle carcasses. *Livestock Science*, *222*(February), 41–48. https://doi.org/10.1016/j.livsci.2019.02.009

Borell, E. H. (2001). The biology of stress and its application to livestock housing and transportation assessment. *Journal of Animal Science*, *79*(E-Suppl), E260. https://doi.org/10.2527/jas2001.79E-SupplE260x

Bozzo, G., Barrasso, R., Marchetti, P., Roma, R., Samoilis, G., Tantillo, G., & Ceci, E. (2018). Analysis of stress indicators for evaluation of animal welfare and meat quality in traditional and Jewish slaughtering. *Animals*, *8*(4), 43. https://doi.org/10.3390/ani8040043

Brcko, C. C., Silva, J. A. R. da, Martorano, L. G., Vilela, R. A., Nahúm, B. de S., Silva, A. G. M., Barbosa, A. V. C., Bezerra, A. S., & Lourenço Júnior, J. de B. (2020). Infrared thermography to assess thermoregulatory reactions of female buffaloes in a humid tropical environment. *Frontiers in Veterinary Science*, *7*. https://doi.org/10.3389/fvets.2020.00180

Burdick, N. C., Carroll, J. A., Hulbert, L. E., Dailey, J. W., Willard, S. T., Vann, R. C., Welsh, T. H., & Randel, R. D. (2010). Relationships between temperament and transportation with rectal temperature and serum concentrations of cortisol and epinephrine in bulls. *Livestock Science*, *129*(1–3), 166–172. https://doi.org/10.1016/j.livsci.2010.01.020

Cain, J. W., Krausman, P. R., Rosenstock, S. S., & Turner, J. C. (2006). Mechanisms of thermoregulation and water balance in desert ungulates. *Wildlife Society Bulletin*, *34*(3), 570–581. https://doi.org/10.2193/0091-7648(2006)34[570:motawb]2.0.co;2

Cannas, S., Palestrini, C., Canali, E., Cozzi, B., Ferri, N., Heinzl, E., Minero, M., Chincarini, M., Vignola, G., & Dalla Costa, E. (2018). Thermography as a non-invasive measure of stress and fear of humans in sheep. *Animals*, *8*(9), 146. https://doi.org/10.3390/ani8090146

Carnovale, F., Xiao, J., Shi, B., Kaart, T., Arney, D., & Phillips, C. J. C. (2021). The effects of vehicle type, transport duration and pre-transport feeding on the welfare of sheep transported in low temperatures. *Animals*, *11*(6), 1659. https://doi.org/10.3390/ani11061659

Casas-Alvarado, A., Martínez-Burnes, J., Mora-Medina, P., Hernández-Avalos, I., Domínguez-Oliva, A., Lezama-García, K., Gómez-Prado, J., & Mota-Rojas, D. (2022). Thermal and circulatory changes in diverse body regions in dogs and cats evaluated by infrared thermography. *Animals*, *12*(6), 789. https://doi.org/10.3390/ani12060789

Casas-Alvarado, A., Mota-Rojas, D., Hernández-Ávalos, I., Mora-Medina, P., Olmos-Hernández, A., Verduzco-Mendoza, A., Reyes-Sotelo, B., & Martínez-Burnes, J. (2020). Advances in infrared thermography: Surgical aspects, vascular changes, and pain monitoring in veterinary medicine. *Journal of Thermal Biology*, *92*, 102664. https://doi.org/10.1016/j.jtherbio.2020.102664

Chandra, B. S., & Das, N. (2001). The handling and short-haul road transportation of spent buffaloes in relation to bruising and animal welfare. *Tropical Animal Health and Production*, *33*(2), 155–163. https://doi.org/10.1023/A:1005242815540

Chirife, J., & Fontana, A. J. (2007). Introduction: Historical highlights of water activity research. In G. V. Barbosa-Cánovas, A. J. Fontana, S. J. Schmidt, & T. P. Labuza (Eds.), *Water Activity in Foods Fundamentals and Applications* (1st editio, pp. 15–28). Blackwell Publishing.

Chulayo, A. Y., Bradley, G., & Muchenje, V. (2016). Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle. *Meat Science*, *117*(October), 89–96. https://doi.org/10.1016/j.meatsci.2016.03.001

Chulayo, A. Y., & Muchenje, V. (2013). The effects of Pre-slaughter stress and season on the activity of plasma creatine kinase and mutton quality from different sheep breeds slaughtered at a smallholder abattoir. *Asian-Australasian Journal of Animal Sciences*, *26*(12), 1762–1772. https://doi.org/10.5713/ajas.2013.13141

Church, J. S., Hegadoren, P. R., Paetkau, M. J., Miller, C. C., Regev-Shoshani, G., Schaefer, A. L., & Schwartzkopf-Genswein, K. S. (2014). Influence of environmental factors on infrared eye temperature measurements in cattle. *Research in Veterinary Science*, *96*(1), 220–226. https://doi.org/10.1016/j.rvsc.2013.11.006

Cilulko, J., Janiszewski, P., Bogdaszewski, M., & Szczygielska, E. (2013). Infrared thermal imaging in studies of wild animals. *European Journal of Wildlife Research*, *59*(1), 17–23. https://doi.org/10.1007/s10344-012-0688-1

Cockram, M. S., Dulal, K. J., Stryhn, H., & Revie, C. W. (2020). Rearing and handling injuries in broiler chickens and risk factors for wing injuries during loading. *Canadian Journal of Animal Science*, *100*(3), 402–410. https://doi.org/10.1139/cjas-2019-0204

Cook, N. J. (2021). Review on the use of infrared thermography to monitor the health of intensively housed livestock. *Journal of Animal Sciences and Livestock Production*, *5*(April), 1–5. https://www.imedpub.com/animal-sciences-and-livestock-production/

Crudeli, G. A., Konrad, J. L., & Patiño, E. M. (2016). Reproducción en Búfalas. In E. M. Crudeli, G. A.; Konrad, J.L.; Patiño (Ed.), *Tecnología en Marcha* (Vol. 1). Mongolia Ediciones.

Crudeli, J. F., Patiño, G. A., Rebak, E. M., Gladys, I., Rivas, A., Barrientos, P. A., & Gregorio, J. (2001). Composición química y características físicas de la carne de búfalos criados en forma extensiva en la provincia de Formosa. *Facultad de Cs. Veterinarias - UNNE.*, *1*(1), 1–4. http://www.unne.edu.ar/unnevieja/Web/cyt/cyt/2002/04-Veterinarias/V-040.pdf

Cruz-Monterrosa, R. G., Mota-Rojas, D., El-aziz, A. H. A., Rayas Amor, A. A., Díaz, M. D., Guerrero-Legarreta, I., Pérez-Álvarez, J. Á., Ramírez-Bribiesca, E., Ghezzi, M. D., Viuda-Martos, M., Fernández-Pérez, J., Marcelo, R., & Napolitano, F. (2020). Calidad de la carne de búfalo de agua: análisis nutricional, sensorial e inocuidad, conservación, empacado y autenticidad. In F. Napolitano, D. Mota-Rojas, I. Guerrero-Legarreta, & A. Orihuela (Eds.), *El búfalo de agua en Latinoamérica, hallazgos recientes.* (3rd ed., pp. 1279–1319). BM Editores.

Cruz-Monterrosa, R., Mota-Rojas, D., Ramírez-Bibriesca, E., Mora-Medina, P., & Guerrero-Legarreta, I. (2020). Scientific findings on the quality of river buffalo meat and prospects for future studies. *Journal of Buffalo Science*, *9*, 170–180. https://doi.org/10.6000/1927-520X.2020.09.18

Cueto, S. L. (2020). *Factores que influyen en la respuesta productiva y bienestar animal durante el transporte de bovinos* [Universidad Autónoma de Chapingo]. https://repositorio.chapingo.edu.mx/bitstream/handle/20.500.12098/501/dcig-lcsi_20.pdf?sequence=2

Cuthbertson, H., Tarr, G., Loudon, K., Lomax, S., White, P., McGreevy, P., Polkinghorne, R., & González, L. A. (2020). Using infrared thermography on farm of origin to predict meat quality and physiological response in cattle (Bos Taurus) exposed to transport and marketing. *Meat Science*, *169*(April), 108173. https://doi.org/10.1016/j.meatsci.2020.108173

Dalla Costa, O. A., Dalla Costa, F. A., Feddern, V., Lopes, L. dos S., Coldebella, A., Gregory, N. G., & de Lima, G. J. M. M. (2019). Risk factors associated with pig pre-slaughtering losses. *Meat Science*, *155*, 61–68. https://doi.org/10.1016/j.meatsci.2019.04.020

Damtew, A., Erega, Y., Hussen, E., Tsegaye, S., & Desiye, M. (2018). The effect of long distance transportation stress on cattle: a review. *Biomedical Journal of Scientific & Technical Research*, *3*(3). https://doi.org/10.26717/BJSTR.2018.03.000908

Di Palo, R., Campanile, G., Ariota, B., Vecchio, D., Grassi, C., Neri, D., Varricchio, E., & Rendina, M. (2007). Milk flow traits in Mediterranean Italian Buffaloes. *Italian Journal of Animal Science*, *6*(sup2), 1319–1322. https://doi.org/10.4081/ijas.2007.s2.1319

Díaz-Luis, A., Díaz, F., Diñeiro, Y., González-Blanco, L., Arias, E., Coto-Montes, A., Oliván, M., & Sierra-Sánchez, V. (2020). Nuevos indicadores de carnes DFD: estrés oxidativo, autofagia y apoptosis. *Informacion Tecnica Economica Agraria*, *117*, 3–18. https://doi.org/10.12706/itea.2020.006

Diaz-Piedra, C., Gomez-Milan, E., & Di Stasi, L. L. (2019). Nasal skin temperature reveals changes in arousal levels due to time on task: An experimental thermal infrared imaging study. *Applied Ergonomics*, *81*(November 2018), 102870. https://doi.org/10.1016/j.apergo.2019.06.001

DOF. (1995). NOM-051-ZOO-1995- Trato humanitario en la movilización de animales. *Diario Oficial de La Federación*, 1–23. http://publico.senasica.gob.mx/?doc=531

DOF. (1996). NOM-031-ZOO-1995- Campaña nacional contra tuberculosis bovina (Mycobacterium bovis). *Diario Oficial de La Federación*, 1–28. https://www.gob.mx/senasica/documentos/nom-031-zoo-1995

DOF. (2012). ACUERDO por el que se establece la Campaña Nacional para el control de la garrapata Boophilus spp. *Diario Oficial de La Federación*. http://www.dof.gob.mx/nota_detalle.php?codigo=5267408&fecha=10/09/2012&print=true

DOF. (2018). Ley federal de sanidad animal. *Diario Oficial de La Federación*, 1–55. http://www.diputados.gob.mx/LeyesBiblio/pdf/LFSA_160218.pdf

England, E. M., Scheffler, T. L., Kasten, S. C., Matarneh, S. K., & Gerrard, D. E. (2013). Exploring the unknowns involved in the transformation of muscle to meat. *Meat Science*, *95*(4), 837–843. https://doi.org/10.1016/j.meatsci.2013.04.031

Ewart, S. (2020). Termorregulación. In B. G. Klein (Ed.), *Cunningham Fisiología veterinaria* (6th ed., pp. 596–607). Elsevier.

Fabela Morón, M. F., Díaz-Ramírez, M., Jiménez-Guzmán, J., Cruz-Monterrosa, R. G., Rayas-Amor, A. A., León-Espinosa, E. B., Mena-Martínez, M., & García-Garibay, M. (2020). Evaluación de la calidad sensorial y nivel de aceptación de formulaciones de sopa de zanahoria microemulsionada con Inulina y Concentrado de Proteína de Suero de Leche (WPC). *Agro Productividad*, *13*(7), 101–106. https://doi.org/10.32854/agrop.vi.1688

Fabio, N., Strappini, A., Ramírez-Bribiesca, E., Alarcón-Rojo, A. D., Cruz-Monterrosa, R., Guerrero Legarreta, I., Rayas-Amor, A. A., García-Galicia, I. A., Ghezzi, M. D., Becerril-Herrera, M., Lázaro de la Torre, C., Mora-Medina, P., Lemus-Flores, C., Bertoni, A., Torres-Bernal, F., Gómez-Prado, J., & Mota-Rojas, D. (2020). Estresores previos a la muerte y su efecto en la calidad de la carne del búfalo de agua. In & O. A. Napolitano F., Mota-Rojas D., Guerrero-Legarreta I. (Ed.), *El búfalo de agua en Latinoamérica, hallazgos recientes.* (3rd ed., pp. 1178–1233). BM Editores.

Failla, S., Vincenti, F., Saltarelli, E., Contò, M., Ballico, S., & Ficco, A. (2007). Aging time effect on quality meat from buffaloes fed on two different diets. *Italian Journal of Animal Science*, *6*(sup2), 1178–1181. https://doi.org/10.4081/ijas.2007.s2.1195

Flores-Peinado, S., Mota-Rojas, D., Guerrero-Legarreta, I., Mora-Medina, P., Cruz-Monterrosa, R., Gómez-Prado, J., Guadalupe Hernández, M., Cruz-Playas, J., & Martínez-Burnes, J. (2020). Physiological responses of pigs to preslaughter handling: infrared and thermal imaging applications. *International Journal of Veterinary Science and Medicine*, *8*(1), 71–84. https://doi.org/10.1080/23144599.2020.1821574

Gallo, C. B., & Huertas, S. M. (2016). Main animal welfare problems in ruminant livestock during preslaughter operations: a South American view. *Animal*, *10*(2), 357–364. https://doi.org/10.1017/S1751731115001597

Gallo, C., Lizondo, G., & Knowles, T. G. (2003). Effects of journey and lairage time on steers transported to slaughter in Chile. *Veterinary Record*, *152*(12), 361–364. https://doi.org/10.1136/vr.152.12.361

Gallo, C., Tarumán, J., & Larrondo, C. (2018). Main factors affecting animal welfare and meat quality in lambs for slaughter in Chile. *Animals*, *8*(10), 165. https://doi.org/10.3390/ani8100165

García-Segovia, P., Andrés-Bello, A., & Martínez-Monzó, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (M. pectoralis). *Journal of Food Engineering*, *80*(3), 813–821. https://doi.org/10.1016/j.jfoodeng.2006.07.010

Ghassemi Nejad, J., Kim, B.-W., Lee, B.-H., & Sung, K.-I. (2017). Coat and hair color: hair cortisol and serotonin levels in lactating Holstein cows under heat stress conditions. *Animal Science Journal*, *88*(1), 190–194. https://doi.org/10.1111/asj.12662

Ghezzi, M. D. (2018). *¿Cómo medimos el bienestar animal?* 1–9.

Giro, A., Bernardi, A. C. de C., Barioni Junior, W., Lemes, A. P., Botta, D., Romanello, N., Barreto, A. do N., & Garcia, A. R. (2019). Application of microchip and infrared thermography for monitoring body temperature of beef cattle kept on pasture. *Journal of Thermal Biology*, *84*, 121–128. https://doi.org/10.1016/j.jtherbio.2019.06.009

Gonçalves, A. A., Garcia, A. R., Rolim Filho, S. T., Silva, J. A. R. da, Melo, D. N. de, Guimarães, T. C., Tavares, H. R., Silva, T. V. G., Souza, E. B. de, Santos, S. do S. D., & Ohashi, O. M. (2021). Scrotal thermoregulation and sequential sperm abnormalities in buffalo bulls (Bubalus bubalis) under short-term heat stress. *Journal of Thermal Biology*, *96*(December 2020), 102842. https://doi.org/10.1016/j.jtherbio.2021.102842

González-Lozano, M., Mota-Rojas, D., Orihuela, A., Martínez-Burnes, J., Di Francia, A., Braghieri, A., Berdugo-Gutiérrez, J., Mora-Medina, P., Ramírez-Necoechea, R., & Napolitano, F. (2020). Review: Behavioral, physiological, and reproductive performance of buffalo cows during eutocic and dystocic parturitions. *Applied Animal Science*, *36*(3), 407–422. https://doi.org/10.15232/aas.2019-01946

Gonzalez-Rivas, P. A., Chauhan, S. S., Ha, M., Fegan, N., Dunshea, F. R., & Warner, R. D. (2020a). Effects of heat stress on animal physiology, metabolism, and meat quality: A review. *Meat Science*, *162*, 108025. https://doi.org/10.1016/j.meatsci.2019.108025

Gonzalez-Rivas, P. A., Chauhan, S. S., Ha, M., Fegan, N., Dunshea, F. R., & Warner, R. D. (2020b). Effects of heat stress on animal physiology, metabolism, and meat quality: A review. *Meat Science*, *162*, 108025. https://doi.org/10.1016/j.meatsci.2019.108025

González, L. A., Schwartzkopf-Genswein, K. S., Bryan, M., Silasi, R., & Brown, F. (2012). Space allowance during commercial long distance transport of cattle in North America. *Journal of Animal Science*, *90*(10), 3618–3629. https://doi.org/10.2527/jas.2011-4771

Grandin, T., & Shivley, C. (2015). How farm animals react and perceive stressful situations such as handling, restraint, and transport. *Animals*, *5*(4), 1233–1251. https://doi.org/10.3390/ani5040409

Guerrero-Legarreta, I., Mota-Rojas, D., Napolitano, F., Ghezzi, M. D., Mora-Medina, P., Otero, J. L., Bertoni, A., Díaz, M. D., Ciocca, J. R. P., Rodríguez-González, D., José-Pérez, N., Domínguez-Oliva, A., Ballerio, M. O., & Álvarez-Macías, A. (2022). El búfalo de agua en la producción de carne: una visión internacional. In F. Napolitano, D. Mota-Rojas, A. Orihuela, A. Braghieri, D. Hufana-Duran, A. C. Strappini, A. M. F. Pereira, M. D. Ghezzi, I. Guerrero-Legarreta, & J. Martínez-Burnes (Eds.), *El búfalo de agua en las Américas: Comportamiento y productividad* (4 edition, pp. 165-198.). BM Editores. https://www.researchgate.net/publication/362544910_Chapter_5_Quality_of_water_buffalo_meat_An_international_perspective_El_bufalo_de_agua_en_la_produccion_de_carne_una_vision_internacional_Book_EL_BUFALO_DE_AGUA_EN_LAS_AMERICAS_Fourth_edition_2022_165

Guerrero-Legarreta, I., Napolitano, F., Cruz-Monterrosa, R., Mota-Rojas, D., Mora-Medina, P., Ramírez-Bribiesca, E., Bertoni, A., Berdugo-Gutiérrez, J., & Braghieri, A. (2020). River buffalo meat production and quality: sustainability, productivity, nutritional and sensory properties. *Journal of Buffalo Science*, *9*(October), 159–169. https://doi.org/10.6000/1927-520X.2020.09.17

Gupta, S., Earley, B., & Crowe, M. A. (2007). Effect of 12-hour road transportation on physiological, immunological and haematological parameters in bulls housed at different space allowances. *The Veterinary Journal*, *173*(3), 605–616. https://doi.org/10.1016/j.tvjl.2006.03.002

Hagenmaier, J. A., Reinhardt, C. D., Bartle, S. J., Henningson, J. N., Ritter, M. J., Calvo-Lorenzo, M. S., Vogel, G. J., Guthrie, C. A., Siemens, M. G., & Thomson, D. U. (2017). Effect of handling intensity at the time of transport for slaughter on physiological response and carcass characteristics in beef cattle fed ractopamine hydrochloride. *Journal of Animal Science*, *95*(5), 1963. https://doi.org/10.2527/jas2016.0821

Hassan, M., H.S. Abdel-Naeem, H., M.H. Mohamed, H., & A. Yassien, N. (2018). Comparing the physico-chemical characteristics and sensory attributes of imported Brazilian beef meat and imported Indian buffalo meat. *Journal of Microbiology, Biotechnology and Food Sciences*, *8*(1), 672–677. https://doi.org/10.15414/jmbfs.2018.8.1.672-677

Hemsworth, P. (2003). Human–animal interactions in livestock production. *Applied Animal Behaviour Science*, *81*(3), 185–198. https://doi.org/10.1016/S0168-1591(02)00280-0

Hernández-Avalos, I., Flores-Gasca, E., Mota-Rojas, D., Casas-Alvarado, A., Miranda-Cortés, A. E., & Domínguez-Oliva, A. (2021a). Neurobiology of anesthetic-surgical stress and induced behavioral changes in dogs and cats: A review. *Veterinary World*, *14*(2), 393–404. https://doi.org/10.14202/vetworld.2021.393-404

Hernández-Avalos, I., Mota-Rojas, D., Mendoza-Flores, J. E., Casas-Alvarado, A., Flores-Padilla, K., Miranda-Cortes, A. E., Torres-Bernal, F., Gómez-Prado, J., & Mora-Medina, P. (2021b). Nociceptive pain and anxiety in equines: Physiological and behavioral alterations. *Veterinary World*, *14*(11), 2984–2995. https://doi.org/www.doi.org/10.14202/vetworld.2021.2984-2995

Hong, H., Lee, E., Lee, I. H., & Lee, S. R. (2019). Effects of transport stress on physiological responses and milk production in lactating dairy cows. *Asian-Australasian Journal of Animal Sciences*, *32*(3), 442–451. https://doi.org/10.5713/ajas.18.0108

Hovinen, M., & Pyörälä, S. (2011). Invited review: Udder health of dairy cows in automatic milking. *Journal of Dairy Science*, *94*(2), 547–562. https://doi.org/10.3168/jds.2010-3556

Hultgren, J., Segerkvist, K. A., Berg, C., Karlsson, A. H., Öhgren, C., & Algers, B. (2022). Preslaughter stress and beef quality in relation to slaughter transport of cattle. *Livestock Science*, *264*(October), 105073. https://doi.org/10.1016/j.livsci.2022.105073

IDF. (2020). *The World Dairy Situacion* (p. Bulletin 506).

Idris, M., Uddin, J., Sullivan, M., McNeill, D. M., & Phillips, C. J. C. (2021). Non-invasive physiological indicators of heat stress in cattle. *Animals*, *11*(1), 71. https://doi.org/10.3390/ani11010071

Ijaz, M., Jaspal, M. H., Hayat, Z., Yar, M. K., Badar, I. H., Ullah, S., Hussain, Z., Ali, S., Farid, M. U., Farooq, M. Z., & Sardar, A. (2020b). Effect of animal age, postmortem chilling rate, and aging time on meat quality attributes of water buffalo and humped cattle bulls. *Animal Science Journal*, *91*(1). https://doi.org/10.1111/asj.13354

Ijaz, M., Li, X., Zhang, D., Hussain, Z., Ren, C., Bai, Y., & Zheng, X. (2020a). Association between meat color of DFD beef and other quality attributes. *Meat Science*, *161*(October), 107954. https://doi.org/10.1016/j.meatsci.2019.107954

INEGI. (2021). *Clima Veracruz de Ignacio de la Llave*. Información por entidad. https://cuentame.inegi.org.mx/monografias/informacion/ver/territorio/clima.aspx?tema=me&e=30

International Committee Veterinary Gross Anatomical Nomenclature. (2017). *Nomina Anatómica veterinaria*. 160.

Isola, J. V. V., Menegazzi, G., Busanello, M., dos Santos, S. B., Agner, H. S. S., & Sarubbi, J. (2020). Differences in body temperature between black-and-white and red-and-white Holstein cows reared on a hot climate using infrared thermography. *Journal of Thermal Biology*, *94*, 102775. https://doi.org/10.1016/j.jtherbio.2020.102775

Jaspal, M. H., Badar, I. H., Amjad, O. Bin, Yar, M. K., Ijaz, M., Manzoor, A., Nasir, J., Asghar, B., Ali, S., Nauman, K., Rahman, A., & Wara, U. U. (2021). Effect of wet aging on color stability, tenderness, and sensory attributes of longissimus lumborum and gluteus medius muscles from water buffalo bulls. *Animals*, *11*(8), 2248. https://doi.org/10.3390/ani11082248

Jeleníková, J., Pipek, P., & Staruch, L. (2008). The influence of ante-mortem treatment on relationship between pH and tenderness of beef. *Meat Science*, *80*(3), 870–874. https://doi.org/10.1016/j.meatsci.2008.04.004

Joele, M. R. S. P., Lourenço, L. F. H., Lourenço Júnior, J. B., Araújo, G. S., Budel, J. C. C., & Garcia, A. R. (2017). Meat quality of buffaloes finished in traditional or silvopastoral system in the Brazilian Eastern Amazon. *Journal of the Science of Food and Agriculture*, *97*(6), 1740–1745. https://doi.org/10.1002/jsfa.7922

José-Pérez, N., Mota-Rojas, D., Ghezzi, M. D., Rosmini, M. R., Mora-Medina, P., Bertoni, A., Rodríguez-González, D., Domínguez-Oliva, A., & Guerrero-Legarreta, I. (2022). Effects of transport on water buffaloes (Bubalus bubalis): factors associated with the frequency of skin injuries and meat quality. *Journal of Animal Behaviour and Biometeorology*, *10*(2216). https://doi.org/https://doi.org/10.31893/jabb.22016

Kandeepan, G., Mendiratta, S. K., Shukla, V., & Vishnuraj, M. R. (2013). Processing characteristics of buffalo meat-a review. *Journal of Meat Science and Technology*, *1*(1), 1–11. www.jakraya.com/journal/jmst

Kataoka, N., Hioki, H., Kaneko, T., & Nakamura, K. (2014). Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown adipose tissue thermogenesis and hyperthermia. *Cell Metabolism*, *20*(2), 346–358. https://doi.org/10.1016/j.cmet.2014.05.018

Khan, M. I., Jo, C., & Tariq, M. R. (2015). Meat flavor precursors and factors influencing flavor precursors—A systematic review. *Meat Science*, *110*, 278–284. https://doi.org/10.1016/J.MEATSCI.2015.08.002

Kinlein, S. A., Wilson, C. D., & Karatsoreos, I. N. (2015). Dysregulated hypothalamic pituitary adrenal axis function contributes to altered endocrine and neurobehavioral responses to acute stress. *Frontiers in Psychiatry*, *6*(MAR), 19–22. https://doi.org/10.3389/fpsyt.2015.00031

Kinsella, K. J., Sheridan, J. J., Rowe, T. A., Butler, F., Delgado, A., Quispe-Ramirez, A., Blair, I. S., & McDowell, D. A. (2006). Impact of a novel spray-chilling system on surface microflora, water activity and weight loss during beef carcass chilling. *Food Microbiology*, *23*(5), 483–490. https://doi.org/10.1016/j.fm.2005.05.013

Kiran, M., Naveena, B. M., Reddy, K. S., Shahikumar, M., Reddy, V. R., Kulkarni, V. V., Rapole, S., & More, T. H. (2016). Understanding tenderness variability and ageing changes in buffalo meat: biochemical, ultrastructural and proteome characterization. *Animal*, *10*(6), 1007–1015. https://doi.org/10.1017/S1751731115002931

Knizkova, I., & Kunc, P.; Gurdil, G.; Pinar, Y.; Selvi, K. (2007). Applications of infrared thermography in animal production. *J. Fac. Agric. OMU*, *22*(3), 329–336.

Krakoff, L. R. (1988). Glucocorticoid Excess Syndromes Causing Hypertension. *Cardiology Clinics*, *6*(4), 537–545. https://doi.org/10.1016/S0733-8651(18)30475-2

Lan, Q., Cao, Z., Yang, X., & Gu, Z. (2022). Physiological and proteomic responses of dairy buffalo to Heat Stress Induced by Different Altitudes. *Metabolites*, *12*(10), 909. https://doi.org/10.3390/metabo12100909

Law, S., Problems, C., & Law, A. (2007). *Legislation , and trade farm-animal welfare ,*. *70*(1), 325–358.

Lees, A. M., Sejian, V., Wallage, A. L., Steel, C. C., Mader, T. L., Lees, J. C., & Gaughan, J. B. (2019). The impact of heat load on cattle. *Animals*, *9*(6), 322. https://doi.org/10.3390/ani9060322

Lei, M. C., Félix, L., Cardoso, R., Monteiro, S. M., Silva, S., & Venâncio, C. (2023). Non-invasive biomarkers in saliva and eye infrared thermography to assess the stress response of calves during transport. *Animals*, *13*(14). https://doi.org/10.3390/ani13142311

Lendez, P. A., Martinez Cuesta, L., Nieto Farias, M. V., Vater, A. A., Ghezzi, M. D., Mota-Rojas, D., Dolcini, G. L., & Ceriani, M. C. (2021). Alterations in TNF-α and its receptors expression in cows undergoing heat stress. *Veterinary Immunology and Immunopathology*, *235*(February 2020), 110232. https://doi.org/10.1016/j.vetimm.2021.110232

Liotta, L., Costa, L. N., Chiofalo, B., Ravarotto, L., & Chiofalo, V. (2007). Effect of lairage duration on some blood constituents and beef quality in bulls after long journey. *Italian Journal of Animal Science*, *6*(4), 375–384. https://doi.org/10.4081/ijas.2007.375

Liu, P., Guo, L., Mao, H., & Gu, Z. (2020). Serum proteomics analysis reveals the thermal fitness of crossbred dairy buffalo to chronic heat stress. *Journal of Thermal Biology*, *89*, 102547. https://doi.org/10.1016/j.jtherbio.2020.102547

Liu, Y., & Chen, Y. R. (2001). Analysis of visible reflectance spectra of stored, cooked and diseased chicken meats. *Meat Science*, *58*(4), 395–401. https://doi.org/10.1016/S0309-1740(01)00041-9

Longo, V., Lana, A., Bottero, M. T., & Zolla, L. (2015). Apoptosis in muscle-to-meat aging process: The omic witness. *Journal of Proteomics*, *125*(2), 29–40. https://doi.org/10.1016/j.jprot.2015.04.023

Lorenzo, J. M., Cittadini, A., Munekata, P. E., & Domínguez, R. (2015). Physicochemical properties of foal meat as affected by cooking methods. *Meat Science*, *108*, 50–54. https://doi.org/10.1016/j.meatsci.2015.05.021

Lowe, G., McCane, B., Sutherland, M., Waas, J., Schaefer, A., Cox, N., & Stewart, M. (2020). Automated collection and analysis of infrared thermograms for measuring eye and cheek temperatures in calves. *Animals*, *10*(2), 292. https://doi.org/10.3390/ani10020292

Lowe, G., Sutherland, M., Waas, J., Schaefer, A., Cox, N., & Stewart, M. (2019). Infrared Thermography—a non-invasive method of measuring respiration rate in calves. *Animals*, *9*(8), 535. https://doi.org/10.3390/ani9080535

Ma, Q., Hamid, N., Oey, I., Kantono, K., & Farouk, M. (2020). The impact of high-pressure processing on physicochemical properties and sensory characteristics of three different lamb meat cuts. *Molecules*, *25*(11), 2665. https://doi.org/10.3390/molecules25112665

Mackenzie, A. ., Drennan, M., Rowan, T. ., Dixon, J. ., & Carter, S. . (1997). Effect of transportation and weaning on humoral immune responses of calves. *Research in Veterinary Science*, *63*(3), 227–230. https://doi.org/10.1016/S0034-5288(97)90025-4

Malik, A. H., & Sharma, B. D. (2010). Comparison of hurdle treatments for buffalo meat. *International Journal of Food Science & Technology*, *45*(8), 1552–1563. https://doi.org/10.1111/j.1365-2621.2010.02291.x

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science*, *71*(1), 100–121. https://doi.org/10.1016/j.meatsci.2005.03.003

Marai, I. F. M., Daader, A. H., Soliman, A. M., & El-Menshawy, S. M. S. (2009). Non-genetic factors affecting growth and reproduction traits of buffaloes under dry management housing (in sub-tropical environment) in Egypt. *Livestock Research for Rural Development*, *21*(3).

Marai, I. F. M., & Haeeb, A. A. M. (2010). Buffalo's biological functions as affected by heat stress — A review. *Livestock Science*, *127*(2–3), 89–109. https://doi.org/10.1016/j.livsci.2009.08.001

Marques da Silva, D. . (2019). *Termografia Infravermelho e Medidas de Eficiência de Bubalinos de Três Grupos Genéticos Sob Condições Tropicais.* Universidade Estadual Paulista.

Marrone, R., Salzano, A., Di Francia, A., Vollano, L., Di Matteo, R., Balestrieri, A., Anastasio, A., & Barone, C. M. A. (2020). Effects of feeding and maturation system on qualitative characteristics of buffalo meat (Bubalus bubalis). *Animals*, *10*(5), 899. https://doi.org/10.3390/ani10050899

Matarneh, S. K., England, E. M., Scheffler, T. L., & Gerrard, D. E. (2017). The conversion of muscle to meat. In *Lawrie´s Meat Science* (pp. 159–185). Elsevier. https://doi.org/10.1016/B978-0-08-

100694-8.00005-4

Matias, B. F., Okano, W., Pértile, S. F. N., Barreto, J. V. P., Andreola, D., & Cunha Filho, L. F. C. da. (2019). Economic losses due to hematoma in bovine carcasses from the north central mesoregion of Paraná. *Acta Veterinaria Brasilica*, *13*(3), 114–119. https://doi.org/10.21708/avb.2019.13.3.8327

Matsuno, S., Mizuno, T., Asano, H., Mito, K., & Itakura, N. (2018). Estimating autonomic nerve activity using variance of thermal face images. *Artificial Life and Robotics*, *23*(3), 367–372. https://doi.org/10.1007/s10015-018-0436-z

Mehdid, A., Martí-De Olives, A., Fernández, N., Rodríguez, M., & Peris, C. (2019). Effect of stress on somatic cell count and milk yield and composition in goats. *Research in Veterinary Science*, *125*, 61–70. https://doi.org/10.1016/j.rvsc.2019.05.015

Mitchell, G., Hattingh, J., & Ganhao, M. (1988). Stress in cattle assessed after handling, after transport and after slaughter. *Veterinary Record*, *123*(8), 201–205. https://doi.org/10.1136/vr.123.8.201

Mota-Rojas, D., Broom, D., Orihuela, A., Velarde, A., Napolitano, F., & Alonso-Spilsbury, M. (2020b). Effects of human-animal relationship on animal productivity and welfare. *Journal of Animal Behaviour and Biometeorology*, *8*(3), 196–205. https://doi.org/10.31893/jabb.20026

Mota-Rojas, D., Ghezzi, M. D., Napolitano, F., Hernández-Ávalos, I., Rosmini, M. R., Thielo de la Vega, L., Cruz-Monterrosa, R., Cajiao, M. N., Miranda Cortés, A., Torres-Bernal, F., Lezama-García, K., Lemus-Flores, C., Soto-Franco, P., Guerrero- Legarreta, I., & Moreno-Avila, N. (2020a). Calidad del aturdimiento en búfalos: reflejos y signos de retorno a la sensibilidad durante la muerte. In F. Napolitano, D. Mota-Rojas, I. Guerrero-Legarreta, & A. Orihuela (Eds.), *El búfalo de agua en Latinoamérica, hallazgos recientes.* (3rd ed., pp. 1072–1115). BM Editores.

Mota-Rojas, D., Ghezzi, M. D., Napolitano, F., Hernández-Ávalos, I., Rosmini, M. R., Vega, L. T. de la, Cruz-Monterrosa, R., Cajiao, M. N., Miranda Cortés, A., Torres-Bernal, F., Lezama-García, K., Lemus-Flores, C., Soto-Franco, P., Moreno-Avila, N., & Guerrero-Legarreta, I. (2020). Calidad del aturdimiento en búfalos: reflejos y signos de retorno a la sensibilidad durante la muerte. In F. Napolitano, D. Mota-Rojas, I. Guerrero-Legarreta, & A. Orihuela (Eds.), *El búfalo de agua en Latinoamérica, hallazgos recientes.* (3rd ed., pp. 1073–1177). BM Editores.

Mota-Rojas, D., Guerrero-Legarreta, I., Rodríguez-González, D., Cruz-Monterrosa, R. G., Álvarez-Macias, A., Bertoni-Mendoza, A., & Domínguez-Oliva, A. Napolitano, F. (2023). Behavioral, productive, and reproductive aspects of the water buffalo in Mexico. *Journal of Animal Behaviour and Biometeorology*, *11*(3), 2023021. https://doi.org/10.31893/jabb.230021

Mota-Rojas, D., Martínez-Burnes, J., Casas-Alvarado, A., Gómez-Prado, J., Hernández-Ávalos, I., Domínguez-Oliva, A., Lezama-García, K., Jacome-Romero, J., Rodríguez-González, D., & Pereira, A. M. F. (2022a). Clinical usefulness of infrared thermography to detect sick animals: frequent and current cases. *CABI Reviews*, *2022*(040). https://doi.org/10.1079/cabireviews202217040

Mota-Rojas, D., Napolitano, F., Braghieri, A., Guerrero-Legarreta, I., Bertoni, A., Martínez-Burnes, J., Cruz-Monterrosa, R., Gómez, J., Ramírez-Bribiesca, E., Barrios-García, H., José, N., Álvarez, A., Mora-Medina, P., & Orihuela, A. (2021c). Thermal biology in river buffalo in the humid tropics: neurophysiological and behavioral responses assessed by infrared thermography. *Journal of Animal Behaviour and Biometeorology*, *9*(1), 1–12. https://doi.org/10.31893/jabb.21003

Mota-Rojas, D., Napolitano, F., Strappini, A., Orihuela, A., Ghezzi, M. D., Hernández-Ávalos, I., Mora-Medina, P., & Whittaker, A. L. (2021). Pain at the slaughterhouse in ruminants with a focus on the neurobiology of sensitisation. *Animals*, *11*(4), 1085. https://doi.org/10.3390/ani11041085

Mota-Rojas, D., Pereira, A. M. F., Martínez-Burnes, J., Domínguez-Oliva, A., Mora-Medina, P., Casas-Alvarado, A., Rios-Sandoval, J., de Mira Geraldo, A., & Wang, D. (2022a). Thermal imaging to assess the health status in wildlife animals under human care: Limitations and perspectives. *Animals*, *12*(24). https://doi.org/10.3390/ani12243558

Mota-Rojas, D., Pereira, A. M. F., Wang, D., Martínez-Burnes, J., Ghezzi, M., Hernández-Avalos, I., Lendez, P., Mora-Medina, P., Casas, A., Olmos-Hernández, A., Domínguez, A., Bertoni, A., & Geraldo, A. de M. (2021b). Clinical applications and factors involved in validating thermal windows used in infrared thermography in cattle and river buffalo to assess health and productivity. *Animals*, *11*(8), 2247. https://doi.org/10.3390/ani11082247

Mota-Rojas, D., Rosa, G. De, Mora-Medina, P., Braghieri, A., Guerrero-Legarreta, I., & Napolitano, F. (2019). Dairy buffalo behaviour and welfare from calving to milking. *CAB Reviews*, *035*, 1–9. https://doi.org/10.1079/PAVSNNR201914035

Mota-Rojas, D., Titto, C. G., Orihuela, A., Martínez-Burnes, J., Gómez-Prado, J., Torres-Bernal, F., Flores-Padilla, K., Carvajal-de la Fuente, V., & Wang, D. (2021d). Physiological and behavioral mechanisms of thermoregulation in mammals. *Animals*, *11*(6), 1733. https://doi.org/10.3390/ani11061733

Mota-Rojas, D., Wang, D., Titto, C. G., Gómez-Prado, J., Carvajal-de la Fuente, V., Ghezzi, M., Boscato-Funes, L., Barrios-García, H., Torres-Bernal, F., Casas-Alvarado, A., & Martínez-Burnes, J. (2021). Pathophysiology of fever and application of infrared thermography (IRT) in the detection of sick domestic animals: recent advances. *Animals*, *11*(8), 2316. https://doi.org/10.3390/ani11082316

Motaghifar, A., Akbari-Adergani, B., Rokney, N., & Mottalebi, A. (2021). Evaluating red meat putrefaction in long term storage in freezing condition based on co-variation of major biogenic amines and Total Volatile Nitrogen. *Food Science and Technology*, *41*(suppl 1), 123–128. https://doi.org/10.1590/fst.08120

Mounier, L., Dubroeucq, H., Andanson, S., & Veissier, I. (2006). Variations in meat pH of beef bulls in relation to conditions of transfer to slaughter and previous history of the animals. *Journal of Animal Science*, *84*(6), 1567–1576. https://doi.org/10.2527/2006.8461567x

Muñoz, D., Strappini, A., & Gallo, C. (2012). Indicadores de bienestar animal para detectar problemas en el cajón de insensibilización de bovinos. *Archivos de Medicina Veterinaria*, *44*(3), 297–302. https://doi.org/10.4067/S0301-732X2012000300014

Murata, H., Shimada, N., & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. *The Veterinary Journal*, *168*(1), 28–40. https://doi.org/10.1016/S1090-0233(03)00119-9

Nakamura, K. (2015). Neural circuit for psychological stress-induced hyperthermia. *Temperature*, *2*(3), 352–361. https://doi.org/10.1080/23328940.2015.1070944

Napolitano, F., Bragaglio, A., Braghieri, A., El-Aziz, A. H. A., Titto, C. G., Villanueva-García, D., Mora-Medina, P., Pereira, A. M. F., Hernández-Avalos, I., José-Pérez, N., Casas-Alvarado, A., Lezama-

García, K., Domínguez-Oliva, A., Rodríguez-González, D., Bertoni, A., & Mota-Rojas, D. (2023). The effect of birth weight and time of day on the thermal response of newborn water buffalo calves. *Frontiers in Veterinary Science*, *10*. https://doi.org/10.3389/fvets.2023.1084092

Napolitano, F., Mota-Rojas, D., Alvarez, A., & Braghieri, A. (2020). Factores productivos y su incidencia en el bienestar de la búfala lechera en sistemas de producción extensivos e intensivos. *Soc Rur Prod Med Amb*, *20*, 155–173. https://www.researchgate.net/publication/349704511_Factores_productivos_y_su_incidencia_en_el_bienestar_de_la_bufala_lechera_en_sistemas_de_produccion_extensivos_e_intensivos_Soc_Rurales_Prod_Med_Ambiente_2020_20_40155-173

Napolitano, F., Mota-Rojas, D., Guerrero-Legarreta, I., & Orihuela, A. (2020). *El búfalo de agua en Latinoamérica, hallazgos recientes.* (3rd ed.). BM Editores. https://www.lifescienceglobal.com/journals/journal-of-buffalo-science/97-abstract/jbs/4550-el-bufalo-de-agua-en-latinoamerica-hallazgos-recientes

Naveena, B., Mendiratta, S., & Anjaneyulu, A. S. (2004). Tenderization of buffalo meat using plant proteases from Cucumis trigonus Roxb (Kachri) and Zingiber officinale roscoe (Ginger rhizome). *Meat Science*, *68*(3), 363–369. https://doi.org/10.1016/j.meatsci.2004.04.004

Naveena, B. M., & Kiran, M. (2014). Buffalo meat quality, composition, and processing characteristics: Contribution to the global economy and nutritional security. *Animal Frontiers*, *4*(4), 18–24. https://doi.org/10.2527/af.2014-0029

Neath, K. E., Del Barrio, A. N., Lapitan, R. M., Herrera, J. R. V., Cruz, L. C., Fujihara, T., Muroya, S., Chikuni, K., Hirabayashi, M., & Kanai, Y. (2007). Difference in tenderness and pH decline between water buffalo meat and beef during postmortem aging. *Meat Science*, *75*(3), 499–505. https://doi.org/10.1016/j.meatsci.2006.08.016

Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Schmidt, C. G., Michel, V., Miranda Chueca, M. Á., Roberts, H. C., Sihvonen, L. H., Spoolder, H., Stahl, K., Velarde, A., Viltrop, A., Candiani, D., Van der Stede, Y., & Winckler, C. (2020). Welfare of cattle at slaughter. *EFSA Journal*, *18*(11). https://doi.org/10.2903/j.efsa.2020.6275

Nuraini, H., Lesa Aditia, E., & Brahmantiyo, B. (2019). Meat quality of Indonesian local cattle and buffalo. *Bovine Science- A Key to Sustainable Development*, *February 2019*. https://doi.org/10.5772/intechopen.79904

Oka, T. (2018). Stress-induced hyperthermia and hypothermia. In *Handbook of Clinical Neurology* (Vol. 157, pp. 599–621). https://doi.org/10.1016/B978-0-444-64074-1.00035-5

Oka, T., Oka, K., & Hori, T. (2001). Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosomatic Medicine*, *63*(3), 476–486. https://doi.org/10.1097/00006842-200105000-00018

Olivier, B., van Bogaert, M., van Oorschot, R., Oosting, R., & Groenink, L. (2005). Stress-induced hyperthermia. In *Techniques in the Behavioral and Neural Sciences* (Vol. 15, Issue PART 2). Elsevier B.V. https://doi.org/10.1016/S0921-0709(05)80053-7

OMSA. (2021). Transporte de animales por vía terrestre. *Código Sanitario Para Los Animales Terrestes*, 1–16. https://www.oie.int/es/que-hacemos/normas/codigos-y-manuales/acceso-en-linea-al-codigo-terrestre/

Ortega Torres, J., & Ariza Botero, M. F. (2012). El mecanismo de muerte celular programada y su importancia en el proceso de maduración de la carne bovina. *Revista de Medicina Veterinaria*, *1*(23), 83. https://doi.org/10.19052/mv.77

Ouali, A., Herrera-Mendez, C. H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L., & Sentandreu, M. A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Science*, *74*(1), 44–58. https://doi.org/10.1016/j.meatsci.2006.05.010

Panel, E., & Ahaw, W. (2011). Scientific opinion concerning the welfare of animals during transport. *EFSA Journal*, *9*(1), 1966. https://doi.org/10.2903/j.efsa.2011.1966

Peaston, R. T., & Weinkove, C. (2004). Measurement of catecholamines and their metabolites. *Annals of Clinical Biochemistry*, *41*(1), 17–38. https://doi.org/10.1258/000456304322664663

Peeters, E., Deprez, K., Beckers, F., De Baerdemaeker, J., Auben, A. E., & Geers, R. (2008). Effect of driver and driving style on the stress responses of pigs during a short journey by trailer. *Animal Welfare*, *17*(2), 189–196.

Pérez-Linares, C., Sánchez-López, E., Ríos-Rincón, F. G., Olivas-Valdéz, J. A., Figueroa-Saavedra, F., & Barreras-Serrano, A. (2013). Factores de manejo pre y post sacrificio asociados a la presencia de carne DFD en ganado bovino durante la epoca cálida. *Revista Mexicana De Ciencias Pecuarias*, *4*(2), 149–160. http://www.scielo.org.mx/pdf/rmcp/v4n2/v4n2a2.pdf

Pérez, M., & Ponce, E. (2013). Tecnología de carnes. *Revista Salud Pública de México*, *1*(1), 27–73. http://publicacionescbs.izt.uam.mx/DOCS/carnes.pdf

Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*, *48*(2), 175–187. https://doi.org/10.1016/j.neuron.2005.09.025

Picard, K., Thomas, D. W., Festa-Bianchet, M., Belleville, F., & Laneville, A. (1999). Differences in the thermal conductance of tropical and temperate bovid horns. *Ecoscience*, *6*(2), 148–158. https://doi.org/10.1080/11956860.1999.11682515

Pircher, A., Bauer, F., & Paulsen, P. (2007). Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. *European Food Research and Technology*, *226*(1–2), 225–231. https://doi.org/10.1007/s00217-006-0530-7

Puolanne, E., & Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science*, *86*(1), 151–165. https://doi.org/10.1016/j.meatsci.2010.04.038

Rebez, E. B., Sejian, V., Silpa, M. V., & Dunshea, F. R. (2023). Heat stress and histopathological Changes of vital organs: A novel approach to assess climate resilience in farm animals. *Sustainability*, *15*(2), 1242. https://doi.org/10.3390/su15021242

Rensis, F. De, & Scaramuzzi, R. J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow—a review. *Theriogenology*, *60*(6), 1139–1151. https://doi.org/10.1016/S0093-691X(03)00126-2

Reséndiz-Cruz, V., García-López, E., Ramírez-Bribiesca, J. E., Hernández-Sánchez, D., Guerrero-Legarreta, I., Mota-Rojas, D., Sánchez-Torres, J. E., & Cruz-Monterrosa, R. G. (2022). Profile of nitrogenous compounds and bacterial proliferation in rabbit meat stored cold with three types of packaging. *Revista Científica de La Facultad de Ciencias Veterinarias*, *XXXII*(single), 1–6. https://doi.org/10.52973/rcfcv-e32121

Rey, J. F., & Povea, I. E. (2012). Water buffalo (Bubalus bubalis) and their technological advantages for the design in healthy meat product. *Journal of Buffalo Science*, *10*, 183–187. https://doi.org/10.6000/1927-520X.2012.01.02.09

Robertson, J. K., Mastromonaco, G., & Burness, G. (2020). Evidence that stress-induced changes in surface temperature serve a thermoregulatory function. *Journal of Experimental Biology*, *223*(4), jeb213421. https://doi.org/10.1242/jeb.213421

Rocha, L. M., Devillers, N., Maldague, X., Kabemba, F. Z., Fleuret, J., Guay, F., & Faucitano, L. (2019). Validation of anatomical sites for the measurement of infrared body surface temperature variation in response to handling and transport. *Animals*, *9*(7), 425. https://doi.org/10.3390/ani9070425

Rodríguez-González, D., Mota-Rojas, D., Napolitano, F., Mora-Medina, P., Ghezzi, M. D., Martínez-Burnes, J., & Guerrero-Legarreta, I. (2023a). *Perspectivas y desafíos del MVZ en la producción bufalina, aseguramiento alimentario y salud pública*. Entorno Ganadero.

Rodríguez-González, D., Guerrero Legarreta, I., Cruz-Monterrosa, R. G., Napolitano, F., Titto, C. G., Abd El-Aziz, A. H., Hernández-Avalos, I., Casas-Alvarado, A., Domínguez-Oliva, A., & Mota-Rojas, D. (2023b). Assessment of thermal changes in water buffalo mobilized from the paddock and transported by short journeys. *Frontiers in Veterinary Science*, *10*. https://doi.org/10.3389/fvets.2023.1184577

Rodríguez-González, D., Guerrero-Legarreta, I., Mora-Medina, P., Cruz-Monterrosa, R., Domínguez-Oliva, A., Flores-Peinado, S., & Mota-Rojas, D. (2023c). *El rol del médico veterinario zootecnista en la producción animal, la seguridad e inocuidad alimentaria*. Ganadería. https://www.ganaderia.com/destacado/el-rol-del-medico-veterinario-%0Azootecnista-en-la-produccion-animal-la-seguridad-e-inocuidad-alimentaria

Rodríguez-González, D., Guerrero Legarreta, I., Chay-Canul, A., Hernández-Avalos, I., Napolitano, F., García-Herrera, R., Pereira, A. M. F., Domínguez-Oliva, A., Casas-Alvarado, A., Reyes-Sotelo, B., & Mota-Rojas, D. (2023). Thermal balance in male water buffaloes transported by long and short journeys. *Animals*, *13*(20), 3274. https://doi.org/10.3390/ani13203274

Rodríguez-González, D., Napolitano, F., Hufana-Duran, D., Ghezzi, M. D., Domínguez-Oliva, A., Álvarez-Macías, A., Guerrero-Legarreta, I., Mora-Medina, P., Morales-Canela, A., José-Perez, N., & Mota-Rojas, D. (2022a). Legislación sobre el bienestar animal durante el proceso de movilización: el caso del búfalo de agua. In F. Napolitano, D. Mota-Rojas, A. Orihuela, A. Braghieri, D. Hufana-Duran, A. C. Strappini, A. M. F. Pereira, M. D. Ghezzi, I. Guerrero-Legarreta, & J. Martínez-Burnes (Eds.), *El búfalo de agua en las Américas: Comportamiento y productividad* (Fourth edi, pp. 353–380). BM Editores. https://www.researchgate.net/publication/363404793_Chapter_13_Legislacion_sobre_el_bienestar_animal_durante_el_proceso_de_movilizacion_el_caso_del_bufalo_de_agua_Book_EL_BUFALO_DE_AGUA_EN_LAS_AMERICAS_Fourth_edition_2022_353-380

Rodríguez-González, D., Napolitano, F., Guerrero-Legarreta, I. G., Mora-Medina, P., Ghezzi, M., José-Pérez, N., Domínguez-Oliva, A., & Mota-Rojas, D. (2022b). Critical aspects of legislation and their impact on the welfare of water buffaloes during transport. *Journal of Animal Behaviour and Biometeorology*, *10*(4), 1–13. https://doi.org/10.31893/jabb.22032

Rodríguez-González, D., Minervino, A. H. H., Orihuela, A., Bertoni, A., Morales-Canela, D. A., Álvarez-Macías, A., José-Pérez, N., Domínguez-Oliva, A., & Mota-Rojas, D. (2022c). Handling and physiological aspects of the dual-purpose water buffalo production system in the mexican humid tropics. *Animals*, *12*(5), 608. https://doi.org/10.3390/ani12050608

Rodríguez-González, D., Mota-Rojas, D., Ghezzi, M. D., Cruz-Monterrosa, R., & Guerrero-Legarreta, I. (2021). *Factores postmortem en la calidad de la carne del Búfalo de agua*. Entorno Ganadero. https://www.researchgate.net/publication/361206617_Postmortem_factors_that_affect_t%0Ahe_physicochemical_characteristics_of_water_buffalo_meatFactores_postmortem_en_la_%0Acalidad_de_la_carne_del_Bufalo_de_agua_Suplemento_Bufalo_de_agua_2021_19_114_9%0A3-101

Rodríguez;, A., & Romero, E. (2016). Evaluación del efecto de diferentes dosis de la fertilización orgánica en la calidad nutricional del pasto camelote (Paspalum fasciculatum willd). *Tlatemoani: Revista Académica de Investigación*, *1*(23), 1–14.

Romanovsky, A. A. (2014). Skin temperature: Its role in thermoregulation. *Acta Physiologica*, *210*(3), 498–507. https://doi.org/10.1111/apha.12231

Sakakibara, S., Arai, S., Hukui, Y., & Sawada, H. (2014). Fluctuation of body surface temperature in calves during transportation using infrared thermography. *Journal of the Japan Veterinary Medical Association*, *67*, 125–128.

Sanchez Hernández, C. S., Sanchez Hernandez, M. Á., Gonzalez Montiel, L., & Pinacho Vicente, A. J. (2017). Propiedades fisicoquímicas del mole negro de Oaxaca. In *Educando en la transversalidad para un conocimiento multidisciplinario.* (p. 206). Universidad del Papaloapan.

Sanhouri, A. A., Jones, R. S., & Dobson, H. (1992). Effects of xylazine on the stress response to transport in male goats. *British Veterinary Journal*, *148*(2), 119–128. https://doi.org/10.1016/0007-1935(92)90103-8

Sapolsky, R. M. (2000). How do glucocorticoids influence stress responses? integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55–89. https://doi.org/10.1210/er.21.1.55

Sartorelli, P., Dominoni, S., & Agnes, F. (1992). Influence of duration of simulated transport on plasma stress markers in the calf. *Journal of Veterinary Medicine Series A*, *39*(1–10), 401–403. https://doi.org/10.1111/j.1439-0442.1992.tb00198.x

Schaefer, A. L., Jones, S. D. M., Tong, A. K. W., & Vincent, B. C. (1989). Infrared thermography of pigs with known genotypes for stress susceptibility in relation to pork quality. *Can. J. Anim. Sci*, *69*, 491–495.

Schmitz, L., Ebinghaus, A., Ivemeyer, S., Domas, L., & Knierim, U. (2020). Validity aspects of behavioural measures to assess cows' responsiveness towards humans. *Applied Animal Behaviour Science*, *228*, 105011. https://doi.org/10.1016/j.applanim.2020.105011

Schwartzkopf-Genswein, K., Ahola, J., Edwards-Callaway, L., Hale, D., & Paterson, J. (2016). S ymposium Paper : Transportation issues affecting cattle well-being and considerations for the future Presented at the cattle transportation symposium sponsored by the Beef Checkoff Program, National Cattlemen's Beef Association, and Colorado State U. *The Professional Animal Scientist*, *32*(6), 707–716. https://doi.org/10.15232/pas.2016-01517

Soerensen, D. D., Clausen, S., Mercer, J. B., & Pedersen, L. J. (2014). Determining the emissivity of pig skin for accurate infrared thermography. *Computers and Electronics in Agriculture*, *109*, 52–58. https://doi.org/10.1016/j.compag.2014.09.003

Soerensen, D. D., & Pedersen, L. J. (2015). Infrared skin temperature measurements for monitoring health in pigs: a review. *Acta Veterinaria Scandinavica*, *57*(1), 5. https://doi.org/10.1186/s13028-015-0094-2

Sommavilla, R., Faucitano, L., Gonyou, H., Seddon, Y., Bergeron, R., Widowski, T., Crowe, T., Connor, L., Scheeren, M., Goumon, S., & Brown, J. (2017). Season, transport duration and trailer compartment effects on blood stress indicators in pigs: Relationship to environmental, behavioral and other physiological factors, and pork quality traits. *Animals*, *7*(12), 8. https://doi.org/10.3390/ani7020008

Stewart, M., Schaefer, A., Haley, D., Colyn, J., Cook, N., Stafford, K., & Webster, J. (2008). Infrared thermography as a non-invasive method for detecting fear-related responses of cattle to handling procedures. *Animal Welfare*, *17*(4), 387–393. https://doi.org/10.1017/S0962728600027895

Stewart, M., Stookey, J. M., Stafford, K. J., Tucker, C. B., Rogers, A. R., Dowling, S. K., Verkerk, G. A., Schaefer, A. L., & Webster, J. R. (2009). Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. *Journal of Dairy Science*, *92*(4), 1512–1519. https://doi.org/10.3168/jds.2008-1578

Stewart, M., Verkerk, G. A., Stafford, K. J., Schaefer, A. L., & Webster, J. R. (2010). Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *Journal of Dairy Science*, *93*(8), 3602–3609. https://doi.org/10.3168/jds.2010-3114

Stewart, M., Webster, J. R., Verkerk, G. A., Schaefer, A. L., Colyn, J. J., & Stafford, K. J. (2007). Non-invasive measurement of stress in dairy cows using infrared thermography. *Physiology & Behavior*, *92*(3), 520–525. https://doi.org/10.1016/j.physbeh.2007.04.034

Stewart, M., Wilson, M. T., Schaefer, A. L., Huddart, F., & Sutherland, M. A. (2017). The use of infrared thermography and accelerometers for remote monitoring of dairy cow health and welfare. *Journal of Dairy Science*, *100*(5), 3893–3901. https://doi.org/10.3168/jds.2016-12055

Strappini, A. (2012). Problemas y errores comunes encontrados en Chile durante el manejo del Ganado. In D. Mota-Rojas, S. Huertas, I. Guerrero-Legarreta, & M. E. Trujillo (Eds.), *Bienestar animal: Productividad y calidad de la carne* (2nd ed., pp. 331–341). Elsevier.

Strappini, A. C., Frankena, K., Metz, J. H. M., Gallo, C., & Kemp, B. (2012). Characteristics of bruises in carcasses of cows sourced from farms or from livestock markets. *Animal*, *6*(3), 502–509. https://doi.org/10.1017/S1751731111001698

Strutzke, S., Fiske, D., Hoffmann, G., Ammon, C., Heuwieser, W., & Amon, T. (2019). Technical note: Development of a noninvasive respiration rate sensor for cattle. *Journal of Dairy Science*, *102*(1), 690–695. https://doi.org/10.3168/jds.2018-14999

Tapia, M. S., Alzamora, S. M., & Chirife, J. (2020). Effects of water activity (aw) on microbial stability as a hurdle in Food Preservation. In *Water Activity in Foods* (pp. 323–355). Wiley. https://doi.org/10.1002/9781118765982.ch14

Tattersall, G. J. (2016). Infrared thermography: A non-invasive window into thermal physiology.

Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, *202*, 78–98. https://doi.org/10.1016/j.cbpa.2016.02.022

Taylor, C. R. (1966). The vascularity and possible thermoregulatory function of the horns in goats. *Physiological Zoology*, *39*(2), 127–139. https://doi.org/10.1086/physzool.39.2.30152426

Teja, A., Sakthivel, J., Ananda Rao, K., Kumaresan, A., Ramesha, K. P., Krishnaswamy, N., Gowtham Varma, C., Sivaram, M., Lavanya, M., Gowdar Veerappa, V., Kataktalware, M. A., Das, D. N., Majumder, K., & Rajbangshi, N. (2023). Digital infrared thermal imaging of udder skin surface temperature: a novel non-invasive technology to monitor calving process in Murrah buffalo (Bubalus bubalis). *Scientific Reports*, *13*(1), 13207. https://doi.org/10.1038/s41598-023-40447-4

Temizkan, M. C., Bayraktaroglu, A. G., & Kahraman, T. (2019). Differential expression analysis of meat tenderness governing genes in different skeletal muscles of bovines. *Journal of the Science of Food and Agriculture*, *99*(7), 3240–3245. https://doi.org/10.1002/jsfa.9434

Thomson, D. U., Loneragan, G. H., Henningson, J. N., Ensley, S., & Bawa, B. (2015). Special Report of finished feedlot cattle following transportation. *Journal of the American Veterinary Medical Association*, *247*(1), 66-72.

Tornberg, E. (2005). Effects of heat on meat proteins – Implications on structure and quality of meat products. *Meat Science*, *70*(3), 493–508. https://doi.org/10.1016/j.meatsci.2004.11.021

Turan, A., Yalcintan, H., Orman, A., & Ekiz, B. (2021). Effects of gender and slaughter age on meat quality of Anatolian water buffaloes. *Tropical Animal Health and Production*, *53*(4), 415. https://doi.org/10.1007/s11250-021-02835-8

Upadhyay, R. C., Singh, A. K., Devi, R., Kumar, Y., & Kumar, P. (2014). Physiological changes and blood flow in Murrah Buffaloes during summer and winter season. *Journal of Buffalo Science*, *3*(2), 63–69. https://doi.org/10.6000/1927-520X.2014.03.02.6

Valadez Noriega, M., & de la Lama, G. C. (2020). Implications, trends, and prospects for long-distance transport in cattle. Review. *Revista Mexicana De Ciencias Pecuarias*, *11*(2), 517–538.

Valkova, L., Vecerek, V., Voslarova, E., Kaluza, M., & Takacova, D. (2021). The welfare of cattle, sheep, goats and pigs from the perspective of traumatic injuries detected at slaughterhouse postmortem inspection. *Animals*, *11*(5), 1406. https://doi.org/10.3390/ani11051406

Van Engen, N. K., & Coetzee, J. F. (2018). Effects of transportation on cattle health and production: a review. *Animal Health Research Reviews*, *19*(2), 142–154. https://doi.org/10.1017/S1466252318000075

Vásquez Morán, Y., Poblete Vilches, H., Arenas Salinas, M., & Vergara Jaque, A. P. (2013). *Efectos estructurales y energéticos de residuos críticos (Y671 Y K639) en la capacidad de transportar iones en el poro del canal receptor de potencial transitorio v vaniloide tipo 1 (TRPV1)* (Vol. 1) [Universidad de Talca (Chile)]. http://dspace.utalca.cl/handle/1950/10895

Verduzco-Mendoza, A., Bueno-Nava, A., Wang, D., Martínez-Burnes, J., Olmos-Hernández, A., Casas, A., Domínguez, A., & Mota-Rojas, D. (2021). Experimental applications and factors involved in validating thermal windows using infrared thermography to assess the health and thermostability of laboratory animals. *Animals*, *11*(12), 3448. https://doi.org/10.3390/ani11123448

Wang, L., Liu, F., Luo, Y., Zhu, L., & Li, G. (2015). Effect of acute heat stress on adrenocorticotropic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. *Biomedical Reports*, *3*(3), 425–429. https://doi.org/10.3892/br.2015.445

Warren, L. A., Mandell, I. B., & Bateman, K. G. (2010). Road transport conditions of slaughter cattle: Effects on the prevalence of dark, firm and dry beef. *Canadian Journal of Animal Science*, *90*(4), 471–482. https://doi.org/10.4141/cjas09091

Weschenfelder, A. V., Saucier, L., Maldague, X., Rocha, L. M., Schaefer, A. L., & Faucitano, L. (2013). Use of infrared ocular thermography to assess physiological conditions of pigs prior to slaughter and predict pork quality variation. *Meat Science*, *95*(3), 616–620. https://doi.org/10.1016/j.meatsci.2013.06.003

Wong, S. S., Sun, N. N., Keith, I., Kweon, C.-B., Foster, D. E., Schauer, J. J., & Witten, M. L. (2003). Tachykinin substance P signaling involved in diesel exhaust-induced bronchopulmonary neurogenic inflammation in rats. *Archives of Toxicology*, *77*(11), 638–650. https://doi.org/10.1007/s00204-003-0485-4

Wong, S. S., Sun, N. N., Lantz, R. C., & Witten, M. L. (2004). Substance P and neutral endopeptidase in development of acute respiratory distress syndrome following fire smoke inhalation. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *287*(4), L859–L866. https://doi.org/10.1152/ajplung.00388.2003

Yerpes, M., Llonch, P., & Manteca, X. (2021). Effect of environmental conditions during transport on chick weight loss and mortality. *Poultry Science*, *100*(1), 129–137. https://doi.org/10.1016/j.psj.2020.10.003

Young, R., Lefevre, L., Bush, S. J., Joshi, A., Singh, S. H., Jadhav, S. K., Dhanikachalam, V., Lisowski, Z. M., Iamartino, D., Summers, K. M., Williams, J. L., Archibald, A. L., Gokhale, S., Kumar, S., & Hume, D. A. (2019). A gene expression atlas of the domestic water buffalo (Bubalus bubalis). *Frontiers in Genetics*, *10*(JUN), 1–14. https://doi.org/10.3389/fgene.2019.00668

Zhang, C., Xiao, D., Yang, Q., Wen, Z., & Lv, L. (2020). Review: Application of infrared thermography in livestock monitoring. *Transactions of the ASABE*, *63*(2), 389–399. https://doi.org/10.13031/trans.13068