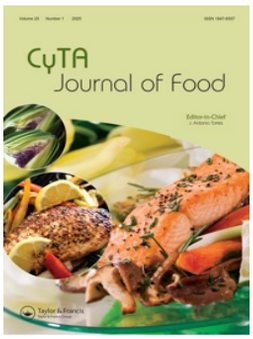

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Effects of preslaughter management on oxidative stress, Warner-Bratzler shear force, heat shock protein 27 expression, and apoptosis index of *Longissimus thoracis et lumborum* in goats

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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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KEYWORDS
Transport and lairage; oxidative stress; heat shock protein 27; apoptosis index; meat tenderness

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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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 ± 1.07°C (Mean ± SE), with the max recorded as 34.03 ± 1.06°C, and the mean relative humidity was 72.63 ± 3.79% (Mean ± SE), with the max recorded as 84.35 ± 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° 58'59.0" N 101° 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 m^2/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

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/cm^2 (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):

$$\text{Apoptosis Index} = \frac{\text{Total count of TUNEL} - \text{positive cells}}{\text{Total counted nucleated cells}} \text{x100}$$

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 postmortem ageing ($p < .05$).

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.

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

	HSP27						
Ageing Days	TS2L3 ($n = 3$)	TS2L6 ($n = 3$)	TS2L12 ($n = 3$)	TS6L3 ($n = 3$)	TS6L6 ($n = 3$)	TS6L12 ($n = 3$)	SEM
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/cm^2) of *longissimus et thoracis lumborum* (LTL) muscles subjected to different lairage, transportation, and ageing days.

	Shear Force (kg/cm$^{2)}$						
Ageing days	TS2L3 ($n = 3$)	TS2L6 ($n = 3$)	TS2L12 ($n = 3$)	TS6L3 ($n = 3$)	TS6L6 ($n = 3$)	TS6L12 ($n = 3$)	SEM
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	$1.13^{b,x}$	$1.55^{d,y}$	$1.16^{b,y}$	$1.31^{c,y}$	$1.26^{c,y}$	$1.03^{a,x}$	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.

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.

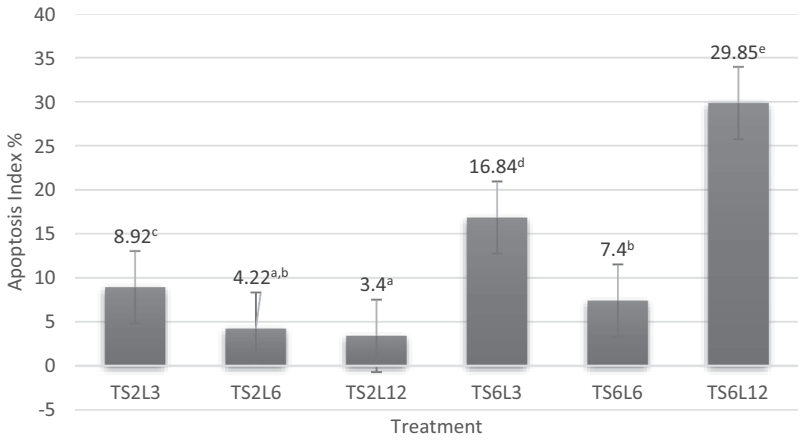

Figure 1. Apoptosis index (%) (mean ± S.E.) of LTL muscles subjected to different lairage, transportation, and ageing days.
[a,b,c,d,e]Values with different superscripts at the same column are significantly different ($P < 0.05$).

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

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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