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

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ABSTRACT

There is a concern regarding the possible decline of nutritional value of meat with an increasing selection for lean meat yield. The selection for reduced fatness reduces muscle aerobicity and possible subsequent mineral concentrations. Average concentrations of iron and zinc of 5625 lamb *longissimus* muscles were 2.03 and 2.43 mg/100 g, qualifying as a good source claim for the majority of the population. Reduced post-weaning fat depth was associated with decreased concentrations of iron but not zinc, whereas post-weaning eye muscle depth and weaning weight were not associated with either mineral. These results confirm that the impact of lean meat yield selection 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.

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 the nutrient content of lamb, in particular the iron and zinc concentrations. The selection of animals for lean growth has been shown to increase the proportion of glycolytic type IIX muscle fibres (Greenwood, Gardner, & Hegarty, 2006; Wegner et al., 2000), resulting in a less oxidative muscle type with a paler (whiter) appearance (Gardner, Pethick, Greenwood, & Hegarty, 2006). This was further confirmed in a study of Kelman, Pannier, Pethick, and Gardner (2014), who demonstrated that selection for lean meat yield reduced oxidative capacity via myoglobin levels and isocitrate dehydrogenase activity (ICDH). Muscles with lower oxidative capacity have been shown to contain reduced iron (Pearce et al., 2009) and zinc concentrations (Kondo, Kimura, & Itokawa, 1991), possibly due to lower concentration of myoglobin, less vascularisation (Choi & Kim, 2009; Lefaucheur, 2010) and fewer mitochondria (Hoppeler, 1985) within these muscles. Therefore, factors that influence muscle fibre type and muscle oxidative capacity are also likely to affect the iron and zinc concentrations.

Animal age is one factor that influences muscle oxidative capacity and hence iron and zinc concentrations, with older animals having been associated with a more oxidative muscle fibre type (Brandstetter, Picard, & Geay, 1998; Greenwood, Harden, & Hopkins, 2007) and increased oxidative capacity (Gardner et al., 2007), and a higher concentration of iron and zinc (Pannier et al., 2010). In contrast, female lambs and Terminal sired lambs have been shown to have more glycolytic type IIX muscle fibres (Greenwood et al., 2007) and may therefore have lower amounts of iron and zinc. Nutrition has also been shown to influence fibre type as demonstrated in lambs on a restricted diet which had more glycolytic type IIX muscle fibres (Greenwood et al., 2006) and a decreased oxidative capacity with lower levels of the aerobic marker myoglobin (Gardner et al., 2006). As such restricted nutrition is also likely to cause lower iron and zinc concentrations.

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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 muscularity 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 IIX 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, McGavin, & 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 new traits such as the iron and zinc concentrations. Given that INF lambs flocks are present across different 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, Turretfield SA, Hamilton VIC, and Rutherglen VIC). The lambs (Merino × Merino, Maternal × 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 post-mortem, 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 200.3 (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, Turretfield, Katanning), year (2007, 2008, 2009), sex (wether, female), birth-rearing type (term representing animals born as single, twin or triplet and reared as single, twin or triplet; 11, 21, 22, 31, 32, 33), sire type (Merino, Maternal, 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 Akaike's information criterion value (AIC) was minimised.