Associations of genetic and non-genetic factors with concentrations of iron and zinc in the *longissimus* muscle of lamb

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1. Introduction

Consumers demand lamb meat that is lean, palatable and has good nutritional attributes, and hence these three key drivers influence the purchase and “willingness to pay” decisions of consumers (Pethick, Banks, Hales, & Ross, 2006). Lamb has been shown to contain high levels of a number of nutrients that are important for human health, such as iron and zinc (Pannier et al., 2010). For this reason the nutritional qualities represent a key marketing tool that is used to compete against other meats and non-meat foods.

An additional key productivity driver throughout the supply chain is lean meat yield, which has rapidly increased in the last decade as lambs have been selected for rapid lean growth (Gardner et al., 2010). However there is a concern regarding the impact of selecting for lean meat yield on these minerals is minimal, but should be monitored to avoid lower levels. Both minerals had a positive relationship with age at slaughter, highlighting age as a key determinant of the concentration of these nutrients. The magnitude of the positive associations of isocitrate dehydrogenase and myoglobin with iron was larger than for zinc, but they strongly indicated the association of these aerobic makers with both minerals.

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In Australia, indirect selection for lean meat yield is targeted using Australian Sheep Breeding Values (ASBVs) for post weaning weight (PWWT), eye muscle depth (PEMD) and c-site fat depth (PFAT). Lambs selected from sires with reduced PFAT have reduced whole carcass fatness and increased loin muscle weight (Gardner et al., 2010). Similarly, lambs selected from sires with higher PEMD breeding values have increased musclearity in high valued cuts mainly located in the animal’s saddle region (Gardner et al., 2010). The selection for reduced PFAT and increased PEMD has previously been reported to be associated with a higher proportion of type IX muscle fibres (Greenwood et al., 2006) and a less oxidative muscle type (Gardner et al., 2006), hence it is likely that the iron and zinc concentrations will also be lower. Lambs selected for high PWWT will be faster growing (Hall, 2000) due to a larger mature size (Huisman & Brown, 2008) and will therefore be less mature and leaner at the same slaughter weight. Maturity has been associated with increasing oxidative capacity (Suzuki & Cassens, 1983; White, Mc Gavin, & Smith, 1978) and more mature lambs might have higher iron and zinc concentrations compared to less mature lambs. Given the impact of these ASBVs on rapid lean growth it is likely that they will affect muscle oxidative capacity and therefore decrease iron and zinc. These effects of these ASBVs on the iron and zinc contents in lamb needs to be examined to ensure that these mineral levels are sufficient to reach the recommended dietary guidelines as explained in Pannier et al. (2010).

The Australian Cooperative Research Centre (CRC) for Sheep Industry Innovation has compiled an Information Nucleus Flock (INF) which produces approximately 2000 slaughter lambs each year. Some of the INF objectives are to measure a diverse range of phenotypic traits, and to produce heritability estimates and genetic correlations for a range of production regions of Australia (Fogarty, Banks, van der Werf, Ball, & Gibson, 2007; van der Werf, Kinghorn, & Banks, 2010), the environmental effects on these nutrients can be determined. Results from three years of progeny (2007–2009) from the INF are presented here. As such this paper is an extension of the first year progeny (2007) analysis reported previously (Pannier et al., 2010). We hypothesised that factors which lead to decreasing oxidative capacity in muscle will be associated with lower iron and zinc concentrations. We therefore hypothesised that lambs from sires with reduced PFAT breeding values, increased PEMD and PWWT breeding values, female lambs and Terminal sired lambs will have lower iron and zinc concentrations in the longissimus muscle. In addition, we also hypothesised that animal age will greatly impact on these mineral levels, with older animals having higher muscle iron and zinc concentrations compared to younger animals, and that muscle aerobic markers ICDH and myoglobin will have a positive association with both minerals.

2. Material and methods

2.1. Experimental design and slaughter details

The design of the Sheep CRC INF is detailed elsewhere (Fogarty et al., 2007; van der Werf et al., 2010). Briefly, approximately 6000 lambs were produced over a 3 year period (year of birth 2007–2009) at eight research sites across Australia (Katanning WA, Cowra NSW, Trangie NSW, Kirby NSW, Struan SA, Turretfied SA, Hamilton VIC, and Rutherglen VIC). The lambs (Merino × Merino, Merino × Merino, Terminal × Merino and Terminal × Border Leicester–Merino) were the progeny of ~100 key industry sires each year, representing the major production types in the Australian sheep industry. The sires included Terminal sires (Hampshire Down, Ile De France, Poll Dorset, Southdown, Suffolk, Texel, White Suffolk), Maternal sires (Bond, Booroola, Border Leicester, Coopworth, Corriedale, Dohne Merino, East Friesian, Prime SAMM, White Dorper), and Merino sires (Merino, Poll Merino). Lambs were mainly maintained under extensive pasture grazing conditions, but were fed grain, hay or feedlot pellets when feed supply was limited at some sites (Ponnampalam et al., 2014). Lambs were yarded the day before slaughter, held for 6 h and then weighed and transported to one of five commercial abattoirs where they were held in lairage overnight and slaughtered the following day at an average carcass weight of 23 kg. For each site lambs were consigned to smaller groups which were killed at the same day (kill groups) to enable carcass weight targets to be achieved. All carcasses were subjected to a medium voltage electrical stimulation (Pearce et al., 2010) and trimmed according to AUS-MEAT specifications (Anonymous, 2005). Carcasses were chilled overnight (3–4 °C) before sampling. All lambs were measured and sampled for a wide range of carcass, meat and growth traits.

2.2. Sample collection and measurements

Hot carcass weight was measured after slaughter. At 24 h postmortem, the longissimus lumborum muscle was excised from the carcasses. Subcutaneous fat and silver skin (epimysium) were removed, and approximately 40 g of diced muscle was collected for mineral and intramuscular fat (IMF) analyses. The samples were then frozen at −20 °C and freeze-dried using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, Blenheim, New Zealand). Approximately 0.2 g dry matter per sample was weighed out for subsequent mineral analysis. Samples were prepared according to the USEPA method 2003 (USEPA, 1991). Iron and zinc concentrations were determined on a Vista AX CCD simultaneous ICP-AES (Varian Australia Pty Ltd.).

IMF was determined using a near infrared procedure (NIR) in a Technicon InfraLyser 450 (19 wavelengths) (Perry, Shorthose, Ferguson, & Thompson, 2001). NIR readings were validated with chemical fat using solvent extraction (chloroform). IMF was expressed as percentage of fresh weight.

For myoglobin, approximately 1 g finely diced muscle was collected and stored at −20 °C. Roughly 0.2 g muscle tissue was homogenised in 0.04 M phosphate buffer (pH 6.5) using a polytron (Kinematica Polytron, probe PT 10–35; Kinematica Gmbh, Luzern, Steinhofhalde, Switzerland) at full speed for 20 s. Samples were centrifuged for 10 min at 3000 rpm (805 G) and the supernatant was collected. Triton X–100 (10%) and 65 mM sodium nitrite were added to the supernatant, following a 60 min incubation at room temperature. The myoglobin assay was performed using a Beckman DU650 spectrophotometer and the myoglobin concentration was estimated using the method of Trout (1991). The absorbances were read at 730 (turbidity) and 409 nm (oxidised pigment).

For isocitrate dehydrogenase (ICDH), within 2 h post-mortem a muscle sample of approximately 1 g over the 12th rib was collected. Subcutaneous fat was removed and samples were finely diced and stored in liquid nitrogen. The activity of ICDH (ICDH; EC 1.1.1.42) was determined by the method of Briand (1981).

2.3. Statistical analysis

The iron and zinc concentrations were analysed using linear mixed effects models (SAS Version 9.1, SAS Institute, Cary, NC, USA). Initially a base model was established including fixed effects for site (Kirby, Trangie, Cowra, Rutherglen, Hamilton, Struan, Turretfied, Katanning), year (2007; 2008; 2009), sex (wether, female), birth-rearing type (terminal, terminal × terminal, terminal × terminal × terminal), dam breed within sire type (Merino × Merino, Border Leicester × Merino, Terminal × Merino, Terminal × Border Leicester–Merino) and kill group within site by year. Sire identification, and dam identification by year were included as random terms. All relevant first order interactions between fixed effects were tested and non-significant ($P > 0.05$) terms were removed in a stepwise manner until the Akaikes information criterion value (AIC) was minimised.
To determine the effect of animal age on iron and zinc concentrations, age at slaughter was included as a covariate and the kill group within site by year term was removed as a fixed effect from the base model, and was included as a random term. Within each site by year, age was confounded by kill group (i.e., there were no lambs of the same age within a separate kill group, and the variation in age within each kill group was less than 10 days). Therefore kill group accounted for the slaughter-age effect, and removing this term as a fixed effect enabled the association of age at slaughter to be estimated. All relevant first order interactions between fixed effects and covariate were tested, as was the covariate quadratic effect. Non-significant ($P > 0.05$) terms were removed in a stepwise manner until the AIC value was minimised.

To test for associations between the mineral levels and aerobic markers such as myoglobin and ICDH, the base model was maintained and myoglobin and ICDH were individually included as phenotypic covariates. Hot carcass weight and IMF were also individually tested as covariates in the base model. Again, these models included all relevant first order interactions between fixed effects and covariates as well as the quadratic effect for each covariate. Non-significant ($P > 0.05$) terms were removed in a stepwise manner until the AIC value was minimised.

The associations between the iron and zinc concentrations and sire ASBVs for PWWT, PEMD and PFAT were also tested in the base model. Initially all 3 ASBVs were included as covariates in the model, as well as their quadratic effect and first order interactions with other terms, and non-significant ($P > 0.05$) terms were removed in a stepwise manner until the AIC value was minimised. Due to the correlations that exist between these ASBVs in this data set (PWWT vs PEMD = 0.3; PWWT vs PFAT = 0.3; PFAT vs PEMD = 0.2) this process was repeated with the ASBVs included one at a time to test the independence of their effects. In addition the 3 ASBV model, for both iron and zinc, were also tested with the inclusion of myoglobin, ICDH, IMF and hot carcass weight as a covariate (one at a time) to assess whether the observed ASBV effects were associated with their correlated impacts on these covariates. The residuals for both iron and zinc were assessed and demonstrated normality and consistent variance between the sub-sets of data defined by the fixed effects of the base model.

3. Results

Data from a total of 5625 animals were available for the mineral analyses and lamb counts per site for the main fixed effects in the base model are given in Table 1. The raw mean for the mineral concentrations, age at slaughter, IMF, myoglobin, ICDH and hot carcass weight are presented in Table 2.

3.1. Iron and zinc concentrations

The average concentration of iron in lamb muscle was 2.03 mg/100 g (Std dev = 0.36), with values ranging from 0.81 to 3.99 mg/100 g and for zinc the average was 2.43 mg/100 g (Std dev = 0.44) with values ranging from 1.18 to 4.49 mg/100 g. Table 3 presents the required concentrations needed (based on the minimum recommended intakes) of iron and zinc in lamb to consume a ‘source’ or ‘good source’ of these minerals as explained in Pannier et al. (2010), and demonstrates how many of the INF lambs reach these recommended guidelines.

3.2. Effect of genetic and non-genetic factors on iron and zinc

The base model outcomes are presented in Table 4. The model used 5625 observations of the total 5847 available (after dropping animals with missing data) and described 49% and 33% of the total variance in iron and zinc. Iron differed between sites ($P < 0.01$) with lambs at the Kirby site (2.27 ± 0.02) having the highest iron levels, contrasting with lambs at the Cowra site (1.98 ± 0.02) which had the lowest iron levels. On average lambs born in 2008 (2.16 ± 0.02) had the highest iron levels, however there was no consistent pattern for 1 year to always have the most or the least iron levels across all sites. The variation between years ($P < 0.01$) differed between sites from as little as 0.04 mg/100 g iron for the Trinity site, to as much as 0.34 mg/100 g iron for the Hamilton site. Female lambs (2.11 ± 0.02) had about 3% higher ($P < 0.01$) iron concentration than wethers (2.05 ± 0.01). Terminal sired lambs (2.01 ± 0.02) had about 3% and 6% less ($P < 0.01$) iron compared to the Maternal (2.08 ± 0.03) and Merino (2.15 ± 0.04) sired lambs. This differed slightly between the birth type-rear types ($P < 0.01$), with Terminal sired lambs born as triplets and reared as twins or triplets having about 1% and 2% more iron compared to the same birth type-rear types of the Maternal sired lambs.

For zinc, lambs at Rutherglen (2.27 ± 0.02) had the lowest zinc levels while lambs at Trangie (2.81 ± 0.02) had the highest levels ($P < 0.01$). Similar to iron, lambs from year 2008 (2.55 ± 0.02) had the highest zinc levels ($P < 0.01$), however this differed across all sites from as little as 0.06 mg/100 g zinc for the Kirby and Turrettfield site, to as much as 0.50 mg/100 g zinc for the Cowra site. Maternal sired lambs (2.51 ± 0.02) had about 3% and 2% more ($P < 0.05$) zinc compared to Merino (2.44 ± 0.03) and Terminal (2.46 ± 0.01) sired lambs.

At each site, the iron and zinc concentrations differed between kill groups ($P < 0.01$) (individual data not shown for the kill groups), with a general trend for the older kill groups to have higher levels of iron and zinc. The effect of age was further demonstrated when age at slaughter was included as a covariate and the kill group within site by year term was included as a random term. The effect of the age covariate was highly significant ($P < 0.001$; Fig. 1) for both minerals and increased iron and zinc concentrations by 0.75 mg/100 g and 0.57 mg/100 g, across the 380 day age range.

3.3. Association of intramuscular fat, hot carcass weight, myoglobin and ICDH with iron and zinc

When IMF was included as a covariate ($P < 0.01$), reduced levels of IMF were associated with reduced iron and zinc levels. For both
minerals the effect was curvilinear and the magnitude of the effect decreased by 0.13 and 0.14 mg/100 g for iron and zinc between 2 and 6% IMF (Fig. 2). In both cases iron and zinc plateaued beyond 6% IMF.

When hot carcass weight was included as a covariate, heavier carcasses had more iron \((P < 0.01)\), increasing by about 0.23 mg/100 g across the 27 kg range in hot carcass weight. This relationship differed between sire types \((P < 0.05)\), with Terminal sired lambs having 0.17 and 0.19 mg/100 g less iron than Maternal and Merino sired lambs. For zinc, heavier carcasses had about 0.09 mg/100 g more zinc \((P < 0.01)\) between 13 and 27 kg hot carcass weight, after which it plateaued.

Both myoglobin and ICDH included as covariates individually were highly significant \((P < 0.01)\). Myoglobin had a positive association with both iron and zinc, increasing the minerals by 0.94 for iron, and 0.30 mg/100 g for zinc across the 2–12.5 mg/g myoglobin range (Fig. 3). For ICDH, iron and zinc increased by 0.47 and 0.30 mg/100 g across the 2–9 μmol/min/g ICDH activity range (Fig. 4).

### 3.4. Effect of sire and sire breeding values on iron and zinc

In the base models for iron and zinc, sire as a random term was significant \((P < 0.01)\) with 95% of sire estimates for iron lying between 1.87 and 2.17 mg/100 g within Terminal, 1.93–2.23 mg/100 g within Maternal, and 2.00–2.29 mg/100 g within Merino sires (Fig. 5), and 95% of sire estimates for zinc lying between 2.26 and 2.66 mg/100 g within Terminal, 2.31–2.70 mg/100 g within Maternal, and 2.24–2.64 mg/100 g within Merino sires.

When the 3 sire ASBVs for PWWT, PEMD and PFAT were included simultaneously in the base model for iron, only PFAT \((P < 0.01; \text{Z-value} = 5.98)\) had an effect (Fig. 5), with a 5 unit reduction in PFAT reducing the iron levels by 0.11 mg/100 g. The magnitude of this effect did not change when PFAT was included independent of the other two ASBVs. Neither PEMD nor PWWT demonstrated an impact on iron. When the 3 ASBV model was corrected for hot carcass weight or IMF, the magnitude of the PFAT effect described above remained unchanged. However, when myoglobin was included, the magnitude of the PFAT effect was diminished by about one third. When ICDH was included the PFAT effect disappeared.

For zinc there were no associations observed with any of the sire ASBVs. The mean and range for these ASBVs are summarised in Table 5.

### 4. Discussion

#### 4.1. Association of sire ASBVs with iron and zinc

Reducing sire PFAT ASBV was associated with a small reduction in the iron content of lamb, aligning well with our hypothesis. Yet in contrast to our hypothesis, a similar result was not evident for zinc. The selection for reduced PFAT has been shown previously to increase the proportion of type IIX muscle fibres (Greenwood et al., 2006), which would align with reduced aerobicity and myoglobin levels (Gardner et al., 2006). This might explain this result given that the haem-iron oxygen carrying protein myoglobin is a key source of iron. However in the study of Gardner et al. (2006), PFAT had no effect on myoglobin, which contrasts the results of Kelman et al. (2014) who demonstrated a 1.07 mg/g tissue myoglobin reduction when reducing PFAT. However the latter was only evident in ewe lambs. Thus it is possible that the association of PFAT with the muscle iron content is partly delivered through its impact on myoglobin, and this was confirmed within our study when the PFAT impact on iron was diminished when including myoglobin in the model.

Kelman et al. (2014) also demonstrated a reduction in ICDH activity of 0.46 μmol/min/g tissue when reducing PFAT, and it appears that the association of PFAT with iron is delivered through this correlation given that the PFAT effect dropped out when ICDH was included in the model. The PFAT impact on muscle iron remained the same when hot carcass weight or IMF was included, illustrating that both covariates are not solely responsible for the observed PFAT effect. However, this trait requires attention because of the current focus by some breeders within the lamb industry to select sires with low PFAT to increase lean meat yield. Given this relationship between PFAT and iron, information on the genetic correlation between carcass fatness and iron levels in lamb is required if iron levels are to be maintained while reducing carcass fatness and improving muscularity through genetic selection.

PEMD did not impact on muscle iron content, not supporting our hypotheses that sires with a higher muscling potential would produce progeny with lower iron levels. This hypothesis was based upon previous studies which demonstrated that increased genetic potential for muscling decreased the proportion of oxidative muscle fibre types (Greenwood et al., 2006), which is likely to align with reduced myoglobin (Gardner et al., 2006) and iron concentration. The premise for this hypothesis appears to be correct, as within these same lambs Kelman and Greenwood et al. (2006) demonstrated a reduction in ICDH activity when reducing PFAT.
et al. (2014) found a negative association between PEMD and oxidative capacity. This was demonstrated by a reduced ICDH activity of 0.50 μmol/min/g tissue across all sire types and a reduced myoglobin concentration of 0.49 mg/g tissue present within the progeny of Terminal sires. Although in contrast to this trend the Merino sired lambs, demonstrated increased myoglobin (1.36 mg/g tissue) with higher PEMD values (Kelman et al., 2014). The inconsistency of the myoglobin response between sire types may in part explain why there was no overall PEMD effect on iron, however it is more likely that the correlation between ICDH/myoglobin and iron (Figs. 3–4) is not strong enough to lead to an impact on iron within this data set. Nonetheless, due to this correlation, long term selection for PEMD producing greater extremes in this breeding value, and impacting more heavily on muscle oxidative capacity, is likely to reduce iron levels.

Neither PFAT nor PEMD showed any association with zinc, not supporting our hypothesis for this mineral. This suggests that the selection for lean meat yield via these ASBVs will not affect the zinc concentration in lamb.

PWWT was not associated with either iron or zinc levels, which is contrary to our hypothesis for both minerals. This hypothesis was based upon the premise that PWWT would reflect maturity, with the progeny of high PWWT sires having a larger mature size (Huisman & Brown, 2008) and therefore being less mature at the same slaughter age (i.e., kill group corrects for age in this analysis — Fig. 1). Although age itself has been shown to have a large effect on both minerals, a variation in maturity at a given age caused by PWWT is clearly too small to affect iron or zinc concentration.

### 4.2. Association of age at slaughter with iron and zinc

Age at slaughter demonstrated a strong association with iron and zinc in the m. longissimus lumborum, supporting our initial hypothesis. However, one concern with the current data set and analysis is that the oldest animals slaughtered at each site were predominantly sired by Merinos because of their slower growth to target slaughter weight. The average slaughter age for Merinos was 372 days, compared to an average of 248 days for the Terminal and Maternal sired lambs. As such, the apparent age effect could have been a Merino sire type effect. To confirm the association with age, an additional analysis was conducted in which all kill groups which consisted of only Merino sired lambs were included. In this case the age effect was still significant for iron and zinc, and the magnitude of these responses were the same, slightly increased, than the magnitude when the Merino only kill groups were included. This confirms that the Merino lambs were merely following the same age continuum as the Maternal and Terminal sired lambs. In further support of this notion, a study by Gardner et al. (2007) demonstrated that, when compared at the same age, Merino lambs did not have greater myoglobin levels than crossbred lambs, and were likely to have had the same iron levels. This impact of age has important implications for the effect of PWWT. Although this ASBV was shown to have no effect on iron and zinc, this was demonstrated in an age corrected model (i.e., corrected for age by the kill group term). None-the-less, the progeny of high PWWT sires will grow faster and therefore reach slaughter weight at a younger age, resulting in lower muscle iron and zinc concentrations.

### 4.3. Association of aerobic markers, intramuscular fat and hot carcass weight with iron and zinc

As hypothesised, both aerobic markers ICDH and myoglobin had a significant positive association with iron and zinc (P < 0.01). However the magnitude of this association differed, with iron increasing four-fold more than zinc across the same range of myoglobin concentration and two-fold more than zinc across the same range of ICDH. Furthermore, the phenotypic and genetic correlations between ICDH and iron (0.21; 0.45) and between myoglobin and iron (0.35; 0.99) were higher compared to the phenotypic and genetic correlations between ICDH and zinc (0.13; 0.20) and between myoglobin and iron (0.09; 0.22) (Mortimer et al., 2014). Thus iron appear to associate more strongly with muscle oxidative capacity than zinc. This is likely due to the direct involvement of iron as a key component of myoglobin which is a haem-iron oxygen storage protein. Hence it is not surprising that iron itself aligns more strongly with a variation in myoglobin than ICDH. Although PFAT had a small association with iron and PEMD had no association on either mineral, these breeding values have been
shown to be associated with ICDH activity and myoglobin concentration (Kelman et al., 2014). Therefore long term selection for these breeding values is likely to result in greater PEMD and PFAT extremes, impacting more heavily on muscle oxidative capacity, and ultimately impacting on iron and zinc given their association with these markers. As such, independent selection for iron and zinc may eventually be required to offset the indirect effect of selection for high PEMD and reduced PFAT breeding values.

When IMF was tested as a covariate within the iron and zinc models, both iron and zinc levels declined with reducing IMF levels, however these effects of IMF on mineral levels were indirect. This was supported by the work of Mortimer et al. (2014) who demonstrated a low positive phenotypic and genetic correlations between IMF and iron (0.05; 0.04) and between IMF and zinc (0.04; 0.09). The relationship between IMF and aerobic markers (ICDH) has been demonstrated previously in cattle (Hocquette et al., 2003) and aligns well with the results in our lambs which also indicated a strong decrease in ICDH activity and myoglobin concentration of 0.72 μmol/min/g and 0.61 mg/g with decreasing IMF levels (Kelman et al., 2014). Our findings again highlight the concerns about increased selection for lean meat yield which reduces IMF (Pannier et al., 2013) and the proportion of oxidative fibre types (Greenwood et al., 2006) and subsequent iron and zinc concentrations due to a less aerobic muscle type (more glycolytic muscle) (Gardner et al., 2006).

Hot carcass weight demonstrated positive associations with iron and zinc. With hot carcass weight at a constant slaughter age (i.e., kill group corrects for age—see Fig. 1) reflecting growth rate within our study, the heavier animals would have been growing faster and would be closer to their mature size. Therefore they would have proportionately deposited more iron and zinc in muscle, aligning well with our results. As thus, our observed differences in growth rate are more likely to be due to environmental effects (better nutrition and possibly reduced worm burden) as opposed to genetic and mature size effects.

4.4. Production factors impacting on iron and zinc

There were differences in iron and zinc concentrations between sites. Lambs from the Cowra and Rutherglen site had the lowest iron and zinc levels, respectively, and in the case for Cowra this was most likely due to the relatively young mean slaughter age of the animals (195 days) compared to the mean slaughter age for the other sites (277 days). In contrast, Kirby had high iron levels and also had a high mean slaughter age of 327 days. For both the Cowra and the Kirby sites their alignment with age was confirmed when age was used as a covariate in the analysis, as this correction eliminated the differences between Cowra and Kirby and the other sites. Increasing age has been associated with a more oxidative muscle type (Greenwood et al., 2007) and greater expression of myoglobin (Gardner et al., 2007). Given that myoglobin accounts for the largest proportion of iron in muscle (Hazell, 1982), and that zinc is associated with antioxidant cascades and the electron transport chain (Powell, 2000), all of which are elevated in more aerobic muscles, levels of both of these minerals would be likely to increase with age. Alternatively, lambs from the Trangie site had fairly high iron and zinc levels, however this was not
explained by age as the lambs from this site had a low mean slaughter age of 208 days. This implies that other environmental factors are impacting at this site. It is difficult to explore this further, given the information available, however we can speculate that nutrition may be a factor. This has been shown before where restricted nutrition increased glycolytic type IIX muscle fibres (Greenwood et al., 2006) and decreased oxidative capacity with lower levels of the aerobic marker myoglobin (Gardner et al., 2006). Hence unrestricted nutrition at the Trangie site may explain the lower iron and zinc concentrations at that site. From the perspective of human nutritional requirements, all sites exceeded the iron levels required to claim lamb as a ‘good source’ of iron and zinc (RDI = 8 mg for both minerals) with the exception of Cowra (1.98 ± 0.019) where the iron levels fell just below this threshold (Table 3). The differences between kill groups within each year at each site partly reflects animal age (as discussed earlier on), with a general trend for iron and zinc levels to increase in older kill groups (see Fig. 1). These kill group effects would also reflect other environmental variables such as different seasons, and processing variations within the abattoir, all of which are beyond the control of this study.

In contrast to our hypothesis, female lambs had higher levels of iron compared to the wethers. This finding also contradicts previous studies showing that female lambs have more glycolytic type IIX muscle fibres (Greenwood et al., 2007) and have a lower oxidative capacity (Gardner et al., 2007), and were expected to have lower iron (Pearce et al., 2009) and zinc concentrations (Kondo et al., 1991). An analysis of the aerobic markers ICDH and myoglobin demonstrated higher levels of myoglobin for the female lambs compared to the wethers (Kelman et al., 2014) which might explain these results for iron, given the high correlation between iron and myoglobin in this study. However, when the model was corrected for myoglobin the magnitude of the difference between sex effect remained unchanged, indicating that this effect is not solely explained by differences in myoglobin. Furthermore, it was also shown that whether lambs had higher ICDH levels compared to female lambs (Gardner et al., 2007; Kelman et al., 2014), which however does not support the above finding that female lambs might have a more aerobic muscle type, but highlights again the different impacts of both markers on both minerals.

As hypothesised Terminal sired lambs had the lowest iron levels and the Merino sired lambs had the highest iron levels. Terminal sired lambs have been shown to have more glycolytic type IIX muscle fibres (Greenwood et al., 2007) which have lower myoglobin levels (Lefaucheur, 2010), and this may reflect their lower iron levels. Furthermore, in the study of Kelman et al. (2014), the levels of myoglobin were the lowest for the Terminal sired lambs, as were the ICDH levels, possibly reflecting the long term selection pressure for muscularity and reduced fatness within this sire type. In addition,
Kelman et al. (2014) demonstrated the highest levels of myoglobin in the Merino (no difference between Merino and Maternal, but both were higher than Terminal) sired lambs which might explain our results for iron within this breed. The higher iron concentration of the Merino sired lambs could also be a reflection of their older age, however this difference remained when the model was corrected for age, again highlighting the independent effects of sire type and age on the iron levels.

In contrast to the effects in iron, the levels for zinc were similar for the Merino and Terminal sired lambs, with the Maternal sired lambs having the highest levels. There is little other evidence comparing muscle zinc concentrations between breeds, and within our data set we have no ability to explain this result.

4.5. Comparison of effects on iron and zinc

For iron, age at slaughter demonstrated the largest non-genetic effect with a magnitude about two and a half times bigger than the magnitude of the differences between sites. The site effect was about three times larger compared to the effect of PFAT, and two times larger compared to the effect of year and sire type. Whilst PFAT does impact on the iron levels, the age at slaughter and the environment effect on iron are much more profound. For zinc, age at slaughter had also the largest effect however its magnitude was close to the magnitude of the site effect and the year effect. However in contrast to iron, no ASBVs were associated with zinc indicating the larger impact of age at slaughter and the environment and production factors on zinc compared to the selection for lean meat yield via the use of ASBVs.

4.6. Current iron and zinc levels in prime lamb

Based on the average concentrations of iron and zinc for all INF lambs tested in this study, Australian lamb can be claimed as a ‘good source’ of iron (2.03 mg/100 g; Table 3) for men of all ages and women over 50 years old, and can be claimed as a ‘source’ of iron for women less than 50 years old. These nutritional claims are based on a recommended daily intake (RDI) of 8 mg for men and women over 50 years old and 18 mg for younger women in one serving of food which is 65–100 g of cooked meat (The Australian Guide to Healthy Eating 1998; NHMRC, 2003) or 93–143 g of fresh meat, assuming a 30% moisture loss during cooking (Huffman, Britton, & Cordray, 1982). For zinc (2.43 mg/100 g; Table 3), lamb can be claimed as a ‘good source’ for women of all ages, but only as a ‘source’ for men. Because a ‘good source’ claim cannot be made for iron in younger women (RDI = 18 mg) and zinc in all men (RDI = 14 mg), this would suggest that there is room for improvement. Given the large number of animals used in this study, compared to other studies in which only 10–30 animals were sampled (Williams, 2007; Williams, Droulez, Levy, & Stobas, 2007), our values for iron and zinc for the longissimus lumborum muscle will be good estimates and hence might differ slightly to what is quoted in the official Australian dietary tables (2.30 mg/100 g iron, 3.40 mg/100 g zinc; NHMRC, 2003). Despite the fact that official Australian Food Composition tables (www.foodstandards.gov.au) and other dietary tables report on multiple lamb cuts (Hoke, Buege, Ellefson, & Maly, 1999; Williams et al., 2007), compared to our study in which only one muscle was analysed, they are often based on the analysis of a small number of retail samples. Such small sample numbers of lamb cuts are insufficient to identify genetic and production effects (particularly age) on levels of nutrients in muscle and are therefore unlikely to provide a particularly reliable estimate of these nutrient levels.

5. Conclusions

Our results demonstrated that selection for lean meat yield through the use of ASBVs will impact slightly on iron levels only through the selection of sires with low PFAT but will not impact on the zinc levels. These results confirm that the effect of lean meat yield selection on iron and zinc is minimal, however effort needs to be made to avoid concentrations dropping any lower. Age was a strong determinant of the mineral content and so were the aerobic markers IDCH and myoglobin, highlighting the link with muscle aerobicity which is reduced in older, leaner and more muscular animals. Achieving levels of iron and zinc that comply with recommended dietary guidelines is a key marketing tool for red meat and hence we can conclude that prime Australian lamb contains substantial concentrations of both minerals. However, there is some room for improvement for iron for younger women and for zinc for men. This is particularly important for differentiating sheep meat from other types of meat for marketing purposes.

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