Factors affecting the colour of lamb meat from the *longissimus* muscle during display: The influence of muscle weight and muscle oxidative capacity

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**A B S T R A C T**

Spectrophotometric measures were used to determine the redness:browness (R630/R580) of 4238 lamb *longissimus* muscle after 3 days under simulated display. The results were analysed using linear mixed effects models. Environmental factors represented by effects such as kill group and site of production produced the greatest variation of up to 2.76 units in R630/R580. Isocitrate dehydrogenase activity, reflecting muscle oxidative capacity, reduced R630/R580 by 0.5 units. Selection for high muscling sires increased R630/R580 by 0.27 units, likely due to changes in muscle oxidative capacity. Lamb carcass weight also increased R630/R580 by 0.5 units. Analysis of genotypic factors influencing lamb size and growth rate could result in reduced R630/R580. Our findings suggest that breeding for increased growth rate and increased muscle weight could result in Australian lamb meat retaining its red colour for extended periods whilst on display.

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**1. Introduction**

The colour of lamb meat is crucial to ensuring customer appeal and strongly contributes to the value of the product. Consumers associate a brown meat colour with a lack of freshness and quality (Faustman & Cassens, 1990). Anecdotal evidence suggests that lamb meat currently has a retail shelf life of only 2 days in Australia, with product exceeding this display period often discounted or minced, representing a major economic limitation to the Australian lamb industry. With surface brown meat colour often discounted or minced, representing a major economic limitation to the Australian lamb industry. With surface this display period often discounted or minced, representing a major economic limitation to the Australian lamb industry. With surface...
important traits for lamb production such as growth rate and increased muscle weight.

One important intrinsic muscle factor that could link meat colour to other carcass traits is muscle oxidative capacity. Muscle oxidative capacity refers to the proportion of oxidative type I, oxidative/glycolytic type IIA and glycolytic type IIX myofibres within a muscle. Muscles with higher proportions of oxidative myofibres, such as *Musculus semimembranosus*, are darker and redder initially with higher myoglobin and iron concentrations. These more oxidative muscles are then prone to more rapid discoloration after slicing for display than the more glycolytic muscle types such as *Musculus semitendinosus* (O’Keeffe & Hood, 1982; Renner & Labas, 1987). King et al. (2011) demonstrated that the initial oxygen consumption rate and reducing capacity of beef during display contributed to animal variation in colour stability, however the extent to which animal variation in muscle oxidative capacity changes the colour of a particular muscle during the display of lamb meat is unknown (Gardner et al., 2007; Warner et al., 2007).

A relatively simple way of comparing the oxidative capacity of a muscle between different animals is to measure the activity of isocitrate dehydrogenase. This enzyme is crucial in the oxygen-dependent citric acid cycle of mitochondria, which are larger and more abundant in oxidative myofibres (Hoppeler, 1985). Gardner et al. (2007) found a correlation between isocitrate dehydrogenase and myofibre type, allowing measures of isocitrate dehydrogenase activity to be used as an indicator of muscle oxidative capacity for research purposes that is less difficult to determine and more economical than fibre typing. Furthermore post mortem mitochondrial activity has been linked to retail colour as this influences the oxygen consumption rate of meat (Tang et al., 2005).

Variation between animals in the oxidative capacity of a muscle could be influenced by a range of factors such as selection for increased muscle weight. Increased muscling has been shown to increase expression of type IIX glycolytic myofibres (Greenwood et al., 2006; Wegner et al., 2000) and could therefore impact meat colour by effectively changing the myofibre composition of lamb meat. Increased muscle weight in lambs has been achieved in Australia by selecting for sires with a high Australian Sheep Breeding Values (ASBVs) for post-weaning weight in lambs (Anonymous, 2005), and then chilled overnight at 3°C to 4°C before samples were collected. The carcasses had an average deep muscle temperature of 3°C at the time of sample collection, measured via a probe into the loin muscle at the level of the 12th rib.

All lambs were measured and sampled for a wide range of carcass, meat and growth traits. Carcasses were weighed after slaughter to determine the whole carcass weight (HCWT) of each lamb. At 24 h post mortem the entire *Musculus longissimus thoracis* (12th rib region) et *lumborum* (lumbar sacral junction) or loin muscle and overlaying subcutaneous fat were removed and weighed separately (short loin muscle weight and short loin fat weight). To determine intramuscular fat a 40 g sample of loin muscle was excised from the caudal (lumbar sacral) aspect, all subcutaneous fat and silverskin were removed off the sample before it was diced and stored in 50 ml collection tubes at −20°C until subsequent freeze drying using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, NZ). Intramuscular fat content was then determined using a near infrared procedure in a Technicon InfraFyen 450 (19 wavelengths) using the method described by Perry et al. (2001) and expressed as a percentage of wet tissue weight. pH measurements were taken 24 h post mortem (pH4.5) using a probe inserted into the centre of the loin muscle at the level of the 12th rib, as described by Pearce et al. (2010).

In order to determine isocitrate dehydrogenase activity 1 g portions of loin muscle were sampled from the carcass within 5 h post mortem. The samples were taken above the 12th rib, snap-frozen in liquid nitrogen and stored at −80°C until isocitrate dehydrogenase activity was measured according to the method of Briand (1981). After excision of the loin muscle from the carcass at 24 h post mortem additional small

2. Material and methods

2.1. Experimental design, slaughter and carcass measurement details

Data were collected from 4238 lambs produced in the Cooperative Research Centre information nucleus flock over a 4 year period (2007–2010) at multiple sites across Australia (Table 1), and has been comprehensively described previously (Fogarty et al., 2007; van de Werf et al., 2010). Colour after display was measured from lambs reared at 5 sites across Australia; Trangie NSW, Coorwa NSW, Hamilton VIC, Rutherglen VIC, and Katanning WA.

The lambs were the progeny of 350 different sires (around 90 sires were used each year across all sites) comprising Terminal sire types (Hampshire Down, Ile De France, Poll Dorset, Southdown, Suffolk, Texel and White Suffolk), Maternal sire types (Bond, Booroola, Border Leicester, Coopworth, Corridale, Dohne Merino, East Friesian, Prime SALLM and White Dorper) and Merino sire types (Merino and Poll Merino). Sireman from all three sire types was used to artificially inseminate Merino dams, while semen from Terminal sires only was used to inseminate crossbred ewes (e.g. Border Leicester × Merino dams). Hence the lambs were of four sire type and dam breed combinations; Maternal ram and Merino ewe, Merino ram and Merino ewe, Terminal ram and Merino ewe or Terminal ram and crossbred Merino ewe.

The lambs were maintained on extensive pasture grazing, with grain, hay or feedlot pellets supplemented when pasture supply was limited. Further details of breed types used, lamb feeding and management are described elsewhere (Ponnampalam et al., 2014; van de Werf et al., 2010). At each site lambs were consigned to smaller groups to be killed on the same day (kill groups) to enable the average carcass weight target of 21–22 kg to be achieved. Given selection for slaughter was made based on weights, the lambs ranged from 134 to 503 days of age at the time of slaughter. Within individual kill groups the age of lambs varied by as little as 5 days and by up to 36 days of age. The day prior to slaughter the lambs were yarded, held for 6 h and then weighed and transported to one of six commercial abattoirs. They were then held in lairage overnight and slaughtered the following day. All carcasses were subjected to medium voltage electrical stimulation (Pearce et al., 2010) and trimmed according to AUS-MEAT specifications (Anonymous, 2005), and then chilled overnight at 3–4°C before samples were collected. The carcasses had an average deep muscle temperature of 3°C at the time of sample collection, measured via a probe into the loin muscle at the level of the 12th rib.

All lambs were measured and sampled for a wide range of carcass, meat and growth traits. Carcasses were weighed after slaughter to determine the whole carcass weight (HCWT) of each lamb. At 24 h post mortem the entire *Musculus longissimus thoracis* (12th rib region) et *lumborum* (lumbar sacral junction) or loin muscle and overlaying subcutaneous fat were removed and weighed separately (short loin muscle weight and short loin fat weight). To determine intramuscular fat a 40 g sample of loin muscle was excised from the caudal (lumbar sacral) aspect, all subcutaneous fat and silverskin were removed off the sample before it was diced and stored in 50 ml collection tubes at −20°C until subsequent freeze drying using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, NZ). Intramuscular fat content was then determined using a near infrared procedure in a Technicon InfraFyen 450 (19 wavelengths) using the method described by Perry et al. (2001) and expressed as a percentage of wet tissue weight. pH measurements were taken 24 h post mortem (pH4.5) using a probe inserted into the centre of the loin muscle at the level of the 12th rib, as described by Pearce et al. (2010).

In order to determine isocitrate dehydrogenase activity 1 g portions of loin muscle were sampled from the carcass within 5 h post mortem. The samples were taken above the 12th rib, snap-frozen in liquid nitrogen and stored at −80°C until isocitrate dehydrogenase activity was measured according to the method of Briand (1981). After excision of the loin muscle from the carcass at 24 h post mortem additional small
portions of loin were sampled from the posterior section (at the level of the 12th rib) and frozen for subsequent measurement of myoglobin, iron and zinc concentrations. For myoglobin concentration, a 1 g sample of loin muscle was excised, finely diced and stored at −20 °C in a 5 ml tube until analysis using the method of Trout (1991). For iron and zinc, loin samples were frozen at −20 °C, freeze-dried using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, Blenheim, New Zealand) and then approximately 0.2 g dry matter per sample was weighed and prepared according to the USEPA method 200.3 (USEPA, 1991). Iron and zinc concentrations were then determined using a Vista AX CCD simultaneous ICP-AES (Varian Australia Pty Ltd).

2.2. Colour sample collection and measurement

After excision at 24 h post mortem a full cross section at least 50 mm in length, 50 mm in width and 30 mm in depth was cut from the anterior portion of the loin muscle, to the posterior of the 12th rib. Each individual sample was then vacuum packaged in clear gas-impermeable plastic (20/80 μm, transparent polyamide air impermeable exterior, polyethylene food approved interior, water vapour transmission rate measured at 23 °C and 85% R.H. – 2.6 g/m²·24 h, oxygen permeability measured at 23 °C and 0% R.H. – 50 cm³/m²·24 hr – bar) and aged in a chiller at 3 °C for 5 days. After this 5 day ageing period the loin samples were removed from their packaging and re-sliced to a length of 30 mm perpendicular to the long axis of the loin sections. The samples were then placed with the freshly cut meat surface facing upwards on black Sytrofoam trays (12 × 12 cm) and wrapped with oxygen-permeable polyvinal chloride film (Resinite “DHW” Meat AEP, 15 μm, oxygen transmission rate of 35650 – 46500 cc/m²·24 h). The loin meat samples were then placed under display, where conditions of light and temperature have been designed to simulate those commonly encountered in Australian retail stores. The samples were displayed on a flat horizontal surface in a walk-in chiller (3.8 × 3 × 4 m) set to 4 °C with no defrost cycle. The temperature at these settings fluctuated between 2 and 6 °C. An overhead light source (3.8 × 3 × 4 m) set to 4 °C with no defrost cycle. The temperature at these settings fluctuated between 2 and 6 °C. An overhead light source was set at horizontal surface in a walk-in chiller

2.3. Statistical analysis

The R630/R580 data were analysed using linear mixed effects model tests for site, year of birth, sire type, sex within sire type, dam breed within sire type and kill group within site by year as well as relevant first order interactions. Sire identification and dam by year identification were included as random terms. Non-significant (P > 0.05) terms were removed in a step-wise fashion (see Table 3 for final model).

The base model described above was then used to test for associations between R630/R580 and various phenotypic covariates (see Table 2). These included HCWT, intramuscular fat, pH24, isocitrate dehydrogenase and muscle pH24. The table below shows the descriptive statistics of R630/R580, the covariates and breeding values analysed.

Table 2

<table>
<thead>
<tr>
<th>Covariates (units)</th>
<th>Mean</th>
<th>St. Dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCWT (kg)</td>
<td>22.7</td>
<td>3.37</td>
<td>12.5–36.0</td>
</tr>
<tr>
<td>Intramuscular fat (%)</td>
<td>4.16</td>
<td>1.05</td>
<td>1.50–9.83</td>
</tr>
<tr>
<td>pH24</td>
<td>5.66</td>
<td>0.14</td>
<td>5.28–6.67</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase (μmol/min/g)</td>
<td>5.18</td>
<td>1.66</td>
<td>4.41–11.4</td>
</tr>
<tr>
<td>Myoglobin (mg/g muscle)</td>
<td>6.51</td>
<td>1.64</td>
<td>2.15–15.6</td>
</tr>
<tr>
<td>Iron (mg/100 g muscle)</td>
<td>20.0</td>
<td>3.44</td>
<td>8.12–45.1</td>
</tr>
<tr>
<td>Zinc (mg/100 g muscle)</td>
<td>24.0</td>
<td>4.52</td>
<td>11.8–44.9</td>
</tr>
<tr>
<td>Short loin muscle weight (g)</td>
<td>354</td>
<td>64.7</td>
<td>157–661</td>
</tr>
<tr>
<td>Short loin fat weight (g)</td>
<td>175</td>
<td>79.8</td>
<td>10.0–590</td>
</tr>
<tr>
<td>Age (days)</td>
<td>251</td>
<td>73.0</td>
<td>134–503</td>
</tr>
<tr>
<td>Maternal sire ASBV (Australian sheep breeding value) estimates:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEMD (post-weaning eye muscle depth)</td>
<td>0.18</td>
<td>0.68</td>
<td>−1.44–1.82</td>
</tr>
<tr>
<td>PFAT (post-weaning fat depth)</td>
<td>0.04</td>
<td>0.86</td>
<td>−1.26–2.52</td>
</tr>
<tr>
<td>PWWT (post-weaning weight)</td>
<td>5.04</td>
<td>2.57</td>
<td>−3.66–10.5</td>
</tr>
<tr>
<td>Merino sire ASBV estimates:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEMD</td>
<td>0.03</td>
<td>1.03</td>
<td>−2.02–2.69</td>
</tr>
<tr>
<td>PFAT</td>
<td>−0.17</td>
<td>0.71</td>
<td>−1.89–1.5</td>
</tr>
<tr>
<td>PWWT</td>
<td>1.79</td>
<td>3.06</td>
<td>−4.99–8.39</td>
</tr>
<tr>
<td>Terminal sire ASBV estimates:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEMD</td>
<td>1.08</td>
<td>1.20</td>
<td>−2.90–4.52</td>
</tr>
<tr>
<td>PFAT</td>
<td>−0.82</td>
<td>0.76</td>
<td>−2.44–2.27</td>
</tr>
<tr>
<td>PWWT</td>
<td>12.2</td>
<td>2.47</td>
<td>1.13–18.1</td>
</tr>
</tbody>
</table>
dehydrogenase activity, myoglobin, and iron and zinc concentration, were incorporated one at a time in the base model along with all relevant first order interactions with fixed effects. Non-significant (P > 0.05) terms were removed in a stepwise manner. This process was then repeated with each phenotypic covariate also fitted with HCWT to test whether the observed association was simply a reflection of HCWT. Lastly, shortlamb muscle weight and shortlamb fat weight were incorporated into the base model (separately) along with HCWT representing the impact of composition (muscling or fatness) on R630/R580. This same approach was taken to test the impact of lamb age at slaughter on R630/R580, with age included as a covariate. However in this case the kill group within site by year term was fitted as a random term.

R630/R580 was also tested for associations with sire ASBVs for PWWT, PEMD and post-weaning c-site fat depth (PFAT). Initially all 3 ASBVs were included as covariates in the model, as well as their first order interactions with other terms, and non-significant (P > 0.05) terms were removed in a stepwise manner. Due to the correlations that exist between these ASBVs in this data set (PWWT vs PEMD = 0.3; PWWT vs PFAT = 0.3; PEMD vs PFAT = 0.1) this process was repeated with the ASBVs one at a time to test the independence of their effects. In addition, the effects of the ASBVs were also tested in models corrected for HCWT, shortlamb muscle weight, shortlamb fat weight, intramuscular fat, P₃₅₀, isocitrate dehydrogenase activity, myoglobin, iron, zinc and lamb age. These models contained the ASBVs plus one phenotypic covariate at the time, as well as their first order interactions with other terms. Non-significant (P > 0.05) terms were removed in a stepwise manner.

The mean, standard deviation and range for the R630/R580 data, all covariates tested and the ASBVs for each sire type are shown in Table 2.

### 3. Results

#### 3.1. Effect of industry production factors on R630/R580 — base model

The average R630/R580 for all lambs over four years was 3.05 ± 0.01 (±SE) (Table 2) and the base model (Table 3) described 60% of the variance in R630/R580. Sites differed markedly in their R630/R580 (P < 0.01; Table 3), with these differences varying each year (Fig. 1). Generally lambs from Katanning had the highest average R630/R580 across all years (3.21 ± 0.04) compared to Trangie which had the lowest average R630/R580 (2.69 ± 0.04). Less variation was observed between years, with higher R630/R580 recorded in 2007 (3.00 ± 0.04) and 2008 (3.06 ± 0.04), than in 2009 (2.84 ± 0.04) and 2010 (2.82 ± 0.04).

Within each year at each site there were also marked differences in R630/R580 between kill groups (P < 0.01; Table 3), which demonstrated no consistent trend and varied by up to 2.76 units. The average kill group R630/R580 ranged from as little as 2.17 units in Rutherglen in 2008 to as high as 4.93 units in Katanning in 2008.

Terminal (3.12 ± 0.02) and Maternal (2.94 ± 0.03) sired lambs had a 0.4 and 0.22 unit higher R630/R580 (P < 0.01; Table 3) compared to Merino sired lambs (2.72 ± 0.04). Progeny of Border Leicester–Merino dams had a higher R630/R580 (3.22 ± 0.02) than lambs from Merino dams (3.02 ± 0.02) (P < 0.01; Table 3).

#### 3.2. Effect of phenotypic carcass traits on R630/R580

R630/R580 was shown to be improved with higher lamb HCWT (P < 0.01). Increasing HCWT across a range of 15 to 30 kg produced a 0.50 unit increase in R630/R580 (Table 4). Increasing shortlamb muscle weight and shortlamb fat weight (separately) also increased (P < 0.01) R630/R580 when analysed with HCWT incorporated into the model. Increasing shortlamb muscle weight and shortlamb fat weight from 100 to 400 g produced a 0.61 and 0.23 unit increase in R630/R580 respectively (Table 4). Intramuscular fat reduced R630/R580 (P < 0.01), with increasing intramuscular fat from 3 to 7% producing a 0.41 unit reduction in R630/R580.

High isocitrate dehydrogenase activity reduced R630/R580 (P < 0.01). Increasing isocitrate dehydrogenase activity from 3 to 7.5 μmol/min/g of muscle corresponded with a 0.50 unit drop in R630/R580 (Fig. 3; Table 4). This association was not changed when HCWT was included in the model. High iron concentrations also reduced R630/R580 (P < 0.01). Increasing iron content from 15 to 35 mg/100 g muscle produced a 0.27 unit decrease in R630/R580 (Table 4), which was reduced to a 0.20 unit change when HCWT was included in the model. In contrast, increasing the myoglobin content from 4 to 12 mg/g of muscle increased R630/R580 (P < 0.05) by 0.04 units (Table 4). When HCWT was accounted for in the model, high myoglobin then decreased R630/R580 by 0.05 units. Variation in muscle zinc concentration did not have a significant (P > 0.05) impact on R630/R580.

High pH₂₄ of the loin muscle produced a marked decrease (P < 0.01) in R630/R580 after display. Increasing pH₂₄ from 5.4 to 6 reduced R630/R580 by 0.88 units (Fig. 4; Table 4). The magnitude of this effect was not altered when HCWT was included in the model.

When age at slaughter was added into the model with the kill group within site and year term fitted as a random effect, increasing age from 140 to 500 days reduced R630/R580 by 0.61 units (P < 0.01; Fig. 2).

#### 3.3. Effect of sire and sire breeding values on R630/R580

Within the base model sire type had a significant impact on R630/R580 (P < 0.01; Table 3). The R630/R580 of lamb loins produced by individual sires ranged from 2.84 to 3.61 units for Terminal sires, 2.79 to 3.25 units for Maternal sires and 2.62 to 2.85 units for Merino sires (Fig. 5).

When the sire ASBVs for PWWT, PEMD and PFAT were included simultaneously as covariates in the base linear mixed effects model, only PEMD affected (P < 0.05) R630/R580. Increasing PEMD from −2.2 to 4.2 units increased R630/R580 by 0.27 units (Fig. 5). When PEMD was incorporated individually in the base model its impact on R630/R580 remained the same. When the phenotypic covariates were included one at a time in the PEMD model, the inclusion of isocitrate dehydrogenase activity accounted for most of the PEMD effect.

### 4. Discussion

In line with our hypothesis, increasing isocitrate dehydrogenase activity in the loin muscle was associated with a reduction in R630/R580. This supports the notion that the colour of lamb meat during display has worsened with increasing muscle oxidative capacity. The importance of our finding is that the difference was found between lambs in one muscle rather than between different muscles as previously described (Lanari & Cassens, 1991; Madhavi & Carpenter, 1993; O’Keeffe & Hood, 1982; Reinerer & Labas, 1987). Selection objectives that change the oxidative capacity of muscles such as growth rate and increased muscling could therefore also change the colour of meat on display.

High sire ASBVs for PEMD markedly increased the R630/R580 of lamb meat after 3 days of display in this study. This positive relationship supports our hypothesis that selection for muscling would improve meat colour during display. Gardner et al. (2007) demonstrated that selection for muscling reduced oxidative capacity of muscle in sheep.
which would lead to the observed improvement in colour. Likewise, Kelman et al. (2014) demonstrated within the same lambs used in the present study that higher PEMD breeding values were associated with reduced isocitrate dehydrogenase activity. Thus it appears that the increase in R630/R580 by 0.27 units seen with high PEMD breeding values is delivered via its correlated reduction in muscle oxidative capacity. This notion is further supported because PEMD had a reduced impact on R630/R580 when the model was corrected for isocitrate dehydrogenase activity. These findings suggest that selecting sires for muscling using the ASBV for PEMD will cause a relative increase in the R630/R580 of their progeny's meat whilst on display by up to 24 h [Jacob et al., 2013]. Given 3 days is the end of the display period used in domestic markets, selecting for muscling should improve the shelf life of lamb meat in this context.

In this study increased HCWT represents increased growth rate, given that our model was corrected for kill group and lambs within kill groups were of similar ages. Thus the positive association between HCWT/growth rate and R630/R580 supported our hypothesis. Alternatively, increasing growth rate via selection of sires with high PWWT estimates did not have an impact on the colour of lamb loins after display, contrary to our hypothesis. The basis of both of these hypotheses was that faster growth rates were likely reflective of genotypes with a larger mature size. Therefore faster growing lambs when compared at the same age would be less mature and their muscle would be less oxidative (Brandstetter et al., 1998), resulting in improved meat colour. While increasing PWWT has been linked with increased mature size (Huisman & Brown, 2009), its lack of impact on R630/R580 casts some doubt on the fibre type/maturity linked premise of our hypothesis. Likewise the phenotypic variation in HCWT may not necessarily imply variation in maturity and subsequent altered fibre type. This is further supported by the fact that there was no change to the association between R630/R580 and HCWT when we corrected the model for isocitrate dehydrogenase activity. Finally, Kelman et al. (2014) demonstrated no consistent association between isocitrate dehydrogenase and HCWT within these same lambs. Therefore it appears that the impact of phenotypic growth rate on meat colour is not delivered via altered muscle oxidative capacity, but perhaps by another alteration to muscle metabolism that impacts myoglobin oxidation or reduction processes.

The progeny of Terminal and Maternal sires had better meat colour than those of Merino sires. Likewise, progeny from Border–Leicester–Merino dams had better colour than those from pure Merino dams. These results align well with our hypotheses regarding selection for muscling, with the greater muscling potential of the Terminal and Maternal genotype lambs when compared to Merino lambs likely to have impacted muscle fibre type reducing oxidative capacity (Greenwood et al., 2007, 2006; Hawkins et al., 1985). This assertion was supported by correcting the model for isocitrate dehydrogenase activity which accounted for these genotype differences. This result is unlikely to reflect variation in age. Although Merino lambs are slowly growing and were therefore often grouped together in later kill groups, there were a number of kill groups (n = 55) where Terminal and Maternal lambs were slaughtered together with Merino lambs enabling the direct comparison of these sire types without confounding with age.

Importantly, in this study meat R630/R580 after 3 days of display was more strongly associated with isocitrate dehydrogenase activity than iron or myoglobin concentrations. While lower isocitrate dehydrogenase activity was associated with a marked increase in R630/R580, the association with R630/R580 for iron was only half this magnitude across its range, and almost non-existent for myoglobin. This supports the work of Mortimer et al. (2013) that found a phenotypic correlation of −0.12 between meat R630/R580 and isocitrate dehydrogenase activity, compared to a correlation of only 0.04 and −0.02 with myoglobin and iron concentrations respectively. In this study high iron concentration reduced meat R630/R580 by 0.27 units, supporting that more oxidative muscles brown more rapidly, given that higher iron levels are found in more oxidative muscles (Lefaucheur, 2010). In contrast, the small positive impact of myoglobin on R630/R580 by 0.04 units was unexpected given that myoglobin is also more concentrated in more oxidative muscles (Lefaucheur, 2010). An explanation for this may be the positive association demonstrated by Kelman et al. (2014) between myoglobin and HCWT. Within this study growth rate is reflected by HCWT at the same slaughter age (i.e. model corrected for kill group) and was positively associated with R630/R580 of meat colour. Thus in the context of meat colour, myoglobin content appears to be more strongly reflecting variation in growth rate than variation

![Fig. 1. Effect of site and year of birth on R630/R580, where IN02 represents Trangie NSW, IN03 Cowra NSW, IN04 Rutherglen VIC, IN05 Hamilton VIC and IN08 Katanning WA.](image)

### Table 4

Effect of covariate phenotypic traits on R630/R580 across their range.

<table>
<thead>
<tr>
<th>Covariate (unit)</th>
<th>Range</th>
<th>Associated change in R630/R580</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH24</td>
<td>5.4–6</td>
<td>−0.88</td>
<td>105.85*</td>
</tr>
<tr>
<td>Age at slaughter (days)</td>
<td>140–500</td>
<td>−0.61</td>
<td>21.71*</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase (μmol/min/g)</td>
<td>3–7.5</td>
<td>−0.5</td>
<td>16.38*</td>
</tr>
<tr>
<td>Intramuscular fat (%)</td>
<td>3–7</td>
<td>−0.41*</td>
<td>4.86**</td>
</tr>
<tr>
<td>Iron concentration (mg/100 g)</td>
<td>15–35</td>
<td>−0.27</td>
<td>15.77**</td>
</tr>
<tr>
<td>Myoglobin concentration (mg/100 g)</td>
<td>4–12</td>
<td>0.04</td>
<td>1.42**</td>
</tr>
<tr>
<td>Shortloin fat weight (kg)</td>
<td>0.1–0.4</td>
<td>0.23*</td>
<td>15.58**</td>
</tr>
<tr>
<td>HCWT (kg)</td>
<td>15–30</td>
<td>0.5</td>
<td>47.00*</td>
</tr>
<tr>
<td>Shortloin muscle weight (kg)</td>
<td>0.1–0.4</td>
<td>0.61*</td>
<td>17.03**</td>
</tr>
</tbody>
</table>

* represents the effect of a covariate analysed with HCWT included into the model
* represents P < 0.01 whilst ** represents P < 0.05.
in muscle oxidative capacity. In support of this assertion high myoglobin reduced R630/R580 by 0.05 units when the model was corrected for HCWT.

Increasing pH24 of loin muscle also had a strong impact on meat colour, dramatically reducing the R630/R580 across the pH24 range. Mortimer et al. (2013) also found negative phenotypic (−0.16) and genetic (−0.37) correlations between meat colour and pH24. The exact mechanisms underpinning this relationship have been poorly defined, but may relate to the impact of pH24 on oxygen exchange at the meat surface. A high pH24 reduces the amount of oxygen able to penetrate through the meat surface due to water binding and swelling of proteins (Lawrie, 1958). Additionally, a high pH24 enhances utilisation of oxygen by mitochondria, with both factors resulting in less oxygen available to form the red pigment oxymyoglobin (Lawrie, 1958). These results are consistent with Jacob et al. (2013), who also identified pH as a key carcass trait needing to be minimised to prevent detrimental effects on lamb meat colour during display.

Intramuscular fat content reduced the R630/R580 of the lamb loin muscle after display. This supports the phenotypic (−0.14) and genetic (−0.31) correlations reported by Mortimer et al. (2013). Studies have demonstrated a synergistic relationship exists between lipid and myoglobin oxidation (Faustman et al., 2010), though it is not known whether extracellular lipid peroxidation could promote intra-fibre myoglobin oxidation. Alternatively, significant correlations have been found between intramuscular fat and intracellular lipid content (Essén-Gustavsson et al., 1994). Thus increasing intramuscular fat could reflect an associated increase in intracellular lipid, where lipid peroxidation would more directly impact on myoglobin oxidation. It is also ambiguous whether the close relationship between intramuscular fat and meat colour relates to the dependence of both processes on similar optimal conditions and substrates such as oxygen and iron, or whether myoglobin oxidation is directly promoted by primary and secondary reactive products of lipid oxidation (Faustman et al., 2010). These results point out the need for careful management of intramuscular fat.

Fig. 2. Effect of lamb age at slaughter (days) on R630/R580. Lines represent predicted means for age at slaughter (+SE). Icons represent each residual from the predicted means.

Fig. 3. Effect of isocitrate dehydrogenase activity in μmol/min/g of muscle on R630/R580. Lines represent predicted means ± SE. Icons represent each residual from the predicted means.
fat and colour. For example intramuscular fat is a positive consumer trait since it improves tenderness and eating quality (Mortimer et al., 2013; Pannier et al., 2014) while on the other hand intramuscular fat potentially reduces the R630/R580 of meat during display.

There was a strong association (Fig. 2) evident between lamb age and meat colour in this study. The possibility of a biased estimate for the magnitude of age cannot be completely discounted given that it is difficult to entirely differentiate an age effect from kill day effects in this data-set. The 0.61 unit reduction in R630/R580 seen over the increasing age range is likely underpinned by increased muscle oxidative capacity associated with increasing maturity.

There was significant variation in meat colour in this study between lambs produced at different sites, over different years and between different kill groups. Given the great variation in geographic regions and climates between sites, we anticipate great variation in factors such as nutrition and the age of lambs reaching slaughter between different production sites and years. With nutrition and age both influencing a lamb’s muscle oxidative capacity and growth rate (Brandstetter et al., 1998; Moody et al., 1980), these factors could account for some of the variation evident between sites and years. However, correction for growth rate, muscle oxidative capacity and age via inclusion of HCWT, isocitrate dehydrogenase activity and kill group terms in the model demonstrated that these factors only account for about 20% of the variation between sites and years. This demonstrates that there is more to nutrition than just its impact on muscle oxidative capacity and growth rate, with changes in antioxidant status likely to be central to the observed changes in meat colour.

Previous work by Khliji et al. (2010) has demonstrated that R630/R580 values equal to or above 3.3 units are required for customer acceptance of meat. In this study the average R630/R580 measured in lamb loins after 3 days of simulated retail display failed to reach this level, with approximately 70% of the lambs measured falling under 3.3

Fig. 4. Effect of pH24 on R630/R580. Lines represent predicted means ± SE. Icons represent each residual from the predicted means.

Fig. 5. Effect of sire ASBVs for PEMD on R630/R580. Lines represent predicted means ± SE for each sire type. Icons represent individual sire estimates taken from the base model plus the sire type predicted mean for: □, Terminal sires; Δ, Maternal sires; ○, Merino sires.
units. Yet importantly, this work has demonstrated significant variation in meat colour and identified some of the key factors influencing it. Different kill groups from the same site within the same year created the largest magnitude change in R630/R580 of up to 2.56 units, while there was up to 0.52 units difference between sites over the four year period and 0.24 units difference observed between years at any given site. The phenotypic carcass traits with the largest impact on R630/R580 were pH24 and shortloin muscle weight, producing a 0.88 unit reduction and 0.61 unit increase in R630/R580 respectively. Isocitrate dehydrogenase activity, lamb age at slaughter and HCWT each accounted for approximately 0.50 unit change in R630/R580 ratio. There was a 0.40 unit difference between sire types and a 0.20 unit difference between dam breeds. The ASBV for PEMD produced a 0.27 unit change in R630/R580, while there was a 1.0 unit difference in R630/R580 between sire estimates for different individual sires.

5. Conclusions

Aligning with our hypotheses, this comprehensive study supported general thinking that muscle oxidative capacity is one of the key drivers of lamb meat colour on display, selection for increased muscling improved the R630/R580 of meat after display, likely driven by changes in muscle oxidative capacity. Increasing carcass weight, representative of growth rate, also increased R630/R580, though independent of muscle oxidative capacity. Despite environmental factors such as kill group accounting for more of the observed variation in meat colour, the genotypic effects demonstrated are comparatively large and suggest the potential to produce more lamb meat of an acceptable colour at 3 to 4 days of display. The potential for genetic manipulation is supported by the work of Mortimer et al. (2013) that found the R630/R580 after 2 days of display to have correlation coefficients of 0.34 for phenotype, 0.10 for genotype and a heritability of 0.30 (±0.04). While the potential to develop a sire estimated breeding value encompassing retail meat colour is promising, establishing the interactions between carcass traits and colour are also important for industry prediction and monitoring purposes; in order to ensure that meat redness and thus shelf life are not inadvertently reduced with selection pressures for different meat carcass traits such as intramuscular fat or lean meat yield.

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References


