BLEEDING EFFICIENCY AND MEAT OXIDATIVE STABILITY IN GOATS SUBJECTED TO TRADITIONAL HALAL SLAUGHTER AND SLAUGHTER FOLLOWING MINIMAL ANAESTHESIA

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Abstract – This study compared the effects of conscious halal slaughter and slaughter following minimal anesthesia on bleeding efficiency in goats and oxidative stability of lipid and myofibrillar proteins of *Longissimus lumborum* muscle during refrigerated storage. Ten Boer cross bucks were divided into two groups and subjected to either halal slaughter without stunning (HS) or minimal anaesthesia prior to slaughter (AS). The result showed that slaughter method had no effect on blood loss, residual haemoglobin and oxidative stability of lipids and myofibrillar proteins. However, lipid and protein oxidation increased as postmortem storage progressed. It can be concluded that subjecting goats to minimal anesthesia prior slaughtering or halal slaughter did not affect bleeding efficiency and oxidative stability of goat meat.

Key Words – goat meat, residual blood, storage stability, slaughter method.

I. INTRODUCTION

In livestock production and processing chain, slaughtering plays crucial role in animal welfare, meat quality and safety. Although slaughtering takes quite a short period, it is a delicate process whose mishandling could destroy producers' efforts made amid longer durations of growth and fattening [1]. Furthermore, the amount of blood retained in meat is one of the most critical factors influencing the quality changes and deterioration. Blood components, particularly hemoglobin, are compelling lipid oxidation promoters which in turn destabilizes the myofibrillar structure by oxidation or degradation of myofibrillar proteins [2] and may reduce the shelf-life of meat. Protein oxidation is a significant issue in meat quality assessment as muscle tissues involve high amounts of proteins playing a significant role in meat quality [3].

The amount of blood loss during slaughter depends on the slaughtering method. Slaughtering methods include religious (halal and Kosher) and conventional (stunning prior slaughter) method or combination of religious and conventional methods e.g electrical stunning followed by halal throat cut. Halal method has been accentuated to provide a considerable bleeding when the heart is still beating, which might be beneficial for shelf-life or meat quality [1]. Although several works have been conducted on the efficacy of different slaughter methods on bleeding efficiency and storage stability of meat, most information originates from research in conventional slaughter methods with limited comparison to specifically halal slaughter method. This is due to the limited access to religious slaughter without stunning in most developed countries due to legal and welfare reasons. Animals subjected to minimal anesthesia has been accepted as a humane model to study noxious stimuli associated with animal slaughter, particularly in countries where pre-slaughter desensitization and stunning are mandatory [4]. Pain comprised of both sensory and affective components [5]. When animals are anaesthetized prior slaughter, only sensory pain would be felt. When animals are slaughtered fully conscious without any form of stunning, both sensory and affective pain would be felt [5]. Thus, physiological/sensory pain and its associated stress changes could affect bleed out, residual blood content and oxidative stability of meat.

This objective of this study was to compare the effect of minimal anesthesia prior slaughter and halal slaughter on bleeding efficiency in goats, and oxidative stability of lipid and myofibrillar protein of *longissimus lumborum* muscle during postmortem refrigerated storage.

II. MATERIALS AND METHODS

This study was conducted following the animal ethics guidelines of the Research Policy of Universiti Putra Malaysia. A total of 10 Boer cross bucks weighing 23.15 ± 1.42 kg and about 7 months old were obtained from a local farm in Selangor. The goats were randomly allotted into two groups consisting of 5 animals each and subjected to either conscious halal slaughter without stunning or slaughter following minimal anesthesia. Slaughtering was carried out at the Department of Animal Science research abattoir, Faculty of Agriculture, Universiti Putra Malaysia. In the halal method, the animals were humanely slaughtered according to halal slaughtering procedure as outlined in the MS1500: 2009 [6]. In the anesthesia process, animals were anaesthetized using 5mg/kg propofol administered by rapid injection into cephalic vein and maintained with halothane in 100% oxygen, slaughtered and subsequently bled [7].

After evisceration, the *longissimus lumborum* (LL) muscle was separated into two parts, the first part was snap frozen in liquid nitrogen (Malaysian Oxygen Bhd., Malaysia) before being stored at -80 ºC until subsequent determination of TBARS and protein oxidation at 0 day and haemoglobin determination. The carcasses were hung in the cold room at 4 ºC until when the next sampling was done at either 7 or 14 days *postmortem*. On each sampling day, the chops were snap frozen in liquid nitrogen and stored at -80 ºC until subsequent determination of TBARS and protein oxidation at 7 and 14 d *postmortem*.

The amount of blood loss was estimated as the difference between pre-slaughter weight and post-slaughter weight once the animal is dead (based on ECG). Residual blood haemoglobin content in *Longissimus lumborum* (LL) muscle was quantified using a modified kinetic technique of Goyal and Basak [8] in which, haem act as a chemical catalyst to break down hydrogen peroxide into water and nascent oxygen. Lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) using QuantiChromTM TBARS Assay Kit (DTBA-100, BioAssay Systems, USA) following the manufacturer's description of the colorimetric protocol. Protein oxidation quantified as loss of thiol groups and degradation of Myosin heavy chain, actin and troponin-T according to the method of Morzel *et al.* [9]. Data were analyzed by one-way (Table 1) or two-way (Tables 2 and 3) ANOVA using the GLM procedure of Statistical Analysis System (SAS) package Version 9.2 software. Significance was set at $p<0.05$. Duncan's multiple range test was used to separate means.

III. RESULTS AND DISCUSSION

Table 1 shows the result of blood loss obtained from goats subjected to the halal slaughter without stunning (HS) and slaughter following anesthesia (AS). There was no significant difference ($p>0.05$) in blood loss between HS and AS. Our observation is in line with the findings of Anil *et al.* [10], in which slaughter method (conventional with stunning *versus* no stunning) did not affect blood loss in sheep and cattle. Also, there was no significant difference ($p>0.05$) in muscle residual hemoglobin content between the HS and AS group (Table 1). This observation could be explained by the similarity in blood loss between the two slaughter methods. The present finding is in agreement with those of Alvarado *et al.* [11] who reported similarity in the residual hemoglobin contents in the breast muscle of non-stunned broiler chickens compared to stunned broilers.

Table 1 Blood loss and haemoglobin content of goats subjected to halal slaughter and slaughter following minimal anesthesia

Parameter	Treatment	
	HS	AS
Blood loss (%)	4.99 ± 0.09	4.73 ± 0.07
Haemoglobin (mg/100 g)	0.85 ± 0.04	0.90 ± 0.05

HS - Halal slaughter without stunning; AS- Slaughter following minimal anaesthesia.

The results of lipid and protein oxidation during 14 days postmortem are shown in Table 2. Slaughter method had no effect on goat meat lipid oxidation at 0, 7 and 14 d *postmortem*. These values are consistent with the results for haemoglobin in LL muscle. Haemoglobin is powerful promoter for lipid oxidation and a stronger pro-oxidant than myoglobin. Studies have shown that TBARS, peroxide values and hexanal during storage were greatest for haemoglobin as compared to myoglobin. In general, lipid

oxidation increased ($p<0.05$) with ageing time in both groups. However, no group (HS or AS) had TBARS value that reached detectable concentration for humans as established by Insausti *et al.* [12]. The absence of significant difference in lipid oxidation observed in the present study corroborates the report of Linares *et al.* [13] in lambs, which showed that slaughter method had no effect on meat lipid oxidation at 1 and 7 d postmortem. The free thiol content of fresh myofibrils was 40.94 and 40.35 nmole/mg protein for HS and AS, respectively. At 7 and 14 d postmortem, the thiol concentration in the meat produced by AS was 35.06 and 27.96 nmole/mg protein while that in HS, was 34.64 and 27.31 nmole/mg protein, respectively. Slaughter method had no effect on myofibril free thiol content. However, the protein thiols reduced significantly ($p<0.05$) as protein oxidation increased with increasing postmortem aging

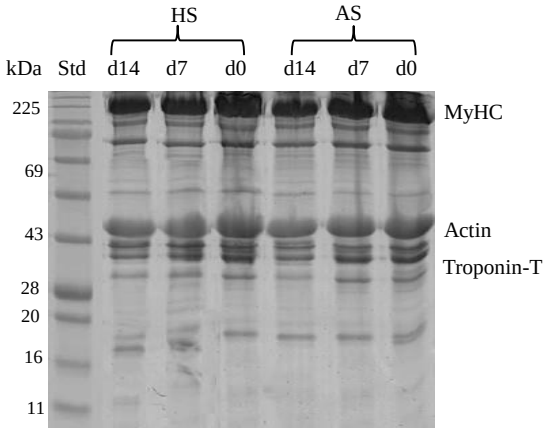
Table 2 Malondialdehyde and thiol content of LL muscle in goats subjected different slaughtering methods during 14 days of refrigerated storage

Parameter	Days postmortem	Treatment	
		HS	AS
Malondialdehyde (mg/kg meat)	0	$0.50^{c} \pm 0.03$	$0.46^{c} \pm 0.05$
	7	$1.14^{b} \pm 0.04$	$1.15^{b} \pm 0.06$
	14	$2.09^{a} \pm 0.05$	$2.11^{a} \pm 0.05$
Thiol (nmole/mg protein)	0	$40.35^{a} \pm 1.23$	$40.94^{a} \pm 0.70$
	7	$34.64^{b} \pm 1.33$	$35.06^{b} \pm 1.07$
	14	$27.31^{c} \pm 2.34$	$27.96^{c} \pm 1.47$

HS - Halal slaughter; AS - slaughter following minimal anaesthesia.
[a-c] Indicate significant differences ($p<0.05$) among the different ageing times within each slaughter group.

Electrophoresis was performed in order to observe modifications in goat meat myofibrillar proteins during *postmortem* aging. In both slaughter methods, the SDS-PAGE patterns showed a decrease of band intensities corresponding to myosin heavy chains and troponin-T as *postmortem* days increased (Figure 1). The actin band was relatively stable. The observed decrease in band intensity is indicative of protein degradation (ageing) by proteolytic enzymes. The absence of significant difference observed between HS and AS with regards to the myofibrillar protein degradation could be explained by the protein thiol levels. Increase in oxidation can enhance protein degradation by proteases. Additionally, oxidation of proteins due to attack by reactive oxygen species including hydroxyl radicals (HO•) can cause degradation of meat and reduce its shelf life or results in the formation of potentially hazardous substances [14]. The degradation of troponin-T and myosin heavy chain increased with increasing *postmortem* aging while the degradation of actin was relatively low (Table 3). Similar to the present findings, Xue *et al.* [14] reported that increased bovine myofibrillar proteins denaturation was associated with degradation of myosin heavy chain but with little influence on degradation of actin.

Figure 1 a representative gel showing pattern of myofibrillar proteins of LL in goats subjected to different slaughtering methods during 14 days of refrigerated storage.



Equal amount of protein (25 µg) of each sample was loaded and electrophoresed on a separate 12% SDS-PAGE under 120 V of constant voltage for about 90 min. The gels were then stained with coomassie blue staining for 60 min and destained with destaining solution for 45 min. The bands of myofibrillar proteins were visualised using GS-800 Calibrated Imaging Densitometer.

Table 3 Reflective density/mm^2 of myofibrillar proteins of LL muscle in goats subjected different slaughtering methods during 14 days of refrigerated storage

Parameter	Days postmortem	Treatment	
		HS	AS
MyHC	0	$59.98^{a} \pm 0.35$	$58.79^{a} \pm 1.94$
	7	$51.35^{b} \pm 1.12$	$49.49^{b} \pm 1.98$
	14	$45.43^{c} \pm 1.07$	$42.16^{c} \pm 2.66$
Actin	0	$16.81^{a} \pm 0.53$	$16.14^{a} \pm 0.59$
	7	$16.67^{a} \pm 3.86$	$16.06^{a} \pm 3.30$
	14	$15.94^{a} \pm 2.45$	$15.22^{a} \pm 4.02$
Troponin-T	0	$14.23^{a} \pm 0.65$	$14.75^{a} \pm 0.61$
	7	$11.64^{b} \pm 0.36$	$10.60^{b} \pm 0.84$
	14	$7.10^{c} \pm 0.46$	$6.34^{c} \pm 0.53$

HS - Halal slaughter; AS - slaughter following minimal anaesthesia.
[a-c] Indicate significant differences ($p<0.05$) among the different ageing times within each slaughter group.

In oxidative conditions, interaction of proteins with other biomolecules can lead to cross-linking/polymerization. For example, aldehydic lipid oxidation products (malondialdehyde or 4-hydroxynonenal) can react with amino groups of proteins to form fluorescent aggregates known as lipofuscin or ceroid. According to Estévez et al. [2], the onset of lipid oxidation in meat system seems faster than the oxidative degradation of myofibrillar proteins, thus it is more likely that lipid-derived radicals and hydroperoxides promote protein oxidation than *vice versa*. Soyer *et al.* [15] reported a good correlation between lipid and protein oxidation in chicken meat during frozen storage. However, in the present study, there was no correlation between lipid oxidation (as measured by TBARS) and protein oxidation (as measured by sulphydryl content) in goat muscles ($r = -0.50$, p value = 0.39 and $r = -0.31$, p value = 0.69, for HS and AS, respectively at d 14). In the present study, as the MDA concentration increased, the thiol groups reduced, which indicates increase in lipid protein and protein oxidation.

IV. CONCLUSION

The results of the present study showed that blood loss, residual blood content and lipid-protein oxidation of goat meat were not affected by slaughter method. Postmortem ageing decreased the oxidative stability of lipid and myofibrillar proteins.

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