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Effects of knife sharpness on muscle biochemistry, physicochemical and meat quality attributes in cattle

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ABSTRACT

This study evaluated the effect of knife sharpness on bleeding, physicochemical and meat quality parameters in cattle. A total of 20 cattle (Brahman crossbreed steers, 420.6 ± 24.60 kg were divided into two groups and slaughtered by using a sharp knife (ANAGO sharpness score above 8.0) and a commercially/less sharp knife (LSK) (minimum ANAGO score of 7.8). The carcases were evaluated for residual haemoglobin, myoglobin, pH, muscle glycogen, muscle lactate, myofibrillar fragmentation index, sarcomere length, water holding capacity, cooking loss, shear force, protein carbonyls, thiobarbituric acid reacting substances and microbial quality. Animals slaughtered in the LSK group exhibited higher ($p < 0.0001$) lactate, shear force, sarcomere length and myofibrillar fragmentation index than the sharp knife group. Determining the carbonyl and thiol content revealed that the protein oxidation increased with ageing time but was not affected by the slaughter knife. The findings indicate that less-sharp knives caused substantial physiological stress responses, compromising animal welfare and meat quality.

HIGHLIGHTS

The study brings to the world knowledge findings highlighting the significance of knife sharpness:

- The study is novel as it depicts the effects of knife sharpness on physicochemical meat quality metrics, solely focusing on bleeding efficiency.
- The precise effects of knife sharpness on muscle biochemistry and structure, a rare mechanistic insight.
- The study differentiates the influences of ageing and slaughter techniques, enhancing the understanding of post-mortem meat quality dynamics.

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Introduction

Animals are inevitably handled during various operations during slaughter, thus affecting their welfare status. Animal welfare during slaughter has taken centre stage in meat production, as it is directly correlated with meat quality and consumer acceptance (Kumar et al. 2023b). During the slaughtering process at the slaughterhouse, along with gentle restraints, the sharpness of the slaughter knife plays a vital role in reducing pain and stress, thereby improving animal welfare (Kumar et al. 2023a).

The pain and stress in animals during slaughter, as induced by the inappropriate sharpness of the knife, may cause adverse effects on the physiological state of animals due to the initiation of sympathoadrenal (SPA) and hypothalamic–pituitary–adrenal axis (HPA) (Kumar et al. 2023b). These changes have an adverse effect on carcase and meat quality (Loudon et al. 2019). The use of a sharp knife during cattle slaughter (ANAGO score of 8.0 or higher) resulted in significantly reduced pain and stress, as evidenced by lower post-slaughter levels of adrenaline ($p < 0.0001$), glucose, creatine kinase and

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lactate dehydrogenase, compared to those slaughtered with a commercial sharp knife (Imlan et al. 2020). The study reported significantly lower median frequency (F50) and total power (ptot) of electroencephalogram (EEG) in cattle slaughtered with a sharp knife compared to those slaughtered with a commercial sharp knife ($p < 0.0001$) (Imlan et al. 2020).

Grandin (1994) suggested that the design of the knife and the cutting procedures are crucial in preventing the animal from reacting to the cut. A knife should have a razor-sharp edge and be free from nicks and scratches to reduce pain by preventing the dragging and grasping of the flesh (Pleiter 2010). The cut should not reach the spine and must be made at a ventral neck position near the lower jaw, severing both carotid arteries, jugular veins, trachea and oesophagus to allow blood flow (Nakyinsige et al. 2013). One of the significant advantages of using an extremely sharp knife is that the cut is smooth and less painful, with proper vessel severing, which allows for adequate bleeding and rapid loss of consciousness, rendering the animal insensitive to stress and pain (Kumar et al. 2023a). In addition, incomplete bleeding also causes adverse effects on the meat quality by negatively impacting the meat colour, increasing oxidative changes, and microbial growth (Gregory et al. 2012).

Bleeding is paramount during slaughter to produce good quality meat by improving colour and extending shelf-life. Therefore, inefficiency in bleeding out animals at slaughter deteriorates the meat quality (Aghwan et al. 2016). Poor bleeding of animals affects the appearance and meat acceptability (Gregory et al. 2012). Blood, a perfect medium for microbial growth, also affects meat quality. For facilitating rapid blood flow and immediate loss of consciousness, the use of an extremely sharp knife is recommended by animal welfare scientists and religious scholars (Kumar et al. 2023a, 2023b). The halal method of slaughter or the use of a razor-sharp knife provides considerable bleeding, which is beneficial for the extension of the storage life of meat and the maintenance of its quality (Farouk et al. 2014).

The use of a sharp knife is well described and given utmost importance in the Kosher and Halal slaughter of animals. There is still a lack of scientific studies highlighting the role of sharp knives on physicochemical and meat quality attributes in beef. Therefore, this study aims to determine the effect of knife sharpness on the various physicochemical and meat quality parameters in steers.

Materials and methods

Ethical note

The study was conducted following the animal ethics guidelines of the Research Policy of Universiti Putra Malaysia (UPM/IACUC/R028/2016).

Animals and slaughtering procedure

Twenty crossbreed steers with a live weight of 420.60 ± 24.60 kg were procured from Katherine, Northern Territory, Australia, and transported by sea over 14 days from Port Darwin, located at Latitude 12° 25′ S and Longitude 130° 47′ E, to Johor Port Berhad Pasir Gudang, Malaysia, situated at Latitude 1° 26′20″ N and Longitude 103°53′25″ E. The steers were transported by road from Pasir Gudang Port to Universiti Putra Malaysia, Serdang, Selangor. They were housed at the ruminant facility of the Institute of Tropical Agriculture and Food Security at Universiti Putra Malaysia (latitude 2° 59′ 06.5″ N, longitude 101° 43′ 40.7″ E) for five months for fattening purposes. The animals were transported 30 km by road to the Ruminant Commercial Abattoir, Department of Veterinary Services, located in Shah Alam, Selangor, Malaysia, for slaughter. The steers were slaughtered as per the descriptions of Imlan et al. (2020).

Experimental design

Victorinox® Fibrox Swiss Army knives, with a length of 30.48 cm, were obtained from Switzerland for the experiment. Cattle were divided into two groups of ten each. One group was slaughtered using a sharp knife, resulting in an ANAGO score exceeding 8.0, while the other group was severed with a less sharp knife (LSK), with an average ANAGO score of 7.8. The knives' blades were sharpened using a machine following each killing. Imlan et al. (2020) demonstrated that the common practice of not sharpening or manually sharpening dull knives yielded a maximum sharpness score of 7.80. The experimental knives were new and exhibited an unboxed sharpness score of 7.8, the minimum standard for a commercial slaughterman's knife. The pH, lactic acid, sarcomere length and glycogen content were assessed in muscle samples at intervals of 0, 1 and 7 d of ageing. Colour values were measured at 0, 1, 7 and 14 d of storage, while drip loss was computed at 1, 3, 7 and 14 d. During the 0–7 and 14-day ageing periods, we assessed shear force, cooking loss, myofibrillar fragmentation index, protein

oxidation and meat lipid content, and conducted microbiological examinations.

Residual haemoglobin and myoglobin analysis

Following the descriptions outlined by Nakyinsige et al. (2014) method for extracting LTL muscle haeme protein utilising an ice-cold buffer consisting of 80 mM KCl and 50 mM Tris–HCl at pH 8.0. The haemoglobin concentration was assessed through a modified kinetic method that utilised haem to decompose hydrogen peroxide into water and oxygen. The emerging oxygen oxidised o-tolidine, resulting in a green-blue colouration. The absorbance of haem proteins was assessed at 364 nm with a UV–visible spectrophotometer (Varian Australian, PTY LTD, Australia) and compared to a standard solution of bovine blood haemoglobin (H262G, Sigma-Aldrich, St. Louis, MO). Following the removal of haemoglobin from the supernatant *via* ammonium sulphate (0.525 g/mL), centrifugation (2000 *g*, 21 °C for 45 min), and filtration (0.45 μm diameter, Denville® syringe filter), myoglobin levels were assessed using a modified kinetic method involving o-toluidine. A standard solution was prepared using an equine heart solution (Sigma-Aldrich, M1882). The same procedure applies to total haem quantification O'Brien et al., (1992). . The haem underwent oxidation with a 100 μM K3Fe(CN)6 stock solution. The Soret band at 420 nm, along with absorbance measurements at 540 and 580 nm, was utilised to quantify haem protein levels. Oxidised equine myoglobin exhibits extinction coefficients of 3.19/g cm at 540 nm, 1.95/g cm at 580 nm and 35.7/g cm at 420 nm.

Myofibrillar fragmentation index analysis

The myofibrillar fragmentation index of LTL muscle was determined as described by Hopkins et al. (2000). The pulverised muscle sample was homogenised for 60 s in 30 mL of 20 mm ice-cold KH2PO4 buffer containing 100 mm KCl, 1 mm EDTA and 1 mm MgCl2. This was followed by centrifugation at 1000 *g* for 15 min at 2 °C, after which the supernatant was removed, and the procedure was repeated. After removing the connective tissues by filtration through 50 mL conical tubes using 1.0 mm polyethene strainers, followed by rinsing with 15 mL of the buffer, the concentration of protein for the final suspension was determined using the Bio-Rad Protein Assay Kit II 500-0002 following the colourimetric analytical method.

Meat quality assessment

Muscle glycogen, lactate and pH. The LTL muscle glycogen and lactate content were assessed using kits EnzyChromTM Glycogen Assay Kit (Cat# E2GN-100) and EnzyChrom™ Lactate Assay Kit (# ECLC-100), respectively, following the protocols recommended by the manufacturers. The muscle pH was estimated using a pre-calibrated portable pH metre (Mettler-Toledo AG, Zürich, Switzerland) at pH 4.0 and 7.0. Approximately 0.5 g of muscle samples were homogenised in 10 mL of ice-cold deionised water for 30 s. During homogenisation, 5 mm sodium iodoacetate was added to stop glycolysis.

Drip loss measurement. The drip loss of the samples was assessed as per Honikel (1998). A 20 g fresh sample was weighed (W1), and the sample was stored in a 4 °C chiller for 14 d under vacuum in a polyethylene bag. After the designated time interval, the meat samples were removed from the bags, gently blotted to dry, and weighed (W2). The percentage drip loss was estimated using the following formula: Drip loss (%) = [(W1- W2) ÷ W1] × 100.

Colour profile testing. The meat colour (CIE, L*, a*, b*) was recorded using a Colour Flex spectrophotometer (Hunter Lab, Reston, Virginia, VA). The instrument was set to a 10° standard observer, D56 illuminant, and a 400–700 nm reflectance range. Before use, the equipment was calibrated against black and white tiles supplied with the equipment. The muscle samples were incubated for 30 min. The triplicate reading was taken for each sample by rotating the sample at 90° in the following two observations (the cup rotates).

Cooking loss. The cooking loss of meat samples was measured by observing the weight of the meat samples before (W1) and after cooking (W2). The samples were cooked in a preheated water bath at 80 °C for 10 min after reaching an internal temperature of 78 °C.

The percentage of cooking loss was estimated using the following formula:

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

Shear force and sarcomere length measurement. The shear force of the cooked sample (1 cm height × 1 cm width × 2 cm length) was determined by using a texture analyser (TA. HD plus®, Stable Micro System, Surrey, UK) equipped with a Volodkevitch bite jaw as per the method followed by Sazili et al. (2013). The instrument was standardised at a 5 kg weight and a

10 mm/s speed. The sarcomere length of the samples was evaluated by fixing the muscle tissue in 30 mL fixative solution (5 mm EDTA, 0.039 M boric acid, and 0.1 M KCl in 2.5% glutaraldehyde) for 2 h, followed by incubation at 4 °C for 24 h (Kandeepan et al. 2009). The fixed muscle tissue was homogenised in 14 mL 0.25 M sucrose solution for 15 s at 6000 rpm with a homogeniser (T18 digital ULTRA-TURRAX® - IKA, Germany), and a drop of resultant homogenate was put on a glass slide and examined under an image analyser microscope (Olympus, BX. 51. TF, Tokyo, Japan).

Lipid oxidation measurement. Lipid oxidation, as measured by 2-thiobarbituric acid reactive substances (TBARS) in meat samples, was measured using QuantiChrom™ TBARS Assay Kit (DTBA-100, BioAssay Systems, Hayward, CA). The standard colourimetric protocol prescribed with the kit was followed to calculate the TBARS value.

The determination of free thiols. The free thiol content was determined following Ellman's technique of using 2, 2-dithiobis (5-nitropyridine), DTNP. With slight modifications as outlined by Morzel et al. (2006). Washed pellets containing 4 mg of myofibrillar proteins were dissolved in 3 mL of 100 mM phosphate buffer at pH 8 containing 8 M urea. About 30 μL of 10 mm DTNP (stock solution in ethanol) was added, followed by incubation for 1 h at room temperature. The absorbance at 386 nm was measured using a spectronic®20 GENESYSTM spectrophotometer (Spectronic Instruments, Waltham, MA) against a blank buffer without protein. The absorbance of the blank was subtracted, and the thiol concentration was estimated using an absorption coefficient of $14\,mm^{-1}\,cm^{-1}$. The final results were expressed in nanomoles of free thiol per milligram of protein.

The determination of protein carbonyls. Carbonyl groups were estimated using the Protein Carbonyl Colourimetric Assay Kit, cat # 10005020 (Cayman, Ann Arbor, MI), following the manufacturer's instructions. Concisely, samples were crushed manually in liquid nitrogen. Approximately 0.25 g of frozen crushed muscle tissue was rinsed with phosphate-buffered saline to eliminate red blood cells or clots. After rinsing, the tissue was homogenised (T18 digital ULTRA-TURRAX® - IKA, Germany) for 30 s on ice in 1.5 mL of 50 mm ice-cold MES buffer at pH 6.7 containing 1 mm EDTA. The homogenate was then centrifuged using a microcentrifuge (Eppendorf Centrifuge, Mikro 22 R Hettich, Germany) at 10,000 *g* for 15 min at 4 °C. Exactly 200 μl of the samples were transferred to two labelled 2 mL microcentrifuge tubes (sample and control tubes). To each sample and control tube, 800 μL of DNPH and 2.5 M HCl were added, and the mixture was incubated in the dark at room temperature for an hour with brief vortexing of the samples every 15 min. One millilitre of 20% TCA was added to each sample, and the control tube was placed on ice and incubated for 5 min. This was followed by centrifugation at 10,000 *g* for 10 min at 4 °C. The pellets were washed twice with 1 mL ethanol/ethyl acetate mixture (1:1), and the centrifugation was repeated. After the final washing, 500 μL of guanidine hydrochloride was pipetted into all tubes, followed by vortexing. The protein suspensions were then centrifuged to remove the remaining debris. Lastly, 220 μL of supernatant from the samples and controls were loaded in duplicate into wells of a transparent flat-bottom 96-well plate, and the optical density (OD) was immediately determined at 370 nm using an auto UV Xenon flash lamp microplate reader (infinite M200, Tecan, Austria). The protein carbonyls (nmol/mL) concentration of the samples was calculated using the following equation:

$$\text{Protein carbonyls (nmol/mL)} = [(\text{CA}/0.011\,\mu M^{-1})]\,(500\,\mu L/200\,\mu L)$$

where CA is subtracting the average absorbance of controls from the average absorbance of the sample.

The proteins lost during the final washing steps were determined by transferring 100 μL of the sample and Control from the wells to a 1 mL quartz cuvette and adding 900 μL of guanidine hydrochloride. Absorbance was measured at 280 nm wavelength using a spectrophotometer (Cary 50 probe UV–visible spectrophotometer, Varian Australia, PTY LTD, Australia). A bovine serum albumen (BSA) standard curve (0.25–2.0 mg/mL) dissolved with the guanidine hydrochloride and read using a spectrophotometer at 280 nm was used to calculate the amount of protein. The amount of protein lost was measured using the formula:

$$\text{Protein concentration (mg/ml)} = [\text{A280} - \text{y-intercept})/\text{slop}] \times 2.5 \times 10.$$

where 2.5 is the correction factor that returns the result to the original sample, 10 is a dilution factor. The final concentration of carbonyl in LL muscle was measured according to the following equation and was expressed as nanomoles of DNPH per milligram of protein:

$$\text{Carbonyl content (nmole/mg)} = \text{carbonyls (nmole/ml)/protein (mg/ml)}.$$

Microbiological analysis. Microbiological counts (Total aerobic count, Enterobacteriaceae, *Pseudomonas,* and Lactic acid bacteria) of *Semitendinosus* muscle samples were assessed by following the methods described by Spec (1984). The Petri plates were incubated at 32 °C for 72 h for all estimated microbial counts, except for *Pseudomonas* spp, and incubated at 25 °C for 72 h (Bórnez et al. 2009). The total bacterial count was calculated by counting the number of colonies and multiplying this number by the dilution factor to get the log cfu/g value. A colony counter (Stuart®, USA) was used for counting.

Statistical analysis

The experiment was conducted in a factorial design. The data were analysed using the GLM procedure of the Statistical Analysis System (SAS) package version 9.2 software (SAS Inc., Cary, NC)(SAS, 2007), and statistical significance was set at $p < 0.05$. Duncan's multiple range test was used to elucidate significantly different means.

Results and discussion

pH values

Table 1 reveals that knife sharpness did not impact muscle pH. Muscle samples from animals slaughtered with sharp and dull knives did not differ. However, both groups experienced a significant ($p < 0.0001$) drop in muscle pH throughout ageing. In contrast to D'Agata et al. (2009), pH somewhat stabilised from d 2 to d 6 in conventional slaughter but rose significantly in ritual slaughter.

Muscle glycogen content

Table 1 shows that muscle glycogen content was unaffected by knife sharpness and sampling duration. The glycogen content of meat samples from animals slaughtered with sharp and less sharp knives was similar on day 0 ($p > 0.05$). Muscle glycogen content was significantly higher ($p < 0.05$) in meat samples from animals slaughtered with sharp knives on day 1 compared to those with less sharp knives.

Also, sharp knives may preserve muscular glycogen stores better than less sharp knives due to less stress and effort. Halal-slaughtered rabbits with restraint and sharp knives had decreased glycogen levels (Nakyinsige et al. 2014).

Additionally, post-mortem glycogen content decreased significantly with ageing (Regenstein 2020).

Muscle lactate concentration

Table 1 shows that a significant interaction was recorded between knife sharpness and the sampling point for lactate. Muscle lactate content was significantly higher ($p < 0.05$) in meat samples from animals slaughtered with less sharp knives on day 1 than those with sharp knives. In both groups, animals slaughtered using sharp and less sharp knives showed a significant ($p < 0.05$) increase in muscle lactate from 0 to 7 d of ageing. Proper preslaughter handling reduces animal stress. Animals handled well before slaughter exhibit decreased serum lactate and stress (Warriss and Brown 2000).

Stress caused considerably higher lactate content in meat samples from animals slaughtered with a LSK. Also, the study found that animals with less-sharpened knives accumulate more lactic acid. During post-mortem ageing, slaughter knife sharpness impacted lactate concentration at days 1 and 7. Lactate rises on days 1 and 7 with glycogen synthesis. Consistent with Ferguson and Gerrard (2014), lactate levels rose ($p < 0.05$) during storage due to glycogen breakdown.

Glycolytic potential

Table 1 shows the glycogen potential, indicating all components in the muscle that can be converted into lactic acid. It refers to the potential lactate formation in the muscles at an exsanguination state. No significant interaction was observed between knife sharpness and the sampling point for glycolytic potential. In this study, neither the sharpness nor the ageing period affected glycolytic potential.

Protein thiols

Table 1 shows that the application of sharp and less sharp knives during the slaughtering of cattle was not observed to have a significant interaction between treatment and sampling. The thiol content decreased significantly ($p < 0.05$) from 0 to 7 d. No difference was observed in thiol values for the meat samples collected on days 1 and 7 from the animals slaughtered with sharp and less sharp knives.

Meanwhile, thiol values were higher ($p = 0.0167$) on d1 in the samples from animals with less sharp knives than those with sharp blades. Similar findings were reported by Nakyinsige et al. (2014) in halal *vs.* gas-stunned rabbits and (Sabow et al. 2015) in stunned *vs.* non-stunned goats.

Table 1. Effect of knife sharpness and ageing period on pH, glycogen, L-lactate, glycogen potential, carbonyl and thiol content in *Longissimus Dorsum et lumborum* muscle of cattle.

		Sampling period				
Parameter	Treatment	D0	D1	D7	p-value	Trt*period
pH	Sharp knife	6.51 ± 0.05^{ax}	5.91 ± 0.06^{bx}	5.69 ± 0.01^{cx}	<.0001	
(unit)	Less sharp knife	6.58 ± 0.04^{ax}	5.93 ± 0.03^{bx}	5.66 ± 0.01^{cx}	<.0001	0.62
	p-value	0.41	0.82	0.06		
Glycogen	Sharp knife	7.48 ± 0.42^{ax}	6.39 ± 0.05^{bx}	6.31 ± 0.26^{bx}	0.0119	
(mg/g meat)	Less sharp knife	6.75 ± 0.51^{ax}	5.62 ± 0.20^{ay}	4.25 ± 0.18^{by}	0.0001	0.07
	p-value	0.28	0.0004	<.0001		
L-lactate	Sharp knife	11.33 ± 0.12^{cx}	12.96 ± 0.42^{by}	14.32 ± 0.09^{ay}	<.0001	
(mg/g meat)	Less sharp knife	11.51 ± 0.22^{cx}	14.95 ± 0.22^{bx}	17.64 ± 0.04^{ax}	<.0001	<.0001
	p-value	0.48	0.002	<.0001		
Glycogen potential[1]	Sharp knife	26.31 ± 0.81^{ax}	25.75 ± 0.44^{ax}	26.95 ± 0.54^{ax}	0.4042	
(mg/g meat)	Less sharp knife	25.01 ± 0.95^{ax}	26.20 ± 0.27^{ax}	26.15 ± 0.39^{ax}	0.3234	0.38
	p-value	0.3184	0.4577	0.30		
Carbonyl	Sharp knife	1.36 ± 0.07^{cx}	2.17 ± 0.18^{bx}	3.79 ± 0.32^{ax}	<.0001	0.84
(nmol/mg protein)	Less sharp knife	1.41 ± 0.04^{cx}	2.29 ± 0.06^{bx}	4.03 ± 0.11^{ax}	<.0001	
	p-value	0.52	0.52	0.49		
Thiol	Sharp knife	44.13 ± 0.35^{ay}	40.19 ± 1.04^{bx}	37.72 ± 1.09^{bx}	0.0005	0.75
(nmol/mg protein)	Less sharp knife	45.31 ± 0.21^{ax}	41.75 ± 0.15^{bx}	38.30 ± 0.19^{cx}	<.0001	
	p-value	0.02	0.17	0.61		

[a,b,c]Means within the same row with different superscripts differ significantly at $p < 0.05$.
[x,y]Means that they are within the same column, with different superscripts, and are significantly different at $p < 0.05$.
[1]Glycolytic potential = 2 × glycogen + lactate (Zhang et al. 2009).

Carbonyl content

Several effects, such as protein carbonylation, fragmentation or polymerisation, initiate protein oxidation. It negatively affects the meat's sensory qualities and water-holding capacity. Protein carbonyl content is a non-enzymatic and irreversible modification of proteins, resulting in the formation of carbonyl moieties induced by oxidative stress and associated mechanisms (Berlett and Stadtman 1997).

Table 1 shows that carbonyl content increased significantly ($p < 0.05$) as the post-mortem time increased from d 0 to d 7 in the meat samples from animals slaughtered with sharp as well as less sharp knives. No difference was observed in carbonyl values for the meat samples collected on days 0, 1 and 7 from the animals slaughtered with sharp and less sharp knives. The result of this study conforms with the observations of Berlett and Stadtman (1997), who observed that the amount of carbonyl groups significantly increased as the meat aged.

Residual haemoglobin and myoglobin concentration

The residual haemoglobin and myoglobin content in the muscle indicates the extent of the haemorrhage.

Table 2 shows that this study noticed no significant difference ($p = 0.585$) in residual haemoglobin and myoglobin content among the treatments. The slaughter process that causes intense struggle and stress in animals could lead to excessive haemorrhages in muscles, thereby leading to higher haemoglobin content in the muscle (Alvarado et al. 2007).

Table 2. Differences in haemoglobin and myoglobin concentration in *Longissimus Dorsum et lumborum* muscle during postmortem in cattle subjected to knife sharpness.

	Treatment		
Parameter	Sharp knife	Less sharp knife	p-value
Haemoglobin (mg/100 g)	0.161 ± 0.10	0.162 ± 0.001	0.59
Myoglobin (mg/100 g)	475.20 ± 19.88	476.20 ± 26.59	0.87

Alvarado et al. (2007) reported that the blood content of animals slaughtered by cutting of throats was significantly lower than that of the animals that were slaughtered by methods with no bleeding step. Although different methods of slaughtering, which had bleeding steps, showed no significant difference in Hb content in the muscle. The higher haem proteins (haemoglobin and myoglobin) in meat make it more prone to oxidative changes due to its iron content.

Colour values (CIE L*, a* and b*)

Table 3 shows no significant knife sharpness-sampling point interaction for L*, a* and b* colour values. No significant difference was seen in meat samples from animals slaughtered with sharp and less sharp knives, and neither sharpness nor ageing affected meat lightness (L*). The post-mortem meat samples from animals slaughtered with sharp and less sharp knives showed much-reduced redness (a*). Yellowness (b*) was significantly reduced in meat from animals

Table 3. Effect of knife sharpness and ageing period on colour, shear force, cooking loss, MFI, and sarcomere length of *Longissimus dorsum et lumborum* in cattle.

		Sampling period					
Parameter	Treatment	D0	D1	D7	D14	p-value	Trt*Period
Lightness	Sharp knife	29.25 ± 1.21^{ax}	30.45 ± 0.70^{ax}	31.65 ± 1.07^{ax}	32.22 ± 1.82^{ax}	0.37	0.98
(L*)	Less sharp knife	29.62 ± 0.98^{ax}	29.81 ± 1.46^{ax}	31.46 ± 0.69^{ax}	31.98 ± 1.26^{ax}	0.38	
	p-value	0.83	0.66	0.90	0.92		
Redness	Sharp knife	15.07 ± 0.69^{ax}	14.84 ± 0.53^{abx}	11.02 ± 0.69^{cx}	12.67 ± 0.52^{bcx}	<.0001	0.79
(a*)	Less sharp knife	16.00 ± 1.39^{ax}	14.84 ± 0.54^{abx}	11.53 ± 0.79^{bx}	12.16 ± 0.45^{bx}	0.0031	
	p-value	0.52	0.99	0.63	0.50		
Yellowness	Sharp knife	15.75 ± 0.64^{ax}	15.01 ± 0.42^{ax}	13.86 ± 0.48^{ax}	14.26 ± 0.60^{ax}	0.09	0.89
(b*)	Less sharp knife	15.42 ± 0.44^{ax}	15.31 ± 0.68^{abx}	13.50 ± 0.39^{bx}	13.78 ± 0.35^{abx}	0.01	
	p-value	0.71	0.69	0.60	0.55		
Shear force	Sharp knife	1.80 ± 0.03^{ax}	1.52 ± 0.03^{by}	1.23 ± 0.02^{cy}	1.02 ± 0.01^{dy}	<.0001	0.01
(kg)	Less sharp knife	1.83 ± 0.04^{ax}	1.76 ± 0.04^{ax}	1.46 ± 0.02^{bx}	1.19 ± 0.02^{cx}	<.0001	
	P-value	0.53	0.0003	<.0001	<.0001		
Cook loss	Sharp knife	26.08 ± 0.46^{ax}	25.62 ± 0.66^{ax}	25.37 ± 0.38^{ax}	25.14 ± 1.27^{ax}	0.85	0.99
(%)	Less sharp knife	27.00 ± 1.20^{ax}	26.75 ± 0.59^{ax}	26.03 ± 0.58^{ax}	25.86 ± 1.58^{ax}	0.85	
	P-value	0.42	0.25	0.34	0.72		
MFI	Sharp knife	27.40 ± 0.24^{dx}	39.42 ± 0.32^{cx}	70.05 ± 0.25^{bx}	92.59 ± 0.25^{ax}	<.0001	<.0001
	Less sharp knife	27.51 ± 0.30^{dx}	37.48 ± 0.23^{cy}	64.55 ± 0.33^{by}	78.53 ± 0.18^{ay}	<.0001	
	P-value	0.77	0.0003	<.0001	<.0001		
Sarcomere	Sharp knife	1.11 ± 0.00^{bx}	1.55 ± 0.02^{ax}	1.61 ± 0.01^{ax}	<.0001		
(μm)	Less sharp knife	1.10 ± 0.00^{bx}	1.33 ± 0.05^{ay}	1.35 ± 0.04^{ay}	0.05	<.0001	
	p-value	0.12	0.0006	<.0001			

[a,b,c]Means within the same row with different superscripts differ significantly at $p < 0.05$.
[x,y]Means that they are within the same column, with different superscripts, and are significantly different at $p < 0.05$.

slaughtered with less sharp knives. In contrast, sharp knife animals aged similarly post-mortem ($p < 0.05$).

The results of this study are in agreement with (D'Agata et al. 2009), who reported a significant effect of storage time on redness (a*) and yellowness (b*) of beef as influenced by slaughtering methods. The substantial reduction in redness value during meat ageing could be attributed to myoglobin oxidation (Rowe et al. 2004).

Drip loss

As can be seen in Figure 1, the interactions between knife sharpness and sampling point were comparable. Meat samples from animals slaughtered with less sharp knives had considerably higher drip loss ($p < 0.05$) on day 1 post-mortem than those slaughtered with sharp knives. Drip loss considerably increased ($p < 0.05$) in meat samples from animals slaughtered with sharp knives from day 1 to 14 post-mortem. A similar trend was noted in animals slaughtered with less sharp knives from day 1 to 14 post-mortem. The higher drip loss in meat from cattle slaughtered with a LSK could be due to higher stress levels leading to increased anaerobic glycolysis. This resulted in pH decline and protein denaturation (Bertram et al. 2004), thereby decreasing the WHC of the meat. Similarly, significantly lower drip loss was reported in meat from conventionally slaughtered animals than in meat from kosher animals.

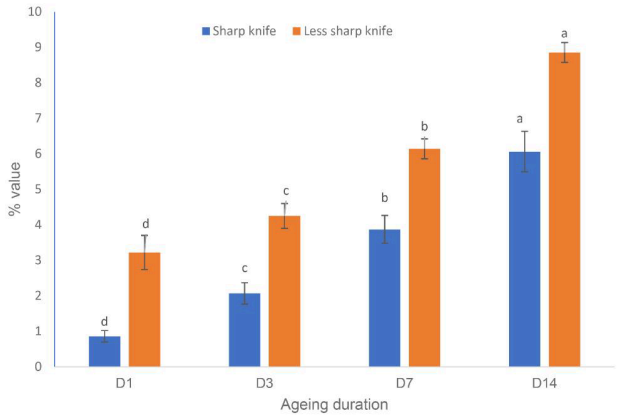

Figure 1. Effect of knife sharpness and ageing period on cattle's *longissimus dorsum et lumborum* drip loss.

Shear force

Table 3 shows that knife sharpness and an ageing period followed a significant ($p = 0.007$) interaction for shear force values. On day 0, knife sharpness did not significantly affect shear force ($p > 0.05$) at day 0. However, significantly higher shear force was observed in meat samples from animals with less sharp knives than those with sharp knives on day 1. With increasing ageing duration, the shear force value significantly ($p < 0.05$) decreased in cattle slaughtered with a sharp and a LSK.

Similar findings of more tender meat from the kosher method (with an extremely sharp knife) compared to conventional slaughter were reported (Agbeniga et al. 2014). This could be attributed to the

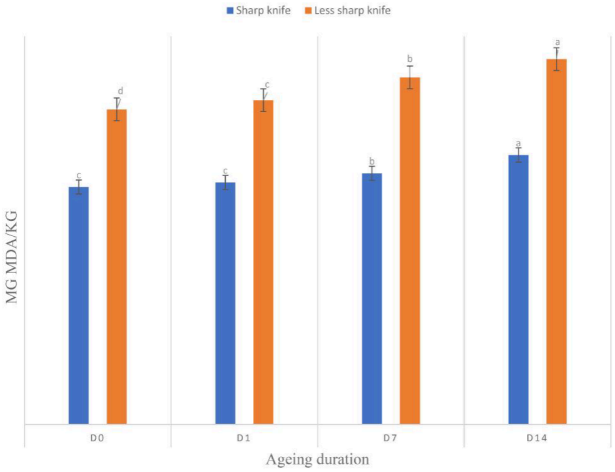

Figure 2. Effect of knife sharpness and ageing period on the malondialdehyde content (mg/kg) of *longissimus dorsum et lumborum* muscle in cattle.

action of endogenous protease enzymes on myofibrillar proteins during ageing. Further, the ultimate pH (above 6.0) and the lower water content, as indicated by the drip loss, might have played a role in the higher shear force in the meat from cattle slaughtered by a LSK.

Cooking loss

Table 3 shows that this study did not report any significant interaction between treatment and sampling points for cooking loss. Neither treatment nor the ageing period affected cooking loss. This could be linked with the meat samples' drip loss and ultimate pH. Our results agreed with those of Hassan and Manap (2015), reporting non-significant differences in meat slaughtered by different methods.

Myofibril fragmentation index (MFI)

Table 3 shows that MFI indicates the degradation of myofibrillar proteins (proteolysis) with a significant ($p < 0.001$) interaction between treatment and sampling point recorded for MFI in this study. Table 3 indicates that significantly higher MFI was observed in samples from animals slaughtered with sharp knives than in those slaughtered with a LSK on day 1, whereas sharpness had no effect on MFI on day 1. MFI values increased significantly ($p = 0.0001$) with an increased ageing period from day 0 to day 14 in both the groups of animals slaughtered with a sharp and a LSK.

Similar to our findings, halal-slaughtered rabbits had considerably greater MFI ($p < 0.05$) than gas-stunned rabbits (Nakyinsige et al. 2014). The shear force decreases as structural proteins disintegrate greater MFI (Hou et al. 2014). Sharp knife-slaughtered beef may have a greater MFI due to a rapid pH decrease, which enhances muscle proteolysis. MFI may rise with ageing due to myofibrillar protein breakdown around the Z-disk. Cows Hou et al. (2014) and rabbits Nakyinsige et al. (2014) similarly increased MFI during PM storage.

Sarcomere length

Table 3 shows a significant interaction between sampling points and treatments for sarcomere ($p < 0.0001$) was observed. Knife sharpness had no significant ($p > 0.05$) effect on sarcomere length on day 0, whereas samples from the animal slaughtered with a sharp knife had higher values for the sarcomere length than those slaughtered with a LSK on day 1.

Post-mortem ageing substantially affected the sarcomere length, decreasing contractile force and increasing overlap between the A and I bands (Hou et al. 2014). Similar findings of higher sarcomere length upon post-mortem ageing in the *pectoralis major* poultry muscle under acute stress were also reported by Wang et al. (2017).

Lipid oxidation

As can be seen in Figure 2, TBARS, as an indicator of lipid oxidation, was recorded as significantly higher in samples from animals slaughtered with less sharp knives than those slaughtered with sharp knives on days 0 post-mortem. TBARS increased substantially with the ageing period and were significantly higher ($p < 0.0001$) on days 7 and 14 than on days 0 and 1 in both animal groups slaughtered with a sharp and a LSK.

The present findings agree with those of Niu et al. (2016), who observed that pre-slaughter immunological stress could increase body oxidative damage and lipid oxidation, affecting meat quality. The TBARS values also reported an increasing trend with the advancement of ageing time.

Microbiological quality

Table 4 shows the microbial quality of beef during ageing. This study observed no significant interaction between slaughter positions and post-mortem ageing for the total aerobic count, Enterobacteriaceae, lactic acid bacteria and *Pseudomonas spp*. In both groups, an increase in the microbial count was observed with the increase in the ageing duration. However, the microbial counts were recorded well within the acceptable limit. The microbial population was

Table 4. Effects of knife sharpness on microbiological quantification of *Semitendinosus* muscle during post-mortem ageing periods in cattle.

		Sampling period						
Parameter	Treatment	D0	D1	D3	D7	D14	p-value	Trt*period
Total Aerobic Count	Sharp knife	2.99 ± 0.10cx	3.08 ± 0.01cx	3.17 ± 0.07cx	4.20 ± 0.08bx	5.92 ± 0.12ax	<.0001	0.99
(Log 10 cfu/g)	Less sharp knife	3.00 ± 0.15cx	3.08 ± 0.05cx	3.10 ± 0.07cx	4.19 ± 0.04bx	5.92 ± 0.07ax	<.0001	
	p-value	0.97	0.97	0.50	0.91	0.97		
Enterobacteriaceae	Sharp knife	1.72 ± 0.05ex	3.09 ± 0.07dx	4.10 ± 0.02cx	4.56 ± 0.02bx	5.13 ± 0.01ax	<.0001	0.99
(Log 10 cfu/g)	Less sharp knife	1.73 ± 0.03ex	3.09 ± 0.15dx	4.10 ± 0.01cx	4.56 ± 0.02bx	5.13 ± 0.02ax	<.0001	
	p-value	0.90	0.99	0.96	0.80	0.93		
Pseudomonas	Sharp knife	1.16 ± 0.04ex	2.54 ± 0.02dx	2.92 ± 0.12cx	3.85 ± 0.05bx	5.11 ± 0.01ax	<.0001	0.9997
(Log 10 cfu/g)	Less sharp knife	1.15 ± 0.04ex	2.53 ± 0.07dx	2.92 ± 0.09cx	3.85 ± 0.05bx	5.12 ± 0.03ax	<.0001	
	p-value	0.90	0.90	0.98	0.93	0.71		
Lactic acid bacteria	Sharp knife	1.10 ± 0.03ex	2.20 ± 0.05dx	3.52 ± 0.04cx	4.44 ± 0.13bx	5.14 ± 0.04ax	<.0001	0.08
(Log 10 cfu/g)	Less sharp knife	1.08 ± 0.03ex	2.49 ± 0.07dx	3.52 ± 0.08cx	4.44 ± 0.02bx	5.14 ± 0.02ax	<.0001	
	p-value	0.71	0.54	0.98	0.97	0.99		

[a,b,c]Means within the same row with different superscripts differ significantly at $p < 0.05$.
[x,y]Means that they are within the same column, with different superscripts, and are significantly different at $p < 0.05$.

comparable during the 14-d post-mortem ageing period between samples from animals slaughtered with a sharp knife and a LSK group.

The spoilage of meat occurs when the total viable counts and Enterobacteriaceae cross 7–8 log cfu/g (Insausti et al. 2001). The non-significant difference between the two groups could be due to comparable blood loss. The amount of blood remaining in the carcase is considered the most significant factor in determining the meat's degree of contamination and microbial quality (Sabow et al. 2015).

Conclusion

The study's results indicate that most physical and chemical characteristics, blood loss or residual blood content, lipid oxidation and the microbiological quality of meat from cattle slaughtered using a sharp knife vary from those slaughtered using a LSK. Irrespective of the slaughter knife sharpness, physicochemical properties, lipid oxidation and microbiological quality of beef varied by post-mortem ageing. Thus, this study supports that using sharp knives may also be among other factors influencing welfare and meat quality in cattle.

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Disclosure statement

'None'.

Data and model availability statement

The model was not deposited in an official repository.

Declaration of generative AI and AI-assisted technologies in the writing process

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