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Association of blood glucose, blood lactate, serum cortisol levels, muscle metabolites, muscle fiber type composition, and pork quality traits

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ABSTRACT

The objective of this study was to investigate the relationship of blood glucose levels with blood lactate, serum cortisol levels, postmortem muscle glycogen and lactate content, muscle fiber type composition, and pork quality traits. Compared to pigs with lower blood glucose levels, pigs with higher blood glucose levels showed higher blood lactate and serum cortisol levels at exsanguination, and they had lower residual glycogen and higher lactate content in the muscle at 45 min postmortem. In addition, pigs with higher blood glucose levels had higher type IIB and lower type I area composition and finally exhibited lower muscle pH, paler color, and excessive loss of fluid on surface. These results imply that measuring blood glucose levels at exsanguination can be useful to indicate early glycolytic rates during postmortem and thus may be of value in the identification of pork with undesirable quality traits.

1. Introduction

Pre-slaughter stress can have significant influence on pork quality (D'Souza, Dunshea, Warner, & Leury, 1998). Avoiding stress just before slaughter is important not only for improving animal welfare but also for maintaining pork quality (Edwards et al., 2010; Hambrecht, Eissen, Newman, Smits, den Hartog et al., 2005; Hambrecht, Eissen, Newman, Smits, Vertegen et al., 2005; Hambrecht et al., 2004). Under normal circumstances, muscle pH declines gradually until the onset of rigor mortis during postmortem (Bendall, 1973; Lawrie, 1966). Under stressful situations, the physiological response may influence the rate and extent of muscle metabolism (D'Souza, Dunshea, Warner, & Leury, 1998; Warriss, 2010), resulting in detrimental pork quality (Edwards et al., 2010; Hambrecht, Eissen, Newman, Smits, den Hartog, et al., 2005; Hambrecht, Eissen, Newman, Smits, Vertegen, et al., 2005; Hambrecht et al., 2004). Stress may activate the hypothalamic–pituitary–adrenocortical axis, resulting in the secretion of cortisol into the blood (Minton, 1994). Circulating levels of cortisol in the blood respond less rapidly and recover more slowly compared to other indices, thus, may reflect the quality of pre-slaughter handling processes (Warriss, Brown, Adams, & Corlett, 1994). Many previous studies (Choi, Jung, Choe, & Kim, 2012; Hambrecht, Eissen, Newman, Smits, den Hartog, et al., 2005; Hambrecht, Eissen, Newman, Smits, Vertegen, et al., 2005; Hambrecht et al., 2004; Shaw, Trout, & McPhee, 1995; Warriss et al., 1994) have shown the relationships between cortisol levels and meat quality and generally considered as the primary biomarker of stress (Russell, Koren, Rieder, & van Uum, 2012).

Circulating lactate levels rapidly elevated by physical effort including behavioral reactions to stress (Edwards et al., 2010; Hambrecht, Eissen, Newman, Smits, den Hartog, et al., 2005; Hambrecht, Eissen, Newman, Smits, Vertegen, et al., 2005; Hambrecht et al., 2004; Warriss, 2010; Warriss et al., 1994). Several studies (Edwards et al., 2010; Hambrecht, Eissen, Newman, Smits, den Hartog, et al., 2005; Hambrecht, Eissen, Newman, Smits, Vertegen, et al., 2005; Hambrecht et al., 2004; Warriss et al., 1994) have shown that higher blood lactate levels may be associated with poor pork quality. Changes in blood glucose levels may also be associated with stress (Mota-Rojas et al., 2012), partly due to the activation of the sympatho-adrenal system (Warriss, 2010). In addition, Choe et al. (2009) have shown that a higher blood glucose level at exsanguination is related to inferior pork quality and that area composition of muscle fiber type may influence blood glucose levels. Muscle fiber characteristics, especially fiber type composition, can be a major factor for variation in the metabolites content (Choe et al., 2008). Therefore, the objective of this study was to investigate the relationship of blood glucose levels with blood lactate, serum cortisol levels at exsanguination, postmortem muscle glycogen and lactate content, muscle fiber type composition, and pork quality traits at the same time.

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2. Materials and methods

2.1. Animals, pre-slaughter handling, and slaughter procedure

All experimental conditions respected the Korean Animal Protection Act, including detailed provisions for transport and slaughter. Treatment and handling conditions as well as experimental procedures were controlled and/or approved by the Ministry of Agriculture, Food, and Rural Affairs of South Korea. The slaughter procedure was under the supervision of the Korea Institute for Animal Products Quality Evaluation.

A total of 111 Yorkshire pigs (pure breed, castrated male) were evaluated. All pigs were selected on the basis of certain criteria: non-carrier for the halothane gene and clinically healthy. The treatment conditions, both before and after slaughter, were the same for all pigs. The pigs were divided randomly into different pens on a commercial farm, with a stocking density of 1.0 m^2 per pig. The temperature was maintained at 20 ± 2 °C, and artificial light was provided from 0900 to 2100. Water and food were available *ad libitum* at nipple drinkers and food dispensers. In addition, all pigs were fed the same commercial diet in accordance with the National Research Council (1998). When the weight of the live pigs reached 115 ± 5 kg, they were transported to a commercial slaughter facility. Approximately 4 h prior to transport, feed was withdrawn. The angle for loading and unloading was as horizontal to the surface of the ground as possible. Electric prods were not used during loading or unloading. The truck had two straight decks, and a hydraulic lift raised the floor of the deck to create the second deck. The truck was equipped with natural ventilation, and the stocking density was approximately 0.47 m^2 per pig. The pigs were transported for 1 h to the slaughter facility after loading. The pigs were not mixed with unfamiliar pigs during transport and lairage. To reduce stress, the pigs were showered with water, and water was available for drinking *ad libitum* during lairage. After the pigs had rested for approximately 12 h at the slaughter facility, they were slaughtered. All pigs were slaughtered during the winter period in 4 batches (30, 30, 30, and 21 pigs per batch). The pigs were slaughtered with an electrical stunning (2–4 s at over 1.25 A) and scalding–singeing process. The carcasses were split into the left and right sides and then cooled in the chilling room (4 °C). Within 45 min postmortem, three to five pieces (0.5 × 0.5 × 1.0 cm) were taken from the longissimus dorsi muscle at the 8th and 9th thoracic vertebra for histochemical analysis. At 45 min postmortem, muscle sample for the analysis of glycogen and lactate content was taken from the longissimus dorsi muscle at the 9th and 10th thoracic vertebra. The samples for muscle glycogen and lactate contents and histochemical analysis were promptly frozen in liquid nitrogen and stored at −80 °C until subsequent analyses. After 24 h chilling, muscle samples were taken from the longissimus dorsi muscle at the 10th and 15th thoracic vertebra for the measurements of muscle glycogen and lactate content and pork quality traits. The collection of all muscle samples and pH measurements were made at the right side of each carcass.

2.2. Blood samples and measurements

Blood samples were collected from each pig within 30 s during exsanguination after electrical stunning and sticking. The blood samples were collected using two types of tubes: the first tube was treated with potassium oxalate/sodium fluoride (BD Vacutainer fluoride tube; Becton Dickinson) to inhibit further glycolysis, and the second tube was treated with a thrombin-based clot activator and polymer gel (BD Vacutainer rapid serum tube; Becton Dickinson) for rapid clotting and easy separation of serum.

Blood glucose and lactate levels were measured using hand-held devices (blood glucose: OneTouch Ultra, LifeScan, Inc.; blood lactate: Lactate Scout, EKF Diagnostics) from the first tubes (potassium oxalate/sodium fluoride-treated tubes). All measurements were completed within 10 min of exsanguination at the slaughter facility. The blood glucose levels were expressed as mg/dL, and the blood lactate levels were expressed as mmol/L.

Serum was separated from each rapid serum tube (second tubes), transferred to Eppendorf tubes, and the tubes were stored at −80 °C until cortisol measurement. The cortisol concentration was determined using the electrochemiluminescence immunoassay (ECLIA, Elecsys E170, Roche Diagnostics, Switzerland), and the results were calculated as μg/dL.

2.3. Muscle glycogen and lactate content measurements

Glycogen content of muscle was measured via the method described by Dreiling, Brown, Casale, and Kelly (1987). Approximately 1.5 g of tissue was minced, suspended in 10 mL of 9% cold perchloric acid (PCA), and thoroughly homogenized for 30–45 s with a mechanical tissue disrupter. After centrifugation (15,000 ×*g* at 4 °C), the supernatants were decanted and saved for glycogen determination. An iodine color reagent was prepared by the combination of 1.3 mL of a solution containing 0.26 g of iodine and 2.6 g of potassium iodide (in 10 mL distilled water; fresh daily) with 100 mL saturated $CaCl_2$. The reagent (2.6 mL) was added to the glycogen standard or tissue extract (0.4 mL). Glycogen standard curves were developed for each set of samples. Linear regression equations were used to determine the glycogen concentration in the corresponding samples.

Lactate content was determined spectrophotometrically (340 nm) using a commercial kit for the determination of lactic acid (Boehringer-Mannheim, Germany). Approximately 500 mg of muscle was homogenized for 30 s in 2 mL of 1 M PCA. KOH (2 M) was added to neutralize the solution, and the final volume was made to 10 mL with distilled water. Following 20 min of refrigeration and centrifugation, the lactic acid concentration was measured. The lactate change value was obtained by the difference between the lactate content at 24 h and 45 min postmortem.

2.4. Histochemical analysis

Within 45 min postmortem, muscle samples were cut into 0.5 × 0.5 × 1.0 cm pieces, promptly frozen in liquid nitrogen, and stored at −80 °C until subsequent analyses. Using a cryostat (CM1850, Leica, Germany) at −20 °C, serial transverse muscle sections (10 μm thickness) were obtained from each sample and mounted onto glass slides. Myofibrillar adenosine triphosphatase (mATPase) staining was used to classify the fiber types of samples (Brook & Kaiser, 1970a; Lind & Kernell, 1991) as follows: (1) unfixed muscle sections were pre-incubated at room temperature for 10 min in a buffer consisting of 100 mM potassium chloride in 100 mM sodium acetate, adjusted to pH 4.7 with acetic acid; (2) sections were then washed for 30 s in 20 mM glycine buffer (pH 9.4) containing 20 mM $CaCl_2$; (3) sections were subsequently incubated at room temperature for 25 min in 40 mM glycine buffer (pH 9.4) containing 20 mM $CaCl_2$ and 2.5 mM ATP disodium salt; (4) next, muscle sections were washed in three 30 s changes of 1% $CaCl_2$; (5) sections were then kept in 2% $CoCl_2$ for 3 min and washed in three 30 s changes of distilled water; and (6) muscle sections were finally immersed in 1% yellow ammonium sulfide solution for 30 s, washed in distilled water, and then embedded in glycerol jelly. All histochemical samples were examined by an image analysis system with an operational system consisting of an optical microscope equipped with a CCD color camera (IK-642 K, Toshiba, Japan) and a standard workstation computer that controlled the entire system (Image-Pro Plus, Media Cybernetics, L.P., USA). Approximately 600 muscle fibers without signs of tissue disruption and freeze damage were analyzed. The muscle fibers were divided into types I, IIA, and IIB following the nomenclature system of Brook and Kaiser (1970a, 1970b). The cross-sectional area of the muscle fiber was determined as the ratio of the total measured area to the total number of fibers. The area composition of muscle fiber type was calculated as