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Initial Bloom Color is a Poor Predictor of Retail Color Stability in Lamb Loin Meat

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Abstract: The rapid browning of lamb meat on retail display reduces its appeal to consumers and thus the marketability of lamb meat. Predicting the rate of meat browning on retail display would allow retailers to effectively manage this issue. The ability of bloomed meat color at the start of retail display to predict meat browning over subsequent retail display was investigated in lamb loin meat. Mixed breed lambs ($n = 4404$) produced at 5 sites over 5 yr were slaughtered at ~23kg carcass weight and measured for loin pH at 24 h, myoglobin, iron and zinc concentrations, isocitrate dehydrogenase activity and intramuscular fat. Loin meat was aged for 5 d before being re-sliced and overwrapped for color measurement over a 72 h simulated retail display. Meat redness (R630/R580) was measured after blooming and every 24 h across display using a Hunterlab spectrophotometer. Simple and partial correlation coefficients between initial and subsequent R630/R580 measures over the display were low (≤ 0.4). Accounting for key muscle traits influencing meat color such as pH_{24}, myoglobin, iron or intramuscular fat concentration did not improve these correlations between bloomed meat color and subsequent meat color over retail display. Therefore bloomed meat color at the start of display is not a useful predictor of meat browning after 24 h of retail display. Alternatively, correlations between 24, 48, and 72 h R630/R580 were > 0.8, suggesting that meat color measured from 24 h of display can accurately predict subsequent retail meat browning.

Introduction

Consumers demand that displayed lamb meat has a bright red color. The rapid change in lamb meat color from red to brown on retail display reduces its appeal to consumers and thus the marketability of lamb meat (Jeyamkondan et al., 2000). Substantial variation exists in the color stability of lamb meat from different animals (Calnan et al., 2014; Jacob et al., 2014), preventing retailers from predicting and effectively managing the browning of lamb meat on retail display.

Lamb meat develops a bright red color with blooming, as oxygen binds with myoglobin to form the red pigment oxymyoglobin. Though oxidative metabolism in muscle postmortem is limited, it has important effects on meat color. Meat with high proportions of oxidative muscle fibers contain more mitochondria that compete with myoglobin for oxygen, reducing the depth of oxygen penetration with blooming (Faustman and Cassens, 1990). By generating free-radical species and depleting oxygen concentrations in meat, postmortem oxidative metabolism also favors myoglobin oxidation into the brown pigment

metmyoglobin (Hood and Riordan, 1973). Muscle oxidative metabolism therefore influences both the bloomed color of lamb meat and its color stability over retail display, and it would be reasonable to expect that color stability may be associated with bloomed meat color at the start of retail display. If this association could be demonstrated, measures of bloomed meat color early in the postmortem period may be useful to predict meat color stability without the need for exposing meat to a simulated display period.

Accounting for variation in animal factors that influence myoglobin oxidation in meat may improve the ability of bloomed meat color to predict color stability on retail display. Increasing ultimate meat pH (pHu), intramuscular fat and markers of muscle oxidative capacity [muscle isocitrate dehydrogenase (ICDH) activity, myoglobin and iron concentration] have been associated with increased browning of lamb meat on retail display (Atkinson and Follett, 1973; Calnan et al., 2014; Hood, 1980). High pHu is associated with increased metabolic activity, oxygen consumption and production of oxidative free-radicals that trigger myoglobin oxidation (Faustman and Cassens, 1990). Ultimate pH can be approximated in lamb carcasses by measuring pH at 24 h postmortem (pH_{24}). Meat with high oxidative capacity consumes more oxygen, generates more free-radicals and thus browns at a faster rate (O'Keeffe and Hood, 1982; Renerre and Labas, 1987). Though it is impractical for industry to measure oxidative indicators including ICDH activity, myoglobin or iron concentration, the age of a lamb at slaughter may be used to estimate muscle oxidative capacity given that the oxidative capacity of muscles increases with lamb age (Kelman et al., 2014). Increasing intramuscular fat also increases lamb meat browning (Calnan et al., 2014; Renerre and Labas, 1987), potentially via increased lipid peroxidation and free-radical generation (Faustman et al., 2010). Measuring intramuscular fat is not currently practicable in the lamb industry, despite the value of this measure to predict eating quality and potentially to predict meat browning on display (Thompson, 2004). However, technology to measure intramuscular fat is rapidly advancing and may soon be available to industry. Therefore there is the potential for retailers to use information on meat pH_{24}, lamb age and intramuscular fat to improve predictions of lamb meat browning.

The extent of meat browning may be inferred using spectrophotometric light reflectance to measure the redness or red: brown (R630/R580) of a meat surface (Hunt, 1980). These ratios progressively reduce as lamb meat browns over retail display (Hopkins et al., 2013; Jacob et al., 2014; Ripoll et al., 2008), where R630/

R580 values below 3.3 units will cause consumer rejection of lamb meat based on its color (Khliji et al., 2010). The objective of this study was to determine if lamb age, pH_{24} and intramuscular fat concentration in addition to bloomed meat redness (R630/R580) at the start of retail display may provide enough information for retailers to predict the rate of lamb meat browning on subsequent retail display. Retailers could then predict when the threshold for color acceptability may be reached and maximize the retail display period of premium valued product. We hypothesized that knowledge of lamb age, pH_{24}, intramuscular fat concentration and surface R630/R580 at the start of retail display would allow the prediction of lamb loin redness (R630/R580) at the end of a 72 h simulated retail display period.

Material and Methods

This study was approved by the Department of Primary Industries and Regional Development, Animal Ethics Committee (No: 1–10–1), Western Australia.

Lamb production

The Sheep Cooperative Research Center produced 4404 lambs at 5 sites across Australia between 2007 and 2011 as part of the information nucleus flock experiment, which has been comprehensively described previously (Fogarty et al., 2007; van der Werf et al., 2010). Climatic conditions vary substantially between the sites of production which are up to 3,400 km apart. A total of 451 industry-proven Terminal, Maternal and Merino sires were used across all sites of production to artifically inseminate Merino and crossbred Merino ewes. Lambs were maintained on extensive or broadacre pasture grazing at each site, with grain, hay or feedlot pellets supplemented when pasture supply was limited. Further details of lamb management, nutrition and genetic design of this experiment have been published previously (Ponnampalam et al., 2014; van der Werf et al., 2010).

Slaughter details

At each production site lambs were consigned to smaller groups to be killed on the same day (slaughter groups). Lambs were designated to slaughter groups based on their live weights, with the aim to achieve an average carcass weight of 22 to 23 kg. A total of 73 groups of lambs were slaughtered. The group size ranged from 23 to 131 lambs, containing 60 lambs on average. Each site produced 2 to 5 slaughter groups each

year, most commonly 3 to 4. The time of year that lambs were slaughtered varied considerably between slaughter groups and between sites and years of production.

The average age of the lambs in a slaughter group varied substantially between groups. Lambs were aged 8 mo (or 248 d) at slaughter on average, though ranged from 134 to 503 d old. In contrast the range of lambs ages within slaughter groups was small, as lambs were bred via artificial insemination and therefore were born within a short period of time. The age range of lambs within slaughter groups was 11 d on average, and varied from as little as 5 d and up to 36 d age difference.

The day prior to slaughter, lambs were yarded and fasted for 6 h before being weighed and transported to a commercial abattoir. The distance from the site of production to the abattoir ranged from 12 to 430 km, though was consisent for each site. At the abattoir, lambs were held in lairage overnight with access to water only before slaughter the following day. Lamb carcasses were electrically stimulated on medium voltage (Pearce et al., 2010), trimmed according to AUS-MEAT specifications (Anonymous, 1992) and hung within a 3 to 4°C chiller overnight.

Carcass sampling

Lambs were measured and sampled for a wide range of carcass and muscle traits (Table 1). The hot carcass weight (HCWT) of each lamb was measured in kg on the chain following slaughter. At 20 to 25 h postmortem the *M. longissimus thoracis et lumborum* or loin muscle (from the level of the 12th rib to the lumbar sacral junction) and overlaying subcutaneous fat were removed from the left side of each carcass. These components were weighed separately as short-loin muscle weight (g) and shortloin fat weight (g). The absolute shortloin muscle and fat weights were analyzed in combination with the hot carcass weight (kg) of each lamb to represent relative shortloin muscle weight and relative shortloin fat weight. The excised loin muscle was then subsampled for chemical measurements and sprectrophotometric meat color measurement over a simulated retail display.

Loin muscle temperature and pH (pH_{24}) were measured in the center of the loin muscle, adjacent to the 12th rib. A TPS WP-80 pH and temperature meter (with a Mettler Toledo puncture pH probe- LoT406-M6-DXK-S7/25) was calibrated at pH 4 and 7 within the chiller before insertion into the muscle, as further described by Pearce et al. (2010).

Chemical measurements

ICDH activity was measured in the loin muscle of lambs produced in 2007 and 2008 ($n = 1804$, Table 1). A 1 g portion of loin muscle adjacent to the 12th rib was collected at 3 to 4 h postmortem, snap-frozen in liquid nitrogen and stored at –80°C until the activity of ICDH could be measured in µm per min per g of wet muscle, according to the method of Briand (1981).

Intramuscular fat, myoglobin, iron and zinc concentrations were measured in the loin muscle of lambs

Table 1. The mean, standard deviation and range of R630/R580 at 0, 24, 48, and 72 h of simulated retail display and for the carcass covariates tested

Trait	Number	Mean	St. dev	Range
R630/R580[1]: 0 h	4537	5.38	1.07	2.44 – 15.9
24 h	4538	4.22	1.06	2.00 – 11.6
48 h	4538	3.52	0.85	1.87 – 8.24
72 h	4404	3.04	0.68	1.81 – 8.05
Covariates, units:				
pH_{24}	4393	5.66	0.15	5.23 – 6.67
Myoglobin, mg/g wet muscle	2623	6.43	1.65	2.15 – 15.6
Iron mg/100 g wet muscle	2625	20.0	3.39	8.12 – 45.1
Isocitrate dehydrogenase activity, µmol/min/g of wet muscle	1804	5.03	1.56	1.43 – 11.3
Zinc mg/100 g wet muscle	2625	24.1	4.50	12.0 – 44.9
Intramuscular fat, %	2628	4.10	1.01	1.59 – 9.59
Hot carcass weight, kg	4355	22.7	3.37	12.5 – 36.0
Shortloin muscle weight, g	4400	360	95.6	157 – 1110
Shortloin fat weight, g	4397	171	76.0	10.0 – 590
Age d	4403	248	76.0	134 – 503

[1]Higher R630/R580 represents a redder meat color and lower R630/R580 represents a browner meat color. R630/R580 < 3.3 is considered too brown for consumer approval of lamb meat color (Khliji et al., 2010).

produced from 2007 to 2009 (Table 1). A 40 g muscle sample was excised from the caudal (lumbar sacral) aspect of the loin to determine intramuscular fat concentration. All subcutaneous fat and silverskin were removed before the sample was diced and stored at –20°C until freeze drying using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry, NZ). Intramuscular fat concentration (%) was determined using a near infrared procedure in a Technicon Infralyser 450 (19 wavelengths) via the method described by Perry, Shorthose, Ferguson and Thompson (2001) and was expressed as a percentage of wet tissue weight.

Small muscle portions were sampled from the caudal section of the loin and frozen for measurement of myoglobin, iron and zinc concentrations. Myoglobin concentration was determined using a 1-g sample excised, finely diced and stored at –20°C until analysis using the method of Trout (1991) and was expressed as mg per g of wet loin muscle weight. For iron and zinc, loin samples were frozen at –20°C and freeze-dried (Cuddon FD 1015, Cuddon Freeze Dry, Blenheim, New Zealand), before 0.2 g dry matter per sample was prepared according to the USEPA Method 200.3 (USEPA, 1991) for determination of iron and zinc concentration using a Vista AX CCD simultaneous ICP–AES (Varian Australia Pty Lt, Sydney, Australia). Iron and zinc concentrations are expressed as mg per 100 g of wet loin muscle weight.

Color sample collection and measurement

After excision of the loin muscle at around 24 h postmortem, a full cross-section at least 50 mm in length was cut from the cranial aspect of the loin muscle. This portion of muscle was originally located directly caudal to the 12th rib and was cut perpendicular to the long axis of the loin. The width of the sample was trimmed to 50 mm, while the depth of the muscle sample was determined by the depth of the whole loin muscle (around 30 mm). Samples were individually vacuum packaged using an 11 L/min vacuum (Orved Eco Vacuum pro), clear gas-impermeable packaging (20/80 to 100 microns, transparent polyamide air impenetrable exterior, polyethylene food approved interior, water vapor transmission rate measured at 23°C and 85% R.H– 2.6 gr/mq– 24hr, oxygen permeability measured at 23°C and 0% R.H– 50 cm^3/mq– 24hr– bar) and were stored within a dark chiller at 3 to 4°C for 5 d.

The muscle samples were briefly removed from the chiller for re-slicing, re-packaging and blooming within a boning room at 18 to 20°C, mimicking standard retail preparation of lamb meat. The loin muscle samples were removed from their vacuum packaging and re-sliced using a butterfly cut (perpendicular to the long axis of the loin muscle) to create a fresh meat surface at least 30 mm in length. The freshly sliced sample was re-packaged on black Styrofoam trays (12 × 12 cm) and wrapped with oxygen-permeable polyvinal chloride film (Resinite "DHW" Meat AEP, 15 μm thickness, oxygen transmission rate of 35,650 – 46,500 cc/m2 per 24 hr). The freshly cut meat surface was facing upward and in full contact with the overwrapped film. The samples were bloomed for 30 min before being placed under simulated retail display for 72 h. For the simulated display, samples were displayed on a flat horizontal surface in a walk-in chiller (3.8 × 3 x 4 m) set to 4°C with no defrost cycle. Temperatures within the chiller fluctuated between 2 and 6°C on these settings. An overhead light was suspended 1.5 m above the samples to provide a light intensity of 1,000 Lux, as measured with a Dick Smith Electronics Light meter Q1367. This light source consisted of 8 Nelson Fluorescent Meat Display BRB Tubes, 58 W and 1520 mm in length. A diffuser was fitted to the lights to achieve an even distribution of light and an actual color temperature of 4,000 K.

The color of each meat surface was measured within the chiller using a Hunterlab spectrophotometer XE Plus (Cat. No. 6352, model 45/0-L, aperture of 3.18 cm) held flush with each overwrapped meat surface. The light source was set at D65, the observer was set to 10° and the instrument was calibrated using black glass and white ceramic tiles according to Hunterlab (Hunter Associates Laboratory, Inc.) directions. The redness or red:brown color of a meat surface (R630/R580) is determined by the % of light reflectance at 630 nm/% reflectance at 580 nm (American Meat Science Association, 2012). Loin R630/R580 was measured twice at each time point across the simulated retail display (0, 24, 48, and 72 h). The 2 measures were captured at 90 degrees on the horizontal plane to each other and were averaged for statistical analysis.

Statistical analysis

Correlations between R630/R580 values at 0, 24, 48, and 72 h of simulated retail display were analyzed in SAS (SAS Version 9.1, SAS Inst. Inc., Cary, NC). Simple correlations were estimated using the PROC CORR command, and partial correlations were estimated using a multivariate analysis. In the multivariate analysis the correlations between R630/R580 values at different time points were corrected for fixed effects including site, year, slaughter group within site by year, and sex and dam breed within sire type. The same data set was used to calculate simple and partial correlations.

The correlations between R630/R580 time point measures were then tested in a multivariate analysis with different phenotypic covariates (pH_{24}, myoglobin, iron, ICDH activity, zinc, intramuscular fat, HCWT, relative shortloin muscle and fat weight, lamb age) accounted for in the model. Each phenotypic covariate was tested separately in each model, along with their squared term and interactions with fixed effects, before nonsignificant terms ($P > 0.05$) were removed. Lamb age, pH_{24} and intramuscular fat were also incorporated together, along with their interactions with fixed effects, while shortloin muscle and fat weight were always analyzed with HCWT to represent the impact of relative shortloin muscle and fat weight.

R630/R580 values at each time point (0, 24, 48, and 72 h) were then analyzed separately using linear mixed effects models. Fixed effects for site, year, slaughter group within site by year, sex and dam breed within sire type were included in these models along with random terms for sire and dam by year. Nonsignificant ($P > 0.05$) terms were removed in a step-wise fashion to form a base model for R630/R580 at each time point. These base models were then used to test the association between the phenotypic carcass covariates and each R630/R580 measure over simulated retail display. The phenotypic covariates were incorporated one at a time into the linear mixed effects models for R630/R580 at 0, 24, 48, and 72 h display, along with their squared term, interactions with fixed effects and random terms. Nonsignificant terms ($P > 0.05$) were removed to form a final model that was used to predict the mean R630/R580 across the covariate range. The magnitude impact of each covariate is determined by the unit change in predicted mean R630/R580 across the listed covariate range. The impact of lamb age at slaughter on R630/R580 was also regressed in a linear mixed effects model, however given the small variation in lamb age within slaughter groups and substantial variation in age between slaughter groups, in these models, slaughter group within site by year was fitted as a random term.

Linear mixed effects models were then used to assess the associations between R630/R580 values at different time points of simulated retail display. In these models fixed terms for production site, year and slaughter group within site by year were not included, given that this information is very unlikely to be known to a retailer interested in predicting lamb meat browning on display. R630/R580 at 0 h was incorporated as a covariate into the base models for R630/R580 at 24 and 72 h, along with its squared term and any interactions with sex and dam breed within sire type, with the same random effects and process of step-wise removal of nonsignificant effects (P

> 0.05). The same process was used to incorporate R630/R580 at 24 and 48 h separately as covariates into the model of R630/R580 at 72 h. These models were used to determine the coefficient of determination (R-Square) and root mean square error for each association.

These same models were then used to test the influence of phenotypic covariates (pH_{24}, myoglobin, iron, ICDH activity, zinc, intramuscular fat, HCWT, shortloin muscle and fat weight, lamb age) on the associations between R630/R580 at different time points. The covariates were incorporated one at a time into each model along with their squared term and interaction with sex and dam breed within sire type. Nonsignificant ($P > 0.05$) terms were removed. This process was then repeated with each phenotypic covariate also fitted with HCWT to ensure the observed association was not simply a reflection of carcass weight.

Given the large range in R630/R580 values measured during display (Table 1), the data set was limited to R630/R580 values within 3 standard deviations of the mean and the entire statistical analysis was repeated. Restricting the data set did not impact the results, therefore all R630/R580 data was retained in the analysis.

Results

The mean, standard deviation and range in lamb carcass and muscle traits including loin R630/R580 at 0, 24, 48, and 72 h of simulated retail display are shown in Table 1. Carcasses had an average loin muscle temperature of 3.1°C ($\pm$ 1.7) at 24 h postmortem when pH was measured in the loin.

Correlations between lamb loin R630/R580 at 0, 24, 48, and 72 h of simulated retail display

Lamb loin R630/R580 measured at 0, 24, 48, and 72 h of display were positively correlated. However, loin R630/R580 at 0 h had simple correlation coefficients of only 0.20, 0.18 and 0.10 with R630/R580 at 24, 48, and 72 h respectively (Table 2). In contrast, R630/R580 at 24 h had simple correlation coefficients of 0.82 and 0.80 with R630/R580 at 48 and 72 h display (Table 2). Partial correlation coefficients (that accounted for the fixed effects listed in Table 3) increased to 0.40, 0.41, and 0.32 between R630/R580 at 0 h and subsequent R630/R580 measures (Table 2). Accounting for the same fixed effects did not effectively increase the correlations between R630/R580 at 24, 48, and 72 h further (Table 2).

Incorporating each carcass trait (Table 1) into the multivariate models did not substantially alter the par-

Table 2. Simple (upper, above the diagonal) and partial (lower, below the diagonal) correlation coefficients between R630/R580 measures at each time point of simulated retail display

R630/R580	0 h	24 h	48 h	72 h
0 h	–	0.20	0.18	0.10
24 h	0.40	–	0.82	0.81
48 h	0.41	0.83	–	0.85
72 h	0.32	0.80	0.87	–

Table 3. Numerator and denominator degrees of freedom (NDF, DDF), F-values and P-values for the effects of the base linear mixed effects models for lamb m. *longissimus lumborum* R630/R580 at 0, 24, 48, and 72 h of simulated retail display[1]

Effect	NDF, DDF	F-values			
		0 h	24 h	48 h	72 h
Site	4, 776	23.7	88.4	153.7	86.1
Year	4, 3106	94.1	39.5	6.5	16.7
Slaughter group (site × year)	62, 776	58.2	49.1	40.5	43.1
Sex Dam breed (sire type)	5, 776	13.9	35.5	35.8	33.4

[1]All effects have a P-value < 0.001.

tial correlation coefficients between R630/R580 at 0, 24, 48, and 72 h. A maximum correlation coefficient of 0.40 was reached between R630/R580 at 0 and 72 h with incorporation of muscle iron concentration. Incorporating lamb age, intramuscular fat and pH_{24} simultaneously produced a correlation coefficient of only 0.36. The correlation between R630/R580 at 0 and 24 h was also maximized at 0.48 when loin iron concentration was accounted for, and reached 0.46 with incorporation of lamb age, intramuscular fat and pH24. The correlation coefficients between R630/ R580 at 24, 48, and 72 h did not increase by more than 0.04 when lamb age, intramuscular fat, pH_{24}, or any other carcass trait was accounted for.

The effect of animal production factors on R630/R580 at 0, 24, 48, and 72 h of simulated retail display

The influence of production effects (site and year of lamb production, slaughter group within site and year, lamb sex, sire type and dam breed) on loin R630/R580 after 72 h of retail display has been previously published (Calnan et al., 2014). The models for analyzing production effects on lamb loin R630/R80 measured at 0, 24, 48, and 72 h of display are outlined in Table 3. These models describe 62, 55, 58, and 58% of the variance in loin R630/R580 at 0, 24, 48, and 72 h of display.

Carcass trait effects on R630/R580 at 0, 24, 48, and 72 h of simulated retail display

The models described in Table 3 were used to test the association between carcass traits (Table 1) and each measure of R630/R580 across the 72 h simulated retail display (Fig. 1 and 2). The magnitude of impact of each carcass trait on R630/R580 is demonstrated across a standard range of two standard deviations from the carcass trait mean (Table 1, Fig. 1 and 2). Increasing loin pH_{24} across this range of 5.36 to 5.96 had the greatest magnitude impact on R630/R580 at 24, 48, and 72 h display (Fig. 1, $P < 0.0001$). While pH_{24} had a substantial effect on R630/R580 at 0 h, its impact on R630/R580 more than doubled from 0 to 24 h display (Fig. 1). Increasing myoglobin concentration of the loin from 3.13 to 9.73 g/100g muscle was positively associated with R630/R580 measured from 0 to 48 h of display, having the greatest magnitude impact on R630/R580 at 0 h of all traits measured (Fig. 1, $P < 0.0001$). However, the impact of myoglobin concentration on R630/R580 reduced with time on retail display, myoglobin having the smallest impact of all traits on R630/R580 at 48 h ($P = 0.004$) and no impact on color after 72 h display (Fig. 1, $P = 0.13$).

The iron concentration of the loin muscle was associated with R630/R580 only at 0 ($P < 0.0001$) and 72 h ($P = 0.0009$) of display (Fig. 1). Furthermore, the effect of increasing iron from 13.2 to 26.8 mg/100g of muscle differed substantially between these 2 time points (Fig. 1). Increasing ICDH activity was associated with reduced R630/R580 from 24 to 72 h of simulated retail display (Fig. 1, $P < 0.0001$), though did not influence R630/R580 at the start of retail display ($P > 0.05$). Increasing ICDH activity from 2.91 to 8.15 μmol/min per g of muscle had a very similar magnitude impact on R630/R580 from 24 h of retail display onward (Fig. 1). The zinc concentration of the loin muscle was not associated with R630/R580 at any time during the simulated retail display ($P > 0.05$). Inceasing intramuscular fat concentration of the loin from 2.08 to 6.12% slightly increased R630/R580 at the start of display ($P = 0.01$), though had a negative impact on all subsequent R630/ R580 measures (Fig. 1, $P < 0.02$). The magnitude impact of intramuscular fat was relatively small though increased with time on retail display (Fig. 1).

Increasing lamb HCWT from 16.0 to 29.5 kg increased loin R630/R580 by a similar magnitude throughout the simulated retail display (Fig. 2, $P \leq 0.0002$). Increasing relative shortloin muscle weight from 169 to 551 g (reflecting increased carcass muscling) and relative shortloin fat weight from 19 to 323 g (reflecting increased carcass fatness) increased R630/R580
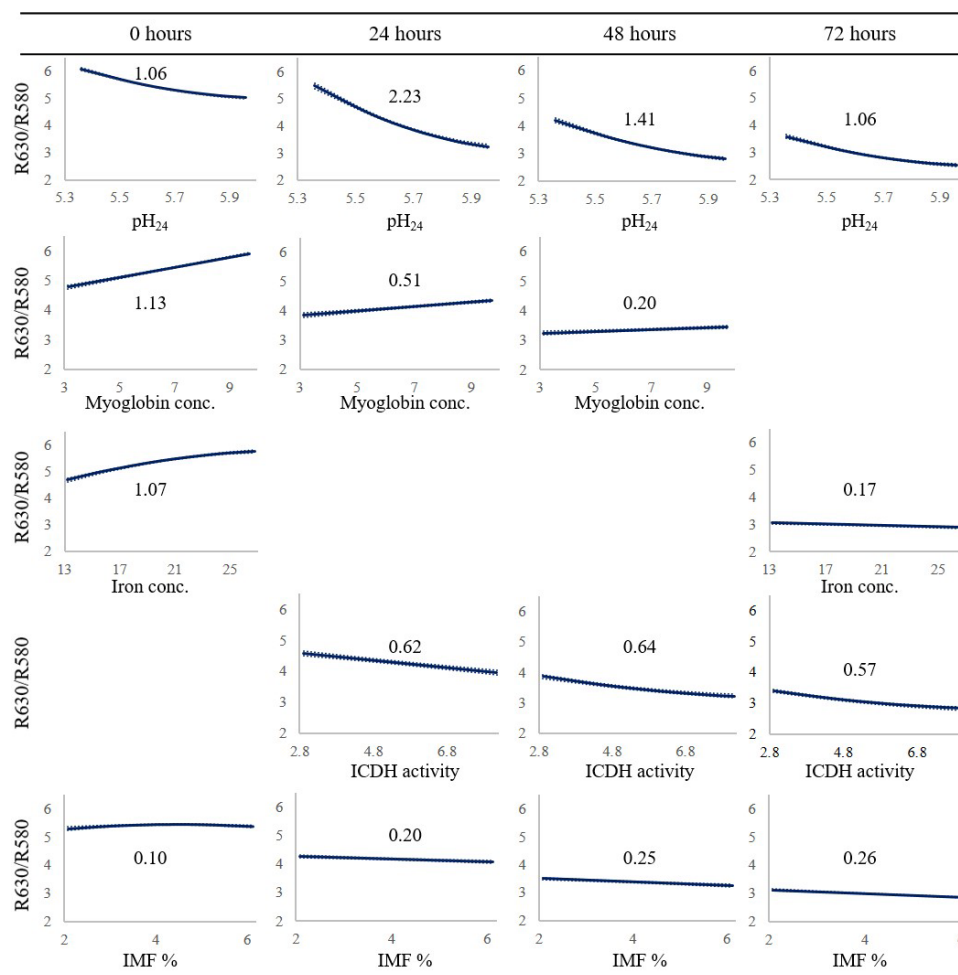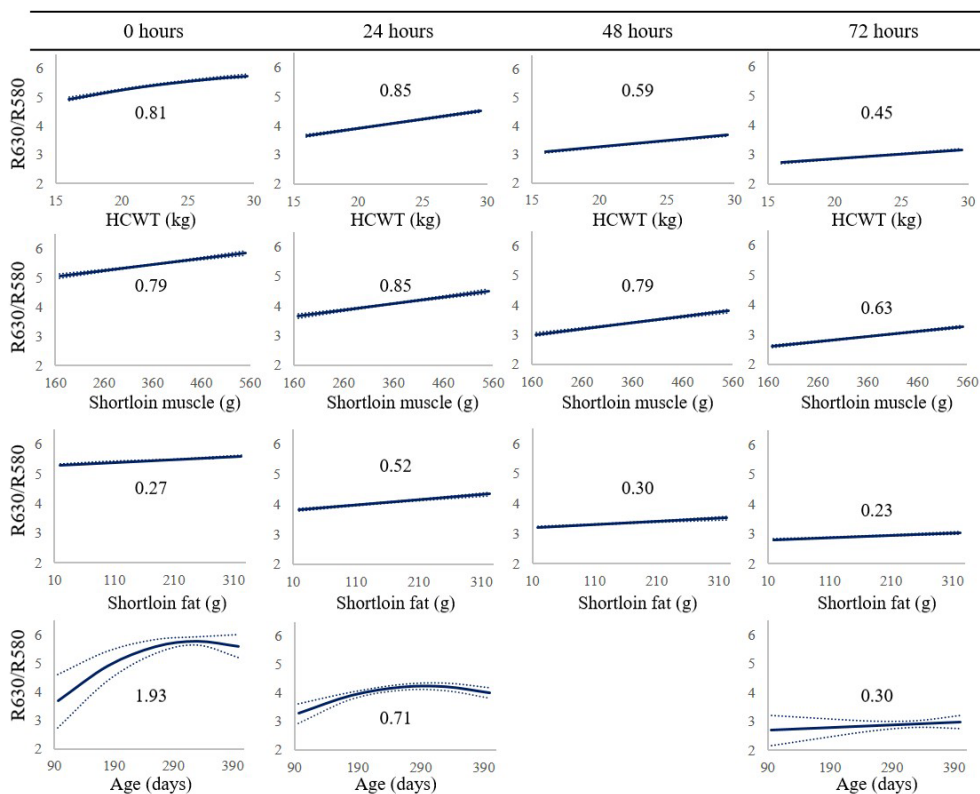
Figure 1. Association between loin R630/R580 (at 0, 24, 48, and 72 h of display) and loin muscle covariates: pH$_{24}$, myoglobin concentration (mg/g wet muscle), iron concentration (mg/100g wet muscle), ICDH or isocitrate dehydrogenase activity (μmol/min/g of wet muscle) and IMF or intramuscular fat %. Solid lines within figures represent the predicted means, dotted lines represent the standard error of the mean (often too small to distinguish dotted lines clearly). The number above each figure represents the unit change in R630/R580 across a covariate range of 2 standard deviations from the mean. All associations represented in this figure are statistically significant ($P < 0.05$).

throughout display (Fig. 2, $P \leq 0.0012$). However, relative shortloin fat weight had only around one third the magnitude of impact of relative shortloin muscle weight for all display times except 24 h (Fig. 2). Increasing lamb age from 96 to 400 d increased R630/R580 substantially at 0 h display ($P < 0.0001$), moderately at 24 ($P = 0.01$) and at 72 h display ($P = 0.04$), yet did not influence R630/R580 at 48 h display (Fig. 2, $P > 0.05$).
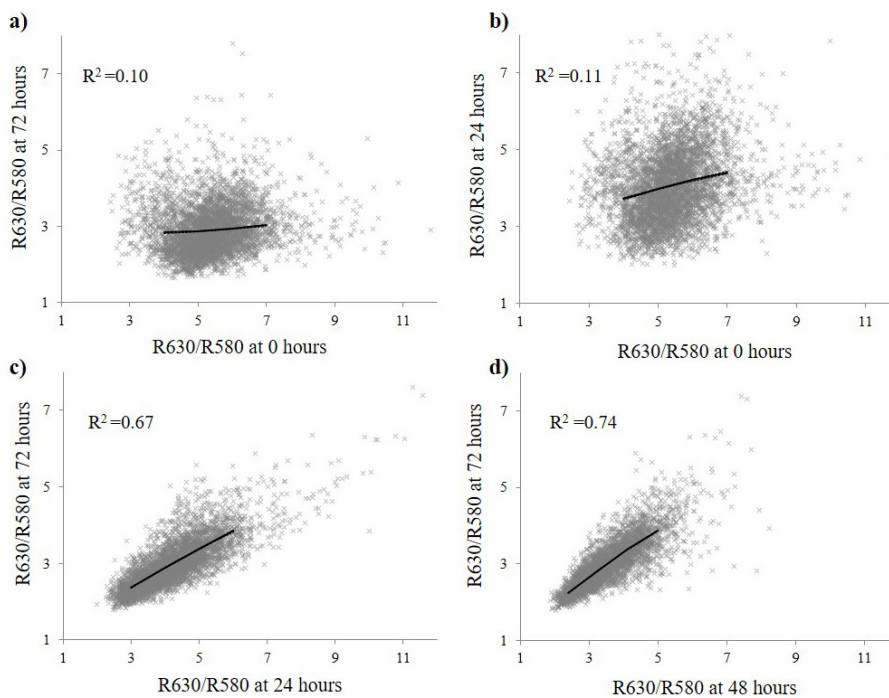
Associations between lamb loin R630/R580 at 0, 24, 48, and 72 h of simulated retail display and the influence of carcass traits on these associations

The associations between (a) R630/R580 at 0 and 72 h, (b) R630/R580 at 0 and 24 h, (c) R630/R580 at 24

and 72 h, and (d) R630/R580 at 48 and 72 h are shown in Fig. 3 ($P < 0.0001$). The coefficients of determination (R-Square) are shown above each association, ranging from 0.10 between R630/R580 at 0 and 72 h, up to 0.74 between R630/R580 at 48 and 72 h of display (Fig. 3). The R-Square and root mean square error of these associations did not change substantially when carcass traits were accounted for in the models (Table 4). The R-square of the association between R630/R580 at 0 and 72 h increased from 0.10 up to 0.18 with incorporation of myoglobin, iron or intramuscular fat concentration, and up to 0.20 with incorporation of relative shortloin fat weight (Table 4). Though incorporation of ICDH activity appeared to have the largest impact increasing the R-square of the association between R630/R580 at 0 and 72 h to 0.22, this is due to ICDH activity being measured in only a subset of lambs, those

Figure 2. Association between loin R630/R580 (at 0, 24, 48, and 72 h of display) and lamb carcass covariates: HCWT or hot carcass weight (kg), shortloin muscle weight (g) relative to carcass weight (representing carcass muscling), shortloin fat weight (g) relative to carcass weight (representing carcass fatness), and lamb age in days. Solid lines within figures represent the predicted means, dotted lines represent the standard error of the mean (often too small to distinguish dotted lines clearly). The number above each figure represents the unit change in R630/R580 across a covariate range of 2 standard deviations from the mean. All associations represented in this figure are statistically significant ($P < 0.05$).

Figure 3. Associations between loin R630/R580 at a) 0 and 72 h of display; b) 0 and 24 h display; c) 24 and 72 h display and d) 48 and 72 h display. Solid lines represent predicted means, dashed lines represent the standard error from the predicted mean (standard error too small to distinguish dotted lines clearly) and individual points represent the residuals values of the lambs (difference of raw value from the predicted mean). The coefficient of determination (R-Square or R^2) for each association is shown above the graph.

Table 4. The influence of carcass traits on the coefficient of determination (R-square) and root mean square error (RMSE) of the associations between R630/R580 at different time points on simulated retail display

Carcass traits added to R630/R580 associations	R630/R580 at 0 and 72 h		R630/R580 at 0 and 24 h		R630/R580 at 24 and 72 h		R630/R580 at 48 and 72 h	
	R-Square	RMSE	R-Square	RMSE	R-Square	RMSE	R-Square	RMSE
R630/R580 association:	0.10	0.64	0.11	1.00	0.67	0.39	0.74	0.35
Carcass trait incorporated:								
pH_{24}	0.17	0.62	0.18	0.96	0.67	0.39	0.74	0.35
Myoglobin, mg/g muscle	0.18	0.64	0.15	1.01	0.71	0.38	0.78	0.33
Iron, mg/100g muscle	0.18	0.64	0.14	1.02	0.71	0.38	0.77	0.34
Isocitrate dehydrogenase activity, µmol/min/g[1]	0.22 (0.20)	0.66 (0.66)	0.16 (0.15)	1.02 (1.03)	0.72 (0.71)	0.39 (0.40)	0.78 (0.77)	0.35 (0.36)
Zinc, mg/100g muscle	0.17	0.65	0.14	1.02	0.67*	0.39*	0.77	0.34
Intramuscular fat, %	0.18	0.64	0.15	1.01	0.71	0.38	0.77	0.34
Hot carcass weight, kg	0.13	0.63	0.16	0.97	0.68	0.38	0.74	0.35
Shortloin muscle weight, g[2]	0.14	0.63	0.18	0.96	0.68	0.38	0.74	0.35
Shortloin fat weight, g[2]	0.20	0.61	0.22	0.94	0.68	0.38	0.74	0.35
Age, d	0.13	0.63	0.13	0.99	0.69	0.38	0.78	0.32

*Zinc did not impact the association between R630/R580 at 24 and 72 h ($P > 0.05$). All other values represent a statistically significant ($P < 0.05$) effect of the carcass trait on the R630/R580 association.

[1]Isocitrate dehydrogenase activity was measured in only 1,804 lambs in this study. R-square values and RSME of the association between R630/R580 measures within this subset of lambs are shown in brackets.

[2]Shortloin muscle and fat weights were analyzed with hot carcass weight accounted for in the model to examine the influence of relative shortloin muscle weight (muscling) and relative shortloin fat weight (fatness) on R630/R580.

produced in 2007 and 2008 (Table 1). In this subset of lambs, the R-square of the association between R630/R580 at 0 and 72 h with no covariates accounted for was 0.20 rather than 0.10 (Table 4).

Discussion

Contrary to our hypothesis, measuring the bloomed color (R630/R580) of loin meat at the start of retail display could not accurately predict meat browning after 3 d of retail display, even when used in combination with measurement of lamb age, loin pH_{24} and intramuscular fat concentration. This was shown in a number of ways. First through the poor correlations between lamb loin R630/R580 measured at the start of display with all subsequent R630/R580 measures across the 3 d simulated display (Table 2). When these correlations were adjusted for fixed production effects (site and year of lamb production, slaughter group within site and year, sex and dam breed within sire type), or for the phenotypic carcass covariates (lamb age, pH24, intramuscular fat, iron and myoglobin concentrations, ICDH activity, HCWT, relative shortloin muscle or fat weight) they were stronger, but not to the level that would suggest that they would be robust predictors of subsequent meat color at 24, 48 or 72 h display (Table 2). Second, through the general linear

model associations between R630/R580 at time 0 versus the later time points (Fig. 3), which described just 10 to 11% of the variation in meat color and weren't considerably improved with the inclusion of fixed production effects or phenotypic carcass covariates in the model (Table 4). This suggests that even with considerable knowledge of animal background and carcass phenotype traits, there is little capacity to use bloomed meat color measured at the start of retail display to then predict meat color at 3, 2, or even 1 d into retail display. This might suggest that blooming takes longer than 30 min to complete or that oxidative metabolism is less important in lamb meat than beef in relation to the variation in color stability.

In contrast to this, there were strong correlations between R630/R580 at 24, 48, and 72 h display, that varied little when corrected for fixed production factors or carcass phenotype measurements. Furthermore R630/R580 at both 24 and 48 h were strongly associated with R630/R580 at 72 h (Fig. 3). In these models where only lamb sex and breed type were accounted for, R630/R580 measures at 24 and 48 h described 67 and 74% of the variation in meat color at 72 h display. While R630/R580 at 24 and 48 h described up to 72 and 78% of 72 h color with the inclusion of carcass phenotype measurements (Table 4). These results suggest that even with no prior knowledge of animal information, meat color measurements taken at 24 h of

retail display represent color change due to metmyoglobin formation and thus would be useful to predict meat color at 48 h display and beyond.

The ability of 24 h meat color rather than initial bloomed color to predict retail meat browning suggests that key factors determining retail meat browning are not manifesting their effects until some point between initial meat blooming and 24 h post-oxygenation. Generally the greatest difference in individual covariate effects on R630/R580 at 0, 24, 48, and 72 h were observed between 0 and 24 h of display (Fig. 1 and 2). For example, lamb age and pH_{24} had substantially different associations and impacts on R630/R580 at 0 h compared to measures taken 24 h later. The magnitude of the impact of increasing pH_{24} on R630/R580 more than doubled from 0 to 24 h display. This suggests that the full effect of pH_{24} on meat redness was not apparent until 24 h into retail display. The different effects of carcass traits on R630/R580 at 0 and 24 h likely relates to these time points representing different meat color traits- the initial development of meat redness with blooming and the subsequent deterioration in meat redness with browning on retail display. Meat pH24 is an important determinant of both these color traits (American Meat Science Association, 2012), though it exerts its influence on initial meat color and on retail browning via different (though interrelated) biochemical mechanisms (Faustman and Cassens, 1990). Increasing pH reduces meat lightness and redness due to changes to the physical structure of meat obstructing oxygen diffusion and due to increased oxygen consumption reducing oxygen availability to bind with myoglobin (Hopkins et al., 2013). Whereas the negative influence of pH on lamb meat color stability is thought to relate to free radical oxidative species that accumulate with postmortem metabolism and trigger metmyoglobin formation (Kropf, 1993). The effects of pH on meat color are incompletely understood and the effect of pH on beef color stability may differ from the effect of pH on lamb color stability.

The effects of muscle myoglobin, iron and ICDH activity on R630/R580 across display may also relate to the different mechanisms underpinning bloomed meat color development and subsequent meat browning on retail display. The substantial effect of myoglobin concentration on R630/R580 at 0 h aligns with other work (McKenna et al., 2005) and suggests the amount of pigment is the most important determinant of bloomed meat color due to less variation in the form of myoglobin present at this time (American Meat Science Association, 2012). Reflectance at 630 nm represents maximal reflection of the red pigment oxymyoglobin while reflectance at 580 nm is the reflectance minimum for pure oxymyoglobin (American Meat Science Association, 2012). The other two myoglobin forms that determine surface meat color, purple deoxymyoglobin and brown metmyoglobin are also reflected at these wavelengths to an extent; with moderate deoxymyoglobin reflection at 630 nm, while metmyoglobin is the principle pigment reflected at 580nm. The reduced impact of myoglobin on R630/R580 at 24 h onward suggests the relative concentrations of the different forms of myoglobin (deoxymyoglobin, oxymyoglobin and metmyoglobin) vary more at these times than at the start of display, so the amount of myoglobin pigment is less predictive for redness from 24 h on retail display (Calnan et al., 2014; McKenna et al., 2005; O'Keeffe and Hood, 1982). These results aligns with previous research reporting myoglobin concentration to have a small negative effect (King et al., 2010) or no effect on beef color stability (McKenna et al., 2005). The close association between myoglobin and iron concentration in muscle may in turn account for the moderate influence of iron on R630/R580 at the start of display, its lack of effect at 24 and 48 h of display, and the small effect of iron at the end of display when the free-radical byproducts of oxidative metabolism accumulate and trigger myoglobin oxidation (Faustman and Cassens, 1990). ICDH activity is more closely associated with oxidative metabolism (Brandstetter et al., 1998) than myoglobin pigmentation, which may explain its failure to influence initial R630/R580 measures and its moderate effect on subsequent meat color across display. As with pH_{24}, the influence of myoglobin, iron and ICDH activity on bloomed meat color development and subsequent meat browning are interrelated and difficult to fully distinguish.

Meat color measured shortly after blooming and 24 h later representing different meat color traits may account for the weak correlations between R630/R580 at 0 h and all subsequent measures of R630/R580, and the strong correlations between R630/R580 at 24, 48, and 72 h of display. Based on these results, measuring meat color at 24 h display could provide an excellent prediction of meat browning in the subsequent days of retail display. However, measuring meat color after 24 h of display is unrealistic in a commercial retail setting. Having only measured R630/R580 at 24 h intervals in this study, the possibility remains that R630/R580 measured earlier in retail display could better predict subsequent retail meat browning. For example, measuring R630/R580 6 h post-blooming may prove more feasible in a retail setting. Until work has been undertaken to determine if R630/

R580 measured between 0 and 24 h could accurately predict meat browning, the value of R630/R580 may be limited to the use of 24 h measures in driving genetic improvement of lamb meat color stability via the development of a retail color breeding value for lamb meat.

Conclusions

Despite common mechanisms influencing bloomed lamb meat color and its stability over retail display, initial bloomed loin color was poorly correlated with subsequent meat color measures over a 72 h simulated retail display. Accounting for key muscle traits influencing meat color such as pH_{24}, myoglobin, iron or intramuscular fat concentration did not substantially increase the correlations between bloomed meat color and subsequent meat color measured over retail display. The influence of carcass traits on meat color differed over the simulated retail display, initial bloomed meat color being principally determined by the concentration of myoglobin pigment present, while meat color after 72 h display was influenced by factors such as pH and ICDH activity, presumably due to their effects on myoglobin oxidation. Measuring bloomed meat color along with key carcass traits is therefore unlikely to be a useful tool for predicting color stability in a commercial application. In contrast, meat color measured at 24 h display can accurately predict the extent of lamb meat browning at 2 to 3 d of retail display. While measuring meat color after 24 h on display is not practical in a retail setting, these measures may prove valuable to the lamb industry in the development of a retail color breeding value to reduce the rate of lamb meat browning on retail display.

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