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Effect of sex and time to slaughter (transportation and lairage duration) on the levels of cortisol, creatine kinase and subsequent relationship with pork quality

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ABSTRACT

The study determined the effect of sex and time to slaughter on cortisol and creatine kinase levels, and pork quality in commercial crossbred pigs. Saliva samples were before collected transportation, on arrival at the abattoir, and after a 20 hour lairage period. Cortisol levels from saliva (SC), serum (SeC) and urine (UC) were determined. Creatine kinase (CK) levels were determined from serum samples. Fifteen boars vs. 15 gilts were immediately slaughtered on arrival (SOA), and the other 15 boars vs. 15 gilts were rested for 20 h before slaughter. Meat quality parameters were also determined. In both sexes, SC significantly increased in response to time to slaughter. There was a significant interaction of sex and time to slaughter on SeC. Gilts had higher CK levels and lower muscle L* values than boars. There were correlations among baseline SC, SeC, UC and most meat quality parameters. Time to slaughter influenced levels of SC, UC, CK and pork quality between boars and gilts.

1. Introduction

According to Schonreiter and Zanella (2000) and Averos, Herranz, Sanchez, Comella, and Gosalvez (2007), human–animal interactions can modify an animal's physiology. However, limiting pig exposure to stress during handling en route to the abattoir, and carefully assessing the effects of different *ante-mortem* actions can help pigs to recover from the previous encountered stress (Gajana, Nkukwana, Marume, & Muchenje, 2013). Though responses to travel and lairage events are to a certain extent, gene-dependent factors; they can be influenced among other factors, by substandard transport and/or lairage durations or mixing of animals (Aziz, 2004; Correa et al. 2010).

According to Bennett et al. (2008) and Escribano, Fuentes-Rubio, and Cerón (2012), the psychological or physiological stress encountered by pigs during their switch to pork can be evaluated on the status of the hypothalamic–pituitary–adrenal (HPA) axis. This was supported by Smith and French (1997) and Van de Perre, Permentier, De Bie, Verbeke, and Geers (2010) who stated that the activity of the HPA axis and sympathetic nervous system (SNS) can be increased due to human–animal interactions and or exposure to unfriendly settings/environments. This may lead to a great increase of stress initiated secretions in the circulatory system which are known to have a glycogenolysis and gluconeogenesis effect (Hoffman & Laubscher, 2011). This can impair pork quality through anaerobic glycolysis and the production of hydrogen ions (measured as ultimate pH) and thus, decrease eating quality (Choi, Jung, Choe, & Kim, 2012; Geverink, Bradshaw, Lambooij, Wiegant, & Broom, 1998). High secretion of stress-related indicators (cortisol and creatine kinase) can thereafter cause vasoconstriction, especially in high temperature seasons (in excess of 15 °C, as found in Mediterranean like climate), that can hinder heat dissipation (Romero & Butler, 2007). Findings by Gonzalez et al. (2007) supported this and these authors also noted that in addition to exhausted metabolic muscle and dehydration, heat stress is a consequence of transport, especially with high stocking densities and poor ventilation. According to Pérez et al. (2002), the muscle of a stress-susceptible pig can be over-reactive to stressful handling. Such muscle can be prone to undue catabolic activities which activate glycolysis and block glucose uptake by peripheral tissues. The pig can therefore develop hyperthermia and lethal blood metabolites (viz. hyperglycaemia) (Vasconcellos et al. 2011).

Assessment of stress in pigs has traditionally relied on faecal, serum and urine samples, measuring heart rate and or behavioural parameters (Escribano et al. 2012; Muchenje, Dzama, Chimonyo, Strydom, & Raats, 2009; Seshoka, Kanengoni, Siebrits, & Erlwanger, 2013), ignoring concentrations in saliva. Yet, saliva sampling is a less invasive and less stressful method compared to other routes which may be impractical, time taxing or pose various problems (viz. complicates result

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interpretations or may be unethical) due to the lack of superficial blood vessels in the species (Bennett et al. 2008; Hillmann, Schrader, Mayer, & Gygax, 2008). The objective of this study was, therefore, to assess the influence of time to slaughter (transport and lairage duration) between boars and gilts under warm Mediterranean climatic conditions on the levels of cortisol and creatine kinase and subsequent relationship with pork quality in a less invasive sampling method.

2. Materials and methods

During the course of the study, routine farm to abattoir practices and conditions were maintained and all experimental procedures were according to the ethical principles of experimentation established by the Committee of Ethics on Animal Use of the Society for the Prevention of Cruelty to Animals (SPCA).

2.1. Site description

The study was conducted at the University of Fort Hare Farm's Piggery Trust unit. The farm is located 32° 48′ S (latitude) and 26° 53′ E at 520 m above sea level (Eastern Cape Province, South Africa). The average rainfall at the farm is approximately 480 mm per year, and mostly comes in summer. Mean temperature of the farm is about 18.7 °C per year. The topography of the area is generally flat with a few steep slopes.

2.2. Animal management

Sixty 22 weeks old commercial crossbred pigs (Duroc boars × Dutch Landrace-Large White gilts) were used in the study. All pigs were reared on the farm, given drinking water via nipple drinkers and a commercial diet *ad libitum*. On Sunday (day before slaughter), all pigs were given a light feed and easy access to water. Pigs were allotted to a 2.5 m width × 5.4 m length fully slatted pens (15 pigs per pen) on the basis of sex and housed in adjacent pens with concrete floor, in enclosed and temperature-controlled (19.5 °C; 60 to 70% relative humidity) room. Artificial light was provided from 07:00 to 17:00, with no daylight visible in the room. All the pigs were handled at the farm (Fort Hare Piggery Trust) under identical conditions.

2.3. Transportation details

Sixty crossbred pigs (30 boars vs. 30 gilts) were transported to East London commercial abattoir under the same handling conditions on Monday. Briefly, they were all handled and loaded from Fort Hare Piggery Trust farm using an electric prodder sparingly and were off-loaded at the East London commercial abattoir at 12:00 pm after a two hour drive. The pigs were stocked at an average loading density of 0.8 m^2 per animal (Warriss, 1998). There were no separations by sex or loading by pen which thus meant that full mixing (from different pens) occurred. A tailgate lift for loading and an off-loading ramp of 15° slope were used. A rigid chassis type 2011 Isuzu (single-decker truck,) equipped with four pens (2.0 m length × 1.6 m width × 1.2 m height), spring suspension, natural ventilation, ABS braking system, slatted sides and a grid of cross slating floor (made from metal) was used. The truck had neither drinking nipples nor showers. The driver was driving at a mean speed of 60 km/h on a tarred road to the East London commercial abattoir which is close to 120 km away from the farm. There was a 15 minute stop and go for road maintenance. Temperature and relative humidity at loading and off-loading were 23.5 °C and 61%, respectively.

2.4. Lairage details and slaughter procedure

On arrival, 30 pigs (15 per sex) were randomly selected within their sex and slaughtered in accordance with approved commercial procedures of the abattoir. The other 30 (15 per sex) were held in the roofed lairage pen (8.0 × 4.0. × 2.5 m, width × length × height, respectively) for 20 h with no feed given, but only potable water *ad libitum*. The live slaughter weight for gilts was approximately 76.9 ± 5.7 kg while boars weighed on average 78.3 ± 5.3 kg. An electric prodder was used sparingly to move the pigs from the pens to the slaughter area. The 15 pigs by sex were placed in a room, stunned with a head-only electric stunner (110 V and 8 A) for 3–5 s, shackled and then bled by sticking within 30 s. The carcasses were immersed in warm water with a regulated temperature of 60 °C. The hair from the carcasses was removed by mechanical tumbling, followed by evisceration and inspection of the carcasses by the authorized meat inspection personnel.

2.5. Sampling procedures and chemical analysis

2.5.1. Saliva sampling

Three saliva samples per pig were taken: one basal sample from all pigs on Sunday (a day before transportation between 07:00 and 10:00), on arrival at the abattoir on Monday from the 30 pigs selected to be slaughtered on arrival, and on Tuesday before stunning from the other 30 pigs. Saliva samples were taken using Salivette tubes (Sarstedt AG & Co, Germany), by allowing individual pigs to chew cotton balls attached to pieces of string for 1–2 min until the balls were thoroughly moistened (Seshoka et al. 2013). The cotton balls were then placed in a tube, transported (for 90 min.) on ice (4 °C) from the abattoir to the University of Fort Hare laboratory. The samples were centrifuged at 20 °C for 10 min at 3550 x g and stored at -20 °C until analyzed for cortisol.

2.5.2. Blood collection, Serum separation, and Urine collection

Blood samples were collected at the slaughter plant during exsanguination using 6.0 ml Vacutainer® tubes treated with anticoagulant. The samples were put on ice (4 °C) and transported to the laboratory. To remove debris and minimise the turbidity which can negatively impact on the accuracy of analysis (Mohamed, Campbell, Cooper-White, Dimeski, & Punyadeera, 2012; Salimetrics, 2012, chap. 1), the samples were centrifuged at 20 °C for 10 min at 3550 ×*g*. Serum samples were transferred to Eppendorf tubes (1.5 ml) and stored at -20 °C until analyzed for cortisol and creatine kinase. Urine samples were taken from the bladder of pigs on the slaughter line into hand-held plastic cups, and labelled with the individual's number and sex. They were stored at -20 °C in separate vials until analysis for cortisol.

2.5.3. Measurement of cortisol levels in saliva, serum and urine

After the samples were stored at −20 °C, the levels of cortisol were determined using a commercial cortisol enzyme immunoassay (EIA) kit for the *in-vitro* diagnostic quantitative determination of cortisol in plasma (Palme & Möstl, 1997) according to the manufacturer's instructions. Saliva (SC) and serum (SeC) and urine cortisol (UC) concentrations were expressed in ng/ml, and nmol/L, respectively. The inter-assay coefficient of variance ranged from 16.06% to 16.34%, and the intra-assay coefficient of variance ranged from 9.5% to 11.0%.

2.5.4. Measurement of creatine kinase level in serum

The levels of creatine kinase (CK) (for synthesis energy-providing molecules, and marker of damage of CK-rich tissues) in the stored serum were determined using a commercial colorimetric diagnostic kit (CK; IL Test kit, No. 181,605–90) on the Monarch 2000 Chemistry system (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium). Concentrations of CK in serum were expressed in units per litre (U/L). The inter-assay coefficient of variance was 5.15%, and the intra-assay coefficient of variance was 11.13%.

2.6. Pork samples and meat quality determination

For pork quality analyses, a 2 cm-thick *Longissimus dorsi* (LD) muscle of the left side of the carcass was sectioned (before cooling) at the level