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Phenotypic blood glutathione concentration and selenium supplementation interactions on meat colour stability and fatty acid concentrations in Merino lambs

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Abstract

The interaction between blood glutathione (GSH) and supplementation of selenium (Se, 2.5 mg/kg diet) on meat colour and fatty acids concentrations was studied. Forty eight Merino lambs selected for high blood GSH (HGSH) or low GSH (LGSH) concentration were used. They were fed individually with or without Se supplement for 8 weeks. There were interactions ($P < 0.05$) between GSH and Se on the colour stability (as w630nm/w580nm ratio) of m. longissimus (LD), m. semimembranosus (SM) and m. semitendinosus. Without Se supplementation the ratio was higher in HGSH than LGSH group. However, the difference was reduced with Se supplement. Polyunsaturated and n-3 fatty acids in SM and LD were higher in HGSH than in LGSH group ($P < 0.05$), and did not change with Se supplement. Se supplementation increased Se content in LD ($P < 0.001$) and the lungs ($P < 0.05$), but had no influence in the heart.

Key words: antioxidants, redox state, myoglobin, lipid peroxidation, lamb, meat quality

1. Introduction

During retail display fresh red meat changes in colour from red to brown. A common practice in meat retailing is to discount meat that fails to sell within 48 hours of retail display to ensure that all meat is sold before the colour change is noticeable to consumers. While the change of meat colour involves complex processes of chemical and biochemical reactions, it is generally accepted that the colour change is closely associated with spontaneous autoxidation of myoglobin (Gray et al., 1996; Trout, 2003).

Myoglobin is the principle heme protein responsible for meat colour, and serves as an oxygen storage and oxygen delivery function in living cells (Livingston et al., 1983). Myoglobin is composed of an iron atom and the heme iron can exist in a reduced ferrous (Fe^{2+}) or oxidised

ferric (Fe^{3+}) form (Faustman and Cassens, 1990). Reduced myoglobin (Fe^{2+}) can be deoxygenated or oxygenated. Deoxymyoglobin shows purplish-red in colour, and oxymyoglobin shows a bright cherry red colour (Mancini and Hunt, 2005). These colours are synonymous with freshness and consumers consider it attractive (Renerre, 1990). Oxidised (Fe^{3+}) myoglobin only exists in the deoxygenated form, and is brown in colour (Mancini and Hunt, 2005). This suggests that maintaining heme iron in its reduced state is critical for colour stability of meat products.

Oxidative species include hydroxyl radicals, peroxyl radicals, hydrogen peroxide, superoxide anions and nitric oxide generated in oxidative processes. These can result in heme protein oxidations (Burton and Traber, 1990). The reducing-oxidative property in the muscle could thus to be responsible for the oxidative processes of myoglobin. Maintaining a reducing state in tissues could be achieved by supplementation of exogenous antioxidants into diets for feedlots to increase their concentrations in muscles. For example, vitamin E as an antioxidant is currently recommended to feedlot operators to include in diets of cattle prior to slaughter to stabilise meat colour by preventing post-mortem oxidation (Faustman et al., 1998). The level required is approximately an extra 8 mg vitamin E per kg meat or about 8 times higher than non-supplemented animals.

Mammals have strong endogenous antioxidant systems to scavenge oxidative species and hydrogen peroxide. Amongst these systems, the glutathione (GSH) antioxidant system predominates and can be found in high concentrations compared to most of other antioxidants in tissues. Because of its high reducing potential (Jones, 2006), GSH can regenerate other antioxidants, such as vitamins E and C in biological tissue (Meister and Anderson, 1983; Li et al., 2001). Natural between-animal variation in GSH concentration in blood is high. In our research using Merino sheep, blood GSH levels varied 7-fold between individuals (Liu et al.,

2005). Recent research in New Zealand with Romney sheep gave heritability estimates for blood GSH concentration of 0.34 (±14) (Hohenboken et al., 2004). It has been shown that there are two types of gene variations that result in low GSH concentration. One is abnormally low activity of γ-glutamyl cysteine synthetase, the first enzyme responsible for GSH biosynthesis (Meister and Anderson 1983). The concentration trait appeared to be controlled by a pair of autosomal alleles which are dominant for high concentration (Tucker et al., 1976). Another type of naturally occurring variation is a lesion in the erythrocyte amino acid transport system. This defect results in an impaired entry of cysteine into the erythrocytes (Tucker et al. 1976; Young et al., 1976). Because of its dominant role in the endogenous antioxidant system, genetic variation in GSH concentration could be associated with the redox state in tissues. This may determine reducing capacity post-mortem.

We hypothesised that high GSH concentration in tissue reduces post-mortem oxidation of myoglobin and unsaturated fatty acids and therefore, is associated with colour stability of meat. Selenium (Se) is the co-factor for GSH peroxidase, an enzyme that catalyses reduction of peroxides with GSH. Dietary supplementation of Se can enhance GSH peroxidase, and could affect the GSH redox state. To test the hypothesis we used lambs selected for high or low whole blood GSH concentration and then supplemented with Se for 8 weeks. Sheep were slaughtered and muscle samples taken to measure the colour and colour stability, and concentrations of fatty acids and GSH in the muscles. The effects of blood GSH and Se supplementation on colour stability and fatty acids are reported.

2. Materials and Methods

2.1 Animals, managements and dietary treatment

The use of the animals and the procedure of the experimental protocol were approved by the CSIRO Floreat Animal Ethics Committee.

A base flock of 440 12-month old Merino wether lambs, at the CSIRO Yalanbee Experimental Station were initially sampled to measure blood GSH concentration. The lambs were ranked according to their GSH concentrations and the top 24, ie, high GSH group, and the bottom 24 lambs, ie, low GSH group, were selected. The selected sheep were transported into the animal house and housed in individual pens. Upon arrival they were drenched with an anthelmintic to remove gastrointestinal parasites (Virbac^{TM}, Abamectin, 0.2 mg/kg liveweight, and Scanda^{TM}, Oxfendazole, 4.5 mg/kg liveweight and Levimasole, 6.9 mg/kg liveweight). The lambs were fed a hay/lupin/barley pelleted diet (approximately 1.1 kg per head per day) for 3 weeks to allow for acclimatization. Each GSH group was then split into one of two sub-groups, and one subgroup was fed a diet containing 2.5 mg selenium/kg as sodium selenite for 8 weeks (supplementation period). This formed a 2 (high or low blood GSH) × 2 (with or without Se supplementation) factorial design. The lambs were fed once per day in the morning, and feed supply was controlled to allow the lambs to gain weight at about 100 g/d on average. The feed intake was recorded daily. Fresh water was freely accessed by sheep via individual drinkers.

2.2 Sampling procedures and meat colour measurement

Blood samples were taken at the end of the acclimatisation period, and then at fortnightly intervals during the supplementation period. The blood samples were processed immediately for determination of whole blood GSH concentration.

The lambs were weighed every fortnight. By the end of the experiment, the lambs were shorn, and the fleece was weighed, and sampled for measuring clean fleece weight. The sheep were then slaughtered at the end of the supplementation period. The animals were killed by the 'captive bolt' technique. The internal organs (heart, lungs, liver and kidneys) were separated and weighed. Approximately 10 g muscle samples were taken immediately from *m. longissimus dorsi* (LD), *m. semimembranosus* (SM) and *m. semitendinosus* (ST), and also from the lungs and heart, cut into pieces, frozen in liquid N, and stored at -80°C. The samples were used to analysis of the concentrations of GSH, Se and fatty acids. The hot carcass was then weighed, and hung in a cool room for 24 hours at 4°C.

The cold carcass was weighed the next day. Triplicate samples (about 3×3×1 cm dimension) of SM, ST and LD were taken, placed on a black foam tray, wrapped with plastic film, and heat sealed. The sample was stored in a display cabinet under fluorescence light (100-1500 Lux) at 4°C for 5 days. Colour and reflectance readings from wave length of 400nm up to 700nm at 10 nm intervals for each muscle sample were determined using a Hunter Lab Mini Scan (tm) XE Plus (Model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA., USA) at 6 h after the sampling, and then at 12 hourly intervals until 96 h post-sampling. The instrument was calibrated on a white tile and black glass as manufacturer's specifications. Colour parameters, ie, L*, a*, b* values, and the reflectance at 580nm (w580) and 630nm (w630) were reported in this paper. The wavelength 580nm is the absorption peak for oxymyoglobin (Fe^{2+}) while 630nm is the absorption peak for metmyoglobin (Fe^{3+}) (Hunt, 1980), so the ratio of w630 to w580 provides valuable information on the amount of surface oxymyoglobin relative to metmyoglobin in meat (Stewart et al., 1965).

2.3 Chemical analysis

2.3.1 Blood and muscle GSH

GSH concentration in fresh blood sample was determined using the method as originally described by Sedlak and Lindsay (1968). Briefly, about 0.5 g blood sample was transferred into a tube pre-filled with 2 mL of 0.04M EDTA solution, and the sample weight was recorded. The mixture was thoroughly vortexed, and 2.5 mL 10% trichloroacetic acid (TCA) added and vortexed again. The solution was centrifuged for 15 min at 2800×g to precipitate the protein. Approximately 300 µl of supernatant was then transferred into a microcentrifuge tube, and centrifuged for 5 min at 18,000×g. The supernatant was used to determine GSH concentration using 5,5'-bisdithio(2-nitroenzoic acid) (DTNB) as the reagent in a Cobas Mira Diagnosica System (F. Hoffmann-La Roche, Switzerland). Fresh GSH standard solutions were prepared for each batch of the sample by dissolving reduced GSH standard (Sigma Aldrich, Product No. G4251. Australia) in a solution that contained 0.04M EDTA and 5% TCA.

To determine GSH concentration in the LD and SM muscles, 0.3 g muscle was homogenized using a Ystral homogenizer (Model X-10/25, Ystral gmbh, Ballrechten-Dottingen, Germany) in 3×1 mL 0.02M EDTA, and 2.5 mL ice cooled 10% TCA was immediately added. The sample was vortexed thoroughly and centrifuged for 15 min at 4000×g at 4°C for deproteinisation. The supernatant was processed as described above for blood samples.

2.3.2 Fatty acid analysis

Fatty acid concentrations in the LD and SM muscles were determined as their methyl ester derivatives (FAME) in a Perkin-Elmer gas chromatographer (GC, Perkin-Elmer, Melbourne, Australia) using a method as described by O'Fallon et al. (2007).

About a 0.5 g muscle sample was ground to a fine powder in a mortar and pestle in liquid nitrogen. The sample was transferred into a hydrolysis tube, and the sample weight was recorded. Then 0.1 mL of internal standard (tridecanoic acid, 10 mg/mL), 0.7 mL 10M KOH and 5.3 mL methanol were added, and incubated with rigorous hand-shaking every 20 min for 1 h at 55°C for hydrolysation of lipids. Then, 0.58 mL 24N H_2SO_4 was added and incubated with rigorous hand-shaking every 20 min for 1.5 h at 55°C for methylation of free fatty acids. The ester derivatives of fatty acids were then extracted into 1 mL of hexane, and used in GC analysis.

The GC system was fitted with a split injector, a HP-Innowax column (Part No. 19091N-236, 60m length × 0.25µm ID, 0.50µm thick film. J&W Scientific, USA) and an FID detector. The carrier gas was helium. The temperature of the injector and the detector was set at 210°C and 250°C respectively. The GC temperature program was initially at 120°C for 1 min, then increased to 250°C at a rate of 20 °C/min, and held for 20 min. Peak areas were integrated, and used to calculate fatty acid concentrations against the fatty acid ester standards (Supelco, Product No. 18919. Sigma Product No. D5679. Fluka Product No. 43959). Fatty acid concentration in the muscles was expressed as mg per 100 g wet tissue.

2.3.3 Se analysis

Se concentration in the LD muscle, lungs and heart were determined using the fluorometric method as described originally by Watkinson (1966).

About 0.3 g of the muscle or tissue sample was weighed, and placed into a digestion tube. Then 3.5 mL of HNO_3-$HClO_4$ (2.5 : 1) mixture was added, and left overnight. Next day the sample was digested by heating the tube to 150°C in a temperature controlled heating block, and held at this temperature until the volume was reduced to less than 3.5 mL. The

temperature was increased to 190°C, and held until the solution became clear. The tube was then cooled at roomed temperature and 0.2 mL HCl (1 : 1) was added. The temperature of the heating block was reduced to 160°C, and the tube was heated again for 20 min to reduce selenate to selenite. The temperature of the heating block was then reduced to 130°C. Two blanks and three standards (Sodium selenite, Sigma Aldrich, Australia) were processed in the same way. After cooling at room temperature, 2 mL of 0.04M EDTA in NH_4OH solution (1 : 1) containing 0.001% bromocresol purple was added to the tube, and then reheated at 130°C until the ammonia was boiled off, and the solution became distinctly yellow. The tube was cooled at room temperature, and 0.01M HCl was added to make the solution up to 10 mL (marked on the tube). An aliquot of 2 mL was then mixed with 0.32 mL of 0.05% (w/v) 2,3-diaminonaphthalene in 0.1M HCl and vortexed to form fluorescent derivative. The derivative was extracted into 2 mL cyclohaxane and the fluoroscence was then meausured in a Perkin-Elmer Fluorescence spectrophotometer (Model 1000, Perkin—Elmer, Beaconsfield, Buckinghamshire, UK) with Excitation at 376nm and Emission at 520nm. Se concentration was calculated and expressed as μg/kg wet tissue.

2.4 Statistical analysis

To detect the effects of the treatments (GSH×Se) on the parameters of meat colour stability, ie, the time-related changes in meat colour, the meat colour data from 6 h until 96 h was analysed using a residual maximum likelihood model (REML) for repeated measures. In this model, GSH, Se and time (Hour) were included as factors, plus all their interactions, and individual animals were treated as random variables for the repeat. We noticed that the ratio of w630 to w580 and b* values showed exponentially decay patterns with time, and there was heterogeneity across time in their variations. Therefore, a logarithm transformation (Ln) of the ratio (Ln (Ratio)) or b* (Ln b) was performed before the analysis. The results are reported

in this paper. In this study we focused on the colour stability, so the time-related trends for the ratio of w630 to w580 are presented in Fig 1. The statistical significances (P values), not the actual values of the means, for all the colour parameters are reported in Table 2. If the treatment (GSE, Se and GSE×Se) effects were significant, their values averaged over the period of 96 h, not the values for each time points, are reported.

As for single measures, ie, feed intake, live weights, fleece weight, organ weights, GSH, fatty acids and Se concentration in the muscles and tissues, the effects of the treatments were examined in a model of two-way analysis of variance (ANOVA), where GSH and Se were factors, and their interaction was included in the model. Correlation analysis was performed for GSH concentrations between whole blood and the muscle.

For all the data, least square means and standard error of the means are reported. All the analyses were performed using computing software GenStat (Version 11.1, VSN International Ltd, UK).

3. Results

3.1 Feeding results, carcass and organs

Live weights and weight gains of the sheep, feed intake, the weights of carcass and internal organs are presented in Table 1. The average feed intake over the 8 weeks was controlled to be same between the 4 groups. The carcass weight and dressing rate were significantly higher in high GSH group compared with low GSH group ($P < 0.05$), whereas there were no significant differences ($P > 0.05$) between GSH groups in live weights, weight gain, wool growth, and the weights of the internal organs. Se supplementation had no significant effects ($P > 0.05$) on all the measures except for the weight of the lungs ($P < 0.05$).

There was no significant interaction ($P > 0.05$) of GSH and Se supplementation on any measures.

3.2 Colour and colour stability of the muscles

Colour stability, measured as time-related changes in the ratio of w630 to w580, and colours (L*, a*, b*) of LD, SM, and ST muscles are shown in Figs 1, 2, 3 and 4. The statistical significance of the effects of GSH and Se supplementation, interactions of GSH×Se, and GSH×Se×Hour, on all the colour parameters for each of the muscles are presented in Table 2. Time had significant influences on all the parameters ($P < 0.001$), and its interaction with GSE×Se was not significant ($P > 0.05$) except for Ln(Ratio) for *m. semitendinosus* ($P < 0.05$).

There were significant influences of GSH on Ln(Ratio) and b* ($P < 0.05$) for *m. longissimus dorsi*. The value of Ln(Ratio) and b* were significantly higher ($P < 0.05$) in high GSH group than low GSH group, and the values of b* were 19.95 and 19.46 (SEM 0.298) respectively. Selenium supplementation had a significant influence on w580 ($P < 0.05$), and the values were 5.796 and 5.312 (SEM 0.2964) with and without Se supplementation. There were no significant influences of either GSH, or Se supplementation on the other parameters. There was no significant influence of GSH×Se interactions on any of the parameters ($P > 0.05$).

There were significant interactions between GSH and Se on w630/w580 ratio, Ln(Ratio), w580, and a* ($P < 0.05$) for *m. semimembranosus*, but not on w630, L* and b*. As shown in Fig 1, w630/w580 ratio was consistently higher for high GSH group than low GSH group without Se supplementation, and the difference diminished with Se supplementation. Selenium supplementation had no influence on w630/w580 ratio for high GSH group, but resulted in higher ratios for low GSH group. The interaction between GSH×Se

supplementation on w580 and a* were also significant ($P < 0.05$). The w580 values were 5.41 and 5.26 for high GSH with or without Se supplementation over the 96 h, and 15.77 and 15.22 for low GSH group respectively (SEM 0.424). The values for a* were 15.62 and 15.85 for high GSH with or without Se supplementation, and 15.77 and 15.22 for low GSH group respectively (SEM 0.605).

There were significant interactions between GSH and Se supplementation on w630/580 ratio, w630 and a* ($P < 0.05$) for *m. semitendinosus*. The w630/w580 ratio appeared to be consistently higher for the high GSH group than low GSH group without Se supplementation, whereas with Se supplementation the ratio turned to be higher for low GSH group after 36 h. Within the high GSH group, the ratio was lower without Se supplementation, whereas Se supplementation resulted in higher w630/w580 ratios in low GSH group after 36 h. The high GSH group had a significant low value of L* (43.82 vs 44.27, SEM 0.545. $P < 0.05$), but had no significant influence on the other parameters. Selenium supplementation had a significant influence on L* (44.30 vs 43.90, SEM 0.549. $P < 0.05$) and b* (20.29 vs 20.10, SEM 0.201. $P < 0.05$) values, but not on a* value and w580.

3.3 Fatty acids concentrations in the muscles

Fatty acid concentrations in *m. longissimus dorsi* and *m. semimembranosus* are shown in Table 3. The concentration of polyunsaturated fatty acids (PUFA) and n-3 fatty acids were significantly higher in the high GSH group than in the low GSH group ($P < 0.05$) in both the muscles. The ratio of omega-6 fatty acid to omega-3 fatty acid (n-6 : n-3) was significantly lower and docosahexaenoic acid (DHA) concentration significantly higher in *m. semimembranosus* for the high GSH group ($P < 0.05$), but in *m. longissimus dorsi* the differences were not significant ($P > 0.05$). The DHA concentration in muscle *m.*

longissimus dorsi was lower in Se supplementation group compared without the supplementation ($P < 0.05$). Otherwise, there were no significant differences ($P > 0.05$) in saturated fatty acids, monounsaturated fatty acids, conjugate fatty acids, n-6, eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA) between GSH groups, and with or without Se supplementation. There were no significant influences of the interaction between GSH and Se supplementation on any fatty acid concentration.

3.4 Se concentration

Se concentration (µg/kg wet tissue), as shown in Table 4, in *m. longissimus dorsi* was significantly higher in sheep supplemented with Se compared with no supplementation (202 vs 65 µg/kg wet tissue, SEM 15.4, $P < 0.001$). The concentration was also significantly higher in high GSH sheep than low GSH sheep (152 vs 114 µg/kg, SEM 15.4, $P < 0.05$). There was no significant interaction ($P > 0.05$) on Se concentration in the muscle between GSH and Se supplementation.

There was a significant interaction between GSH and Se supplementation on Se concentration in the lungs. The mean values were 548 and 352 µg/kg for the high GSH groups, and 488 and 387 µg/kg for the low GSH groups, with or without Se supplementation (SEM 23.2 µg/kg, $P < 0.05$).

Se concentration in the heart ranged from 140 to 148 µg/kg (SEM 22.1) between the four groups. There were no significant differences between high and low GSH levels, with or without Se supplementation, and their interaction ($P > 0.05$).

3.5 GSH concentration in whole blood and muscles (Table 4)

During the acclimatisation period when all the lambs were fed the same diet. The concentrations of blood GSH at the end of the acclimatisation period were 0.974 and 0.394 mM (SEM 0.025, $P < 0.001$) for the high and low GSH groups respectively. When all the repeated measures of blood GSH were analysed, there was no significant ($P > 0.05$) time on GSH concentration. At the end of the supplementation period the final concentrations of blood GSH were 0.866 and 0.471 mM, remaining significantly different (SEM 0.036, $P < 0.001$) respectively. Se supplementation did not have a significant effect ($P > 0.05$) on GSH concentration during the supplementation period. There were no significant interactions between GSH, Se supplementation and time on GSH concentration ($P > 0.05$). As shown in Fig 5(a), there was a significant correlation between the initial and the final concentration in all the lambs ($r = 0.915$, $P < 0.001$).

The concentrations of GSH in muscle *m. longissimus dorsi* were 0.519 and 0.385 mmol/kg wet tissue (SEM 0.056, $P < 0.01$) for high and low GSH groups, and 0.488 and 0.416 mmol/kg (SEM 0.056, $P > 0.05$) with or without Se supplementation. There was no significant interaction between GSH and Se supplementation ($P > 0.05$).

The concentrations of GSH in *m. semimembranosus* were 0.496 and 0.446 mmol/kg wet tissue (SEM 0.070, $P > 0.05$) for the high and low GSH groups, and 0.460 and 0.482 mmol/kg (SEM 0.070, $P > 0.05$) with or without Se supplementation. There was no significant interaction between GSH and Se supplementation ($P > 0.05$).

The relationships between the final blood GSH and GSH concentrations in muscles of *m. longissimus dorsi* or *m. semimembranosus* are shown in Fig 5(b) and Fig 5(c). For muscle *m. longissimus*, the correlation including all the animals was significant ($r = 583$, $P < 0.001$), and

the regression relationship differed between high and low GSH groups with the slope values of 0.848 (SE 0.193) and 0.150 (SE 0.156) respectively ($P < 0.05$). The correlation including all the animals was significant for *m. semimembranosus* ($r = 0.345$, $P < 0.05$) however the regression relationship was not significantly different between high and low GSH groups ($P > 0.05$).

4. Discussion

4.1 Interactions of GSH and Se supplementation on colour and colour stability

Our results indicate that blood GSH concentration and Se supplementation had an interaction on colour stability, shown as time-course of the w630/w580 ratio, in meat from Merino lambs. Without Se supplementation, the high ratio of w630 to w580 was associated with high GSH levels in blood, though the differences in the ratios between high and low GSH groups varied between the three muscles: the greatest difference was in *m. longissimus dorsi*, then *m. semimembranosus*, with only a small difference in *m. semitendinosus*. The difference was reduced by Se supplementation and the effects of the supplementation depended on blood GSH level: resulting in lower w630/w580 ratios for high GSH group, whereas higher ratios for low GSH group. The results indicate there were complex mechanisms for the apparent association between blood GSH level and meat colour stability. To our knowledge, this is the first time that the relationship between phenotypic variation in blood GSH and meat colour stability has been examined.

Various factors can alter the colour stability of meat, however the change in the redox state of the iron atoms in myoglobin is dominant (Gray et al., 1996; Mancini and Hunt, 2005). Discolouration results from the oxidation of ferrous myoglobin (Fe^{2+}) to ferric myoglobin or metmyoglobin (Fe^{3+}). Metmyoglobin is located under the surface of meat, and its formation

depends on the meat's reducing activity, oxygen pressure, pH and temperature (Mancini and Hunt, 2005). We hypothesised that preventing ferrous myoglobin from oxidation or promoting the reduction of metmyoglobin is a critical factor for maintaining colour stability.

It is well known that ferrous ion can be oxidized to ferric iron by H_2O_2. This is the Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\bullet} \qquad (1)$$

This reaction occurs mostly in anaerobic conditions (Qian and Buettner, 1999). GSH is a highly powerful reducing molecule (donating an electron), and also has metal chelation capacity (Jocelyn, 1972). It can reduce H_2O_2 and other peroxides through a reaction (Meister, 1989):

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

$$2GSH + ROOH \rightarrow GSSG + ROH + H_2O \qquad (2)$$

where GSSG is oxidized glutathione. These two reactions form the chemical foundation for our hypothesis that a high level of GSH in tissues is associated with a high reducing capacity. This could reduce the formation of H_2O_2, and then oxidation of the ferrous iron in turn maintains the colour stability of the meat. Schafer and Buettner (2001) used the Nernst equation to calculate the redox potential for each redox couple, and concluded that the GSSG/2GSH couple is the most abundant redox couple in a cell and, therefore, the GSH redox state can serve as an indicator of redox environment in cells and in plasma (Jones et al., 2000; Jones, 2002). The concentration of total glutathione (GSH + GSSG) reduced continuously during the storage of beef (Renerre et al., 1996), and apparently correlates with the change of meat colour. Thus, GSH concentration may be an indicator that could associate with the colour stability of meat. Even so, further investigation is required on associations between the GSH redox state in muscles and the iron redox state in myoglobin postmortem.

The beneficial effect of high GSH on colour stability of meat has been reported in various studies. Kortz (1973) studied colour stability in fresh pork meat and recorded a high correlation between water soluble –SH groups and meat colour stability. The study also demonstrated that GSH reduced a portion of metmyoglobin to oxymyoglobin *in vitro* and increased colour saturation with the addition of reduced GSH to pork samples (Kortz, 1973). Tang et al. (2003) investigated the effect of GSH on oxymyoglobin oxidation in bovine skeletal muscle cytosol and found that there was less metmyoglobin formation in the presence of GSH comparative to the controls. This response was concentration dependent with 0.8mM of GSH decreasing metmyoglobin formation by 72% at pH 5.6, after 48h storage at 4°C (Tang et al., 2003).

Selenium specifically affects the GSH redox state. The reaction of GSH with peroxides is catalysed by GSH peroxidase (GSHpx), an enzyme characterised by Se as part of the active site. Oxidized GSH formed in this reaction is reduced to GSH and the reaction is catalysed by GSH reductase (Meister and Anderson, 1983). It would be expected that changes in activities of these two enzymes would affect the ratio of GSH to GSSG, and therefore the GSH redox state. The activity of GSHpx is closely associated with Se concentration. Dietary supplementation of Se can substantially increase Se concentrations in blood and tissues (Costa et al., 1993) and GSHpx activity. We anticipated that the increased GSHpx activity in muscles would accelerate the reaction of GSH with peroxides, which would reduce the peroxide concentration and then prevent oxidation of ferrous irons. Interestingly however, the results were not as expected. Depending on the blood GSH level, the dietary supplementation of Se reduced the ratio of w630/w580 in the three types of muscles, indicating that the supplementation reduced myoglobin to metmyoglobin ratio. It is probable that the enhanced GSHpx activity from Se supplementation increased the oxidation of GSH to GSSG, while the

reduction of GSSG to GSH probably did not match the oxidation rate either because hydrogen donor, NADPH was rapidly exhausted post-mortem (Bekhit and Faustman, 2005), or GSH reductase activity did not increase correspondingly. If so, the GSH to GSSG ratio would have been reduced, and the redox state became less negative which does not favour the reduction of metmyoglobin to myoglobin (Mancini and Hunt 2005). The result suggests that manipulating a single enzyme activity that is involved in the complex redox system may alter the global redox state. A change in the global redox state could affect many reactions. At this stage, it is still not clear where the focus should be in altering the global redox state in muscles or manipulating a single redox component. This warrants a further investigation.

4.2 Effects of GSH and Se on polyunsaturated fatty acids in meat

We observed that a higher blood GSH level was significantly associated with higher n-3 fatty acids and PUFA in the muscles of the lambs, increasing by 15-29%, whereas Se supplementation did not affect n-3 fatty acid concentrations in the muscles. The n-3 fatty acid concentration in tissues reflects the balance of the absorption of the fatty acids from the digestive tract, a conversion of long chain (C18, n-3) to very long chain (C20, C22, C24) n-3 fatty acids, and their breakdown in the tissue. The breakdown includes the fatty acids lost through both processes of the β-oxidation and peroxidation. It is unlikely that blood GSH could affect the intake of the fatty acids. We are not aware of any literature that shows blood GSH could influence the β-oxidation, and the activities of Δ6- and Δ5-desaturases. However, it has been well known that PUFA's, including both n-3 and n-6, are more vulnerable to peroxidation (Niki et al., 2005), usually termed lipid peroxidation. Reactive oxygen species or free radicals that have a one-electron reduction potential (E°′) greater than +600 mV for PUFA, including $HO^{\bullet}$, $ROO^{\bullet}$, $HOO^{\bullet}$, $NO_2^{\bullet}$ and $O_2^{\bullet}$ (Buettner, 1993), but not $NO^{\bullet}$

(Venkataraman et al., 2004), can cause the initiation of lipid peroxidation in tissues. It is expected that reductants that can reduce these radicals can prevent lipid peroxidation.

It has been demonstrated that dietary supplementation of antioxidants can reduce lipid peroxidation. Vitamin E is widely used for both monogastric and ruminant animals, and vitamin C is also used in conjunction with vitamin E in monogastric animals. Lambs supplemented with 1 g α-tocopherol per kg feed for 9 weeks significantly increased α-tocopherol concentration from 0.8 to 5.3 mg/kg in *m. longissimus*, and improved both lipid and colour stability (Guidera et al., 1997). For grain-finishing cattle this high level of vitamin E concentration in the muscle could be achieved by a supplementation of vitamin E, while cattle finishing on green pasture already have high concentrations of vitamin E (Lanari et al., 2004). Eikelenboom et al. (2000) pointed out that although supplementing vitamin E increases concentration in muscle and effectively reduces lipid peroxidation, the supplementation has limited effect on improving colour stability.

In the endogenous antioxidant system, GSH is a primary antioxidant in tissues because it is more highly concentrated than other endogenous antioxidant compounds, and its greater redox potential (Buettner, 1993) can reduce the oxidants that cause lipid peroxidation. This is a probable explanation of our finding that the high blood GSH, and higher GSH in the muscles, reduced peroxidation of PUFA, and thus resulted in a relatively higher concentration of PUFA. This was also supported by the lack of significant differences in the monounsaturated fatty acid (MUFA) concentration between GSH groups, as the peroxidation does not occur in MUFA. Reduced lipid peroxidation was also correlated with a great colour stability of the muscle, and this has been reviewed by Morrissey et al. (1998) and Bekhit et al. (2003). Other research supports the effect of GSH on lipid peroxidation eg, more oxidative

types of muscle had a relatively high GSH (Renerre et al., 1996), or red muscles had high GSH than white muscle in rats (Purucker et al., 1991). In the rat model the GSSG/GSH ratio showed a marked increase when accompanying an increase in lipid peroxidation (Purucker *et al*., 1991), suggesting that depletion of this pair of cellular antioxidant is the first event and lipid peroxidation is secondary.

We did not observe significant effect of the 2.5 ppm Se supplementation on fatty acid concentrations in the muscles, except for relatively lower DHA in *m. longissimus dorsi* of the lambs. In the report by Yu et al. (2008), a dietary supplementation of 2 ppm Se in crossbred lambs did not affect the concentrations of total saturated fatty acids and MUFA in either the liver and plasma, whereas it had significant effects on PUFA concentration in the liver. The effect was oil-source related i.e. increased in sheep fed a sunflower oil diet but no change in sheep fed a linseed oil diet (Yu et al., 2008). However, Haug et al. (2007) found that a higher dietary Se level (0.84 ppm), when compared to a lower level (0.50 ppm), was associated with higher concentrations of EPA, DPA and DHA, but not α-linolenic acid (C18, 3n-3) in muscles of broilers. Schafer et al. (2004) observed reduced n-3 fatty acid concentrations in the liver of rats fed a Se deficient diet. Haug et al. (2007) noted that the supplementation-resulted in a higher Se^{2-} to S^{2-} ratio that enhanced stability of Fe^{2+}. This is thought to link to the reduction of the rate of reactive oxygen species production and lipid peroxidation in tissues. The difference in the above results from to other reports could be related to the different levels of Se and lipid in the diets. 2-2.5 ppm Se in the diet is a high concentration for animals. As we have discussed already, Se supplementation not only affects GSHpx activity, but also may alter the GSH redox state through changes to the ratio of GSSG to GSH, and presumably the global redox state in tissues. These effects might compromise each other.

Therefore, the mechanism of dietary Se supplement on lipid peroxidation needs further investigation.

4.3 Se concentration in tissues in response to Se supplementation

Recent studies have shown that there is an inverse relationship between Se status and cancer (Clark et al., 1996), and cardiovascular diseases (Rayman, 2000). For cancer patients, it is suggested that supplementation with Se alters GSHpx activity, and enhances the immune system. Selenium induces the apoptosis that removes mutated or damaged cells, and affects the production of testosterone (Gronberg, 2003). The role of Se in preventing cardiovascular diseases is due to its ability to prevent lipid peroxidation and hence the formation of atherosclerotic plaques (Rayman, 2000). In a 27 year follow-up study of Swedish men, serum Se was inversely related to F_2-isoprostanes, a pre-eminent indicator of lipid peroxidation and oxidative stress. The conclusion was that high concentrations of serum Se predict reduced levels of oxidative stress in a male population (Helmersson et al., 2005). Plasma or serum Se concentration is good biomarker of Se status in the body, which is mainly determined by Se contents in foods and in turn by Se in the soil. Therefore, Se intake from foods in the regions where there is low Se concentration in soils, eg, in the UK (Mishra et al., 2007) and New Zealand (Russell et al., 1999) has drawn a strong interest, and increasing Se content in foods is proposed in preventive health programs. In Australia, deficiency or sub-clinic deficiency of Se in farm animals (eg, while muscle disease) is common (Tinggi, 2003), indicating a low Se content in Australian soils.

Supplementation of Se in diets for domestic animals can be an effective approach to increasing Se content in meat, e.g. in lambs (Zachara et al., 1993), in pigs (Mahan et al., 1999) and in beef cattle (Hintze et al., 2002). In our research, the supplementation of 2.5 ppm Se for

8 weeks increased Se concentration in the muscle three fold, and by 26-56% in the lungs depending on the blood GSH level, but there was no change in the heart. The results suggest that muscle seemed to be a tissue with a substantially strong response to dietary Se levels and, therefore, can be a significant source of food Se when animals are fed high Se diets. We can also hypothesise that the muscle may be more vulnerable to Se deficiency, whereas the heart may be tolerant to variations in dietary Se intakes. These tissue-specific characteristics in Se status in response to Se intake should be considered when applying Se supplementation for preventive health. We also observed that the response of Se concentration in the muscle and lungs to the Se supplementation varied with the blood GSH level, and that a stronger response was obtained in sheep with higher GSH. We would conclude that this was related to high GSH induced high GSHpx activity and in turn a high Se content.

5. Conclusions

In conclusion, we used the GSH system as a model to investigate the relationship between the endogenous redox system and meat colour and colour stability and fatty acid compositions as a better understanding of this relationship could identify heritable biochemical characteristics that would enhance the genetic approach to improve meat colour stability. Interactions between various redox couples and related redox enzymes make the relationship very complex and thus the research focus needs to be narrowed to the key indices that closely relate to the myoglobulin redox state.

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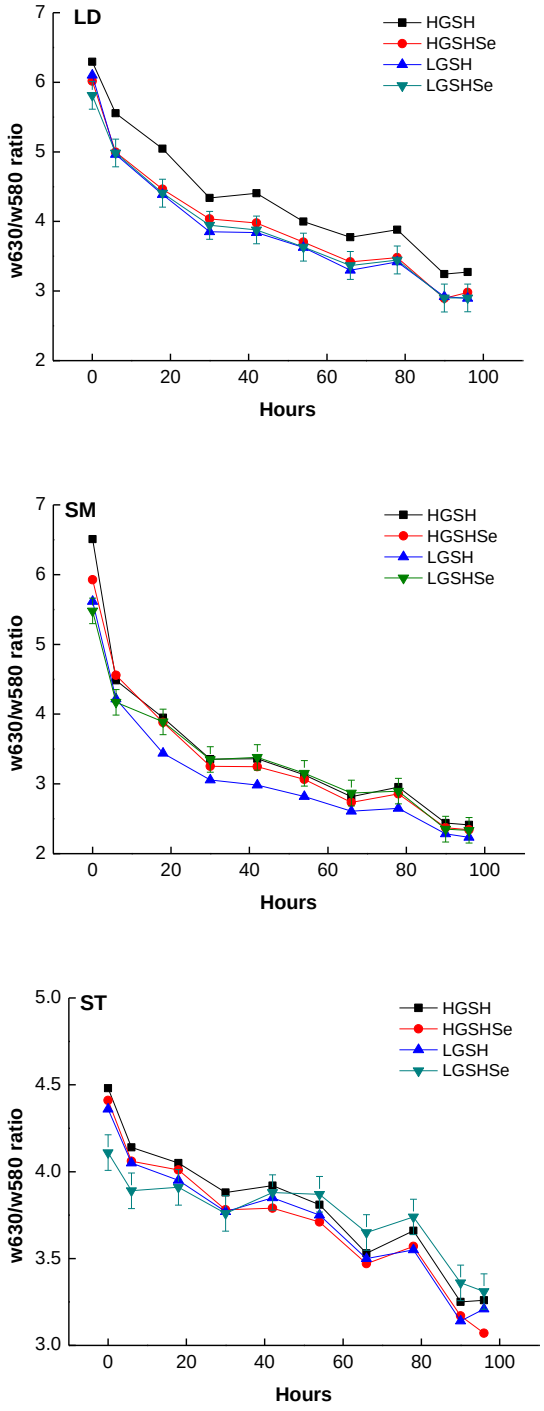

Fig. 1 Colour stability, shown as the ratio of reflectance reading at 630nm (w630) to the reading at 580nm (w580), of *m. longissimus dorsi* (LD), *m. semimembranosus* (SM), and *m. semitendinosus* (ST) of 12 months old Merino lambs selected for high blood GSH (HGSH) or low GSH (LGSH) concentration across without (0ppm) or with dietary supplementation of 2.5ppm Se (HGSHSe, LGSHSe). Each group had 12 animals. The error bar is standard error of the means.

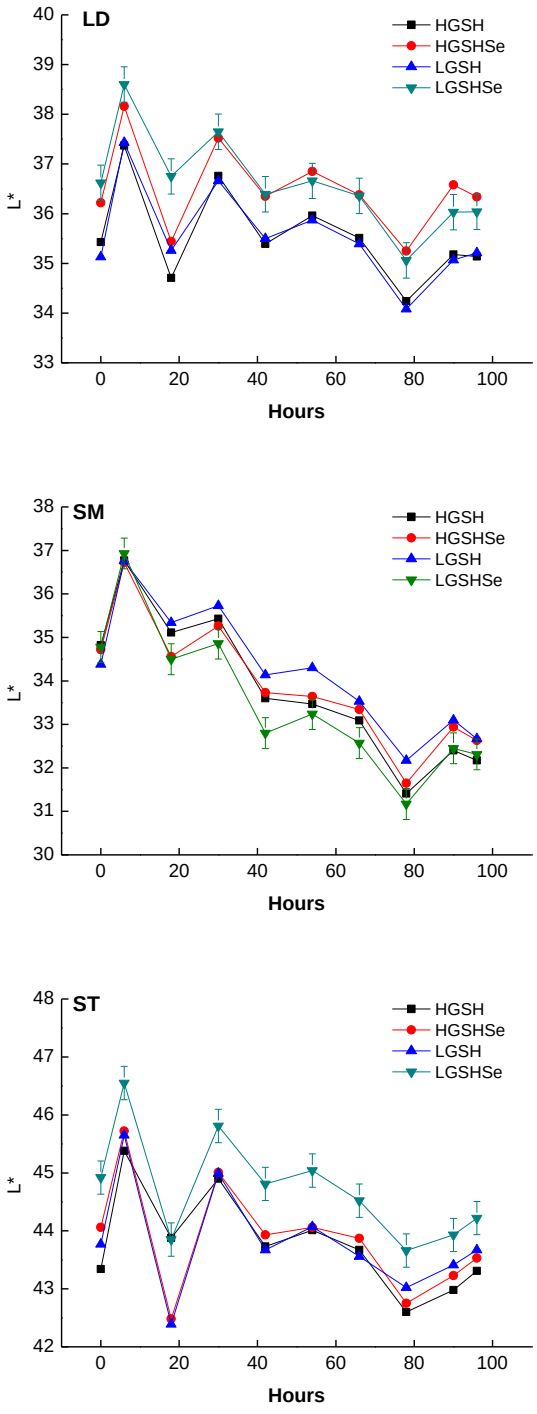

Fig. 2 L* values of *m. longissimus dorsi* (LD), *m. semimembranosus* (SM), *and m. semitendinosus* (ST) of 12 months old Merino lambs selected for high blood GSH (HGSH) or low GSH (LGSH) concentration across without (0ppm) or with dietary supplementation of 2.5ppm Se (HGSHSe, LGSHSe). Each group had 12 animals. The error bar is standard error of the means.

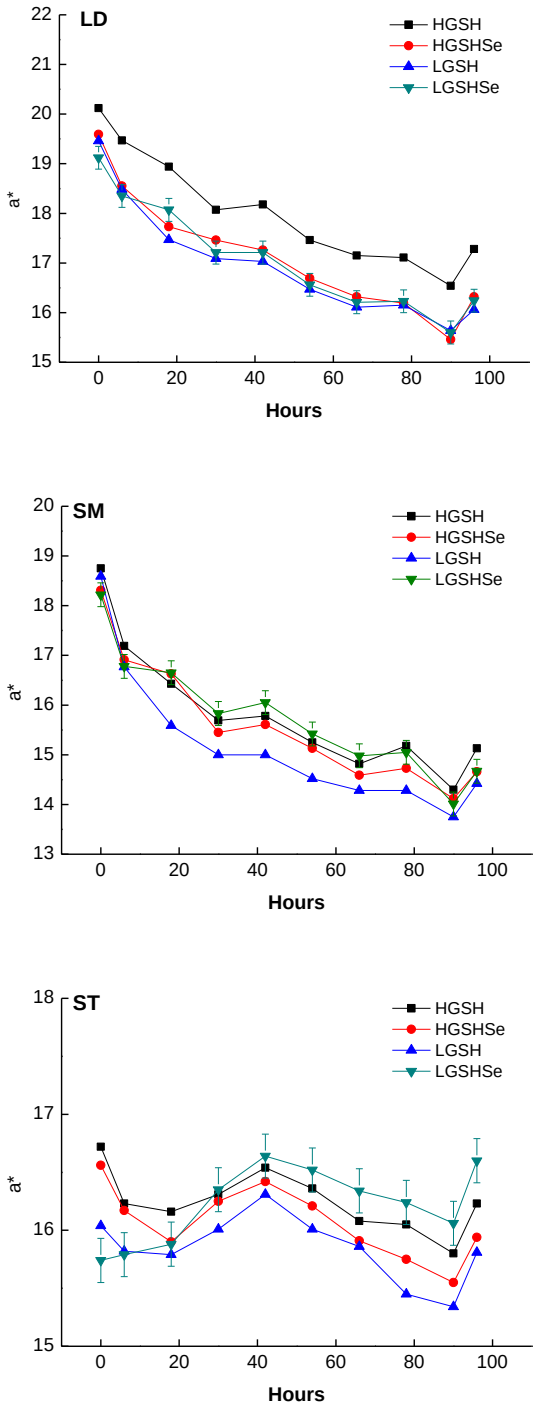

Fig. 3 a* values of *m. longissimus dorsi* (LD), *m. semimembranosus* (SM), *and m. semitendinosus* (ST) of 12 months old Merino lambs selected for high blood GSH (HGSH) or low GSH (LGSH) concentration across without (0ppm) or with dietary supplementation of 2.5ppm Se (HGSHSe, LGSHSe). Each group had 12 animals. The error bar is standard error of the means.

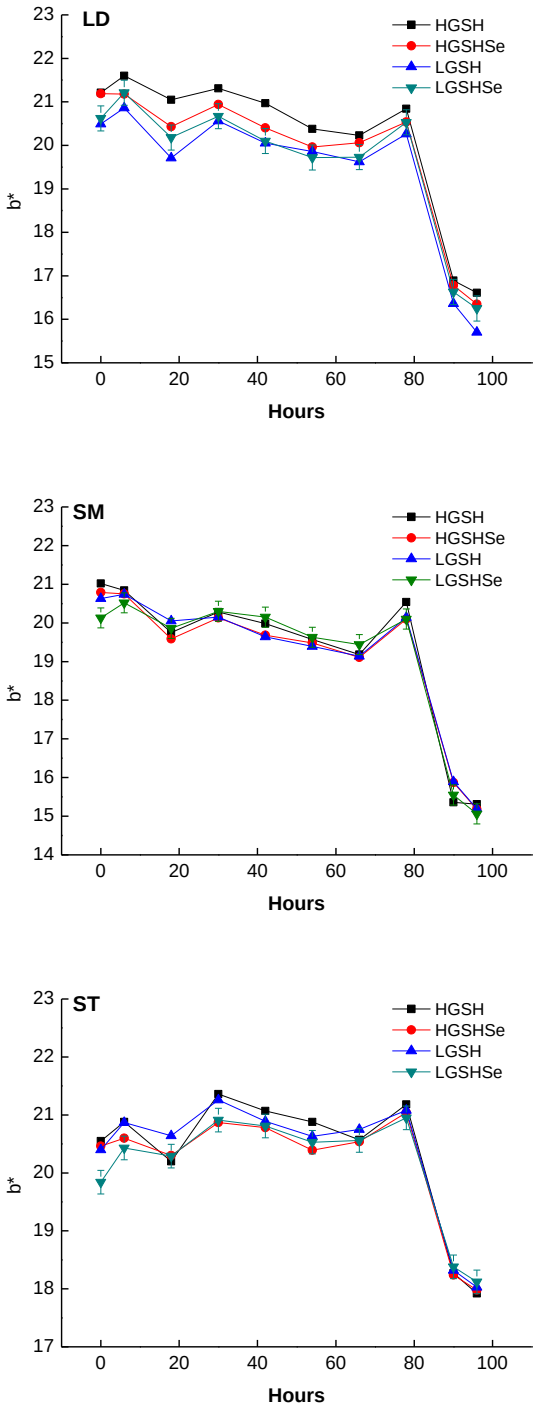

Fig. 4 b* values of *m. longissimus dorsi* (LD), *m. semimembranosus* (SM), *and m. semitendinosus* (ST) of 12 months old Merino lambs selected for high blood GSH (HGSH) or low GSH (LGSH) concentration across without (0ppm) or with dietary supplementation of 2.5ppm Se (HGSHSe, LGSHSe). Each group had 12 animals. The error bar is standard error of the means.

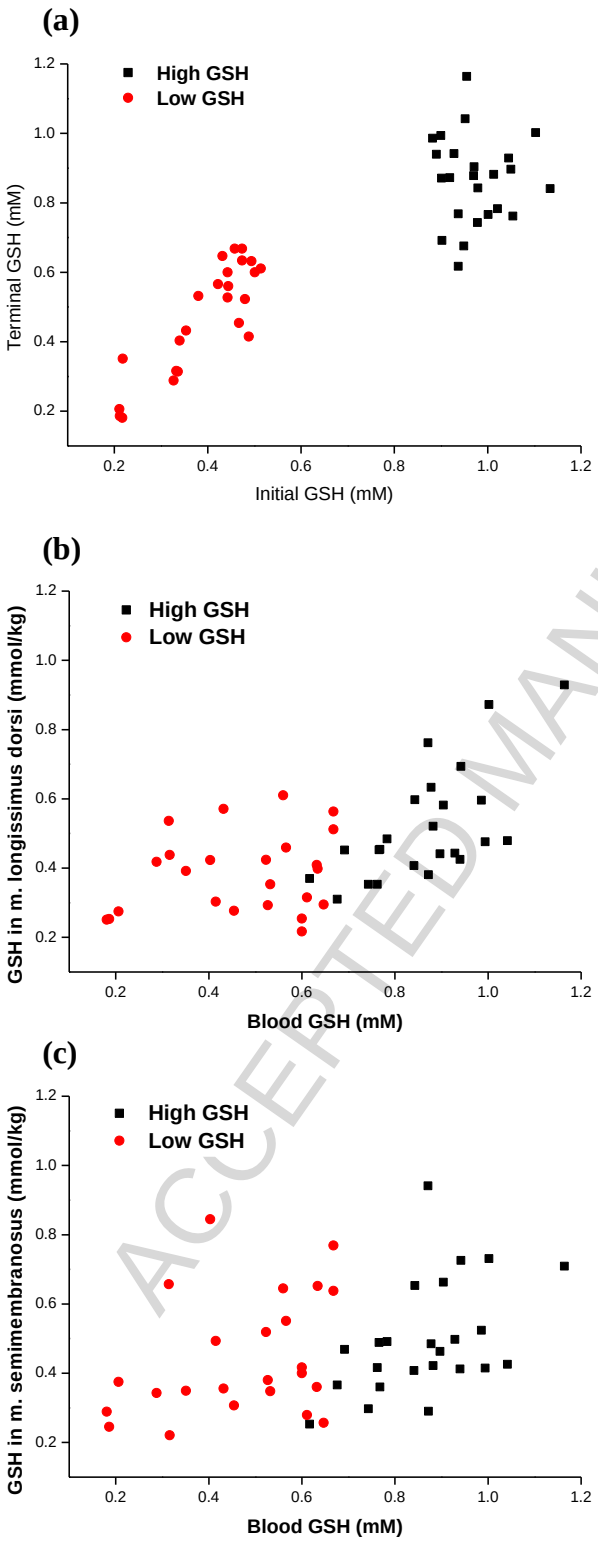

Fig. 5 The relationships between the initial and terminal blood GSH concentrations (a), and between the terminal blood GSH and GSH concentration in *m. longissimus dorsi* (b) or *m. semimembranosus* (c) of 12 month old Merino lambs selected for high blood GSH or low GSH concentration across without or with dietary supplementation of 2.5ppm Se. One spot represents one animal.

Table 1
Live weight gain, carcass and organ weights of Merino lambs with high or low blood GSH and supplemented with 0 or 2.5 ppm Se supplementation.

	High GSH		Low GSH		SEM	Significance (P values)		
	0ppm	2.5ppm	0ppm	2.5ppm		GSH	Se	GSH×Se
Feed intake (g/d)	1168	1165	1167	1168	2.8	0.88	0.77	0.53
Initial live weight (kg)	36.2	35.2	35.6	36.0	0.67	0.91	0.64	0.30
Terminal live weight (kg)	42.4	42.4	43.3	42.2	0.69	0.83	0.13	0.98
Live weight gain (g/d)*	103	103	110	92	5.3	0.73	0.11	0.09
Clean wool weight (g)	1371	1405	1408	1386	40.5	0.84	0.89	0.49
Cold carcass weight (kg)	19.0	18.5	18.1	17.8	0.37	**0.037**	0.30	0.71
Dressing rate	0.451	0.451	0.431	0.436	0.005	**0.001**	0.72	0.60
Heart (g)	208	206	209	206	4.9	0.84	0.62	0.93
Lungs (g)	449	413	458	409	16.5	0.88	**0.013**	0.69
Liver (g)	555	527	551	529	15.0	0.96	0.11	0.83
Kidneys (g)	113	111	113	123	3.2	0.069	0.19	0.055

*: Live weight gain was calculated using a regression method using all the live weights during the experimental period.

Table 2
The effects of blood GSH and Se supplementation (2.5ppm) on colour stability of the muscles of 12 months old Merino lambs

	Significant levels of the effects (P values)			
	GSH	Se	GSH×Se	GSH×Se×Hour
		m. longissimus dorsi		
L*	0.21	0.10	0.72	0.92
a*	0.11	0.18	0.12	0.67
b*	**0.02**	0.40	0.13	0.98
Ln(b*)	0.07	0.64	0.32	0.83
W580	0.52	**0.048**	0.67	0.81
W630	0.20	0.31	0.24	0.57
W630/w580 Ratio	0.07	0.30	0.06	0.92
Ln(Ratio)	**0.04**	0.29	0.07	0.95
		m. semimembranosus		
L*	0.89	0.63	0.90	0.15
a*	0.50	0.067	**0.024**	0.59
b*	0.82	0.61	0.57	0.93
Ln(b*)	0.90	0.59	0.55	0.93
W580	0.30	0.086	**0.029**	0.58
W630	0.46	0.20	0.41	0.88
W630/w580 Ratio	0.57	0.079	**0.046**	0.38
Ln(Ratio)	0.53	0.075	**0.012**	0.50
		m. semitendinosus		
L*	**0.02**	**0.007**	0.81	0.86
a*	0.33	0.12	**0.047**	0.11
b*	0.52	**0.008**	0.65	0.93
Ln(b*)	0.50	**0.008**	0.64	0.94
W580	0.67	0.69	0.69	0.40
W630	**0.039**	0.15	**0.005**	0.42
W630/w580 Ratio	0.43	0.51	**0.045**	0.068
Ln(Ratio)	0.45	0.77	0.55	**0.012**

Table 3
Fatty acid concentrations (mg/100 g wet tissue) in *m. longissimus dorsi* and *m. semimembranosus* of Merino lambs with high or low blood GSH across with 0 or 2.5 ppm Se supplementation.

	High GSH		Low GSH		SEM	Significance (P values)		
	0ppm	2.5ppm	0ppm	2.5ppm		GSH	Se	GSH×Se
			m. longissimus dorsi					
SFA	979	1075	1058	936	107.1	0.78	0.90	0.32
MUFA	855	873	828	730	86.8	0.33	0.65	0.50
PUFA	269	279	261	233	13.5	**0.05**	0.52	0.17
CLA	4.63	3.87	3.60	2.09	1.297	0.29	0.39	0.78
n-3	25.8	28.6	24.4	22.8	1.77	**0.047**	0.75	0.23
n-6	62.9	64.7	61.8	60.2	2.19	0.21	0.94	0.44
n-6 : n-3 ratio	2.71	2.45	2.57	2.69	0.177	0.78	0.72	0.28
EPA	5.64	6.87	5.45	5.66	0.378	0.071	0.066	0.19
DPA	5.62	7.01	5.91	6.05	0.482	0.49	0.12	0.20
DHA	3.28	3.12	3.55	2.69	0.163	0.72	**0.031**	0.14
			m. semimembranosus					
SFA	1246	124	1214	1071	171.1	0.53	0.70	0.66
MUFA	1120	999	930	840	131.3	0.19	0.43	0.91
PUFA	313	274	267	245	17.5	**0.037**	0.091	0.64
CLA	8.40	7.33	6.66	5.17	1.606	0.23	0.43	0.90
n-3	45.8	46.3	35.1	36.5	3.47	**0.005**	0.79	0.89
n-6	65.3	61.3	59.7	60.2	2.02	0.104	0.39	0.29
n-6 : n-3 ratio	1.51	1.43	1.79	1.72	0.112	**0.014**	0.48	0.98
EPA	5.76	6.54	5.27	5.80	0.357	0.092	0.072	0.74
DPA	9.22	9.80	8.18	9.26	0.557	0.16	0.14	0.65
DHA	3.23	2.90	2.65	2.43	0.256	**0.047**	0.29	0.84

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: conjugated linolenic acids; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid.

Table 4
Se concentrations in *m. longissimus dorsi*, lungs and heart, and GSH concentrations in whole blood and the muscles of Merino lambs with high or low blood GSH across with 0 or 2.5 ppm Se supplementation.

	High GSH		Low GSH		SEM	Significance (P values)		
	0ppm	2.5ppm	0ppm	2.5ppm		GSH	Se	GSH×Se
			Se concentrations (µg/kg wet tissue)					
m. longissimus dorsi	74	232	57	171	15.4	**0.02**	**0.001**	0.16
Lungs	352	548	387	488	23.2	0.58	**0.001**	**0.046**
Heart	159	122	136	159	22.1	0.74	0.76	0.18
			GSH concentration					
Initial blood sample (mM)	0.987	0.961	0.386	0.403	0.025	**0.001**	0.85	0.40
Final blood samples (mM)	0.847	0.873	0.446	0.478	0.036	**0.001**	0.42	0.93
m. longissimus dorsi (mmol/kg wet tissue)	0.476	0.563	0.356	0.414	0.056	**0.002**	0.078	0.72
m. semimembranosus (mmol/kg wet tissue)	0.479	0.513	0.484	0.407	0.070	0.31	0.67	0.27