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K. Nakyinsige, A.Q. Sazili, I. Zulkifli, Y.M. Goh, F. Abu Bakar, A.B. Sabow

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INFLUENCE OF GAS STUNNING AND HALAL SLAUGHTER (NO STUNNING) ON RABBITS WELFARE INDICATORS AND MEAT QUALITY

K. Nakyinsige[a,f], A. Q. Sazili[a,b*], I. Zulkifli[a,b,c], Y. M. Goh[c,d], F. Abu Bakar[a,e] and A. B. Sabow[b,g]

[a] Halal Products Research Institute, [b] Department of Animal Science, Faculty of Agriculture, [c] Institute of Tropical Agriculture, [d] Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, [e] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

[f] Department of Food Science and Nutrition, Islamic University In Uganda, 2555, Mbale, Uganda

[g] Department of Animal Resource, Faculty of Agriculture, University of Salahaddin, Karkuk Street , Runaki 235 n323, Erbil, Kurdistan Region, Iraq.

Corresponding author: Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel.: +603-89474870; Fax: +603-893810244.
E-mail: awis@upm.edu.my (Awis Qurni Sazili).

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1. Introduction

Animals may be at great risk of fear during the procedures that take them to new situations, such as pre-slaughter handling, which implies an important additional stress (Duncan, 2004). It is important to note that each animal perceives, at slaughter, several signals of danger, such as odours, sights and sounds. In fact for these animals, vision, audition, and particularly olfaction constitute a very rich perceptive universe which is used to regulate social and sexual behaviours and to ensure the survival in dangerous situations (Micera, Albrizio, Surdo, Moramarco, & Zarrilli, 2010). In order to determine the changes produced a few seconds after receiving the stimulus, as is the case at the moment prior to slaughter, it is important to evaluate the changes produced within the sympathetic-adrenomedullary system, with the liberation of catecholamines to the bloodstream.

Recently, there has been increasing interest in the measurement of stress at slaughter as an indicator of animal welfare status (Gupta, Earley & Crowe, 2007). Stress reactions to the slaughter procedure influence ante- and post mortem muscle metabolism and, consequently, the rate and extent of glycogen breakdown and pH decline. Because there exists a relationship between the pre-slaughter handling of animals and meat quality (Nowak, Mueffling & Hartung, 2007; Terlouw, 2005; Hambrecht, Eissen, Nooijen, Ducro, Smits, den Hartog & Verstegen, 2004; Kannan, Kouakou, Terrill, & Gelaye, 2003; Sañudo, Sanchez, & Alfonso, 1998; Gregory, 1994), it strengthens the hypothesis that a lower animal stress during the slaughtering phase

improves meat and meat products quality with positive economic and qualitative influences (Casoli, Duranti, Cambiotti & Avellini, 2005). For instance, minimizing stress at slaughter ensures yielding meat with optimum ultimate pH and minimizes incidences of dark, firm and dry (DFD) and pale, soft and exudative (PSE), thus producing meat products with the desired colour, texture, myofibrillar fragmentation index (MFI) and juiciness. The welfare of animals at slaughter time is protected by the Humane Slaughter Act of 1958, which makes stunning prior to slaughter mandatory in order to ensure that animals are unconscious and do not suffer unnecessarily. However, for human rights and freedom of worship purposes, the law permits slaughtering in accordance with ritual requirements of any religious faith that prescribes a method of slaughter whereby the animal suffers loss of consciousness by severance of the carotid artery with a sharp instrument (Nakyinsige, Che Man, Aghwan, Zulkifli, Goh, Abu Bakar, Al-Kahtani & Sazili, 2013a). Although there has been some research on the effect of slaughter method on meat quality (Channon, Payne & Warner, 2002; Hambrecht et al., 2004; Henckel, Karlsson, Jensen, Oksjerg & Petersen, 2002; Kim, Lee, Jung, Lim, Seo, Lee, Jang, Baek, Joo & Yang, 2013; Lafuente & Lopez, 2000; Savenije, Schreurs, Winkelman-Goedhart, Gerritzen, Korf & Lambooij, 2002), most information originates from work in conventional slaughter methods with limited comparison to religious slaughter (Anil, 2012). Recently, to ensure animal welfare and optimum meat quality, carbon dioxide (CO_2) gas stunning is considered a valid alternative system to stun animals such as pigs, poultry and sheep (Linares, Bórnez & Vergara, 2007; Nowak et al., 2007; Gregory, 2005; Vergara, Linares, Berruga & Gallego, 2005). However, the method is not often practiced in rabbit slaughtering because its effect on the welfare of rabbits has not been satisfactorily scientifically investigated (EFSA, 2006).

Halal slaughter without stunning has been associated with delayed loss of consciousness (Gregory et al., 2010) and a noxious stimulus in the period following the ventral neck incision (Gibson et al., 2009). However, in rabbits, Lopez et al (2008) observed no reaction to the throat cut, no vocalization, spasms or movements were observed during the hanging phase or after halal slaughtering and the rabbits' bodies remained totally relaxed and floppy on the chain from the beginning. On the other hand, CO_2 stunning is said to be advantageous as it requires less handling, particularly eliminating the necessity of restraining the animals, and more than a single animal can be stunned simultaneously (Nowak et al, 2007; Niel and Weary, 2006; EFSA, 2004). However, in rabbits, exposure to high concentrations of carbon dioxide has been recognized to often trigger severe aversive reactions during most experimental investigations (EFSA, 2005). Never the less, according to Hertrampf and von Mickwitz, 1979 cited by EFSA (2006) rabbits are rather tolerant to carbon dioxide; they could be stunned if body size and breed are taken into account, and stunning them in groups would avoid unnecessary stress. In an earlier study involving lowering rabbits individually into gas-filled containers at a commercial slaughter plant, Dickel, 1976 cited by EFSA (2006) showed that exposure of rabbits to a CO_2 concentration of 60-70% by volume for 20 to 25 sec was optimal to achieve a reflexless narcosis and concentrations higher than 70% tended to stun kill. Thus this study aimed at assessing the effect of CO_2 gas stunning which has not been conducted until now in comparison with slaughter without stunning on physiological stress responses and meat quality in rabbits.

2. Materials and Methods

2.1 Ethical Note

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

2.2 Experimental animals, stunning and slaughter

A total of 80 male New Zealand white rabbits weighing between 1800 g and 2000 g were obtained from a commercial farm (East Asia Rabbit Corporation) located in Semenyih, West Malaysia.The rabbits were divided into two groups of 40 animals each and subjected to either gas stunning (GS) or halal slaughter (HS). The slaughter procedure was conducted at the Department of Animal Science abattoir, Faculty of Agriculture, Universiti Putra Malaysia. In the halal method (HS), the 40 animals were humanely slaughtered according to halal slaughter procedure as outlined in the Malaysian Standard MS1500: 2009 (Department of Standards Malaysia, 2009). The animals were slaughtered by a licensed slaughter man by severing carotid artery, jugular vein, trachea and oesophagus. The vagus nerve was also severed. In order to carry out gas stunning (GS), groups of ten rabbits were placed in a gas chamber containing 61.4 % CO_2, 20.3 % O_2 and 18.29 % N_2 for 5 min. All the 40 animals were subsequently bled to drain excess blood from the carcass.

2.3 Blood sampling

To determine the basal values of the analysed parameters, blood was collected from the ear vein of ten randomly chosen animals assigned as the control group. The animals were comfortably

restrained in a commercial rabbit restrainer and 5 ml of blood were collected from the ear vein using 21 gauge needles. At exsanguination, 5 ml of the sticking blood were obtained from the jugular venipuncture of ten randomly chosen animals per treatment from both HS and GS. Ten representative blood samples per treatment for hematological parameters were collected in lithium heparin tubes, pre-chilled and transported to the Hematology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia within less than two hours. Samples for hormone analysis were collected in EDTA tubes, pre-chilled before centrifuged at 800 g for 15 min at 4^{o}C. The resultant plasma were divided into aliquots and stored at -80^{o}C until subsequent analysis.

2.4 Carcass sampling

After evisceration and carcass dressing, approximately 20 g of *Biceps femoris* (BF) muscle from the left hind limbs were collected, properly labeled, vacuum packaged and stored in a 4^{o}C chiller for drip loss determination (Honikel, 1998). The left *Longissimus lumborum* (LL) between the 6th and 8th lumbar vertebra was removed and divided into two, and snap frozen in liquid nitrogen before being stored at -80°C for subsequent determination of pH (pre-rigor) and glycogen content, and myofibrillar fragmentation index (MFI) at d 0. The carcasses were then hung in the 4°C chiller and after trimming off any visible connective tissue, the right LL muscle was dissected (6th to 8th, 9th to 10th and 11th-12th lumbar vertebra) at 3 specific periods, that is, 1, 7 and 14 d post mortem, respectively, vacuum packed and stored in a – 80^{o}C freezer until subsequent analyses of pH, colour, shear force and cooking loss. The left LL muscle from the 9th to 12th lumbar vertebra was dissected into three portions at specific periods of 1, 7 and 14 d post mortem for subsequent analysis of MFI.

2.5 Determination of physiological stress responses

Physiological stress responses (animal welfare indicators) were determined through plasma catecholamines (adrenaline and noradrenaline) as well as biochemical and haematological parameters. Biochemical and haematological parameters were determined using the method of Nakyinsige, Sazili, Aghwan, Zulkifli, Goh & Abu Bakar (2013b). Biochemical parameters (Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), glucose, lactate, urea, total protein and calcium) were determined using an automatic analyzer (Automatic analyzer 902 Hitachi, Germany). All reagents used were from Roche (Hitachi). Total haemogram (packed cell volume (PCV), haematocrit, haemoglobin, red blood cells (RBC), white blood cells (WBC), and lymphocytes) was determined using an automatic haematology analyzer (CELL DYN® 3700, Abbot, USA.) using Veterinary Package soft ware. The quantitative analysis of adrenaline (epinephrine) content in blood was carried out using Adrenaline Plasma Enzyme-Linked ImmunoSorbent Assay (ELISA) High Sensitive kit # BA E-4100 (LDN®, Germany) while noradrenaline (norepinephrine) quantification was carried out using Noradrenaline Plasma ELISA High Sensitive kit # BA E-4200 (LDN®, Germany). The competitive ELISA kits used the micro titer plate format where the hormone is extracted from a plasma sample using a cis-diol-specific affinity gel, acylated and then modified enzymatically. The antigen is bound to the solid phase of the micro titer plate and the derivatized standards , controls, samples as well as the solid phase bound analytes compete for a fixed number of anti serum binding sites.

2.6 Determination of meat quality traits

Both the pre and post rigor pH of the meat was determined by the indirect method using a portable pH meter (Mettler Toledo, AG 8603, Switzerland). The samples were removed from -80^{o}C storage and manually pulverized in liquid nitrogen. Approximately 0.5 g of each crushed muscle sample was homogenized (Wiggen Hauser® D-500, Germany) for 30 s in 10 ml ice cold deionized water in the presence of 5 mM sodium iodoacetate (Merck Schuchardt OHG, Germany) to prevent further glycolysis. The pH of the resultant homogenates was measured using the electrode attached to the pH meter.

The meat colour determination was conducted by Color Flex spectrophotometer (Hunter Lab Reston, VA, USA) using International Commission on Illumination (CIE) Lab-values (also known as L*, a*, b*) with D56 illuminant and 10˚ standard observer, tristimulus values (X,Y,Z) and reflectance at specific wavelength (400-700) nm to express the meat colour data. The device was calibrated against black and white reference tiles prior to use. The frozen muscle samples of approximately 10 mm of thickness (AMSA, 2012) from day 0, 1 and 7 were transferred from -80°C freezer into a 4°C chiller and stored overnight. The thawed samples were unpacked and bloomed for 30 min, and were placed with the bloomed surface in contact with the base of the Color Flex cup. For each sample, a total of three readings (the cup rotates 90˚ in the second and third reading) of L*, a* and b* values were recorded and then averaged (Hunt, 1980).

The water holding capacity (WHC) of the meat was determined in terms of drip loss and cooking loss according to the methods described by Honikel, (1998). For drip loss, fresh meat samples

dissected from the *Biceps femoris* (BF) muscle from the left hind limbs were individually weighed (approximately 20 g) and recorded as initial weight (W1). The weighed samples were placed into polyethylene plastic bags, properly labeled, vacuum packaged and stored in a 4°C for 7 days. After the 7 d storage, the samples were removed from the bags, gently blotted dry using paper towels, weighed and recorded as W2. The drip loss was calculated and expressed as the percentage of differences of sample initial weight and sample weight after 7 d storage divided by sample initial weight (% drip loss = [(W1- W2) ÷ W1] × 100) (Honikel, 1998). The samples that were used for color determination were collected and used for determining cooking losses. After colour determination, the samples were individually weighed and recorded as initial weight (W1), placed in water-impermeable polyethylene plastic bags and vacuum packed. The samples were then cooked in a pre heated water bath set at 80°C. When the internal temperature of the samples reached 78°C as monitored using a stabbing temperature probe (HI 145-00 thermometer, HANNA® instruments, USA) inserted into the geometric centre of the sample, the cooking was continued for another 10 min. The cooked samples were then removed from the water bath, equilibrated to room temperature, removed from the bag, blotted dry using paper towels without squeezing, and reweighed (W2). The cooking loss percentage was calculated using the following equation:

Cooking loss (%) = [(W1- W2) ÷ W1] × 100 (Honikel, 1998).

The samples used for cooking loss determination were collected and used for determining tenderness of the rabbit meat. The textural assessment was conducted using the TA.HD plus® texture analyser (Stable Micro System, Surrey, UK) equipped with a Volodkevitch bite set. The

equipment was calibrated at 5 kg for weight, 10 mm return distance for height and the blade speed was set at 10 mm/s. Sample preparation was conducted following the procedure previously described by Sazili, Parr, Sensky, Jones, Bardsley & Buttery (2005). From each sample, at least 3 replicate blocks (1 cm × 1 cm × 2 cm) were cut as parallel to the direction of the muscle fibres as possible and each block was sheared in the centre and perpendicular to the longitudinal direction of the fibres. Shear force values were reported as the average peak positive force of all blocks value of each individual sample.

2.7 Determination of glycogen content

Glycogen content of the LL muscles was determined using Glycogen Assay Kit # K646-100 (Bio Vision, USA) following the manufacturer's instructions for the colorimetric assay.

2.8 Myofibril fragmentation index measurement

MFI was measured according to the turbidity method of Hopkins, Littlefield & Thompson (2000) with some modifications. In duplicate, 2.5 g of pulverized muscle samples were mixed with 30 ml cold buffer (100 mM KCl, 20 mM potassium phosphate, 1 mM EDTA,1 mM $MgCl_2$, pH 7.0 at 4°C) and homogenized on ice using an Ultra- Turrax T5FU (IKA- Labrortechnik Staufen, Germany) for 60 s. The homogenate was centrifuged at 1000 g, 2°C for 15 min using an Avanti® J-26XPI centrifuge (BECKMAN COULTER®, USA). The supernatant was discarded with the pellet re-suspended in 25 ml buffer following which, the centrifugation was repeated. The resulted supernatant was discarded and the pellet suspended in 15 ml of buffer, followed by vortexing. The myofibril suspensions were then filtered into 50 ml centrifuge tubes through 1.0

mm polyethylene strainers to remove any remaining connective tissue. The total protein concentration of the final suspension was determined using the Bio-Rad Protein Assay Kit II 500-0002 from Bio-Rad (USA) following the micro plate protocol for colorimetric analytical procedure, with Bovine Serum Albumin used for the standard curve and absorbance was measured at 595 nm using a RAyto RT- 2100C microplate reader (Rayto, China). In triplicate, aliquots of myofibril suspensions were diluted in the buffer to a final protein concentration of 0.5 ± 0.05 mg/ml, vortexed and poured into cuvettes. Absorbance was immediately measured at 540 nm with a spectronic®20 GENESYS^{TM} spectrophotometer (Spectronic instruments, USA). The mean of the triplicate absorbance readings was multiplied by 150 to obtain the MFI (Hopkins et al., 2000).

2. 9 Data analysis

The experiment was of a completely randomized design. Data analysis was performed using the GLM procedure of Statistical Analysis System package (SAS) Version 9.1.3 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA) and statistical significance was set at $P<0.05$. A Duncan multiple range test was used to test the significance of variance between the means of the studied parameters.

3 Results and Discussion

3.1 Effect of slaughter method on blood biochemical parameters

Analysis of the sticking blood is one way to obtain information on the animal's pain as this blood provides information on the type and degree of stress to which the animal was subjected during stunning and sticking (Nowak et al., 2007). Changes in biochemical and haematological constituents of rabbit blood after slaughter are shown in Table 1. All variables were significantly higher than the basal values ($P<0.05$). Results indicate that the killing of animals was associated with hypercalcemia, hyperglycemia, lactic acidemia, and increases in haematocrit and activities of liver enzymes Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT), and Creatine kinase (CK), leucocytosis and lymphocytopenia. There was no significant difference on the effects of both slaughter methods on dehydration in rabbits as evidenced by the lack of significance difference in total protein, haematocrit and packed cell volume (Table 1). Both slaughter methods caused hypercalcemia. However, GS resulted in significantly higher concentration of calcium ions in blood compared to HS. The killing procedure was generally associated with lymphocytopenia although there was no significant difference between GS and HS.

There are no specific plasma indicators for pain. However, if the pain causes muscle activity or fear, some plasma indicators of those reactions can be monitored instead (Gregory, 1998). Both slaughter methods caused hyperglycemia. However, GS exhibited significantly higher levels of blood glucose than HS. Quantification of glucose is a useful tool for assessing stress owing to its involvement in energy metabolism during stressful situations (Nakyinsige et al., 2013b). During stressful situations, the secretion of catecholamine and glucocorticoids stimulates hepatic

glycogenolysis leading to an increase in glucose levels (Pollard, Littlejohn, Asher, Pearse, Stevenson-Barry, Mcgregor, Manley, Duncan, Sutton, Pollock & Prescott, 2002; Knowles & Warriss, 2000; Shaw and Turne, 1992). When the rabbits were stunned in the gas chamber, they were not physically restrained. The chances of physical activity or rather struggling to look for oxygen is higher and thus a larger percentage of alteration in glucose levels in comparison to the baseline values. Gas stunning is achieved through a neuronal function caused by hypercapnic hypoxia and diminishing pH in the central nervous system (Niel & Weary, 2006; Raj, 2004; Velarde, Gispert, Faucitano, Manteca & Diestre, 2000; Warriss, 2000; Kohler, Meier, Busato, Neiger-Aeschbacher & Schatzmann, 1999). In addition, stunning in the CO_2 chamber increases the anaerobic oxidative metabolism that increases glucose levels in the blood stream (Becerril-Herrera et al., 2009).

Lactate levels in the blood can also be used to assess pre-slaughter stress shortly before or during slaughter and/or stunning (Nowak et al., 2007; Hambrecht et al., 2004; Brown, Warriss, Nute, Edwards & Knowles, 1998; Jensen-Waern & Nyberg, 1993). Pre-slaughter stress was reported to be correlated to high lactate levels in the blood of slaughtered pigs (Nowak et al., 2007; Brown et al., 1998; Hambrecht et al., 2004). In the present study, the levels of lactate after slaughter were significantly higher than the basal levels. With regard to the slaughter methods, the level of lactate was higher in gas stunned (GS) rabbits than in the halal group (HS) but the values were not significantly different. Slaughter without prior stunning has also been implicated in increased blood lactate as a result of rapid anaerobic glycolysis (Grandin, 1998). On the other hand, stunning in the CO_2 chamber increases the anaerobic oxidative metabolism, stimulates the respiratory rate and may lead to respiratory distress (Becerril-Herrera et al., 2009). Stunning with

80% CO_2 for 70 or 100 s induced stress as evidenced through higher lactate levels in pigs (Nowak et al., 2007). Mota-Rojas, Becerril-Herrera, Roldan-Santiago, Alonso-Spilsbury, Flores-Peinado, Ramírez-Necoechea, et al. (2012) reported a threefold increment in lactate levels compared to the baseline after stunning pigs with 80% CO_2. In line with Velarde et al. (2000), these authors also explained that CO_2 stunning is caused by a depression of the neuronal function followed by hypercapnic hypoxia and decreased pH in the central nervous system. Moreover, CO_2 stunning increases anaerobic oxidative metabolism that rises lactate in the bloodstream (Becerril-Herrera et al., 2009), leading to metabolic acidosis. Becerril-Herrera et al. (2009) attributed the high lactate levels in pigs to the atmospheric change of CO_2, which forces the pig to use alternative metabolic routes (like the lactate one) for ATP production. An upsurge in blood lactate occurs as a result of anaerobic glycolysis, during which pyruvate is reduced to lactate by the liver enzyme lactate dehydrogenase.

The slaughter procedure generally increased the activities of liver enzymes ($P<0.05$). Compared to halal slaughter, gas stunning caused significantly higher activities of enzymes LDH, AST, and CK. The level of ALT activity for the two slaughter methods was not different ($P>0.05$). The increased activity of liver enzymes is indicative of weariness, tissue damage and muscle fatigue. Elevated levels of LDH in serum are indicative of stress and muscle fatigue (weariness). Elevated levels of transaminases are indicative of damage to internal organs. Elevated CK activity is an indicative of cell muscle damage and muscle fatigue (EFSA, 2004).

In the present study, the slaughter procedure generally caused hypercalcemia, hyperglycemia, lactic acidemia, an increase in haematocrit, increase enzyme activity, leukocytosis and

lymphocytopenia. These biochemical and hematological changes in rabbits at the slaughter time indicated an intense stress response from animals in order to cope to this situation. Noteworthy, none of the parameters exceeded the normal physiological range for rabbits. This is in line with the arguments of Becerril-Herrera et al. (2009), Hartung, von Müffling & Nowak (2008) and Shaw & Turne (1992) that after sacrifice, most stunning methods lead to an increase in critical blood constituents like catecholamines, lactate, glucose, calcium, magnesium, and proteins although these alterations may not necessarily translate into compromising animal welfare.

3.2 Effect of slaughter method on catecholamines levels

Changes in the amount of catecholamines (adrenaline and noradrenaline) are as presented in Table 2. Generally, there was a highly significant increase in the amount of both adrenaline and noradrenaline following the slaughter procedure. Under normal (non-stressful) physiological conditions, catecholamines are released from the adrenal medulla to regulate certain body functions like maintenance of blood pressure. However, under stressful situations, high concentrations of catecholamines are discharged into the blood stream to prepare the body in the case that rapid energy expenditure was required (Shaw & Tume, 1992). When an animal bleeds out, there is a fall in pressure and this activates the sympathetic adrenal medullary nervous system resulting in the release of noradrenaline from the sympathetic endings and the adrenal medulla along with adrenaline (Gregory, 1998). Authors observed a five times rise in adrenaline amongst HS animals and a ten times rise among the GS animals. The noradrenaline was seven times higher in the sticking blood than basal values for HS while the value was twelve times higher for GS. In horses, Micera et al. (2010) also observed an increment in catecholamines after captive bolt stunning, when compared to the level measured during the lairage. Nowak et al.

(2007) and Hambrecht et al. (2004) indicated over hundred fold increment in adrenaline epinephrine and noradrenaline in the plasma of pigs stunned with CO_2. Hartung, Nowak, Waldmann & Ellenbrock (2002) also reported an extreme increase in catecholamine levels in blood after CO_2 gas stunning in pigs. Forslid (1988) suggested that the CO_2 gas could be involved in the process of respiratory acidosis which is an important and potent sympathetic-adrenal stimulus factor promoting noradrenaline release. Conversely, Forslid (1988) observed that levels of catecholamines during CO_2 stunning did not differ from those recorded post-transportation. In lambs, Linares, Bórnez & Vergara (2008) also found no significant effect of stunning on noradrenaline levels. Noteworthy, the increment in catecholamines may not necessarily indicate slaughter-induced stress as some authors have indicated that high levels of catecholamines in the sticking blood are due more to the stunning technique itself than an indication of the amount of stress (Nowak et al., 2007; Hambrecht et al., 2004; Troeger & Woltersdorf, 1991). For instance, Hambrecht et al. (2004) found that the levels of both catecholamines in the sticking blood of pigs were about 10 times lower after electrical stunning although the blood also contained indicators of stress, particularly cortisol and lactate concentrations.

3.3 Effect of slaughter method on meat quality

3.3.1 Muscle glycogen content

Muscle glycogen content at the time of slaughter is one of the most influential factors of ultimate pH (Rosenvold, Petersen, Laerke, Jensen, Therkildsen, Karlsson, et al., 2001). When glycogen reserves are low at the time of slaughter, a small amount of lactic acid is formed during rigor development resulting in high ultimate pH. The results for the effect of slaughter method on

muscle glycogen content are shown in Table 3. Before the onset of *rigor mortis*, the concentration of glycogen in the muscle was not different for the two slaughter methods (P>0.05). After *rigor mortis*, the LL muscles from the HS group presented higher glycogen than those from the GS group although the values were only significant on day 7. Contrary to the findings of Channon et al., (2002) who, in pigs, reported that stunning method (head to brisket, head only and CO_2 stunning) did influence muscle glycogen concentrations post rigor. In the present study, rabbits from the GS group had less muscle glycogen compared to those from the HS group. This could be explained by the way in which the slaughter procedure was performed. In halal slaughter, the rabbits were carefully restrained and slaughter was performed by a well trained slaughter man using a very sharp knife. Lopez, Carrilho, Campo & Lafuente (2008) reported that the halal slaughtered rabbits had no reaction to the throat cut and no vocalization, spasms or movements was observed during the hanging phase or after slaughtering. They observed that the rabbits' bodies remained totally relaxed and floppy on the chain from the beginning. The reduced glycogen reserves could also be explained by the increased anoxic convulsion observed in gas stunned rabbits which causes increased utilization of adenosine triphosphate (ATP) by the muscles. The anaesthetic effect of gas stunning has been shown to be responsible for the increasing rate of glycogen metabolism (Savenije et al., 2002). Additionally, CO_2 stunning has previously been shown to be responsible for a decrease in the glycogen level (Henckel et al., 2002).

3.3.2 Muscle pH values

The results for the effect of slaughter method on muscle pH are presented in Table 3. The pre rigor pH was significantly different, with HS having lower pH compared to GS (6.53 and 6.73, respectively). At d 1 and 7 post mortem, the statistical significance was absent although the pH for HS was numerically lower than that of GS (6.19 vs. 6.29 and 6.04 vs. 6.12, respectively). Although the stunning of rabbits affects meat quality by influencing post mortem muscle acidification, these differences in muscle pH during early rigor development may not affect the ultimate muscle pH (Lafuente & Lopez, 2000; Dal Bosco, Castellini & Bernardini, 1997). Though there were not described any statistical differences, the post rigor pH of GS was higher that HS. The numerically high value of pH in GS could be related with a high level of catecholamines as reported by Foury, Devillers, Sanchez, Griffon, Le Roy & Mormede (2005) who also explained that catecholamines increase the glycogenolysis and therefore reduce the lactic acid production post mortem. High levels of adrenaline and noradrenaline have been associated with the stunning technique itself more than the amount of stress (Nowak et al., 2007; Hambrecht et al., 2004). In the present study, both adrenaline and noradrenaline were almost two times higher in GS than HS animals.

3.3.3 Muscle colour values

The effect of slaughter method on the colour of rabbit LL muscle is shown in Table 3. On d 1, GS showed significantly greater lightness than HS. However, on d 7, the lightness of LL muscles from both HS and GS did not differ. No significant differences were observed in meat redness. GS showed significantly greater yellowness than HS on d 1. However, the yellowness values did not differ significantly on d 7. The results of the present study show that meat from the HS group was darker (lower L* value) than that from GS group. This finding disagrees with the earlier

findings of Onenc & Kaya (2004) and Channon et al. (2002) but agrees with the findings of Linares et al. (2007) who also reported darker meat in un- stunned lambs as compared to the CO_2 stunned ones. Kim et al. (2013) also found higher lightness of bovine *Longissimus* muscle in CO_2 gas stunning treatment than in captive bolt stunning. These authors attributed the high L* value to the possible high level of stress hormones. In the present study, the GS group had significantly higher catecholamines than HS. The redness of the meat did not differ significantly subject to slaughter method. Channon et al. (2002) also reported that for pork, the a* and b* values were not influenced by stunning method. The possible explanation for this is the lack of variation in the myoglobin content of the muscles. Myoglobin is the major heme protein responsible for the red colour of meat (AMSA, 2012).

3.3.4 Drip loss, cooking loss and shear force values

As shown in Table 3, the drip loss of *Biceps femoris* muscle of rabbits subjected to HS and GS did not differ (1.50 % vs. 1.44 %, $P>0.05$). Upon cooking, the WHC of the *Longissimus lumborum* muscle was significantly different. The cooking loss for HS was significantly lower than that of GS (23.27 % vs. 25.70 % for d 1 and 20.43 % vs. 24.51 % for d 7). The lack of significant difference in the drip loss of *Biceps femoris* muscles from HS and GS can be attributed to the lack of variation in the ultimate pH. Water holding capacity is influenced by muscle pH decline and temperature post mortem. In agreement with the present findings, Onenc & Kaya (2004) also found no significant effect of slaughter method on the WHC of beef. Agbeniga, Webb & O'Neill (2013) reported no significant difference in drip loss of beef from Kosher and conventionally slaughtered (pneumatic captive bolt gun stunning for approximately 45 seconds before the neck cut) animals. In poultry, gas stunning affected water holding capacity

to a lesser extent (Savenije et al., 2002) while in lambs, Vergara & Gallego (2000) also found no difference in drip loss between electrically stunned and non-stunned animals.

Slaughter method had a significant effect on cooking loss, with HS exhibiting a lower cooking loss than GS. In light lamb, Linares et al. (2007) reported a lower cooking loss in the non-stunned animals compared to the CO_2 stunned and electrically stunned animals. According to Gregory (2008), most meat researchers would accept that meat quality in stunned animals is comparable to that from animals slaughtered without stunning. However, there are some researches that pointed out that meat from un-stunned animals had lower cooking losses (Agbeniga et al., 2013; Linares et al., 2007; Onenc & Kaya, 2004). Loss of water together with other soluble substances such as vitamins and minerals may also adversely affect the nutritional quality of the meat.

Table 3 also shows results for shear force. On d 1, HS exhibited lower shear force values compared to GS ($P<0.05$). The shear force values reduced with aging and at d 7, there was no significant difference between the two methods. Shear force is inversely related to tenderness and it is considered as one of the most important factors affecting consumer acceptability of meat (Sazili et al., 2005). In this study, meat samples from HS were comparable to those from the GS group in terms of tenderness. This result disagrees with the findings of Linares et al. (2007), in lambs, who reported that meat from the gas stunned group was more tender than that from the un-stunned group. However, in beef, Agbeniga et al. (2013) reported that meat from the kosher method was more tender than that from the conventional slaughter (pneumatic captive bolt gun stunning for approximately 45 s before the neck cut) method. The statistical and numerical differences observed at d 1 and d 7, respectively could be attributable to the higher water loss of

meat from the GS group during cooking. A similar explanation was given by Agbeniga et al. (2013) who attributed the higher shear force values of meat from the conventionally slaughtered group to significantly higher cooking loss.

3.4 Myofibril fragmentation index (MFI)

The turbidity method, which involves measuring the absorbance at 540 nm and multiplying the value by a constant, which is either 200 (Culler, Parrish, Smith & Cross, 1978) or 150 (Hopkins et al., 2000; Hopkins, Martin & Gilmour, 2004) is most commonly used method to obtain MFI. The result obtained using this method is as presented in Table 4. Accordingly, meat samples from GS exhibited significantly lower MFI than those from the HS animals. Determining the extent of fragmentation of myofibrils when subjected to homogenization is an indication of the degradation of muscle myofibrillar proteins under post mortem conditions and the MFI is a useful indicator of the extent of proteolysis reflecting the degradation of key structural proteins, particularly rupture of the I-band and breakage of intermyofibril linkages (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995). Degradation of intermyofibril linkages occurs as meat ages (Taylor et al. (1995). Hopkins et al. (2004) using the turbidity method, showed that samples aged for 1 day gave significantly lower values of MFI than those aged five days regardless of the type of homogenizer and speed of homogenization. In our study the MFI increased from 73.63 ± 0.51 at d 0 to 196.89 ± 1.61 at 14 and from 70.53 ± 0.91 to 178.35 ± 2.10 for HS and GS, respectively. Marino et al. (2013) reported a strong negative correlation ($r = -0.98$, $p<0.001$) between MFI and shear force whereas Karumendu et al. (2009) and Lametsch et al. (2007) reported weak negative correlations (-0.38 and -0.34, respectively). In the present study, MFI

was also weakly correlated to shear force (r = -0.24, p = 0.30 and r = -0.27, p = 0.26 for HS and GS, respectively).

3.5 The relationship between slaughter-induced stress and meat quality

Amongst stress-induced changes, adrenaline is most likely to play an important role in the determination of meat quality (Terlouw, 2005). Stress induces release of adrenaline into the blood stream. An early work showed that adrenaline injections before slaughter resulted in higher ultimate pH (Hedrick, Parrish & Bailey, 1964). In our study, no significant correlation was found between adrenaline and ultimate pH values (r = 0.25, P>0.05; and r = 0.09 P>0.05) for HS and GS, respectively) which is suggestive that stress experienced by the rabbits was below the threshold required to adversely affect meat quality. Besides, the ultimate pH values (6.19 and 6.29 for HS and GS, respectively) recorded in the present study falls within the normal range for rabbits.

Conclusion

To the best of our knowledge, this work constitutes the first physiological approach to compare the effects of gas stunning and halal slaughter without stunning on the welfare of rabbits. For both gas stunning and halal slaughtering, the studied welfare indicators in the sticking blood were significantly higher than their basal values taken at farm. The results revealed that both slaughter methods caused hypercalcemia, hyperglycemia, lactic acidemia, leukocytosis, lymphocytopenia and an increase in haematocrit and activities of enzymes LDH, ALT, and CK. Noteworthy, there was a five times and ten times increment in adrenaline amongst HS and GS

animals, respectively. The noradrenaline was seven times higher in the sticking blood than basal values for the former and twelve times higher for the later. These biochemical and hematological changes in rabbits at the slaughter time indicated an intense stress response from animals in order to cope to this situation even though it may not necessarily translate into compromising of animal welfare. The study is also enriched by comparing the two methods in the terms of meat quality. Ultimate pH, which is the commonly used parameter in studies assessing ante mortem factors was not variable and fell within the normal range for rabbit meat. The use of gas mixtures for stunning of rabbits can reduce the stress of pre-slaughter handling and probably increase throughput in slaughter plants. However, there is need for more studies bout the use of different gas mixtures.

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TABLE CAPTIONS

Table 1: Differences in Blood Biochemical and Hematological Parameters of Rabbits Subjected to Halal Slaughter and Gas Stunning

Table 2: Differences in the Amount of Catecholamines Released into the Blood Stream during Halal Slaughter and Gas Stunning

Table 3: Effect of Halal Slaughter and Gas Stunning on Meat Quality of New Zealand White Rabbit LL Muscle

Table 4: Effect of Halal Slaughter and Gas Stunning on Myofibril Fragmentation Index of Rabbit Meat

Table 1

Parameters	C	HS	GS	SEM	NPR
Glucose (mmol/L)	4.99^{c}	7.47^{b}	13.95^{a}	0.61	4.16-8.60mmol/L*
Lactate (mmol/L)	7.74^{b}	9.67^{a}	9.78^{a}	0.27	
Creatine kinase (U/L)	599.90^{c}	1917.00^{b}	2783.50^{a}	183.46	140-372*
Alanine aminotransferase ((U/L)	44.71^{c}	48.74^{ba}	51.14^{a}	1.38	45-80*
Aspartate aminotransferase (U/L)	23.90^{c}	37.12^{b}	51.72^{a}	1.5	35-130*
Lactate dehydrogenase (U/L)	309.70^{c}	574.20^{b}	738.60^{a}	13.90	
Calcium (mmol/L)	2.92^{c}	3.48^{b}	3.79b^{a}	0.07	2.75-3.50mmol/L*
Urea (µmol/L)	127.31^{c}	162.49^{b}	189.34^{a}	3.41	3320-7470 µmol/L *
Total protein (mmol/L)	64.99^{c}	69.54^{a}	68.56^{a}	0.92	5.4-7.5g/dl*
Haematocrit	28.28^{b}	31.57^{a}	31.36^{a}	0.47	33-50%$^{\#}$
Packed cell volume (L/L)	0.22^{b}	0.30^{a}	0.31^{a}	0.00	33-50%$^{\#}$
White blood cells (x10^{9}/L)	5.11^{c}	8.26^{a}	6.73^{b}	0.31	5-12.5$^{\#}$
Lymphocytes (x10^{9}/L)	4.07^{a}	1.77^{b}	1.86^{b}	0.13	1.6-10.6$^{\#}$
Red blood cells (x10^{12}/L)	4.70^{b}	5.21^{a}	5.10^{ab}	0.07	5-8$^{\#}$
Haemoglobin (g/L)	94.26^{b}	105.22^{a}	104.54^{a}	1.56	10-17× 10 g/L$^{\#}$

C = Control (basal blood parameters before slaughter).
HS = Halal slaughter.
GS = Gas stunning.
NPR = Normal Physiological Range
SEM = Standard error of mean.
*Melillo, A. (2007). Rabbit Clinical Pathology. *Journal of Exotic Pet Medicine, 16(3), 135-145*
$^{\#}$Merck Sharp & Dohme Corpration (2011). The Merck Veterinary Manual. Retrieved 19/02/2013 from http://www.merckvetmanual.com/mvm/htm/bc/tref6.htm
^{a,b,c} Least square means within the same row with different superscripts differ significatly at$P<0.05$.
Number of samples = 10

Table 2

Parameters	C	HS	GS	RMSE	Level of significance
Adrenaline (ng/ml)	27.6 ± 1.42^{c}	138.0 ± 1.99^{b}	275.9 ± 2.36^{a}	6.20	***
Noradrenaline (ng/ml)	38.4 ± 1.54^{c}	268.8 ± 1.70^{b}	460.8 ± 1.73^{a}	5.25	***

C = Control ((basal blood parameters before slaughter).
HS = Halal slaughter.
GS = Gas stunning.
RMSE = Root mean square error.
^{a,b,c} Least square means within the same row with different superscripts differ significatly at $P< 0.05$.
Number of samples = 10.
*** = significantly different at $p<0.0001$.

Table 3

Parameter		Days post mortem	HS	GS	SEM
		0	1.01[a]	1.01[a]	0.01
Glycogen (mg/kg)		1	0.87[a]	0.86[a]	0.01
		7	0.49[a]	0.45[b]	0.01
	Pre-rigor	0 (less than 15 min)	6.53[b]	6.73[a]	0.04
pH (unit)	Post rigor	1	6.19[a]	6.29[a]	0.05
		7	6.04[a]	6.12[a]	0.09
	L*	1	45.6[b]	47.5[a]	0.50
		7	43.6[a]	44.2[a]	0.50
Colour	a*	1	8.90[a]	8.80[a]	0.70
values		7	6.90[a]	8.40[a]	0.50
	b*	1	14.96[a]	13.05[b]	1.03
		7	13.97[a]	12.96[a]	0.46
Drip loss (%)		7	1.50[a]	1.44[a]	0.11
		1	23.27[b]	25.70[a]	0.78
Cooking loss (%)		7	20.43[b]	24.51[a]	0.98
		1	0.82[b]	1.190[a]	0.10
Shear force (kg)		7	0.81[a]	0.91[a]	0.06

HS-Halal slaughter
GS- Gas stunning
SEM- Standard error of mean
L* - lightness; a* - redness; b* - yellowness.
[a, b] Least square means within the same row with different superscripts differ significantly at $P<0.05$.
Number of samples = 40.

Table 4

Days post mortem	HS	GS	RMSE	Significance
0	73.63 ± 0.51^{a}	70.53 ± 0.91^{b}	2.33	*
1	96.67 ± 0.79^{a}	91.48 ± 0.97^{b}	2.80	**
7	169.32 ± 1.70^{a}	144.41 ± 1.08^{b}	4.51	***
14	196.89 ± 1.61^{a}	178.35 ± 2.10^{b}	5.92	***

HS-Halal slaughter
GS- Gas stunning
RMSE = Root mean square error.
[a, b] Least square means within the same row with different superscripts differ significantly at P<0.05.
Number of samples = 40.
* = significantly different at p<0.05, ** = significantly different at p<0.01, *** = significantly different at p<0.0001.

Highlights

>There is an increasing use of gas stunning due to the perceived improvement in meat quality. >However the method has not been practiced in rabbits due to lack of scientific studies on its effects on animal welfare. >We studied the effects of gas stunning on animal welfare and meat quality in rabbits. >The welfare indicators fell within the normal physiological ranged for rabbits and the meat quality was comparable to that of the unstunned animals.