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Influence of gas stunning and halal slaughter (no stunning) on rabbits welfare indicators and meat quality

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ABSTRACT

This study assessed the effect of gas stunning which has not been conducted until now in comparison with slaughter without stunning on the welfare and meat quality of rabbits. Eighty male New Zealand White rabbits were divided into two groups of 40 animals and subjected to either halal slaughter without stunning (HS) or gas stunning using 61.4% CO_2, 20.3% oxygen and 18.3 % nitrogen (GS). Analysis of the sticking blood revealed that both slaughter procedures caused a substantial increase in the levels of catecholamines, hypercalcemia, hyperglycemia, lactic acidemia and an increase in enzyme activities. The ultimate pH of the Longissimus lumborum muscle did not differ between treatments. GS exhibited higher lightness and cooking loss, and lower glycogen and MFI than HS. This indicates that both GS and HS can be significant stressors although the amount of stress may be below the threshold to negatively affect rabbit meat quality.

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 odors, 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 behaviors 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 (Gregory, 1994; Hambrecht et al., 2004; Kannan, Kouakou, Terrill, & Gelaye, 2003; Nowak, Mueffling, & Hartung, 2007; Sañudo, Sanchez, & Alfonso, 1998; Terlouw, 2005), 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 color, 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

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instrument (Nakyinsige, Che Man, et al., 2013). Although there has been some research on the effect of the slaughter method on meat quality (Channon, Payne, & Warner, 2002; Hambrecht et al., 2004; Henckel, Karlsson, Jensen, Oksjerg, & Petersen, 2002; Kim et al., 2013; Lafuente & Lopez, 2000; Savenije et al., 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 (Gregory, 2005; Linares, Bórnez, & Vergara, 2007; Nowak et al., 2007; 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, Fielding, Von Wenzlawowicz, & Von Holleben, 2010) and a noxious stimulus in the period following the ventral neck incision (Gibson et al., 2009). However, in rabbits, Lopez, Carrilho, Campo, and Lafuente (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 (EFSA, 2004; Niel & Weary, 2006; Nowak et al., 2007). 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 s 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 the 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 the carotid artery, jugular vein, trachea and esophagus. 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 analyzed 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 was collected from the ear vein using 21 gauge needles. At exsanguination, 5 ml of the sticking blood was 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 2 h. Samples for hormone analysis were collected in EDTA tubes, pre-chilled before centrifuged at 800 *g* for 15 min at 4 °C. The resultant plasma were divided into aliquots and stored at −80 °C until subsequent analysis.

2.4. Carcass sampling

After evisceration and carcass dressing, approximately 20 g of the *Biceps femoris* (BF) muscle from the left hind limbs was collected, properly labeled, vacuum packaged and stored in a 4 °C chiller for drip loss determination (Honikel, 1998). The left *Longissimus lumborum* (LL) between the 6th and 8th lumbar vertebrae 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 vertebrae) at 3 specific periods, that is, 1, 7 and 14 d post-mortem, respectively, vacuum packed and stored in a −80 °C freezer until subsequent analyses of pH, color, 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 hematological parameters. Biochemical and hematological parameters were determined using the method of Nakyinsige, Sazili, et al. (2013). 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 hemogram (packed cell volume (PCV), hematocrit, hemoglobin, red blood cells (RBCs), white blood cells (WBCs), and lymphocytes) was determined using an automatic hematology analyzer (CELL DYN® 3700, Abbot, USA) using Veterinary Package software. 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.