Meat and Muscle Biology™

Breed and Nutrition Effects on Meat Quality and Retail Color after Lamb Pre-Slaughter Stress

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Abstract: The main objective of this research was to investigate the interactions between breed, nutrition and pre-slaughter stress, and the potential impacts on the meat quality and retail color/shelf life of lamb meat. Forty-eight Merino and second cross (Merino × Border Leicester) × Poll Dorset lambs were allocated to 2 dietary treatments: low energy diet (LE; 7.8 MJ ME/kg and 12% CP) vs. high energy (HE; 11.8 MJ ME/kg and 12% CP). Samples of *longissimus lumborum* (LL), and *rectus femoris* (RF) were taken 15 min post-slaughter for glycogen and lactate determination. pH and temperature of muscle samples were recorded at 0.5, 1.5, 3, 4.5, 6, and 24 h post-mortem. Muscle LL samples from the same side were collected at 24 h post slaughter for tenderness, cooking loss and retail color assessment. For retail color stability, the selected portion of muscle LL was sliced to create a fresh surface, placed on a tray and over wrapped. Color measurements were made fresh (after a 30 min bloom at 4°C, d 1) and then on d 3 and 6 of display, using a Hunter lab spectrophotometer XE Plus. Second-cross lambs on HE had the highest muscle glycogen at slaughter for both LL and RF. For the RF, the pHu values were elevated in Merinos on both LE and HE diets and 2X on the LE diet. Only for 2X lamb was the HE diet sufficient to reduce the pHu. HE diet reduced blood lactate in 2X lambs and elevated in Merinos. Merinos produced tougher meat at 6 d post-slaughter, which was also reflected by higher cooking losses. Both breeds of lambs on the LE diet had longer color shelf-life (higher R630/580) than the lambs on the HE diets.

Introduction

Meat quality is determined by both color and appeal on the retail shelves, and the consumer eating quality (tenderness, flavor, overall acceptability; Warner et al., 2010). Color contributes significantly to the consumer perception of meat quality and value, and discoloration compromises its appearance resulting in price discounting or removal of meat from display (Channon et al., 2005). Meat discoloration is an oxidative process that occurs in post-mortem muscle leading to conversion of oxymyoglobin to metmyoglobin (Faustman et al., 2010). The concentration of vitamin E in the muscle has been known to play a crucial role in delaying lipid peroxidation and in meat color stability (Suman et al., 2014; Ponnampalam et al., 2012a; Jose et al., 2016). We (Warner et al., 2017) have recently further shown that retail color stability of lamb meat is influenced by breed, muscle type, packaging, and iron concentration. However, the interactions between breed, nutrition and pre-slaughter stress, and the potential impacts on the meat quality and retail color of lamb meat needs to be further elucidated.

Meat quality development is driven by the post-mortem muscle glycolysis, which is one of the major biochemical processes that regulate the pH decline, and ultimate pH (pHu) to some extent (England et al.,

2013; Jacob et al., 2005). Ultimate pH is well known to influence meat quality development, which in turn is influenced by the level of muscle glycogen at slaughter (Gardner et al., 2014). Glycogen is the main source of energy in the post-mortem muscle and determines the post-mortem muscle glycolysis and pH decline from 7.2 to 5.5 as a result of lactate and hydrogen ion accumulation (Scheffler et al., 2011), under normal conditions. Muscles with low muscle glycogen at slaughter usually have limited pH decline and result in higher pHu (Van Laack et al., 2001). Meat with a high pHu (> 5.7) is called dark-cutting (Tarrant 1989), which is inferior in meat quality and more prone to bacterial spoilage, otherwise known as dark, firm and dry meat (DFD). A low ultimate pH of 5.5 is desirable to achieve the acidification of muscle that is associated with better meat quality, however the extent of pH decline may vary among different muscle types in different breeds of sheep.

Sheep meat industry in Australia utilizes different breeds (Merino, Border Leicester, Poll Dorset, and Suffolk etc.) of sheep and crossbreds (first and second crosses) that may have variable glycogen levels at slaughter. The interactions between animal genetics, age and nutrition have been reported to impact the sheep meat quality (Warner et al., 2007a; Thompson et al., 2006). Merinos have been suggested to produce higher ultimate pH meat as compared to other purebred (Young et al., 1993) and crossbred lambs (Hopkins and Fogarty 1998b; Hopkins et al., 2007; Gardner et al., 1999). These differences in pre-slaughter muscle glycogen levels could stem from the greater depletion of muscle glycogen between farm and slaughter in Merinos plausibly due to their greater sensitivity to pre-slaughter stress (Gardner et al., 1999), which in general is the major factor responsible for muscle glycogen depletion during pre-slaughter transportation and handling processes (Channon et al., 2000; Warriss 1990; Ferguson and Warner 2008). The differences in glycogen levels also exist between the muscles as the glycolytic muscles contain greater glycogen as compared to oxidative muscles that are more reliant on oxidative metabolism and contain greater proportion of type I fibers (Pethick et al., 1995a; Jacob et al., 2005), however could be influenced by the nutritional status and the physical exercise pre-slaughter (Ponnampalam et al., 2012b).

Nutritional (metabolic energy in particular) status of the animal pre-slaughter can influence the muscle glycogen levels at slaughter and eventually the meat quality (Knee et al., 2007). Various studies (Pethick and Rowe 1996; Tudor et al., 1996) investigating the impact of nutritional factors on muscle glycogen have shown a direct relationship between metabolizable energy intake and muscle glycogen levels. The seasonal variations in muscle glycogen and incidence of dark cutters due to lower quality or quantity of pastures during winter and late summer is also explained to some extent by this relationship (Knee et al., 2004; Pethick et al., 2000; Ponnampalam et al., 2017). Merinos have been known to have greater tendency to have higher pHu in the knuckle, but not in the loin (Warner et al., 2007b). This finding, however, needs further investigation to understand the potential mechanisms that may either affect the deposition of muscle glycogen and/or depletion of muscle glycogen due to the nutritional or genetic differences in susceptibility to pre-slaughter stress, respectively. Therefore, the aim of this research was to investigate the interactions between breed, nutrition and pre-slaughter stress, and the potential impacts on the meat quality and retail color of lamb meat. We hypothesized that:

1. Merinos are more susceptible to pre-slaughter stress than crossbred lambs.

2. Feeding a diet high in energy will increase the muscle glycogen content.

3. Exercise stress at 2 h pre-slaughter will deplete muscle glycogen, particularly in Merinos.

4. Increasing the muscle glycogen content of the knuckle will reduce the pHu and improve the shelf-life.

Materials and Methods

All procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004) and was approved by the Animal Ethics Committee (AEC # 2694) Department of Economic Development, Jobs, Transport and Resources.

Experimental design, animals, and diets

Thirty (30) Merino and 30 second cross (2X) wether lambs, were purchased from farms in Southern NSW and Central Victoria, respectively. The genotypes of dam and sire for Merino and 2X lambs were Merino × Merino and (Merino × Border Leicester) × Poll Dorset. After 2 wk acclimatization to the group pens, 24 of the heaviest lambs within each breed were selected, and then housed in individual pens at Animal Facilities, Werribee, Victoria. Within the breed, lambs were

allocated to 2 dietary treatments (low energy vs. high energy; 24 lambs per diet) by stratified randomization based on live weight, and the extra animals were returned to the farm. After an adaptation period of 7 d to oaten chaff: lucerne chaff mix with experimental diets, lambs were allocated to dietary treatments. Animals on low energy (LE) diet received a mixed ration containing 7.8 MJ ME/kg and 12% CP on dry matter (DM) basis. Lambs on high energy (HE) diet received a mixed ration containing 11.8 MJ ME/kg and 12% CP. Within each dietary treatment, lambs were balanced for breed. Animals were fed daily at ad libitum amounts for the first 4 wk and then on restricted intakes for the following 3 wk to maintain similar growth rates in animals consuming the same diet across the two breeds.

Throughout the 7-wk period, intake and refusals for individual lambs were recorded. Refusals were collected and weighed daily before allocation of fresh diets every day. Clean water was available for drinking at all times.

Pre-slaughter exercise stress

At the end of 7 wk feeding trial, the lambs were individually subjected to a pre-slaughter exercise stress regime at 2 h pre-slaughter to deplete their muscle glycogen. Lambs of each breed were exercised and slaughtered in batches of 8 over 3 slaughters ($n = 16$ lambs per slaughter). In the morning, the 8 lambs from each breed had their feed removed but had access to water. On the day of slaughter, at 2 h prior to slaughter, lambs were individually removed from their pens and subjected to an exercise-stress treatment for 15 min. This exercise regime has been previously described in Warner et al. (2005b) and has been demonstrated to induce muscular fatigue. Individual lambs undergoing exercise treatment were removed from their pens and subjected to constant movement in isolation from the lamb herd, without audio or visual contact. The exercise regime was conducted in a diversionary paddock, where individual lambs were exercised for 15 min by a stock handler. The exercise regime was designed to achieve strenuous exercise and muscular fatigue. The period of isolation did not exceed 15 min. Lambs were then herded directly to the abattoir pens for slaughter. None of the lambs was left alone in a pen either at the abattoir or in the shed by using the 'spare' animals to accompany them.

Slaughter and sample collection immediately post-slaughter

The lambs were slaughtered using the standard commercial stunning procedure. Lambs were slaughtered by head-only electrical stunning, while restrained in a V-Restrainer, followed by exsanguination and low voltage electrical stimulation was applied at 1 min post slaughter using a rectal probe and electrode clip applied to the stick wound. A square bipolar wave form was applied, providing 157-mA peak to peak (28 to 33V) with a frequency of 14 Hz for a duration of 60 s. Stunning was done, and a blood sample was collected post-slaughter in the V-restrainer prior to exsanguination. The blood samples were collected into heparinized tubes, immediately placed on ice until the plasma was separated by centrifugation at 2,000 g for 10 min at room temperature. The serum was decanted into Eppendorf tubes and frozen at –20°C until analysis of glucose and lactate. Once the skin was removed, a 2-g sample of the muscles *longissimus lumborum* (LL) and *rectus femoris* (RF) was taken at 15 min post-slaughter, using the drill borer, from the left side of the carcass, for measurement of glycogen, glucose and lactate content at slaughter. The muscle samples were trimmed to remove any fat and snap frozen in liquid nitrogen. Additional muscle samples (2 g) of the LL and RF were also taken from the same side, at 1.5 and 6 h post-slaughter for measurement of glycogen and lactate. The pH and temperature of muscles were also recorded from the left side of the carcass at 0.5, 1.5, 3, 4.5, 6, and 24 h post-slaughter, using a Micrometer pH vision model 6007 portable pH meter (Jenco Electronic Ltd, San Diego CA) fitted with a polypropylene spear type gel electrode (IJ42, Ionode, Brisbane, Queensland).

Muscle samples collected at deboning

Carcasses were chilled over a 24 h period to a final temperature of 3 to 4°C and muscle *longissimus lumborum* and *rectus femoris* (RF; undamaged) were excised from each carcass. A full cross-section of LL was cut into 6 portions and samples were taken for retail display color (60 mm), tenderness (WBSF, 65 g), and glycogen (10 g) and lactate (10 g) measurements. The portions of the LL muscle collected for retail color, tenderness and purge were vacuum packaged and aged at 2°C for 5 d. The packaging used was Henkovac AC 3000 ('s-Hertogenbosch, The Netherlands) and the vacuum pouch used were Viking Food Solutions 70 um PA/PE (Epping, Australia), size 165 × 250 mm. The level of vacuum was approx. 1 mbar. Muscle samples of RF were also collected for glycogen and lactate (10 g). All the samples thus collected for biochemical analysis were stored at –80°C till further analysis. Final measurements of WBSF, surface color, and purge were taken at Day 6 post-slaughter. Samples of LL collected for color evaluation were subjected to

retail display and measurements of color conducted at d 1, 3, and 6 of display under retail display conditions.

Packaging, simulated retail display, and color measurements

For retail color stability, the selected portion of muscle LL was sliced to create a fresh surface (30 mm thickness), placed on a black Styrofoam tray (12 × 12 cm) and over wrapped with oxygen-permeable PVC film (15 μm) as described previously (Warner et al., 2017). The packaged trays were maintained at 3 to 4°C under fluorescent light (1,000 lux) to simulate retail display for 6 d. Packages were displayed on a display cabinet having 3 shelves with appropriate ventilation and lighting. Packages were not rotated during the display period. Light reflectance measurements were made fresh (after a 30 min bloom at 4°C, d 1) and then on d 3 and 6 of display, using a Hunter lab Miniscan XE Plus 45/10 (Reston, VA) with light source set to D65/10 (daylight, 10° observer). The surface "redness" was calculated as per the previously reported method (Hunt and King, 2012). Briefly, redness was calculated as percent of light reflected at the wavelength 630 nm divided by the percent of light reflected at wavelength 580 nm and is subsequently described as R630/580.

Analytical methods

Glycogen, glucose, and lactate were measured from the muscle samples collected 15 min post slaughter. The concentration of glycogen in muscle was measured enzymatically, as per the previously reported method (Chan and Exton, 1976) with slight modification (Gardner et al., 2001). Muscle glucose (Sigma Aldrich, Pty, Ltd., St. Louis, MO; Cat. 510A) and lactate (Sigma Aldrich, Cat. 735–10) concentration was analyzed using enzymatic kits with slight modification for microplate reader.

Measurement of shear force and cook loss

The shear force measurement was conducted according to the methodology reported by Warner et al. (2005b). Briefly, frozen LL (65 g) samples were cooked in plastic bags in a water bath at 70°C for 30 min. From each block, 6 × 1 cm^2 subsamples were cut parallel to the direction of the muscle fibre and shear force was measured by an Instron texture analyzer fitted with an inverted V blade and cross head speed of 300 mm/min perpendicular to fiber orientation. For calculating the cook loss, the cooked samples were cooled in

cold running water for 30 min. After cooling, samples were dried with paper towel and weighed. The cooked weight was divided by the frozen weight, and the value was subtracted from 100 and expressed in percentage to calculate % cook loss.

Statistical analysis

The data were analyzed by subjecting to 2 × 2 factorial design with REML, using GenStat (VSN International UK, 16th edition). Main effects observed for significance were breed and dietary energy levels, and the interaction of breed and diet was analyzed. The variables tested were plasma glucose and lactate concentration. Similarly, meat quality attributes (pH, color, shear force, cook loss) were analyzed with a model that included the effects of breed and dietary energy levels, and the 2-way interaction between treatments. For the pre-rigor muscle pH values, muscle was tested in the analysis and was found to be nonsignificant at each time point ($P > 0.05$). Individual muscle samples were identified as the experimental unit. All data are presented as least squares means and SED. Differences between different means were evaluated using the SED × the t test and a significant effect was established at $P \leq 0.05$.

Results

There were both main effects (breed, $P = 0.009$; diet, $P = 0.047$) and an interaction ($P < 0.005$) between breed and diet on muscle glycogen content, such that glycogen content of both the muscles (LL and RF) at the time of slaughter, was highest in 2X lambs on HE diet, whereas the 2X lambs on the LE diet and Merinos on LE and HE diets had similar muscle glycogen contents (Table 1).

There was no significant effect of diet or breed on the pH 24 (pHu) values for the LL muscle. For the RF, the pHu values were elevated (> 5.7, dark-cutting) in the Merinos on both LE and HE diets, and the 2X lambs on the LE diet. Only for the 2X lambs was the HE diet sufficient to reduce the pHu, which is also reflected in the muscle glycogen levels.

There was significant ($P = 0.002$) effect of diet on plasma glucose levels of lambs, and lambs on HE diet showed the greater concentration of plasma glucose compared to LE diet lambs. Merino lambs on the HE diet demonstrated greatest plasma glucose at slaughter followed by 2X HE lambs, while the LE lambs of both the breeds had similar plasma glucose levels.

Table 1. Effect of breed [(Poll Dorset × Border Leicester) × Merino, second cross, vs. Merino] and diet (High energy, HE vs. Low energy, LE) on plasma metabolites at slaughter, muscle glycogen, cook loss, ultimate pH (pHu) and Warner Bratzler shear force (WBSF) on the *longissimus lumborum* (LL) and *rectus femoris* (RF)

Metabolites and meat quality attributes	Second cross		Merino		SED	P-value		
	HE	LE	HE	LE		Breed	Diet	Breed × diet
Total muscle glycogen, umol/g LL	140[a]	116[b]	113[b]	117[b]	6.70	0.009	0.047	0.005
RF	105[a]	79.8[b]	88.2[c]	89.6[c]	5.89	0.393	0.006	0.002
Plasma Glucose, mmol/L	6.38	5.31	7.31	5.75	0.55	0.088	0.100	0.540
Plasma lactate, mmol/L	3.32[a]	4.75[b]	6.21[c]	4.38[b]	0.70	0.016	0.700	0.002
Cook loss, % - d 1	18.6	19.5	20.1	20.7	1.19	0.120	0.410	0.870
d 6	18.5[a]	17.0[a]	20.1[b]	18.8[b]	1.03	0.024	0.058	0.930
WBSF, kg - d 1	3.67	3.14	3.97	3.67	0.31	0.066	0.062	0.600
d 6	1.87[b]	1.64[b]	1.97[a]	2.01[a]	0.13	0.014	0.320	0.160
pHu- LL	5.63	5.66	5.67	5.66	0.06	0.610	0.740	0.610
RF	5.88	6.05	6.05	6.03	0.10	0.300	0.300	0.190
pH30- LL	6.78	6.79	6.78	6.83	0.04	0.580	0.370	0.590
RF	6.72	6.80	6.82	6.84	0.05	0.065	0.170	0.460

[a–c]Values in rows with different superscripts differ significantly.

Merinos on the HE diet had significantly ($P = 0.0016$) higher blood lactate levels at slaughter, relative to Merinos on LE and relative to the 2X lambs. There was a significant ($P = 0.002$) interaction between breed and diet such that lambs of both breeds on the LE diet had similar blood lactate at slaughter, whereas HE diet lambs had lower blood lactate in 2X lambs, and elevated blood lactate in the Merinos.

There was a main effect of breed ($P = 0.014$) on meat tenderness such that Merinos produced tougher meat at d 6 post-slaughter, as indicated by the Warner-Bratzler shear force values (Table 1). Breed also influenced cooking loss ($P = 0.024$) as Merinos had higher values for cooking loss on d 6 post-slaughter (Table 1), which supports the increased toughness, as cooking loss and Warner-Bratzler shear force are often related (Hughes et al., 2014).

The relationship between glycolytic potential and muscle ultimate pH was calculated and has been presented in Fig. 1. Since there was no effect of diet or breed on ultimate pH, the data were pooled to compare any differences between LL and RF muscle types. As was expected, LL had higher glycolytic potential and greater decline in ultimate pH as compared to RF muscle.

The pH decline early post-mortem (0 to 6 h) was only recorded for the LL and has been presented in Fig. 2. Second-cross lambs on the HE diet tended ($P = 0.069$) to have greater decline in muscle pH at 30 min post-slaughter as compared to Merinos and 2X lambs on LE diet. At 4.5 h post-slaughter, there was significant effect of diet, and the lambs on the HE diet showed a greater decline in pH as compared to lambs on the LE diet.

The effect of breed and diet on the retail color stability was investigated by measuring the R630/580 (oxymyoglobin/metmyoglobin) ratio at 1 to 6 d of simulated display and has been presented in Fig. 3. There was significant ($P = 0.05$) effect of diet such that the lambs on the LE diet irrespective of the breed, produced more desirable color (higher R630/580) than the lambs on the HE diet.

Discussion

Muscle glycogen, pH decline, glycolytic potential, and ultimate pH of meat

Merino lambs in general had lower glycogen content in both LL and RF muscles at the time of slaughter, as compared to 2X lambs, although this was further affected by the level of nutrition. Finishing lambs on a HE diet demonstrated higher glycogen content in 2X lambs at slaughter but not in Merino lambs. The lower muscle glycogen content of Merinos could be attributed to their higher sensitivity to pre-slaughter stress leading to greater muscle glycogen depletion during pre-slaughter exercise stress, as the positive linear relationship between metabolizable energy intake and muscle glycogen content is well known (Pethick and Rowe, 1996). Our data reconfirm the association of Merino genetics with pre-slaughter stress sensitivity and are in agreement with previous work by Gardner et al. (1999), where the authors demonstrated the greater depletion of muscle glycogen by the Merino lambs between the farm (muscle biopsies at farm) and commercial slaughter as compared to 1X and 2X lambs. It is speculated that the lambs finished on a
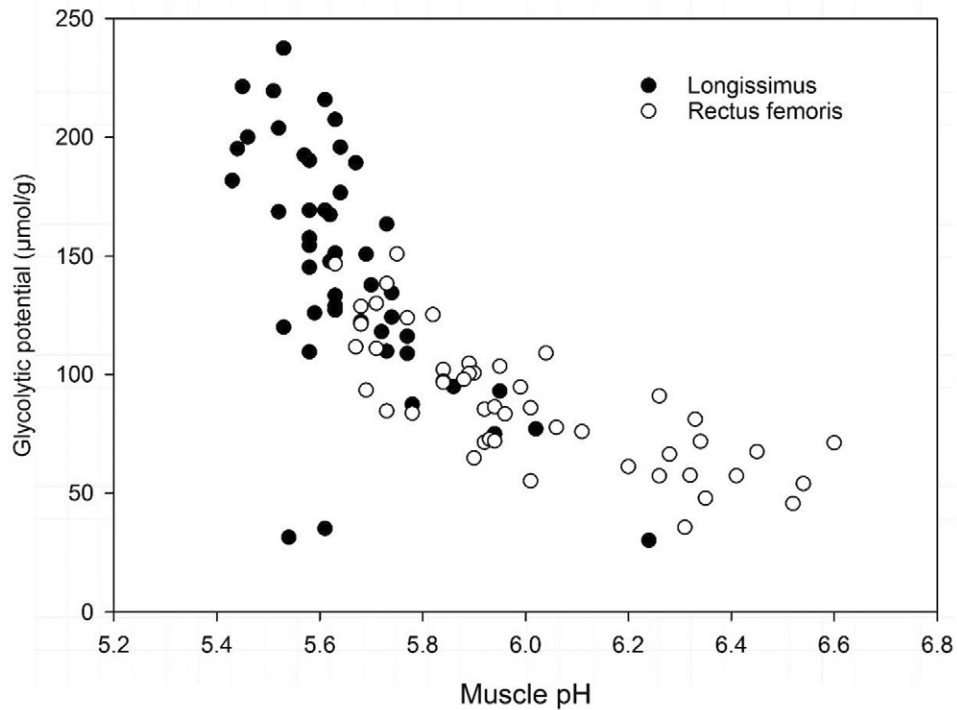
Figure 1. The relationship between glycolytic potential and muscle ultimate pH for the *longissimus lumborum* (LL) and *rectus femoris* (RF).
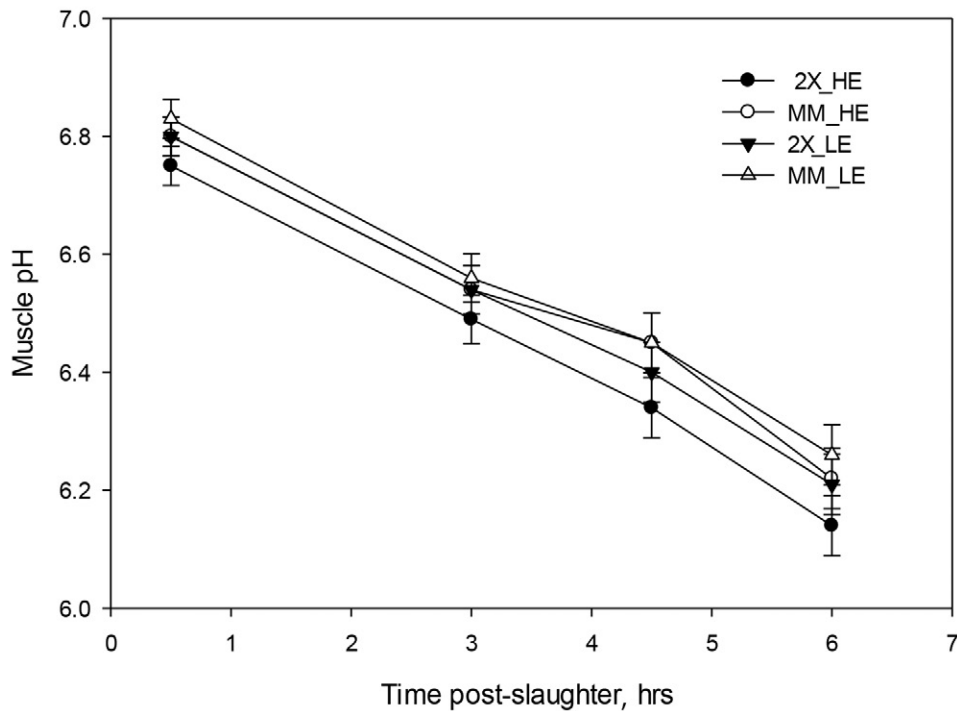

Figure 2. The effect of breed (Poll Dorset × Border Leicester) × Merino, 2X, vs. Merino, MM) and diet (High energy, HE vs. Low energy, LE) on the muscle pH at times of 0.5, 3, 4.5, and 6 h post-slaughter (time), averaged across both *longissimuss lumborum* and *rectus femoris* (there was no difference between muscles at each time point; $P > 0.05$). Significant P-values and trends were; Diet (4.5 h, $P = 0.023$; 6.0 h, $P = 0.070$) and Breed (30 min, $P = 0.069$).
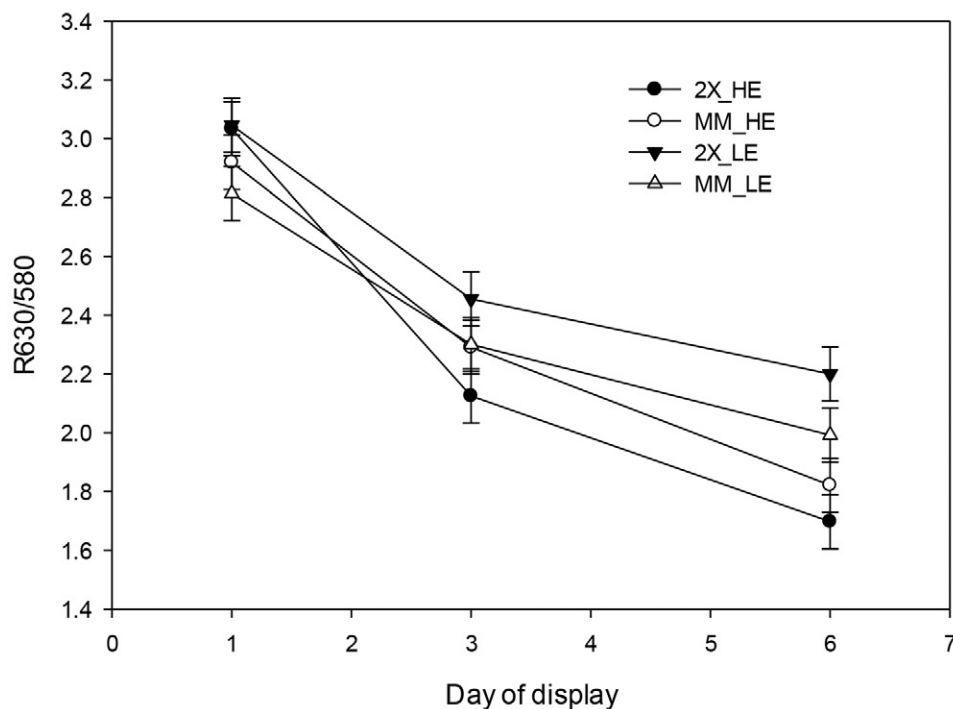
Figure 3. The effect of breed [(Poll Dorset × Border Leicester) × Merino, 2X, vs. Merino, MM] and diet (High energy, HE vs. Low energy, LE) on the R630/580 (oxymyoglobin/metmyoglobin) ratio for the *longissimus thoracis et lumborum* at d 1, 3, or 6 of simulated retail display (d). Significant P-values were; Diet 0.020, d < 0.001, Breed. diet 0.050, diet. d 0.058.

high energy diet in this study had increased glycogen concentration in liver and muscle in both the breeds. However, Merino lambs depleted significant amounts of muscle glycogen because of exercise stress. This could be due to the basal and hormone stimulated differences in the use of energy metabolites between Merino and 2X breeds (Ponnampalam et al., 2012b) and lambs could not replenish muscle glycogen before slaughter. It usually takes 48 to 72 h to replenish muscle glycogen following exercise in lambs, depending on muscle type and nutrition (Gardner et al., 2001; Jacob, Gardner, and Pethick 2009).

As per the expectation, RF contained lower glycogen content compared with LL in both breeds, although second-cross lambs on HE diet had RF glycogen levels comparable to LL glycogen levels in Merinos and second-cross lambs on the LE diet. This explains the greater extent of pH decline and lower pHu of RF as observed in HE 2X lambs, as compared to Merinos and LE 2X lambs. Interestingly, although RF glycogen levels in Merino and LE second-cross lambs were above the threshold levels required for normal post-mortem muscle glycolysis and pH decline (Immonen and Puolanne, 2000), still the pH decline in RF was prematurely arrested above the normal pHu leading to dark-cutting meat (pHu > 5.7). Our data indicate that there are some other factors, possibly inherent muscle characteristics, that determine the

extent of pH decline and pHu, rather than just muscle glycogen. This observation is consistent with previous work (Van Laack et al., 2001) suggesting that glycogen concentration only accounts for 40% of the variation in pHu. This was supported by Jacob et al. (2005), who observed some incidences of increased pHu in lamb *semimembranosus and semitendinosus* muscle without concomitant changes in muscle glycogen concentration. Nevertheless, muscle glycogen concentration is important and influences the post-mortem pH decline, but poorly explains the extent of pH decline and ultimate pH.

This discrepancy in ultimate pH and muscle glycogen concentration has been partially explained, at least in in vitro studies (England et al., 2016) that utilized reconstituted muscle glycolytic buffer system or Scope system (Scopes 1973). In that study by England et al. (2016), inclusion of excess glycogen in the muscle glycolyzing buffer system did not resolve the high pHu of oxidative muscle and the pH decline stopped prematurely at higher pHu, even in the presence of enough glycogen in the system. The glycolytic capacity or a lack thereof, was suggested to terminate post-mortem pH decline and resulted in an elevated pHu in oxidative muscle (England et al., 2016). Our data on glycolytic potential of LL and RF demonstrate the lower glycolytic capacity and higher ultimate pHu of RF and suggest that lower glycolytic

capacity of RF may be responsible for higher pHu. These data suggest potential opportunities to remedy the problem of dark cutters in ruminant meat. Selecting animals for greater percentage of fast glycolytic fibers may help to resolve the high pHu of some of these muscles in ruminants, however, vigilance must be observed to examine the impact on overall quality of meat.

Plasma lactate and glucose concentration at slaughter

The higher plasma lactate concentration at the time of slaughter observed in Merinos in this study also reconfirms the greater sensitivity of Merino lambs to exercise stress as compared with 2X lambs. Somewhat intriguing is that these Merino lambs had lower basal plasma lactate and a lower plasma lactate response to epinephrine than the 2X lambs (Ponnampalam et al., 2012b), which is counter to the response at the time of slaughter. However, the stress around slaughter increased peak lactate to a much greater extent (ca. 2 to 4x) than the response to epinephrine. Elevated serum lactate level following stress treatment has been previously reported in lambs (Apple et al., 1995), and mostly is the end result of increased anaerobic metabolic activity, however also can occur from aerobic metabolism (Connett et al., 1984). The greater rate of lactate production exceeding the rate of uptake by muscle and liver ultimately leads to elevated circulating lactate levels (Stanley et al., 1985). This could possibly explain the higher lactate levels observed in the present study for Merino lambs on high energy. The exercise stress on Merino lambs possibly accelerated the rate of hepatic and muscle glycogenolysis and glycolysis in the active muscle fibers leading to increased production of lactate, which is transported in blood ("lactate shuttle") and metabolized either in liver for glucose production (Pethick et al., 1995a) or oxidized by other active or inactive muscle fibers with high respiratory rates (Brooks 1986). On the other hand, lower lactate levels in Merino on LE could be explained by lower "lactate shuttle" because of lower glycogenolysis and glycolysis rate, limited by the lower glycogen accumulation in muscle on LE diets.

Similar to lactate, higher plasma glucose concentration at slaughter in Merino lambs indicated greater response to exercise stress, given that basal plasma glucose concentrations were lower in Merinos as compared to second-cross lambs (Ponnampalam et al., 2012b). The data for plasma glucose and lactate concentration at slaughter indicate that Merino lambs finished on high energy and exposed to stress show an acceleration in post-mortem glycogenolysis, likely

due to an increase in gluconeogenesis in the liver to increase glucose production. The glucose produced in the liver is transported via blood to active muscles, likely explaining the observed increased plasma glucose levels. The glucose in turn is metabolized through anaerobic glycolysis, as is reflected by the higher plasma glucose and concomitant increase in lactate concentration observed in this study. On the other hand, 2X lambs on high energy diet appear to metabolize glucose aerobically following glycogenolysis as is reflected by the increase in plasma glucose level without the commensurate increase in lactate levels. The greater glucose concentration observed in Merinos on high energy as compared to 2X lambs, again articulates the greater stress responsiveness of the former, as increased adrenaline secretion following stress has been known to induce hyperglycemia (Apple et al., 1995; Cryer, 1980). However, the possibility of lower glucose clearance from circulation in Merinos cannot be excluded, as the stress has been implicated in peripheral insulin resistance/ lower insulin responsiveness of peripheral tissues.

Muscle tenderness and cooking loss

Previous research has shown inconsistent differences in tenderness between different genotypes (Hopkins et al., 2005; Purchas et al., 2002; Hopkins and Fogarty, 1998a). Although, meat from the Merino breed has been reported to have higher ultimate pH consistently (Hopkins and Fogarty, 1998a; Gardner et al., 1999), the differences in tenderness are not evident. We observed the significant differences in meat tenderness between the 2 breeds as indicated by the Warner-Bratzler shear force values. Merinos produced tougher meat at d 6 post-slaughter, which is also reflected by the higher cooking losses in Merinos. This discrepancy in data from the previous work (Hopkins and Fogarty, 1998a; Hopkins et al., 2005) and our data could be due to the exercise stress which was the focus of this study, and was not inflicted on lambs in earlier studies. However, as reported previously by Purchas et al. (2002), the differences in LL shear force can occur between the breeds, and the aging of meat as practiced in the industry may eliminate these differences.

In the current study, high energy diet tended to increase (P = 0.062) meat toughness on d 1 samples (WBSF was higher for high energy diet group) compared with the LE diet group, which was similar to that observed with lupin diet in Ponnampalam et al. (2002). Lupin diet provided high energy and was shown by increased carcass weight and carcass fatness compared with other groups. But this effect

was diluted or disappeared with tenderisation process during ageing as noted in meat tenderness of d 6 samples that did not differ between HE and LE groups. Increased muscle deposition and reduced fat deposition as assessed by carcass component gain and GR fatness with fish meal feeding had no effect on meat tenderness compared with a low energy basal diet (Ponnampalam et al., 2003).

Lack of consistency in tenderness has been known (Warner et al., 2005a) and so has the effect of preslaughter stress on muscle tenderness. Previously Warner et al. (2005b) had observed that exposing lambs to acute exercise pre-slaughter caused high ultimate pH and increased tenderness. In agreement with this observation, Sutherland et al. (2016) also reported increased tenderness of m. longissimus lumborum of lambs that were intensively handled preslaughter. However, cattle subjected to acute preslaughter stress produced meat which the consumer rated as tougher with inferior quality, which was independent of muscle pH and temperature (Warner et al., 2007c). On the contrary, Geesink et al. (2001) had ruled out the effect of stress independent of ultimate pH on tenderness. While the possible differences in the response to stress between breeds may be responsible for the discrepancies in tenderness, further investigations are required to better understand the effects of preslaughter stress on muscle tenderness.

Retail color stability and shelf life

The meat from both breeds finished on the LE diet showed better shelf-life (higher R630/580) than the meat from sheep fed HE diet. Furthermore, the meat from 2X lambs on LE diet showed the greatest color stability on d 6 of display as compared to meat from Merinos and 2X lambs on HE diet. These findings indicate that diet has more influence than genetic background. We have previously seen that meat from 2X lambs fed low quality pasture had better retail shelf-life than those lambs fed hay and cereal grain (Ponnampalam et al., 2010). These data agree with our recent work (Warner et al., 2017), and previous work (Warner et al., 2007b; Hopkins et al., 2005) that have shown differences in meat color and retail shelf life in different genotypes of sheep. We recently have shown that the lamb meat color stability increases as the proportion of Merino genotypes declines (Warner et al., 2017). The reduced color stability of Merinos could be attributed to differences in basal and hormone stimulated metabolism and possible differences in muscle physiology (Ponnampalam et al., 2012b) as compared to 2X lambs, as more aerobic and

less glycolytic muscles in Merinos are more likely to experience the oxidative stress (Warner et al., 2007b; Greenwood et al., 2007). Other possible explanation for less stable Merino meat could be the differences in mitochondrial activity post-mortem again leading to higher oxidative stress (Seyfert et al., 2006), resulting in shorter time to discoloration of meat.

The effect of diet on meat color stability is quite intriguing and could again stem from the differences in metabolic and biochemical status of muscle at the time of slaughter, as many factors can interfere with the myoglobin oxidation and color deterioration (Ponnampalam et al., 2012a). Lambs on LE diet presumably had lower metabolic and mitochondrial activity resulting in decreased oxidative stress pre-slaughter and better color stability and shelf life of lamb meat as reflected by more redness in meat due to delayed metmyoglobin formation (i.e., greater R630/580 = oxymyoglobin/metmyoglobin) over the 6 d simulated retail display.

Conclusions

Finishing lambs on high energy diet accumulates higher muscle glycogen content in 2X lambs at slaughter but not in Merino lambs, which may contribute to lower RF pHu in former. Higher sensitivity of Merinos to stress leads to greater glycogen depletion pre-slaughter and produces tougher meat post-slaughter, which is also reflected by the higher cooking losses in Merinos. Lambs from both breeds finished on low energy diet produced meat with better shelf-life compared with lambs fed the high energy diet.

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