

**REPUBLIC OF THE PHILIPPINES
UNIVERSITY OF SOUTHERN MINDANAO
KABACAN, COTABATO**

GRADUATE SCHOOL



S.1.1. For a Master degree program in Teacher Education at least 5 full-time faculty members who have published works in professional journals;

**Master of Science in
Animal Science**

PARAMETER A

Academic Qualifications and
Professional Experience

SYSTEM-INPUTS AND PROCESSES

S.1.1. For a master's degree
program in the Graduate
Teacher

Education, at least 5 faculty
members have published
works in professional
refereed journals

Faculty	Title	Authors	Name of Journal and link to the website	Date Published	Indexed/Peer Reviewed
Department of Animal Science (15)					
Jurhamid C. Imlan (14)	Chelated Minerals, Vitamins, and Electrolytes Improve Early Production Performance and Profitability of Broiler Chickens	Novelito B. Ybañez & Jurhamid C. Imlan	Tropical Animal Science Journal https://doi.org/10.5398/tasj.2026.49.2.160	Feb 2026	Scopus
	Effects of preslaughter management on oxidative stress, Warner-Bratzler shear force, heat shock protein 27 expression, and apoptosis index of Longissimus thoracis et lumborum in goats	Razlina Rag hazli, Azalea-Hani Othman, Ubedullah Kaka, Ahmed A Abubakar, Jurhamid C Imlan , Hazilawati Hamzah, Awis Q Sazili, Goh Yong-Meng	CyTA Journal of Food: Vol 23 (1) https://doi.org/10.1080/19476337.2024.2435880	Feb 2025	Scopus

	Impact of stocking densities and road transport distance on meat quality and malondialdehyde levels in semitendinosus and infraspinatus muscles of Brahman crossbred heifers in a tropical climate	Ahmed A. Abubakar, Zulkkifli Idrus, Yong M. Goh, Ubedullah Kaka, Azad B. Sabow, Jurhamid C. Imlan , Azalea H. Othman, Razlina Raghazali & Awis Q. Sazili	Italian Journal of Animal Science https://doi.org/10.1080/1828051X.2024.2432544	Nov 2024	Scopus

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Chelated Minerals, Vitamins, and Electrolytes Improve Early Production Performance and Profitability of Broiler Chickens

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ABSTRACT

Feed costs account for over half of total production costs in broiler farming; thus, minimizing them without compromising performance is essential. This study aimed to evaluate the effects of dietary feed additives containing zinc and manganese chelates, vitamins C and D, and sodium bicarbonate (NaHCO₃) on growth performance, carcass traits, and footpad dermatitis, and to conduct a cost-benefit analysis. A total of 144 male day-old Cobb 500 broiler chicks were randomly assigned to a completely randomized design (CRD) with four treatments, each replicated four times with nine birds per replicate. The dietary treatments consist of a basal diet without any additive (T1) and the basal diet supplemented with the feed additive at three inclusion levels: 0.5 g/kg (T2), 1 g/kg (T3), and 2 g/kg (T4). These inclusion levels supplied with 5, 10, and 20 mg/kg of chelated Zn and Mn; 424.5, 849, and 1,698 mg/kg of sodium bicarbonate; 4, 8, and 16 mg/kg of vitamin C; and up to 325, 650, and 1,300 IU/kg of vitamin D, respectively. The results showed that supplementing the basal diet with 2 g of Zn and Mn chelates, vitamins C and D, and a NaHCO₃ mixture per kg significantly enhanced broiler performance, especially in body weight, feed intake, and weight gain during the second week. Clear linear effects appeared in the early production stages. Additionally, a cost-benefit analysis indicated that higher supplementation was economically beneficial. Furthermore, no adverse effects were observed on carcass characteristics, skin tearing, or footpad dermatitis. In conclusion, increased supplementation with these nutrients improved broiler chicken performance during the early production phase.

Keywords: broiler performance; mineral chelates; vitamin C; vitamin D; sodium bicarbonate

INTRODUCTION

Due to the growing global population, demand for meat, particularly poultry, continues to surge rapidly (Dong *et al.*, 2014). Poultry meat derived from broilers is the most widely produced globally (Mramba & Mapunda, 2024). Improved genetics that contribute to a rapid growth rate and enhanced feed efficiency in broilers are considered economically advantageous. However, feed cost remains the primary determinant of profitability in broiler production (Abdalgil, 2025). For this reason, producers seek sustainable solutions that support efficient growth, preserve meat quality and profitability, and help broilers better withstand heat stress. More recently, broiler nutritionists have focused on alternative nutritional strategies, including the use of chelated trace minerals and vitamins in broiler diets (Alagawany *et al.*, 2021; Faghih-Mohammadi *et al.*, 2022). Moreover, because broilers are raised primarily for meat production, growth performance and carcass quality remain key economic traits (Tavárez *et al.*, 2016).

Beyond nutrition, factors such as management, genetics, and post-slaughter handling also play essential roles in determining meat quality in broilers (Mir *et al.*, 2017).

Trace minerals are critical for normal physiological and metabolic function in chickens (Byrne & Murphy, 2022). Inorganic trace minerals are less digestible than their organic and chelated counterparts, despite their extensive use (Lee & Kim, 2025). Therefore, to improve the absorption of trace minerals, the chelated form is preferred (Broom *et al.*, 2021). Different trace minerals, such as manganese (Mn) and zinc (Zn), have been studied to assess their effects on broiler chickens. Manganese supports metabolism, collagen synthesis, and antioxidant defenses, helping to prevent leg deformities and improve bone health (Olgun, 2017; de Carvalho *et al.*, 2021). On the other hand, zinc (Zn) plays a crucial role in the metabolism of energy, proteins, nucleic acids, and carbohydrates (Faghih-Mohammadi *et al.*, 2022). Its positive effects on growth performance are well established, and it is also involved in the regulation of key hormones such as glucagon, insulin, and sex hormones

(Abd El-Hack *et al.*, 2018). Moreover, Zn has been shown to enhance immune function, increase daily weight gain, and improve feed utilization efficiency in broiler chickens (Hidayat *et al.*, 2021). When combined with manganese in organic chelated forms, these trace minerals further improve growth performance and carcass yield compared with their inorganic counterparts (Nguyen *et al.*, 2025). Meanwhile, Pacheco *et al.* (2021) found that during the second week of production, chelated Zn and Mn considerably increased broiler body weight and feed conversion ratio. Additionally, recent research indicates that supplementing these minerals in broiler diets improves intestinal health (Bortoluzzi *et al.*, 2020; Franklin *et al.*, 2022).

Vitamins, both fat-soluble and water-soluble vitamins, are vital to broilers' metabolic processes. As a fat-soluble vitamin, vitamin D is especially crucial for controlling the absorption of calcium and phosphorus, which supports immunological function and bone mineralization (Setiyaningsih *et al.*, 2023). On the other hand, by preventing oxidative damage and boosting immunological responses, vitamin C, a water-soluble vitamin, aids in cellular repair (Shakeri *et al.*, 2020). Additionally, Setiyaningsih *et al.* (2023) found that broiler development performance at the later production stage was strongly impacted by the combined supplementation of vitamins C and D. While vitamin C supplementation by itself does not always enhance growth performance, it is essential for controlling broiler reactions to different stressors (Yu *et al.*, 2021). Furthermore, this vitamin is essential for mitigating the adverse effects of heat stress in broilers (Attia *et al.*, 2017; Del Barrio *et al.*, 2020; Al-Khalafah *et al.*, 2025). Moreover, a diet containing electrolytes, specifically sodium bicarbonate, reduces mortality and also mitigates the adverse effects of heat stress in broilers (Livingston *et al.*, 2022).

Although chelated trace minerals, vitamins, and electrolytes have individually demonstrated positive effects on broiler performance, existing studies have evaluated mainly these nutrients in isolation or in limited combinations. Moreover, no work has simultaneously assessed growth performance, carcass traits, footpad health, and economic outcomes under tropical conditions. Having said this, the mixtures of these feed additives had not been explored; thus, the authors hypothesized that higher inclusion rates of mineral chelates, vitamins, and electrolytes would improve broiler performance and provide economic benefits without detrimental effects on carcass yield or foot pad health. Hence, the objectives of this study were to investigate the growth performance, carcass traits, incidence of footpad dermatitis, and economic efficiency in broilers fed diets enriched with chelated Zn and Mn, vitamins C and D, and sodium bicarbonate.

MATERIALS AND METHODS

Ethical Approval

The study was conducted at the Poultry Project, University of Southern Mindanao, Kabacan, Cotabato,

Philippines. All handling protocols and ethical procedures for broiler chickens in this study were thoroughly reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern Mindanao under Protocol No. 2024-0055.

Preparation of Experimental Animals

This study utilized 144 male day-old Cobb 500 broiler chicks, each with an average initial weight of 47.82 grams. All chicks were acquired from a recognized hatchery in Davao City, where they received vaccinations against infectious bronchitis and Newcastle disease. To help reduce transport-related stress, the chicks were given a 5% sugar solution for three hours after arriving at the experimental facility (Broiler Production Committee, 2006).

Each chick was individually weighed to facilitate proper randomization. For identification, lightweight safety pins (1.9 cm in length) were used as wing bands, a method shown to cause minimal distress compared with conventional identification techniques (Dennis *et al.*, 2008). Each pin was fitted with a paper tag bearing a unique identification number (1–144) and secured with transparent tape. During the banding procedure, the chick was gently held in one hand, with the head kept upright and the body fully supported to minimize handling stress. After stabilizing the wing, the safety pin was inserted through the wing web from the ventral side and secured. All chicks were consistently banded on the same wing, preferably the left wing.

To minimize variation in initial body weight while preserving randomization, individual chick weights and corresponding wing-band numbers were recorded in an Excel spreadsheet. Chicks were then stratified into upper and lower weight categories. Each replicate consisted of nine birds, with an equal representation of heavier and lighter chicks to ensure comparable starting weights across replicates. After stratification, replicates were randomly assigned to their respective pens.

Sixteen pens measuring 1.0 × 0.75 m were thoroughly cleaned and disinfected two weeks prior to chick placement (Cobb-Vantress, 2021). Each pen was equipped with a feeder trough, a waterer, and a 25-W brooder heater. To regulate airflow, a Sakoline burlap curtain was installed following the method described by Hidayat *et al.* (2021), and newspapers were used as the initial litter material. Brooder bulbs were switched on four hours before the chicks arrived to stabilize the brooding temperature. Throughout the study, standard biosecurity protocols were strictly observed.

Brooding Management

Ambient temperature during the growing period was maintained between 28 and 32 °C. Continuous lighting was provided during the first two weeks to support early chick growth. After the brooding phase, the lighting program was adjusted to provide illumination from 6:00 p.m. to 6:00 a.m., mimicking natural dusk-dawn conditions. This intermittent lighting schedule

promoted feed intake during nighttime hours while allowing adequate rest periods, which are essential for proper digestion and overall health (Broiler Production Committee, 2006; Hu *et al.*, 2021).

Feeding Management

Formulated diets were provided throughout the experiment and were classified into brooder, starter, and grower phases. Broiler chicks were fed *ad libitum* throughout the study, with clean and potable drinking water available at all times. The feed ingredients and nutrient composition of the experimental diets are presented in Table 1. The experiment was conducted with four treatment groups, each replicated four times. There were nine birds per replicate. The dietary treatments consist of a basal diet without any additives (T1) and the basal diet supplemented with the feed additives at three inclusion levels: 0.5 g/kg (T2), 1 g/kg (T3), and 2 g/kg (T4). These inclusion levels supplied

with 5, 10, and 20 mg/kg of chelated Zn and Mn; 424.5, 849, and 1,698 mg/kg of sodium bicarbonate; 4, 8, and 16 mg/kg of Vitamin C; and up to 325, 650, and 1,300 IU/kg of Vitamin D, respectively (Table 2).

The formulated basal diet samples (1 kg each) were submitted to Lipa Quality Control Center Inc. (LQCCI, 2025) for analysis. The proximate analysis of poultry feeds was conducted according to the official methods recommended by the Association of Official Analytical Chemists (AOAC, 2016) to determine crude protein, crude fiber, crude fat, moisture, and ash content (Table 1).

Health Care and Sanitation

Strict biosecurity measures were followed throughout the study. Poultry pens were cleaned daily, with newspapers used as bedding during the first week and replaced each day. From day 10 onward, sanitized rice hulls replaced newspapers and were changed every

Table 1. Composition and nutrient profile of the basal diet formulated for booster, starter, and grower phases (in 100 kg diet) for broiler chickens

Feedstuff	Booster	Starter	Grower
	(1-10 days old)	(11-20 days old)	(21-30 days old)
Corn (%)	57.87	59.17	62.74
L-Lysine (%)	0.3	0.25	0.3
L-Threonine (%)	0.2	0.1	0.18
DL Methionine (%)	0.4	0.35	0.35
US soya HP (%)	35	33	29
Coco Oil (%)	1.5	3	3.5
Salt (%)	0.35	0.35	0.35
MDCP (%)	2.4	1.5	1.5
Limestone (%)	1.15	1.4	1.2
Toxin binder (%)	0.2	0.2	0.2
Choline 60% (%)	0.2	0.2	0.2
Vitamin concentrate premix (%)	0.03	0.03	0.03
Mineral premix (%)	0.1	0.15	0.15
Calcium formate (%)	0.3	0.3	0.3
Nutrient analysis ^a			
ME (kcal/kg)	2952.586	3061.944	3129.636
Moisture (%)	12.31	12.25	11.43
Ash (%)	6.55	05.13	05.27
Crude protein (%)	22.22	20.86	20.40
Crude fiber (%)	2.34	02.81	02.37
Crude fat (%)	3.08	04.38	05.98
Nitrogen Free Extract (%)	53.50	54.57	54.55

Note: ^aObtained from a laboratory analysis of samples submitted to Lipa Quality Control Center Inc. (LQCCI, 2025), except for metabolizable energy (ME).

Table 2. Composition of Zn and Mn chelates, Vitamins C and D, and NaHCO₃ mixtures used for broiler chickens

Nutrient composition	Amount ^a	Composition per treatment		
		0.5g/kg ^b	1 g/kg ^b	2 g/kg ^b
Chelated Zinc	10 g/kg	5 mg/kg	10 mg/kg	20 mg/kg
Chelated Manganese	10 g/kg	5 mg/kg	10 mg/kg	20 mg/kg
Sodium bicarbonate	849 g/kg	424.5 g/kg	849 g/kg	1.698 g/kg
Vitamin C	8 g/kg	4 mg/kg	8 mg/kg	16 mg/kg
Vitamin D, IU/kg	< 650,000	up to 325	up to 650	up to 1300

Note: ^aThe compositions were based on the analysis by the Vethealth Corporation. ^bObtained by manual computation following the formula: Nutrient/kg feed = (Inclusion Rate in g/kg) * (Nutrient content in g/kg) / 1000000g (McDonald *et al.*, 2011).

4 days to reduce odors and fly activity. Rice hulls were chosen due to their availability in the area and the proven positive effects on reducing ammonia and the occurrence of footpad dermatitis in broiler (Şahin & Çelen, 2021).

Sampling and Analysis

Body weight (g). The average body weight of the broiler chickens in each pen was recorded weekly using a digital scale, with a maximum capacity of 14 kg and a minimum readability of 2 g.

Weight gain (g/bird). This was determined by taking the difference between the birds' final and initial total weights, then dividing that figure by the number of birds in each cage, as illustrated in the formula below.

$$\text{Weight Gain (g/bird)} = (\text{Total final body weight} - \text{Total initial body weight}) / \text{No. of birds per cage}$$

Average feed intake (g). Feed intake was determined by accurately recording the amount of feed offered and the feed remaining for each experimental group. These data were used to calculate total feed consumption using the following formula:

$$\text{Average feed intake (g/bird)} = [\text{Feed offered (g)} - \text{Feed refused (g)}] / \text{No. of birds per cage}$$

Feed conversion ratio. Feed conversion ratio (FCR) was calculated as the ratio of average weekly feed intake to average weekly weight gain, using the following formula:

$$\text{Feed conversion ratio} = \text{Average feed intake (g/bird)} / \text{Average weight gain (g/bird)}$$

Carcass Yield and Quality

A total of 32 birds (2 per replication) were selected to determine the carcass characteristics listed below. The researchers ensured that the selected birds per pen were closest to the average weight, as the method described by Taylor *et al.* (2021). Before slaughter, all birds were fasted for 12 hours (Schneider & Gewehr, 2023). By following the procedure described by Faria *et al.* (2010), the birds were slaughtered by severing the carotid artery and the external jugular vein close to the occipital bone and atlas to facilitate bleeding. After bleeding, the birds were immersed in a scalding tank

at approximately 60 °C for up to 2 minutes. Manual defeathering was simultaneously followed.

Carcass weight (g). Carcass weight was obtained after the removal of the head, neck, feathers, shank, and visceral parts except the lungs. The weight was obtained using a digital weighing scale.

Carcass yield percentage. This was calculated using the following formula (Faria *et al.*, 2010; Bulkaini *et al.*, 2022):

$$\text{Carcass percentage} = (\text{Carcass weight} / \text{Body weight at slaughter}) \times 100$$

Percentage of offal. According to the procedure described by Bulkaini *et al.* (2022), offal weight was measured by weighing the cleaned intestines, liver, heart, and gizzard. The offal percentage was calculated by dividing the total offal weight by the bird's live weight and multiplying by 100%.

Percentage of edible offal. The weight of edible offal (foot, neck, and head) was determined by weighing each part individually. The edible offal percentage was determined by dividing the total weight of these parts by the bird's live weight, then multiplying the result by 100 (Bulkaini *et al.*, 2022).

Skin resistance test. At the end of the study, eight birds from each treatment group were slaughtered. A 1 cm incision was made between the thigh and back, and its length was measured with a caliper before scalding and defeathering. After defeathering, the incision was measured again, and the increase in length was recorded as skin tearing (Rossi *et al.*, 2007; Zaghari *et al.*, 2022).

Occurrence of Footpad Dermatitis

Footpad dermatitis was visually assessed at slaughter in a total of 80 birds (20 birds per treatment) using the scoring system adapted from the European Union Reference Centre for Animal Welfare for Poultry (EURCFAW, 2020). Lesions were scored on a scale from 0 to 2, as illustrated in Figure 1.

Cost-Benefit Analysis

The production cost (expressed in Philippine peso, PHP) was determined by accounting for expenses for

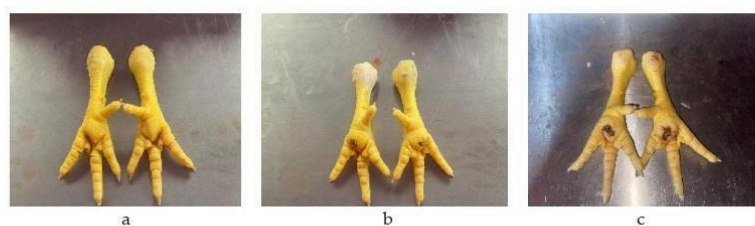


Figure 1. The footpad dermatitis scale was used in the study. The researchers randomly selected these images from slaughtered broilers. a. scale of 0 (no lesion); b. scale of 1 (mild lesion); c. scale of 2 (moderate to severe lesion).

chicks, feed, and feed additives (Table 5). The cost was based on local prices in the Philippines for January 2025, with an exchange rate of 58.39 PHP per US dollar (Bangko Sentral ng Pilipinas, 2025). The overall production cost was computed on both a per-bird and per-kilogram basis for broilers. In addition, the extra expenses associated with the test ingredients were incorporated into the cost-benefit analysis following the method first described by Khatun *et al.* (2019).

Feed intake (kg/bird) and final weight (kg/bird) were determined from the average measurements collected over the 30-day feeding trial. Feed price was computed based on the composite cost of all feedstuffs used in the formulation. The cost of the feed additives was incorporated using the actual supplier pricing provided by Vethealth Corporation. The total feed cost per kilogram was obtained by adding the basal feed price and the corresponding treatment cost. Feed cost per bird was then calculated by multiplying feed intake by the total feed cost per kilogram.

Production cost included expenses for chicks, feed, and feed additives. These expenses were summed to obtain the total cost of production per broiler. The sale price per broiler was computed by multiplying the average final liveweight by the prevailing liveweight price of PHP 150/kg. Moreover, the cost of production per kilogram was determined by dividing the total production cost by the average final weight. Profit per broiler was calculated as: Profit/broiler = Sale price/broiler - Total cost of production/broiler. On the other hand, the profit per kilogram liveweight was computed by dividing the profit per bird by the final weight. Lastly, to determine the profit PHP/kg (over control), the profit per kilogram for Treatments 2, 3, and 4 was compared with that of the control group.

Statistical Data Analysis

Data were analyzed using analysis of variance (ANOVA) with the Statistical Tool for Agricultural Research (STAR version 2.0.1) software developed by the International Rice Research Institute (2022). The experiment followed a Completely Randomized Design (CRD), and orthogonal polynomial contrasts (linear and quadratic) were applied to evaluate treatment trends, particularly for broiler performance parameters. Mean differences among treatments were further compared using Tukey's Honestly Significant Difference (HSD) test at the 5% and 1% levels of significance. In addition, the percent occurrence of footpad dermatitis was analyzed using the Kruskal-Wallis test at a 5% significance level.

RESULTS

Growth performance of broilers fed diets supplemented with a mixture of chelated zinc, manganese, vitamins, and sodium bicarbonate was assessed using key performance indicators, including body weight, feed intake, livability, and feed conversion ratio. To establish baseline values, all birds were weighed upon arrival on December 6, 2024, and

subsequent body weight measurements were recorded weekly up to day 30 to monitor growth patterns throughout the experimental period.

Body Weight and Weight Gain of Broiler Chickens (g)

During the early growth phase (1–21 days), body weight (BW) and weight gain (WG) showed a significant linear response ($p < 0.05$), with broilers receiving Treatment 4 (2 g/kg) exhibiting greater body weights. Nevertheless, neither the latter growth phase (22–30 days) nor the entire study period (1–30 days) showed any significant changes ($p > 0.05$) (Table 3).

Feed Intake of Broiler Chicken (g)

Feed intake (FI) was monitored daily by recording the amount of feed offered and refused. A highly significant linear effect ($p < 0.01$) was observed during days 8–14, indicating increased feed consumption as the levels of Zn and Mn chelates, vitamins C and D, and NaHCO_3 supplementation increased (Table 3). In addition, a significant linear response ($p < 0.05$) was detected over the entire 30-day experimental period. During the second week, broilers in Treatment 4 consumed significantly more feed ($p < 0.05$) than those in the other treatments, with a mean intake of 433.19 g.

Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) exhibited a significant linear response ($p < 0.05$) during the 1–7-day and 15–21-day periods, with the lowest FCR values observed in broilers supplemented with 2 g/kg of Zn and Mn chelates, vitamins C and D, and NaHCO_3 (T4), averaging 1.42 and 0.97, respectively. No significant effects ($p > 0.05$) were detected outside these periods (Table 3).

Livability (%)

As presented in Table 3, livability exhibited a significant linear response ($p < 0.05$) throughout the experimental period, indicating improved survival with increasing levels of supplementation.

Carcass Characteristics

The results are summarized in Table 4, which presents data on carcass weight, dressing percentage, edible offal percentage, and skin resistance to tearing. No significant differences ($p > 0.05$) were observed among treatments for carcass weight, carcass yield, offal percentage, or edible offal percentage. Mean offal and edible offal values were 8.67% and 7.87%, respectively, and did not vary significantly across treatments. Similarly, no significant differences ($p > 0.05$) were detected in skin resistance to tearing (Table 4). These findings indicate that dietary supplementation with Zn and Mn chelates, vitamins C and D, and NaHCO_3 did not adversely affect carcass characteristics in broiler chickens.

chelates, Vitamins C and D, and NaHCO₃ mixture (Figure 2), but no significant difference ($p>0.05$) was observed among the treatments. The same significance levels were observed in mild cases. Results revealed that supplementation with the said nutrients is comparable to the control treatment in terms of footpad health in broiler chickens.

Cost-Benefit Analysis

Cost-benefit analysis revealed that higher supplementation at 2 g/kg provided the highest profitability, achieving a net profit of ₱117.86 per bird and ₱75.07 per kg liveweight. At an exchange rate of 58.39 pesos per US dollar in January 2025, the net profit per bird is 2.02 US dollars, and 1.29 US dollars per kg liveweight. Lower supplementation levels (0.5 and 1 g/kg) failed to improve profitability compared with the control, mainly because feed costs increased without a corresponding increase in final body weight. Thus, the addition of 2 g of Zn and Mn chelates, Vitamins C

and D, and NaHCO₃ mixture per kg was economically advantageous (Table 5).

DISCUSSION

Chelated minerals are widely recognized for their higher bioavailability compared with inorganic sources, resulting in improved nutrient absorption and enhanced growth performance (Bhagwat *et al.*, 2021). Meshreky *et al.* (2015) further demonstrated that zinc and manganese supplementation markedly enhanced both humoral and cell-mediated immune responses, highlighting the advantages of chelated minerals over inorganic forms. In the present study, treatment effects became most evident after the first week of growth, a period characterized by rapid physiological development. During this stage, broilers have increased nutritional demands to support skeletal growth, immune maturation, and metabolic activity. Zinc plays a crucial role in bone mineralization and collagen synthesis (Zhang *et al.*, 2018), and its supplementation

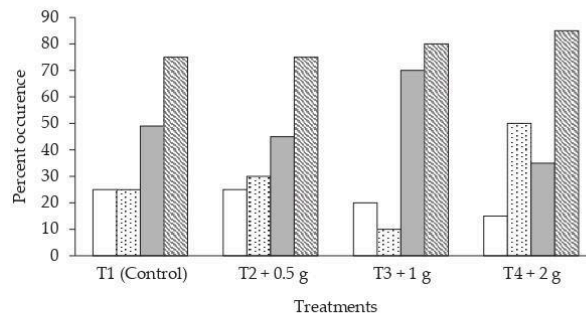


Figure 2. Percent occurrence of footpad dermatitis in broilers fed a diet with a mixture of chelated zinc, manganese, vitamins, and sodium bicarbonate. Note: □ No lesion; ▤ Mild; ■ Moderate to severe; ▨ All with lesion. The dietary treatments consist of a basal diet without any additives (T1) and the basal diet supplemented with the feed additives at three inclusion levels: 0.5 g/kg (T2), 1 g/kg (T3), and 2 g/kg (T4).

Table 5. Cost-benefit analysis for feeding diets supplemented with chelated zinc and manganese, vitamins, and sodium bicarbonate in broiler chickens. The cost was based on local prices in the Philippines in January 2025 and expressed in Philippine peso

Variables	Treatments			
	T1 (Control)	T2 + 0.5 g/kg	T3 + 1 g/kg	T4 + 2 g/kg
Feed intake (kg/bird)	2.75	2.78	2.77	2.91
Final weight (kg/bird)	1.46	1.46	1.45	1.57
Feed price (kg)	26.07	26.14	26.22	26.36
Treatment used	0.00	0.17	0.33	0.66
Total feed cost/kg	26.07	26.31	26.55	27.02
Total feed cost/bird	71.69	73.15	73.53	78.64
Chick price (PHP)	39.00	39.00	39.00	39.00
Total cost of production PHP/broiler	110.69	112.15	112.53	117.64
Total cost of production PHP/kg	75.81	76.81	77.61	74.93
Sale price PHP/broiler at 150/kg liveweight	219.00	219.00	217.50	235.50
Profit PHP/broiler	108.31	106.85	104.97	117.86
Profit PHP/kg	74.19	73.19	72.39	75.07
Profit PHP/kg (over control)	0.00	-1.00	-1.79	0.89

Note: * Zn and Mn chelates, Vitamins D and C, and NaHCO₃ mixture; PHP – Philippine peso; / - per; kg – kilogram. The dietary treatments consist of a basal diet without any additives (T1) and the basal diet supplemented with the feed additives at three inclusion levels: 0.5 g/kg (T2), 1 g/kg (T3), and 2 g/kg (T4).

Table 3. Growth performance of broiler fed with a mixture of chelated zinc, manganese, vitamins C and D, and sodium bicarbonate in their diet

Variables	Treatments						CV (%)			
	T1 (Control)	T2 + 0.5 g/kg	T3 + 1 g/kg	T4 + 2 g/kg	SEM	Tukey's		Orthogonal		
						HSD Test		polynomial contrasts		
						P-Value ^a		P-Value		
							Linear	Quadratic		
1 day of age										
IW (g)	47.48	47.28	47.05	49.48	1.66	0.72		0.45	0.44	6.94
1-7 days of age										
BW (g)	135.88	135.02	138.11	164.15	8.68	0.10		0.04*	0.14	12.11
FI (g)	148.89	139.25	137.88	162.08	8.01	0.18		0.30	0.06	10.89
WG (g)	88.39	87.74	91.05	114.66	7.59	0.08		0.03*	0.13	15.89
FCR	1.71	1.60	1.53	1.42	0.08	0.12		0.02*	0.96	9.98
8-14 days of age										
BW (g)	385.64 ^b	390.14 ^b	400.39 ^b	475.15 ^a	21.53	0.04*		0.01*	0.13	10.43
FI (g)	369.99 ^b	374.81 ^b	391.33 ^b	433.19 ^a	11.82	0.01*		<0.01**	0.14	6.03
WG (g)	338.16 ^b	342.86 ^b	353.33 ^b	425.66 ^a	20.40	0.03*		0.01*	0.12	11.18
FCR	1.11	1.10	1.11	1.02	0.03	0.25		0.14	0.30	6.23
15-21 days of age										
BW (g)	813.13	837.83	845.50	953.92	37.00	0.08		0.02*	0.28	8.58
FI (g)	792.06	800.84	802.80	874.14	28.17	0.20		0.07*	0.29	6.89
WG (g)	765.65	790.55	798.45	904.44	36.11	0.08		0.02*	0.28	8.86
FCR	1.04	1.01	1.01	0.97	0.02	0.17		0.04*	0.78	4.35
22-30 days of age										
BW (g)	1462.97	1464.69	1448.67	1567.82	45.25	0.43		0.22	0.20	5.76
FI (g)	1440.95	1460.91	1440.24	1431.17	16.19	0.63		0.50	0.39	2.24
WG (g)	1415.49	1417.41	1401.67	1518.33	41.79	0.92		0.14	0.19	5.81
FCR	1.03	1.03	1.03	0.94	0.03	0.21		0.11	0.19	6.47
1-30 days of age										
FW (g)	1462.97	1464.69	1448.67	1567.82	42.79	0.08		0.22	0.20	5.76
FI (g)	2751.89	2775.81	2772.24	2900.59	40.99	0.09		0.03*	0.23	2.93
WG (g)	1415.34	1417.04	1401.61	1519.23	41.83	0.22		0.14	0.19	5.81
FCR	1.95	1.96	1.98	1.91	0.03	0.54		0.46	0.28	3.51
L (%)	91.67	94.44	100.00	100.00	3.10	0.20		0.04*	0.66	6.43

Note: ^aP-value less than 0.05 is significantly different; Means with the same letter are not significantly different; * means significant ($p < 0.05$) linear effect; ** means highly significant ($p < 0.01$) linear effect; IW (g) – initial weight; FW (g) – final weight; BW (g) – body weight; FI (g) – feed intake; WG (g) – weight gain; g – gram; FCR – feed conversion ratio; L (%) – livability; SEM – standard error of the means; CV – coefficient of variation. The dietary treatments consist of a basal diet without any additives (T1) and the basal diet supplemented with the feed additives at three inclusion levels: 0.5 g/kg (T2), 1 g/kg (T3), and 2 g/kg (T4).

Table 4. Carcass characteristics of broiler fed with a diet with a mixture of chelated zinc, manganese, vitamins, and sodium bicarbonate

Variables	Treatments					SEM	CV (%)	P-value ns
	T1 (Control)	T2 + 0.5 g/kg	T3 + 1 g/kg	T4 + 2 g/kg	SEM			
Carcass weight, g	1147.75	1137.00	1069.25	1180.00	56.17		9.90	0.58
Carcass percentage	74.57	74.66	73.63	74.11	0.35		0.95	0.20
Percentage of offal	9.14	8.20	8.46	8.88	0.50		11.60	0.57
Percentage of edible offal	7.63	7.54	8.00	8.33	0.26		6.51	0.17
Skin resistance test, cm	1.62	1.50	1.39	1.05	0.19		27.42	0.22

Note: ns – no significant difference ($p > 0.05$); SEM – standard error of the means; g – gram; cm – centimeter. The dietary treatments consist of a basal diet without any additives (T1) and the basal diet supplemented with the feed additives at three inclusion levels: 0.5 g/kg (T2), 1 g/kg (T3), and 2 g/kg (T4).

Footpad Dermatitis Assessment

A total of 80 birds (20 per treatment) were used to assess the presence of footpad dermatitis using the scale shown in Figure 1. In the visual assessment conducted, the percentage of birds with footpad dermatitis (mild

to severe) in each Kruskal-Wallis test revealed no significant difference ($p > 0.05$) with mean values of 75% (T1), 75% (T2), 80% (T3), and 85% (T4) in all treatments, as shown in Figure 2.

For moderate to severe cases, a notably high incidence was observed in birds fed 1 g of Zn and Mn

Footpad health is widely recognized as an indicator of both nutritional adequacy and effective management practices in broiler production. In the present study, the footpad condition did not differ significantly among treatments, indicating that all inclusion levels of the feed additives produced outcomes comparable to those of the control group. This finding is consistent with the report of Zhao *et al.* (2010), who likewise observed no significant effect of chelated trace minerals on footpad lesion incidence. However, these results contrast with those of Chen *et al.* (2017), who demonstrated that higher dietary concentrations of certain trace minerals, particularly zinc and manganese, enhanced protection against footpad dermatitis by promoting lesion healing and reducing severity over time. The discrepancy between studies may be partly explained by differences in mineral balance, as the present study did not include additional trace minerals that may support tissue repair. Interactions among dietary minerals are complex, and both antagonistic and synergistic effects can influence the absorption and utilization of zinc and manganese (Swiatkiewicz *et al.*, 2017). Elevated levels of certain nutrients may impair mineral bioavailability, potentially limiting their effectiveness in supporting lesion healing. In addition to nutritional factors, litter management plays a critical role in the development of footpad dermatitis. In this study, frequent litter replacement was implemented following the recommendations of Alabi *et al.* (2023), who emphasized that regular litter management, regardless of litter type, is essential for minimizing the incidence and severity of footpad lesions in broilers.

The economics of using Zn and Mn, Vitamin C and D, and sodium bicarbonate were presented in the cost-benefit analysis table (Table 5). Results revealed that a higher inclusion rate of these feed additives in a 2 g/kg diet provided an economic advantage compared to the control and other inclusion levels. This treatment has the highest selling price of 235.50 pesos or 4.03 dollars. The use of organic trace minerals in microchelated forms has consistently shown economic advantages (Khatun *et al.*, 2019). Although Treatment 4 slightly increased feed cost per kilogram, the resulting gain in productivity lowered the overall production cost per kg and yielded the highest profit per bird (₱117.86). This agrees with Levy (2017), who reported that profitability in broiler systems rises when biological performance, especially FCR, growth rate, and survival, improves, as feed accounts for most production costs.

CONCLUSION

Higher supplementation (2 g/kg) of broiler diets with a mixture of Zn and Mn chelates, Vitamins C and D, and NaHCO₃ led to improved broiler chicken performance during the early stages of production. It also increased broiler survivability throughout the production cycle. Additionally, these nutrients did not negatively affect carcass characteristics or the occurrence of footpad lesions. Moreover, this inclusion rate provides an economic benefit to farmers. Significant linear effects indicate that higher inclusion

levels offer advantages in growth performance during early stages, along with economic benefits. Therefore, we recommend that future studies investigate supplementation levels beyond 2 g/kg and include assessments of intestinal morphology to better understand the physiological mechanisms behind these responses.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used Grammarly v4 to improve the readability and language of the manuscript. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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has been demonstrated to enhance feed efficiency and average daily gain by supporting enzymatic processes involved in protein synthesis and energy metabolism (Hidayat *et al.*, 2019). Likewise, manganese is essential for carbohydrate and lipid metabolism, contributing to the energy requirements of broilers during the early rapid growth phase (Shokri *et al.*, 2021). Organic chelated forms of Zn and Mn are more efficiently absorbed and utilized than inorganic sources, particularly during periods of accelerated growth (Sunder *et al.*, 2013). In addition, Mn is critical for cartilage development and bone matrix formation, although its direct impact on overall growth may be less pronounced (Pacheco *et al.*, 2017). The significant linear increases in body weight and weight gain observed during the second and third weeks of production suggest that higher inclusion levels of these feed additives enhance economically important traits, consistent with the findings of de Carvalho *et al.* (2021) on different manganese sources in broilers.

Sodium bicarbonate has also been reported to increase feed consumption in broiler chickens by helping maintain optimal physiological conditions that encourage feeding behavior (Osman *et al.*, 2015; Turner *et al.*, 2025). Earlier research indicates that supplementing with sodium bicarbonate can improve feed intake, promote weight gain, and enhance the feed conversion ratio. However, exceeding recommended levels can have adverse effects, including increased mortality rates (Yasooob & Tauqir, 2017). In broilers, sodium bicarbonate acts as a buffer, maintaining blood pH and counteracting metabolic acidosis, particularly under stress or when diets are high in protein or certain minerals (Martínez *et al.*, 2021). Chicks during the first 14 days of life are less efficient at thermoregulation, and sodium bicarbonate can improve their ability to cope with heat stress by promoting water intake and supporting electrolyte balance, thus reducing stress-related mortality and improving overall viability (Osman *et al.*, 2015).

Another factor contributing to the observed responses during early growth may be the inclusion of vitamins C and D in the dietary treatments. The first 14 days of a broiler's life are characterized by rapid growth, organ development, and immune system maturation, making this stage particularly responsive to nutritional interventions (Ferronato *et al.*, 2024). During the starter phase, chicks undergo intensive skeletal and metabolic development, processes that rely heavily on adequate vitamin D for calcium and phosphorus utilization, as well as vitamin C for antioxidant protection and immune support (Shojadoost *et al.*, 2021). Most studies evaluating vitamin C supplementation in broiler diets have focused on its role under heat stress conditions (Van Hieu, 2022) or its interaction with other feed additives (Hajati *et al.*, 2015; Attia *et al.*, 2017; Jahejo *et al.*, 2019; Kumar *et al.*, 2021). Amer *et al.* (2021) reported that dietary ascorbic acid at 400 mg/kg significantly improved growth performance during the starter phase, although greater benefits were observed when vitamin C was combined with safflower oil during the grower and finisher phases. These findings generally align with the present study, except for differences in feed intake responses. Similarly, Chand *et al.* (2014) demonstrated that combined

supplementation of vitamin C and zinc enhanced growth performance and health status in broilers. Variations between previous studies and the present results may be partly explained by differences in environmental conditions; in the current study, uniform ventilation likely reduced heat stress, whereas birds in the study by Chand *et al.* (2014) were subjected to heat stress during the fourth and fifth weeks of production.

Overall, no notable differences in growth performance parameters, including body weight, weight gain, and feed conversion ratio, were observed across treatments over the entire experimental period. These findings are consistent with several studies reporting limited or no effects of dietary zinc (Salim *et al.*, 2012; Sunder *et al.*, 2013; Zakaria *et al.*, 2017; Yang *et al.*, 2017; Foltz *et al.*, 2017; Zhang *et al.*, 2018), manganese (Ghosh *et al.*, 2016; Khakpour Irani *et al.*, 2019; Sun *et al.*, 2021), or mineral chelates (Yang *et al.*, 2017; Pacheco *et al.*, 2017) on overall broiler growth performance. In contrast, Zhao *et al.* (2010) reported significant improvements in body weight following supplementation with chelated copper, zinc, and manganese. This discrepancy may be attributed to differences in mineral composition and experimental duration, as the present study evaluated chelated zinc and manganese in combination with vitamins and sodium bicarbonate, without copper, over a shorter 30-day period, whereas Zhao *et al.* (2010) conducted a 52-day trial. Moreover, Rezapour *et al.* (2024) observed significant improvements in body weight during the brooder phase when broilers were supplemented with a mixture of zinc and manganese. Similarly, the present study detected significant responses during the early growth stage, with higher inclusion levels of chelated zinc and manganese outperforming lower levels. Despite differences in mineral sources, inclusion rates, and experimental conditions, these findings consistently indicate that the benefits of mineral chelates are more pronounced during the early stages of broiler growth.

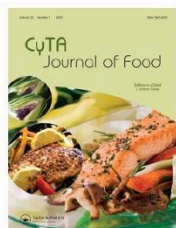
The chelated trace minerals and vitamins used in this study had no adverse effect on carcass characteristics, particularly yield, offal percentage, and skin-tear resistance. This finding is consistent with the results of Zhao *et al.* (2010), which revealed that chelated trace minerals did not significantly improve carcass quality in broilers. Similarly, Sun *et al.* (2021) reported that manganese supplementation had no notable impact on carcass traits. In the current study, the average carcass weight was 1133.50 g, with an average dressing percentage of 74.24%, indicating that the birds maintained uniform carcass quality across treatments. Moreover, this result aligns with the study by Amer *et al.* (2021), which also found that ascorbic acid supplementation had no significant effect on carcass yield. Regarding zinc supplementation, previous studies have shown that it can significantly enhance skin collagen, with male broilers exhibiting thicker skin compared to females (Salim *et al.*, 2012). However, the same study noted that zinc supplementation did not affect the skin's resistance to tearing, suggesting that while zinc contributes to skin development, other factors may also influence skin strength and integrity.

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DNAJA1 in the LT muscles of bulls and the tenderness of their meats (Bernard et al., 2007).

Another gene (HSPB1) encoding a 27 kDa heat shock protein (HSP27) was down-regulated. HSP27 belongs to the small heat shock protein (hsp20) family. It involves stress resistance and actin organisation (Bernard et al., 2007). Guay et al. (1997) demonstrated that the decrease in HSP27 expression resulted in the formation of actin polymers, hence increasing the durability of actin microfilaments. Consequently, reducing its expression may promote the disarray or breakdown of actin. The observed down-regulation in T+ muscles may lead to decreased toughness or increased breakdown of actin microfilaments with meat ageing, which could explain the higher tenderness scores associated with the meat. Indeed, previous studies have demonstrated that the breakdown of actin after death plays a role in the tenderisation of pork (Bernard et al., 2007). HSP27 is a protein that prevents cell death (apoptosis) by interacting with essential parts of the cell's signalling pathway that activate caspases and initiate apoptosis (Bernard et al., 2007).

Conclusion

However, lairage has greatly enhanced the tenderness of most meats during transit stress. Ageing affected WBSF, too. Lairage and transit durations become unnoticeable after three to seven days of ageing. This study shows that transit and lairage significantly affected the WBSF value in goats subjected to transportation. These data also show that 2- and 6-h trip lengths did not substantially affect the MDA levels or HSP27 expression. However, transportation and lairage showed increased apoptotic levels in goats' pre-rigour. The apoptosis index rose in goats transported for 6 h instead of 2 h. The most prolonged transit and lairage duration groups had the highest apoptotic score, indicating a more significant stress reaction. The absence of MDA and HSP27 responses and elevation of apoptosis show that apoptosis detection is a more accurate stress indicator than oxidative stress and HSP27 expression. The findings showed that the meat apoptosis index can be utilised to develop a new stress assessment method after the animal is slaughtered.

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Authors contribution

RR sampling, lab work, and draft writing; AHO sampling and lab work; UK sampling, analysis, and writing; AAA sampling, lab work, and writing; JCI sampling and lab work; HH, supervision and research design; AQS supervision, research design, and lab work; YMG sampling, supervision, statistical analysis, and fund acquisition of research.

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Intramuscular fat plays a crucial role in determining the tenderness of meat. However, goat meat generally has very little or no marbling. Therefore, our research did not focus on the intramuscular fat content of goat meat, as this factor is insignificant. In addition, goats have minimal amounts of subcutaneous fat, unlike beef and lamb. According to McMillin and Brock (2005), health-conscious consumers prefer goat meat because it contains less fat and has a healthier composition of fatty acids than beef and lamb. Furthermore, goat carcasses commonly experience cold shortening because they lack enough fat under the skin and have smaller carcass sizes (Gadiyaram et al., 2008). The tenderness of meat development can be affected by sarcomere shortening and reduced proteolytic activity, which occurs due to similar postmortem circumstances.

Heat shock protein-27

In the current study, it was observed that no difference was detected in the level of HSP27 between treatment groups throughout the ageing days. However, a study on goats after transport showed an increased HSP27 expression in goats' parenchymatous organs (Hu et al., 2020). These differences showed the different levels of responsiveness of various organs to stressors, in which the parenchymatous organs were observed to be more sensitive to stress than muscle. The low expression of HSP27 in muscle could likely be due to the muscle not being vulnerable to transportation stress. It could also be postulated that goats in this study did not experience heat stress because of the environmental conditions since they were provided shade during transport and lairage. According to a report by Salama et al. (2014), goats have the physiological ability to be resilient to heat stress. The variation in muscle contractile and metabolic properties and the HSP27 volume also contribute to the difference in HSP27 expression (Terlouw et al., 2021). Although insignificant, there was a decreasing trend of the HSP27 concentration throughout the ageing days. The current findings reported are consistent with those of other studies that found a decreasing trend of the concentration of HSP27 throughout the ageing days, which could be due to the limited time point of the protective effect of small-HSP before rigour mortis (Huang et al., 2016; Lomiwes, Farouk, et al., 2014). These findings would explain the depletion of the HSP27 in the muscle tissue from the day of slaughter (D0) until day 7 (D7) on postmortem days.

Apoptosis

The apoptosis index is the percentage of apoptotic cells over the total number of nucleated cell counts. The higher the index, the more cells had gone through apoptosis, possibly due to stress – the apoptosis index of LTL muscles from the 2-h transported goats decreased as the lairage time increased. In the 6-h transported goats, the apoptosis index fluctuated from high at 3 h of lairage, decreased at 6 h, and increased again after overnight lairage. These findings show that more than 3 h of lairage was needed in the TS2 goats, while longer lairage and 6 h were required by the TS6 goats for the cells to return to their normal condition. However, excessively lengthy lairage duration has been shown to impose another stress set on the goats. The low level of oxidative stress in goats' cells did not trigger the

overexpression of HSP27 in the muscle tissues after transportation and lairage. At the same time, stress at slaughter could likely contribute to the muscle tissue's high apoptotic index on the day of slaughter (D0). Overall, the effect of stress can be seen in the increased level of apoptosis, especially when goats did not have sufficient rest or were put in a lengthy lairage.

The relationship between lipid peroxidation, HSP27 expression, and the duration of transportation, lairage, and ageing with meat tenderness was evaluated in the current study. Results obtained from this study found that transportation, lairage duration, and length of the ageing days profoundly affected meat tenderness. According to Ferguson and Warner (2008), animals with less stress produced more tender meat than highly stressed animals. In this study, animals transported for a longer duration required longer lairage time to recover; thus, insufficient recovery time produced tougher meat. Twelve-hour lairage has a significant impact on improving meat tenderness after both transport durations. There are several explanations for this result. First, meat tenderness from the 12 h lairage animals at D0 might be attributable to feed and water deprivation.

Similarly, lambs' meat was more tender after being subjected to one day of fasting rather than not fasting or being put in a more extended fasting period (Ilian et al., 2001). Liquid loss in cooked meat would produce dry and tougher meat, increasing the WBSF values. According to a study by Devine et al. (2006), following preslaughter handling and on-farm stress, animals should be allowed sufficient lairage time to enable the animals to restore their muscle glycogen content, lowering the WBSF values and producing more tender meat. It was observed that meat tenderness was not influenced by the concentration of HSP27 in the muscle tissue. The protective effect of the HSP27 in muscle is limited within the meat's ultimate pH. Since there was a significant increase in muscle tenderness, it is safe to conclude that the HSP27 did not contribute to the muscle tenderness in this study, contrary to the previous findings that detected the opposite relationship between tenderness and HSP27 expression (Gagaoua et al., 2020). This contradictory result may be due to the differences between the initial HSP27 levels in both studies.

Goats from all groups showed increased meat tenderness throughout the ageing days. Similar results were shown in many studies using various species and breeds (Marino et al., 2013). There were significant differences between D3 and D7 for both TS2L6 and TS2L12 and no difference between D3 and D7 for the TS6L12 group, possibly due to the proteolysis process involved, as highlighted by Lana et al. (2015) in their study. Although some studies suggested that programmed cell death (PCD) will induce meat tenderisation (Sierra & Oliván, 2013), this study found otherwise. The WBSF data obtained from day 0 showed that the apoptotic rate did not influence meat tenderness, which may be due to the rapid cell death rate causing interference with the tenderisation process of the meat (García-Macia et al., 2014).

A novel hypothesis proposed that apoptosis is the first stage of meat ageing (Chen et al., 2005). The anti-apoptotic activity of DNAJA1 may play a role in inhibiting cellular apoptosis during the process of muscle-to-meat conversion, hence reducing meat tenderisation. This observation elucidates the correlation between the expression levels of

days on WBSF values showed that all treatment groups had the highest WBSF values at day 0 and significantly ($p < .05$) differed between the two transport groups and within treatment groups. Similarly, the WBSF values within treatment groups differ significantly ($p < .05$) except for TS2L3 and TS6L12, where no differences ($p > .05$) were observed on days 3 and 7. All groups generally had their highest WBSF values at day 0 and decreased significantly ($p > .05$), with ageing days reaching the lowest WBSF values after day 7.

Apoptosis

Figure 1 shows the apoptosis index (%) in the LTL muscle of goat's pre-rigour. Results from the analysis show that transport ($F(1,12) = 182.72$, $p = .00$) and lairage ($F(2,12) = 46.9$, $p = .00$) duration plays a significant role in the apoptosis rate of the LTL muscle. This study had a significant interaction between transport and lairage durations ($F(2,12) = 58.8$, $p = .00$) for the apoptosis index. The L3 group animals had the highest within TS2 for apoptosis index. The apoptotic index ($p < .05$) decreased significantly in the TS2L6 and TS2L12 groups. Similarly, a significant ($p < .05$) difference in the apoptotic index percentage was observed between the TS6 and TS2 groups. The apoptotic index percentages decreased significantly ($p < .05$) in the TS6L6 group compared to the TS6L3 group and increased again in the TS3L12 group, which differ significantly ($p < .05$). Additionally, there was a significant ($p < .05$) difference between groups across treatments.

Discussion

Lipid peroxidation

According to Montilla et al. (2014); and Wang et al. (2019), malondialdehyde (MDA) is a byproduct and marker to measure lipid peroxidation in the muscle. Although previous findings show that lipid oxidation develops rapidly after slaughter (Linares et al., 2007), in the current study, a slight increase was observed, which was not significant post-transport (2h and 6 h) on day 0, and lairage duration matched similar to those observed in earlier studies on lambs (De La Fuente et al., 2010) and calves (Burke et al., 2009). However, this finding is inconsistent with earlier research, which found that the MDA concentration was only significant at 12 h and 48 h after transportation (Nazifi

et al., 2009; Piccione et al., 2013). After postmortem ageing, on day 1, transportation and lairage duration significantly impacted MDA concentration. The differences in MDA concentration between treatments were only seen on day 1 postmortem, while a significant increase in MDA concentration within treatment groups was only observed on days 3 and 7 postmortem periods.

On day 1, the TS6L12 goats showed the highest MDA concentration, suggesting that the increase in MDA concentration was only established after more than 18 h from the beginning of the transportation. The significant difference in MDA concentration between the TS2L3 and TS6L12 goats suggests that long transport and excessive lairage duration are stressful due to feeding, water deprivation, and heat stress, thus increasing the MDA concentration in muscle tissues (De La Fuente et al., 2010). Simultaneously, increased physical activity during transportation and lairage has been shown to induce oxidative stress in animals, boosting reactive oxygen species (ROS) production (Altan et al., 2003). On the other hand, results from the current study showed no difference in the MDA concentration between treatments as the ageing days increased. The present findings seem consistent with those of Sabow et al. (2016), who reported that MDA concentration increased throughout the ageing days within each treatment group.

Warner-Bratzler shear force

The examination of Warner-Bratzler shear force (WBSF) is a method used widely for assessing the tenderness of meat (Novaković & Tomašević, 2017). Muscle tenderness was determined based on the WBSF values. According to Warner et al. (2022), meat exhibits toughness when it possesses a high WBSF value, whereas meat tenderness is associated with a low WBSF value. The findings showed that WBSF values increased with increasing road transport distances across ageing days except for TS6L12 on day 0, which was lower than those obtained from other treatment groups. A report by (Naldurtiker et al., 2022) observed an increase in WBSF values with increasing stress, suggesting that the extent of muscle development (inherent high pH of goats can hinder the activities of endogenous proteolytic enzymes, which affects the tenderising process) corresponds to the length and amount of stress-induced.

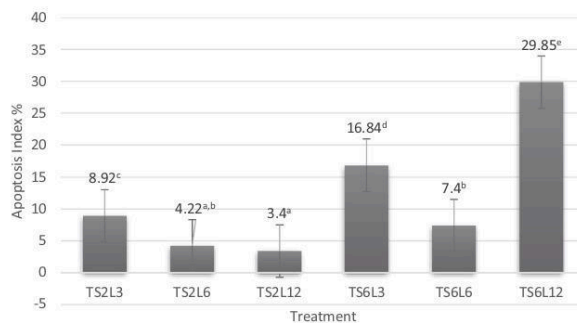


Figure 1. Apoptosis index (%) (mean $\pm$ S.E.) of LTL muscles subjected to different lairage, transportation, and ageing days. ^{a,b,c,d,e} Values with different superscripts at the same column are significantly different ($P < 0.05$).

Table 1. Effects of preslaughter management on the concentration of malondialdehyde (MDA) of *Longissimus et thoracis lumborum* (LTL) muscles subjected to different lairage, transportation, and ageing days.

Ageing Days	mg MDA/kg meat						SEM
	TS2L3 (n = 3)	TS2L6 (n = 3)	TS2L12 (n = 3)	TS6L3 (n = 3)	TS6L6 (n = 3)	TS6L12 (n = 3)	
D0	0.26 ^a	0.26 ^a	0.25 ^x	0.29 ^x	0.27 ^x	0.29 ^x	0.01
D1	0.21 ^{abx}	0.34 ^{ab,x}	0.31 ^{ab,x}	0.33 ^{ab,x}	0.36 ^{ab,x}	0.47 ^{ab,xy}	0.03
D3	0.31 ^y	0.59 ^y	0.44 ^{xy}	0.45 ^y	0.45 ^y	0.52 ^{xy}	0.04
D7	0.77 ^z	0.65 ^z	0.72 ^y	0.74 ^y	0.72 ^y	0.72 ^y	0.02
SEM	0.13	0.09	0.10	0.10	0.10	0.09	

^{a,b}Values with different superscripts in the same row are significantly different ($p < .05$).

^{x,y,z}Values with different superscripts in the same column are significantly different ($p < .05$).

Significant level * $p < .05$, NS = not significant.

T: Treatments; D: Ageing days; SEM: Standard error of the means.

Heat shock protein-27

Table 2 illustrates the result of various transport groups and lairage durations on the concentration of HSP27, which did not differ ($p > .05$) significantly. Furthermore, the concentration of HSP27 expression from days 0 to 7 indicates a decreasing trend in all the treatment groups with ageing days, and no significant ($p > .05$) differences were observed for the periods.

Meat toughness

The value of the Warner-Bratzler shear force of the LTL muscle of goats in each group was measured on various ageing days, as presented in Table 3. The study demonstrated a significant ($p < .05$) transport effect between the two transportation groups on day 0. Specifically, goats in TS6 had a significantly ($p < .05$) higher WBSF value than those in TS2 after being lairaged for 3h or 6 h. Nevertheless, no significant difference was observed between the TS2 and TS6 goats following the 12-h lairage (L12), and transport time had an observable impact on goats from both transport groups on days 1 and 3, following the

L3 and L12. Nonetheless, the outcomes derived from the two lairage durations exhibit contrasting characteristics. No interaction was recorded.

Following L3, the results from the TS6 group showed a significantly higher ($p < .05$) WBSF value than the TS2. Again, after L12, the TS2 had a considerably higher WBSF value than the TS6. No observable differences ($p > .05$) from transportation duration on the WBSF value on day 7. Following lairage, the result in both transportation groups from day 0 had a significantly ($p < .05$) higher WBSF value for L3 and L6 and significantly ($p > .05$) lower for those at L12 for the same period. The TS2 and TS6 groups showed different trends in the WBSF value of meat sampled on D1, D3, and D7. On D1, the WBSF value from TS2 meat was the lowest during L3, increased significantly ($p < .05$) differing with L6, and decreased further L12, which was significantly ($p < .05$) different from L3 and L6.

Similarly, the WBSF value of the TS6 group on day 1 decreased significantly ($p < .05$) as the lairage time increased. On days 3 and 7, the WBSF value for the TS2 goat recorded the highest value at L6, significantly ($p < .05$) different from L3 and L12. As for the TS6 group, the WBSF values decreased as the lairage time increased, which significantly ($p < .05$) differ from each other (L3, L6, and L12). The effect of ageing

Table 2. Effects of preslaughter management on heat shock protein 27 (HSP27) expression in *Longissimus et Thoracis Lumborum* (LTL) muscles subjected to different lairage, transportation, and ageing days.

Ageing Days	HSP27						SEM
	TS2L3 (n = 3)	TS2L6 (n = 3)	TS2L12 (n = 3)	TS6L3 (n = 3)	TS6L6 (n = 3)	TS6L12 (n = 3)	
D0	2.31	2.42	2.46	2.27	2.41	2.2	0.04
D1	2.25	2.37	2.29	2.04	2.22	2.18	0.05
D3	2.18	2.24	2.24	1.94	2.21	2.05	0.05
D7	1.87	2.04	1.95	1.81	2.17	1.81	0.06
SEM	0.10	0.08	0.11	0.10	0.05	0.09	

^{a,b}Values with different superscripts in the same row are significantly different ($p < .05$).

*Values with different superscripts in the same column are significantly different ($p < .05$).

Significant level * $p < .05$, NS = not significant.

T: Treatments; D: Ageing days; SEM: Standard error of the means.

Table 3. Effects of preslaughter management on Warner-Bratzler shear force (kg/cm²) of *longissimus et thoracis lumborum* (LTL) muscles subjected to different lairage, transportation, and ageing days.

Ageing days	Shear Force (kg/cm ²)						SEM
	TS2L3 (n = 3)	TS2L6 (n = 3)	TS2L12 (n = 3)	TS6L3 (n = 3)	TS6L6 (n = 3)	TS6L12 (n = 3)	
D0	1.78 ^{b,z}	1.77 ^{b,z}	1.56 ^{a,z}	1.91 ^{c,w}	1.82 ^{b,c,w}	1.63 ^{a,z}	0.05
D1	1.38 ^{b,y}	1.67 ^{d,z}	1.5 ^{c,z}	1.52 ^{c,z}	1.42 ^{b,z}	1.21 ^{a,y}	0.06
D3	1.13 ^{b,x}	1.55 ^{d,y}	1.16 ^{b,y}	1.31 ^{c,y}	1.26 ^{b,y}	1.03 ^{a,x}	0.07
D7	1.07 ^{b,x}	1.31 ^{d,x}	1.07 ^{a,b,x}	1.17 ^{c,x}	1.15 ^{c,x}	1.01 ^{a,x}	0.04
SEM	0.16	0.10	0.12	0.16	0.15	0.14	

^{a,b,c,d}Values with different superscripts in the same row are significantly different ($p < .05$).

^{w,x,y,z}Values with different superscripts in the same column are significantly different ($p < .05$).

Significant level * $p < .05$, NS = not significant.

T: Treatments; D: Ageing days; SEM: Standard error of the means.

determine the shear force, lipid peroxidation, heat shock protein-27 (HSP27), and apoptotic cells until further analysis.

Determination of lipid peroxidation

About 4 ml of 1.15% KCl solution was added to 1 g of crushed meat samples. The samples were homogenised using a T18 digital Ultra-Turrax disperser (I.K.A., Germany) at medium speed for 1 min. It was then mixed with 300 µL water, 2 ml of TBA solution, 165 µL SDS, and 35 µL BHT and heated for 60 min in a 95°C pre-heated water bath (Kumari Ramiah et al., 2014). The foundation of the thiobarbituric acid reactive substance (TBARS) assay is the reaction between the thiobarbituric acid (TBA) with MDA at high temperature to form a pink MDA-(TBA)₂ complex (Lykkesfeldt & Svendsen, 2007; Sochor et al., 2012). The mixture was then put under running water to cool it down. A volume of 3 ml of n-butyl alcohol was added to the mixture, and the substance was later centrifuged at 5000 rpm for 10 min. The butanol layer was separated and read at 532 nm against n-butyl alcohol using a spectrophotometer. Standard curves of 1,1,3,3-tetraethoxypropane were created, and the TBARS value was calculated and expressed as mg MDA/kg meat (Kumari Ramiah et al., 2014).

Determination of Warner-Bratzler shear force

A texture analyser (Stable Micro System, Surrey, UK) equipped with Volodkevich bite jaws was utilised to analyse the shear force of the meat. Before utilisation, the device underwent calibration to ensure a weight of 5 kg, while the blade speed distance was set at a constant value of 10 mm/s. Before use in this experiment, the samples from the previous cooking loss measurement were allowed to stand at room temperature for 3 h. Two cores of 2 cm × 1 cm × 1 cm (length × width × height) of each sample were cut following the longitudinal orientation of the muscle fibres. The cores were then sheared once horizontally at the centre using the texture analyser. The maximum force needed to cut the meat was recorded as the Warner-Bratzler shear force value (WBSF). Results were expressed as a load in kg/cm² (Li et al., 2018; Sabow et al., 2017).

Determination of heat shock protein-27

Meat samples were homogenised in 10 volumes of PBS buffer of pH 7.4 on ice. Samples were later homogenised using an Ultra-Turrax homogeniser (I.K.A., Germany). The homogenates were centrifuged at 12,000 rpm for 20 min at 4°C (Shen et al., 2009). The clear supernatant was collected and used for HSP27 analysis. The analysis of HSP27 was performed using a commercially available Caprine Heat Shock Protein 27 (HSP27) ELISA kit (Qayee-Bio, Shanghai, China). The assay starts with preparing reagents and standards supplied according to the manufacturer's instructions. Later, the assay was performed by adding standards and samples to the wells. Standards and samples were run in duplicates and measured using a standard microplate reader (450 nm) (Tecan Infinite M2000, Switzerland). The detection range of the HSP27 assay was 450 nm. The expression of HSP27 was expressed as U/L.

Determination of apoptotic index using TUNEL assay

The terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay technique observed apoptotic cells in the muscle (Didenko, 2010). A commercial In-Situ Cell Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany) was utilised for the experiment. The methodology employed in this study relies on the identification of DNA strand fragmentation within apoptotic cells. The DNA polymerase and terminal deoxynucleotidyl transferase (TdT) were used to label the fragmented DNA strand. According to Ansari et al. (1993), the tissue slide preparation was done. First, goat muscle tissues were fixed in four percent buffered formaldehyde and were later embedded in paraffin. The tissue was cut to ~5 µm and mounted on glass slides. The tissue sections were dewaxed at 60°C for 30 min and dipped in xylene and ethanol (100%, 95%, 90%, 80%, 70%) for rehydration. It was then air-dried and deparaffinised. Before being stained with the TUNEL reaction, the slides were treated with the permeabilisation solution of Triton x-100 and sodium citrate and rinsed with PBS solution. The slides were then incubated for 60 min at 37°C in a dark, humidified chamber. The TUNEL-positive cells were counted under a 20 × magnification fluorescence microscope at the excitation wavelength of 450–500 nm and a detection wavelength of 515–562 (green). Ten microscopic fields were randomly selected to detect the TUNEL-positive cells for each slide (Borbely et al., 2012; Li et al., 2017; Maidana et al., 2015). The determination of apoptosis index for each sample was calculated based on the following formula by (Wang et al., 2011):

$$\text{Apoptosis Index} = \frac{\text{Total count of TUNEL - positive cells}}{\text{Total counted nucleated cells}} \times 100$$

Statistical analysis

Statistical analysis was performed using SPSS Statistic 26 software (SPSS Inc., Chicago, IL, U.S.A.). A two-way ANOVA model was used to examine the interaction between the transport and lairage duration and its effects on meat tenderness and apoptosis rate in goats' meat. One-way ANOVA was conducted to analyse the differences between treatment groups when the main effects or interactions were detected. Duncan's multiple range test was used to elucidate the significantly different means. All the values were expressed as Mean ± SE, and the confidence level was 95%.

Results

Lipid peroxidation

Table 1 shows the MDA concentration in the LTL muscle; at day 0, no significant differences ($p > .05$) were observed between and within treatment groups. On day 1, significant ($p < .05$) differences within and between treatment groups. On days 3 and 7, significant ($p < .05$) differences between treatments were seen following ageing. The investigation found statistically significant differences between the TS2L3 group (0.21 ± 0.02 mg MDA/kg meat) and the TS6L12 group (0.47 ± 0.11 mg MDA/kg meat) ($p < .05$). The concentration of MDA exhibited a significant rise with the progression of ageing days ($p < .05$). After one day of ageing, notable alterations in the concentration of MDA were observed in the TS2 group. Similarly, the MDA concentration in the TS6 group showed an elevation after three days of post-mortem ageing ($p < .05$).

meat during the ageing period (Sabow et al., 2017; Scheffler et al., 2013).

The dark, firm, and dry (DFD) is characterised by a high ultimate pH and water content. However, the water is firmly bound, which makes the meat seem dry (Chulayo et al., 2016; Loudon et al., 2018). The determination of meat tenderness relied on the meat trait and the biochemical process during the pre-slaughter, slaughter, and post-slaughter process, together with the storage methods (Guillemin et al., 2012). Typically, the meat will be kept for the ageing process, a procedure meant to improve meat tenderness (Marino et al., 2013).

Scientists have addressed cellular stress response in the last decade as part of animals' stress indicators (Hu et al., 2016). Another gene (HSPB1) encoding a 27 kDa heat shock protein (HSP27) was down-regulated. HSP27 belongs to the small heat shock protein (hsp20) family. It involves stress resistance and actin organisation (Bernard et al., 2007). Guay et al. (1997) demonstrated that the decrease in HSP27 expression resulted in the formation of actin polymers, hence increasing the durability of actin microfilaments.

Consequently, reducing its expression may promote the disarray or breakdown of actin. The observed down-regulation of troponin-T in muscles may lead to decreased toughness or increased breakdown of actin microfilaments with meat ageing, which could explain the higher tenderness scores associated with the meat. According to Lomiwes, Lomiwes, Hurst, et al. (2014), HSP expression may be induced in muscle cells in response to environmental stress, physiological circumstances, or animal slaughtering. HSP27 and other heat shock proteins (HSPs) can attach to and safeguard myofibrillar proteins from being broken down by calpain, thereby stabilising proteins that have undergone denaturation. Additionally, HSPs act as competitive inhibitors of the proteolytic enzymes.

There is a growing interest in linking oxidative stress changes and the expression of HSP27 with the rate of apoptosis and how they relate to meat quality, especially meat tenderness in goat meat. This study utilised the apoptosis rate in meat as a stress parameter to detect the effect of preslaughter management in goats.

Therefore, this study's objective was to investigate the oxidative stress level through the malondialdehyde (MDA) concentration, HSP27 expression, and the potential biomarkers of apoptotic index in the *Longissimus thoracis et lumborum* (LTL) and how they can be related to the preslaughter management associated with meat tenderness in goats' subjected to different transport durations and lairage time in a tropical climate.

Materials and methods

The research protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Universiti Putra Malaysia (approval no: AUP: R042/2017).

Experimental conditions, animals, and treatments

Due to its proximity to the equator, Malaysia experiences consistent and abundant sunlight throughout the year, accompanied by ample rainfall. The experiment was carried out during February and March. Throughout the testing period, the weather fluctuated between intense heat and

rainfall. The mean temperature was $32.25 \pm 1.07^\circ\text{C}$ (Mean $\pm$ SE), with the max recorded as $34.03 \pm 1.06^\circ\text{C}$, and the mean relative humidity was $72.63 \pm 3.79\%$ (Mean $\pm$ SE), with the max recorded as $84.35 \pm 3.52\%$. Eighteen male Boer crossbred goats aged 6–8 months were sourced from a commercial farm in Selangor, Malaysia. Goats were grouped based on the two transport durations ($n=9$), which were the 2 h (TS2) and 6 h (TS6), and three distinct lairage periods (L3 = 3 h, L6 = 6 h, and L = 12 h) for each transport duration. The test power based on the transport duration was determined at (1-beta) of 0.8 (or 80%). The duration of transport was chosen based on the EU guidelines (Council Regulation (EC) No 1/2005 of 22 December 2004 on the Protection of Animals during Transport and Related Operations and Amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97, 2005), a 6-h transport was chosen to define a short transport duration. Simultaneously, the 2-h journey was selected to represent the ultrashort transport duration since no significant temperature and relative humidity changes existed. Both durations are in line with Malaysia's geographical and environmental conditions.

Transportation, lairage, and slaughter procedure

All goats were subjected to the same handling procedure and personnel during transportation, starting from the farm until reaching the research abattoir. All goats in each transportation group were transported at once on a flatbed lorry from the farm to the Research Abattoir of the Animal Science Department, Faculty of Agriculture, Universiti Putra Malaysia ($2^\circ 58'59.0''$ N $101^\circ 44'06.4''$ E). The space allowance for goats on the lorry adheres to the guidelines determined by the EU guidelines. According to the guidelines, a goat of less than 35 kg requires space between 0.20 and 0.30 m²/animal. Goats were transported at about 8 a.m. The animals from the TS2 transportation group arrived at about 10 a.m. at the abattoir, while goats in the TS6 transport group arrived at 2 p.m. The transportation truck had a canvas roof and a wooden truck bed. Animals were transported on a flat surface road with an average 50 km/h speed. A sudden braking style was avoided to prevent animals from unnecessary stress caused by reckless driving. During transportation, no feed or water was given. The loading and unloading process was done cautiously to reduce the stress caused by rough handling. Goats were kept in lairage in a roofed holding area with natural light, a concrete floor, and a solid fence beside the slaughter area. Water was made available throughout the time in lairage. However, no feed was given to minimise gut contamination during slaughter. All goats were slaughtered at the end of each lairage period according to the Malaysia Standard MS 1500:2009 (Halal Food: Production, preparation, handling, and storage- General Guidelines).

Efforts have been made to lessen the stress experienced by the goat during the slaughter process. The slaughter area was cleaned after every slaughter process before the next animal was brought in. Goats were dressed soon after exsanguination. Muscles were trimmed to remove any fat and connective tissue. The LTL muscle was cut immediately within 15 min post-slaughter upon completing the carcass dressing, divided, vacuum packed, labelled as day 0 (D0 pre-rigour samples), and subsequently stored in a chiller to

Effects of preslaughter management on oxidative stress, Warner-Bratzler shear force, heat shock protein 27 expression, and apoptosis index of *Longissimus thoracis et lumborum* in goats

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ABSTRACT

This study examines how preslaughter management affects Warner-Bratzler shear force, oxidative stress, heat shock protein 27, and apoptosis index in goats in tropical climates of Malaysia. Three hours (L3), six hours (L6), and twelve hours (L12) of lairage were given to goats before slaughter following two different road transportation distances, two hours (TS2) and six (TS6) hours, respectively. Increased transit length significantly ($p < .05$) affects apoptotic index and Warner-Bratzler shear force (WBSF) in goat meat, with TS6 animals resulting in tougher meat than in TS2 animals. The impact of stress on meat tenderness decreased significantly ($p < .05$) after 12 h of lairage – further, ageing of goat meat for three to seven days diminished transport stress's impact in all groups. Transit durations did not affect ($p > .05$) malondialdehyde (MDA) concentration and HSP27 expression. Transport and lairage duration interacted significantly with the apoptosis index in this study. In summary, apoptosis detection is a more sensitive stress parameter.

HIGHLIGHTS

Study on goat stress response

- Oxidative stress triggers a cellular stress response in goats.
- HSP27 and apoptosis rate affect meat tenderisation.
- The lairage period, determined based on the transport duration, allowed animals to rest before slaughter.
- Apoptosis detection is more sensitive than oxidative stress and HSP27 expression.

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Introduction

Increasing populations and demand for quality proteins necessitate the movement of livestock for meat processing and consumption via different transportation systems and contribute to pre-slaughter stressors, including handling, novelty, environmental temperature, journey, and lairage duration that cannot be underestimated (Díaz et al., 2014; Ekiz et al., 2012; Ferguson & Warner, 2008). Lairage resulted in relaxation and alleviating transportation stress in goats by lowering cortisol and glucose levels (Bórnez et al., 2009; Jama et al., 2016; Kannan et al., 2000). However, animals could not recuperate from transportation stress after insufficient lairage, while excessive lairage duration predisposed animals to other stressors, such as feed deprivation or the novelty of the environment (Vergara et al., 2017; Yalcintan et al., 2018). This increased stress has detrimental effects on the goats' meat quality, leading to economic losses for farmers (Kannan et al., 2002; Terlouw et al., 2021).

Earlier studies demonstrated that the inadequate oxygen supply after slaughter would trigger cells to engage in cell suicide mechanisms (apoptosis) to protect their overall

function and homeostasis. The apoptosis in muscle tissue started right after slaughter, decreasing after 24 hours and escalating abruptly on day four (Becila et al., 2010; Cao et al., 2010). Thus, it is most important to look at the apoptosis rate within 24 hours to determine the impact of pre-slaughter stress on the animals. Apoptosis would cause muscle shrinkage and expansion in the extracellular space, resulting in tenderer meat (García-Macia et al., 2014; Ouali et al., 2006). Nevertheless, rapid cell deterioration and death will interfere with meat tenderisation.

In physically stressed animals, metabolic exhaustion causes ATP disruption, resulting in increased muscle glycogenolysis and muscle glycogen depletion (Terlouw et al., 2021). In live animals, lactate transportation from the breakdown of glucose to the liver is from the peripheral tissue, which will eventually be converted back to glucose. However, in dead animals, lactic acid accumulation will increase the pH of the muscles, causing the separation of the filaments and contraction of muscle fibres and decreasing the ATP supplies (Lana et al., 2015; Pearce et al., 2011). The decrease in meat pH was often recorded due to the proteolysis process in

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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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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

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors will make data available on request.

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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-Lonerger 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), discoloration (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

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.

Treatments	MDA, g/kg meat				p Value
	Day 0	Day 1	Day 7	Day 14	
<i>Transport distance</i>					
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	
<i>Stocking density</i>					
200 kg/m ²	0.44 ± 0.00az	0.58 ± 0.00cy	1.19 ± 0.01cx	1.72 ± 0.02cw	<.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	
<i>Muscle type</i>					
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	
<i>DIS × DES (two-way interaction)</i>					
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 ± 0.02dw	<.0001
DIS450 × DES600	0.46 ± 0.00dz	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	
<i>DIS × DES × MUTYP (three-way interaction)</i>					
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/m² than pig muscle transported with 236 kg/m² and 251 kg/m² (Pereira et al. 2015). Similarly, Kim et al. (2004) found that pigs at a high density of 0.31 m²/100 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 m²/100 kg body weight) and low stocking densities (0.39 m²/100 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

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/m² and lower WBSF values in animals subjected to a livestock density of 400 kg/m² 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.

Treatments	Shear force, g			p Value
	Day 1	Day 7	Day 14	
<i>Transport distance</i>				
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	
<i>Stocking density</i>				
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	
<i>Muscle type</i>				
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	
<i>DIS × DES (two-way interaction)</i>				
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.

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.

Treatments	Cooking loss, %			p Value
	Day 1	Day 7	Day 14	
<i>Transport distance</i>				
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	
<i>Stocking density</i>				
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	
<i>Muscle type</i>				
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	
<i>DIS × DES (two-way interaction)</i>				
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	
<i>DIS × DES × MUTYP (three-way interaction)</i>				
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.

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.

Treatments	Glycogen				p Value
	Day 0	Day 1	Day 7	Day 14	
<i>Transport distance</i>					
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	
<i>Stocking density</i>					
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	
<i>Muscle type</i>					
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	
<i>DIS × DES (two-way interaction)</i>					
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	
<i>DIS × DES × MUTYP (three-way interaction)</i>					
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.

Treatments	pH				p Value
	Day 0	Day 1	Day 7	Day 14	
<i>Transport distance</i>					
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	
<i>Stocking density</i>					
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	
<i>Muscle type</i>					
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	
<i>DIS × DES (two-way interaction)</i>					
DIS450 × DES200	5.91 ± 0.01fx	5.42 ± 0.01dz	5.60 ± 0.01dy	6.41 ± 0.01fw	.0006
DIS450 × DES400	5.99 ± 0.01ex	5.44 ± 0.01dz	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	
<i>DIS × DES × MUTYP (three-way interaction)</i>					
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 ± 0.01cd	6.41 ± 0.03cd	6.72 ± 0.04c	<.0001
DIS850 × DES600 × IF	6.56 ± 0.06a	5.76 ± 0.01a	6.41 ± 0.02cd	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.

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

Treatment	Lightness (L*)			Redness (a*)			Yellowness (b*)			p Value		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14			
Transport distance	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	3523	
	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	1274	
	p Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		
Stocking density	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	0598	
	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	0527	
	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 ax	.0798	16.19 ± 0.24 ax	16.62 ± 0.36 ax	5813	
	p Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		
Muscle type	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	4328	
	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	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	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	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	5269
	DIS850 × DES200	35.30 ± 0.37 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	5045
	DIS850 × DES400	35.66 ± 0.39 az	38.20 ± 0.22 by	41.80 ± 0.31 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	3963
	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	.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	6273
	DIS450 × DES400 × ST	34.06 ± 0.18 g	36.62 ± 0.09 e	37.62 ± 0.15 e	<.0001	14.22 ± 0.13 e	14.12 ± 0.08 h	14.13 ± 0.08 h	.8322	14.25 ± 0.26 ef	14.07 ± 0.15 fg	.3597
	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	<.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	.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–h) differ significantly. At a significance level of $p < .05$, means in the same row with distinct letters (w–z) differ significantly.

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

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 polyethylene 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:

$$\text{Cooking loss (\%)} = \left[\frac{W_1 - W_2}{W_1} \right] \times 100$$

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:

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

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 x 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 x 3 x 2 (distance x stocking x 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.

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 carcasses 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 carcasses 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.

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 carcass 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 m² 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/m² (high), 400 kg/m² (medium) and 200 kg/m² (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,

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/m², medium density of 400 kg/m² and high density of 600 kg/m². 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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Brahman cattle; meat
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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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animals



Review

Importance of Knife Sharpness during Slaughter: Shariah and Kosher Perspective and Scientific Validation

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Simple Summary: A sharp knife of appropriate dimension (blade length) is very important during halal and kosher slaughtering of animals without stunning for a rapid and clean neck severance. It improves bleeding and alleviates pain and stress in animals by early onset of unconsciousness. An efficient bleed-out improves meat quality and food safety. With the ever-increasing demand for halal and kosher meat due to its awareness, authenticity, nutritive value, and animal welfare compliance, there is an urgent need to emphasize the role of knife sharpness during slaughter as per the prescribed religious practices. Other issues such as neck cut positions, blade length of the knife, proper training of slaughterhouse workers, infrastructure, and constant monitoring of the slaughtering process also need to be addressed to improve animal welfare and meat quality.

Abstract: Halal and kosher slaughter have given the utmost importance to the sharpness of knives during the slaughter of animals. A sharp knife of appropriate dimension (blade length) makes slaughter less painful during neck severance and facilitates desirable bleeding. The role of knife sharpness has not been given due credit from an animal welfare perspective and is likely ignored by the people involved in slaughterhouses. A neat, clean, and efficient neck cut by an extremely sharp knife reduces the pain. It improves the bleeding out, thus making animals unconscious early without undergoing unnecessary pain and stress. It also helps in improving meat quality and food safety. A slight incremental improvement in knife sharpness could significantly improve the animal welfare, productivity, efficiency, and safety of meat plant workers. The present review critically analyzed the significance of knife sharpness in religious slaughter by reducing stress and pain and improving meat quality and food safety. The objective quantification of knife sharpness, proper regular training of slaughterers, and slow slaughter rate are the challenges faced by the meat industry.

Keywords: religious slaughter; knife sharpness; neck cut position; slaughter skill; restraint; animal welfare

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knowledge of animal sentience, pain, and distress associated with slaughter without stunning and various ways to alleviate the pain and distress during slaughter. Sufficient human resources with expertise in slaughter techniques and proper knowledge of religious values should be employed to reduce the work pressure in slaughterhouses. Alternatively, the slaughterhouse management may provide a set of sharp knives for slaughtering a group of animals in a day or a shift depending on usage and need. At a point of slaughter, more than one slaughterer should be appointed so that when one person is slaughtering, another will inspect and sharpen the knife and, later, vice versa. The management should also practice regular interviews/interactions with workers to assess their compassion and empathy towards animals and the intrinsic value of various guidelines prescribed in religious slaughter. Whenever needed, they should intervene accordingly.

9. Conclusions

Knife sharpness plays a crucial role in rapid and clean neck severance, alleviating pain and stress in animals for producing good quality meat following religious practices. With the ever-increasing demand for halal and kosher meat due to its authenticity, nutritive value, and animal welfare compliance, it is of utmost importance to emphasize knife sharpness during slaughter as per the prescribed religious practices. Manufacturing design of slaughter knives for ergonomics, maintenance, sharpness, and assessment must be conducted for food safety and animal welfare, as well as for the sensory appeal of meat such as the palate knives and perceived healthiness.

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than using the desired ones [62]. One survey conducted over ten years duration reported that over 80% of abattoir workers used an inappropriately sharpened knife [89]. The slaughterers performing repetitive tasks continuously for hours could also result in the cutting of the corners of the blades [45]. Using blunt/less sharpened knives has been associated with lower production and increased injuries due to greater force during meat cutting [90].

A razor-sharp knife requires lower grip force, cutting time, and cutting moments, whereas a blunt knife was projected to require 25% more cutting moments and musculoskeletal disorders (MSDs) [91]. Further, Grandin [92] described how using an inadequately sharp knife results in a higher force applied by the operator and an increase in cutting time. Further, a sharp knife is more productive by increasing the cutting speed by 1.5 times [92]. Thus, a small increment in knife sharpness could result in a significant gain in overall productivity by improving the speed of production and the safety and quality of the meat.

There is an urgent need to make people aware of the importance of knife sharpness and the role played by a sharp knife in improving and maintaining high standards, particularly in religious slaughter without stunning. There should be proper emphasis on regular training and refresher courses for the personnel involved in this meat production. A study by Claudon et al. [91] with 196 respondents in France revealed that 42% of the 196 butchers complained that the blade being used was not sharp enough and only 16% stated having been trained in sharpening and maintenance of knives. There should be national-level training programs for livestock handlers, slaughterhouse workers, and staff. The personnel employed in meat production should have a certificate of competence.

Islam and Judaism have given utmost importance to knife sharpness, but the specific detailing of the knife is not mentioned for halal slaughter, as that mentioned in kosher slaughter. Thus, it is recommended to develop proper specifications for the knife used for halal slaughter [27]. There is a need to make slaughter workers and butchers aware of the importance of knife sharpness and its crucial role in ensuring the slaughter of animals with minimal pain. This follows the intrinsic principles of animal welfare prescribed in Islam and Jewish.

Further, slaughterhouse management should be more concerned with proper facilities and the gentle handling of animals during slaughter. The butchers/shochet should undergo regular training or refresher course to update them about the importance of gentle handling, knife sharpness, and a neat-clean neck cut. This will protect from scotoma (factory blindness) and compassionate fatigue in slaughterers due to a monotonous work profile. There is a need for regular auditing and inspection of slaughterhouses for proper compliance with knife sharpness and other factors that affect animal welfare during slaughter.

There is a requirement for proper monitoring of the whole process of kosher and halal slaughter. Various outcome-based measures or variables should be continuously monitored to improve the process. The high levels of vocalization (moo or bellow) during handling and restraint are associated with physiological stress due to aversive conditions such as excessive pressure from a restraint device, the sharp edge of a restraint sticking into an animal, or the use of electric goads [93–95]. Cattle undergoing ritual slaughtering in a Weinberg pen (in which the animal is inverted during slaughtering) were recorded to spend eight times longer time and have significantly higher cortisol and hematocrit values as compared to cattle slaughtered conventionally or in an ASPCA (American Society for Prevention of Cruelty to Animals) pen (in which the animal remains standing during slaughtering) [96]. Time to collapse, loss of posture, or eye rollback is an important indicator of animal welfare and should be continuously monitored [88]. The loss of consciousness should occur within 30–40 s after the neck cut [88]. In addition, there is a need for constant supervision and monitoring of knife sharpness by the slaughterhouse management as people tend to become sloppy when monitoring and oversight by management are reduced.

The authors believe there should be improved knife sharpening devices, and slaughter persons should be trained in knife sharpening. The slaughter persons should have basic

shackled and the neck cut. In mechanically turned restraints, fewer cutting movements were reported by the authors in the same study. The higher number of cuts directly influences the pain felt by animals during slaughter. Gregory [85] reported a sudden nociceptor discharge lasting for 4 s upon throat cut.

Thus, proper training/technical knowledge for gentle handling of animals to handlers, less stressful restraint devices during immobilization, elimination of distractions, and appropriate neck cutting with extremely sharp knives would all maintain high animal welfare standards while producing high-quality meat with fewer instances of petechial hemorrhages [9,32].

7.3. Restraints and Slaughter Position

Gentle stretching of the neck during neck cutting with proper restraint improves the quality of the neck cut. The wounds should be left open to alleviate pain and facilitate bleeding. After the throat cut, the animal should be released from restraint. An inverting-conscious animal could aggravate the fear and distress in animals as well as the aspiration of ruminal fluids coupled with compression of internal thoracic organs exerting pressure and inhibiting respiration [86]. During neck cutting, the neck should be properly within reach of slaughterers for neck cutting in a swift movement. Velarde et al. [59] also observed fewer cuts (three cuts) performed during the slaughter of cattle at 90° position on their sides as compared to higher cuts (five cuts) in cattle slaughtered at 180° position on their back as well as nine cuts performed in slaughter in the upright position.

During kosher and halal slaughter in Italy, cattle are restrained in the upright position [84]. Stressful restraint causes struggling, thereby masking the behavioral response to neck cuts. The restraints should not provide excess pressure and avoid jerky movements, which could make animals excited and agitated, thereby affecting the throat cut and slaughter process. A calm animal loses consciousness earlier than an agitated animal [83]. Animals should not be suspended by limbs except for poultry and there must not be any injury inflicted to restrain animals such as the cutting of tendons [1]. Small ruminants suspended on a shackle were reported as having significantly higher struggling as compared to manual restraints on their side [87]. Act of abusive and rough handling such as beating, poking with pointed sticks, dragging, leg clamping, shackling, and hoisting is also strictly prohibited under animal welfare and slaughter legislation [9,22].

The restraint equipment used for halal and kosher slaughter should hold the animal in a comfortable upright position before and during the slaughtering process. The same standards should be applied if a rotating box is used for restraining the animals [88]. Velarde et al. [59] observed that cattle in halal slaughter without stunning were restrained by four different methods, namely, by turning the animal at 45°, turning on their side (at 90°), turning on their back (180°), and upright positions with modified ASPCA pens. The authors [59] observed vocalization in fewer cattle in upright restraint boxes as compared to inverted restraints/turned on their backs. The cattle restrained by turning on their side lost posture early followed by cattle that were turned 45° and slaughtered in the upright position, with the longest time to loss of posture recorded in cattle turned on their back [59].

8. Prospects and Challenges

Maintaining the proper level of knife sharpness in modern slaughterhouses with higher slaughter rates is quite challenging. In the case of religious slaughter, it is required to slaughter as per the specific requirement of animal handling, knife specification, and good neck cut as the recommended practice of halal or kosher to make it safe and fit for eating to that particular community. For maintaining the desirable slaughter line speed, more infrastructure and human resources are required in religious slaughter. This will result in increasing the cost of production of meat.

The knife size and sharpness sometimes do not match the prescribed guidelines in cases of religious slaughter. There are more incidences reported in various slaughterhouses regarding the use of shorter and poorly sharpened knives during the slaughter of animals

7. Determinants of Knife Sharpness

The overall impact and outcome of the knife sharpness are affected by several factors, viz., training and expertise of the slaughterers, neck cut position, restraints, and slaughter position. These factors improve the efficiency of knife sharpness, neat-clean neck cut, and improve bleeding efficiency, thereby maximizing animal welfare compliance while alleviating pain and distress.

7.1. Neck Cut Position

The neck incision under kosher slaughter is usually done at the position of cervical vertebra C2–C4. However, if done at C1, there are fewer incidences of false aneurysm formation, premature blockage of blood loss, and accumulation of blood in the upper and lower respiratory tract, later associated with unpleasant sensory signals [19]. Neck cut position is a key determinant in the onset of a false aneurysm [79] usually appearing between 7–21 s of the neck cut [80]. However, the case of a higher cut (C1) may potentially result in a cut to the larynx, including the associated bones, which may damage the knife's sharpness and result in the rejection of the meat due to non-compliance with religious practices, particularly in Kosher slaughter [38]. Furthermore, cattle slaughtered with C1 neck cuts produced a different sound than those with the neck cut at C2–C4 [19]. Similarly, Gibson et al. [81] also noted a lower time to final collapse in anima, reducing suffering, upon performing a high neck cut. It could be due to the increased branching of carotid arteries at a higher neck position (above tracheal ring 2), thereby minimizing or preventing the retraction of carotid arteries within the connective tissue sheath [41,81].

The neck severance at the C1 would further reduce the potential risk of irritation associated with blood infusion into the respiratory tract due to the cutting of laryngeal nerves (transferring signals from the upper respiratory tract) and the vagus nerve (transmitting signals from the lungs and inferior trachea) [19,82]. In a controlled trial with captive bolt stunning of cattle, Gregory et al. [80] observed that making a neck cut at the C3 position had four times higher chances of early arrested blood flow and a 2.5 times higher frequency of false aneurysm formation compared to neck cutting at the C1 position. Gregory et al. [80] attributed the benefits of a sharp and clean cut at C1 using a very sharp knife with rapid blood loss and lower incidences of the formation of the false aneurysm to the following factors, viz.,

- (1) Higher branching of the carotid artery at the C1 position lowers the chances of sealing all carotid artery branches.
- (2) The C1 neck cut needs stretching of the chin, thereby stretching the artery with less chance of their retraction within the connective tissue.
- (3) The presence of less connective tissue at the C1 position.

7.2. Skill and Training of Slaughterers

Under kosher slaughter, it is mandatory for a shochet to undergo rigorous training and education to obtain a license to slaughter animals. However, such training and skill requirements are not mandatory in halal slaughter. A trained slaughterer cuts both carotid arteries and jugular veins more effectively by making a fast swift cut close to the jaw, thereby ensuring efficient bleeding and early onset of unconsciousness. Grandin [83] observed a very short collapse time for cattle (5 s) if kosher slaughtered by a good shochet whereas a collapse time of up to 1 min in the case of a throat cut by a poor shochet. Further, a proper cut also ensures lower incidences of false aneurysms.

During neck cutting, the average numbers of cutting movements vary with the skill of the slaughterer and the restraint system used for immobilizing the animal. Cenci-Goga et al. [84] observed variations in the number of cutting movements between halal and kosher-shackled and slaughtered sheep due to the difference in the skill of the slaughterers during neck cutting. The authors [84] in a survey on religious slaughter in Italy observed 25.2 cutting movements for halal-slaughtered cattle in the upright position, 2.9 for halal-slaughtered sheep shackled and the neck cut, and 1.25 for kosher-slaughtered sheep

In present-day slaughterhouses with high throughput, it is quite challenging to quantify the sharpness of knives every time. ANAGO[®], New Zealand has developed an instrument called an ANAGO[®] sharpness tester for the objective of accurate and reliable assessment of the sharpness of knives as well as to assist in effectively controlling and improving the sharpness to the desired level. This helps in measuring knife sharpness and achieving the desired sharpness [76]. The equipment can be applied to ensure sharpness in slaughterhouses on a large scale. This instrument can also detect the presence of nicks on the blade.

The ANAGO[®] sharpness score presents a sharpness profile of the knife from the tip to the heel based on the relative force required to cut. The sharpness score ranges from 2.0 (42 times as much force required) to 10.0 (no force required while cutting). For measuring the knife sharpness score, sections of 20 mm blocks in length on the blade are divided from the tip to the end [76]. A sharpness score of 8.0 could be judged as in the sharp category, while most of the knives used in commercial slaughterhouses were reported to have an average of 7.80 [51]. Additionally, to maintain the sharpness level to a score of 8.0 or above, it is necessary to sharpen the knife by machine after every slaughter. In contrast, manual sharpening of the knife after every slaughter can achieve a maximum score of 7.80 [51] as presented in Figure 3.



Figure 3. Knives used for the halal slaughter of cattle. Source: [77]. Copyright permission was obtained from the Universiti Putra Malaysia.

Figure 4 presents Chalaf used for the kosher slaughter of animals.



Figure 4. Chalaf used for the kosher slaughter of animals. Source: [78]. Copyright permission was obtained from the figure owner on 19 May 2023.

It is highly preferable to use a sharp knife with a durable edge. The sharpness of the blade is determined by several factors, such as the properties of the steel, the relative movements of the blade and target material, the curvature and edge angle of the blade, grinding, and finish [74]. A good sharpness condition needs a cutting force of 25 N and a blunt condition with a 75 N cutting force. Moreover, McGorry et al. [74] concluded that while a sharp blade finish lowers the force needed for elongated cuts through different tissues, remarkably, the finish has a comparable effect for shorter cuts through muscle only as a tendency.

6. Quantifying the Sharpness

The objective quantification of the sharpness of the knife is quite challenging. During religious slaughter, it is carried out by the slaughterman prior to religious slaughter. It is also recommended to sharpen the knife and inspect its sharpness before halal or kosher slaughter by Muslims and Jewish authorities. As sharpness is directly linked with animal welfare compliance and meat quality, there is, thus, an urgent need to objectively quantify the sharpness of knives used in religious slaughter. The objective quantification of the sharpness of the knife is quite challenging. During religious slaughter, it is carried out by the slaughterman prior to the religious slaughter. Thus, it is quite challenging to standardize the sharpness measurement under various slaughtering conditions. The current measurement of knife sharpness is basically done by visual inspection/assessment, which is very subjective and varies with individual experience, training, and expertise. Commercial slaughterhouses have very high slaughter rates, so the step of knife sharpness assessment and maintaining the desired level of knife sharpness could slow down the slaughter rate and warrants extra labor and capital investment.

The traditional method to test the knife sharpness in slaughterhouses is based on a paper test under which a piece of paper is held by one corner, and if the knife can cut the hanging-dangling part of the paper, it is considered to be sharp, and if it fails to do so, then blunt. A sharp knife (in a dry state) should slice a standard paper (A4 printer paper, 80 g weight) hanging by one corner. It is a rapid and cost-saving method but can only detect the sharpness in the center of the knife, leaving untested the other commonly used area of the knife during slaughter operations [75]. Under kosher slaughter after sharpening and in between the slaughter of animals, the shochet carefully checks the knife for roughness and nicks by running his fingernails up and down on the edge of the *Chalaf* (Figure 2). Keeping in mind the sharpness of the *Chalaf*, this should be carried out with the utmost precautions and after proper training.



Figure 2. *Chalaf* sharpness test by shochet during kosher slaughter. Source: [60]. Copyright permission was obtained from the figure owner on 18 May 2023.

slaughtered with a sharp knife (sharpened with a machine with ANAGO sharpness score of 8.0) [51]. Further, the authors observed a significant increase ($p < 0.0001$) in catecholamines (adrenaline), glucose, CK, and LDH post-slaughter in comparison to pre-slaughter in the commercial sharp knife group as compared to the sharp knife group. Increased LDH and CK in the blood indicate muscle damage, fatigue, and stress in animals [4,6,64,65]. Similarly, the release of catecholamines (epinephrine/adrenaline and nor-epinephrine/nor-adrenaline) in the blood indicates an initial reaction to stress/fear [17,66].

An electroencephalogram presents the electrical activity of neurons rapidly, accurately, and objectively [67,68]. These variables are well interrelated with animals' physiological and biochemical parameters [3]. On analyzing electroencephalogram (EEG) variables, a significant increase was noted in the median frequency (F_{50}) ($p < 0.0001$) and total power (Ptot) ($p < 0.0001$) in the animals slaughtered with the sharp commercial knife as compared to those slaughtered with the sharp knife [51]. These parameters could be correlated with pain and stress observed during neck cuts [17,57,69,70]. The significantly increased alpha, beta, delta, and theta waves in the EEG spectrum in cattle after slaughter increased could be attributed to use of razor-sharp knives resulting in a mild behavioral response in animals being non-painful to the animal [38].

A sharp change in the EEG spectrum upon neck cutting is depicted in Figure 1.

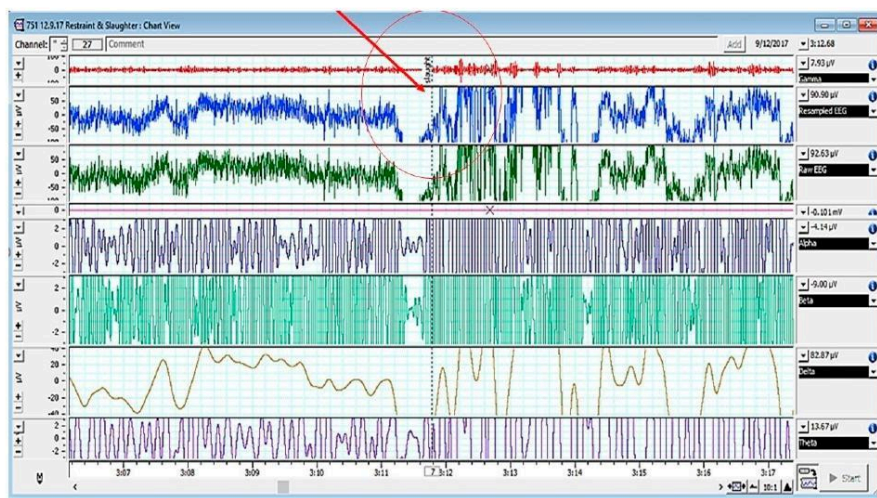


Figure 1. EEG spectrum of cattle pre- and post-slaughter (Note: encircled/red arrow showing the point of slaughter). Adopted from [3].

5. Mechanical Attributes of the Knife

In general, knife sharpness refers to the design, fitness, and quality of cutting-edge design and is measured in terms of the forces needed for cutting [71]. Sharpness can be defined by the level of force exerted by the knife during the cutting of the material [72] or the area of the cutting edge [73]. The main parameters determining the efficiency of all cutting operations depend on the durability, thickness, consistency, and speed accomplished by blades and correlate to the suitable application. In various meat-cutting operations, professional workers have duly acknowledged that the knife blade's sharpness affects the workers' productivity and the product's quality [72]. Further, the grip forces and cutting actions were greater with blunt but workable knife blades vs. recently sharpened knives [72] with a 20–30% decline in grip force, cutting actions, and time with sharper contrast with blunter blades.

Grandin [58] observed that a short knife used in the halal slaughter of cattle indicates that digging the end of the knife blade into the throat caused intense reaction and pain. Muslim scholars recommended a sufficient length of knife used in Halal slaughter with a minimum of 18 cm for cows and 24 cm for buffaloes. Likewise, Velarde et al. [59] observed that the blade length used for halal slaughter was 29.6 ± 1.79 cm, whereas size and shape were more uniform for kosher slaughter (approx. 40 cm *Chalaf* knife). The blade length of knives used for the halal slaughter of sheep (without stunning) was 22.2 ± 1.82 cm and 25 cm in case of kosher slaughter [59]. Table 1 summarizes the blade length of knives used in halal and kosher slaughtering.

Table 1. Blade length (approx. cm) of knife used for halal and kosher slaughtering.

Species	Halal Slaughter	Kosher Slaughter
Cattle	29.6 ± 1.79 cm (Also varies from 18 cm for cow to 24 cm for buffalo)	40.0
Poultry	—	13.5
Sheep	22.2 ± 1.82	25.4

(Source: [59,60]).

Perfect sharpness and proper height of the knife ensure the severing of all jugular and carotids with a rapid stroke resulting in a sudden drop of arterial pressure to the brain and fast and massive blood loss [18]. In a survey of slaughterhouses in Italy regarding kosher slaughter practices, Bozzo et al. [35] observed the majority of rejection (2.4% of total samples) due to failure to comply with a pause (*Shehiyah*) followed by non-compliance to the pressure (*Derasah*) and stabbing (*Halad*) rule. The authors also did not notice any rejection due to non-compliance to slanting and tearing. This further strengthens the requirement for sharp knives of the appropriate dimension for kosher slaughter.

In addition, Abd El-Rahim [61] advocated that the minimum length of the sharp edge of a knife used to slaughter animals should be at least 12 cm. Further elaborating, Helmut [28] recommended that the knife be constantly sharpened and free from scratches and faults as it may cause pain by dragging and grasping the tissues during a neck cut. According to personal observation by Leffert [45], the blades used for religious slaughter in the USA were of 6–18 inch size with significant variations in the degree of sharpness [45]. This could have affected the variation in the onset of unconsciousness in animals slaughtered by religious methods from 10–60 s [45] in the slaughterhouses visited by the author in the USA.

The shorter knife takes more time to perform neck cuts as its tip may gouge the wound and become stuck in the cut, with sawing motion during the cutting procedure. This would stimulate accessible nerve endings in the skin and at the cut edge of the skin leading to potential pain perception [19]. There have been reports of using blunt knives in halal slaughter in various countries [19,62], consequently requiring more pressure and more attempts during neck incisions, potentially provoking pain. In water buffalo, in lateral recumbency, neck twisting is impossible due to the horns' breadth. In such cases, a blade is inserted into the skin, and after the animal has settled down, the blade is repositioned in the wound, and several vertical cuts (up to 7–19 cuts) are made, as observed by Gregory et al. [20]. There is a need for specific legislation, guidelines, and training programs for handlers to improve the welfare of water buffaloes during slaughter [63].

4.3. Sharpness on Blood Biochemical and Electroencephalogram (EEG)

Imlan et al. [51] evaluated the effect of knife sharpness on stress and pain perception in Brahman crossbred steers in terms of blood enzymes, plasma catecholamines, and electroencephalogram (EEG) changes. The authors reported a significant difference between the concentration of pre- and post-slaughter glucose, creatine kinase (CK), and lactate dehydrogenase (LDH) concentration in steers slaughtered with a sharp commercial knife (ANAGO sharpness score of 7.8) commonly used in slaughterhouses as compared to those

unconsciousness, thus reducing the pain during the whole process. In addition to this, it also improves meat quality by reducing petechial hemorrhages. Stressed and excited animals usually take more time to become unconscious than calm animals, thus not meeting the higher animal welfare standards [9,32].

To preserve animal welfare, religious slaughter without stunning requires improved administration. The animal remains conscious during the neck cut, and it will take some time (varies with species or neck cut) to undergo a state of unconsciousness. Thus, the animal remains sensitive to pain and stress between this neck cut and unconsciousness. It was observed that if religious slaughter without stunning was performed with a proper razor-sharp knife on a calm and rested animal, restrained properly/comfortably, then animals showed very little (flinch) or no reaction to the neck cut [43]. This reaction/flinch was noticed even less than an ear tag punch, a metal clanging noise, air hissing, followed by no further reaction afterwards [43]. Similarly, Barnett et al. [44] also observed mild physical response to neck cutting in some birds (100 birds) whereas no response was observed in the majority of birds (592 birds) during kosher slaughter. The authors [44] also observed the presence of physical response to touching the eye or eyelid in birds up to 5 s after a neck cut, which disappeared after 15 s of the neck cut. The loss of posture and presence of involuntary muscular contraction was noticed after 12–15 s of neck cutting and the loss of 40% of total blood within 30 s of neck severance. However, some animal welfare scientists believe that the pain during religious slaughter could be reduced if religious slaughter is performed correctly [45]. Further, low behavioral responses after a neck cut may not necessarily indicate the pain-free status of the animal [45,46].

As blood is an excellent medium for the growth of various microorganisms, maximum possible drainage is recommended for improving the quality of meat. Further, the presence of blood in carcasses could also make the appearance of the carcass dark, which consumers prefer less. Kosherization has also been reported to improve meat quality by reducing *Salmonella* and coliform counts [47]. The hemoglobin in the blood is a potent lipid prooxidant, and efficient bleeding is also recommended to improve the meat's oxidative stability. Further slaughter and bleeding methods have been reported to affect meat color and sensory properties [48,49]. Koshering (slating and washing) also has an effect on meat quality by significantly reducing shear force and drip loss [50].

4.1. Knife Sharpness on Pain Sensation

Very few studies have evaluated the effect on animal welfare and meat quality associated with the sharpness of slaughter knives [51]. If restrained properly without pressure and gently done, cattle were observed to have little or no reaction to the throat cut in three kosher slaughterhouses [43]. When the blade touched the skin, cattle showed a slight flinch which was less vigorous than the animals' reactions to an ear tag, and cattle were observed to remain calm as the cut proceeded [43]. Further, the wound should be open during the incision to prevent pain. Further, the knife should be of sufficient length, so that its tip remains outside the neck during the cut [52].

The presence of nicks on blades is considered to cause irritation while cutting blood vessels and associated tissues during halal and kosher slaughter. Gibson et al. [16,53–57] noted that the electroencephalogram spectrum varies with neck cutting and could be correlated with pain sensation. However, the size of the knife (10 inches) and its machine sharpening used in the study make it difficult to conclude pain and stress in animals during religious slaughter, particularly kosher slaughter [34,38]. Further, the short knife even sharpened on a grindstone used in these studies could cause the tip to gouge the throat [52].

4.2. Sharpness and Blade Length

Sharp knives need a lower force requirement than blunt knives. This lower brute force lowers the damage to the meat. Knife sharpness is correlated with the forces produced and energy needed during the cutting process, the cutting edge, and the cutting surface [39].

width of the neck of the animal to be slaughtered). It is essential for decreasing stress and suffering during animal slaughter [33]. The sharpness and size of the knife (*Chalaf*, also known as *Chalef/Chalof/Chalif*), along with the rigorous training and inspection by Jewish authorities, ensure a proper supply of kosher meat and its production [34].

Kosher slaughter is completed in five phases, viz., selection of animal (cloven hooves and ability to ruminate as per The New International Version of the Bible 2011, Leviticus XI), health inspection of the animal, slaughter, inspection (*bedika*), and cleaning (*nikkur*) of meat, and koshering (washing and salting meat for removing blood) [27]. *Shechitah* is performed by making a clean incision at the front of the neck and cutting the trachea, esophagus, carotid arteries, and jugular veins by using a *Shechitah* knife (*Chalaf*, derived from the Hebrew word meaning ‘to change’) with a *Shochet* (authorized slaughter man) as per fundamental commandment, conveyed via the Oral Law and dating back to the time of Moses [18].

The *Chalaf* is designed to have exquisite sharpness and is repeatedly inspected between animals to avoid imperfections [18]. There are five principles of halachic (traditional body of Jewish law) during the *Shechitah* viz. *Shehiyah*/pause (uninterrupted incision), *Derasah*/pressure (no pressing of the blade against the neck), *Halad*/stabbing (adequate length of the blade so it does not get covered with wool, feathers, or hide), *Hagramah*/slanting (severing neck at the appropriate point for neat, clean, and efficient incision), and *Ikkur*/tearing (no tearing of tissues) [18,35]. An animal is deemed unfit if any problem is found with the knife or the neck cut [36].

Chalaf should be perfectly smooth and razor-sharp without any nicks or serrations to facilitate the slaughter process as painlessly as possible. It is twice the length of the neck of the animals going to be slaughtered (poultry: 14–16 cm, sheep and goats: 25 cm, cattle: 40–45 cm) [37]. Before and after *Shechitah*, *chalaf* must be inspected along with fingernails to ensure compliance with Jewish slaughter regulations during the cut, and any presence of a nick makes the meat unfit for consumption (*terefa/terefah*) or rejection [34]. In chicken slaughter, the *Chalaf* may be inspected following the killing of all animals in a group and checked for nicks, with the provision that all slaughtered birds are considered *terefah*/rejected if nicks are discovered [38].

Further, detecting a nick is tricky as a trained *shochet* may detect a nick that generally goes unnoticed by the trained sharpener [38]. It is mandatory to check the neck of animals to ensure the absence of any materials that may damage the knife. In case of potential knife-damaging materials such as dirt, dust, soil, etc., the animal should be washed. This process during kosher slaughter slows down the slaughtering process in addition to being a cause of stress. In religious slaughter, washing animals while entering the lairage is widely followed so that animals will have sufficient time to recover from stress [38]. However, proper care should be taken in cold climatic conditions, as it may lead to severe cold stress in animals.

4. Knife Sharpness in Animal Welfare during Slaughter

Sharpness significantly affected the forces generated and energy required during the cutting process, the cutting-edge durability, and the surface finish [39]. In the USA, Section 1902 of the Humane Methods of Livestock Slaughter Act of 1978 stipulates religious slaughter by a method in which “the animal undergoes unconsciousness by the lack of blood to the brain due to the instantaneous severing of carotid arteries with a sharp instrument and proper handling in accordance with such slaughter” [40].

The knife design and neck cut process were crucial in preventing the animal from reacting to the cut, ensuring rapid blood flow [28,41]. The knife’s sharpness and a clean and uninterrupted cut also influence vasoconstriction, clotting, and ballooning resulting in carotid occlusion/false aneurysm [42] due to constriction of the caudal end of the severed carotid arteries. While the sharpness of the knife influences neck cutting, the method of neck cutting and the number of cuts influence pain perception [19]. A rapid and efficient cut by a razor-sharp knife is crucial for maximum blood loss and early onset of a state of

حَدَّثَنَا عُمَرُو بْنُ عَلِيٍّ، حَدَّثَنَا يَحْيَى، حَدَّثَنَا سُفْيَانُ، حَدَّثَنَا أَبِي، عَنْ عُبَايَةَ بْنِ رِفَاعَةَ بْنِ رَافِعِ بْنِ خَدِيجٍ، عَنْ رَافِعِ بْنِ خَدِيجٍ، قَالَ قُلْتُ يَا رَسُولَ اللَّهِ إِنَّا لَأَقُو الْعُدُوَّ غَدًا، وَلَيْسَتْ مَعَنَا مُدَى فَقَالَ اغْجَلْ أَوْ أَرِنْ مَا أَنْهَرَ الدَّمَ وَذَكِّرْ ائِمَّ اللَّهَ فُكُلٌ، لَيْسَ الْبِسنَ وَالظَّفَرُ، وَسَأُحَدِّثُكَ، أَمَّا الْبِسنَ فَمُعْظَمٌ، وَأَمَّا الظَّفَرُ فَمُدَى الْحَبَشَةِ وَأَصْبَنًا نَهَبَ إِبِلٍ وَعَنَمٌ قَدْ مَبُتَّهَا بَعِيدٌ، فَرَمَاهُ رَجُلٌ بِسَهْمٍ فَخَبَسَهُ فَقَالَ رَسُولُ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ إِنَّ لِهَذِهِ الْإِبِلِ أَوَابِدَ كَأَوَابِدِ الْوَحْشِ، فَإِذَا غَلَبَكُمْ مِنْهَا عُنَى، فَافْعَلُوا بِهِ هَكَذَا ،

The incision should be instantaneous, with one uniform directional movement, and achieved without interruptions, uncertainty, or force [28].

Furthermore, the cut must be made from the ventral position of the neck near the lower jaw and just before the spine [28]. This is following hadith that “the jugular veins and the carotid arteries (*wadaja’an*), in addition, to the throat (*hulqum*) and the trachea (*marī*)”, but the vertebral or spinal cord must not be cut.” The head must not be wholly separated from the remaining body during slaughter.

“The knife must be razor sharp and without blemishes and damage. For animals with normal necks, the slaughter must begin with an incision on the neck just before the glottis, and for animals with long necks, such as chickens, turkeys, ostriches, camels, etc., the incision must be before the glottis”.

“... must be done once only. The slaughtering implements must not be lifted off the animal during slaughtering. Any lifting is construed as one act of slaughter. Multiple acts of slaughter on one animal are prohibited”.

Under Halal, acceptable animals and birds are slaughtered with a razor-sharp knife to have a swift, deep incision cutting the front of the esophagus, trachea, jugular veins, and carotid arteries [29]. In Halal slaughter, proper emphasis is given to the knife’s sharpness to facilitate rapid and efficient blood drain and the onset of unconsciousness.

3. The Kosher Perspective of Knife Sharpness

Kosher slaughter/*Shechitah*/*Shechita* is the animal slaughter method followed by the Jewish community derived from a *mitzvà* (commandment) mentioned in the book of Deuteronomy 12:21

“And ye shall be men of holy calling unto Me, and ye shall not eat any meat that is torn in the field” (Exodus XXII:30)

“...thou shall kill of thy herd and of thy flocks, which the Lord hath given thee, as I have commanded thee...” (Deuteronomy XII:21). “[...] you may slaughter animals from the herds and flocks the Lord has given you, as I have commanded you, and in your towns, you may eat as much of them as you want”.

Rapid blood loss and maximum bleed-out is recommended in Kosher slaughter as

“Only be sure that thou eat not the blood: for the blood is life” (Deuteronomy 12:23)

In Kosher meat production, three factors, viz., permitted animals, strict prohibition of blood, and mixing meat with milk, are vital considerations [30]. Wild birds and pigs are considered impure in kosher diets [31]. In Jewish law (*Halacha*), importance is given to compliance with animal welfare and avoiding pain and stress during slaughtering (Ha Levi A. 13th cent, Karo 1563d) [30]. During *Shechitah*, Jewish law (*Halacha*) emphasizes the suitability of a knife (sharpness and size), immobilization of animals, and neck cut (correct knowledge of anatomy and physiology, and skill [32].

In kosher slaughter, the knife’s sharpness, its absence of nicks, a pre-slaughter examination of the knife’s sharpness, and its size are all given careful consideration (double the

A clean neck cut at the proper position using a very sharp knife facilitates rapid blood loss and fewer incidences of false aneurysm formation, thereby leading to the early onset of unconsciousness. Specific details about sharpness and its dimensions for the *Chalaf* are mentioned for kosher slaughter. However, this very critical factor is largely overlooked in scientific literature. In this context, this paper critically reviews the significance of knife sharpness in religious animal slaughter without stunning under the broad ambit of animal welfare principles by alleviating stress and pain, this ultimately improving meat quality. The manuscript also reviewed various factors that could render knife sharpness as an important determinant of animal welfare during slaughter such as the position of the neck cut, knife dimension, training/expertise of the slaughterman, restraints, and presentation of the animal during slaughter.

2. Sharia's Perspective of Knife Sharpness

A sharp knife is recommended for halal and kosher slaughter for efficient bleeding, alleviating pain, and producing quality meat [26,27]. Islam prescribes proper guidelines for ensuring the sharpness of knives and the slaughter of an animal by complying with animal welfare principles mentioned in the Holy Quran and Hadiths.

“Certainly Allah has decreed proficiency in all things. Thus ... if you perform slaughter (zabih), perform it well (painlessly). Let each of you sharpen his knife/blade and let him minimize suffering to the animal he slaughters (zabiha die painlessly/peacefully).”

“Allah calls for mercy in everything, so be merciful when you kill and when you slaughter; sharpen your blade to relieve its pain.” (Al-Qaradawi, 1994).

“Allah has commanded you to treat all creatures with kindness. When you slaughter an animal, do so kindly. Sharpen the knife well and give comfort to the animal being slaughtered.”

The Islamic tradition strongly advocates the humane slaughter of animals to alleviate pain and suffering. For example, *Sahih Muslim (Book 21, Chapter 11, Number 4810)* records Prophet Mohammad (PBUH) saying:

“Verily Allah has enjoined goodness to everything; so when you kill, kill in a good way, and when you slaughter, slaughter in a good way. So every one of you should sharpen his knife and let the slaughtered animal die comfortably.”

“When one of you slaughters, let him complete it.”

حَدَّثَنَا أَبُو بَكْرِ بْنُ أَبِي شَيْبَةَ، حَدَّثَنَا إِسْمَاعِيلُ بْنُ أَبِي عُلَيْيَةَ، عَنْ خَالِدِ بْنِ الْحَذَّاءِ، عَنْ أَبِي قِلَابَةَ، عَنْ أَبِي الْأَشْعَثِ، عَنْ شَدَّادِ بْنِ أَوْيسٍ، قَالَ ثُبَّتَانِ خَفِظَتُهُمَا عَنْ رَسُولِ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ قَالَ إِنَّ اللَّهَ كَتَبَ الْإِحْسَانَ عَلَى كُلِّ شَيْءٍ فَإِذَا قَتَلْتُمْ فَأَحْسِنُوا الْقِتْلَةَ وَإِذَا ذَبَحْتُمْ فَأَحْسِنُوا الذَّبْحَ وَلْيُجِدْ أَعْدَكُمْ شَفَرَتَهُ فَلْيُرَخَّ دَبِيحَتَهُ

In accordance with the Prophet's (PBUH) commandment that at the point of slaughter, the knife's cutting edge must be well sharpened (Jama'ulFawa'id). In Islam, using bones, claws, teeth, nails, and alike is strictly forbidden. This implies less pain to the animals during slaughter.

“Use everything to slaughter which allows blood to flow, except for teeth and nails, and all else is permissible” (Sahih Bukhari, p 827; Sunan Abu Dawood).

1. Introduction

The World Organization for Animal Health (WOAH) defines animal welfare as “the physical and mental state of an animal concerning the condition in which it lives and dies” [1]. It consists of the responses of an animal to socio-physiological factors, escapes or avoidance behavior, animal physiology, and biochemistry [2]. Each society has its interpretation of animal welfare based on its moral and ethical values and welfare standards [3]. With increasing awareness, and education, consumers prefer food not only for good nutritive quality, sustainability, and processing but also for ethical and spiritual quality. Consumers are more concerned about handling animals and management practices involved during meat production, such as stunning prior to slaughter, and free-range chicken production. The animal welfare issue has taken center stage in global meat production and marketing [4–9]. Further, a well-established link between pain or stress during slaughter and its negative impact on meat quality warrants the immediate attention of researchers and policy-makers for proper animal welfare compliance during animal slaughtering [3].

The pre-slaughter handling and slaughter process is crucial from an animal welfare perspective as it comprises converting the live animal into edible pieces of meat for human consumption. Several pre-slaughter factors cause an animal stress, fear, pain, and distress, thus compromising their welfare [10,11]. Pain is “an unpleasant sensory and emotional experience associated with or resembling real or potential tissue injury” [12]. Stress is a complex physiological state that comprises a range of integrative and behavioral changes in response to a real or perceived threat to homeostasis. In contrast, fear denotes a condition of danger perception or potential harm that could compromise an animal’s safety [13]. Distress denotes a negative and aversive state under which the ability of an animal to cope and adapt is impaired [14]. Pre-slaughter handling practices have a significant effect on animal stress, pain, distress, and fear, such as transportation conditions, loading and unloading, stocking density, water and feed availability, attitude and training of animal handlers, and slaughtering practices (such as stunning, restraints, knife sharpness, and training of butchers). Knife characteristics, especially knife sharpness, are very crucial among these factors.

During kosher and halal slaughter, animals should be restrained by using minimum stressful methods to hold the animals for neck cuts. Gentle handling and less stressful restraints lower the issue of delayed periods of consciousness after a neck cut and improve animal welfare [15]. These considerations also affect the reaction of the animal to a throat cut [16–18], the issue of prolonged consciousness after the neck cut [19], and the perfusion of blood in the respiratory tract [20]. Tight/robust restraints cause stress—especially in cases where animals are turned on their side or back in a rotatory casting pen—substantial tissue damage, and prolonged time to reach the stage of unconsciousness, thereby feeling pain, distress, anxiety, and suffering [21]. The issue of gentle handling and less stressful restraining methods are more critical in the slaughter of cattle without stunning as compared to sheep. This could be due to the larger body size of cattle [15] and anatomical differences in the blood supply to the brain; thereby taking longer time to reach the stage of unconsciousness in cattle as compared to sheep [22,23]. The World Organization for Animal Health (WOAH, previously known as OIE) recognized fully inverted, upright, and lateral/sideway restraints for animals, with suspending only allowed in poultry [24].

Even though religious authorities (both Islam and Judaism) and sacred texts emphasize the importance of using a sharp knife and maintaining its sharpness during slaughter, there is very little scientific evidence available to corroborate this element, especially in the context of ritual slaughter (halal and kosher slaughter). Religious slaughter implies the slaughter of permissible animals by severing the trachea, esophagus, and blood vessels using a sharp blade and following laws as prescribed by rituals (blessings/invocations) that characterize its purity/ethical value [25]. Islam and Judaism have given utmost importance and zero tolerance towards animal welfare compliance during slaughter and strongly advocate benevolent and compassionate treatment of animals.

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