



Lamb loin tenderness is not associated with plasma indicators of pre-slaughter stress

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ABSTRACT

The purpose of this study was to test if associations exist between plasma indicators of acute and chronic stress and lamb loin Warner Bratzler Shear Force (WBSF). Blood was collected at exsanguination from 2877 lambs from the Meat and Livestock Genetic Research flock with a suite of indicators analysed. Loin (*M. longissimus lumborum*) WBSF was measured after 5 days aging. Plasma indicators of stress did not relate to WBSF, however a positive association was found between WBSF and kill order, indicating that immediate pre-slaughter factors may be causing reduced tenderness in lamb. In addition, selection for decreased fat depth (PFAT) was associated with increased loin WBSF, indicating that genetic selection for increased carcass leanness is negatively affecting lamb loin tenderness.

1. Introduction

Tenderness is a key driver of consumer acceptance of lamb due to its impact on eating quality (Pannier et al., 2014), yet even the higher quality cuts such as the loin (*M. longissimus lumborum*) are known to vary markedly in tenderness. This was demonstrated in a study by Pannier et al. (2014) whom showed that approximately 33% of lamb loins rated as good every day (3 star) and 7% as unsatisfactory (2 star) by untrained consumers under the Meat Standards Australia grading system.

Previous research in beef has indicated that stress prior to slaughter is linked with a reduction in tenderness (Warner, Ferguson, Cottrell, & Knee, 2007). Warner et al. (2007) simulated acute stress by subjecting cattle to electric prodders immediately prior to slaughter, resulting in decreased consumer tenderness scores for the grilled loin. Gruber et al. (2010) also found that cattle with more excitable temperaments and higher flight scores were correlated with higher Warner Bratzler shear force (WBSF) values. Similarly, Pighin et al. (2015) found that loin hardness was greater in cattle under conventional immediate pre-slaughter handling compared to reduced stress handling.

Plasma indicators reflecting stress are well established, but the association between stress and tenderness has yet to be fully explored in lamb. Most recently work by Stewart, McGilchrist, Gardner, and Pethick (2014) showed that lambs have elevated plasma glucose, lactate and non-esterified fatty acid (NEFA) concentrations at slaughter, reflecting adrenergic stress resulting in increased glycogenolysis and

lipolysis (Martin, McGilchrist, Thompson, & Gardner, 2011). Cortisol is one of the most common stress indicators measured (Shaw & Tume, 1992) and has been shown to be elevated above basal at slaughter (Pighin et al., 2015; Probst et al., 2014). Increases in circulating creatine kinase (CK) and aspartate aminotransferase (AST) can be seen with unaccustomed exercise, transport handling stress and low-level trauma or bruising (Fisher et al., 2010; Pettiford et al., 2008; Sutherland, Bryer, Davis, & McGlone, 2009; Tarrant, 1990; Tollersrud, Baustad, & Flatlandsmo, 1971). Stress may prevent animals from drinking in lairage (Hogan, Petherick, & Phillips, 2007), which can result in dehydration and elevated plasma total protein and sodium concentrations (Jacob et al., 2006; Radostits, Gay, Hinchcliff, & Constable, 2007). Haptoglobin, an acute phase protein is normally present in very low levels in healthy animals (Ceciliani, Ceron, Eckersall, & Sauerwein, 2012) but increases in response to infection, inflammation, tissue damage (Cray, Zaias, & Altman, 2009). More recently, haptoglobin has been used as a marker of stress in livestock (Lomborg, Nielsen, Heegaard, & Jacobsen, 2008; Salamano et al., 2008). Conversely, high plasma magnesium levels have been shown to attenuate the stress response (Hubbard, 1973) by reducing catecholamine and glucocorticoid secretion (Classen et al., 1986; Kietzmann & Jablonski, 1985).

The objective of this study was to examine if an association exists between lamb tenderness, as measured by Warner Bratzler Shear Force (WBSF) and plasma stress indicators. It was hypothesised that increasing plasma lactate, glucose, NEFA, cortisol, CK, AST, sodium, total protein and haptoglobin concentrations at slaughter will be associated

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with increased WBSF values in lamb loin. In addition, it was hypothesised that increased plasma magnesium concentrations at slaughter will be associated with a reduction in loin WBSF.

2. Materials and methods

This study was approved by the Department of Agriculture Western Australia Animal Ethics Committee #2-13-07.

2.1. Experimental design and slaughter details

The design of Meat and Livestock Australia Genetic Resource flock (formerly known as the Sheep CRC Information Nucleus Flock (INF)) has been described previously (Fogarty, Banks, Van der Werf, Ball, & Gibson, 2007; Van der Werf, Kinghorn, & Banks, 2010). Wether and female lambs ($n = 2877$) were produced from artificial insemination of Merino, Border Leicester x Merino (BLM) and Commercial Maternal (CM) dams over a two year period (2013 and 2014) at the Katanning, WA and Kirby, NSW research sites. The lambs were the progeny of 394 different sires, which comprised Terminal sire types (Ile De France, Poll Dorset, Suffolk, Texel, Charolais and White Suffolk), Maternal sire types (Booroola, Border Leicester, Coopworth, Dohne Merino and Prime SAMM) and Merino (Merino and Poll Merino) sires, representing the major production types in the Australian sheep industry. These sires were chosen to represent the full range of Australian Sheep Breeding Values (ASBVs) for key traits within each sire type. Semen from all three sire types was used to artificially inseminate Merino dams, while only semen from Maternal and Terminal sires was used to inseminate cross-bred (BLM and CM) ewes. Maternal lambs sent to slaughter comprised very few females (which were retained for breeding purposes), meaning effective comparisons between sexes could only be made within the Terminal and Merino sired lamb groups and Maternal sired lambs from Merino dams. The lambs were maintained on extensive pasture grazing, with grain, hay or feedlot pellets supplemented when pasture supply was limited.

For each site, lambs were assigned to smaller kill groups ($n = 17$) of between 48 and 307 lambs to be killed on the same day to enable carcass weight targets to be achieved. Given selection for slaughter was made based on weights, the average age of lambs in each kill group varied between 193 and 416 days old at slaughter, however within individual kill groups the age range was smaller, varying as little as 16 days and by up to 33 days of age. Lambs were yarded on farm the day before slaughter and were taken off feed and water for between 5 and 18 h. Lambs were then weighed and transported by truck to one of three commercial abattoirs (1 in WA and 2 in NSW). At the Katanning site transportation lasted for 0.5 h compared to 1.5 to 2.5 h for the Kirby site. Lambs were held overnight in lairage with free access to water and slaughtered the following day after electrical stunning.

2.2. Blood collection

Blood samples were collected into 9 mL lithium heparin Vacuette® tubes (Greiner bio-one, Austria) from each lamb at slaughter, immediately following exsanguination. Tubes were immediately placed in ice for between 2 and 5 h until centrifugation at 3000 rpm for 15 min. Following centrifugation, plasma samples were pipetted in two separate aliquots and stored in 2 mL tubes at -80°C until processing.

Once samples were thawed, each sample was gently inverted several times before a 100 μL sample was pipetted into 1.7 mL sample cups (Greiner Bio-one, Kremsmuster, Austria). Laboratory analyses of plasma were carried out as a batch samples using the Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd., Melville, NY) and commercially available reagent kits at Murdoch University, Perth, WA or otherwise stated. For each commercial kit, the correlating control and calibration sera was used.

Laboratory analyses of plasma were carried out as a batch samples.

Plasma lactate, glucose, NEFA, magnesium, total protein, CK and AST were analysed using commercial available reagent kits (Olympus Diagnostics, Tokyo, Japan). Plasma sodium was analysed using commercial available sodium kit (Randox Laboratories kit, County Antrim, UK). Samples were analysed using the Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd., Melville, NY). For each commercial kit, the correlating control and calibration sera was used.

Plasma haptoglobin and BHOB were analysed by the Western Australian Department of Agriculture (DAFWA) Animal Health Laboratories, South Perth. Plasma β -hydroxybutyrate was analysed using the commercial reagent kit (Randox Laboratories kit, County Antrim, UK) Plasma haptoglobin was determined using an in-house method, based on the method described by Eckersall et al. (1999). Plasma Cortisol levels were determined from a subset of 500 lambs in 2013 using chemiluminescent immunoassay performed using an Immulite® 2000 Immunoassay system (Siemens, Germany) at Vetpath Veterinary services, (Perth, Australia).

2.3. Carcass measurements

Following slaughter, lambs were dressed according to AUS-MEAT standards and hot carcass weight (HCWT) was recorded at an average of 23.2 kg (Std Dev = 2.94). All carcasses underwent medium voltage electrical stimulation to optimise pH decline such that the carcass loin temperature at pH 6 lies between 18 and 25 $^{\circ}\text{C}$ (Pearce et al., 2010) and were chilled overnight (3–4 $^{\circ}\text{C}$) before sampling.

At 24 h post-mortem *M. longissimus lumborum* (loin) pH (pH24LL) was measured as described by Pearce et al. (2010) on the left section of the muscle at the lumbar-sacral junction, where a small 4 cm incision was made to identify the caudal end of the loin muscle. Muscle pH was measured using an Orion 250A pH meter (cat. no. 0250A2, Orion Research Inc., Boston, MA USA) fitted with a glass body, spear tipped probe (cat. no. 8163BN, Orion Research). The pH meter was regularly calibrated using buffers with known pH of 4 and 7.

From the carcass saddle region the left short loin (AUS-MEAT 4480) (Anonymous, 2005) up to the 12th rib was removed. From this, the *M. longissimus lumborum* (loin) was prepared by removing sub-cutaneous fat and connective tissue (epimysium). Loin samples of approximately 65 g were collected from the cranial aspect of the loin muscle. Samples were vacuum packed, aged for 5 days at 1 $^{\circ}\text{C}$ and then frozen at -20°C until subsequent testing. Packaged frozen samples were cooked in a water bath at 71 $^{\circ}\text{C}$ for 35 min and then cooled in running water for 30 min after cooking. Six cores (approximately 3–4 cm long, 1 cm^2) from each loin sample were cut and Warner-Bratzler shear force (WBSF) was measured on each core sample using a Lloyd texture analyser with a Warner-Bratzler shear blade fitted (Hopkins, Toohey, Warner, Kerr, & van de Ven, 2010). Laboratory processing of loin samples and measurement of WBSF was performed at the University of New England Meat Science Department (Armidale, New South Wales, Australia).

2.4. Data analysed

Data from lambs ($n = 2877$) from the Kirby site in 2012 ($n = 1128$) and 2013 ($n = 721$) and Katanning sites in 2012 ($n = 524$) and 2013 ($n = 504$) were used in the analysis. Details of sex, dam breed and sire type across each year are shown in Table 1. Of the total lambs with WBSF data available, the base model used 2609 lambs that had all production data available. Table 2 shows the raw data means, standard deviation, minimum and maximum values for WBSF and plasma indicators analysed. Haptoglobin data had a skewed distribution and was log transformed to normalise the data.

Of the 394 sires used within this study, 59 Maternal, 138 Merino and 197 Terminal sires had Australian Sheep Breeding Values (ASBV) for Post Weaning Weight (PWT), Post Weaning Eye Muscle Depth (PEMD) and Post Weaning Fat Depth (PFAT). The breeding values for PEMD and PFAT are based upon live animal ultrasound measurement

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