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 w630 nm/w580 nm 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.

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1. Introduction

During retail display a 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 h 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, Gomaa, & Buckley, 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, Lamar, & Brown, 1983). Myoglobin is composed of an iron atom and the heme iron can exist in a reduced ferrous (Fe^{2+}) or oxidized ferric (Fe^{3+}) form (Faustman & 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 & Hunt, 2005). These colours are synonymous with freshness and consumers consider it

attractive (Renerre, 1990). Oxidized (Fe^{3+}) myoglobin only exists in the deoxygenated form, and is brown in colour (Mancini & 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 & 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, Chan, Schaefer, & Havens, 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 (Li, Cobb, Hill, Burk, & May, 2001; Meister & Anderson, 1983). 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 & Anderson 1983). The concentration trait appeared to be controlled by a pair of autosomal alleles which are dominant for high concentration (Tucker, Kilgour, & Young, 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, Ellory, & Tucker, 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 were 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™, Abamectin, 0.2 mg/kg liveweight, and Scanda™, 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 sub-group 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) $\times$ 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/day 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 acclimatization 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 for analysis of the concentrations of GSH, Se and fatty acids. The hot carcass was then weighed, and hung in a cool room for 24 h at 4 °C.

The cold carcass was weighed the next day. Triplicate samples (about $3 \times 3 \times 1$ 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 lx) at 4 °C for 5 days. Colour and reflectance readings from wave length of 400 nm up to 700 nm 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, i.e., L*, a*, b* values, and the reflectance at 580 nm (w580) and 630 nm (w630) were reported in this paper. The wavelength 580 nm is the absorption peak for oxymyoglobin (Fe^{2+}) while 630 nm 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, Zipser, & Watts, 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 0.04 M 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 $\times g$ to precipitate the protein. Approximately 300 µL of supernatant was then transferred into a microcentrifuge tube, and centrifuged for 5 min at 1800 $\times g$. 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 0.04 M 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 0.02 M EDTA, and 2.5 mL ice cooled 10% TCA was immediately added. The sample was vortexed thoroughly and centrifuged for 15 min at 4000 $\times g$ at 4 °C for deproteinisation. The supernatant was processed as described previously 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, Busboom, Nelson, and Gaskins (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 10 M KOH and 5.3 mL methanol were added, and incubated with rigorous hand-