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Consequences of long-distance transport on the behavior and health of young-bulls that may affect their fitness to adapt to feedlots

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HIGHLIGHTS

- The consequences of long-distance transport on the fitness of young bulls to adapt to the feedlot are unknown.
- We studied the post-arrival consequences of 32-hour journeys and the trailer compartment in 104 young bulls.
- We found post-transport fatigue (PTF) during the first two weeks' post-arrival.
- We found persistent signs of bovine respiratory disease (BRD) from day 5 to day 60 post-arrival.
- Transporting young bulls in the belly was linked to fatigue after transport, while transport in the deck was associated with respiratory problems.

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ABSTRACT

Some studies have shown that long distance journeys and the type of trailer compartment have consequences on farm animal welfare. However, there is little evidence to indicate how these consequences affect the fitness of young bulls to adapt to a novel and challenging environment such as an intensive industrial feedlot. Therefore, the objective of our study was to evaluate the consequences of long-distance transport and trailer compartment on the behavior and health of young bulls during the first 60 days after arrival to the feedlot. An evaluation protocol was conducted to record individual behavior (scan sampling) and health indicators of young bulls from days 1-10 and 51-60 after arrival. In addition, three ocular thermal images were taken per animal in a chute during weighing, one each on day 0, day 2 (48 h) and day 50. From our results, the thermophysiological profile, maintenance behaviors and health indicators suggest that there are two distinct groups of consequences affecting animal fitness during the first two months in the feedlot. The first was linked to post-transport fatigue (PTF) that seemed to disappear after the 10 days post-arrival at feedlot. The second was related to signs of bovine respiratory disease (BRD) that began 6 days post-transport and persisted until day 60. In addition, the trailer compartments known as the belly and the deck were shown to be problematic for animal welfare, where the transport in the belly was linked to fatigue after transport, while transport in the deck was associated with respiratory problems. Our study underscores the importance of applying preconditioning practices in cow-calf rearing systems at least a couple of months prior to the long-distance journey, in addition to implementing good loading practices to select which animals are best suited for a given compartment. Our results may be useful to minimize the impacts of PTF and BRD, to propose best practices for livestock transport in countries with similar production systems and agroecosystems.

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1. Introduction

For economic and logistical reasons, the transport of live animals is an integral part of the global meat production chain, with direct and indirect impacts on the price of meat, safety, traceability, food safety, marketing, trade barriers and public opinion, as well as having important implications for animal welfare (Miranda-De La Lama et al., 2014). Actually, in many regions/parts of the world the production flow of beef cattle starts with the animals being born in a cow-calf system, weaned, and hauled to a sale barn where they are sold and transported to a stocker operation (Estévez-Moreno et al., 2021). There they are grown for a period of time, transported to a feedlot for the finishing phase, and then finally transported to a slaughter facility (Schuetze et al., 2017). Specifically, in the Mexican case, the cow-calf system is tropical extensive and/or semi-extensive in the southeastern states of the country, followed by finishing in intensive feedlots located in the semi-arid regions of the Bajio and northern part the country (Valadez-Noriega, et al., 2020). Therefore, journeys longer than 30 hours are more the rule than the exception in the Mexican beef industry. Since Mexico, Canada and the U.S. signed the North American Free Trade Agreement (NAFTA) 30 years ago, beef transportation in Mexico has been gradually modernized by replacing the national fleet of single-deck trucks with pot-belly trailers (Valadez-Noriega et al., 2018). This is because the pot-belly design is suitable for long-distance transport and has greater load capacity (typically 30-45 young bulls or 60-75 calves), resulting in a low transportation cost per animal (Kannan et al., 2016). This type of trailer is characterised by a rigid aluminium chassis with symmetrical perforations (passive ventilation), double deck, two to four axles, five compartments and internal ramps (Schwartzkopf-Genswein and Grandin, 2014). The term "pot-belly" refers to the design of the trailer belly which, when loaded, increases stability (reducing the potential for rollover accidents) and allows for better utilisation of space (Shearer, 2021). However, this type of trailer has been criticized for the difficulties involved in handling livestock due to the need for the animals to negotiate multiple internal ramps and poor autonomy for loading/unloading, because they do not have hydraulic unloading ramps and tend to rely on loading/unloading docks at farms, feedlots and slaughterhouses. In addition, some compartments during transport have been linked to heat stress (belly) and cold stress (doghouse), which could affect the health and welfare of cattle upon arrival at the feedlot (González et al., 2012a; Goldhawk et al., 2014).

Even under favorable conditions, cattle are exposed to a number of potential stressors that can compromise their welfare, health and performance during transportation and logistics operations, due to changes in the thermal micro-environment, weather conditions, changes in the social structure (separation or/and mixing), handling, withdrawal of feed and water, vibration and acceleration and associated fatigue, exposure to new pathogens, position in the truck, loading and unloading stress and injury, noise and environmental pollutants (Miranda-de la Lama et al., 2014). The interaction between these stressors, with pre-existing health and welfare conditions and individual differences, can limit or affect an animal's fitness during each journey (Miranda-de la Lama et al., 2020). Consequently, transportation is a critical logistic period in the beef cattle industry, and the annual economic losses (i.e. mortality, dark meat, carcass bruising) caused by transport stress are substantial (Zhao et al., 2021). These losses are especially important for calves and young bulls transported from their initial farms or feedlots to finishing feedlots (Roberts et al., 2016). Most of the animals sent to feedlots are healthy and physically fit, but, depending on the length and quality of the journey, transport can pose a significant challenge even for fit and healthy animals (Cockram, 2019). Several studies have shown that stress caused by long-distance transport in cattle is associated with increased incidence and severity of bovine respiratory disease (BRD), commonly referred to as "transport fever" (Earley et al., 2017).

BRD is a worldwide welfare, health and economic concern and is the number one disease of stocker, backgrounder, and feedlot cattle in North America (Hilton, 2014). It is a multifactorial infectious disease, caused by a complex interaction between different viral and bacterial pathogens, and stress from environmental factors that suppress host defense mechanisms, facilitate the spread of the pathogen, or both (Lowie et al., 2021). Although the disease can occur in cattle of any age, they are more likely to be affected soon after arrival at the feedlot as they are exposed to a wide range of respiratory pathogens (Timsit et al., 2016). Signs of the disease include fever, anorexia, and lethargy, in addition to disease-specific clinical signs such as nasal discharge, coughing, and abnormal breathing (Toaff-Rosenstein et al., 2016). Traditionally, the diagnosis of BRD is based on the detection of some signs by pen-checkers (i.e. depression, anorexia, fever), although this diagnostic approach is not always accurate because cattle are prey animals and therefore often mask signs of disease, especially in the presence of people (Weary et al., 2009; Timsit et al., 2016). Aditionally, post-transport fatigue (PTF) is a condition of physiological and emotional exhaustion related to the effort an animal has been subjected to during a journey of prolonged duration or high intensity (vibration, high densities, sudden temperature changes, social regrouping) (Miranda-de la Lama et al., 2020; EFSA Panel on Animal Health and Welfare AHAW., 2022). The most common signs of this condition are decreased behavioural motivation, lethargy and loss of muscle coordination (Broom and Kirkden, 2004). Despite the numerous studies that have been conducted to understand the factors and their interactions that cause BRD and PTF disorders, there is no information on the role of the trailer compartment in long distance transport, and its possible effect on animal fitness upon arrival and while adapting to a new feedlot. Therefore, the aim of our study was to evaluate the consequences of long-distance transport and trailer compartment on the behavior and health of young bulls during the first 60 days after arriving to a feedlot.

2. Material and Methods

The study was conducted in October 2017-Febraury 2018 (winter) to study the consequences of long-distance transport and trailer compartment on the behavior and health of commercial young bulls during two long-distance journeys. The animals were transported from an extensive commercial farm located in the municipality of Tizimín (21°08′33 "N, 88°09′53 "W), Yucatán state (in the Southeastern part of the country) to a commercial feedlot in the municipality of Ezequiel Montes (20°42′26.4 "N, 99°54′48.5 "W) in the state of Querétaro (Bajio region in the North-central part of the country). The municipality of Tizimin is located 20 m above sea level and a mean temperature of 25ºC (range of 16ºC to 35ºC). The Köppen classification describes the climate of this municipality as warm sub-humid AWo (x') (i') g with a short, hot and partly cloudy summer and winters that are hot, humid and mostly clear. The average annual precipitation is 1154 mm. Cattle from this region are normally raised in the state and sold to fatteners in the center and north of the country when they are grown to more than 350 kg. These cattle are considered medium-large in the market and due to their characteristics are considered medium fattening cattle. This means that when they are introduced into an intensive fattening system, they can reach the desired slaughter weight (between 550 and 600 kg) in a period of 90 to 120 days. Meanwhile, the municipality of Ezequiel Montes is located at 1978 m above sea level and a mean temperature of 18.5ºC (rage of -5ºC to 27ºC). The Köppen climate classification system describes the municipality as cold semiarid climate (BSk), with hot summers and cool winters, and most rainfall occurring in the cooler months, and an average annual precipitation of 573mm. This feedlot was chosen because of its installed capacity and because it is located in one of the largest cattle fattening regions in Mexico. All study procedures were conducted within the guidelines of approved local official norms for animal care (NOM-015-ZOO, 2002: Humanitarian care of animals during transport), and the study protocols were approved (DC-2022/3-1) by the Institutional Animal Ethics Committee for the Care and Animal Use (SICUAE) of the National Autonomous University of Mexico (UNAM).

2.1. Study description

Post-transport assessments were implemented as a cross-sectional study to assess the behavioral and health consecuences of long-transport distance and trailer compartment in young bulls that entered a commercial feedlot through standard schedules. We used a total of 104 commercial zebu young bulls, from 24 to 30 months of age and an average live weight of 376.9 (±42.8) kg, from two long distance journeys (32h). The young bulls were pre-fed under similar conditions, mainly on forage grasses such as Taiwan (*Pennisetum purpureum*), guinea (*Pannicum maximum*), Star of Africa (*Cynodon niemfluensis*), Brizantha (*Brachiaria brizantha*) and Tanzania (*Pannicum maximum var. Tanzania*), and mineral salt supplements. The two journeys were conducted with a 2-week difference between the first (n=54) and the second one (n=50). The transport distance between the origin (Tizimin) and the destination (Ezequiel Montes) was 1,679km, always on highways. Each journey lasted 32 h (29 h transport + 3 h of loading and unloading, and verification stops at sanitary control posts) and the time between the different procedures was maintained the same. Journeys always started at 14.00 h and arrival was the following day at approximately 22.00 h. The first journey was conducted from October 28 to 29, 2017, recording at origin a temperature of 30.3°C and 77% relative humidity, while at arrival the temperature recorded was 11.4°C and the relative humidity was 66%. The second journey was carried out from November 9 to 10, 2017, recording a temperature at origin of 28.7°C, and a relative humidity of 73%. On arrival at the feedlot, the temperature recorded was 9.9°C and a relative humidity of 60%.

Our study, including unloading and management related to the reception and adaptation of the animals in the feedlot, was performed under commercial conditions, but monitored by project personnel. For both journeys the truck-trailer and driver were the same. Pot-belly trailers have five compartments, known as nose, belly, back, deck and doghouse. In our study only the first four compartments were analyzed (Fig. 1), because the size and weight of the transported animals did not allow to use the doghouse. Not using the doghouse compartment is a common and recommended practice to avoid injury and prevent accidents due to possible loss of balance (Pederson et al., 2018). Upon arrival to the feedlot (day 0), the animals in each compartment were unloaded, using the unloading dock and aisles and introduced one by one into a handling chute, where each animal was imaged using an infrared thermographic camera, weighed and ear-tagged on the right ear (always in that order). A specific color ear tag was used for each compartment (nose: blue; belly: yellow; back: green; and deck: white) and the ear tag was marked with three digits to maintain individual animal identification. After these procedures, animals from the same journey were housed in the same pen for the duration of the study and baiting. The pen was approximately 700 m^2 (14 m^2 per animal), with a roof that covered the whole pen, at a height of 7.30 m. The pen had two water troughs (130 cm long and 60 cm high, made of concrete, 250 L capacity), a lying area (bare soil), and a feeding area with a concrete floor (see Valadez-Noriega et al., 2020).

Immediately upon introduction to the pen, the animals had *ad libitum* access to fresh water and feed (Fig. 2). The diet was formulated according to NRC (2000) and contained (g/kg DM) maize straw (200),

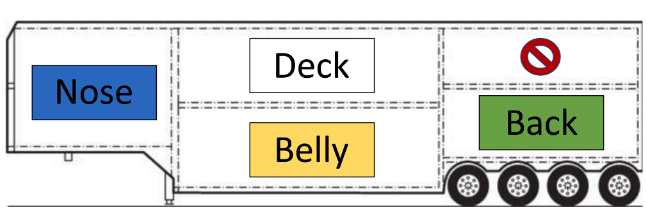

Fig. 1. Distribution of animals in the four compartments of the pot-belly trailer used in our study: Nose, Belly, Back and Deck (draw modified from Schwartzkopf-Genswein and Grandin, 2014).

bakery waste (240), ground corn (430), soybean meal (80), bypass fat (10; Enervit, Zuavit SA de CV, Ecatepec, Mexico), mineral premix (15), buffer (5; containing [mg/kg DM] Na 182, 84 mg), and 6.7 mg/kg of zilpaterol hydrochloride (Zilmax, Intervet; Merck and Co., Inc., Madison, NJ, USA). The chemical composition of the diet was 132 g/kg DM of crude protein, 7 and 3 g/kg DM of calcium and phosphorus, respectively, and 1.9 and 1.29 Mcal/kg DM of net energy for maintenance and body weight gain, respectively. At 48 h post-arrival, the animals were subjected to a reception protocol which consisted of administering a clostridial vaccine (Ultrachoice 8, Zoetis), an anthelmintic (Safeguard, Merck Animal Health, Kenilworth, NJ), a pentavalent modified-live virus respiratory vaccination (containing bovine herpesvirus-1, bovine viral diarrhea virus types 1 and 2, parainfluenza-3 virus, and bovine respiratory syncytial virus; (BoviShield Gold 5, Zoetis, Kalamazoo, MI), and an implant that contained 200 mg of trenbolone acetate and 40 mg of estradiol (Revalor-XS, Merck Animal Health). At 50 d post-arrival, a systemic deworming agent was applied and a new anabolic implant was inserted.

2.2. Thermophysiological measurements and weighing

An infrared thermal camera (FLIR i7, FLIR Systems, Boston, MA) was used to collect ocular thermal images of each animal while in the chute as described by Baier et al. (2020). Three images were taken per animal, one on day 0, day 2 (48 h) and day 50. Each image was taken of the right eye from a distance of approximately 0.914 m, measured by the internal laser beam and the calibrated laser beam of the IR camera in perpendicular alignment to the ocular area of interest. The measuring laser beam was positioned rostral to the eye to avoid direct contact with the eye. All images were taken by the same researcher, while she was immediately to the right of the chute. Images were analyzsed using the FLIR R&T software package. Animals were weighed on the same days after each image was taken, always using the same scale, which was calibrated before weighing.

2.3. Behavioral assessment

Animals were observed on days 1-10 and 51-60 post-arrival, to quantify behavioral changes as a consequence of fatigue from the journey and adaptation to the feedlot. Scan sampling was conducted every 10 minutes for 6 h per day (09:00 AM to 11:00 AM, 12:00 PM to 14:00 PM and 15:00 PM to 17:00 PM) for 20 days (2 periods of 10 days), for a total observation time of 120 h per journey, giving a total of 780 scan samples per animal (104 animals) and 81,120 observations of maintenance behaviors to obtain information on the total time spent lying idle down (recumbent, not supported by legs), standing idle (supported by legs but not performing locomotion), walking (moving from one place to another), ruminating (performing slow chewing movements and regurgitations), feeding (head inside the feeder trough), drinking (head inside the water trough), and self-licking (licking self on the chest, flank or legs).

2.4. Health assessment

An evaluation protocol for recording daily health conditions of young bulls was developed based on an exhaustive literature review and preliminary observations by the research team. The protocol was validated with 50 animals from a pilot journey prior to our study. The evaluation was carried out on days 1-10 and 51-60 post-arrival. Sampling was always performed at 18.00 h in the pens, which included a 3-point scale nasal discharge score, where 1 was clear discharge, 2 was turbid discharge and 3 was bloody discharge. Subsequently we measured ocular discharge (score of 2: presence or absence), coughing (score of 2: presence or absence), muscle tremors (score of 2: presence or absence), apathy (score of 2: presence or absence), bruxism (score of 2: presence or absence), signs of fever (score of 2: presence or absence),

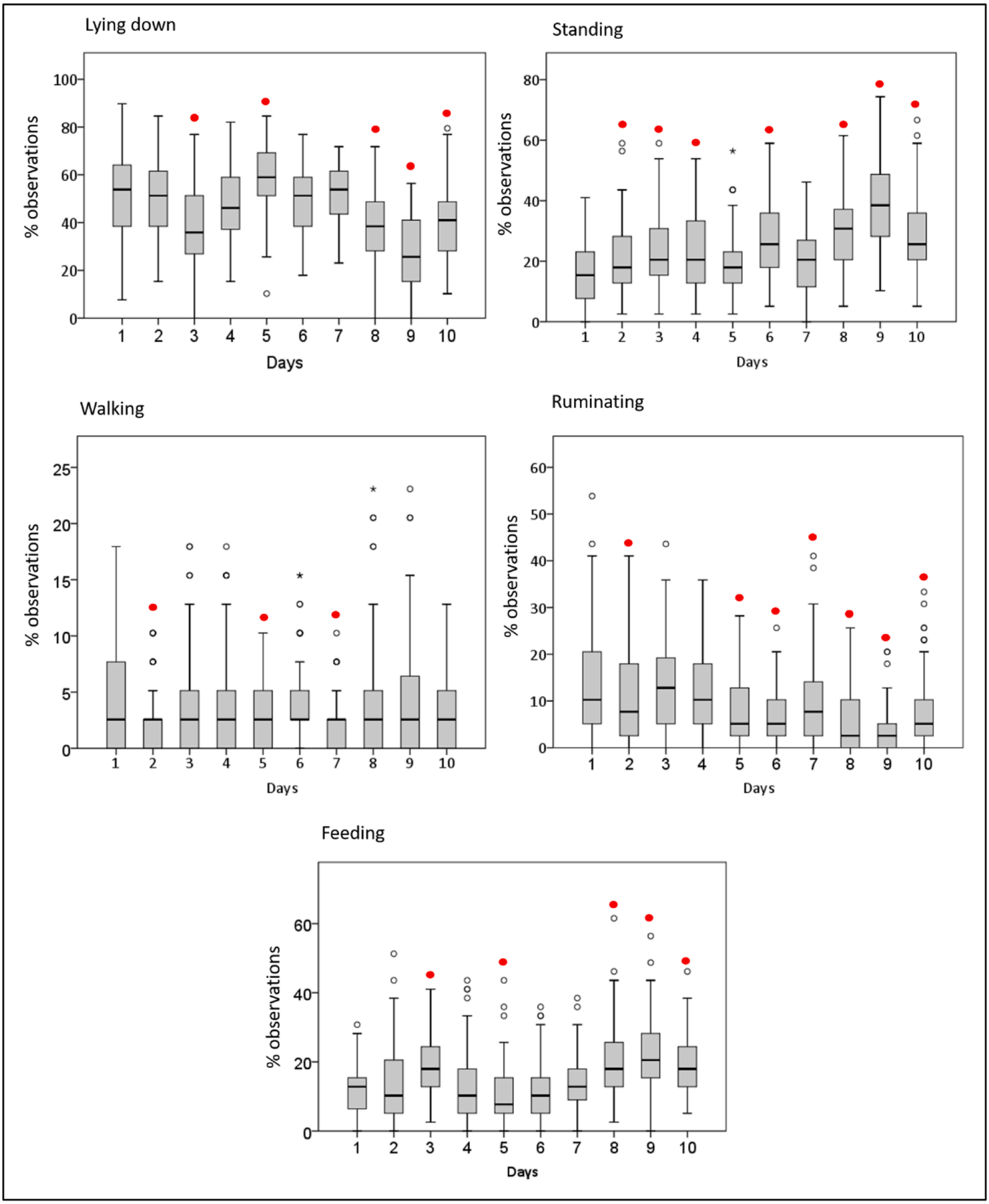

Fig. 2. Development of the percentage of maintenance behaviors/animal during the first day's post-arrival.
● Represents significant differences (P<0.05) between day 1 and days 2-10.

diarrhea (score of 2: presence or absence), lameness (score of 2: presence or absence), wounds (score of 2: presence or absence), and contusions (without bleeding) in locomotor limbs and other body parts (score of 2: presence or absence). The evaluation was always performed by the same observer and in the same order.

2.5. Statistical analyses

Data were entered into Microsoft Excel (Microsoft Corporation, 2010) and then analysed with the SPSS statistical package (IBM SPSS Statistic 22). Differences (P<0.05) in eye temperature, animal weight, and maintenance behaviors during the first two months post-transportation were examined using a one-way repeated measure analysis of variance (ANOVA). The truck compartment where the animals were transported (nose, belly, back, and deck) was included as a fixed categorical factor. In the case of temperature and weight, three moments of measurement were compared (0 h, 48 h, and 50 days), while for the behavior two different analyses were performed: i) comparing the first ten days post transport and 2) comparing four observation periods, each grouping the observations of 5 days (P1= day 1 to 5, P2= day 6 to 10, P3= day 51 to 55, and P4= day 56 to 60). These analyses were supplemented with Friedman's test and Wilcoxon signed-rank test for non-parametric variables. The appearance of clinical signs in young bulls was examined by comparing their prevalence in the four observation periods using Cochran's Q test. Each of the clinical signs was registered as prevalent if it was observed during at least one of the 5 days within each period. When significant differences were detected, multiple McNemar's tests were applied to perform pair-wise comparisons.

3. Results

No animals died during the two journeys or after arrival to the feedlot, nor were there any non-ambulatory animals.

3.1. Thermophysiological measurements and weighing

The mean eye temperature upon arrival to the feedlot was 37.1°C (±2.3). Then, 48 h later the mean temperature was 37.9°C (±1.4) and at 50 days it was 37.3°C (±3.9), with no significant differences between the three measurements. However, when analysing the evolution of temperature in the animals per compartment, the ocular temperature measured upon arrival (36.5°C) was significantly (P<0.05) lower in the belly compartment compared to 48 h and 50 days later (38.1°C and 37.1°C, respectively). The mean weight of young bulls upon arrival was 376.9 (±42.8) kg, then 400.0 (±44.5) kg after 48 h, and 478.3 (±70.2) kg 50 days post-transport. In addition, during the first 48 h post-transport, the average daily weight gain was higher (11.6 ±6.2 kg) compared to between days 3 and 50 (1.7 ±1.1 kg; P<0.01). No significant differences were found when comparing compartments and mean weights over the three measurements.

3.2. Behavioral assessment

Of the seven behavioral categories studied, only drinking and self-grooming remained stable on the first day post-transport (P>0.05). In contrast, the time spent resting was less on days 3, 5 and 8 to 10, while on most days the animals spent more time standing than on the first day. The frequency of walking was similar to the first day except for days 3, 5 and 7. The frequency of ruminating decreased consistently from day 5 onwards, while a higher percentage of time was spent eating on days 3, 5 and 8 to 10. The post-transport maintenance behaviors across the 4 observation periods can be seen in Table 1. No significant differences were observed between observation periods for drinking behavior (P>0.05), while for the remaining six behavioral categories measured there were significant differences (P<0.05). The animals spent more time lying down in periods 1 and 3, compared to periods 2 and 4. For standing behavior, the animals had a lower percentage for this category in period 1, but it increased significantly in the remaining 3 periods. For walking, the animals showed higher activity in periods 1 and 2 compared to periods 3 and 4, although the percentage of animals walking compared to the previous two categories of behavior (lying and standing) was very low (less than 3.5%). In the case of feeding behavior, the percentage was higher in period 2 compared to the other three periods, while rumination had a lower percentage in period 2 compared to the other three periods. Self-grooming was very low throughout the study but significantly higher in period 4 compared to the first three observation periods. In the case of the behavioral consequences of long-distance transport related to the trailer compartment, significant differences were found in all four compartments. In the case of the nose, significant differences were found for the variables of lying, standing, feeding and rumination (P<0.05). For the belly, significant differences were found for lying, standing, walking, feeding, rumination and self-grooming (P<0.05). For the deck, significant differences were observed for lying, standing, rumination and self-grooming (P<0.05), Finally, for the back compartment, there were only significant differences in the four periods for the standing and rumination behavior (P<0.05).

Table 1
Evolution of the post-transport behavioral responses of young bulls during four observation periods (% of daily observations).

Behavior	Observation periods				*P* **
	Period 11-5 d	Period 26-10 d	Period 351-55 d	Period 456-60 d	
Total (n=104)					
Lying down *	49.2 (11.2) a	41.0 (8.5) b	48.4 (8.6) a	42.0 (7.6) b	<0.001
Standing *	20.1 (6.9) a	28.8 (7.7) b	23.9 (6.9) c	29.3 (7.1) b	<0.001
Walking **	3.1 (3.0) a	3.6 (2.6) a	2.6 (2.6) b	2.6 (2.1) b	0.002
Feeding *	13.6 (5.3) a	17.6 (6.1) b	10.9 (3.9) a	13.1 (5.2) a	<0.001
Ruminating *	11.5 (6.7) a	6.6 (0.5) b	11.7 (5.1) a	10.3 (4.7) a	<0.001
Drinking **	1.5 (1.5)	1.0 (1)	1.5 (1.5)	1.0 (1.5)	NS
Self-grooming **	0.5 (0.5) a	0.5 (1) a	0.5 (0.9) a	1.0 (1) b	<0.001
Nose (n=14) *					
Lying down	51.3 (10.0) a	40.8 (14.0) b	47.7 (12.7) ab	40.8 (7.7) b	<0.001
Standing	20.3 (12.7) a	32.1 (9.4) b	24.1 (13.5) a	32.8 (8.5) b	<0.001
Feeding	9.5 (3.0) a	15.9 (4.0) b	10.0 (2.1) a	12.1 (4.5) a	<0.001
Ruminating	10.5 (6.3) a	6.4 (5.0) b	10.8 (3.8) a	9.5 (3.9) a	0.003
Belly (n=41)					
Lying down *	45.6 (19.8) a	40.5 (13.3) b	48.7 (14.4) a	41.5 (10.0) ab	<0.001
Standing *	17.4 (11.3) a	27.2 (10.8) b	22.6 (13.1) a	26.7 (9.0) b	<0.001
Walking **	3.6 (2.8) a	3.6 (3.1) a	2.6 (2.1) b	2.6 (2.1) b	<0.001
Feeding *	14.1 (5.5) a	17.5 (6.9) b	11.0 (4.3) c	13.4 (5.6) a	<0.001
Ruminating *	11.6 (6.8) ac	7.9 (5.3) b	12.0 (4.7) a	10.7 (4.9) c	<0.001
Self-grooming **	0.5 (0.5) a	0.5 (1.0) a	1.0 (1) b	1.03 (1.0) b	<0.001
Deck (n=39)					
Lying down *	47.7 (15.9) a	43.1 (9.2) b	47.7 (9.7) a	41.5 (13.3) b	<0.001
Standing *	20,5 (6.7) a	28.2 (10.3) b	22.6 (8.7) c	28.2 (11.3) b	<0.001
Feeding *	14.0 (5.4) a	18.3 (5.6) b	11.0 (4.2) c	13.0 (5.3) a	<0.001
Ruminating *	11.4 (7.0) a	5.4 (4.1) b	11.5 (5.8) a	10.2 (4.8) a	<0.001
Self-grooming **	0.5 (0.5) a	0.0 (0.5) a	0.5 (1.0) b	1.0 (1.0) b	<0.001
Back (n=10) *					
Standing	17.2 (6.9) a	27.2 (14.4) bc	22.3 (9.7) b	28.2 (15.6) c	0.001
Ruminating	13.3 (6.7) a	6.2 (5.6) b	12.82 (5.7) a	10.2 (5.2) b	0.001

Significant differences were established at *P*<0.05 according to the one-way repeated measure analysis of variance. [a,b,c], upper letters in the same row indicate significant differences (*P*<0.05) between observation periods according to paired sample t-test, and Wilcoxon signed-rank test. Mean.

* (SD),

** Median (IQR). NS: non-significant differences.

3.3. Health assessment

Table 2 shows the prevalence of clinical signs of disease during the first 60 days on the feedlot, divided into 5 day periods (period 1: 1 to 5 days, period 2: 6 to 10, period 3: 51-55; and period 4: 56 to 60 d). There were significant differences for all variables (P<0.05), except bloody nasal discharge, ocular discharge, bruxism, contusions and wounds (P>0.05). Clear discharges were lower on arrival and in the last two observation periods, while turbid discharges were lower on arrival but increased significantly in the following three consecutive periods. Thus, when summing the three categories of nasal discharges we found the same pattern as described for turbid nasal discharge. Coughing was significantly lower in periods 1 and 4 compared to the two intermediate periods (2 and 3). In the case of muscle tremors, apathy and lameness, their prevalence was always significantly higher in periods 1 and 2, compared to periods 3 and 4. The prevalence of diarrhea was low in period 1, but tripled in period 2 and decreased significantly by 40% for periods 3-4.

In the case of compartment effects on the presentation of clinical signs of disease, significant effects were found only for the belly and deck. In the case of the belly, the prevalence between periods was significant (P<0.05) for cough, muscle tremors, diarrhea and apathy; coughing was higher in period 2 (compared to period 1), muscle tremor was higher in period 2 (compared to periods 3 and 4), diarrhea was 8 times and 12 times higher in periods 2 and 3 (respectively) compared to period 1, apathy was higher in the first three periods compared to total absence in period 4. Finally, the trailer deck had significant effects (P<0.05) between periods for total and clear nasal discharge and coughing, muscle tremors and apathy. Total nasal discharge was at least 3 times higher in period 2, 3 and 4, compared to period 1, and a similar pattern was observed for clear nasal discharge. Coughing was 8 times higher in period 2 compared to period 1. Muscle tremor was higher in periods 1 and 2 compared to periods 3 and 4, with not tremors. A similar pattern was observed for the apathy variable.

Table 2
Evolution of the prevalence of health indicators of young bulls during the post-transport observation period (% animals affected).

Health indicators	Observation periods Period 1 1-5 d	Period 2 6-10 d	Period 3 51-55 d	Period 4 56-60 d	*P* **
Total (n=104 young bulls)					
Fatigue-injuries					
Muscle tremor	6.73 ^{a}	7.69 ^{a}	0.00 ^{b}	0.00 ^{b}	0.001
Apathy	13.46 ^{a}	10.58 ^{a}	1.92 ^{b}	0.00 ^{b}	<0.001
Lameness	5.77 ^{a}	3.85 ^{a}	0.96 ^{b}	0.00 ^{b}	0.024
Contusions	1.92	2.88	0.00	0.00	NS
Wounds	3.85	0.00	1.92	0.00	NS
Bruxism	1.92	4.81	8.65	5.77	NS
Gastro-intestinal					
Diarrhea	6.73 ^{a}	23.08 ^{b}	13.46 ^{c}	13.46 ^{c}	0.004
Respiratory					
Nasal discharge total	19.23 ^{a}	48.08 ^{b}	40.38 ^{b}	42.31 ^{b}	< 0.001
Nasal clear discharge	10.58 ^{a}	25.96 ^{b}	18.27 ^{a}	19.23 ^{a}	0.030
Nasal turbid discharge	6.73 ^{a}	28.85 ^{b}	25 ^{b}	25.96 ^{b}	<0.001
Nasal bloody discharge	2.88	2.88	3.85	0.96	NS
Ocular discharge	14.42	9.62	11.54	10.58	NS
Cough	3.85 ^{a}	17.31 ^{b}	12.5 ^{b}	7.69 ^{a}	0.002
Young bulls that journeyed on the belly (n=41)					
Muscle tremor	2.44 ^{ab}	9.76 ^{b}	0.00 ^{a}	0.00 ^{a}	0.035
Apathy	14.63 ^{a}	4.88 ^{a}	2.44 ^{a}	0.00 ^{b}	0.026
Diarrhea	2.44 ^{a}	29.27 ^{b}	19.51 ^{b}	14.63 ^{ab}	0.005
Cough	4.88 ^{a}	24.39 ^{b}	19.51 ^{ab}	9.76 ^{ab}	0.015
Young bulls that journeyed on the deck (n=39)					
Muscle tremor	12.82 ^{a}	10.26 ^{a}	0.00 ^{b}	0.00 ^{b}	0.008
Apathy	10.26 ^{a}	17.95 ^{a}	0.00 ^{b}	0.00 ^{b}	0.002
Nasal discharge total	17.95 ^{a}	56.41 ^{b}	43.59 ^{b}	48.72 ^{b}	0.001
Nasal clear discharge	7.69 ^{a}	43.59 ^{b}	28.21 ^{b}	35.9 ^{b}	0.002
Cough	2.56 ^{a}	20.51 ^{b}	12.82 ^{ab}	5.13 ^{ab}	0.010

*Prevalence of clinical signs was defined as the observation of the sign for at least 1 day within each observation period.

** Significant differences at $P<0.05$ according to the Cochrane Q test. ^{a,b,c}, upper letters in the same row indicate significant differences ($P<0.05$) between observation periods according to the McNemar test. NS: non-significant differences.

4. Discussion

Cattle fattening systems are restrictive with respect to key resources such as shade, thermal comfort, feeding space and surface areas compared to pre-grazing or cow-calf systems (Valadez-Noriega et al., 2020). Therefore, for a young bull from grazing systems, a long-distance transport and placement in a restrictive environment such as a feedlot may present a significant challenge to homeostatic and behavioral mechanisms, with high biological costs regarding animal welfare and health (Marchesini et al., 2018). It is widely accepted that the first two months after arrival at the feedlot represent a crucial period for cattle welfare (Noffsinger et al., 2015). Thus, our study documented the evolution of the thermophysiological profile, maintenance behaviors and prevalence of several clinical signs in young bulls during the first 60 days on a feedlot, after being subjected to long-distance winter transport from Southeastern to North-central Mexico. To our knowledge, this is the first study to focus on the consequences of long-distance journeys during the first 60 days in the feedlot. Our results may be useful to minimize the impacts of PTF and BRD, to propose best practices for livestock transport in the country, and other countries with similar production systems and agroecosystems.

4.1. Thermophysiological measurements and weighing

Infrared thermography is a non-invasive diagnostic technique that measures thermal variations in the surface temperature of animals, because it is able to detect the amount of radiated heat caused by changes in blood flow (LokeshBabu et al., 2018). The thermal response of animals to physical restraint in a farm-level chute is an indicator of an individual's threshold of emotional reactivity to a challenge, and can be used as a predictive proxy measure of how animals cope with challenges throughout their lives (Cuthbertson et al., 2020). Based on the hypothesis that long-distance transport and exposure to the novel environment of the feedlot may affect the magnitude of the stress response of animals over the first two months' post-transport, we measured eye temperature of animals on arrival (day 0), at 48 h (day 2) and at 50 days in the feedlot. Our results show that although there are no differences between the sampling days for the animals on arrival and with the two subsequent sampling periods, a significant hypothermia effect was observed for the animals in the trailer belly compartment on arrival, which disappeared in the two subsequent sampling periods. One possible explanation for this interesting result may be due to stress hypothermia, which occurs when animals are exposed to unavoidable stressors for a prolonged period of time (i.e. such as long-distance transport). Hypothermia may be a passive adaptive response to save energy in a challenging environment (Oka, 2018). Conversely, when animals are exposed to acute stimuli, accentuated and transient stress hyperthermia can occur, which helps to warm up the muscular and central nervous systems, leading to increased physical and cognitive performance to actively cope with a challenge (Lees et al., 2020). Therefore, it is possible to infer that the belly is the compartment that would be most related to the presentation of post-transport fatigue (PTF) which has sequelae in the first two weeks' post-arrival. However, after 50 days in the feedlot the response in the same animals is hyperthermia. This is consistent with the scientific literature which indicates that animals that have been exposed to repeated or chronic stress show enhanced hyperthermic responses to a new stressor (Oka, 2018). Regarding weight gain, our results show that animals on arrival gained more than 11 kg during the first 48 hours in the feedlot regardless of which compartment of the truck they came from; this increase may be due to a compensatory effect stimulated by the simultaneous offer of feed and fresh water on arrival. In this regard, Schwartzkopf-Genswein et al. (2007) found that animals did not consume enough feed if it was not provided in the presence of water on arrival at the feedlot. Finally, long-distance transport and stalls did not appear to affect weight gain over the first 60 days in the feedlot. A possible explanation for these results may be related to the combined effect of the feedlot reception protocol (vaccination, deworming and growth promoters) and a high-energy diet (Sumano et al., 2021) that could counteract any differences in weight produced by long-distance journey (Werner et al., 2013).

4.2. Behavioral assessment

Upon arrival to a destination after a long-distance transport, normal cattle behavior is altered as cattle recuperate (Van Engen and Coetzee, 2018). Habituation is an important factor that may underlie the behavioral changes observed in feedlots during the weeks following arrival, some of which are of interest for animal welfare monitoring (Toaff-Rosenstein and Tucker, 2018). Our results show that, from their arrival until day 60 on the feedlot, calves dedicated a significant percentage of their behavioral budgets (in order of importance) to lying down (>40%), standing (>20%), eating (>11%) and ruminating (>11%). There were some important differences in the first five days (50% lying down and 20% standing), but the order of importance remained similar. These results coincide with previous work that suggests that post-transport resting is effective for decreasing cortisol levels, replenishing muscle glycogen concentrations, reducing hunger, dehydration and body weight loss, recovering from physical and emotional stress caused by transport (Knowles et al., 1999). However, the time spent lying down could be proportional to the time needed to reduce post-transport stress and fatigue (Frese et al., 2016). From our results, it seems that 5 days are sufficient to attenuate post-transport stress and fatigue because in the second observation period (6-10 d) calves spend less time lying, accompanied by an abrupt rise in standing, which interestingly coincides with a significant drop in the time budget for rumination. This result has been described in cows which are deprived of lying time and then spent more time standing without ruminating (Cooper et al., 2007). In our case, the second period may be when animals try to recover their normal activity patterns, but it also coincides with an increase in the clinical signs related to disease, especially to BRD. Possibly, as fatigue decreases, chronic problems start with the disruption of certain behaviors related to cattle health. Normally, rumination is specially affected by the amount and duration of the forage fraction of the diet, and sorting behavior, and is positively correlated with dry matter intake (Byskov et al., 2015). In our case, when calves spent more time feeding, rumination decreased significantly. It is possible that a low level of rumination is related to a sub-clinical presentation of BRD, and may be a tool for early detection of the disease, as reported by Marchesini et al. (2018). Another possible explanation is that feeding behavior (more time spent eating) and decreased rumination are caused by the drastic change in diet, much lower in physically effective fiber, as well as by the administration of anabolic hormones (González et al., 2012b; Beauchemin, 2018).

We did not observe substantial significant variation in water consumption between observation periods. The time budget for drinking (<1.5%) was also noticeably lower compared to other behaviors, including eating. Previous studies in slaughterhouses have shown that despite having the opportunity to rehydrate after arrival, calves tend not to increase their drinking time budgets, as the priority is to lie down and explore the pen (Clariget et al., 2021), which is contrary to what Villarroel et al. (2011) have found for pigs, for example (a preference to rehydrate over resting, after a long journey). Another possible explanation may be that animals drank less since they were unfamiliar with the new environment and distrusted of the water supply and new caretakers, which could lead to increased stress, as seen by Noffsinger et al. (2015). A novel environment will not only decrease water consumption, but also induce diuresis and water losses by inhibiting the renin-angiotensin-aldosterone axis (Hogan et al. 2007). As for self-grooming, we found a significant increase in period 3 compared to the rest of the periods, but it never exceeded 1% of the behavioral budget. A possible explanation for this result is related to a combination of fatigue (period 1), adaptation to the feedlot (observation periods 1 to 4) and the increased prevalence of clinical signs of BRD in periods 2, 3, and 4. This is because self-grooming behavior tends to decrease in animals with allostatic loads or during the loss of homeostaisis, as resources are redirected from behaviors that are not essential in the short term (savings strategy) to the immune response, which is energetically costly (Toaff-Rosenstein and Tucker, 2018). Finally, a compartment effect for maintenance behavioral budgets was observed for all four trailer compartments tested. Our results show that the belly and the deck compartments had more of an effect on behavior, compared to the nose and back. That may be because the belly and deck compartments are more demanding in terms of handling and uncomfortable micro-environmental conditions. These results show that ergonomic limitations in trailer design may have subtle consequences on cattle behavior, However, further studies are needed to mitigate these effects by considering trailer carrying capacity, animal welfare and animal health.

4.3. Health assessment

In general, the clinical signs or health indicators used in this study can be classified as those related to fatigue-injuries, gastrointestinal problems or respiratory issues. Fatigue-injury indicators reflect the immediate impacts of long-distance transport and are especially characterised by lameness, muscle tremors and apathy. Their prevalence was especially concentrated during the first two observation periods and then they decreased or disapperad with time (periods 3 and 4). The development of lameness due to metabolic reasons, accelerated weight gain or low activity has been reported as a prevalent problem in finishing young bulls and can be detected at the slaughterhouse level (Bautista-Fernández et al., 2021; Losada-Espinosa et al., 2021). Therefore, it would appear that the lameness (contusions and injuries) observed in our study is attributable to accidents during transport and loading/unloading operations (leg entrapment, projection against static objects and falls; Minka and Ayo, 2007; Bautista-Fernández et al., 2021). Transportation prevents cattle from lying down and resting, which can increase PTF and the risk of lameness (Noffsinger et al., 2015). Post-transport fatigue is due to an interaction between the effects of transport and high environmental temperatures that manifests clinically with lameness and lethargy, in addition to high serum lactate and creatine kinase concentrations, lower blood pH and a high incidence of muscle tremors (Schuetze et al., 2015). Although the prevalence of teeth grinding or bruxism was relatively low and not significant between the four observation periods, the prevalence was persistent throughout the study. According to Gleerup et al. (2015) bruxism is an important indicator of "gross pain behavior" that should be included in all pain assessment schemes in cattle because it is a sign of intense pain.

Changes in the immune system during long-distance transport are modified mainly by increased levels of circulating corticosteroid hormones, but also by other hormones such as vasopressin and oxytocin (Werner et al., 2013). At the surface level, immunological changes can be observed in clinical signs of acute post-transport disease (Earley et al., 2012). It is important to note that, although the animals in our study were vaccinated within 48 h of arrival at the feedlot (to prevent the presentation of BRD), total nasal discharge (and cloudiness) was highly prevalent from the second observation period onwards, and remained so during periods 3 and 4. Coughing had a similar trend, except for observation period 4. These results agree with several studies that indicate that the presentation of BRD is not immediate upon arrival, and its signs are evident after the first 5 days post-transport (Earley et al., 2017; Cirone et al., 2019). The prevalence of disease or signs of disease in vaccinated animals may be because calves have immune dysfunction induced by previous stress and natural exposure to the pre-existing viral agent(s), so vaccination at weaning is recommended in cow-calf systems (Richeson et al., 2019). Furthermore, Richeson et al. (2015) found that vaccination (with a vaccine similar to that used in our study) had no immediate effect on morbidity or mortality associated with clinical BRE. Under the conditions of our study there was no evidence of previous vaccinations, so the high prevalence of subclinical BRD may be due to pre-existing infections.

The spatial dimensions of compartments holding cattle during transport are important for their welfare (in the horizontal as well as the vertical plane) and lack of space may lead to several negative welfare consequences such as restriction of movement, resting problems and thermal stress (EFSA, 2022). Recent studies have shown that the belly and deck compartments of pot-belly trailers are more problematic for cattle health (Stanford et al., 2011). Our study corroborates this effect, with belly animals having consequences related to post-transport fatigue (PTF) in the first two observation periods and especially diarrhea in periods 2 and 3. The animals on the deck present consequences of fatigue the first two periods, and a clear and high prevalence in respiratory indicators from the second period onwards, which prevails in periods 3 and 4. A possible explanation for these results is that both compartments are physically demanding for the animals due to their location in the truck and during the journey (i.e. vibration, noise), but they are different in microclimatic terms (Goldhawk et al., 2014). Thus, the deck exposes the animals to higher wind currents, while the belly has a lower ventilation gradient and can produce dehydration and diarrhea even during winter journeys (Miranda-de la Lama et al., 2018). Similar to heat stress, cold stress is a result of dehydration from increased metabolic demands of heat generation, as respiratory water loss is increased at a higher metabolic demand (Moak, 2021). Our results on compartment effects are novel, although they have their limitations since only two journeys were sampled in a single season and the number of animals tested per compartment was not balanced, so it is important to conduct further research under commercial conditions with the post-arrival health protocol used in this study. Finally, our study emphasizes the importance of implementing preventive medicine practices such as preconditioning in cow-calf systems or pre-calf farms at least a couple of months prior to long-distance journey. Several evidences have pointed out the efficacy of preconditioning calves to reduce post-transport morbidity and mortality (Bailey et al., 2016). However, preconditioning is often not considered profitable by many producers because it does not pay off in terms of live weight gain. Therefore, it is important to promote a culture of preconditioning among beef cattle industry stakeholders, emphasizing that a preconditioned calf will be more suitable for long-distance journeys, increase its chances of survival, have a better adaptation to the feedlot, and save on future treatments (Hilton, 2015).

5. Conclusion

In general, according to the thermophysiological profile, maintenance behaviors and health signs, our study suggest two main effects on the animals after arrival to the feedlot. The first is related to post-transport fatigue (PTF), which seems to dissipate after the first 10 days post-transport, and the second related to respiratory signs of bovine respiratory disease (BRD), starting at 6 days post-transport and persisting at least until day 60. Additionally, the trailer compartments known as the belly and the deck were shown to be problematic for animal welfare, where the transport in the belly was linked to fatigue after transport, while transport in the deck was associated with respiratory problems. Our study emphasizes the importance of promoting calf preconditioning among producers to prepare calves for transport in the two months preceding the journey. In addition, at the time of loading, handlers should consider the effects of the belly and deck compartments and avoid using them for especially vulnerable animals (i.e. poor body condition, lame or symptoms of respiratory disease).

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Declaration of Competing Interest

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

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