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Selenium Supplementation and Increased Muscle Glutathione Concentration Do Not Improve the Color Stability of Lamb Meat

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In the eyes of the consumer, a red surface color of lamb meat is desirable. This red color is caused by oxymyoglobin; however, under conditions of retail display this pigment slowly oxidizes and turns brown, deterring consumers. The antioxidant activity of both glutathione (GSH) and selenium has been suggested to slow myoglobin oxidation, thus improving color stability. The following experiment was designed to test the hypothesis that high muscle GSH will improve the color stability of lamb meat, and this effect of GSH will be further improved by supplementing animals with selenium. Forty-eight 12-month-old Merino wether lambs were selected from a flock for high (n = 24) or low (n = 24) GSH concentration in whole blood. Each GSH group was then randomly allocated into two selenium treatments (supplemented with or without 2.5 mg of selenium/kg for 8 weeks). The lambs were slaughtered, and samples were taken from m. semimembranosus (SM) and m. longissimus dorsi (LD) to measure muscle GSH, selenium, and vitamin E concentrations. Further samples were taken to measure color stability (as oxy/metmyoglobin ratio, reflectance at 630/580 nm) over 96 h of retail display. There was no effect of muscle GSH concentration or selenium supplementation on oxy/metmyoglobin ratio at 60, 48, or 30 h of retail display, with the only exception being the non-selenium-supplemented SM samples, which actually decreased in ratio as the muscle GSH concentration increased (P < 0.05). There was a poor correlation between blood and muscle GSH, with a correlation coefficient of 0.18 for the SM and 0.026 for the LD. Thus, it is apparent that neither GSH nor selenium improved the color stability of meat from merino lambs.

KEYWORDS: Glutathione; color stability; selenium; metmyoglobin

INTRODUCTION

During retail display the surface color of fresh meat changes from red to brown over several days. A cut that appears red in color is considered to be attractive to consumers (*1*); thus, the retail sector is forced to discount meat that fails to sell within a designated shelf life. This discounting ensures that all meat is sold before a color change is noticeable to consumers; however, it also results in a significant economic loss (*2*). For this reason there is considerable interest within the industry in maintaining a desirable surface color by improving the color stability in fresh red meat.

The change in the surface color of meat is caused by the oxidation of the muscle pigment myoglobin (*3*). Myoglobin is a heme-containing protein and can be present in a reduced ferrous (Fe^{2+}) or oxidized ferric (Fe^{3+}) form (*4*). The reduced form can exist in a deoxygenated (deoxymyoglobin) or an oxygenated state (oxymyoglobin), which differ in color from a purplish-red to a bright cherry red (*5*). Alternatively, the oxidized form of myoglobin, known as metmyoglobin, is brown in color (*5*); thus, it is the ratio of oxymyoglobin to metmyoglobin (*6*) on the surface of red meat that determines whether it appears red or brown.

The oxidation of myoglobin can be accelerated by the presence of pro-oxidants such as hydrogen peroxide (*7*). As a means of protecting themselves against this oxidative damage, cells are equipped with an array of endogenous antioxidants such as glutathione (GSH), which can scavenge hydrogen peroxide and other oxidative species. In mammals, GSH is found in relatively high concentrations compared to other antioxidants and is thus considered to be an important antioxidant system. GSH exists in two redox states, reduced (GSH) and oxidized (GSSG), and can be easily converted between the two forms. However, the potential of GSH to act as an antioxidant is favored when present mainly in the reduced form, where a hydrogen ion is readily available to participate in redox reactions. Additionally, the high reducing potential of GSH (*8*) allows for the regeneration of other antioxidants such as vitamins E and C (*9*, *10*), further enhancing antioxidant defenses in biological tissue. GSH has also been shown to possess the ability to slow the oxidation of bovine and equine myoglobin in vitro (*11*); however, this antioxidant ability has an essential requirement for the activity of the enzyme GSH

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peroxidase, which catalyzes the decomposition of hydrogen peroxide. Furthermore, the mineral selenium is suggested to be an essential component for GSH antioxidant activity as it is a component of the active site for the majority of mammalian GSH peroxidases (*12*); thus, it can be assumed that the antioxidant capacity of GSH is dependent on both selenium and GSH peroxidase activity.

Manipulation of GSH via the diet is difficult, and for this reason the availability of GSH in the muscle is mainly dependent on the level of expression within cells. Because of this, GSH concentrations are likely to vary between animals, a contention supported in previous research using Merino sheep in which blood GSH levels were shown to vary 7-fold between animals (*13*). Assuming that the ability of GSH to slow the formation of metmyoglobin in vitro can be reproduced in meat products, animals with higher muscle GSH are likely to have greater color stability. Therefore, we hypothesize that high muscle GSH will increase the color stability of lamb meat, and this effect of GSH will be further improved by supplementing animals with selenium.

MATERIALS AND METHODS

Animals, Management, and Dietary Treatment. A group of 48 lambs was selected from a flock of 400 12-month-old Merino wether lambs on the basis of blood GSH concentrations. The sheep were ranked according to blood GSH concentration, and the top 24 and the bottom 24 lambs were selected. These 48 lambs were transported 100 km by truck to an animal house, and an anthelmintic drench (Virbac, abamectin, 0.2 mg/kg of live weight; Scanda, oxfendazole, 4.5 mg/kg of live weight, and levimasole, 6.9 mg/kg of live weight) was applied to remove gastrointestinal parasites. Sheep were then maintained in individual pens and fed (approximately 1.1 kg per head per day) a hay/lupin/barley pelleted diet (9.26 MJ ME/kg, 10.8% CP and 7.1% metabolizable protein on DM basis) for 3 weeks to allow for acclimatization. Within the high and low blood GSH treatment groups, sheep were randomly allocated to either the control diet, which was the same used during acclimation, or the selenium-supplemented diet, which also contained 2.5 mg of selenium/kg as sodium selenite (analysis of the control diet showed it contained 0.2 ppm Se). This approach resulted in animals with a range of blood GSH within the selenium-supplemented and nonsupplemented groups. Sheep were weighed weekly throughout the experiment.

Sampling Procedures and Meat Color Measurement. Blood samples were taken at the end of the supplementation period prior to slaughter (final). These samples were processed immediately for determination of whole blood GSH concentration, and this was used as a comparison between blood GSH from before and after the supplementation period. The sheep were slaughtered by captive bolt and exsanguination. Immediately following slaughter, 10 g samples of the m. semimembranosus (SM) and m. longissimus dorsi (LD) were collected, frozen in liquid N, and stored at −80 °C until used for further analysis.

Additional samples of the SM and LD were collected 4 days after slaughter for retail display. Display samples were cut into 2 cm thick slices, trimmed of visible fat, placed on black Styrofoam trays, and overwrapped with 10 μm oxygen-permeable polyvinyl chloride wrap. The meat was stored at 4 °C in a refrigerator fitted with cool white fluorescent lights (OSRAM L36W/20, Germany). Color measurements were taken every 12 h for 96 h using a Hunter Lab Mini Scan XE Plus (model 45/0-L, Hunter Associates Laboratory Inc., Reston, VA), with the light source set to C and the aperture set to 10. The instrument was calibrated on a white tile and black glass according to the manufacturer's specifications.

The wavelength 580 nm is the absorption peak for oxymyoglobin, whereas 630 nm is the absorption peak for metmyoglobin (*6*); thus, the surface myoglobin oxidation was predicted from the oxy/metmyoglobin ratio calculated from the ratio of light reflectance at 630 and 580 nm.

Blood and Muscle GSH Concentration. Two methods were used for the determination of GSH in the muscle and blood. The 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) method was used for the quick determination of blood concentration from which the animals were initially selected. This relatively simple method measures free-form thiols, including GSH, that react with DTNB; thus, it is not specific for GSH. HPLC analysis was then used to more accurately measure the GSH in the muscle and blood samples.

DTNB Chemical Method. The GSH concentration in fresh blood was determined using the method described by Sedlak and Lindsay (*14*) and modified for an autoanalyzer. Briefly, about 0.5 g of blood was transferred into a tube prefilled with 2 mL of 0.04 M EDTA and weighed. A 2.5 mL aliquot of 10% trichloroacetic acid (TCA) was added and then mixed. The solution was centrifuged for 15 min at 4000*g* at 4 °C to precipitate protein. About 300 μL of the supernatant was transferred into a microcentrifuge tube and centrifuged for 5 min at 15000*g*. The supernatant was then combined with DTNB in 0.4 M Tris buffer (pH 8.0) in a Cobas Mira Diagnostica System (F. Hoffmann-La Roche, Switzerland). A GSH standard solution was prepared by dissolving reduced GSH standard (Sigma Aldrich, product G4251, Australia) in a solution that contained 0.04 M EDTA and 5% TCA. This method measures free-form thiols, including GSH, that can react with DTNB.

HPLC Method. The blood and muscle GSH concentrations were determined by the precolumn *o*-phthalaldehyde (OPA) derivative method using HPLC described in ref *15*. For blood, approximately 0.5 g of blood was mixed with 0.45 mL of 0.02 M EDTA and thoroughly mixed to allow for hemolysis. The solution was mixed with 50 μL of 50 mM dithiothreitol (DTT) and left to stand at room temperature for 5 min to allow for reduction of oxidized glutathione. One milliliter of freshly prepared 5% (w/v) metaphosphoric acid (MPA) was added, mixed, and centrifuged for 15 min at 4000*g* at 4 °C for deproteinization. One hundred microliters of the supernatant was then mixed with 2 mL of phosphate buffer (pH 7.0) and 300 μL of OPA solution for derivatization for 1 h at room temperature. The solution was filtered through a 0.2 μm syringe filter into a glass vial for HPLC analysis.

For muscle samples about 0.5 g of muscle was taken out of liquid nitrogen and immediately crushed, collected into a plastic tube, and weighed. The samples were then homogenized in 1 mL of solution of a 0.02 M EDTA solution, after which 1 mL of ice-cold 20% MPA was added, mixed, and then centrifuged for 20 min at 4000*g* at 4 °C. A 100 μL aliquot of the supernatant was mixed with 300 μL of phosphate buffer (pH 7.0) and 400 μL of OPA solution (5 g/L) and left to stand at room temperature for 30 min for formation of the derivative. One milliliter of phosphate buffer was added to the solution, filtered, and used for determining GSH in HPLC. Another 100 μL aliquot of the supernatant was added to 300 μL of phosphate buffer (pH 7.0) followed by 50 μL of 50 mM DTT. This was incubated for 10 min at room temperature to allow for reduction of disulfide, after which 400 μL of OPA solution was added and incubated for a further 30 min at room temperature. Nine hundred and fifty microliters of phosphate buffer was then added, filtered, and used for the determination of total GSH (reduced GSH plus that which was reduced by DTT; thus, the difference is assumed to be that of the oxidized GSSG form) by HPLC. Peak areas were integrated for the calculation of the concentration against that of the standard GSH

A Shimadzu HPLC system was used to determine the concentration of the GSH derivative. The system was equipped with a Shimadzu fluorescence detector (model RF-10A) and an Alltech column (Alltima C18, 5 μm, 250 mm × 4.6 mm, part 88057; Alltech Associations Inc., Deerfield, IL). The temperature of the column was maintained at 30 °C, and the sample injection size was 50 μL. The mobile phase contained 0.1 M sodium acetate (pH 6.20) with 15% acetonitrile, and the flow rate was 0.7 mL/min. A single standard of GSH was prepared using the same procedure as the samples and was analyzed twice daily.

Selenium Analysis. Selenium concentration in the LD muscle was determined using the fluorometric method as described by Watkinson (*16*). Briefly, about 0.3 g of the muscle sample was digested with variable heating in a solution of 3.5 mL of $HNO_3/HClO_4$ (2.5:1). Two blanks and three standards (sodium selenite, Sigma Aldrich, Australia) were processed in the same way. Fluorescence was then meausured in a Perkin-Elmer fluorescence spectrophotometer (model 1000, Perkin-Elmer, Beaconsfield, Buckinghamshire, U.K.) with excitation at 376 nm and emission at 520 nm. Selenium concentration was calculated and expressed as micrograms per kilogram of wet tissue.

Vitamin E Analysis. The vitamin E content of muscle was measured using high-performance liquid chromatography with fluorescence detection as described by McMurray and Blanchflower (*17*). Additionally,

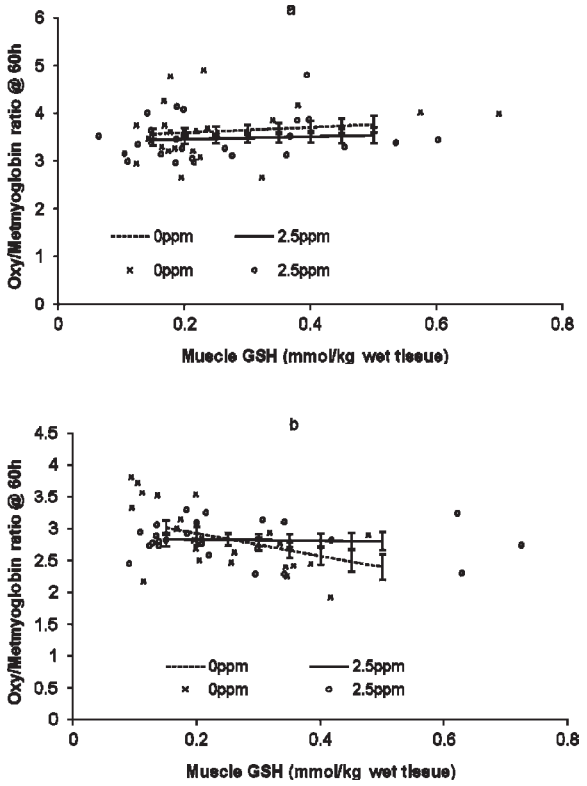

Figure 1. Oxy/metmyoglobin ratio at 60 h of retail display of meat from lambs supplemented or not with selenium in relation to muscle GSH content in the LD (**a**) and the SM (**b**). The raw data are represented as × for the 0 ppm and as ○ for the 2.5 ppm selenium supplementation groups.

samples were saponified according to the method of Bieri et al. (*18*), before extraction with hexane.

Statistical Analysis. The oxy/met ratios at 30, 48, and 60 h for each individual muscle sample were determined by fitting the exponential function described below using the nonlinear procedure in SAS (SAS Institute Inc., Cary, NC).

$$y_t = x_u + (x_i - x_u)^{-xkt}$$

y_t is the oxy/metmyoglobin ratio as a function of time, x_u is the ultimate oxy/metmyoglobin ratio reached, x_i is the initial oxy/metmyoglobin ratio, x_k is the rate of oxy/metmyoglobin ratio decline, and t is time. The oxy/met ratios at 30, 48, and 60 h were then analyzed using a linear mixed-effects model in SAS, with selenium supplementation and muscle as fixed effects, muscle GSH concentration (as determined by HPLC analysis) as a covariate, and animal as the random term.

A simple correlation between blood GSH concentration and tissue GSH concentration with both the LD and SM was also determined using SAS.

RESULTS

Effect of GSH and Selenium Concentration and Muscle on the Oxy/Metmyoglobin Ratio. In almost all cases there was no effect of muscle GSH concentration or selenium supplementation on oxy/metmyoglobin ratio at 60 h of retail display ($P < 0.05$; **Figure 1**). The only exception to this was in the non-selenium-supplemented SM samples, which actually decreased in ratio as the muscle GSH concentration increased ($P < 0.05$). The LD was more color stable than the SM, with an oxy/metmyoglobin ratio about 0.75 unit higher at 60 h ($P < 0.05$; **Figure 1**). Similar effects were seen at 48 and 30 h of retail display ($P < 0.05$; data not shown).

Tissue GSH, Selenium, and Vitamin E Concentrations. There was no difference in GSH concentration between the selenium treatments in all tissues measured (**Table 1**). Additionally, selenium supplementation for 8 weeks did not increase the concentration of GSH in the blood. In the muscle, GSH ranged from 0.064 to 0.699 mmol/kg of wet tissue in the LD and from 0.09 to 0.724 mmol/kg of wet tissue in the SM. The HPLC method also enabled determination of total GSH, which represented the combination of GSH and its oxidized disulfide form (GSSG). Given that total GSH was about 10−11 times higher than GSH in both muscles, this would suggest that in post-mortem muscle the ratio of GSH to GSSG is close to 1:10. There was no difference in muscle GSH or total muscle GSH concentrations between selenium treatments.

Table 1. Glutathione Concentrations in Whole Blood and Muscles of 48 Merino Sheep

	selenium supplementation			
	0 ppm	2.5 ppm	SEM	*P* value
whole blood GSH concentration (mM)				
initial	0.247	0.281	0.0271	0.08
final	0.243	0.279	0.0223	0.13
GSH concentration (mmol/kg of wet tissue) in m. semimembranosus				
GSH	0.237	0.263	0.0386	0.53
total GSH	2.47	2.68	0.343	0.52
GSSG/GSH ratio[a]	10.24	10.57	0.841	0.78
GSH concentration (mmol/kg of wet tissue) in m. longissimus				
GSH	0.239	0.261	0.0399	0.59
total GSH	2.4	2.47	0.296	0.85
GSSG/GSH ratio[a]	10.27	9.5	0.841	0.52

[a] GSH/GSSG ratio calculated via the formula (total GSH − GSH)/GSH.

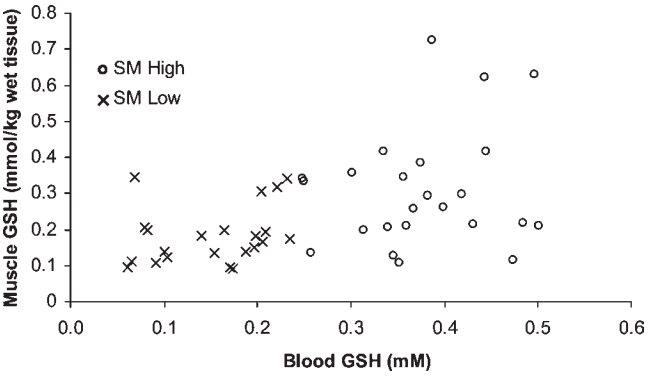

Figure 2. SM GSH concentration versus initial blood GSH concentration, as measured by HPLC. Data are separated by GSH group based on blood GSH concentration as measured by the chemical DTNB method (○, high blood GSH; ×, low blood GSH). $R^2 = 0.18$.

The relationship between blood (initial) and muscle GSH (as determined by HPLC) was weak, with correlation coefficients of 0.18 for the SM and 0.026 for the LD. The initial high and low GSH groups, as selected by the initial DTNB method, can be clearly identified by the segregation in blood GSH at about 0.25 mM blood GSH (**Figure 2**). However, there was a large overlap observed for muscle GSH within these two GSH groups. Nonetheless, a good range in muscle GSH was still achieved independent of the blood GSH selection groups.

Selenium concentration (μg/kg of wet tissue) in LD was significantly higher in sheep supplemented with selenium compared with no supplementation (202 vs 65 μg/kg of wet tissue, SEM = 10.9, $P < 0.001$).

Vitamin E concentration differed ($P < 0.05$) between muscles with values of 0.972 ± 0.05 mg/kg in the LD and 1.185 ± 0.05 mg/kg in the SM. There was no effect of selenium supplementation or GSH on the muscle vitamin E concentration.

DISCUSSION

Increasing muscle GSH concentration did not improve the color stability of meat from merino lambs, in contrast to our initial hypothesis. The only effect of GSH was seen in the SM of the non-selenium-supplemented lambs, but in this case it worsened color stability. Although this effect was overcome by selenium supplementation, there was no observed improvement in the oxy/metmyoglobin ratio due to selenium supplementation per se.

The decreased color stability observed with increasing muscle GSH in the SM without selenium supplementation is difficult to explain. Given that the SM is a more oxidative muscle type compared to the LD (*19*), the intrinsic requirement for antioxidants should be greater. Second, this observed decrease was only seen in the samples without selenium supplementation, which could be accounted for by a reduction in GSH peroxidase activity due to a lower selenium availability (*20*). However, if this were the case, then samples with low muscle GSH and no selenium should also have a low oxy/metmyoglobin ratio, which in fact was higher and no different from the selenium-supplemented samples. Despite this, all of the current samples had a relatively poor color stability when compared to other studies conducted in this laboratory (*21*). Therefore, this observed decrease should not be of concern because GSH did not improve the color stability of meat from merino lambs.

Although GSH is capable of slowing the oxidation of myoglobin in vitro (*11*), the ability of GSH to slow this oxidation in meat seems to be lost, even in the presence of supplemented selenium. Additionally, the activity of GSH peroxidase in post-mortem aging in beef and pork is not altered (*22*), suggesting that this enzyme is not likely to explain the lack of GSH antioxidant activity. GSH is known to have a multitude of physiological functions, and its antioxidant activity is dependent on the interactions with a number of additional antioxidants. For example, the antioxidant activity of GSH is dependent on vitamin B_6, which facilitates the availability of selenium for GSH peroxidase activity (*23*). Furthermore, GSH is responsible for regenerating both vitamins C and E from their oxidized products, an important interaction in relation to studies in meat color because of the positive benefit vitamin E has on color stability in both lamb and beef (*24*, *25*). In the current study the lambs had a relatively low but not deficient muscle vitamin E concentration (*26*). Furthermore, the treatment groups did not deliver a difference in vitamin E; therefore, vitamin E would not be relevant to the current results.

Besides the antioxidant function, GSH also plays a major role in detoxification of cellular toxins. The lipid peroxidation product 4-hydroxynonenal (HNE) is one such toxin, which GSH can conjugate with and, subsequently, detoxify (*27*, *28*). The presence of HNE is an indicator of lipid peroxidation and oxidative stress in the cell (*28*), and such conditions often lead to the rapid depletion of cellular GSH (*29*). However, vitamin E inhibits lipid peroxidation (*24*), hence lowering the amount of cytosolic HNE (*30*) and thus sparing GSH. Therefore, the means by which the antioxidant activity of GSH seems to be inactivated may be through interactions with lipid peroxidation and may be prevented through additional interaction with vitamin E.

Another important consideration is the redox state of GSH and thus total redox potential of the cell. During the antioxidant function of GSH, it is oxidized to the disulfide (GSSG) form. The reduced form can be regenerated via GSH reductase activity with reducing power provided by NADPH. The ratio of GSH/GSSG in resting cells often exceeds 100:1, whereas in conditions of oxidative stress values have been reported between 10:1 and 1:1 (*31*). The current data indicate that this ratio in meat is close to 1:10, largely favoring the oxidized form in contrast to that seen in physiological conditions. Thus, it is likely that the total redox status could potentiate the antioxidant response, and in the conditions in meat this ratio is not favorable. Additionally, this may also be driven by the availability of reducing equivalents (NADPH) available for the reduction of GSSG (*29*), an assertion that requires further investigation.

Blood GSH did not have a strong correlation with the muscle concentration and thus did not represent the actual GSH available in the muscle, which could possibly influence the oxidation of myoglobin. As such, blood GSH levels cannot be used as an indictor of muscle GSH status. However, for the purpose of this study, the selection of animals of high and low blood GSH resulted in a sufficient range of muscle GSH levels across both selenium treatments.

Neither GSH nor selenium improved the color stability of meat from merino lambs. The ineffectiveness of GSH as an antioxidant in meat needs further research. The apparent inactivation of the antioxidant function of GSH may be a result of cellular interactions that could possibly be prevented by the addition of other antioxidants important in manipulating the total cellular redox state. Furthermore, it is likely that there are many other variables between animals, such as genetic potential and redox status, that may limit the antioxidant potential of GSH in meat. These possibilities require further investigation.

LITERATURE CITED

(1) Renerre, M. Review: Factors involved in the discoloration of beef meat. *Int. J. Food Sci. Technol.* **1990**, *25*, 613–630.
(2) Williams, S.; et al. Vitamin E. *Meat Int.* **1992**, *3*, 853.
(3) Gray, J. I.; Gomaa, E. A.; Buckley, D. J. Oxidative quality and shelf life of meats. *Meat Sci.* **1996**, *43*, 111–123.
(4) Faustman, C.; Cassens, R. G. The biochemical basis for discoloration in fresh meat: a review. *J. Muscle Foods* **1990**, *1*, 217–243.
(5) Mancini, R. A.; Hunt, M. C. Current research in meat color. *Meat Sci.* **2005**, *71*, 100–121.
(6) Hunt, M. C. Meat color measurements. *Proc. −Annu. Reciprocal Meat Conf.* **1980**. *33*.
(7) Morey, K. S.; Hansen, S. P.; Brown, W. D. Reaction of hydrogen peroxide with myoglobins. *J. Food Sci.* **1973**, *38*, 1104–1107.
(8) Jones, D. P. Redefining oxidative stress. *Antioxid. Redox Signal.* **2006**, *8*, 1865–1879.
(9) Meister, A.; Anderson, M. E. Glutathione. *Annu. Rev. Biochem.* **1983**, *52*, 711–760.
(10) Li, X.; et al. Mitochondrial uptake and recycling of ascorbic acid. *Arch. Biochem. Biophys.* **2001**, *387*, 143–153.
(11) Tang, J.; et al. Effect of glutathione on oxymyoglobin oxidation. *J. Agric. Food Chem.* **2003**, *51* (6), 1691–1695.
(12) Wilke, B. C.; et al. Selenium, glutathione peroxidase (GSH-Px) and lipid peroxidation products before and after selenium supplementation. *Clin. Chim. Acta* **1992**, *207*, 137.
(13) Liu, S. M.; et al. Biochemical differences in Merino sheep selected for resistance against gastro-intestinal nematodes and genetic and nutritional effects on faecal egg count and egg output. *Anim. Sci.* **2005**, *81*, 149–157.
(14) Sedlak, J.; Lindsay, R. H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **1968**, *25*, 192.
(15) Cereser, C.; et al. Quantitation of reduced and total glutathione at the femtomole level by high-performance liquid chromatography with fluorescence detection: application to red blood cells and cultured fibroblasts. *J. Chromatogr., B: Biomed. Sci. Appl.* **2001**, *752*, 123–132.
(16) Watkinson, J. H. Fluorometric determination of selenium in bilogical material with 2,3-diaminonaphthalene. *Anal. Chem.* **1966**, *38*, 92–97.
(17) McMurray, C. H.; Blanchflower, W. J. Application of a high-performance liquid chromatographic fluorescence method for the rapid determination of α-tocopherol in the plasma of cattle and pigs and its comparison with direct fluorescence and high-performance liquid chromatography−ultraviolet detection methods. *J.Chromatogr., A* **1979**, *178*, 525.

(18) Bieri, J. G.; et al. The determination of α-tocopherol in animal tissue by column chromatography. *Acta Chem. Scand.* **1961**, *15*, 783–790.
(19) Gardner, G. E.; et al. Sheep genotype, age and muscle type affect the expression of metabolic enzyme markers. *Aust. J. Exp. Agric.* **2007**, *47*, 1180–1189.
(20) Whanger, P. D.; et al. Effects of selenium and vitamin E on blood selenium levels, tissue glutathione peroxidase activities and white muscle disease in sheep fed purified or hay diets. *J. Nutr.* **1977**, *107*, 1298–1307.
(21) Jose, C. G.; et al. CT scanning carcases has no detrimental effect on the color stability of M. longissimus dorsi from beef and sheep. *Meat Sci.* **2009**, *81*, 183–187.
(22) Daun, C.; et al. Glutathione peroxidase activity, tissue and soluble selenium content in beef and pork in relation to meat ageing and pig RN phenotype. *Food Chem.* **2001**, *73*, 313–319.
(23) Yin, S. A.; et al. The necessity of dietary vitamin B6 to selenium biopotency for tissue selenium and glutathione peroxidase in rats. *J. Nutr. Sci. Vitaminol.* **1991**, *37*, 509–516.
(24) Wulf, D. M.; et al. Effect of dietary supplementation of vitamin E on storage and caselife properties of lamb retail cuts. *J. Anim. Sci.* **1995**, *73*, 399–405.
(25) Faustman, C.; et al. Beef color update: the role for vitamin E. *J. Anim. Sci.* **1998**, *76*, 1019–1026.
(26) Fry, J. M.; et al. Plasma and tissue concentrations of α-tocopherol during vitamin E depletion. *Br. J. Nutr.* **1993**, *69*, 225–232.
(27) Alin, P.; Danielson, U. H.; Mannervik, B. 4-Hydroxyalk-2-enals are substrates for glutathione transferase. *FEBS Lett.* **1985**, *179*, 267.
(28) Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med.* **1991**, *11*, 81.
(29) Sen, C. K. Nutritional biochemistry of cellular glutathione. *J. Nutr. Biochem.* **1997**, *8*, 660–672.
(30) Yoshino, K.; et al. Formation of aliphatic aldehydes in rat plasma and liver due to vitamin E deficiency. *Chem. Pharm. Bull.* **1986**, *34*, 5184–5187.
(31) Chai, Y. C.; et al. S-Thiolation of individual human neutrophil proteins including actin by stimulation of the respiratory burst: evidence against a role for glutathione disulfide. *Arch. Biochem. Biophys.* **1994**, *310*, 273–281.

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