



## Bleeding Efficiency and Meat Oxidative Stability and Microbiological Quality of New Zealand White Rabbits Subjected to Halal Slaughter without Stunning and Gas Stun-killing

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**ABSTRACT:** A study was conducted to compare the effect of halal slaughter without stunning and gas stun killing followed by bleeding on residual blood content and storage stability of rabbit meat. Eighty male New Zealand white rabbits were divided into two groups of 40 animals each and subjected to either halal slaughter without stunning (HS) or gas stun-kill (GK). The volume of blood lost during exsanguination was measured. Residual blood was further quantified by determination of haemoglobin content in *Longissimus lumborum* (LL) muscle. Storage stability of the meat was evaluated by microbiological analysis and measuring lipid oxidation in terms of thiobarbituric acid reactive substances (TBARS). HS resulted in significantly higher blood loss than GK. HS had significantly lower residual haemoglobin in LL muscle compared to GK. Slaughter method had no effect on rabbit meat lipid oxidation at 0, 1, and 3 d postmortem. However, at 5 and 8 days of storage at 4°C, significant differences ( $p < 0.05$ ) were found, with meat from the GK group exhibiting significantly higher levels of MDA than that from HS. At day 3, greater growth of *Pseudomonas aeruginosa* and *E. coli* were observed in the GK group ( $p < 0.05$ ) with *B. thermosphacta* and total aerobic counts remained unaffected by slaughter method. At days 5 and 7 postmortem, bacterial counts for all tested microbes were affected by slaughter method, with GK exhibiting significantly higher growth than HS. It can be concluded that slaughter method can affect keeping quality of rabbit meat, and HS may be a favourable option compared to GK due to high bleed out. (**Key Words:** Rabbit, Halal Slaughter, Gas Stun Kill, Residual Blood, Storage Stability)

### INTRODUCTION

Meat is considered to be spoiled when it becomes unfit

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Submitted Jul. 19, 2013; Accepted Oct. 29, 2013; Revised Nov. 16, 2013

for human consumption. Spoilage is a subjective judgment by the consumer, normally, influenced by cultural and economic considerations or the sensory acuity of the individual and the intensity of the change (Nychas et al., 2008). However, bacteria levels between  $10^7$  and  $10^9$  cfu/cm<sup>2</sup> during refrigerated storage (Borch et al., 1996) and TBARS values equal to or greater than 5 mg MDA/kg meat (Insausti et al., 2001) comprise the threshold for detecting fitness for human consumption. Microbial growth and lipid oxidation are among important factors influencing shelf life and consequently consumer acceptance of fresh meat. Spoilage of raw meat accounts greatly for major annual losses to processors and retailers (Nattress et al., 2001). The rate of spoilage varies according to the species of microflora present, the characteristics of the meat, processing methods, product composition, and the environment in which the meat is stored (Borch et al., 1996; Ellis et al., 2002; Nychas et al., 2008). Due to its specific composition, meat, particularly, rabbit meat with higher

ultimate pH (Rodríguez-Calleja et al., 2005) comprises an excellent substrate for growth of spoilage bacteria.

The killing of animals for food (slaughter) is a very delicate operation governed by strict regulations related to food hygiene and safety, working conditions and animal welfare. Traditionally, slaughter practices have dealt with factors that affect wholesomeness and quality of meat. For instance, the meat for Muslim consumption is required to be halal and thoyyib (meaning acceptable and wholesome). The industry aims at achieving customer acceptability through the development and control of processes in order to produce wholesome products with high quality and safety (Castro-Giráldez et al., 2011) while consumers expect meat products to have the expected nutritional value, wholesomeness and freshness; all of which are influenced by the animal production system. Slaughtering is such a vital step in the production chain for not only animal welfare, but also meat quality and safety, which despite its quite short duration, poses potential risks that create critical points in the overall process and its mismanagement can ruin efforts made by producers during much longer periods of growth and fattening.

To optimize bleed out at slaughter and reduce carcass and meat defects is a major goal of the meat processing industry, as improved bleeding can improve the quality of the meat during storage (Ali et al., 2007). Inefficient and improper bleeding may cause more blood to be retained in the meat. Blood favours multiplication of spoilage microorganisms and acts as a carrier for food borne pathogens (Lerner, 2009). Additionally, residual blood in the meat equates to retention of more haemoglobin. Haemoglobin is a powerful promoter of lipid oxidation (Everse and Hsia, 1997; Alvarado et al., 2007). Lipid oxidation constitutes a major cause of non-microbial meat spoilage, especially under pro-oxidative conditions such as storage and cooking. It can also occur during refrigeration and frozen storage (Soyer et al., 2010).

According to the federal humane slaughter law, all processors of food animals must render USDA inspected animals insensible to pain before being shackled, hoisted and cut or exsanguinated. However, the law permits slaughtering in accordance with ritual requirements of any religious faith that prescribes a method of slaughter whereby animals suffer loss of consciousness by severance of the carotid artery with a sharp instrument (College of Law, 2011). Due to the sheer number of Muslims globally and the fact that they are very keen to uphold the tenets of their religion, it has become apparent that the market for meat from animals slaughtered by the halal method is a significant proportion of the global production and supply. Unlike other consumers whose decision to purchase meat is often guided by the perception of healthiness, safety and

sensory traits such as colour, tenderness, juiciness and aroma or flavour, Muslims consider the halal status of the meat before any other factor (Nakyinsige et al., 2012). Henceforth, the objectives of this study were to i) compare halal slaughter without stunning and gas stun-killing followed by bleeding with regard to residual blood and ii) to relate this residual blood to storage stability of rabbit meat.

## MATERIALS AND METHODS

This study was conducted following the animal ethics guidelines of the Research Policy of Universiti Putra Malaysia.

### Stunning and slaughter

A total of 80 male New Zealand white rabbits with body weight between 1.8 kg and 2.0 kg were obtained from a commercial farm (East Asia Rabbit Corporation) located in Semenyih, West Malaysia (GPS Coordinates: *Latitude*: 3° 1'49.84"N *Longitude*: 101°51'59.86"E). The rabbits were divided into two groups of 40 animals each and subjected to either halal slaughter or gas stun-kill. The slaughter procedure was conducted 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 slaughter procedure as outlined in the Malaysian Standard; MS1500: 2009 (Department of Standard Malaysia, 2009). The procedure involved severing the carotid artery, jugular vein, trachea and oesophagus. In the gas stun-kill procedure, groups of ten rabbits were dipped in a gas chamber containing 61.4% carbon dioxide, 20.3% oxygen and 18.3% nitrogen for 8 min and subsequently bled.

### Sample handling and storage

After evisceration, the right LL muscle was divided into two sections; the first section was snap frozen in liquid nitrogen (Malaysian Oxygen Bhd., Malaysia) before being stored at -80°C until subsequent determination of TBARS at 0 day. The other section was divided into four chops for subsequent determination of TBARS at 1, 3, 5, and 7 d postmortem. The chops were vacuum packed and kept at 4°C for 1, 3, 5, and 7 d, accordingly. After the aging period, the samples were stored at -80°C until subsequent analysis. The right hind limbs and the fore arms were aseptically packed in stomacher bags and stored at 4°C for microbial enumeration.

### Determination of blood loss

The amount of blood lost during slaughter without stunning and gas stun-killing was measured by collecting

blood in a measuring cylinder for 60 s after exsanguination and recording its volume.

### Haemoglobin quantification

All chemicals used in the analysis; Tris-HCl buffer (Cat # 161-0798, BIO-RAD, USA), sodium acetate (S7670-500G, SIGMA-ALDRICH, USA), potassium phosphate (P3786-500G, SIGMA-ALDRICH, USA), potassium chloride (Bendosen, Malaysia), O-tolidine (Bendosen, Malaysia), Triton-X-100 (R&M Chemicals, UK), ethanol (HmbG Chemicals, Malaysia) and glacial acetic acid (HmbG Chemicals, Malaysia) were of analytical grade.

### Extraction of haemoglobin

The frozen muscle tissues were manually pulverized in liquid nitrogen. Haemoglobin was extracted according to the method described by O'brein et al. (1992). 5 g of sample were mixed with 15 mL of ice cold extraction buffer (80 mM KCl, 50 mM Tris-HCL, pH 8.0) and homogenised (Wiggen Hauser D-500, Germany) for 40 s. The use of ice cold extraction buffer was to prevent denaturation of myoglobin by acid produced during glycolysis whereas KCl mimics intracellular ion concentration (O'brein et al., 1992). The samples were rinsed with additional 5 mL of the buffer and centrifuged (Hitachi Koki, Japan) at 5,000 g for 10 min at 21°C to clarify the suspension. The supernatant was divided into aliquots in 2 mL microcentrifuge tubes to be used as stock sample solution for further evaluation.

### Evaluation of haemoglobin

Evaluation of haemoglobin was carried out following a modified kinetic method of Goyal and Basal (2009) in which heme acts as a chemical catalyst to break down hydrogen peroxide into water and nascent oxygen. Nascent oxygen then oxidizes o-tolidine to give an oxidized product of green-blue colour. The rate of colour development is directly proportional to heme concentration (Goyal and Basal, 2009). O-tolidine stock solution was prepared by dissolving 2 g of o-tolidine in 100 mL of solvent (20 mL of glacial acetic acid (GAA) and 80 mL of ethanol) to make a stock solution. The working reagent (0.4 g/dL) was prepared by diluting stock solution with the same solvent (1:5). A hundred microlitre of Triton-X-100 was mixed with 100 mL of working reagent to increase the linearity of kinetic reaction. A hundred millilitre of 2% (v/v) hydrogen peroxide solution was prepared in deionized water and 2.26 g sodium acetate was added to create a buffering environment with GAA present in the final reaction mixture so as to maintain pH between 3.0 and 3.5 in the final reaction mixture. The prepared solution could only be used within 6 to 8 h.

### Haemoglobin assay

Haemoglobin standard solution was prepared by diluting bovine blood haemoglobin (H262G, Sigma- Aldrich, USA) in Tris-HCl buffer, pH 8.0, at a concentration of 240 mg/L. For the estimation of the enzymatic reaction kinetics, the stock solution was diluted with 50 mM Tris-HCl buffer to make final concentrations of 4.0, 8.0, 12.0, 16.0, 20.0, and 24.0 mg/L of haemoglobin, respectively. Exactly 1.0 mL of working solution and 1.0 mL of H<sub>2</sub>O<sub>2</sub> solution were pipetted into test tubes, vortexed and allowed to stand for 5 min at room temperature. To this mixture was added 10 µL of each sample or standard. Absorbance at 630 nm (A<sub>630</sub>) was measured after 120 s (carry 50 probe UV-visible spectrophotometer, Varian Australian, PTY LTD, Australia).

### Lipid oxidation measurement

Lipid oxidation was measured as 2-thiobarbituric acid reactive substances (TBARS) using QuantiChrom TBARS Assay Kit (DTBA-100, BioAssay Systems, USA) following the colorimetric protocol as described by the manufacturer. Precisely, samples were manually pulverized in liquid nitrogen and 200 mg were mixed with 2 mL ice-cold phosphate buffered saline (PBS) and rapidly homogenized with an Ultra- Turrax T5FU (IKA- Labortechnik Staufen, Germany) for 20 s on ice. Two hundred microlitres of homogenates were mixed with 200 µL of ice-cold 10% trichloroacetic acid (TCA) and incubated for 5 min on crushed ice. The tubes were centrifuged (Eppendorf Centrifuge, Mikro 22R Hettich, Germany) at 21,900 g, 4°C for 5 min. Standards were prepared by mixing 15 µL of the 1.5 mM malondialdehyde (MDA) with 735 µL deionised water to get final concentration of 30 µM MDA. Thereafter, 300, 180, 90, and 0 µL of 30 µM MDA were diluted with 0, 120, 210, and 300 µL of deionised water to generate the final 30, 18, 9, and 0 µM MDA as standards 1, 2, 3, and 4, respectively. 200 µL of samples and standards in labelled 1.5 screw cap glass tubes, were added with 200 µL of thiobarbituric acid reagent and the mixture was incubated in a dry heating block at 100°C for 60 min. Following equilibration to room temperature, 100 µL of standards and samples were loaded in duplicate into wells of a clear flat-bottom 96-well plate (Greiner Bio-One, Germany) and optical density (OD) was determined at 535 nm using auto UV Xenon flash lamp microplate reader (infinite M200, Tecan, Austria). After subtracting the OD of blank (standard 4) from all standard and sample values, a standard curve was obtained by plotting the  $\Delta OD_{535}$  against standard concentrations. TBARS (µM MDA equivalent) concentration of the samples was calculated using the following equation:

$$TBARS = [(R_{\text{sample}} - R_{\text{blank}}) / \text{Slope} (\mu\text{M}^{-1})] \times n$$

Where  $R_{\text{sample}}$ ,  $R_{\text{blank}}$  are the  $OD_{535\text{nm}}$  of the sample and  $H_2O$  blank ( $STD_4$ ) and  $n$  is the sample dilution factor ( $n = 3$  for deproteinated samples).

### Microbiological analysis

On each sampling day, 25 g of meat samples were aseptically weighed, transferred to a stomacher bag containing 225 mL of 0.1% of peptone water (Merk KGaA, Germany) and homogenized using a stomacher (Inter Science, France) for 120 s at room temperature. For microbial enumeration, 0.1 mL samples of serial dilutions (1:10 diluent, and peptone water) of homogenates were spread on the surface of dry media. Tenfold dilutions were spread plated in duplicate. Aerobic plate counts were enumerated on plate count agar (Merk KGaA, Germany) following 2 d incubation at 30°C (Harrigan, 1998). *Pseudomonas aeruginosa* numbers were determined, after 2 d incubation at 25°C on Fluka Analytical 70887-500G Centrimide Agar (SIGMA-ALDRICH, Spain). *Escherichia coli* were enumerated after 24 h incubation on Tryptone Soy Agar (CM0131, Oxoid, England) at 36°C. *Brochothrix thermosphacta* was enumerated on streptomycin sulphate cicloheximide thallos acetate agar (STAA, Oxoid), supplemented with STAA Selective Supplement SR0151 E (Oxoid, England) following 18 h incubation at 26°C.

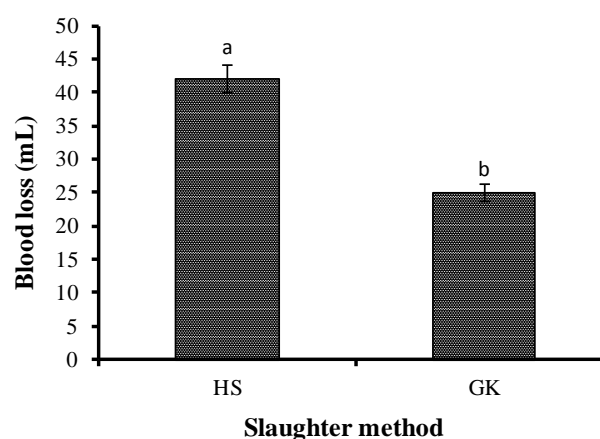
### Statistical analysis

The experiment was of a completely randomized design. All analyses were performed using the GLM procedure of Statistical Analysis System package (SAS) Version 9.2 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA) and statistical significance was set at  $p < 0.05$ . Data were subjected to one-way analysis of variance (ANOVA) using a model that included treatment and animal as possible source of variation. Duncan's multiple range test was used to test the significance of variance between the means of the studied parameters.

## RESULTS AND DISCUSSION

### Blood loss

The results for blood loss are shown in Figure 1. The method of halal slaughter without stunning resulted in significantly higher blood loss than gas stun-killing. Comparatively, the average volume of blood lost during halal slaughtering (HS) was 42.07 mL while the loss following gas stun-killing (GK) was 25.15 mL. The consumption of blood is forbidden (Nakyinsige et al., 2012a; b). Therefore, religious slaughter aims at draining as much blood as possible out of the carcass. In Europe and North America, modern slaughter practices are also based on traditions which require that animals killed for food should be properly bled before consumption. Normally, this



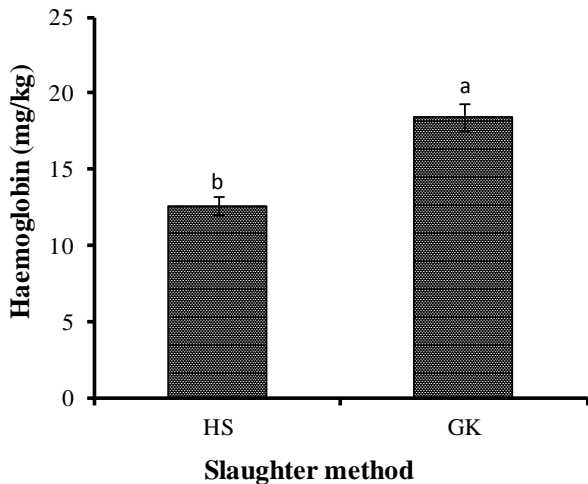
**Figure 1.** Differences in blood loss of New Zealand white rabbits subjected to halal slaughter without stunning and gas stun-killing. HS: Halal slaughter without stunning; GK: gas stun-killing. <sup>ab</sup> Means with different letters differ significantly at  $p < 0.05$ .

is achieved through a bilateral or a unilateral neck cut to sever the carotid arteries and jugular veins. Factors influencing bleeding efficiency at sticking include; i) blood vessels that are severed, ii) size and patency of the sticking wound, iii) cardiac arrest at stunning, iv) orientation of the carcass – positioned horizontally or vertically, v) vasodilation or vasoconstriction in the capillary bed, vi) tonic muscle contractions squeezing blood capillaries and vessels, and vii) clonic activity causing movement of blood towards the sticking wound (Gregory, 2005). Poor bleeding efficiency can negatively affect colour of the meat and is considered to be a major quality defect, which can even cause undesirable discoloration and short shelf life (Griffiths and Nairn, 1984). Residual blood has also been associated with meat flavours. Greater blood loss during halal slaughtering can be due to the fact that when animals are bled without stunning, the greatest percentage of blood loss (between 75% and 85%) occurs while the heart is still bleeding.

Inefficient and improper bleeding may cause more blood (haemoglobin) to be retained in the meat. Haemoglobin is a powerful promoter of lipid oxidation and may decrease the shelf life of meat products. Residual blood is also important in promoting microbiological deterioration of carcasses (Warriss, 2000; Alvarado et al., 2007; Lerner, 2009). In addition to accelerating multiplication of spoilage microorganisms, blood acts as a carrier for food borne pathogens (Lerner, 2009).

### Residual haemoglobin concentration

The results for effect of HS and GK on residual haemoglobin concentration are shown in Figure 2. GK had significantly higher residual haemoglobin in LL muscle compared to HS. This is contrary to the findings of Alvarado et al. (2007) who reported no significant



**Figure 2.** Differences in haemoglobin content of New Zealand white rabbits subjected to halal slaughter without stunning and gas stun-killing. HS: Halal slaughter without stunning; GK: gas stun-killing. <sup>ab</sup> Means with different letters differ significantly at  $p < 0.05$ .

difference between non-stunned and gas-stunned and bled broilers. The high residual haemoglobin content in the present study can be attributed to the lower bleed out in GK when compared with HS. Oellingrath et al. (1990) indicated that the haemoglobin content in meat depends on the extent of vascular bed in the muscles as well as the bleeding of the carcass. The haemoglobin in rabbit LL muscle can be a result of haemorrhaging of blood into the muscle caused by the stunning and slaughter procedure.

### Meat lipid oxidation

Lipid oxidation levels during the first seven days postmortem are shown in Table 1. Generally, lipid oxidation increased ( $p < 0.001$ ) with storage time in both groups. Slaughter method had no effect on rabbit meat lipid oxidation at 0, 1, and 3 d postmortem. However, at days 5 and 8 of storage at 4°C, significant differences ( $p < 0.05$ ) were present, with meat from gas stun-killed animals exhibiting significantly higher levels of MDA than that

**Table 1.** Level of lipid oxidation (mg MDA/kg meat) of rabbit meat obtained by halal slaughter without stunning and gas stun-killing on

Time postmortem (days)	HS	GK	SEM	p value
0	0.0141 <sup>a</sup>	0.0143 <sup>a</sup>	0.0014	0.9228
1	0.0263 <sup>a</sup>	0.0398 <sup>a</sup>	0.0065	0.1596
3	0.0697 <sup>a</sup>	0.0773 <sup>a</sup>	0.0120	0.6597
7	0.1527 <sup>b</sup>	0.1962 <sup>a</sup>	0.0097	0.0057

<sup>ab</sup> Least square means in the same row with different superscripts are significantly different at  $p < 0.05$  for the different types of slaughter at the same time postmortem.

HS = Halal slaughter without stunning; GK = Gas stun-killing. SEM = Standard error of the means.

from the halal slaughtered group. These values were consistent with the results for residual haemoglobin, which indicated that GK had higher residual haemoglobin compared with HS.

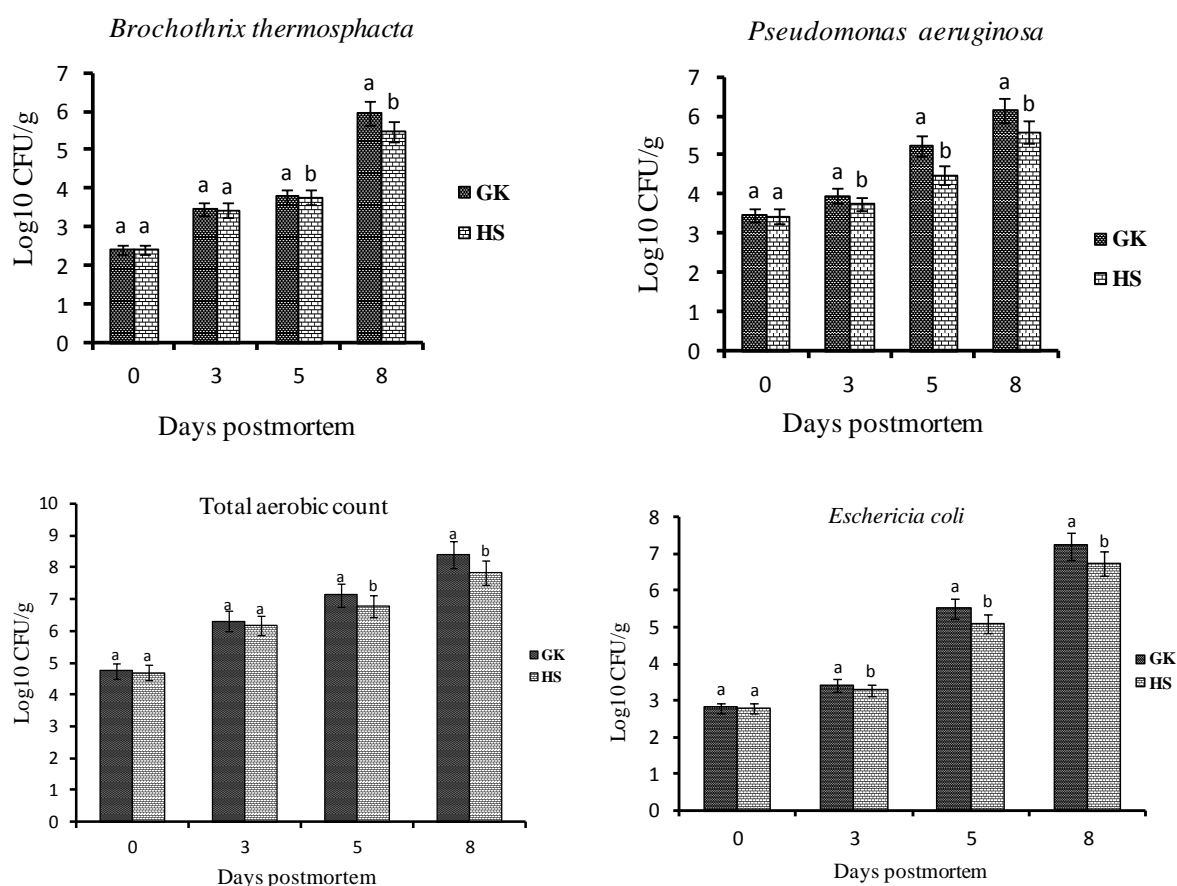
Autocatalytic oxidative processes of lipids constitute a major cause of reduced shelf life in meat, only second to microbial spoilage. These processes begin immediately after slaughter and their magnitude depends partly on the amount of pro-oxidants present in the system. Residual blood in meat increases the concentration of heme proteins and haemoglobin in meat. Haemoglobin is a powerful promoter of lipid oxidation (Everse and Hsia, 1997; Alvarado et al., 2007). This explains why gas stunning that resulted in less blood loss exhibited significantly higher levels of lipid oxidation.

Lipid oxidation has been implicated in the deterioration of flavour (Fernández et al., 1997; Insausti et al., 2001; Jeremiah, 2001; Faustman et al., 2010), formation of rancid odours (Fernández et al., 1997; Santé-Lhoutellier et al., 2008), discolouration (Juncher et al., 2001; Faustman et al., 2010) and production of potentially toxic compounds (Morrissey et al., 1998; Richards et al., 2002) in meat. It has been reported that TBARS values equal to or greater than 5 mg MDA/kg meat comprise the threshold for detecting off-odours and off-taste for humans (Insausti et al., 2001). In this study however, this value was not reached.

The rate and extent of oxidation is under the influence of pre-slaughter events such as stress and post-slaughter events such as early post-mortem pH decline, carcass temperature, cold shortening, and interventions such as electrical stimulation. The presence of transition metals, particularly iron, is pivotal in facilitating the generation of species capable of abstracting a proton from an unsaturated fatty acid (Buckley et al., 1995). Such high molecular weight iron sources as haemoglobin and myoglobin can directly catalyze lipid oxidation (Johns, 1989; Alvarado et al., 2007; Vareltzis et al., 2008; Maqsood and Benjakul, 2011; Thiansilakul et al., 2012a). Their redox chemistry supports their pro-oxidative effect on lipids. During the process of autoxidation, ferrous myoglobin or haemoglobin is converted to ferric metmyoglobin or haemoglobin. The superoxide anion radical liberated in this process can readily be converted to hydrogen peroxide, which enhances the ability of heme proteins to promote lipid oxidation (Thiansilakul et al., 2012). Methaemoglobin (MetHb) or Metmyoglobin (MetMb) reacts with hydrogen peroxide or lipid hydroperoxides to generate ferryl heme protein radicals, which can abstract hydrogen from polyunsaturated fatty acids thus initiating lipid oxidation (Miller et al., 1997).

### Microbiological quality

The microbiological quality of meat is dependent on the physiological status of the animal at slaughter, the spread of



**Figure 3.** Meat microbiological quality of New Zealand white rabbits subjected to halal slaughter without stunning and gas stun-killing. HS: Halal slaughter without stunning; GK: gas stun-killing. <sup>ab</sup> Means with different letters differ significantly at  $p < 0.05$ .

contamination during slaughter and processing, the temperature and other conditions of storage (Koutsoumanis and Sofo, 2004). Figure 3 shows the effect of slaughter method on microbial levels of rabbit meat during the first eight days postmortem. At d 0, slaughter method had no significant effect on microbial growth. However, at d 3 postmortem, greater growth of *Pseudomonas aeruginosa* and *E. coli* were indicated by meat samples obtained from the GK group ( $p < 0.05$ ) with *B. thermosphacta* and total aerobic counts remained unaffected by slaughter method. At d 5 and 7 postmortem, bacterial counts for all tested microbes were affected by slaughter method, with samples from the GK group exhibiting significantly higher growth than the HS. Generally, increased growth of all microorganisms with storage time was observed in meat samples from both slaughter groups and meat samples from the gas stun-killed animals had the highest counts of all microorganisms considered in this study.

The bacterial count was consistent with the amount of residual blood in the carcass. The higher bacterial growth exhibited by the GK group can be attributed to low blood loss due to more readily accessible nutrients available for bacterial growth in the retained blood. Blood favours

multiplication of spoilage microorganisms (Lerner, 2009). In kosher slaughtering, the meat is further soaked in water for about half an hour and then placed on a slanted board and thickly covered with salt on each side for about 20 to 60 min so that the salt draws blood from the meat by osmosis. Hajmmer et al. (1999) reported that kosherisation of beef briskets reduced the aerobic plate count (APC), coliforms, *Escherichia coli* and Salmonella counts on the samples compared to initial counts. One of the most important factors that affect the level of contamination and therefore enhance the extent of the deterioration is the amount of blood left within the carcass after bleeding. Blood is considered to be an excellent medium for bacterial growth due to its high nutritive value, temperature, pH and water activity or relative humidity. Glucose, the substrate preferentially used by many microorganisms such as *B. thermosphacta* (Gill and Newton, 1977) and *Pseudomonas* (Warriss, 2000) when growing in meat, is readily available in blood. Ali et al. (2011) also reported that higher blood loss in halal slaughter was associated with lower bacteria count in minced meat at 48 h postmortem.

Unlike red meats, the shelf life and microbial quality of rabbit meat and the changes it may undergo during storage

have been not fully studied (Sunki et al., 1978; Rodríguez-Calleja et al., 2005; 2006). Bobbitt (2002) studied the shelf life of rabbit carcasses stored at 4°C. He estimated a shelf life of only 3 days for boxed rabbit carcasses. Rabbit carcasses spoil more rapidly due to higher ultimate pH (Rodríguez-Calleja et al., 2005).

## CONCLUSION

Meat is a low acid food with pH ranges close to neutral (5.4 to 7.0) and particularly rabbit meat is very susceptible to spoilage due to its high ultimate pH. Our results indicate that stunning and bleeding methods do affect the shelf life of rabbit meat, and halal slaughter without stunning may be a favourable option compared to gas stun-killing due to high bleed out. This study affirms that religious slaughter can be used successfully as an alternative to conventional stunning methods in order to improve the keeping quality of meat. However, the procedure should be carefully conducted without compromising animal welfare.

## ACKNOWLEDGEMENT

This project was funded by Universiti Putra Malaysia through Research University Grant Scheme, grant no. 02-02-12-1713RU.

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