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Impact of stocking densities and road transport distances on meat quality and malondialdehyde levels in *semitendinosus* and *infraspinatus* muscles of Brahman crossbred heifers in a tropical climate

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ABSTRACT

Transporting cattle in tropical climates substantially impacts oxidative stability and meat quality due to increased stress levels. The objective of this research is to assess the impacts of road transport with two different distances and three stocking densities on meat quality indices and malondialdehyde (MDA) levels in the *infraspinatus* (IF) and *semitendinosus* muscles of Brahman crossbred heifers in a tropical climate. Sixty Brahman crossbred heifers were exposed to two different road travel distances: 450 and 850 km. Each travel distance was divided into three different stocking densities: low density of 200 kg/m^2, medium density of 400 kg/m^2 and high density of 600 kg/m^2. The number of animals in each stocking density was kept equal. Following transit, IF and *semitendinosus* muscle samples were collected and assessed for meat quality characteristics, including colour attributes, pH, muscle glycogen contents, Warner-Bratzler shear force (WBSF), cooking loss and lipid oxidation. The findings showed notable impacts of livestock densities and road transport distances on meat quality parameters and MDA levels in Brahman crossbred heifers' muscles. Similarly, increased livestock densities resulted in higher levels of MDA and alterations in meat quality measures than lower densities following both distances of road transportation. These findings highlight the significance of optimising livestock density and reducing road transport lengths to maintain the oxidative stability of meat quality in heifers.

HIGHLIGHTS

Study on cattle responses to stressors associated with road transportation.

- Transport, distances and densities impact colour, cooking loss and lipid oxidation.
- Stress caused by transportation impacts the quality of meat and the well-being of heifers.
- Monitoring throughout transportation and when held in lairage is essential to welfare.

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Stocking density; distance; Brahman cattle; meat quality; lipid oxidation

Introduction

There is a growing demand for beef worldwide, consequently leading to an increase in the movement of animals to abattoirs for meat production (Greenwood 2021). In addition to trade in beef, live cattle are transported on a large scale by sea route to importing countries, followed by land journeys to various destinations such as farms, feedlots and slaughterhouses. For example, Malaysia imports about 77% of total beef consumed (www.statista.com), and in 2019, it imported 38,373 beef cattle (Zulkifli et al. 2019; Abubakar et al. 2021).

Road transport is inevitable in the livestock sector as it facilitates the movement of animals from farms to feedlots, markets and slaughterhouses (Navarro et al. 2019). However, this process is not without challenges and consequences related to the well-being of animals, the quality and health. The duration of

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transport and the conditions during transit, such as loading density, trailer microclimate and handling quality, are critical factors influencing cattle welfare (Nielsen et al. 2022). Extended transport times have been associated with increased weight loss, alterations in blood composition, and elevated stress indicators (Masmeijer et al. 2019; Kumar et al. 2023).

Road transport of cattle is one of the major stressors encountered in livestock production. Previous studies have investigated issues related to the alleviating stress of cattle during transportation (Van Engen and Coetzee 2018; Nielsen et al. 2022). Road travel impacts cattle far beyond welfare issues and the quality of meat produced. According to Sullivan et al. (2022), transport stress can cause bruises and influence the carcase and meat quality by causing shrinkage pH changes and water losses. Dealing with various stressors during transportation requires a thorough approach to understanding and reducing negative consequences (Van Engen and Coetzee 2018). Nonetheless, there is limited evidence of the change in distances and livestock densities due to transportation by road on post-mortem ageing of different muscles and the oxidative stability of meat from cattle in tropical settings such as Malaysia.

These animals are frequently hauled by sea and road to several destinations, such as farms, feedlots and slaughterhouses. It raises animal welfare concerns due to long voyages and hauling trucks, which cause muscle tension, bruises and injury in some cases, loading, unloading and finally slaughter.

Thus, extensive scientific evidence is needed to formulate a universal set of guidelines for transporting, handling and managing livestock, which would alleviate stress and produce good-quality meat. Nonetheless, evidence of changing distances and stocking densities during transport is scarce due to the post-mortem ageing of different muscles and oxidative stability of beef from cattle in tropical settings such as Malaysia.

The present study offered unique insights into how road transportation under high ambient temperature and high relative humidity conditions affects meat quality and oxidative stress markers among cattle imported into wet and humid equatorial regions. The Brahman cross heifers were originally from subtropical Australia but had been adapted under Malaysian conditions for six months. This simulated the usual practice where animals are sourced from Australia, fattened or grew up under equatorial conditions before being sold for slaughter. The insight gathered would complement the utility of meat quality and welfare markers, data from other studies in subtropical to temperate regions, such as Australia, Southern Europe and many others. The dearth of data on how these animals responded to road transport stress in the equatorial regions necessitated the current study it provides valuable data points to safeguard and better the welfare and meat quality of cattle imported into equatorial regions.

Therefore, the study examined how distances and livestock densities during transportation impact the post-mortem ageing of various muscles, meat quality and malonaldehyde levels from heifers in a tropical environment.

Materials and methods

Animal husbandry practices and management

The animals, precisely 60 heifers of Brahman crosses, were kept at the Animal Research Center of the Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia. The research centre is at Meridian 101°43′40.7″E and Parallels 2°59′06.5″N on Jalan Maklumat. The animals were kept in enclosed pens equipped with a concrete floor and PVC roofing, each accommodating 15 animals. The pens were naturally ventilated. The authorised spatial allocation was 3.5 m^2 per animal. The animals were provided with livestock diets, pellets, straws, unlimited access to drinking water, and continuous lighting for 24 h. The mean temperature within the house was 33.0 ± 1.36 °C daytime and 23.1 ± 1.40 °C at night, accompanied by an average water vapour content of 82.6 ± 1.40%.

Livestock, hauling and treatment

There were 60 heifers, Brahman crosses and around 24 months old. They had an average live weight (LW) of 290.0 ± 36.0 kg. The heifers were divided into two groups based on the distance they were transported. One group consisted of 30 heifers hauled over 450 km a short distance, while the other consisted of 30 heifers hauled over 850 km a long distance. Each transit distance had three distinct livestock densities of equal no of animals assigned ($n = 10$): 600 kg/m^2 (high), 400 kg/m^2 (medium) and 200 kg/m^2 (low). Before the commencement of the experiment, randomisation was ensured by utilising a random number assignment. The heifers were hauled for 9 h (short-distance) or 17 h (long-distance) before being unloaded at Malaysia's Shah Alam Commercial Slaughterhouse Complex (Kompleks Abatoir Shah Alam Jabatan Perkhidmatan Veterinar Malaysia, Shah Alam, Malaysia) positioned on Meridian 101°31′26.8″E and Parallels 3°03′34.9″N, on Lots 6 and 7, Jalan Utas, 15/7, Seksyen 15, 40200,

Shah Alam, Selangor, Malaysia, slaughterhouse for neck cut. Both the exits at the farm and arrivals at the abattoir were recorded. Animals were transported via the highway using a 5-ton truck with roof coverings and a non-slippery floor. Consistency was maintained in both the driver and the route throughout the experiment. The heifers were carried exclusively at night without any breaks for rest or showers and were not provided with any food during the journey. The heifers followed the same route within Selangor, Malaysia, until they reached the desired distance. The travel duration was utilised to compute the differences between arrival and departure times. The farm and other areas in the state have typical daily temperatures ranging from 32 to 35 °C during the day to 22–24 °C at night during transportation. Based on the Malaysian Meteorological Department data (Malaysian Meteorological Department 2024), the experiment reported a vapour content of 84.1%.

The slaughtering technique

Upon the arrival of the heifers at the abattoir, they were immediately unloaded at the lairage area. They remained there for 12 h before their slaughter, during which they had a free supply of water. Slaughtering was conducted at the Large-ruminants Animal unit of the Shah Alam Abattoir Complex; following the lairage, they were moved via the raceway to the slaughter hall, where they were carefully restrained in a modified Mark 4 box equipped with a chin lift. In adherence to the Malaysian standards MS1500:2009 (Department of Standards Malaysia 2009), they were slaughtered following the halal slaughter guidelines. The operation entailed the severed sections of the jugular veins, trachea, oesophagus and two carotid arteries.

The sampling and storage of muscles

Within 15 min of post-slaughter, sampling began. Samples were taken from all 60 animals. Dressed carcases were immediately placed in the 4 °C cold room for sampling and ageing. The samples were divided into two portions. The first portion of *infraspinatus* (IF) and *semitendinosus* (ST) muscle was removed and snap frozen in liquid nitrogen (Malaysian Oxygen Sdn. Bhd., Melaka, Malaysia) for glycogen, pH (pre-rigour) and lipid peroxidation and kept at −80 °C until further analysis. The samples were frozen in liquid nitrogen to prevent muscle deterioration, especially pre-rigour. The carcases were stored at 4 °C until they were sampled at three intervals after post-mortem days 1, 7 and 14. The second portion was stored in a stomacher bag at 4 °C to assess colour, WHC, cooking loss and Warner-Bratzler shear force (WBSF) after 1, 7 and 14 days post-mortem. The sample was collected in a plastic pill box, immersed in liquid nitrogen, and stored at −80 °C until glycogen, malondialdehyde (MDA) and pH analysis.

The evaluation of meat quality assessment

Measurement of colour attributes

A Colour Flex Spectrophotometer, manufactured by Hunter Lab (Reston, VA), was utilised to measure the colour of meat. The device is based on the International Commission on Illumination (CIE) Lab colour space, which uses L^*, a^* and b^* values. The measurements were taken using the 10° standard observer and a D56 illuminant. The spectrophotometer provided tristimulus values (X, Y, Z) and reflectance data at specific wavelengths ranging from 400 to 700 nm. The colourimeter underwent calibration using black and white tiles. Before commencing, samples from frozen IF m. and *semitendinosus* (ST) m. collected on days 1, 7 and 14 were moved from a freezer at −80 °C to a chiller at 4 °C overnight. The samples, which had been thawed and were approximately 12 mm thick, were allowed to bloom for 30 min. Subsequently, they were positioned with the bloomed surface facing downwards towards the base of the colour flex cup. Each sample was measured three times for L^* (lightness), a^* (redness) and b^* (yellowness) values, and the average was calculated.

Muscle pH

Segments of the IF and *semitendinosus* (ST) muscles, upon collection, were submerged in liquid nitrogen (Malaysian Oxygen Sdn. Bhd., Melaka, Malaysia). Subsequently, the collected samples were frozen at −80 °C and stored until further analysis. Muscle samples from the *infraspinatus* (IF) m. and *semitendinosus* (ST) m. were removed from storage at −80 °C. The samples were manually crushed in liquid nitrogen using a crushing utensil. Around 0.5 g of finely ground muscle tissue was gathered and blended for 30 s in 10 mL of cold deionised water (Wiggen Hauser D-500, Berlin, Germany). Sodium iodoacetate, a chemical, was employed to impede the progression of glycolysis by mainly targeting the enzyme glyceraldehyde 3-phosphate dehydrogenase and the synthesis of lactic acid. A pre-calibrated portable pH metre was used to measure the indirect pH of the resultant homogenates.

Cooking loss

In order to ascertain the cooking loss, the *infraspinatus* (IF) m. and *semitendinosus* (ST) m. were weighed (*W*1) and then placed in polyethene bags using a vacuum packaging method. The samples were heated in a water bath to a temperature of 80 °C. After the samples attained an internal temperature of 78 °C, as determined using a stabbing temperature probe (HI 145-00 thermometer, HANNA Instruments, Woonsocket, RI), the cooking process was extended for another 10 min. Once the cooked samples were taken out of the water bath and left to cool down to the room's temperature, they were carefully dried by blotting and then weighed again (*W*2). The subsequent equation was employed to compute the proportion of cooking loss:

$$\text{Cooking loss } (\%) = \left[\frac{W1 - W2}{W1}\right] \times 100$$

where *W*1 is the initial weight of the sample before cooking; *W*2 is the weight after cooking.

The determination of Warner-Bratzler shear force values

The shear force of the IF m. and *supraspinatus* (ST) m. was measured with a texture analyser (TA.HD plus, Stable Micro System, Godalming, UK) equipped with a Volodkevich biting jaw. The device was standardised with a weight of 5000 x g, with the blade speed and distance for height adjusted to 10 mm/s (Hayat et al. 2021). The sample was divided into three equal blocks, each measuring 1 cm in height, 1 cm in width and 2 cm in length, aligned with the muscle fibres. The Volodkevich biting jaw is used to shear each block perpendicular to the longitudinal direction of the fibres. Shear force measurements represent all samples 'blocks' average peak positive force. The tenderness of meat is inversely correlated with the levels of shear stress.

The determination of muscle glycogen content

Segments of the IF and *semitendinosus* (ST) muscles, upon collection, were submerged in liquid nitrogen (Malaysian Oxygen Sdn. Bhd., Melaka, Malaysia). Subsequently, the collected samples were frozen at −80 °C and stored until further analysis. Muscles from the IF m. and *supraspinatus* (ST) m. were manually crushed in liquid nitrogen until finely ground. Glycogen content was assessed by adhering to the manual provided by the manufacturer of the assay EnzyChrom™ Glycogen Assay Kit (Cat# E2GN-100; BioAssays, Hayward, CA) colourimetric instructions. A graph was plotted using 570 nm as the optical density standard against the concentration. The concentration of glycogen in the samples was quantified by employing the standard curve and applying the formula:

$$\text{Glycogen concentration} = \frac{[(R_{\text{sample}} - R_{\text{blank}})]}{\text{Slope } (\int \mu g/mL)}$$

where R_{sample} and R_{blank} are the OD 570 nm values of the sample and blank (standard 5).

The determination of malondialdehyde assay

The measurement of meat lipid oxidation was conducted using the marker MDA. After sample collection, they were immersed in liquid nitrogen to snap freeze and maintained at −80 °C until analysis. The IF m. and *supraspinatus* (ST) muscle samples were retrieved from storage at −80 °C and crushed manually in liquid nitrogen using a crusher and pestle. After adding the reagents, approximately 1 g of pulverised meat (Wiggen Hauser® D-500, Berlin, Germany) was thoroughly mixed. Following the addition of 200 µL of the generated sample, 350 µL of BHT, 165 µL of thiobarbituric acid and 2 mL of deionised water were subsequently added. The samples were agitated using a vortex mixer for 60 s, followed by incubation in a water bath at 95 °C for one hour. The samples were cooled to ambient temperature using a continuous flow of water. Following the cooling process, 3 mL of n-butanol was introduced and vigorously mixed for 60 s using a vortex mixer. The samples were centrifugated for 10 min at a rotational speed of 5000 × *g*. The butanol layer was isolated, and the absorbance at a wavelength of 532 nm was measured compared to pure butanol.

Statistical and data analysis

The experiment followed a 2 × 3 × 2 (distance × stocking × muscle anatomical location) model. Data were analysed by three-way analysis of variance (ANOVA) considering the main effects of two distances, three different stocking densities, and two muscles for heifers and their interactions. The Statistical Analysis System version 9.4 (SAS Institute Inc., Cary, NC) was used for the statistical analysis, utilising the general linear model (GLM) approach. When statistically significant findings were observed, the means were compared using Duncan's multiple range test. A significance level of $p < .05$ was used to determine statistical significance.

Results

Colour attributes

The current study explored the impacts of varying road transport distances and stocking densities on meat quality attributes and MDA levels of IF and semitendinosus muscles of Brahman cross heifers in a hot humid and tropical climate and explains how the welfare of animals during transportation and meat quality are linked. The impact of different distances and stocking densities on the colour attributes in IF m. and *semitendinosus* (ST) muscle throughout distinct post-mortem ageing periods is shown in Table 1. Meat colour is one of the essential organoleptic attributes of meat. Meat colour defines the choice and acceptability in the eyes of the consumer. A two-way significant interactions were observed between distances and livestock density for lightness (L^*) at days 1, 7 and 14 ($p < .05$) post-mortem periods of ageing. Distances affected lightness in heifers on days 1, 7 and 14 post-mortems in both livestock densities ($p < .05$). Additionally, there was a significant interaction three-way that was observed between distances, density and muscle types following ageing in animals subjected to a distance of 450 km, a density of 400 for IF muscle, a distance of 850 km, and a density of 600 for IF and ST in lightness.

Heifers hauled for 450 km recorded no significant differences in redness (a^*) on days 1, 7 and 14 post-mortems. On the other hand, no variations were noticed between days 7 and 14 for those hauled over 850 km, except for day 1. Significant interactions were observed for redness (a^*) in heifers kept at a density of 400, having significantly ($p < .05$) higher a^* values than their counterparts put at low and high livestock densities. Also, significant interactions were observed between distances, density and muscle types following ageing in animals subjected to a distance of 450 km, a density of 400 for IF muscle, a distance of 850 km, and a density of 600 for IF and ST in redness. The lower livestock density group had significantly lower ($p < .05$) values than the two other groups in all distances (450 km and 850 km). The values of a^* were significantly higher ($p < .05$) during lengthy journeys than the short a^* values on days 1, 7 and 14. The low stocking group consistently had significantly ($p < .05$) higher a^* values than the medium or high stocking density groups. Neither livestock density nor distances affected redness on day 14 of post-mortem ageing ($p > .05$).

There were significant interactions ($p < .05$) observed between distances, density and muscle types following ageing in animals subjected to a distance of 450 km, a density of 400 for IF muscle, a distance of 850 km, and a density of 600 for IF and ST for yellowness (b^*). Livestock density affected b^* values in both travel distances across post-mortem ageing. At one-day post-mortem, hauling heifers for the long-distance, high and medium stocking density groups had higher b^* values than those hauled for the lower stocking density. The group with the highest livestock density exhibited a greater b^* value over short distances on days 7 and 14 post-mortem, in contrast to those with medium and low livestock density. Similarly, the high livestock density group had higher b^* values than the medium and low livestock groups in both lengths of journeys. All groups' yellowness values were unaffected by the duration of ageing for short- and long-distance movements.

Muscle glycogen

Table 2 demonstrates the impacts of the journey length and livestock densities on the glycogen levels in the IF m. and *supraspinatus* (ST) muscles at different post-mortem ages. Statistically significant interactions ($p < .05$) between distances and livestock densities were observed. Distances, densities and muscle types significantly interacted on different ageing days. The impacts of the length of journey travel and livestock densities on muscle glycogen concentration were substantially higher, and the significance level differed ($p < .05$). Glycogen levels were considerably greater ($p < .05$) on days 0, 1, 7 and 14 after post-mortem ageing in animals that were transported over a longer distance than those transported over a shorter distance. The glycogen concentrations of the low livestock density group were considerably higher than those of the medium and high livestock density groups, regardless of distance ($p < .05$).

Muscle pH

The impacts of different distances and livestock densities on pH concentrations of the IF and *semitendinosus* (ST) m. at various post-mortem ages are shown in Table 3. The pH of meat is vital as it affects other physicochemical parameters, including colour, tenderness, flavour and shelf-life. The current study's outcome revealed the main effects, and the significant two-way interactions ($p < .05$) between transport lengths and livestock densities were observed irrespective of muscle type. Also observed were significant three-way interactions between distances, density and muscle types following ageing in animals

Table 1. Impacts of distances and livestock densities on colour attributes of *infraspinatus* (IF) and *semitendinosus* (ST) muscles over post-mortem ageing periods in heifers subjected to road transport in a tropical climate.

	Lightness (L^*)				Redness (a^*)				Yellowness (b^*)			
Treatment	Day 1	Day 7	Day 14	*p* Value	Day 1	Day 7	Day 14	*p* Value	Day 1	Day 7	Day 14	*p* Values
Transport distance												
450 km	33.82 ± 0.17 bz	35.77 ± 0.15 by	37.45 ± 0.10 bx	<.0001	14.29 ± 0.08 bx	14.20 ± 0.09 bx	14.25 ± 0.08 bx	.6921	14.51 ± 0.11 bx	14.31 ± 0.12 bx	14.34 ± 0.12 bx	.3523
850 km	35.88 ± 0.22 az	38.50 ± 0.22 ay	41.04 ± 0.18 ax	<.0001	17.96 ± 0.12 ay	18.53 ± 0.15 ax	18.88 ± 0.18 ax	.0511	16.70 ± 0.17 ay	16.84 ± 0.24 ay	17.78 ± 0.30 ax	.1274
p Value	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	
Stocking density												
200 kg/m^2	33.73 ± 0.29 cz	35.64 ± 0.29 cy	37.92 ± 0.25 cx	<.0001	15.35 ± 0.27 cx	15.56 ± 0.36 cx	15.57 ± 0.37 cx	.7212	15.00 ± 0.18 cx	14.53 ± 0.17 cx	15.22 ± 0.31 cx	.0598
400 kg/m^2	34.94 ± 0.23 bz	37.59 ± 0.29 by	39.67 ± 0.35 bx	<.0001	16.32 ± 0.32 bx	16.38 ± 0.34 bx	16.67 ± 0.41 bx	.1195	15.63 ± 0.28 bx	15.62 ± 0.29 bx	16.22 ± 0.44 bx	.0527
600 kg/m^2	35.87 ± 0.24 az	38.18 ± 0.25 ay	40.15 ± 0.30 ax	<.0001	16.72 ± 0.32 ax	17.15 ± 0.38 ax	17.25 ± 0.40 cx	.0798	16.19 ± 0.24 ax	16.62 ± 0.36 ax	16.73 ± 0.38 ax	.5813
p Value	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	
Muscle type												
IF	35.81 ± 0.24 az	37.73 ± 0.29 ay	39.55 ± 0.29 ax	<.0001	16.29 ± 0.26 az	16.73 ± 0.34 ay	17.08 ± 0.39 ax	.9255	16.04 ± 0.24 az	16.04 ± 0.33 ay	16.94 ± 0.41 ax	.4328
ST	33.89 ± 0.14 bz	36.54 ± 0.19 by	38.94 ± 0.25 bx	<.0001	15.96 ± 0.25 bz	15.99 ± 0.25 by	16.04 ± 0.24 bx	.8923	15.17 ± 0.13 bz	15.151 ± 0.13 by	15.185 ± 0.12 bx	.3715
p Value	<.0001	<.0001	<.0001		.0004	<.0001	<.0001		<.0001	<.0001	<.0001	
DIS × DES (two-way interaction)												
DIS450 × DES200	32.17 ± 0.16 ez	34.43 ± 0.07 ey	36.53 ± 0.04 ex	<.0001	13.75 ± 0.11 fx	13.43 ± 0.06 fx	13.58 ± 0.09 fx	.9717	14.13 ± 0.10 dx	13.59 ± 0.09 ex	13.69 ± 0.10 ex	.0584
DIS450 × DES400	34.22 ± 0.12 dz	35.98 ± 0.15 dy	37.54 ± 0.08 dx	<.0001	14.36 ± 0.08 ex	14.32 ± 0.07 ex	14.32 ± 0.07 ex	.6201	14.18 ± 0.15 dx	14.10 ± 0.11 dx	14.10 ± 0.11 dx	.4115
DIS450 × DES600	35.06 ± 0.12 cz	36.90 ± 0.18 cy	38.30 ± 0.11 cx	<.0001	14.77 ± 0.13 dx	14.83 ± 0.11 dx	14.83 ± 0.17 dx	.1908	15.22 ± 0.22 cx	15.25 ± 0.23 cx	15.25 ± 0.23 cx	.5269
DIS850 × DES200	35.30 ± 0.27 cz	36.85 ± 0.35 cy	39.32 ± 0.23 bx	<.0001	16.95 ± 0.14 cy	17.69 ± 0.23 cx	17.94 ± 0.28 cx	.8144	15.87 ± 0.22 by	15.47 ± 0.15 cy	16.76 ± 0.37 bx	.0415
DIS850 × DES400	35.66 ± 0.39 az	39.20 ± 0.22 by	41.80±.31 ax	<.0001	18.27 ± 0.17 by	18.43 ± 0.20 by	19.03 ± 0.32 bx	.3465	17.08 ± 0.28 ay	17.15 ± 0.30 by	18.35 ± 0.57 ax	.0963
DIS850 × DES600	36.69 ± 0.40 az	39.45 ± 0.25 ay	42.00 ± 0.13 ax	<.0001	18.66 ± 0.11 ay	19.66 ± 0.22 ax	19.66 ± 0.53 ax	.08922	17.15 ± 0.31 az	19.46 ± 0.20 ax	18.22 ± 0.52 ay	.0068
p Value	<.0001	<.0001	<.0001		.0014	.0076	.0081		<.0001	<.0001	<.0001	
DIS × DES × MUTYP (three-way interaction)												
DIS450 × DES400 × IF	34.38 ± 0.16 fg	35.34 ± 0.07 f	37.45 ± 0.09 e	<.0001	14.50 ± 0.09 e	14.52 ± 0.09 g	14.51 ± 0.08 g	.1527	14.10 ± 0.17 ef	14.13 ± 0.11 fg	14.13 ± 0.17 f	.6273
DIS450 × DES400 × ST	34.06 ± 0.18 g	36.62 ± 0.09 e	37.62 ± 0.15 e	<.0001	14.22 ± 01.3 e	14.12 ± 0.08 h	14.13 ± 0.08 h	.8322	14.25 ± 0.26 ef	14.07 ± 0.15 fg	14.07 ± 0.38 f	.5397
DIS850 × DES600 × IF	38.44 ± 0.09 a	40.52 ± 0.12 a	42.41 ± 0.15 a	<.0001	18.58 ± 0.08 a	20.10 ± 0.19 a	20.51 ± 0.08 a	.5321	18.42 ± 0.15 a	20.25 ± 0.20 a	20.68 ± 0.09 a	<.0001
DIS850 × DES600 × ST	34.94 ± 0.13 e	38.38 ± 0.09 c	41.58 ± 0.08 b	<.0001	18.75 ± 0.21 a	18.82 ± 0.19 c	18.82 ± 0.19 c	.7556	15.88 ± 0.18 c	15.76 ± 0.54 d	15.76 ± 0.17 d	.4740
p Value	<.0001	<.0001	.0037		.0276	.0156	.7606		<.0001	<.0001	<.0001	

DIS: distance, DES: density, MUTYP: muscle type, IF: *infraspinatus*, ST: *semitendinosus*

At a significance level of $p < .05$, means in the same row with distinct letters (a–e) differ significantly. At a significance level of $p < .05$, means in the same row with distinct letters (w–z) differ significantly.

Table 2. The impacts of distances and livestock densities on glycogen content of *infraspinatus* (IF) and *semitendinosus* (ST) muscles over post-mortem ageing periods in heifers subjected to road transport in a tropical climate.

	Glycogen				
Treatments	Day 0	Day 1	Day 7	Day 14	*p* Value
Transport distance					
450 km	1.67 ± 0.01bw	1.00 ± 0.01bx	0.80 ± 0.01by	0.52 ± 0.00bz	<.0001
850 km	1.86 ± 0.01aw	1.09 ± 0.01ax	0.90 ± 0.01ay	0.47 ± 0.00az	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
Stocking density					
200 kg/m^2	1.80 ± 0.02aw	1.07 ± 0.01ax	0.88 ± 0.01ay	0.52 ± 0.01az	<.0001
400 kg/m^2	1.75 ± 0.02bw	1.04 ± 0.01bx	0.85 ± 0.01by	0.48 ± 0.00bz	<.0001
600 kg/m^2	1.74 ± 0.01bw	1.02 ± 0.01cx	0.82 ± 0.01cy	0.47 ± 0.00cz	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
Muscle type					
IF	1.74 ± 0.01bz	1.03 ± 0.01by	0.84 ± 0.01bx	0.48 ± 0.00bw	<.0001
ST	1.80 ± 0.01az	1.05 ± 0.01ay	0.86 ± 0.01ax	0.50 ± 0.01aw	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
DIS × DES (two-way interaction)					
DIS450 × DES200	1.90 ± 0.01aw	1.11 ± 0.00ax	0.93 ± 0.00ay	0.59 ± 0.00az	<.0001
DIS450 × DES400	1.85 ± 0.01bw	1.09 ± 0.01bx	0.91 ± 0.00by	0.56 ± 0.00bz	<.0001
DIS450 × DES600	1.85 ± 0.01bw	1.07 ± 0.01cx	0.87 ± 0.01cy	0.50 ± 0.00cz	<.0001
DIS850 × DES200	1.71 ± 0.01cw	1.03 ± 0.01dx	0.81 ± 0.01dy	0.50 ± 0.00cz	<.0001
DIS850 × DES400	1.66 ± 0.01dw	1.00 ± 0.01ex	0.80 ± 0.00ey	0.45 ± 0.01dz	<.0001
DIS850 × DES600	1.64 ± 0.01ew	0.98 ± 0.01fx	0.78 ± 0.01fy	0.45 ± 0.00dz	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
DIS × DES × MUTYP (three-way interaction)					
DIS850 × DES400 × ST	1.68 ± 0.01g	1.01 ± 0.01h	0.80 ± 0.01g	0.45 ± 0.00g	<.0001
DIS850 × DES600 × IF	1.62 ± 0.01i	0.96 ± 0.01j	0.78 ± 0.01h	0.44 ± 0.00h	<.0001
DIS850 × DES600 × ST	1.66 ± 0.01h	0.98 ± 0.01i	0.78 ± 0.00h	0.45 ± 0.00g	<.0001
p Value	.0038	.0038	.01359	.0042	

DIS: distance, DES: density, MUTYP: muscle type, IF: *infraspinatus*, ST: *semitendinosus*
At a significance level of $p < .05$, means in the same row with distinct letters (a–e) differ significantly. At a significance level of $p < .05$, means in the same row with distinct letters (w–z) differ significantly.

Table 3. The impacts of distances and livestock densities on pH values of *infraspinatus* (IF) and *semitendinosus* (ST) muscles over post-mortem ageing periods in heifers subjected to road transport in a tropical climate.

	pH				
Treatments	Day 0	Day 1	Day 7	Day 14	*p* Value
Transport distance					
450 km	5.98 ± 0.01bx	5.45 ± 0.01bz	5.65 ± 0.01by	6.44 ± 0.01bw	.0011
850 km	6.50 ± 0.01ax	5.91 ± 0.01ay	6.45 ± 0.01ax	6.70 ± 0.01aw	.0044
p Value	<.0001	<.0001	<.0001	<.0001	
Stocking density					
200 kg/m^2	6.18 ± 0.04cx	5.46 ± 0.01cz	6.04 ± 0.01ay	6.53 ± 0.02cw	.0136
400 kg/m^2	6.23 ± 0.03bx	5.53 ± 0.01by	6.05 ± 0.06ax	6.57 ± 0.02bw	.0022
600 kg/m^2	6.30 ± 0.04ax	5.59 ± 0.01az	6.06 ± 0.06ay	6.63 ± 0.03ax	.0048
p Value	<.0001	<.0001	.2098	<.0001	
Muscle type					
IF	6.23 ± 0.04bx	5.51 ± 0.02bz	6.02 ± 0.06by	6.53 ± 0.01bw	<.0001
ST	6.24 ± 0.03ax	5.53 ± 0.01az	6.08 ± 0.04ay	6.59 ± 0.02aw	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
DIS × DES (two-way interaction)					
DIS450 × DES200	5.91 ± 0.01fx	5.42 ± 0.01 dz	5.60 ± 0.01dy	6.41 ± 0.01fw	.0006
DIS450 × DES400	5.99 ± 0.01ex	5.44 ± 0.01 dz	5.88 ± 0.01cy	6.44 ± 0.01ew	.0018
DIS450 × DES600	6.02 ± 0.01dx	5.50 ± 0.01cz	5.88 ± 0.03cy	6.47 ± 0.01dw	<.0001
DIS850 × DES200	6.45 ± 0.01cx	5.49 ± 0.01cy	6.46 ± 0.02bx	6.64 ± 0.01cw	<.0001
DIS850 × DES400	6.48 ± 0.01bx	5.61 ± 0.01by	6.44 ± 0.01by	6.69 ± 0.01bw	.0079
DIS850 × DES600	6.57 ± 0.01ax	5.68 ± 0.01az	6.49 ± 0.01ay	6.79 ± 0.01aw	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
DIS × DES × MUTYP (three-way interaction)					
DIS450 × DES600 × IF	6.01 ± 0.01g	5.46 ± 0.01efg	5.51 ± 0.01h	6.46 ± 0.01g	<.0001
DIS450 × DES600 × ST	6.03 ± 0.01f	5.53 ± 0.02d	5.86 ± 0.01e	6.48 ± 0.01f	<.0001
DIS850 × DES200 × IF	6.47 ± 0.01c	5.47 ± 0.01ef	6.44 ± 0.01cb	6.64 ± 0.01e	<.0001
DIS850 × DES200 × ST	6.42 ± 0.01e	5.50 ± 0.01e	6.40 ± 0.01d	6.63 ± 0.01e	<.0001
DIS850 × DES400 × IF	6.50 ± 0.01b	5.67 ± 0.01b	6.46 ± 0.01b	6.66 ± 0.01d	<.0001
DIS850 × DES400 × ST	6.44 ± 0.02d	5.56 ± 0.01 cd	6.41 ± 0.03 cd	6.72 ± 0.04c	<.0001
DIS850 × DES600 × IF	6.56 ± 0.06a	5.76 ± 0.01a	6.41 ± 0.02 cd	6.77 ± 0.04b	<.0001
DIS850 × DES600 × ST	6.58 ± 0.03a	5.58 ± 0.01c	6.57 ± 0.02a	6.80 ± 0.02a	<.0001
p Value	<.0001	<.0001	<.0001	.3863	

DIS: distance, DES: density, MUTYP: muscle type, IF: *infraspinatus*, ST: *semitendinosus*
At a significance level of $p < .05$, means in the same row with distinct letters (a–e) differ significantly. At a significance level of $p < .05$, means in the same row with distinct letters (w–z) differ significantly.

subjected to a distance of 450 km, a density of 600 for both muscles, a distance of 850 km, and a density of 600 for IF and ST in redness. Both transport lengths and stocking densities were substantially increased ($p < .05$). Following the post-mortem at 0 days, 1, 7 and 14 ageing days, heifers exposed to a prolonged distance had their pH values differing significantly ($p < .05$) from those with a shorter distance. Regardless of transport length and duration, those kept at a low livestock density had substantially lower pH levels ($p < .05$) than their counterparts at medium and high density across post-mortem periods. Additionally, with ageing, a high pH was discovered in both muscles when distance and livestock density increased, indicating DFD in meat.

Cooking loss

Table 4 shows the cooking loss of IF and *semitendinosus* (ST) muscles in heifers subjected to different distances and livestock densities at various post-mortem ages. two-way interactions ($p < .05$) between transport lengths of 450 km and 850 km and for livestock densities of 200, 400 and 600 were observed irrespective of muscle type within ageing periods on days 1, 7 and 14. Also observed were significant three-way interactions between distances, density and muscle types following ageing in animals subjected to a distance of 450 km, a density of 600 for both muscles, a distance of 850 km, and a density of 200, 400 and 600 for IF and ST for cooking loss. On days 1, 7 and 14 post-mortem, animals stocked at a low and medium livestock density had significantly lower cooking loss percentages in both lengths than those at a high livestock density. The ageing period showed no significant influence on cooking loss in heifers carried over a long distance ($p > .05$) compared to those transported over a short length. Additionally, there was a significant difference between meat types across various ageing periods.

Warner-Bratzler shear force values

Results of the WBSF test on the IF and *semitendinosus* (ST) samples at various post-mortem ages in heifers subjected to road travel over two different journey

Table 4. The impacts of distances and livestock densities (SD) on the cooking loss of *infraspinatus* (IF) and *semitendinosus* (ST) muscles over post-mortem ageing periods in heifers subjected to road transport in a tropical climate.

	Cooking loss, %			
Treatments	Day 1	Day 7	Day 14	*p* Value
Transport distance				
450 km	30.31 ± 0.39bx	28.25 ± 0.31by	25.23 ± 0.25bz	<.0001
850 km	31.52 ± 0.26ax	29.54 ± 0.27ay	26.70 ± 0.34az	<.0001
p Value	<.0001	<.0001	<.0001	
Stocking density				
200 kg/m^2	29.61 ± 0.36cx	27.03 ± 0.30cy	24.69 ± 0.27cz	<.0001
400 kg/m^2	31.14 ± 0.38bx	29.45 ± 0.29by	26.18 ± 0.37bz	<.0001
600 kg/m^2	32.03 ± 0.41ax	30.19 ± 0.32ay	27.01 ± 0.42az	<.0001
p Value	<.0001	<.0001	<.0001	
Muscle type				
IF	28.72 ± 0.18bx	27.15 ± 0.19by	23.99 ± 0.11bz	<.0001
ST	33.13 ± 0.18ax	30.62 ± 0.22ay	27.93 ± 0.25az	<.0001
p Value	<.0001	<.0001	<.0001	
DIS × DES (two-way interaction)				
DIS450 × DES200	28.55 ± 0.50ex	26.10 ± 0.40ey	25.10 ± 0.10ez	.0072
DIS450 × DES400	31.04 ± 0.64cx	28.77 ± 0.37cy	25.34 ± 0.42 dz	<.0001
DIS450 × DES600	31.43 ± 0.71ba	29.87 ± 0.45by	26.24 ± 0.55cz	<.0001
DIS850 × DES200	30.67 ± 0.39dx	27.97 ± 0.34dy	25.29 ± 0.51 dz	<.0001
DIS850 × DES400	31.24 ± 0.44cbx	30.13 ± 0.41by	27.03 ± 0.56bz	<.0001
DIS850 × DES600	32.64 ± 0.41ax	30.51 ± 0.45ay	27.78 ± 0.62az	<.0001
p Value	<.0001	<.0001	.0390	
DIS × DES × MUTYP (three-way interaction)				
DIS450 × DES600 × IF	28.32 ± 0.12g	27.93 ± 0.13f	23.89 ± 0.19e	<.0001
DIS450 × DES600 × ST	34.39 ± 0.11a	31.81 ± 0.13b	28.60 ± 0.17c	<.0001
DIS850 × DES200 × IF	29.08 ± 0.11f	26.48 ± 0.07h	23.10 ± 0.16h	<.0001
DIS850 × DES200 × ST	32.26 ± 0.31d	29.45 ± 0.11d	27.48 ± 0.10d	<.0001
DIS850 × DES400 × IF	29.39 ± 0.11f	28.34 ± 0.11e	24.63 ± 0.19f	<.0001
DIS850 × DES400 × ST	33.07 ± 0.28c	31.92 ± 0.06b	29.44 ± 0.12b	<.0001
DIS850 × DES600 × IF	30.88 ± 0.18e	28.53 ± 0.15e	25.10 ± 0.11e	<.0001
DIS850 × DES600 × ST	34.52 ± 0.11a	32.47 ± 0.10a	30.47 ± 0.10a	<.0001
p Value	.0038	.01359	.0042	<.0001

DIS: distance, DES: density, MUTYP: muscle type, IF: *infraspinatus*, ST: *semitendinosus*
At a significance level of $p < .05$, means in the same row with distinct letters (a–e) differ significantly. At a significance level of $p < .05$, means in the same row with distinct letters (w–z) differ significantly.

lengths (a prolonged distance of 850 and a short distance of 450 km) and livestock densities are depicted in Table 5. After post-mortem ageing at 0 days, 1, 7 and 14 days, a two-way significant interaction ($p < .05$) was observed between transport lengths of 450 km and 850 km and at a livestock density of 200, 400 and 600, irrespective of muscle type. Distance (journey length) affected WBSF values when animals were subjected to a lengthy travel distance at various post-mortem ageing periods of 1, 7 and 14 days. On the other hand, prolonged travel length resulted in lower WBSF values in animals exposed to a livestock density of 200 kg/m^2 and lower WBSF values in animals subjected to a livestock density of 400 kg/m^2 than those subjected to a higher livestock density. Nonetheless, lengthy-distance transport increased the WBSF values in heifers subjected to a high livestock density. On day 7 of post-mortem ageing, the long-distance movement was significantly higher ($p < .05$) than short-distance. Across all lengths, heifers stocked at a low livestock density had significantly lower WBSF values than those at a high livestock density.

Meat lipid oxidation

On days 0 and 7 of post-mortem ageing periods, there were two- and three-way significant interactions ($p < .05$) between various lengths of distances, livestock densities and muscle types on meat lipid oxidation in heifers, as shown in Table 6. Livestock density affected lipid oxidation in animals hauled for shorter durations than the lengthier one on days 0, 1, 7 and 14 of post-mortem ageing periods. However, lipid oxidation was more significant in animals stocked at medium and high-density groups than in the low-livestock density groups across all distances.

Discussion

Transportation of livestock has been associated with several factors that may influence the welfare and quality of meat, including loading, offloading, novelty, feed and water deprivation, novelty and thermal extremes. The current findings examine the impacts of two road-transport distances and three different livestock densities on meat's physicochemical parameters and lipid oxidation (oxidative stability) in Brahman cross heifers in hot and humid tropical conditions. Meat colour attributes, pH, cooking loss, WBSF, glycogen and MDA levels were significantly affected by the distances and livestock densities throughout various post-mortem ageing periods. Furthermore, the outcomes were notably influenced by both the distances and livestock densities.

Colour is a fundamental perceivable quality attribute that affects meat acceptance and influences aesthetic attractiveness and purchasing decisions. A pH alteration affects biochemical changes, muscle structure, oxygen diffusion, the redox state of myoglobin, and meat colour (Ramanathan et al. 2020). In this

Table 5. The impacts of distances and livestock densities on the shear force of *infraspinatus* (IF) and *semitendinosus* (ST) muscles over post-mortem ageing periods in heifers subjected to road transport in a tropical climate.

	Shear force, g			
Treatments	Day 1	Day 7	Day 14	*p* Value
Transport distance				
450 km	1820.89 ± 39.9ax	1710.30 ± 42.1by	1599.47 ± 40.3cz	<.0001
850 km	1835.98 ± 37.2ax	1729.54 ± 39.1by	1588.19 ± 39.0cz	<.0001
p Value	.0955	.0483	.5201	
Stocking density				
200 kg/m^2	1712.32 ± 36.7cx	1634.43 ± 38.7cy	1528.40 ± 38.2cz	<.0001
400 kg/m^2	1837.01 ± 38.6bx	1734.14 ± 40.6by	1587.35 ± 39.2bz	<.0001
600 kg/m^2	1935.96 ± 39.4ax	1791.21 ± 42.1ay	1665.74 ± 42.2az	<.0001
p Value	<.0001	<.0001	<.0001	
Muscle type				
IF	1138.52 ± 6.6bx	1033.30 ± 6.5by	970.97 ± 4.9bz	<.0001
ST	2518.34 ± 6.2ax	2406.55 ± 5.1ay	2216.68 ± 7.1az	<.0001
p Value	<.0001	<.0001	<.0001	
DIS × DES (two-way interaction)				
DIS450 × DES200	1720.17 ± 38.2dx	1610.72 ± 41.5dy	1521.45 ± 38.7cz	<.0001
DIS450 × DES400	1843.19 ± 41.4cx	1734.71 ± 42.1by	1609.25 ± 40.6abz	<.0001
DIS450 × DES600	1899.30 ± 41.1bx	1785.48 ± 43.9ay	1667.70 ± 42.6az	<.0001
DIS850 × DES200	1704.48 ± 36.1dx	1658.13 ± 36.9cy	1535.35 ± 38.7cz	<.0001
DIS850 × DES400	1830.83 ± 36.6cx	1733.56 ± 40.2by	1565.45 ± 38.6bcz	<.0001
DIS850 × DES600	1972.62 ± 38.7ax	1796.95 ± 41.3ay	1663.78 ± 43.0az	<.0001
p Value	<.0001	.0173	.0385	

DIS: distance, DES: density, MUTYP: muscle type, IF: *infraspinatus*, ST: *semitendinosus*
At a significance level of $p < .05$, means in the same row with distinct letters (a–e) differ significantly. At a significance level of $p < .05$, means in the same row with distinct letters (w–z) differ significantly.

Table 6. The impact of distances and livestock densities on malondialdehyde (MDA) content (mg/kg) of *infraspinatus* (IF) and *semitendinosus* (ST) muscles over post-mortem ageing periods in heifers subjected to road transport in a tropical climate.

	MDA, g/kg meat				
Treatments	Day 0	Day 1	Day 7	Day 14	*p* Value
Transport distance					
450 km	0.44 ± 0.00bz	0.59 ± 0.00by	1.19 ± 0.01bx	1.36 ± 0.02bw	<.0001
850 km	0.52 ± 0.00az	0.63 ± 0.00ay	1.30 ± 0.01ax	1.55 ± 0.02aw	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
Stocking density					
200 kg/m^2	0.44 ± 0.00az	0.58 ± 0.00cy	1.19 ± 0.01cx	1.72 ± 38.2cw	<.0001
400 kg/m^2	0.49 ± 0.00bz	0.61 ± 0.00by	1.26 ± 0.01bx	1.51 ± 0.02bw	<.0001
600 kg/m^2	0.51 ± 0.00az	0.63 ± 0.00ay	1.28 ± 0.01ax	1.57 ± 0.02aw	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
Muscle type					
IF	0.49 ± 0.00az	0.61 ± 0.00ay	1.24 ± 0.01ax	1.49 ± 0.02aw	<.0001
ST	0.47 ± 0.00bz	0.61 ± 0.00ay	0.124 ± 0.01ax	1.41 ± 0.02by	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
DIS × DES (two-way interaction)					
DIS450 × DES200	0.41 ± 0.00ez	0.56 ± 0.00ey	1.13 ± 0.01ex	1.18 ± 0.01fw	<.0001
DIS450 × DES400	0.45 ± 0.00ez	0.59 ± 0.00dy	1.21 ± 0.01ex	1.41 ± 0.0dw	<.0001
DIS450 × DES600	0.46 ± 0.00 dz	0.61 ± 0.00cy	1.23 ± 0.01dx	1.47 ± 0.02cw	<.0001
DIS850 × DES200	0.48 ± 0.00cdz	0.60 ± 0.00cdy	1.24 ± 0.01cx	1.36 ± 0.01ew	<.0001
DIS850 × DES400	0.53 ± 0.00bz	0.64 ± 0.00by	1.31 ± 0.01bx	1.61 ± 0.02bw	<.0001
DIS850 × DES600	0.55 ± 0.00az	0.65 ± 0.00ay	1.33 ± 0.01ax	1.66 ± 0.02aw	<.0001
p Value	<.0001	<.0001	<.0001	<.0001	
DIS × DES × MUYP (three-way interaction)					
DIS850 × DES400 × ST	0.55 ± 0.00az	0.64 ± 0.00by	1.31 ± 0.01cbx	1.65 ± 0.03bw	<.0001
DIS850 × DES600 × IF	0.55 ± 0.00az	0.65 ± 0.00ay	1.32 ± 0.02bx	1.65 ± 0.03bw	<.0001
DIS850 × DES600 × ST	0.55 ± 0.00az	0.65 ± 0.00ay	1.34 ± 0.01ax	1.68 ± 0.03aw	<.0001
p Value	.0038	.0038	.01359	.0042	

DIS: distance, DES: density, MUTYP: muscle type, IF: *infraspinatus*, ST: *semitendinosus*
At a significance level of $p < .05$, means in the same row with distinct letters (a–e) differ significantly. At a significance level of $p < .05$, means in the same row with distinct letters (w–z) differ significantly.

study, there were notable interactions between various lengths of distances and livestock densities on different colour attributes of muscles. The colour values were adversely impacted, resulting in a darker colour, as livestock density increased during short- and long-distance transit. The variations in colour attributes of muscle seen in this study can be attributable to the elevated pH values, which are linked to lengthy transit distances and higher livestock densities.

It is worth noting that meat pH increases as livestock density increases. Similar findings of lowered lightness value in muscles in pigs transported with a higher stocking density of 251 kg/m^2 than pig muscle transported with 236 kg/m^2 and 251 kg/m^2 (Pereira et al. 2015). Similarly, Kim et al. (2004) found that pigs at a high density of 0.31 m^2/100 kg body weight decreased the L^* value when the animals moved for longer durations than shorter ones (1 h). On the contrary, at medium (0.35 m^2/100 kg body weight) and low stocking densities (0.39 m^2/100 kg body weight), animals transported longer had higher L^* than those freighted for a shorter time (Kim et al. 2004).

Heifers moved over longer distances had lower amounts of muscle glycogen than those transported over shorter distances. Previous studies have found comparable impacts on muscle glycogen levels in cattle during extended transit periods (Burns et al. 2019). Therefore, these findings support previous studies indicating that the glycogen stores in meat are exhausted over extended periods of transit due to muscle's frequent movement and energy to maintain the animals' equilibrium, as opposed to shorter transport distances. The study found that animals subjected to higher stocking densities had lower glycogen levels than those in medium and low densities.

Ferguson and Warner (2008) observe that insufficient space for farm animals to lie down during transportation amounts to poor welfare and yield of quality meat. Meeting these demands necessitates more energy, which affects the concentration of muscle glycogen and, potentially, the eventual pH. Therefore, the decreased glycogen levels in cattle exposed to higher livestock density may be attributable to elevated energy requirements. The development of meat quality is frequently linked to alterations in the amount and rate of glycolysis, which can result in an unfavourable muscle pH (Immonen et al. 2000). As observed in this study, the pH levels of heifers moved over long distances were significantly higher than those transferred over shorter distances.

The decline in glycogen levels could be attributable to increased physical activity, which forces the animals to tap from their reserves in tissues to ensure homeostasis. Additionally, lower glycogen levels in muscle, as

reported in the current study, could be part of the multifactor reasons why pH and other parameters were affected, which is in agreement with the reports by Loudon et al. (2019), Abubakar et al. (2021) and Terlouw and Bourguet (2022).

Correspondingly, Abubakar et al. (2021) and Burns et al. (2019) reported a higher ultimate pH in cattle, and Lambertini et al. (2006) in rabbits subjected to a long duration of transportation. Therefore, the findings indicate that heifers who travelled farther experienced higher fatigue than those who travelled shorter distances. Moreover, this phenomenon can be elucidated by the heightened process of glycogenolysis, which refers to the metabolic degradation of glycogen into glucose, resulting in elevated pH levels. Moreover, the pH values of the meat were found to be greater in heifers transported at high stocking densities than those transported at medium and low densities. The findings disagree with Lambertini et al. (2006), who found no impact of increased stocking density on muscle pH in steers and rabbits.

The decline in acidity during the ageing process leads to alterations in the colour of meat, its structure, sense of taste, and shear force. A reduction in lightness values is strongly correlated with a change in pH and occurs at a pH level of around 6.0. Animals with low muscle glycogen levels are generally slaughtered and exhibit dark-coloured meat (Ponnampalam et al. 2017). Furthermore, the findings revealed a significant difference in the acidity levels (pH) of meat samples from heifers travelling longer distances compared to those transported over shorter distances before and after rigour mortis.

Our study found notable interactions concerning cooking loss between the distance and stocking density components. Cooking loss was significantly affected by short distances and high stocking density. The present results align with the findings of Lambertini et al. (2006) in rabbits, which demonstrated a decrease in cooking loss as the transit duration increased. The increased water loss in beef with a lower final pH may be attributed to the net charge phenomenon that occurs when muscle proteins reach their isoelectric point (Gajana et al. 2013). Therefore, our findings indicate that animals transported over longer distances experience higher cooking loss.

An absence of observable systemic trajectory or patterns was detected in the shear force interactions. In their study, Kadim et al. (2006) discovered that the meat of goats that had been transported was more resistant to being chewed compared to goats who had not been transferred. The pH of the muscle has a significant and detrimental effect on various qualitative parameters of meat, including colour, water retention and tenderness (Kadim et al. 2006; Teke et al. 2014).

Early post-mortem meat tenderisation is linked to the calpain proteolytic system, which is responsible for myofibrillar proteolysis (Geesink et al. 2000; Ahmed et al. 2013). The μ calpain exhibits optimal action at a pH of 6.5, and its activity is reduced at lesser pH levels. However, the inhibitory activity of calpastatin is not significantly affected by pH (Maddock et al. 2005; Huff-Lonergan and Lonergan 2023). Transportation is a stressor that decreases meat pH by speeding up lactate formation, making the meat less tender (Honkavaara et al. 2003).

Flavour deterioration (Faustman et al. 2010), rancid odour formation (Santé-Lhoutellier et al. 2008), discolouration (Zakrys-Waliwander et al. 2012) and lipid oxidation have been identified as the cause of the generation of potentially hazardous chemicals in meat. Cross-linking or polymerisation may result from the interaction of proteins with other biomolecules under oxidative conditions.

According to Zhang et al. (2013), lipids become susceptible to oxidative changes due to the rapid depletion of naturally occurring antioxidants during meat ageing and storage after slaughter. The substantial pro-oxidative impact of lipids and proteins can be ascribed to the dissociation of haem and iron from myoglobin and haem, respectively (Faustman et al. 2010). Regarding lipid oxidation in lambs transported by road for brief (30 min) or long (5 h) durations at varying stocking densities, De la Fuente et al. (2010), in their study, found no significant interactions between transport time and stocking density.

In contrast, the findings observed notable interactions between journey length and livestock densities, which were significant, with higher livestock density leading to increased lipid oxidation at longer distances. One potential reason for the discrepancy between our results and those reported by De la Fuente et al. (2010) may be attributed to variations in transportation duration and livestock density in the two experiments. The current study found that animals subjected to a higher livestock density had their lipid oxidation levels elevated, which can be attributable to the physiological response in stressful situations.

Conclusions

Finally, transportation of heifers by roads in Malaysia's hot and humid tropical climate impacted various

aspects of meat quality, including WBSF, pH levels, colour attributes, cooking loss, glycogen content and MDA levels. Observed alterations in meat can be linked to distances, livestock densities and high temperatures encountered during transportation. The colour, cooking loss and lipid oxidation in animals transported over long distances are influenced by the distance travelled and the livestock density. The present research reveals that the stress caused by transportation impacts the quality of meat and the well-being of heifers. Therefore, enhancing animal monitoring and oversight throughout transportation and when held in lairage is essential. To minimise the occurrence and regularity of dark, firm and dry meats, giving animals time to adapt following a series of events is crucial.

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Ethical approval

The findings adhered to the Animal Ethics Guidelines of the Research Policy of Universiti Putra Malaysia's Institutional Animal Care and Use Committee with Approval No. AUP-R016/2020.

Disclosure statement

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Data availability statement

The authors will make data available on request.

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