Published August 23, 2018

Meat and Muscle Biology™

Association between Loin Ultimate pH and Plasma Indicators of Pre-Slaughter Stressors in Australian Lamb[1]

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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 ultimate pH. Blood was collected at exsanguination from 2,877 lambs from the Meat and Livestock Australia Genetic Research flock with a suite of indicators analyzed. Ultimate pH was measured in the loin (M. *longissimus lumborum*) at 24 h post-slaughter. There was a positive association ($P < 0.05$) between ultimate pH and plasma glucose and lactate concentrations, which indicates that opportunities exist to reduce variation in ultimate pH by reducing stress in the pre-slaughter period. These effects were small by comparison to production factors, however further understanding of how to best manage lambs in the pre-slaughter period is required to minimize stress and maximize lamb wellbeing and meat quality.

Keywords: lamb, meat quality, stress, ultimate pH, welfare
Meat and Muscle Biology 2(1):254–264 (2018) **doi:10.22175/mmb2017.10.0050**
Submitted 20 Oct. 2017 Accepted 13 June 2018

Introduction

Previous work has demonstrated that pre-slaughter stress has a negative impact on ultimate pH and is frequently attributed to an increased incidence of high pH (> 5.7) meat (Tarrant, 1989; Warriss, 1990). Meat from carcasses with an ultimate pH above 5.7 has a dark color, variable tenderness, poor flavor and cooking attributes (Mendenhall, 1989; Cox et al., 1994) and reduced shelf life (Ferguson et al., 2001; Thompson, 2002).While best practice pathways under the Meat Standards Australia grading systems promote reduced stress during the pre-slaughter period, it is inevitable that stress can still occur (Ferguson and Warner, 2008), therefore further understanding of the link between stress and meat quality is required.

Pre-slaughter stress is multifactorial as lambs are exposed to many different processes from farm to slaughter. Increased handling, novel environments and changes to social structure may cause acute and chronic psychological stress. Lambs also undergo periods of feed and water deprivation and may experience periods of muscular exertion during mustering or transport (McVeigh et al., 1982; Tarrant, 1989; Harman and Pethick, 1994; Apple et al., 2005; Warner et al., 2005; Mach et al., 2008; Jacob et al., 2009). These stress events are associated with a multitude of physiological and metabolite changes in plasma, however limited studies have been conducted in lamb (Sutherland et al., 2016) attempting to relate plasma indicators of stress with ultimate pH.

Acute stress results in the secretion of adrenaline which accelerates pre-slaughter muscle glycogen turnover (Gardner et al., 2014) through its activation of glycogen phosphorylase (Franch et al., 1999) and inhibition of glycogen synthase (Roach, 1990), resulting in elevated plasma lactate concentration. Adrenaline also causes increased rates of adipose tissue lipolysis, liver glycogenolysis and liver gluconeogenesis (Kuchel, 1991), leading to increased circulating glucose and non-esterified fatty acid concentrations. Acute stress

[1]This project was funded by the Australian Meat Processors Corporation, Meat and Livestock Australia and Australian Cooperative Research Centre for Sheep Industry Innovation.

also activates the hypothalamic pituitary axis leading to cortisol release. Many studies have shown elevated cortisol levels in response to pre-slaughter factors such as transport and handling (Warriss, 1990).

The acute phase response is an innate response activated by harmful stimuli such as inflammation, tissue damage and infection (Baumann and Gauldie, 1994; Cray et al., 2009; Piccione et al., 2012). During the acute phase response, cytokines released from macrophages and other cell types are transported to the liver, where they induce synthesis of acute phase proteins, such as haptoglobin in hepatocytes, which then act to remove inflammatory stimuli, promote healing and restore homeostasis (Baumann and Gauldie, 1994). Acute phase proteins such as haptoglobin have been used mainly as indicators of disease and inflammation in livestock, however there is evidence that haptoglobin may be a useful biomarker of stress with previous work showing that transportation and mixing of animals (Arthington et al., 2003; Lomborg et al., 2008; Piccione et al., 2012) causes elevations in blood haptoglobin levels.

Lambs may experience increased physical demands during the pre-slaughter period due to mustering, handling and transport. 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 (Tollersrud et al., 1971; Tarrant, 1990; Pettiford et al., 2008; Sutherland et al., 2009; Fisher et al., 2010) and have previously been associated with increased ultimate pH (Warriss, 1984). In support of this notion, Warner et al. (2005) showed that exercise in the immediate pre-slaughter period caused higher ultimate pH in lamb.

Feed deprivation causes significant increases in circulating non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHOB), indicative of increased lipolysis, yet research tends to show that feed deprivation per se has minimal impact on muscle glycogen and ultimate pH (Daly et al., 2006). Similarly, Lowe et al. (2002) showed that water deprivation even under heat stress, had no effect on ultimate pH or meat color in lamb, while Jacob et al. (2006) showed it slightly improved lamb ultimate pH. Ruminants have been shown to be relatively resilient to periods of water restriction, with minimal changes in indicators of dehydration such as plasma sodium and total protein (Parrott et al., 1996; Jacob et al., 2006; Fisher et al., 2010).

Magnesium has been shown to attenuate the stress response (Hubbard, 1973) by reducing catecholamine and glucocorticoid secretion (Kietzmann and Jablonski, 1985; Classen et al., 1987). Circulating levels of plasma magnesium reflect the nutritional intake of an animal with supplementation shown to reduce stress-mediated glycogen losses in the pre-slaughter period and prevent high ultimate pH (D'Souza et al., 1998; Gardner et al., 2001).

The objective of this study was to assess the association between plasma indicators that reflect acute and chronic stress and lamb ultimate pH. Several hypotheses were tested; 1. Indicators of acute stress including plasma lactate, glucose, cortisol, CK, and AST concentrations at slaughter will be positively associated with an increased ultimate pH at 24 h post-slaughter; 2. Feed deprivation reflected by increased NEFA and BHOB levels, and water deprivation reflected by increased sodium and total protein, will not be associated with ultimate pH; and 3. Increasing plasma magnesium concentration at slaughter will be associated with a decrease in ultimate pH.

Materials and Methods

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

Experimental design, animals, and pre-slaughter management

The design of the Co-operative Research Centre for Sheep Industry Innovation Information Nucleus Flock, now referred to as the Meat and Livestock Australia Genetic Resource flock has been described previously (Fogarty et al., 2007; Van der Werf et al., 2010). Wether and female lambs (n = 2877) were produced from artificial insemination of Merino, Border Leicester × Merino (BLM) and commercial maternal (CM) dams over a two year period (2013 and 2014) at the Katanning, Western Australia (WA) and Kirby, New South Wales (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. Semen from all 3 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 mostly 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 (Table 1). Dam breed comparisons could only be made within maternal sired male lambs and in terminal sired male and female lambs (Table 1). The lambs were maintained on extensive pasture grazing, with grain, hay, or feedlot pellets supplemented when pasture supply was limited.

At both the Katanning and Kirby flocks, lambs were consigned to 17 different kill groups and slaughtered at a target carcass weight of 21 to 22kg. Given selection for slaughter was made based on weight, the average age of lambs in each kill group varied between 193 and 416 d old at slaughter, however within individual kill groups the age range was smaller varying between 16 and 33 d.

Prior to slaughter, lambs were yarded on farm, taken off feed and water for between 5 and 18 h before being transported to 1 of 3 commercial abattoirs (1 in WA and 2 in NSW). For the Katanning flock transportation lasted for 0.5 h compared to 1.5 to 2.5 h for the Kirby flock. Lambs were held overnight in lairage, with free access to water and slaughtered the following day.

Blood collection and processing

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 to 5 h until centrifugation at 3,000 rpm for 15 min. Following centrifugation, plasma samples were pipetted in 2 separate aliquots and stored in 2 mL tubes at –80°C until processing.

Once aliquot samples were thawed, they were gently inverted several times before a subsample (approximately 100 μL) was pipetted into 1.7-mL sample cups (Greiner Bio-one, Kremsmüster, Austria). Plasma lactate, glucose, NEFA, magnesium, total protein, CK, AST, and sodium were analyzed on aliquot one at Murdoch University (Perth, WA) performed using an Olympus AU400 automated chemistry analyzer (Olympus Optical Co. Ltd, Melville, NY). Commercially available reagent kits were used to analyze plasma lactate, glucose, magnesium, total protein, CK, AST (Olympus Diagnostics, Tokyo, Japan), Sodium (Randox Laboratories kit, County Antrim, UK) and NEFA (C Kit Wako Pure Chemical Ind., Osaka, Japan). Aliquot two was used to analyze plasma haptoglobin, BHOB and cortisol. Plasma haptoglobin and BHOB were analyzed at the Western Australian Department of Agriculture Animal Health Laboratories (South Perth, Australia). Plasma β-hydroxybutyrate was analyzed using the commercial reagent kit (Randox Laboratories kit, County Antrim, UK). Plasma haptoglobin was determined using the method described by Eckersall et al. (1999). Analyses were performed using an Olympus AU400 automated chemistry analyzer (Olympus Optical Co. Ltd). Plasma cortisol levels were determined on a subset of samples ($n = 471$) using chemiluminescent immunoassay performed using an Immulite 2000 Immunoassay system (Siemens, Germany) at Vetpath Veterinary services (Perth, Australia).

Carcass processing

Following slaughter, lambs were dressed according to AUS-MEAT standards (Anonymous, 2005) and hot carcass weight was recorded at an average of 23.2 kg (Std Dev = 2.94). All carcasses underwent medium voltage electrical stimulation to optimize pH decline such that the carcass loin temperature at pH6 was between 18 to 25°C (Pearce et al., 2010) and were chilled overnight (3 to 4°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 caudal 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

Table 1. Number of lambs measured for M. *longissimus lumborum* (loin) pH at 24 h post slaughter (pH24LL) at each flock, within each year, sex, dam breed, and sire type

	Year		Sex dam breed (Sire type)[1,2,3,4]											
Flock	2013	2014	F Merino (Mat.)	M Merino (Mat.)	M CM (Mat.)	M BLM (Mat.)	F Merino (Mer.)	M Merino (Mer.)	M Mer. (Ter.)	F Mer. (Ter.)	M CM (Ter.)	F CM (Ter.)	M BLM (Ter.)	F BLM (Ter.)
Kirby	1,128	721	37	98	n/a	43	80	194	273	268	n/a	n/a	311	354
Katanning	524	504	2	118	17	5	34	199	156	215	78	109	8	19
Total	1,652	1,225	39	216	17	48	114	393	429	483	78	109	319	373

[1]F: female lamb; M: male (wether) lamb.

[2]CM: commercial maternal dam breed; BLM: Border Leicester × Merino dam breed.

[3](Mat.): maternal sire type; (Mer.): Merino sire type; (Ter.): terminal sire type.

[4]n/a: not applicable.

muscle. Muscle pH was measured using an Orion 250A pH meter (cat. no. 0250A2, Orion Research Inc., Boston, MA) fitted with a glass body, spear tipped probe (cat. no. 8163BN, Orion Research Inc.). The pH meter was regularly calibrated using buffers with known pH of 4 and 7.

Statistical analysis

Loin ultimate pH (pH24LL) data were analyzed using linear mixed effect models in SAS (SAS Inst. Inc., Cary, NC; v 9.1). The base model included fixed effects for flock, year, kill group within flock by year, sire type, age of dam, sire type by flock, sire type by year, sex and dam breed within sire type, and sire type by flock by year. Sire identification and dam identification by year were included as random terms. All relevant interactions between fixed effects were tested and nonsignificant terms ($P > 0.05$) were removed in a stepwise manner. Plasma indicators and kill order were included separately within the base model as covariate terms along with relevant interactions with other terms in the base model. A subset of animals ($n = 471$) from 1 yr (2013) had plasma cortisol analyzed. This covariate was also tested as described above, however in this case the base model for pH24LL did not include the fixed effect for year, kill group was tested within flock only and the only random effect fitted was sire identification. All relevant interactions between covariates and fixed effects were tested and nonsignificant terms ($P > 0.05$) were removed in a stepwise manner.

Results

Raw means and standard deviations for pH24LL and covariates analyzed are presented in Table 2. Outcomes of the base model are presented in Table 3. The average pH24LL was 5.63 and the proportion of variance in pH24LL described by a base model (Table 3) was 26%. Predicted least square means (± SE) for the significant effects of sex by dam breed within sire type for pH24LL are presented in Table 4.

Flocks differed in their pH24LL values although this varied each year ($P < 0.01$, Table 3). There was greatest variation in the Katanning flock which for the 2013 yr had an average pH24LL of 5.72 ± 0.007, compared to 5.63 ± 0.007 for the 2014 yr. There was less variation in pH24LL at the Kirby flock between years, which differed by 0.03 pH units from 5.64 ± 0.008 and 5.61 ± 0.006 for the lambs killed in 2013 and 2014, respectively.

Within each year at each flock, there was also variation in pH24LL between kill groups, which differed by as much as 0.15 pH units. The pH24LL for kill groups ranged from as high as 5.81 ± 0.010 in Katanning in 2013 to as low as 5.56 ± 0.013 in Kirby in 2013.

Sire type had a small effect on pH24LL, however this effect differed across years with pH24LL being highest in 2013 across all sire types. In 2013 terminal sired lambs had the lowest ($P < 0.05$) pH24LL value of 5.65 ± 0.005 compared to maternal (5.70 ± 0.014) and Merino sired lambs (5.71 ± 0.011). Similarly, in 2014 pH24LL was lowest ($P < 0.05$) in terminal sired lambs (5.61 ± 0.007) compared to Merino sired lambs (5.64 ± 0.009). However there was no difference ($P > 0.05$) between terminal and maternal (5.61 ± 0.013) sired lambs. In both 2013 and 2014 there was no difference ($P > 0.05$) in pH24LL between Merino and maternal sired lambs.

The only impact of sex on loin pH24LL was observed in maternal sired lambs with Merino dams and terminal sired lambs with CM dams ($P < 0.05$, Table 3). In the maternal sired lambs, female lambs had

Table 2. Descriptive statistics including mean, standard deviation and range of lamb M. *longissimus lumborum* (loin) pH at 24 h postmortem (pH24LL) and plasma indicator data analyzed

Variable (units)	Mean	SD	Range
pH24LL	5.63	0.13	5.34–6.88
Covariates (units)			
Non-esterified fatty acid (mmol/L)	1.19	0.54	0.165–3.26
Glucose (mmol/L)	4.67	0.94	1.978–10.503
Lactate (mmol/L)	3.48	2.29	0.467–16.427
Magnesium (mmol/L)	0.90	0.14	0.356–1.627
Creatine Kinase (IU)	463.29	341.57	83.07–3731.8
Aspartate Aminotransferase (IU)	152.63	42.79	61.82–613.73
Total Protein (g/L)	68.65	6.21	30.15–93.32
Haptoglobin (mg/mL)	0.42	0.38	0.01–4.82
β-hydroxybutyrate (mmol/L)	0.43	0.15	0.11–1.04
Cortisol (nmol/L)	152.27	62.57	5.5–395

Table 3. F values, P values and numerator (NDF) and denominator (DDF) degrees of freedom for the base linear mixed effects model of M. *longissimus lumborum* (loin) pH at 24 h postmortem (pH24LL) of lambs

Effect	NDF,DDF	F-values
Flock	1,387	81.14**
Year	1,1798	79.15**
Kill group (flock × Year)	13,387	37.17**
Sire type	2,387	12.35**
Sexdambreed (Sire type)	9,387	2.39*
Flock × Year		39.58**
Sire type × Year		3.60*

* $P < 0.05$.

** $P < 0.01$.

Table 4. Predicted means and standard error (SE) for lamb M. *longissimus lumborum* (loin) pH at 24 h postmortem (pH24LL) for the fixed effects for sex and dam breed within sire type

Category[1,2]	Mean ± (SE)
F Merino (Maternal)	5.69 (0.020) [a]
M Merino (Maternal)	5.63 (0.008) [bc]
M CM (Maternal)	5.63 (0.029) [bc]
M BLM (Maternal)	5.66 (0.018) [bc]
F Merino (Merino)	5.67 (0.012) [d]
M Merino (Merino)	5.68 (0.007) [d]
F Merino (Terminal)	5.63 (0.006) [eg]
F CM (Terminal)	5.62 (0.012) [e]
F BLM (Terminal)	5.61 (0.007) [eh]
M Merino (Terminal)	5.63 (0.006) [fg]
M CM (Terminal)	5.65 (0.014) [f]
M BLM (Terminal)	5.63 (0.008) [fh]

[a–h]Letters that differ between rows within sire type are different ($P < 0.05$).

[1]F: female lamb; M: male (wether) lamb.

[2]CM: commercial maternal dam breed; BLM: Border Leicester x Merino dam breed.

pH24LL values 0.055 units higher than male lambs. The opposite was observed in terminal sired lambs where the pH24LL of female lambs was 0.04 pH units lower than male lambs. There was no effect of dam breed on pH24LL ($P > 0.05$).

An association was observed between pH24LL and kill order, however this was inconsistent, varying across flocks ($P < 0.05$). At the Katanning flock, as kill order increased from 0 to 250 there was a 0.06 pH unit decrease from 5.72 ± 0.011 to 5.66 ± 0.009, after which a plateau was reached up to a kill order of 300. The opposite was observed at the Kirby flock as kill order increased from 0 to 100 there was no change in pH24LL (5.61 ± 0.007 to 5.62 ± 0.006), however from a kill order of 100 to 250 there was a 0.05 pH unit increase from 5.62 ± 0.006 to 5.67 ± 0.013.

Association between plasma indicators and ph24ll

There was a significant positive association between pH24LL and glucose concentration ($P < 0.01$, Fig. 1). As plasma glucose concentration increased from 2 mmol/L to 10 mmol/L, pH24LL increased by 0.16 pH units from 5.60 to 5.76. On average, increasing plasma lactate was associated with an increase in loin pH24LL by 0.09 units across the range of lactate from 1 mmol/L to 15 mmol/L. The magnitude of this effect across the range of plasma lactate varied across flocks and years ($P < 0.05$, Fig. 2), ranging from no association at Katanning in 2013, to an increase of 0.11 units across the 14 mmol/L range of lactate at Kirby in 2014.

There was a curvilinear association between pH24LL and haptoglobin, however this was only observed in 2013 ($P < 0.05$). As haptoglobin increased from 0.1 mg/mL to 1.0 mg/mL there was a 0.06 unit increase in pH24LL from 5.60 ± 0.009 to 5.65 ± 0.010 after which the association plateaued. The association between pH24LL and magnesium was inconsistent and differed between flocks and years ($P < 0.05$, Fig. 2). At the Kirby flock in 2013

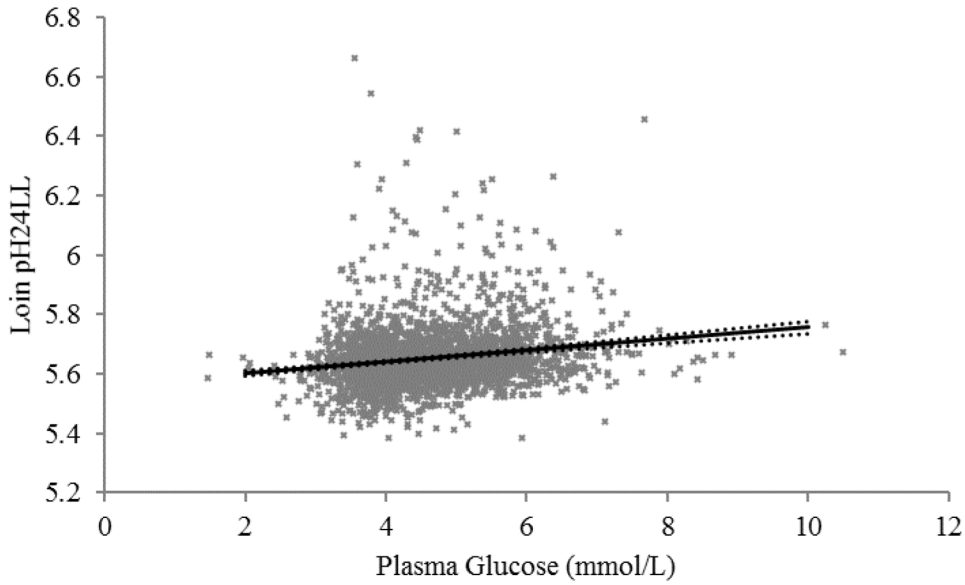

Figure 1. Association between plasma glucose at slaughter (mmol/L) and M. *longissimus lumborum* (loin) pH at 24 h post slaughter (pH24LL). Lines represent ls means ± standard error. Icons (×) denote lamb residuals from the response surface.

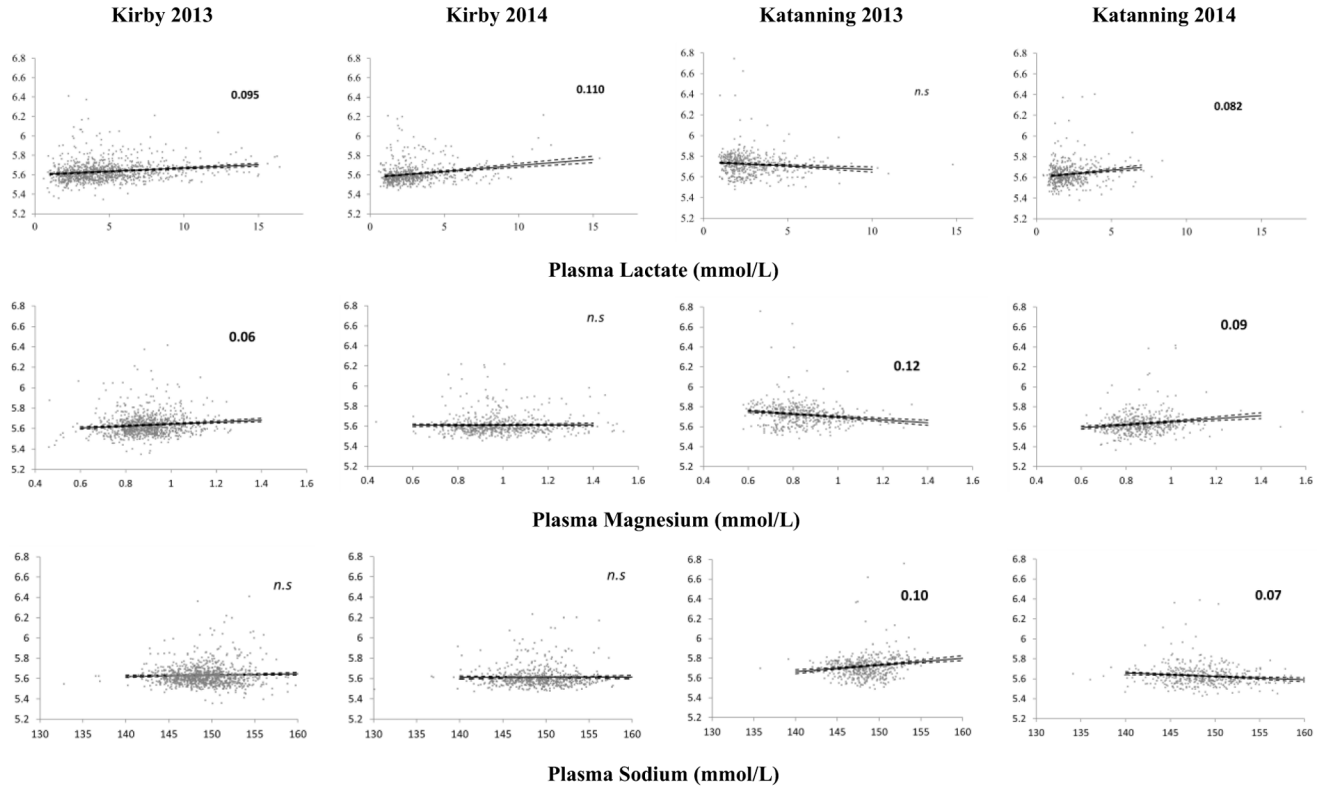

Figure 2. Table of figures showing association between M. *longissimus lumborum* (loin) pH at 24 h post slaughter (pH24LL; *y* axis) and covariate effects: plasma lactate, magnesium and sodium at slaughter (mmol/L) at the Kirby and Katanning flocks in 2013 and 2014. Solid lines within figures represents the predicted least squared means, while dashed lines represent the standard error. Icons (×) denote lamb residuals from the response surface. The number in bold above each figure represents the unit change of pH24LL across the listed range in each covariate. n.s denotes non-significance.

and Katanning flock in 2014 there was a positive association between pH24LL and plasma magnesium. The opposite association was observed at the Katanning flock in 2013 and there was no association ($P > 0.05$) between pH24LL and plasma magnesium at Kirby in 2014.

The association between pH24LL and plasma sodium was only observed at the Katanning flock and varied between years ($P < 0.05$, Fig. 2). The association between pH24LL and BHOB was significant ($P < 0.05$) but inconsistent, varying across flocks ($P < 0.05$). At the Katanning flock, as BHOB increased from 0.1 mmol/L to 0.7 mmol/L there was a 0.09 pH unit increase in pH24LL from 5.61 ± 0.020 to 5.69 ± 0.009 after which a plateau was reached from 0.7 mmol/L to 1.0 mmol/L. The opposite was observed at the Kirby flock as BHOB increased from 0.1 mmol/L to 0.6 mmol/L, pH24LL decreased from 5.68 ± 0.015 to 5.62 ± 0.007. From 0.6 mmol/L to 0.8 mmol/L there was a small increase in pH24LL up to 5.65 ± 0.020 mmol/L. There was an association between pH24LL and AST, however this was only observed at the Katanning flock ($P < 0.05$). As AST increased from 70 IU to 300 IU there was a 0.06 pH unit decrease in pH24LL from 5.68 ± 0.011 to 5.63 ± 0.023. There was a trend ($P = 0.062$) for an association between pH24LL and plasma cortisol, however this was only observed at the Kirby flock. As plasma cortisol increased from 50 nmol/L to 300 nmol/L there was an increase in pH24LL from 5.62 ± 0.018 to 5.68 ± 0.021. There was no association ($P > 0.05$) between plasma NEFA, CK, TP, and concentration with pH24LL.

Discussion

Association between plasma indicators of stress and pH24LL

In line with the hypothesis, increasing plasma lactate and glucose at slaughter were associated with increased loin ultimate pH in lamb, supporting the well-established link between acute stress and ultimate pH (Tarrant, 1981). Muscle glycogenolysis and hepatic glycogenolysis and gluconeogenesis are stimulated by adrenaline (Kuchel, 1991) explaining the associated increases in blood lactate and glucose concentrations. Flock differences in lactate and glucose response are likely to reflect a combination of interacting pre-slaughter animal factors. For example, variation in levels of acute stress and level of muscular activity pre-slaughter (Pethick et al., 1991; Gardner et al.,

1999) are likely to influence the levels of these metabolites in the live animal. The total amount of stored glycogen may also have affected the plasma lactate response. Previous work by Daly et al. (2006) demonstrated an association between increased glycogen storage and more rapid rates of pH decline post-mortem, which is indicative of increased rates of glycogen mobilization and conversation to lactic acid. In a live animal this would be evident as increased lactate entry into plasma (Daly et al., 2006).

Contrary to the hypothesis, there was a significant association between pH24LL and BHOB. However, the magnitude of change in pH24LL across the range of BHOB was small and differed between flocks, suggesting that multiple mechanisms may be driving levels of BHOB as well as the association with ultimate pH. At the Katanning site, the positive association between pH24LL and BHOB may reflect greater lipolysis due to exposure to stress (adrenaline) as a result of elevated NEFA levels (Bassett, 1970). Several authors have reported an association between longer lairage times and incidence of dark cutting (Jacob et al., 2005; Toohey and Hopkins, 2006), due to greater exposure to stress events prior to slaughter. This would increase the mobilization of muscle glycogen (Tarrant, 1989) further elevating pH24LL. At the Kirby flock, there was a weak negative association between pH24LL and BHOB. Circulating levels of BHOB in non-fasted animals also reflect rumen derived butyrate (Bergman, 1990) indicating a more positive energy balance. As such, these lambs may have also had more muscle glycogen, thus exceeding the minimum threshold required to reach an acceptable pH at 24 h post-slaughter. Overall, the multiple confounding factors that could contribute to BHOB concentrations in the pre-slaughter period limit its use as an indicator of ultimate pH in lamb.

The small and inconsistent associations observed between plasma sodium, magnesium, AST, haptoglobin and cortisol with ultimate pH at slaughter suggests that they have limited use as indicators of stress response at slaughter. The levels of sodium found in this study indicate that some animals were dehydrated (Radostits et al., 2007), thus to maximize animal welfare water should be made more readily available. Higher stress in lairage can reduce drinking (Parrott et al., 1987) due to unfamiliarity of the environment or limited access to watering facilities (Thompson et al., 1987; Knowles et al., 1993; Ferguson and Warner, 2008). Further controlled studies are required in this area to fully understand the role of dehydration on lamb meat quality.

The small and inconsistent association between ultimate pH and magnesium may reflect the interaction between on-farm nutrition, mineral supplementation status and stress at slaughter (Gardner et al., 2014). However, as this experiment was not designed to investigate the impact of nutrition it is difficult to draw conclusions about the specific effects of nutritional regimes. Yet, the negative association observed at the Katanning flock in 2013 suggests that in at-risk groups, higher plasma magnesium levels may have a protective effect via reducing the impact of stress mediated glycogen losses (Gardner et al., 2001).

In this study there was a negative association between pH24LL and AST, although only at the Katanning flock. This was unexpected as previous work has shown that elevations in AST are associated with exercise and muscle damage (Kaneko et al., 2008), which would tend to indicate a higher level of pre-slaughter stress. However, plasma AST levels in lambs were within published reference ranges for sheep (Radostits et al., 2007) which suggests that muscle stress may not have been high enough to cause significant turnover of muscle glycogen.

In line with the hypothesis there was a positive association between ph24LL and haptoglobin, however this effect was small and only observed in 2013. Levels of haptoglobin are normally negligible in plasma but increases similar to the current study have been observed as a result of transport, restraint and isolation (Lomborg et al., 2008; Pascual-Alonso et al., 2017). As haptoglobin is a highly sensitive indicator of stress, changes in concentration are likely to occur at a lower stress threshold compared to muscle glycogen turnover and may explain the limited association with loin pH in this study.

Partially supporting the hypothesis, there was a trend for increasing cortisol to be associated with higher ultimate pH. Elevated cortisol levels are associated with acute stress and have been found to be elevated in response to transport and handling (Shaw and Tume, 1992; Knowles et al., 1995). However, natural fluctuations in cortisol concentration due to its circadian rhythm (Chrousos, 1998) as well as individual variation between animals (Moberg, 1987) may have limited its accuracy as an indicator of stress.

The results of this study show that there was an association between plasma indicators of pre-slaughter stress and ultimate pH in lamb. It is well established that to maximize the concentration of muscle glycogen at slaughter and reduce the risk of dark cutting, pre-slaughter stress must be minimized (Tarrant, 1981). Adequate muscle glycogen concentrations are also essential to buffer the effects of pre-slaughter stress (Pethick et al., 2000; Gardner et al., 2014).

Despite elevated levels found for most of the indicators measured, the association between plasma indicators and pH24LL were relatively small in comparison

to the production and environmental factors reported on in this study. To demonstrate this further, the plasma indicators only described 10% of the variation in pH24LL (R^2 = 0.098, RMSE = 0.127). Therefore, it is unlikely that plasma indicators could be used to predict high ultimate pH for individual carcasses. Importantly, a combination of stressors and physiological mechanisms contribute to levels of plasma indicators at slaughter. Thus, the stress response can be non-specific and highly variable (Bray et al., 1989; Broom et al., 1996) and may have contributed to the small effects seen in this study. Moreover, ultimate pH does not increase above 5.5 until pre-slaughter muscle glycogen levels fall below about 0.6 to 0.7%. Given that the level of glycogen in the muscle of lamb can be as high as 2% (Pethick and Rowe, 1996) this suggests that ultimate pH does not change even in the face of substantial mobilization of glycogen from muscle. The practical outcome of this study was to highlight that stress may play a role in determining ultimate pH in lamb on an industry level, with further work required to understand and mitigate the effects of pre-slaughter stress.

Association between environmental factors and pH24LL

Production traits including lamb kill group and flock (site of production) and year had the greatest effect on the ultimate pH of lamb loin (Table 2). These effects remained unchanged when the model was corrected for the plasma indicators of stress which is not surprising given the small and variable associations shown between these plasma indicators and pH24LL. Although this study shows there is evidence that lambs experience stress at slaughter, the multiple confounding factors that impact on these metabolites during the pre-slaughter period may have reduced their accuracy as indicators of immediate pre-slaughter stress. Moreover, it is likely a multitude of interacting factors including animal genetics, production factors and environmental conditions are impacting glycogen turnover and therefore ultimate pH.

Site and year had the largest effect on pH24LL. The Kirby and Katanning flocks in this study were up to 3,800 km apart, meaning substantial climatic variation and differences in pasture types, availability and the type and provision of supplementary feed provided to lambs between different flocks at different times of year. Pre-slaughter factors including handling, time off feed and transport distances to the abattoir also varied substantially between flocks. In addition, there were also differences in carcass processing between flocks, although these should be relatively standardized. Year of production also had a large impact on loin pH24LL. This is likely to reflect seasonal variation between years, particularly related to feeding regimes as well as differences in processing between years.

The slaughter group effect captures variation in slaughter day conditions, including differences in transport and lairage conditions, abattoir processing factors, as well as differences in nutrition during the finishing phase as these groups of lambs were slaughtered at different times across each year. Fluctuations in feed supply, environmental conditions and stress are likely to play a large role in determining ultimate pH through variation in muscle glycogen levels (Pethick et al., 1995).

The impact of lamb breed and sex on pH24LL was small compared to other production factors. Merino and maternal sired lambs had the highest average pH24LL values compared to terminal sired lambs. Although this effect was small, it is in line with previous work by Gardner et al. (2006) that shows that Merinos have a propensity to produce meat with a higher ultimate pH, due to higher stress responsiveness, although we did not find a breed type by stress interaction in this study. In addition, genotype has also been shown to influence muscle glycogen turnover, with the muscle tissue of more muscular genotypes found to be less responsive to adrenaline. This has been demonstrated across 3 different studies, 1 in sheep using lambs sired by high or low muscling potential rams (Martin et al., 2011) and 2 in cattle with the first comparing the progeny of highly muscled Piedmontese with the progeny of Angus and Wagyu sires (Gardner et al., 2009) and the second comparing the extremes in an Angus selection line diverged for muscling (McGilchrist et al., 2011). As terminal genotypes have higher muscling characteristics and a greater proportion of lean in the carcass compared to Merino genotypes (Ponnampalam et al., 2008; Anderson et al., 2015), it may explain why terminal sired lambs had lower pH24LL in this study.

There were small differences between male and female lambs, but they were variable and differed between breed types. In terminal sired lambs, females had marginally lower pH24LL values than male lambs. The opposite result occurred in maternal sired lambs out of Merino dams, with female lambs having slightly higher pH24LL values. These results were unexpected as females have been shown to have more excitable temperaments (Voisinet et al., 1997) and a greater incidence of high ultimate pH (Scanga et al., 1998). This indicates that sex does not play a large role in determining loin ultimate pH in lambs under commercial conditions.

An association was observed between pH24LL and kill order, which differed between flocks. The kill-order term within our model describes the order that

lambs were slaughtered within each kill group. This association was positive in the Kirby flock, indicating that those animals further back had a higher pH24LL. Lambs further back in the kill order may experience greater amounts of intensive handling, which has been shown to cause higher ultimate pH and slower pH declines in lamb (Sutherland et al., 2016). Alternatively, this association may reflect differences in temperament, with those lambs gravitating toward the back of the mob and further back in the kill order possibly being more flighty or of lower hierarchy, which can cause stress (Eldridge et al., 1988; Warriss, 1990; Hemsworth et al., 2011). Therefore, lambs killed later within a kill group may have greater depletion of muscle glycogen stores prior to slaughter underpinning the link between kill order and ultimate pH. It is unclear why an opposite association was observed in the Katanning flock, however this could simply reflect the small impact of kill order in comparison to other production and environmental factors influencing glycogen turnover. Overall, the effect of kill order on pH24LL was small and unlikely to be greatly affecting ultimate pH on a commercial scale.

Approximately 18% of lambs in this study had a pH24LL greater than 5.71. This is significantly higher than 5% non-compliance rate reported for the Meat Standards Australian grading system in beef (McGilchrist et al., 2014), which specifies a cut off for ultimate pH of 5.7. Currently, there is no such cut off for ultimate pH in Australian lamb. Further work is therefore required to understand glycogen metabolism in lamb as well as the production and environmental factors, including stress driving ultimate pH and how this affects lamb eating quality.

Conclusions

The associations found between plasma glucose and lactate and pH24LL indicate that opportunities exist to reduce variation in ultimate pH by reducing stress in the pre-slaughter period. Although these effects were small by comparison to production factors, further understanding of how to best manage lambs in the pre-slaughter period is required to minimize stress and maximize lamb wellbeing and meat quality.

Acknowledgments

Technical staff at Murdoch University, the University of New England and the Katanning and Kirby research stations are thanked for their invaluable assistance in carcass sampling, plasma analysis, and on farm management.

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